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RESEARCH ARTICLE

Effects of varying levels of aflatoxin B_1 and its ameliorant on feed intake and nutrient digestibility in crossbred lactating cows

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Abstract: Twenty-five lactating cows (Karan Fries) were selected from Livestock Research Centre of ICAR-NDRI, Karnal and divided into 5 groups (T_1 to T_5) of 5 animals each based on milk yield, body weight, parity and days in milk (30-50 d). Feeding trial was conducted for 150 days and all the animals were fed as per nutrient requirements (ICAR, 2013). Group T_1 (Control group) was fed basal diet consisting of concentrate, wheat straw and green fodder. Group T_2 (Control + AFB_1@ 20 ppb in diet above control level), T_3 (Control + AFB_1 @ 40 ppb in diet above control level), Group T_4 (T_2 + MOS as ameliorant @ 0.20% of feed) and Group T_5 (T_3 + MOS as ameliorant @ 0.20% of feed). Seven days metabolic trial was conducted towards the end of the feeding trial to study nutrient utilization. Intake of DM, OM, CP, EE and water were found to be similar among all groups. However, DM digestibility (%) was found to be lower ($P<0.05$) in group T_3 (60.59±1.44) as compared to other groups, the digestibility values being 63.69±1.59, 63.04±1.56, 63.47±0.89 and 61.65±1.31 in groups T_1, T_2, T_4 and T_5%, respectively. Also, OM digestibility (%) was lower ($P<0.05$) in group T_3 (63.82±1.74) as compared to groups T_1 (66.78±1.33), T_2 (66.49±1.59), T_4 (67.14±0.93) and T_5 (66.61±1.27). The nitrogen utilisation patterns in terms of N intake, faecal, urinary and milk excretion patterns were similar in all 5 groups. Therefore, supplementing the diets of lactating crossbred cows with mannan-oligiosaccharides @ 0.20% of the feed intake appeared to mitigate the adverse effects of dietary AFB_1 in terms of enhancing DM and OM digestibility.

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Introduction

Mycotoxins are the toxic fungal metabolites which are capable of inflicting deleterious effects on animal and human health. Among them, aflatoxins (AFs) are toxic secondary metabolites or compounds formed by fungal species like *Aspergillus flavus, Aspergillus parasiticus* etc. High ambient temperatures and moisture levels promote the growth of fungi and the formation of mycotoxins (Saleemi et al. 2017, Naseem et al. 2018, Saleemi et al. 2020) and these conditions are prevalent in tropics (Bennit and clitch, 2003). There are 20 different types of aflatoxins wherein aflatoxin B_1 (AFB_1) is the most abundant and potent aflatoxin in naturally contaminated foods and feeds. When ingested by animals and humans, AFB_1 is partly metabolized in the liver to a more polar compound: aflatoxin M_1 (AFM_1) and can be excreted in urine, feces and milk. All livestock species are susceptible to aflatoxicosis and it affects different physiological systems. Acute toxicity of aflatoxins is rare (Etzel, 2014; ALRuwaili et al. 2018) and in chronic cases it is carcinogenic and immunosuppressive (Qian et al. 2014). Aflatoxins primarly target the liver, making aflatoxicosis a condition pre dominantly associated with hepatic dysfunction (Etzel, 2014). When AFB_1 is ingested by dairy cattle through feed, variety of symptoms can occur, like anorexia, subsequent unthriftiness, weight loss, reduced growth rate (Cheng et al. 2017) and decreased milk production (Fink-Gremmels and Van der Merwe, 2019; Rodríguez-Blanco et al. 2020). To alleviate the harmful effects of aflatoxins, various inorganic toxin binders are being used (Rojo et al. 2014; Pate et al. 2018). These inorganic toxin binders have the limitations that they decrease the availability of various minerals like P, Mg, Cu, Zn, Mn, vitamins and may also contain heavy metals. So more focus is now being given on organic toxin binders like yeast cell wall extracts like mannan oligosaccharides (MOS) which have glucomannas as active principals (Khatke et al. 2012). These toxin binders adsorb aflatoxins and thus help in excreting them without being absorbed in GIT. Limited studies on effect of aflatoxin B_1 on feeding efficiency of bovines have been conducted in Indian conditions. So, this study was conducted to investigate the effect

of different levels of aflatoxin B_1 in diet and ameliorative agent on fed intake and nutrient utilization in crossbred lactating cows.

