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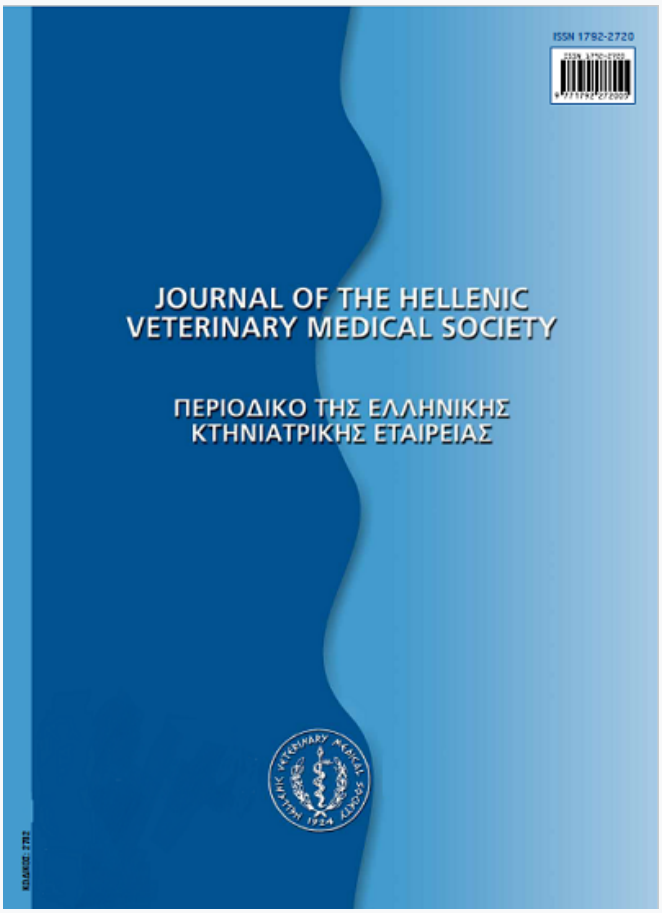

Aflatoxin M1 in Nili-ravi buffaloes and its detoxification using organic and inorganic toxin binders

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Short communication
Σύντομη δημοσίευση

Aflatoxin M1 in Nili-ravi buffaloes and its detoxification using organic and inorganic toxin binders

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ABSTRACT. The present study had two objectives: first, to determine the carry over or excretion percentage of aflatoxin B1 (AFBI) in milk in form of aflatoxin M1 (AFM1) and second, to assess the reduction in excretion of AFM1 in milk using different organic and inorganic toxin binders available in Pakistani market. Lactating Nili-Ravi buffaloes (n=16) were randomly selected and were divided into four treatment groups designated as A, B, C and D. In each treatment 500 µg/Kg of aflatoxin B1 (AFB1) was fed along with no sequestering agent added (control); and three toxin binders: Fixar Viva in group B, Mycosorb in group C and T5X in group D. These toxin binders were added at concentration of 0.25% of dry matter intake of animal. It resulted in 2.13% carryover in milk as AFM1. A significant reduction ($P<0.05$) in dry matter intake, milk production, milk fat and protein percentage was also observed by feeding AFB1. Addition of three toxin binders Mycosorb, Fixar Viva, and T5X at a concentration of 0.25% in ration resulted in 47%, 39%, and 35% reduction in AFM1 secretion respectively. The present study also indicated that percentage carryover of AFM1 in buffaloes is higher than that reported in lactating cows as well as in goats and Mycosorb is capable of reducing the excretion of AFM1 into milk by improving the dry matter intake, milk production and protein contents. These findings may be applicable in field to reduce AFM1 release in milk of Nili-Ravi buffaloes.

