RESEARCH ARTICLE

Ameliorating Effects of Mycotoxcease (Mycotoxin Binder) on Aflatoxin M1 Excretion in Dairy Cows

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Abstract

This study was conducted on 16 crossbred cows to evaluate the effects of mycotoxcease on aflatoxin M1 excretion in milk, nutrient intake and feed efficiency. The cows were divided into two groups based on milk yield, days in milk and parity in T1 (Control) and T2 (Mycotoxcease). The total mixed ration-TMR was fed *ad libitum* to both groups in the morning and afternoon. The T2 cows were fed 50 g of toxin binder in equal halves after mixing with 500 g of concentrate and the same quantity without toxin binder was fed to T1 cows. The dry matter, crude fat, crude protein, and metabolizable energy intake were significantly lower in T2 group. The milk and milk component yield were not affected by toxin binder supplementation. The aflatoxin M1 excretion was 50% lower in T2 than T1 group. Bioconcentration and carry-over percentage were also effectively reduced in T2 group. The feed efficiency and cost of feeding were lower in T2 group. The supplementation of 50 g/day/cow toxin binder reduced aflatoxin M1 excretion, and nutrient intake, with improved feed conversion ratio and lower cost of feeding.

Key words: AFM1 excretion, Crossbred cows, Economics, Feed intake and efficiency, Mycotoxin binder.

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Introduction

Mycotoxins are secondary metabolites of fungal genera like *Aspergillus*, *Fusarium*, and *Penicillium* Iq. Mycotoxins are a group of toxins produced by fungus that contaminate the feed and fodder of dairy cows, posing risks to both animal health and the safety of dairy products for human consumption. The types of mycotoxins are aflatoxins, ochratoxin A, fumonisins, zearalenone, deoxynivalenol (DON), T2 toxin and others are the major ones found in feeds and fodder of dairy cows. Mycotoxins in feed reduce feed intake, nutrient absorption, change nutrient metabolism, and suppress the immunity. Some mycotoxins, like aflatoxins and ochratoxins, can pass through the digestive system of cows and get excreted into their milk, potentially affecting consumers' health. The aflatoxin is further subdivided into B1, B2, G1, and G2, subgroups that are found in feeds, of which aflatoxin B1 is more potent toxic to animals. A certain quantity of AFB1 in the rumen degrades and transforms to aflatoxicol by the rumen microbiota (Tolosa *et al.,* 2021). The remaining quantity is absorbed through passive diffusion in the small intestine, reaching the liver through red blood cells and plasma proteins.

The liver converts aflatoxin to a few metabolites, which are used as biomarkers of animal exposure to mycotoxin and also to evaluate the adsorbent capacity to bond the mycotoxin in a particular time interval (Tolosa *et al.,* 2021). Aflatoxin B1 is hydroxylated in the liver through the cytochrome P450 (Cyt P450) enzyme system to aflatoxin M1, which is excreted in milk along with its presence in blood, milk, tissues, and biological fluids (Tolosa *et al.,* 2021; Campagnollo *et al.,* 2016). Aflatoxin B1 is oxidized to AFB1-8,9-epoxide in the liver and reacts with ribonucleic acids, deoxy ribonucleic acids, and liver proteins, producing hepatocellular carcinomas and liver toxicity. This epoxide is converted into less toxic metabolites like aflatoxin M1, aflatoxin Q1, or aflatoxin P1 (Aslam *et al.,* 2014). The other metabolites excreted in the milk are aflatoxin M2 and M4 at quite low concentrations. Rumen microbiota detoxifies

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mycotoxin but beyond the capacity will appear in milk as aflatoxin M1. In a recent study, the aflatoxin B1 consumed with contaminated feed was excreted as M1 in milk within a few hours (Guo *et al.,* 2021). Thus, aflatoxin M1 content in milk is considered an indicator of the binding capacity of toxin binder. With this background, the experiment was planned to evaluate the effect of mycotoxin binder (Mycotoxcease) supplementation on nutrient intake, body weight changes, milk yield, milk components yield, aflatoxin M1 excretion in milk, feed conversion, and cost of feeding in crossbred cows.

