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RESEARCH ARTICLE

Toxin binders in dairy ration: balancing nutrient utilization and aflatoxin M1 safety in crossbred cows

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Abstract: Mycotoxins are prevalent in every tropical fed ingredients and safety of milk to the consumers a real concern under field conditions. The present study evaluated the comparative efficacy of different dietary toxin binders in mitigating aflatoxin M (AFM) contamination in milk of crossbred lactating cows under natural, non-spiked feeding conditions, without compromising animal performance or health. Sixteen early- to mid-lactation Holstein–Friesian crossbred cows were randomly allotted to four groups (n = 4 per group): control (no binder), sodium bentonite (1%; T1), herbal–clay enzyme premix (0.5%; T2), and a conjugated silicate–yeast–organic acid premix (0.2%; T3). Animals were fed a basal diet consisting of mixed green fodder and concentrate for 60 days. Feed and milk samples were analyzed for aflatoxins using LC–MS/MS, while nutrient intake, digestibility, milk yield, milk composition, and blood biochemical parameters were monitored.

Total aflatoxin levels in the feed were within permissible limit. Dry matter intake, overall nutrient digestibility, milk yield, fat-corrected milk, and milk composition were comparable among treatments (P>0.05), indicating no additional effects of binder supplementation. Blood biochemical indices, including liver and kidney function markers, remained within physiological limits across all groups. AFM concentrations in milk were significantly reduced in binder-supplemented groups compared to control, with the greatest reduction observed in T3. The conjugated binder reduced AFM levels below the European Union permissible limit (0.05 ppb) by the 6th and 8th weeks, achieving an average efficacy of 56–59%. It is concluded that dietary toxin binders effectively reduce AFM transfer into milk under natural contamination conditions, with conjugated silicate–yeast–organic acid binders showing superior efficacy without affecting animal performance or health.

Keywords: Milk, Mycotoxin, Nutrient utilization, Toxin binder

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Introduction

India is the world's largest milk producer, with an estimated production of 247.87 million tonnes in 2024–25 (BAHS, 2025). However, the export of liquid milk remains negligible, largely due to stringent sanitary regulations, particularly restrictions related to aflatoxin M1 (AFM1) contamination (Hattimare et al. 2022; Economic Times, 2023). Mycotoxin contamination is a global concern, affecting nearly 25% of agricultural commodities, with greater prevalence in tropical and subtropical regions where high temperature and humidity favor mold growth (Jelinek et al. 1989; Chilaka et al. 2017). Among mycotoxins, aflatoxins produced mainly by Aspergillus flavus and A. parasiticus are of major concern in dairy production. Aflatoxin B1 (AFB1), the most potent aflatoxin, is metabolized in the liver to AFM1, which is subsequently excreted in milk and poses a direct risk to consumer health due to its heat stability and resistance to common processing methods (Campagnollo et al. 2016; Peles et al. 2019). The carryover of AFB1 to AFM1 in milk varies widely, ranging from 0.6 to 6% in dairy cows (Bervis et al. 2021), prompting strict regulatory limits of 0.5 ppb in the United States and 0.05 ppb in the European Union.

Among available detoxification strategies, dietary supplementation with mycotoxin binders is considered the most practical and effective approach under field conditions. Toxin binders should reduce mycotoxin bioavailability through adsorption in the gastrointestinal tract without compromising feed intake or nutrient utilization (EFSA, 2010). Commercial binders include inorganic clays such as bentonite and HSCAS, organic binders derived from yeast cell walls and lactic acid bacteria, and herbal-based formulations. Although sodium bentonite is widely used due to its cost-effectiveness, comparative evidence on the efficacy of different binder classes

under natural contamination conditions remains limited. Moreover, information on their influence on nutrient digestibility, blood biochemical indices, and milk production is scarce. Therefore, the present study was undertaken to comparatively evaluate three different classes of mycotoxin binders for their ability to mitigate the natural transfer of AFM1 into milk of crossbred cows, while assessing their effects on digestibility, milk yield, and health of the animals.