Keywords: Aflatoxins, Buffaloes, Carry-over percentage, Milk, Toxin binders

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INTRODUCTION

Aflatoxins are secondary metabolites produced by *Aspergillus flavus* and *Aspergillus parasiticus*. The most abundant aflatoxin in naturally contaminated dairy rations is aflatoxin B1 (Soufiani et al., 2016). The occurrence of aflatoxin in feed and feed commodities is more common in countries like Pakistan where temperature and humidity are higher due to optimum growing conditions for molds. Aflatoxins cause liver damage, decrease growth rate and milk production by deteriorating its quality. After ingestion in lactating animals aflatoxin B1 (AFB1) is metabolically bio-transformed by cytochrome P enzyme into hydroxylated form "Aflatoxin M1" that is excreted in milk (Chohan et al., 2016). Contamination of aflatoxin M1 in milk is a matter of serious concern due to its carcinogenic activity as reported by International Agency for Research on Cancer (IARC). The level of AFM1 is also not reduced during pasteurization due to its heat stability; therefore it remains in UHT milk (Diaz et al., 1995) and milk products. Approximately 0.3-6.2% of AFB1 in animal feed is transformed to AFM1 in milk but this carry over may be affected by different factors such as animal species involved. As almost all the previous studies involve cows (Iqbal and Asi, 2013), sheep or goat as experimental animals but not a single study had been conducted on buffaloes that is contributing to 75% of Pakistan's milk yield. Therefore we need to investigate the carry-over percentage of AFM1 in Nili-ravi buffaloes that are the main source of milk in our country.

Moreover, due to high potential risks to human health and economic losses of dairy industry associated with aflatoxins, World Health Organization (WHO) recommends their detoxification to minimum level. Use of toxin binders is reported to be a good practicable method for aflatoxin decontamination. Currently a number of toxin binders for aflatoxins are commercially available in Pakistan. However, there is need to evaluate their effectiveness on carry-over reduction of AFB1 to AFM1 in milk.

Keeping in view the above facts the present study was designed with two objectives; first to determine the excretion percentage of AFM1 in lactating buffaloes and second to uncover the effect of organic and inorganic toxin binders (commercially available in Pakistan) on detoxification of aflatoxin B1 in these animals. The conclusion and recommendations drawn from this study will be helpful for policy makers to implement a strict regulation on AFB1 in food and feed to reduce or avoid the contamination of AFM1 in milk and dairy products.

MATERIALS AND METHODS

Production of AFB1

To harvest AFB1, *Aspergillus flavus* strain NRRL 2999 culture was used as inoculant according to method described by Shotwell et al. (1966). Briefly, 30g of polished rice and 10 mL of distilled water was added to a 300 mL Erlenmeyer flask, autoclaved at 15 psi for 15 min and cooled down to room temperature, and inoculated with culture for fermentation. Further, flasks were placed on an orbital shaker with 130 revolution/min for 10-14 days at 25°C. This process was repeated again and again until required amount of aflatoxin was obtained. Rice was harvested when its color turned brown.

Quantification of AFB1

Brown color fermented rice were collected and mixed in 60 mL of chloroform for 30g of fermented rice. Extraction was made by refluxing for 4hrs and then extract was filtered through cheesecloth. Refluxing was conducted three times to recovered maximum quantity of aflatoxin B1. Then filtrate was dried over thin layer chromatography plate and was transferred in chromatography tank. Methanol (3mL) was added in 97 mL of chloroform and was transferred in tank along with chromatography plate (Gallo et al., 2010). Plates were inspected for aflatoxin B1 in a chromate-viewer to find the components by fluorescence. Quantification of aflatoxin B1 was determined by visual comparison of fluorescence zone with the known quantity of zone formed by the standards of aflatoxin. Aflatoxin B1 was separated by scratching the chromatogram. This was stored in freezer at-20°C to prevent its breakdown.

Experimental design

The experiment was carried out in Livestock Experiment Station, Haroonabad, Pakistan. Lactating Nili-Ravi buffaloes (n=16) were randomly selected and were divided into four treatment groups designated as A, B, C and D. In each treatment 500 µg/Kg of aflatoxin B1 (AFB1) was fed along with no seques-

tering agent added (control); and with three toxin binders: Fixar Viva in group B, Mycosorb in group C and T5X in group D. These toxin binders were added at concentration of 0.25% of dry matter intake of animal. Experiment was divided in two phases. First phase was pre-experimental (6 days) and second was experimental phase (28 days). During the pre-experimental phase buffaloes were fed with ration containing no AFB1 in each treatment. This was done to assure the complete removal of residual aflatoxin M1 in the milk as previous studies show the removal of AFM1 in 3-4 days after withdrawing AFB1 feeding (Kangethe and Langa, 2009). During the experimental phase all lactating buffaloes were offered AFB1 contaminated ration containing Fixar Viva, Mycosorb and T5X at 0.25% of dry matter in treatment B, C and D respectively. Group A was positive control and no toxin binder was added in it. Daily dry matter intake and milk production were recorded. A composite sample was collected from morning and evening milk collection after every 5th day of experiment. A total of 112 samples were collected and were stored in freezer until analyzed for AFM1, milk protein%, milk fat%, lactose% and solid not fat (SNF%). AFM1 was analyzed by competitive ELISA method using Helica Inc. kit.