Materials and Methods

Following approval of the IAEC, the experiment was carried out at Livestock Research Station, Veterinary & Animal Science Research & Extension Unit, Kamdhenu University, Anand (Gujarat, India) for 21 days after adaptive feeding of ten days during hot-humid climate of May-June 2024. During the adaptive phase, all cows were fed a similar total mixed ration without supplementation of toxin binder. The toxin binder - Mycotoxcease - is a blend of Hydrated Sodium Calcium Alimunosilicate (HSCAS), Organic acids, and Mannan Oligo Saccharides - MOS supplied by the Vetmin Nutritions Pvt Ltd, Anand (Gujarat).

A total of 16 crossbred (75% Holstein Friesian x 25% Kankrej) cows were randomly allotted to Control and Treatment groups (p>0.05) in a Completely Randomized Design, with similar initial milk production (13.4±0.92 and 12.7±1.35 kg), days in milk (78.3±6.71 and 77.0±6.08), and lactation number (1.0±0.00 and 1.1±0.13). A total mixed ration (TMR) was formulated by blending compounded concentrate mixture 45 kg, groundnut gotar 28 kg, wheat straw 15 kg, green Hybrid Napier 10 kg, mineral vitamin premix (Amul, Boviplus) 1.0 kg and salt 1.0 kg, and was fed to all the cows *ad libitum* in two parts: the morning (9:00-10:00 h) and the afternoon (14:30-15:30 h). The cost of TMR formulated was worked out to Rs. 23.65/kg DM. The toxin binder (50 g) was mixed with 500 g concentrate mixture and fed to T2 group cows one half each in the morning and afternoon before feeding TMR with additional concentrate mixture as per the level of milk production. The control T1 cows were fed only the same quantity of concentrate mixture without toxin binder. The leftovers in next day morning before milking were collected and weighed to calculate intake.

All cows were housed in a well-ventilated shed with an individual feeding facility. The cows were let loose after morning milking (4:30 to 8:30 h), where potable drinking water was available. Drinking water was also offered at the tying place three times (14:00-15:00 h, 17:00 h, and 21:00 h). All cows were machine milked in the morning (4:30 h) and evening (16:30 h). The weekly milk samples at initial (0), 1, 2, and 3 weeks in proportionate to morning and evening were drawn from each cow to estimate the milk fat, solid not fat, protein content, and aflatoxin M1. The daily composite milk samples were also drawn from all cows of control T1 and treatment T2 groups to evaluate the aflatoxin M1 excretion in milk. The body weight of each cow was recorded on electronic weighing balance before the start and at the end of the study.

The feed and TMR samples were analyzed for proximate constituents as per AOAC (1995). Aflatoxin B1 in feed and M1 in milk samples were estimated at NDDB, CALF laboratory, Anand by HPLC-Fluorescence method using CALF/SOP/CHEM/CCR/Q2-Issue date: 31/07/2014 and CALF/SOP/CHEM/CCR/18, respectively. Milk fat, milk solids not fat-SNF, and milk protein were analyzed as per BIS (1981) using Milkoscreen (FOSS, Denmark). The 4% FCM yield was calculated as per ICAR (2013). The bioconcentration factor (BF) of aflatoxin M1 and carry-over percent of aflatoxin were calculated as per Gou *et al*. (2021) and Zentai *et al*. (2023), respectively.

The data, except body weight, were presented at weekly intervals as Mean with Standard Error. The body weight was presented as initial, final, and changes. The data were analyzed as one-way ANOVA (Snedecor and Cochran, 2014) using WASP 2.0 package developed by Jangam and Wadekar (2004). The means between treatment differing at p<0.05, and p<0.01 were considered as significant and highly significant.

Results and Discussion

The results on total mixed ration composition, body weight of cows, nutrient intake, milk and its components yield, aflatoxin B1 intake and aflatoxin M1 excretion, feed conversion ratio, and cost of feeding are presented in Tables 1 to 4.