Materials and Methods

Experimental location and weather

The experiment was conducted at the Livestock Research Centre, Southern Regional Station, ICAR-NDRI, Bangalore, India. The latitude, longitude, and elevation of the experimental place are 12.947014°N (12° 56 49.2504" N), 77.607679 (77° 36'27.6444" E), and 921m MSL, respectively. The tropical climate is considered to be Aw (Savanna, wet) according to the Köppen-Geiger climate classification, with a mean temperature of 23.6°C and 831mm annual rainfall. The variation in temperatures throughout the year is 6.4°C. During the experimental period, the relative humidity ranged from 59 to 72%, the mean ambient temperature was as low as 15ºC and as high as 31ºC.

Experimental design and diets

Sixteen healthy Holstein–Friesian (HF) crossbred cows (mid-lactation) were randomly assigned to four equal groups after balancing for milk yield (12.34 ± 0.06 kg/d) and DIM (93 ± 3.34 days). Animals were penned in tie-stall, face-to-face housing at the Livestock Research Centre, ICAR–NDRI, Bengaluru, India. All animals were fed at the same time with mixed green fodder (MGF) consisting of fresh para grass (*Brachiaria mutica*), maize (*Zea mays*), and hybrid napier (*Pennisetum glaucum* × *Pennisetum purpurem*) in the proportion of 25:25:50 as basal roughages and supplemented with concentrate supplement (CS) in the ratio of 50:50 and considered as control diet. The Nutrient composition of the diet has been given in Table 1. Diets were formulated according to nutrient requirements by NRC (2001). In addition to the control ration (CG), the treatment rations (TG) were fortified as follows: 1% sodium bentonite (T1); 0.5% premix containing natural clays, toxin-degrading enzymes, and herbal binders (T2); or 0.2% premix comprising silicates, β-glucans, mannans, organic acids, and surfactants (T3). Binders were thoroughly mixed into the concentrate and fed according to the specified dose rates in two daily offerings (morning and evening). Dose rates were set a priori based on a critical review of the literature and aligned with manufacturer specifications regarding source and demonstrated efficacy.

Digestibility trial and chemical analysis

Animals were weighed using a platform weighing machine (Orion Electronic Systems, Pvt Ltd). Animals were weighed every four weeks, once on an empty stomach for two days, to record their body weight. The experiment started from June 2023 to July 2023 (60 days), which included 7 days of adaptation to the diets, and a digestibility trial for 5 days was conducted at the end of the trial. Diet offered, refusal (orts), and feces were weighed daily at a fixed time. After thorough mixing with a gloved hand, 1/100 volume of the faecal sample was taken as a representative sample to estimate the DM. Dried samples were pooled over 5 days. A fresh faecal sub-sample of 1/400 was pooled over 5 days and preserved with known quantities of 10% H_2SO_4 (v/v) in pre-weighed glass bottles to estimate nitrogen. All the samples collected during the digestibility trial were analyzed for proximate and cell wall constituents (CWCs) according to AOAC (2016). Total carbohydrate (TCHO) and non-fibrous carbohydrate (NFC) were derived mathematically (Hall, 2003). Daily milk yield was recorded for each cow, and approximately 50 mL of milk was collected twice weekly for compositional analysis.

Blood metabolites

Blood samples were collected from the jugular vein at the beginning and each fortnight using a vacutainer needle into 10 mL capped EDTA-coated tubes. Tubes were labeled, kept in a flask with an ice gel pack, and stored at 4ÚC. Samples were taken and analyzed using a Mindray auto hematology analyzer (BC-2800 Vet, M/s Mindray Medical International Ltd., Shenzhen, China). Plasma metabolites, viz. plasma triglycerides (GPO-PAP liquid gold kit, Cat. no. 72LS100-60), plasma total protein (Cat. no. 83LS100-60), plasma albumin (Cat. no. 84LS100-60), BUN (Cat. no. 81DP300-72), plasma aspartate transaminase (AST; liquid gold AST kit) and alanine transaminase (ALT; liquid gold ALT kits, Cat. no. 77LS200- 60) and plasma total cholesterol (Cat. no. 71LS200-60), were analyzed using kits obtained from M/s Span Diagnostics Ltd., India, as per the manufacturer's instructions.

