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Effect of supplementing lactating goats fed on aflatoxin contaminated feed with calcium bentonite and activated charcoal on aflatoxin M_1 concentration, excretion and carryover in milk

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Abstract

Aflatoxin is a collective term for a group of toxic and carcinogenic secondary metabolites produced by some strains of A*spergillus flavus* and A*spergillus parasiticus* during growth, on feeds and foods. The fungal spores are found worldwide, in air and soil, and infest both living and dead plants and animals. An experiment was conducted to investigate the concentration, total excretion and carry-over of Aflatoxin B_1 (AFB_1) into milk as Aflatoxin M_1. Nine crossbred lactating goats were divided into three groups of three each, based on the level of milk production. Commercial Aflatoxin B_1 (AFB_1) was administered to all groups at a rate of 100 ppb in the diet. Group I served as control (T_1). In group II (T_2), calcium bentonite (CaB) and in group III (T_3), activated charcoal (AC), were added at the rate of 1% of Dry Matter Intake (DMI). Dry matter intake was not significantly different (P>0.05) among T_1 (1.22), T_2 (1.14) and T_3 (1.13). Daily milk yield was also not significantly different (P>0.05) among treatments T_1 (0.91), T_2 (0.86) and T_3 (1.03) during the experimental period of 14 days. The AFM_1 concentration, excretion and carry-over of AFB_1 in T_1 continued to increase with time, whereas, the same was seen to decline in the adsorbent fed groups T_2 and T_3. The results suggest that supplementation of CaB or AC at 1% of DMI for lactating goats result in a reduction in AFM_1 content in milk and carryover of aflatoxin from feed to milk without causing any change in composition of milk.

Key words: Activated charcoal, aflatoxin, aflatoxin excretion, bentonite, milk contamination

Introduction

Aflatoxin is a collective term for a group of toxic and carcinogenic secondary metabolites produced by some strains of A*spergillus flavus* and A*spergillus parasiticus* during growth, on feeds and foods. The fungal spores are found worldwide, in air and soil, and infest both living and dead plants and animals. Based on fluorescence properties on thin layer plates, four types of aflatoxins (B_1, G_1, B_2,

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G_2) were identified. Among all aflatoxins, B_1 (AFB_1) is synthesized under a wide range of conditions (Wogan, 1977), and is the most potent hepatotoxin. It exhibits a variety of biological effects, such as carcinogenicity, teratogenicity and mutagenicity in farm animals (Applebaum *et al*., 1982). It is well known that AFB_1 can cause chronic diseases in humans and animals, and can present different effects such as hepatotoxicity, genotoxicity and immunotoxicity (CAST, 1989; CAST, 2003). It has been estimated that more than 5 billion people in developing countries worldwide are at risk of chronic exposure to AFB_1 through contaminated foods (Liu and Wu, 2010; Shepherd, 2008; Strosnider *et al*., 2006). The primary disease associated with AFB1 intake is hepatocellular carcinoma, being the third-leading cause of death from cancer globally (WHOSTAT, 2008). With about 550,000–600,000 new cases each year (Dragacci *et al*., 2001; Liu and Wu, 2010), aflatoxin may play a causative role in up to 28% of all global cases of hepatocellular carcinoma (Liu and Wu, 2010).

Moreover, it is now well established that dairy animals consuming rations contaminated with AFB_1 excrete aflatoxin metabolite, aflatoxin M_1 (AFM_1) in milk in concentrations related to feed aflatoxin (Veldman *et al*., 1992; Chopra *et al*., 1999). Upon ingestion by ruminants, AFB_1 is partially destroyed in the rumen, whereas the absorbed AFB_1 rapidly undergoes metabolic processes in the liver to various secondary metabolites (Kuilman *et al*., 1998; Kuilman *et al*., 2000; Kensler *et al*., 2011). Aflatoxin M_1 (AFM_1), a possible human carcinogen (IARC, 2002), is the major oxidized metabolite of AFB_1 and is excreted primarily in the urine and in the milk (Van Egmond, 1989; Prandini *et al*., 2007). The European Union (EU) applies a maximum residue level (MRL) of 0.05 µg AFM_1 kg^{-1} in ruminant milk, and some countries in Africa, Asia and Latin America also enforce this level (Van Egmond, 1989; CAST, 2003; EU, 2006).

Studies in Uganda have shown high levels of Aflatoxin contamination in food and feeds, with levels of more than 1000 ppb in some grains (Alpert *et al*., 1971; Sebunya and Yourtee, 1990; Kaaya and Muduuli, 1992). This exposes humans to high levels of Aflatoxin ingestion and related health hazzards, through food and animal products from contaminated feed. Aflatoxins are also known to cause direct losses in animals, suppression of the immune system, reduced growth rates and lowered feed efficiency (Vincelli *et al*., 1995). It is, therefore, imperative to conduct research leading to reduction in Aflatoxin secretion and carryover in milk so as to save people from consuming such products from carcinogens and also to reduce the country's investment in regulatory and treatment of cancerous patients.

Many approaches (physical, chemical and biological) have been tried to detoxify Aflatoxin (Piva *et al*., 1995). Hydrated sodium-calcium aluminosilicate (HSCAS) is the most thoroughly studied adsorbent in various species, but it has a lower efficacy in reducing the carryover of AFM_1 in milk in compared to bentonite (Veldman, 1992). Activated charcoal (AC) can bind most of the available aflatoxins under *in vitro* conditions (Galvano *et al*., 1996), and reduce the carryover of AFM_1 in milk (Galvano *et al.*, 1996). Earlier, Smith *et al*. (1994) reported reduction of AFM_1 excretion in dairy goats using HSCAS.