Materials and Methods

Culturing of *Aspergillus parasiticus*: aflatoxin producing fungus species *Aspergillus parasiticus var.globosus*- 411 strain was procured from MTCC (IMTECH), Chandigarh in a lyophilized form in a glass ampoule. After receiving, it was stored at 4°C till further use. Culturing of fungus was done over potato dextrose agar balancing pH with tartaric acid. Culturing of fungus was carried out at 25°C and incubated for seven days.

Production of aflatoxin B_1 using crushed maize grains: aflatoxin was produced naturally according to method by Shotwell et al. (1966) and Singh and Shamsudeen (2008). Cracked maize grains were taken in conical flasks in ratio of 1: 2. Suitable numbers of flasks were taken for required quantity of aflatoxin. The flasks were plugged and sealed with nonabsorbent cotton and covered with aluminum foil. After 20 min. of autoclaving at 12°C, the flasks were inoculated with 10 days old spores of *Aspergillus parasiticus*. The inoculated flasks were incubated in a BOD incubator at 30% moisture and 30°C temperature with frequent shaking for 14 days (Singh and Srivastav 2019). Flasks were autoclaved to kill the fungus and content was grounded to powder. Amount of AFB_1 was determined as per IS 16287:2015 (ISO 16050: 2003) and ELISA kit (Elabcience-E-TO-E027) which was used as standard aflatoxin B_1 inoculum in animal feeding trial.

Location of study and animal selection: The study was carried out at Livestock Research Centre, ICAR-National Dairy Research Institute (NDRI), Karnal located at a height of 250 meters above mean sea level with latitude and longitude being $29^0$42" N and $79^0$54" E, respectively. Twenty-five lactating Karan Fries cows were selected from Livestock Research Centre of ICAR-National Dairy Research Institute, Karnal with average body weight (393.7 ± 10.0 kg), average lactation (305 days), average milk yield (3565.2 ± 170.7 kg), average days in milk (74.3 ± 7.4) and average parity (1.8 ± 0.2). The animals were divided homogenously into 5 groups (T_1 to T_5) of 5 animals each based on above mentioned parameters. All the animals were fed rations as per their nutrient requirements (ICAR 2013) which were reformulated fortnightly. All of the animals were fed a 40: 60 (DM basis) ratio of concentrate mixture to fodder. Group T_1 as control group was fed basal diet consisting of concentrate, wheat straw and green fodder with AFB_1 level @ 15 ppb in diet. Group T_2 (Control + AFB_1 @ 20 ppb in diet above control level), T_3 (Control + AFB_1 @ 40 ppb in diet above control level), Group T_4 (T_2 + MOS as ameliorant @ 0.20% of concentrate feed) and Group T_5 (T_3 + MOS as ameliorant @ 0.20% of concentrate). De-worming and timely vaccination of all animals was done to prevent occurrence of diseases. Following 21 days of adaption period, 150 days feeding trial was carried out. Seven days metabolic trial was conducted towards the end of the feeding trial to study nutrient utilization. The experimental proposal was approved by Institutional Animal Ethics Committee with certificate number 48-IAEC-23-12.

Chemical composition and Statistcal analysis

The feed samples for proximate principles viz. DM, OM, total ash, CP and EE (AOAC 2016) and fibre fractions like NDF and ADF (Van Soest et al. 1991) were analysed. Nitrogen in urine and milk was also analysed (AOAC 2016). Feed and fodder samples were ground and analysed for AFB1 content by ELIS kit (Elabcience-E-TO-E027). The data were analyzed using ANOVA, Statistical Package for Social Sciences (SPSS 2020).