Sample collection and analysis

Two feed matrices were sampled for aflatoxin analysis: a MGF and a CS. A representative sample was randomly collected from the entire feedlot after thorough mixing. The bulk sample was finely ground and passed through a No. 20 sieve (FSSAI, 2020), and triplicate subsamples were taken from each feed type (MGF and CS) for mycotoxin analysis. Since the feed contamination was unintentional and uncontrolled, the initial concentration or even the presence of mycotoxins in the samples was unknown. These feed samples were tested for the presence of aflatoxins B1 (AFB1), B2 (AFB2), G1 (AFG1), G2 (AFG2), Zearalenone, and fumonisin B1As the temperature ranged from 24–30 °C and RH was high (e"90%), it was assumed that these conditions favored the growth of fungi and the production of mycotoxins. To monitor the effect of feed contamination on milk safety, sixty-four milk samples were collected from 16 crossbred cows at four different time intervals: 0 weeks, 3 weeks, 6 weeks, and 8 weeks. These milk samples were then tested for aflatoxin M1 (AFM1), the

metabolite of AFB1, to evaluate the transfer of toxins from feed to milk under natural feeding conditions.

Determination of aflatoxins in feed and milk

Aflatoxins B1, B2, G1, and G2, Zearalenone, and Fumonisin B1 in all feed samples and AFM1 concentration of all the collected milk samples were measured using UPLC-ESI-LCMSMS (tandem mass spectrometry) at 3, 6, and 8 weeks after feeding on the above diets. The analytical standards AFB1, AFB2, AFG1, AFG2, Zearalenone, Fumonisin B1, and AFM1 were obtained from Sigma-Aldrich (USA).

Extraction and LC–MS/MS analysis

Aflatoxins in feed and milk were quantified by a modified LC–MS/MS method based on polar multi-residue extraction. Feed samples (H"277 mg) were hydrated with 4 mL water and extracted with 4 mL methanol containing 1% formic acid, followed by centrifugation (4,000 × g, 20 min, 4 °C) and filtration (0.45 µm). Milk samples (H"27 g) were extracted with methanol (1% formic acid), centrifuged, frozen at "20 °C, recentrifuged to remove fat, filtered, and transferred to LC vials.

Mixed standards of aflatoxins (B , B, , G , G, , and M), zearalenone, and fumonisin B were prepared in acetonitrile:water (1:1) and serially diluted to obtain five-point calibration curves. Matrix-matched calibration or isotope-labelled internal standards were applied where necessary.

Chromatographic separation was achieved on a BEH C18 column using a formic-acidified acetonitrile–water gradient with a runtime of 6–10 min. Quantification was performed using multiple reaction monitoring (MRM). Samples (20 µL) were quantified by weighted linear regression (r² e" 0.99), and data processing was carried out using Waters TargetLynx software. Quality control included blanks, matrix spikes, and calibration verification.

Calculation

$$\text{Efficacy (\%)} = \frac{\text{AFM1 incidence in control} - \text{AFM1 incidence in Treatment}}{\text{AFM1 incidence in control}}$$

Statistical procedures

The variance was estimated using CRD using the following ANOVA model.

$$Yij = \mu m + Ti + Bj + eij$$

Where Yij was any observation for which µm was the general mean, Ti was the treatment effect (5 levels), Bj was the block effect (3 levels), and eij was the random error (14 degrees of freedom) component (Das et al. 1991). The significant difference between groups was expressed at probability (p) levels ranging from < 0.10 to 0.01 and a p-value of < 0.10 was demarcated using '•' whereas '*' and '**' were used to denote P values of < 0.05 and 0.01, respectively. The variance between the two means was compared by DMRT (Duncan Multiple Range Test). Data was analyzed using Statistical Package for the Social Sciences (SPSS, ver. 20.0, M/s IBM India Pvt. Ltd.).

Results and Discussion

Estimation of mycotoxins in feed

The total aflatoxins (AFB1, AFB2, AFG1, and AFG2) in the MGF and CS fed to cows in CG and TGs were approximately <20 ppb (µg/kg). We observed a higher concentration of aflatoxins in CS.