TMR- Composition and Intake, Body Weight and Nutrient Intake:

The dry matter content of TMR and concentrate mixture used in the study was 61.43% and 91.64%, respectively. The composition of the total mixed ration on DM basis consisted of crude fat 2.66%, crude fibre 20.82%, crude protein 18.13%, acid soluble ash 4.09%, and Aflatoxin B1 53.35 µg/kg. The corresponding values for the concentrate mixture were 7.44%, 14.52%, 24.85%, 1.27%, and 133.765 µg/kg, respectively. The intake of nutrients, and body weight changes in experimental cows presented in Table 1 indicated that nutrient intake was sufficient to satisfy the nutrient requirement of crossbred cows during the experimental period without influencing the body weight in two groups. The statistical analysis of nutrient intake indicated that dry matter, crude fat, crude protein, and metabolizable energy intake were significantly lower in T2 group of cows than in T1 group. This lower intake of nutrients might be due to better utilization of nutrients owing to the healthier liver in T2 group of cows, as improved antioxidative status of Holstein dairy cows fed diet having 20 and 40 µg aflatoxin B1/kg dry matter added with 0.25% toxin adsorbent (Xiong *et al*., 2015), and better nutrient fermentation in rumen by microbiota and better absorption through rumen wall (Zom *et al*., 2012) has been documented earlier. However, a non-significant effect on dry matter and nutrient intakes was observed earlier in lactating cows and/or buffalo on feeding sodium bentonite clay (Sherasia *et al*., 2023), mycotoxin-sequestering agent (Queiroz *et al*., 2012), calcium montmorillonite clay (Maki *et al*., 2016), diet with added aflatoxin challenge (Sulzberger *et al*., 2017), and AFB1-naturally contaminated feed supplemented with bentonite (Sumantri *et al*., 2012).

Milk and Milk Components Yield

The milk yield (whole milk and 4% FCM) and milk component yield, *viz.*, milk fat, SNF, total solids, protein, and energy (Table 2) were comparable in control (T1) and Mycotoxcease (T2) groups, indicating a non-significant effect of toxin binder supplementation. Similarly, Xiong *et al*. (2015) observed a non-significant effect on lactation performance in Holstein dairy cows fed a diet having 20 and 40 μg aflatoxin B1/kg dry matter added with 0.25% toxin adsorbent. Similarly, several researchers (Kutz *et al*., 2009; Queiroz *et al*., 2012; Sumantri *et al*., 2012; Maki *et al*., 2016; Sherasia *et al*., 2023) also reported non-significant effects on milk and components yield in dairy animals. In contrast, Mehany and Shams (2019) reported significantly higher milk and components yield in Friesian cows on feeding diet with toxin binder (Bentonite and Zeolite), whereas reduced milk yield was reported on feeding a diet contaminated or incorporated with aflatoxin B1 by Ogunade *et al*. (2016).

Aflatoxin Intake and Excretion

The daily total aflatoxin B1 entered was 727.514 and 663.720 μg in the body of cows of control (T1) and Mycotoxcease (T2) group and excretion was 4.399 and 2.200 μg, respectively (Table 3), indicating 50% lower excretion in T2 than T1 group. The lower total intake of aflatoxin B1 in T2 group was due to reduced total feed intake as such and on DM basis (Table 1). An individual aflatoxin M1 excretion in the milk of T1 and T2 group cows (Fig. 1), showed individual variation in excretion while lower excretion was also noted in T2 group cows. This indicated that supplemental feeding of toxin binder (50 g/cow/day) effectively reduced aflatoxin M1 excretion in milk, which might be due to increased binding of B1 in the cows' diet. Similarly, bioconcentration and carry-over percentage were also effectively reduced in T2 group. Patel *et al*. (2011) and Sherasia *et al*. (2023) observed reduced aflatoxin M1 excretion in milk of crossbred cows and buffaloes following sodium bentonite supplementation in the diet. A slightly lower percent excretion (47% and 44%) than 50% found in this experiment was stated by Masoero *et al*. (2009) in Holstein Friesian cows on a feeding diet with sequestering agents. Similarly, Kuboka *et al*. (2022) observed 45% reduction in Aflatoxin M1 excretion in the milk of dairy cows on 1.2% binder (novasil) supplementation. A higher excretion rate (51.3 and 69.7%) was reported by Maki *et al*. (2016) in crossbred

Table. 1: Body weight and feed intake of crossbred cows

Particular	T1 (Control)	T2 (Mycotoxcease)	P-Value
Initial body weight (kg)	390.43±13.02	393.55±16.89	0.886
Final body weight (kg)	397.25±11.80	399.25±16.68	0.923
Change (+/-)	+ 6.82	+ 5.70	0.707
Feed intake kg/d as such basis	20.829^{a}±0.55	18.882^{b}±0.49	0.011
Feed intake kg/d on DM basis	12.946^{a}±0.34	11.750^{b}±0.3	0.011
Crude fat intake kg/day	0.359^{a}±0.01	0.328^{b}±0.01	0.016
Crude fibre intake kg/day	2.666^{a}±0.07	2.417^{b}±0.06	0.011
Crude protein intake kg/day	2.378^{a}±0.06	2.161^{b}±0.05	0.011
ME intake Mcal/day	29.267^{a}±0.75	26.591^{b}±0.68	0.011