Results and Discussion

Chemical composition of feeds and forage

Ingredients and composition of basal concentrate mix feed is shown in Table 1. The chemical composition of ration is presented in Table 2. The total mixed ration contained 12.51% CP and 2.71 % EE, 9.71% , total ash, 47.16% NDF and 27.88% ADF on DM basis. Average AFB1 content was 15 ppb in concentrate feed while it below detectable level in wheat straw and green fodders.

Nutrient intake

The intake of nutrients (DM, digestible DM, OM, CP, DCP) was similar in all the groups. Likewise, water intake from feed (L/d), drinking water intake (L/d) and total water (l/100 kg and (L/kg DMI/d) was found to be similar in all groups ($P < 0.05$). Due to overall high feed intake and relatively low concentrations of aflatoxins in the ration, a dilution effect may have minimized the toxic impact of AFB_1, consistent with observations by Jiang et al. (2020). Compared to monogastric species, ruminants tend to be less susceptible to the effects of mycotoxins, largely owing to the microbial degradation that occurs in the rumen (Haque et al. 2020; Santos and Gremmels 2014). Applebaum et al. (1982)

Table 1 Ingredients and composition of concentrate feed (DM basis)

Ingredient	Parts (%)
Maize grain	25
Barley grain	10
Soybean meal	12
Groundnut cake	06
Mustard oil cake	13
Cottonseed cake decorticated	05
Channa chunni	11
De-oiled rice bran	05
Wheat bran	10
Mineral mixture	02
Common salt	01

showed that when 13 mg of aflatoxin was given to milch dairy cows/d. In a separate study, when lactating cows were administered AFB_1 at 20 ppb and 40 ppb in a total mixed ration, no differences were seen ($P< 0.05$) in daily feed intake or milk output (Wang et al. 2019). The intake of the AFB_1 did not influence DM consumption or milk production (Queiroz et al. 2012). Dairy cows' resistance to mycotoxins may be due to a varied diet which usually consists of a variety of feedstuffs along with substantial body mass (Zain 2011; Mili´cevi´c et. al. 2010). Aflatoxin level in cattle feeds when increased from 0, 26, 56.4, 81.1, and 108.5 µg/kg showed decrease in feed intake in a dose-dependent way (Choudhary et al. 1998). Various in vitro studies using higher levels of AFB_1 have shown negative impact on rumen microbes (Jiang et al. 2012; Sinha and Arora, 1982). Aflatoxin may breakdown and be rapidly absorbed into the bloodstream in in vivo circumstances due to the more diverse rumen microenvironment (Gallo et al. 2008); which has less of an impact on ruminal fermentation. According to Sulzberger et al. (2017), after challenging lactating dairy cows with 100 ~g/kg/d of AFB_1 for three days, the peak ruminal AFB_1 concentration was only

Table 2 Chemical composition of different ingredients of ration (Mean±SE)

Parameter	Concentrate mixture	Maize fodder	Oats fodder	Wheat straw
DM (%)	90.27 ±0.22	20.11 ±0.15	16.92 ±0.44	89.01 ±0.32
OM (%)	91.70±0.49	88.41 ±0.14	89.95 ±0.23	89.70 ±0.69
CP (%)	19.83 ±0.13	10.09 ±0.11	6.92 ±0.32	2.71 ±0.03
EE (%)	4.42±0.14	1.35±0.02	2.44±0.14	0.92±0.05
Total ash (%)	8.30±0.51	11.59 ±0.14	10.05 ±0.23	10.33±0.69
NDF (%)	27.34±0.37	52.65±0.29	53.40±1.27	75.08±1.12
ADF (%)	12.34±0.66	32.29±0.24	30.42±1.09	52.01±0.43

Table 3 Plane of nutrition in different groups during metabolism trial (Mean±SE)