Table 1: Nutrient composition of the diet (% DM basis)

Parameter	Concentrate Supplement	Green fodder		SEM	P value
		Offered	Ort		
Dry Matter	89.19^{a}	20.54^{b}	26.70^{c}	0.75	0.01**
Organic matter	87.5^{b}	88.2^{ab}	89.1^{a}	0.23	0.01**
Crude Protein	13.17^{a}	9.93^{b}	8.50^{b}	0.31	0.01**
Ether extract	3.20^{a}	1.76^{b}	1.55^{b}	0.08	0.01**
Total ash	12.39^{a}	12.02^{ab}	10.9^{b}	0.27	0.01**
Acid insoluble ash	2.51b	2.96^{a}	2.70^{b}	0.09	0.01**
Total Carbohydrates	66.34^{a}	80.92^{a}	71.03^{b}	0.42	0.01**
Cell content	65.32^{a}	31.59^{b}	22.44^{c}	1.00	0.01**
Neutral detergent fiber	34.68^{c}	68.41^{b}	77.56^{a}	1.04	0.01**
Acid detergent fiber	17.61^{c}	47.49^{b}	54.80^{a}	0.60	0.01**
Hemicellulose	17.07^{b}	20.92^{ab}	22.75^{a}	1.20	0.01**
Cellulose	11.22c	38.79^{a}	35.47^{b}	0.79	0.01**
Acid detergent lignin	7.8^{ab}	6.9^{b}	18.2^{a}	0.25	0.01**
Non fibrous carbohydrates	34.97^{a}	12.43^{b}	5.70^{c}	1.18	0.01**

*The values bearing different alpha superscripts in a row differ significantly; ** p< 0.01*

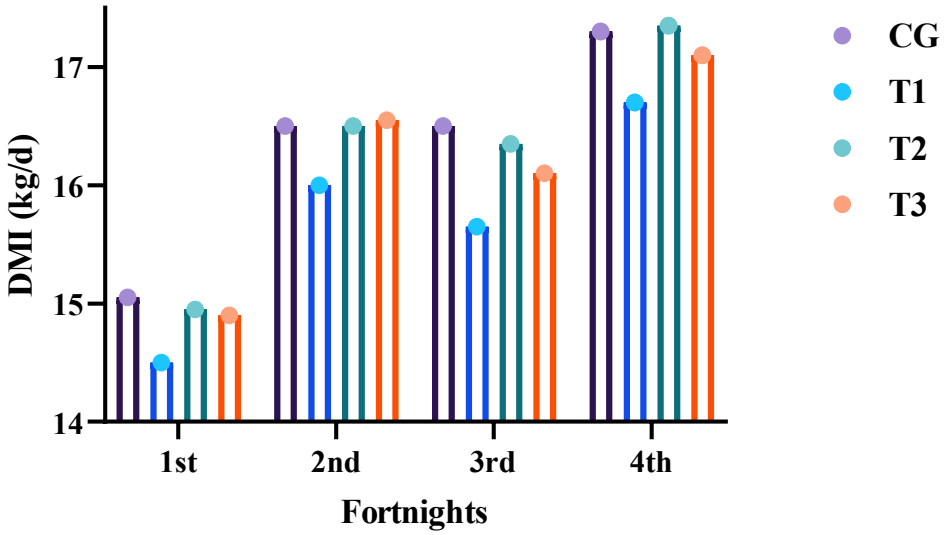


Fig. 1 DMI (kg/d) of animals fed toxin binders in different fortnights

The quantities present in the feed are under natural conditions, unlike earlier studies conducted by spiking the aflatoxins in the feed. The Fumonosin B1 and Zearalenone were below the detection limits of LC-MS/MS.

Dry matter intake (DMI) and digestibility

The total DMI of the experimental animals during different fortnights was similar (P>0.05), as shown in Figure 1. The average DMI was ranged between 13.9 to 17.5 kg/d. The digestibility coefficient of the nutrients is presented in Table 2. Our study revealed significant differences in the digestibility coefficient for EE among different groups. Such a positive impact of toxin binders on nutrient digestibility has been observed by other workers too (Temirayev et al. 2016; Mehany and Shams, 2019).