Milk sample preparation

Half of milk sample was defatted by removing the fat layer after centrifuging samples at 4000 rpm. These defatted samples were analyzed for AFM1 using Enzyme Linked Immunosorbent Assay (ELISA) using microtitre plates (Helica Inc. Pakistan). A total of 112 samples were analyzed for AFM1. Plates were read by using ELISA reader ELX800. Half of the milk was used for analysis of Milk fat%, Protein%, Lactose% and solid non fat% (SNF%) by proximate analysis as described in AOAC 16th edition.

Statistical analysis

Treatment differences were identified by one-way analysis of variance (ANOVA) by the general linear model procedure of SPSS. Statistical significance was considered for $P < 0.05$.

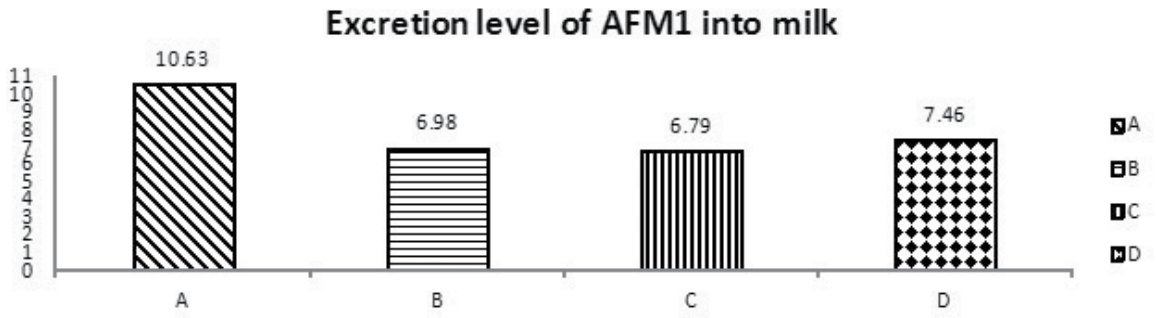
RESULTS AND DISCUSSION

Carry-over percentage of Aflatoxin M1

In pre-experimental phase there was a non significant difference in excretion of AFM1 in all groups. On the other hand at 5th day of experimental phase there was increased excretion of AFM1 in all groups. The highest excretion of AFM1 was observed in control group A (10.031 ±0.29), while in remaining groups AFM1 excretion was lower than control group and minimum AFM1 was observed in group C containing Mycosorb (6.621 ±0.22). On 10th day of experimental phase the level of AFM1 excretion further increased in all groups except in group C where the level of AFM1 did not increase significantly (6.828 ±0.39). While on 15th day of experimental phase a significant decrease in AFM1 excretion was observed in group C (6.73±0.39). This decrease continued on day 25th and 28th of experimental phase with significant values in group C as compared to other groups. Percentage reduction of AFM1 in group B, C and D was 39%, 47% and 35% respectively. However results indicated that this decrease in AFM1 in group B and D was not much significant (Figure 1).

Our results also indicated that a commercially available toxin binder called Mycosorb that pertain glucomannan resulted in increased percentage

Figure 1



reduction of AFM1. Glucomannan is a cell wall derivative of *Sacchromyces cerevisiae* that acts as organic toxin binder in minimizing the adverse effects of aflatoxins. This is in accordance with Murthy and Devegowda (2004) who reported that modified glucomannan readily adsorb several mycotoxins better than inorganic binders in broilers. Similarly a 36 % reduction in carry over from AFB1 in feed to AFM1 in milk was reported in an *in vivo* study by Galvano et al. (1996). Akhtar et al. (2014) also reported its better efficacy in terms of improved hematological and biochemical parameters.

However, our results contradict the findings of Mojtahedi et al. (2013) who reported that inclusion of Mycosorb up to 36g/d (3 times more than recommended dosage) was not effective in reducing AFM1 concentrations in lactating Holstein cows. Similar results were published by Kissell et al. (2013) who described non efficacy of Mycosorb in reducing AFM1 milk concentrations in dairy cows by feeding 10gm Mycosorb per cow per day. This may explain the differential action of organic compounds (glucomannan) in cows and buffaloes.