Table. 2: Milk and milk components yield of crossbred cows

Particular	T1 (Control)	T2 (Mycotoxcease)	P-Value
Milk yield, kg/day	13.754±0.35	13.823±0.47	0.894
4% Fat corrected milk, kg/day	13.381±0.32	13.321±0.54	0.924
Fat yield, kg/day	0.525±0.01	0.519±0.02	0.825
Solid not fat yield, kg/day	1.169±0.03	1.154±0.04	0.771
Total solid yield, kg/day	1.694±0.04	1.673±0.07	0.785
Protein yield, kg/day	0.399±0.01	0.396±0.02	0.886
Milk energy, ME Mcal/day	9.076±0.22	9.022±0.4	0.906

dairy cows fed clay feed additive (0.5 and 1% of dietary DM). The variation in aflatoxin M1 excretion in the milk of dairy animals is due to the dose-dependent manner binding of aflatoxin B1 by clay adsorbent (Sarr, 1995) and different ingredients in the diet (Kutz *et al.*, 2009). In contrast, Wakade *et al.* (2019) reported a slight decrease in milk aflatoxin M1 concentration and Sumantri *et al.* (2012) reported a non-significant carry-over rate of aflatoxin M1 in lactating dairy animals on supplementation of toxin binder in the diet.

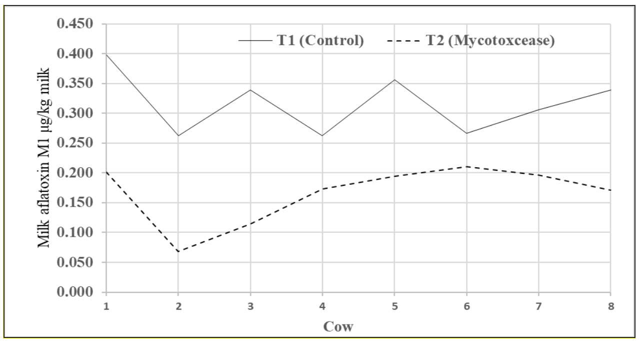

Fig. 1: Aflatoxin M1 excretion in milk of experimental cows

The daily excretion of aflatoxin M1 in milk (Fig. 2) started decline from the second day and it was sharp after one week of supplemental feeding of Mycotoxcease, and the decline of aflatoxin at the end of the experiment (21 days) was 56.16% (from 0.365 to 0.160 µg/kg milk, declined 0.205 µg/kg milk) compared to initial excretion before supplementation (0 day). The supplemental feeding of 50 g/cow/day toxin binder T2 was effective in reducing aflatoxin M1 excretion in milk, this might be due to the binding of aflatoxin B1 in the diet of cows. Similarly, Wakade *et al.* (2019) reported decrease in concentration of aflatoxin M1 in milk from day 0 to day 45 in all the groups in buffaloes. The variation of aflatoxin M1 in milk excretion depends on the absorption of aflatoxin B1 from the diet by toxin binder (Sherasia *et al.*, 2023), rumen microbes' degradation (Tolosa *et al*, 2021), and detoxification by the action of liver microsomal cytochrome P-450 enzyme system (Campagnollo *et al.*, 2016; Tolosa *et al.*, 2021).

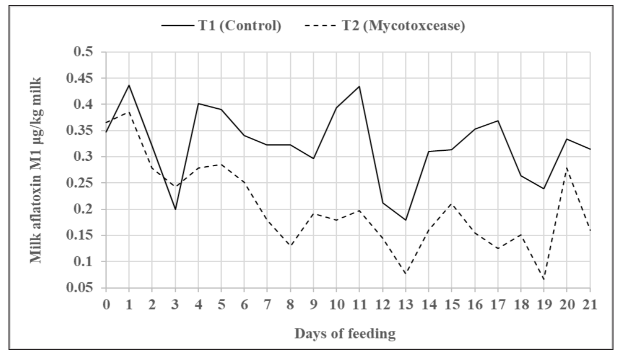

Fig. 2: Average daily excretion of Aflatoxin M1 in milk during the experimental period