Blood metabolites

Blood metabolites were assessed fortnightly and are shown in Table 3**.** Blood glucose levels were below the physiological range in all groups during the experiment. Serum triglycerides remained within normal limits, with initially lower values in the treatment groups. Kidney function markers, BUN and creatinine, stayed within normal ranges; T3 had lower values. Serum total protein, albumin, and globulin showed no significant differences were found among groups. The albumin-to-globulin ratio was slightly low. Liver enzymes, AST and ALT, remained within normal ranges across all groups.

Milk yield and its composition

The average milk yield (kg/d) of all the animals during the experimental period is presented in Figure 2. Milk yield between CG and TGs was statistically comparable throughout the study. However, milk yield increased in all the supplemented groups. The T1 group, which was supplemented with a multi-component toxin binder including herbal additives like turmeric (*Curcuma longa*), showed the highest average milk yield. Fat-corrected milk (4% FCM) yield was also similar among the groups. Milk components, including total solids, fat, and solids-not-fat (SNF),

Table 2: Digestibility coefficient (%) of nutrients from the total diet in different groups

Parameters	CG	T1	T2	T3	SEM	*P*-value
Dry matter	63.82	62.65	64.62	63.64	1.01	0.60
Organic matter	65.70	64.80	66.29	65.33	1.05	0.78
Crude protein	78.75	76.95	78.32	75.49	3.58	0.19
Ether extract	64.19^{b}	69.03^{ab}	70.57^{ab}	73.11^{a}	1.57	0.01*
Total carbohydrates	62.72	61.17	62.46	61.28	1.42	0.81
Cell content	74.98	71.81	75.40	74.19	1.20	0.22
Neutral detergent fiber	53.13	53.96	54.21	53.55	1.19	0.92
Acid detergent fiber	46.31	45.83	48.21	46.69	1.78	0.80
Hemicellulose	64.70	67.77	64.33	65.15	2.21	0.69
Cellulose	57.67	56.81	57.10	57.62	2.44	0.99
Non fibrous carbohydrates	85.65	82.36	84.76	83.90	2.35	0.78

**Means in each row with different superscripts are significantly different (p<0.05); SEM, standard error of the mean.*

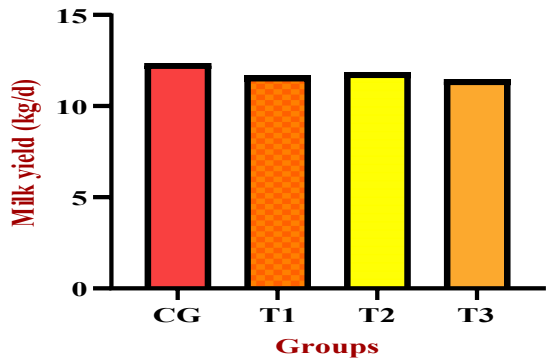


Fig. 2 Average milk yield (kg/d) of different groups fed different toxin binder

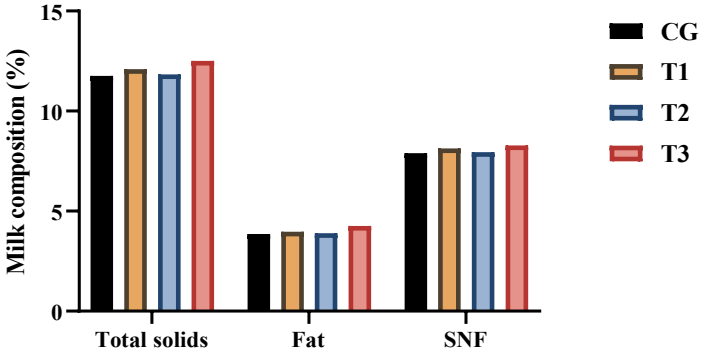


Fig. 3 Effect of dietary toxin binders on milk composition over the experimental period

were analyzed twice weekly for up to 60 days and showed comparable values among the experimental groups (Fig. 3).

Estimation of AFM1 in milk

Milk samples were collected at three different time intervals: day 0 (beginning of the experiment), week 3, and week 8 (completion of the experiment). AFM1 levels were analyzed using the sensitive LC-MS/MS technique. The mean AFM1 (ppb) level in the CG and TGs at different week intervals is presented in Table 4. At day 0, AFM1 concentrations ranged from 0.12 to 0.16 µg/kg in both the CG and TGs. Significant differences among groups were observed at the 6th week ($P<0.05$) and 8th week ($P<0.1$), with the highest concentration of AFM1 detected in the CG.