Parameter	Groups				
	T_1	T_2	T_3	T_4	T_5
Body wt. (kg)	411.00±43.90	405.40±33.24	416.40±8.86	440.40±22.33	456.00±36.91
DM intake (kg/d)	10.32±0.76	10.46±0.75	10.26±0.72	11.32±1.41	10.88±0.36
DM intake (kg/100 kg BW)	2.51±0.04	2.58±0.03	2.46±0.03	2.57±0.05	2.38±0.03
DDMI (kg/d)	6.58±0.55	6.61±0.51	6.234±0.47	7.198±0.37	6.73±0.91
DDMI (kg/100 kg BW)	1.60±0.02	1.63±0.02	1.49±0.00	1.63±0.01	1.47±0.03
OM intake (kg/d)	9.40±0.69	9.53±0.68	9.36±0.64	10.29±0.40	9.90±0.33
OM intake (kg/100 kg BW)	2.28±0.03	2.35±0.03	2.24±0.02	2.33±0.01	2.17±0.02
CP intake (kg /d)	1.44±0.14	1.41±0.08	1.39±0.08	1.45±0.06	1.45±0.20
CP intake (kg/100 kg BW)	0.35±0.007	0.34±0.006	0.33±0.007	0.32±0.004	0.31±0.005
DCPI (kg/d)	0.92±0.11	0.88±0.05	0.83±0.06	0.88±0.13	0.86±0.14
DCPI (kg/100 kg BW)	0.22±0.02	0.21±0.02	0.19±0.03	0.20±0.03	0.19±0.02
Water intake from feed (L/d)	31.39±1.54	29.03±2.62	33.87±1.26	32.25±1.03	30.71±3.50
Drinking water intake (L/d)	48.32±3.84	39.36±4.02	57.73±1.80	45.28±2.20	40.64±3.64
Total water intake (L/d)	79.71±4.65	68.38±6.64	91.59±3.43	77.53±4.34	71.35±7.65
Total water intake (L/100 kg BW)	19.39±2.25	16.86±3.34	21.99±4.30	17.60±2.54	15.64±4.54
Total water intake (L/kg DMI/d)	1.87±0.06	1.61±0.07	2.14±0.08	1.55±0.08	1.43±0.12

Table 4 Effect of aflatoxin B_1 in diet and MOS on nutrient digestibility (%) in crossbred cows (Mean±SE)

Parameter	Groups				
	T_1	T_2	T_3	T_4	T_5
DM	63.69±1. 59^{bd}	63.04±1.56^{bc}	60.59±1.44^{a}	63.47±0.89^{bd}	61.65±1.31^{bc}
OM	66.78±1.33^{b}	66.49±1.59^{b}	63.82±1.74^{a}	67.14±0.93^{b}	66.61±1.27^{b}
CP	62.65±2.98	62.69±1.85	59.84±1.54	60.75±1.32	59.13±2.23
EE	74.34± 0.05	75.96±1.19	73.46±1.88	73.80±2.00	74.20±0.91
NDF	57.06±3.62	57.53±2.13	54.99±1.84	56.98±1.91	55.30±2.20
ADF	38.16±2.02	38.62±3.78	35.74±5.19	37.28±2.68	33.75±3.19

$^{a-d}$ figures in row bearing different superscripts differ significantly (P<0.05)

Table 5 Effect of aflatoxin B_1 and MOS on nitrogen balance in crossbred cows (Mean±SE)

Parameter	Groups				
	T_1	T_2	T_3	T_4	T_5
N intake (g/d)	272.62±14.23	264.14±19.40	273.91±15.84	266.65±17.08	262.41±30.74
N voided in faeces (g/d)	79.95±6.15	84.14±6.83	88.87±5.39	90.57±3.3	93.99±12.36
N voided in urine (g/d)	99.40±5.43	92.40±4.65	94.40±5.87	86.20±3.65	83.20±4.56
N voided in milk (g/d)	77.44±3.23	72.43±3.76	76.58±1.98	75.34±2.34	71.22±3.21
Total N outgo (g/d)	256.79±13.43	248.97±15.54	259.85±16.32	252.11±11.43	248.41±14.87
N absorbed (g/d)	192.67±10.32	180.00±9.55	185.04±9.76	176.08±10.43	168.42±11.32
N balance (g/d)	15.83±1.23	15.17±1.23	14.06±0.99	14.54±1.32	14.00±1.42
Retained N (as % of intake)	5.93±0.67	5.18±0.56	5.04±0.76	5.34±0.56	5.68±0.47
Retained N (as % of absorbed)	8.12±0.38	7.60±0.46	7.31±0.87	7.93±0.62	8.64±0.75

0.20 ~g/L; however, the rumen fluid collection duration in relation to aflatoxin dose was not stated. Jiang et al. (2020) demonstrated that the digestibility of a dairy cow's entire mixed feed and in vitro rumen fermentation were unaffected by 0.75 g/L AFB1.