Our results indicated 2.13% carry-over of aflatoxin M (AFM1) in milk when 500 µg/Kg of aflatoxin B1 was given. This conversion of AFB1 into AFM1 is in accordance with VanEgmond, (1989) who reported the carryover ratio in a range of 1- 4% in cows. However our values were less than that reported by Britzi et al. (2013) in high producing Israeli-Holstein dairy cows. They described a carryover percentage of 5.8% and 2.5%. This may be due to the amount of aflatoxin given as they gave feed containing ~86 µg AFB1 for 7 days. Similarly a number of other factors including species, production level, season as well as feeding and milking routines of animals may affect the carryover percentage of AFM1 (Veldman et al., 1992; Coppock and Christian, 2007; Hussain et al., 2010). Moreover, the differences in ruminal fluid composition of cows and buffaloes may be responsible for its differential carryover in both species. As Upadhaya et al. (2010) reported that aflatoxin B1 degradation in rumen fluid was influenced by the species of animal and types of forage fed to the animals. This may be due to differential biotransformation of aflatoxin in rumen of cattle and buffaloes.

Effect of AFM1 on dry matter intake and milk production

Highest dry matter intake (DMI) and milk production was observed in group C (given Mycosorb) as compared to control and other treatment groups (Table 1). This may be due to organic nature of Mycosob in comparison to Fixer Viva (inorganic) and T5X (mixture of both organic and inorganic). This may be increased efficacy of Mycosorb (containing glucomannan) that improves digestibility and intake in lactating animals (Wohlt et al., 1991).

Effect of AFM1 on milk components

The results of present study indicated that use of Fixar Viva and T5X did not improve protein and fat contents of milk in group B and D. While on other hand, the feeding of Mycosorb significantly increased milk protein and fat contents in group C. This is not in line with the studies of Diaz-Llano and Smith (2006) and Korosteleva et al. (2007) that described no effect of glucomannan mycotoxin absorbent (GMA) on milk composition in dairy cows and lactating sows. This shows a major role of species involved for the effects of toxin binders. In addition, there was not

Table 1. Milk Production and Carry-Over Percentage of Aflatoxin M1 and Its Reduction by Using Toxin Binders in Experimental Groups

Treatments	Toxin Binder (gms) Gm/buffalo/day	Aflatoxin B1 (µg/buffalo/day)	Aflatoxin M1 (µg/kg)	Milk production (kg/day)	Aflatoxin M1 secretion	Carryover %age	Percentage reduction
Group A	0gms	500	1.76	6.04	10.64[a]	2.13%	
Group B	30gms	500	1.07	6.47±0.75	6.98[c]	1.39%	39%
Group C	30gms	500	0.93	7.25±1.33	6.79[b]	1.35%	47%
Group D	30gms	500	1.13	6.70±0.81	7.46[c]	1.49%	35%

Table 2. Percentages of Various Milk Components in Experimental and Control Groups

Parameters	Group A	Group B	Group C	Group D
Dry matter Intake	12.04 ±0.71[a]	12.30 ±0.30[b]	12.83 ±0.28[c]	12.42 ±1.05[b]
Milk Production	6.04 ±0.95[a]	6.47±0.75[a]	7.25± 1.33[b]	6.70 ± 0.81[a]
Protein % in milk	3.61±0.34[a]	3.66±0.63[ab]	3.94±0.35[b]	3.72±0.29[ab]
Fat % in milk	5.37±0.72[a]	5.31±0.76[ab]	5.56±.75[b]	5.56± 0.45[ab]
Lactose % in milk	5.20±0.54[a]	4.96±0.84[a]	5.12±0.35[a]	5.13±0.27[a]

a significant difference in lactose percentage of all groups (Table 2).

CONCLUSIONS

The findings of present study indicate that percentage carryover of AFM1 in buffaloes is higher than that reported in lactating cows as well as in goats and Mycosorb is capable of reducing the excretion of AFM1 into milk by improving the dry matter intake, milk production and protein contents. These findings may be applicable in field to reduce AFM1 release in milk of Nili-Ravi buffaloes.

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