Efficacy of different toxin binders

The efficacy of the toxin binders to bind the natural levels of aflatoxins present in feed during different weeks is presented in Table 5. Compared to the CG, T3, supplemented with a conjugated binder blend of silicates, YCW components, and some organic acids, consistently showed the lowest levels of AFM1 in milk during different intervals. AFM1 acquisition was curtailed to levels below the European standard of 0.05 ppb by the 6th and 8th weeks. The average observed effectiveness ranged from 56% to 59%. The level of acquisition of aflatoxins in T1 (sodium bentonite) and T2 (multicomponent binder) was comparable. AFM1 acquisition into the milk of T1 and T2 was less than 0.5 ppb as per the FSSAI standards in India, but about 10 times higher than the European standard.

Table 3: Blood metabolites in different groups of cows from the 0th day to the end of the trial

Parameter	CG	T1	T2	T3	SEM	P value
Blood glucose (mg/dL)	35.71	28.94	28.99	29.21	3.15	0.33
Triglycerides (mg/dL)	14.37	14.26	13.70	13.18	2.01	0.67
BUN (mg/dL)	14.06	14.48	13.58	13.69	0.89	0.55
Creatinine (mg/dL)	0.90	0.85	0.77	0.73	0.06	0.28
Albumin (g/dL)	2.78	2.79	2.72	2.71	0.08	0.71
Globulin (g/dL)	4.03	3.82	4.57	4.12	0.32	0.45
AST (IU/L)	89.51	87.28	95.31	99.75	5.46	0.44
ALT (IU/L)	42.51	46.82	42.50	46.63	4.76	0.49
AST:ALT	2.11	1.91	2.31	2.11	0.22	0.39

Given the non-significant p-value, the data presentation omits the interaction between treatment and period

Table 4: The level of AFMI (ppb) in different groups of animals at different week intervals

	AFM1 (ppb) in different groups during different week intervals			
Treatment	Zero	III	VI	VIII
Control	0.13	0.94	0.65	0.33
T1	0.12	0.27	0.42	0.11
T2	0.14	0.27	0.42	0.12
T3	0.16	0.01	0.007	0.002

Estimation of mycotoxins in feed

The ambient temperature suitable for Aspergillus growth (82°F or 28°C), unfortunately, overlaps with ideal harvest conditions for green fodder (20-34°C). This mismatch overlap makes freshly cut forage vulnerable to mold contamination. The contamination of aflatoxins in a CS or compound feed may be due to the storage conditions or micro-environment during the day. The pellets are prone to absorb water quickly, and moisture above 11°C is favorable for fungal growth. Since maize, a major ingredient at around 38% in the compound feed (Table 1), is highly susceptible to aflatoxin contamination, the overall risk of aflatoxin in the feed was significant. The contamination of Fusarium species is common in stored forages rather than in fresh forages.

Dry matter intake (DMI) and digestibility

The comparable DMI across treatments may be attributed to the naturally occurring mycotoxin levels being within permissible safety limits. Additionally, all animals received well-balanced rations meeting their nutritional requirements for maintenance and production. Similar studies (Abd El-Baki et al. 2001; Salem et al. 2001) found no significant improvement in dry matter, organic matter, and feed components with the addition of clay minerals. In contrast, Mehany and Shams (2019) reported an increase in feed intake with the supplementation of sodium bentonite. The reason for increased DMI is likely attributed to the presence of multiple components in mycotoxin binders. These components, including clay, yeast products, phytogenics, and herbs, work together to improve absorbability, immune function, and gut health, and reduce oxidative stress, ultimately leading to higher feed intake (Holanda and Kim, 2020). The severity of contamination with aflatoxin, when high, can lead to reduced feed intake in animals (Peles et al. 2019).