Nutrient utilisation

Dry matter digestibility (%) was found to be lower (P<0.05) in group T_3 (60.59±1.44) as compared to other groups, the digestibility (%) values being 63.69±1.59 63.04±1.56, 63.47±0.89 and 61.65±1.31 in groups T_1, T_2, T_4 and T_5, respectively (Table 4). Also, OM digestibility (%) was lower (P<0.05) in T_3 (63.82±1.74) as compared to T_1 (66.78±1.33), T_2 (66.49±1.59), T_4 (67.14±0.93) and T_5 (66.61±1.27). Digestibility of other nutrients like CP, EE, NDF and ADF were similar in all the groups. No observable effects were found with respect to AFB_1 level in ration on N balance (Table 5). Jiang et al. (2020) examined effects on ruminal digestibility using in vitro techniques keeping the dose rate close to commonly found concentration of AFB_1 in feedstuffs i.e. @ 75 µg/kg of feed DM. They found that, in comparison to the aflatoxin-free control diets, those infected with aflatoxin had higher concentrations of NH_3-N and acetate and lower DM digestibility. Amaro et al. (2023) did not observe any differences on nutrient digestibility, fermentation and N flows when aflatoxin B_1 was taken @ 50 to 150 ppb of the diet during in vitro trial. Higher dosages of AFB_1 (649-1920 µg/kg) were associated with lower levels of VFA and NH_3-N (Jiang et al. 2012). In vitro DM digestibility of lucerne hay dropped by 50% after 3 h of incubation with a very high dose of 2,000,000 µg of AFB_1/kg of hay (Westlake et al. 1989). However, in vitro DM digestibility TMR's reduced by 4% at a normally occurring dose (Jiang et al. (2020) which is similar to current study where reduction in DM digestibility of T_3 group was 4.87% as compared to control group. Reduced microbial activity and growth as a result of AFB_1 toxicity might be the cause of AFB_1 detrimental effects on DM and OM digestibility as also observed by Westlake et al. (1989) and Jiang et al. (2020). Ruminal microbial development is influenced by a number of variables including OM digestibility (Clark et al. 1992). Singh et al. (2020) found that truly degradable DM and OM, gas production and microbial biomass production values were lower (P<0.05) in aflatoxin contaminated group than MOS fed groups under in vitro conditions. This decrease in DM digestibility could be explained by impaired ruminal activity which lowers the synthesis of volatile fatty acids and the digestion of fibre (Helferich et al. 1986 a, b). About 79% of the aflatoxins were bound or sequestered when 0.05% MOS was added to a meal containing 200 ppb aflatoxins in vitro. The MOS obtained from S. cerevisiae cell walls had a significantly greater binding ability for AF up to 95% (Mahesh and Devegowda 1996) which is similar to current study where AFB_1 effects were reversed by MOS in feed.

Khatke et al. (2012) found that aflatoxin's effects were largely or totally mitigated in a dose-dependent manner by the use of binders MOS and S. cerevisiae (@ 0.05%, 0.1%, and 0.2%) in poultry feed. Jiang et al. (2018) found addition of yeast fermentation products to have supplementary effect to inorganic toxin binders in reducing harmful effects in dairy cows. In general, MOS enhanced gut health by competing for attachment sites and substrates in the GI tract by acting as prebiotics.

Conclusions

Chronic intake of AFB_1 upto 55 ppb of diet did not influence the feed and water intake. Dry matter and organic matter digestibility were reduced ($P<0.05$) by the AFB_1 present in the diet at higher doses in diet @ 55 ppb. Supplementing the diets of lactating crossbred cows with mannan oligiosaccharides at 0.20% of the feed appeared to mitigate the adverse effects of dietary AFB_1 in terms of enhancing DM and OM digestibility.

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