Conversion Efficiency of Feed to Milk and Cost of Feeding:

The feed-dry matter efficiency conversion to milk, daily feed cost, and feed cost per unit milk production reduced significantly ($p<0.05$) in T2 (Mycotoxcease) group cows compared to T1 (Table 4). The energetic efficiency-metabolizable energy conversion to milk energy was also better (P=0.098) on 50 g/cow/day Mycotoxcease

Table. 3: Aflatoxin intake from diet and excretion in milk in crossbred cows

Particular	T1 (Control)	T2 (Mycotoxcease)	P-Value
Aflatoxin M1, µg/kg milk	0.316^{a} ±0.02	0.166^{b} ±0.01 (-47.4%)	0.000
Total aflatoxin M1 excretion in milk, µg/day/cow	4.399^{a} ±0.30	2.200^{b} ±0.16 (-50.0%)	0.000
Aflatoxin B1 in feed, µg/kg feed	56.242^{b}± 0.08	56.535^{a}± 0.09	0.015
Total aflatoxin B1 intake, µg/day	727.514^{a} ±17.94	663.720^{b}± 16.14	0.011
Bioconcentration = Aflatoxin M1 Conc. in milk/Aflatoxin B1 Conc. in feed	0.006^{a}±0.00	0.003^{b}±0.00	0.000
Carry-over % = (Total Aflatoxin M1 in milk/Total Aflatoxin B1 in feed)*100	0.605^{a}±0.04	0.339^{b}±0.03	0.000

Table. 4: Feed conversion and cost of feeding in crossbred cows

Particular	T1 (Control)	T2 (Mycotoxcease)	% Change over control	P-Value
Feed conversion, DMI kg/kg Milk	0.948^{a}±0.02	0.863^{b}±0.03	-8.97	0.022
Protein utilization efficiency, CPI kg/kg milk CP	6.006±0.15	5.738±0.25	-4.46	0.356
Energetic efficiency, MEI Mcal/milk ME Mcal	3.244±0.07	3.027±0.11	-6.69	0.098
Feed cost ₹/day	311.28^{a}±7.95	283.00^{b}±7.16	-9.09	0.011
Feed cost ₹/kg milk	22.81^{a}±0.56	20.79^{b}±0.65	-8.86	0.023

Where DMI= Dry matter intake, CPI= Crude protein intake, MEI= Metabolizable energy intake, Mcal= Megacalories, ₹= Indian rupees supplemental feeding. The descriptive statistics indicated an 8.97% improvement in dry matter, 4.46 % improvement in crude protein, and 6.69% improvement in metabolizable energy utilization efficiency to milk, milk protein, and milk energy production, respectively. The daily feed cost (₹/day) and feed cost per unit milk (₹/kg milk) reduced significantly in T2 (Mycotoxcease) group of cows compared to a control group (T1) (Table 4). The descriptive statistic revealed a 9.09 % lower in daily feed cost and an 8.86% lower feed cost per unit of milk yield in T2 compared to T1 group. Mehany and Shams (2019) reported improved feed conversion and feed cost per unit 4% FCM yield in Friesian cows fed a diet with 2% bentonite, which aligns with the present findings. On the contrary, Queiroz *et al.* (2012) reported that dietary mycotoxin-sequestering (toxin binder) treatments did not affect feed efficiency in dairy cattle.

Conclusions

The daily supplemental feeding of toxin binder (Mycotoxcease; 25 g each morning and evening for 21 days) to cows significantly reduced dry matter and nutrient intake without affecting milk and milk components yield. The excretion of aflatoxin M1 in milk showed a significant decrease (47.5%) in comparison to control feeding, when compared with initial excretion the reduction was 56.16%. The quantity of dry matter, protein, and energy required to produce each unit of milk, milk protein, and milk energy was reduced significantly and the reduction was 4.5 to 9%. The daily feed cost and feed cost per unit of milk were also reduced significantly and the reduction was 8.9-9.1%. In conclusion, supplementation of 50 g/day/cow toxin binder (Mycotoxcease) in dairy cows reduced aflatoxin M1 excretion in milk, nutrient intake, and improved feed conversion ratio with better cost of feeding.

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