The highest EE digestibility was observed in T3, followed by T2 and T1 ($P< 0.01$). Kihal et al. (2022) observed that the observed improvement in digestibility is likely due to a reduced mycotoxin load in the gastrointestinal tract and an increased nutrient absorption surface area facilitated by the toxin binder. Toxin binders consist of clay minerals along with additives such as prebiotics, herbal supplements, and organic acids, which contribute to restoring gut health by encouraging the growth of beneficial bacteria while inhibiting harmful ones (Azghadi et al. 2024). Herbal supplements provide antioxidant and anti-inflammatory benefits that support intestinal mucosa integrity, and prebiotics such as Mannon oligosaccharides (MOS), a component of YCW, selectively stimulate beneficial bacteria, all of which contribute to improved nutrient utilization. Therefore, the combined effect of these components explains the observed enhancement in the digestibility coefficients of EE.

Blood metabolites

Blood glucose levels were below the physiological range in all groups during the experiment. T2 showed a numerical increase. Serum triglycerides remained within normal limits, initially lower in treatment groups. Kidney function markers (BUN and creatinine) remained within normal ranges; T3 had lower values. Serum total protein, albumin, and globulin showed no significant differences. The albumin-to-globulin ratio was slightly low. Liver enzymes (AST, ALT) remained within normal ranges across all groups (Ghamsari et al. 2022). T2 showed the lowest AST at the end. AST to ALT ratios did not suggest liver damage. Overall, toxin binders, especially in T2 and T3, supported metabolic health and organ function without adverse effects.

Milk yield

In this study milk yield did not differ significantly among treatments, indicating that toxin binder supplementation did not adversely affect productive performance under natural feeding conditions. The numerically higher milk yield observed in the CG and the slight reduction in TGs are consistent with reports showing variable production responses to mycotoxin deactivators (Gallo et al. 2020), although other studies have reported no effect on milk yield (Maki et al. 2016; Sulzberger et al. 2017). Such inconsistencies may be attributed to differences in mycotoxin load, ration composition, and physiological status of the animals. Notably, cows in T1 exhibited the highest average milk yield among binder-supplemented groups, possibly due to the presence of herbal additives such as turmeric, which have been associated with improved lactation performance (Hashemzadeh et al. 2014; Jaguezeski et al. 2018).

Estimation of AFM1 in milk

The concentration of AFM1 in milk differed significantly among groups at specific time points, with the control group showing the highest levels. Variations in aflatoxin ingestion may occur

Table 5: Efficacy (%) of different toxin binders to reduce the AFM1 in milk at different week intervals

	Efficacy (%) of different binders at different week intervals		
Treatment	III	VI	VIII
T1	72.70	38.84	68.63
T2	72.40	39.70	65.01
T3	98.90	99.00	99.51
S.E.M	15.24	14.48	6.22
P value	0.00**	0.001**	0.00**

day-to-day depending on mold growth on fresh grasses and environmental conditions favorable to fungal proliferation.

Efficacy of different toxin binders

Many researchers (Kutz et al. 2009; Xiong et al. 2015; Mirzaei et al. 2020) have observed that adding YCW binders to aflatoxin-contaminated feed effectively reduces AFM1 levels in milk. The β-glucans and MOS in YCW provide multiple specific binding sites exhibiting high affinity for AFB1, enabling effective adsorption of the toxin. This aligns with findings from Mirzaei et al. (2020), who also reported that YCW-based binders generally outperform sodium bentonite. Despite containing various ingredients such as clay minerals, organic matter, herbal detoxifiers, and liver potentiators, the multicomponent binder was not as effective at binding aflatoxin as the conjugated binder in reducing aflatoxin levels. Considering the inconsistent results against aflatoxin throughout the study, the multicomponent binder might have offered other benefits to the animal's health.

Conclusion

This study confirms that dietary toxin binders can effectively reduce aflatoxin M contamination in milk under natural, non-spiked feeding conditions. Supplementation of binders did not adversely affect feed intake, nutrient utilization, milk yield, or animal health. Among the evaluated products, the conjugated silicate–yeast–organic acid binder showed the highest efficacy, reducing AFM concentrations below the European Union permissible limit. The results emphasize that binder composition plays a critical role in aflatoxin mitigation. Overall, conjugated toxin binders offer a practical nutritional intervention to improve milk safety without compromising dairy cow performance.

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Compliance with ethical standards

The study was approved by the committee of ethics in Animal Experimentation vide approval reference CCSEA/IAEC/LA/SRS-ICAR-NDRI-2023/No.05.

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