This present study was designed to investigate the effect of supplementation

of calcium bentonite (CaB) or AC on aflatoxin carryover in milk, of lactating goats fed AFB_1 contaminated ration.

Materials and methods

Nine lactating crossbred goats were maintained on *Brachiaria* cv. mulato hay as the basal diet, and were supplemented with a concentrate mixture comprising of 73, 24, 2.5 and 0.5% of maize bran, cotton seed cake, mineral premix and salt, respectively. The feed ingredients used in formulating the concentrate mixture were analysed for aflatoxin content and efforts were made to ensure that feed ingredients with no or extremely low levels of aflatoxins were used.

Before initiating the experiment, the experimental animals were subjected to a feed adaptation period of 14 days. During this period, the animals were fed the basal diet, supplemented with the concentrate mixture, to determine the dry matter intake for both the basal diet and concentrate mixture for each of the animals. The dry matter intake estimates for the basal and concentrate mixture for each animal, formed the basis for the dry matter quantities given to the individual animal during the experimental period. After determining the dry matter intake of the concentrate mixture, CaB and AC were added to the concentrate mixture at a rate of 1% DM, to come up with three treatments, namely T_1 (control – no CaB or AC added), T_2 (concentrate with CaB at 1%DM) and T_3 (concentrate with AC at 1% DM).

After completion of the feed adaptation period (14 days), the goats were divided, on the basis of milk yield, into three groups of three goats each, and the three treatments were randomly allocated to the animals, following a completely randomised block design (CRBD).

All the nine goats were administered with 100 ppb of commercial aflatoxin-B1 (manufactured by Sigma Chemical Company, USA) externally daily during the experimental period (14 days). The doses of aflatoxin-B1 were fixed based on the goat's dry matter intake during the preliminary period.

Dry matter intake (DMI) of each animal was recorded daily, during the experimental period, by deducting the dry weight of feeds not consumed by each animal, from the total weight of feed given to the animal. Daily milk yield of each animal was recorded in the mornings and evenings separately. Milk samples from all goats were collected individually on 0, 3, 7, 10 and 14th day, and were analysed for aflatoxin-M_1 contents according to procedures described by Rao and Chopra (2001). Milk samples collected on 0, 7 and 14th day were analysed for protein, fat, solids not fat and ash. The data were analysed using mixed model procedures (PROC MIXED) of SAS considering the 'animal effect" as a random effect.

Results

Dry matter intake (DMI) and daily milk yield

There was no significant difference ($P>0.05$) in DMI during the experimental period with 1.22, 1.14 and 1.13 kg day^{-1} in T_1, T_2 and T_3, respectively. The statistical differences between treatment and period were not significant. Similarly, the average daily milk yield during the experimental period was 0.91, 0.86 and 1.03 (kg per day) in T_1, T_2 and T_3, respectively, and were not significant.

Aflatoxin M_1 in milk

Supplementing lactating goats fed on aflatoxin B_1 contaminated ration, with nutritionally inert adsorbents (calcium bentonite or activated charcoal) reduced the concentration of aflatoxin M_1 in milk (Table 1). On the 14th day, the aflatoxin M_1 concentration was significantly ($p<0.05$) higher in T_1 compared to T_2 and T_3. Also, the change in AFM_1 concentration was significantly higher ($P< 0.05$) and positive in T_1 (225) than in T_2 (-44) and T_3 (-50). Although the difference in the change between T_2 and T_3 was not significant, the lowest change in aflatoxin M_1 concentration was observed in T_3. The total AFM_1 excretion in milk followed the same trend. The aflatoxin M_1 excretion was significantly ($p<0.05$) higher in T_1 compared to T_2 and T_3. Still, the change in AFM_1 excretion was significantly higher ($P< 0.05$) in T_1 (223) in comparison to T_2 (-48) and T_3 (-58.6). The AFM_1 content and total excretion in milk in T_1 continued to increase with time; whereas, the same declined in the adsorbent fed groups (Figs. 1 and 2).

Carryover of aflatoxin into milk (B_1 to M_1)

The carryover of AFM_1 in milk in T_1 increased with time, but the same declined in the adsorbent fed groups (Table 1 and Fig. 3). However, the differences between groups were not significant ($P> 0.05$) (Table 1). The change in carryover (%) was significantly higher ($P<0.05$) in T_1 (172.7) than at T_2 (-47.6) and T_3 (-57.1).

Composition of milk

The milk samples collected on 0, 7 and 14th day were not significantly ($P>0.05$) different for fat, SNF, protein and ash between T_1, T_2 and T_3 (Table 2).

Table 1. Effect of supplementing calcium bentonite and activated charcoal on aflatoxin M_1 concentration, excretion and carryover in milk of lactating goats fed aflatoxin B_1 contaminated ration in Uganda

Parameter	Treatment	3rd day	14th day	Change (%) from3rd to 14th day
AFM_1 concentration (µg/l)	T_1 (control)	0.12	0.39[a]	225[a]
	T_2 (CaB)	0.19	0.11[b]	-44[b]
	T_3 (AC)	0.18	0.09[b]	-50[b]
AFM_1 excretion (µg per day)	T_1 (control)	0.18	0.6	223[a]
	T_2 (CaB)	0.27	0.14	-48.1[b]
	T_3 (AC)	0.29	0.12	-58.6[b]
Carryover (%)	T_1 (control)	0.11	0.30	172.7[a]
	T_2 (CaB)	0.21	0.11	-47.6[b]
	T_3 (AC)	0.21	0.09	-57.1[b]

[a]Values with different letters in a column differ significantly ($P<0.05$). ($T_{1=}$ Control, $T_{2=}$ Calcium Bentonite, $T_{3=}$ Activated Charcoal, AFM_1 = Aflatoxin M--$_1$)

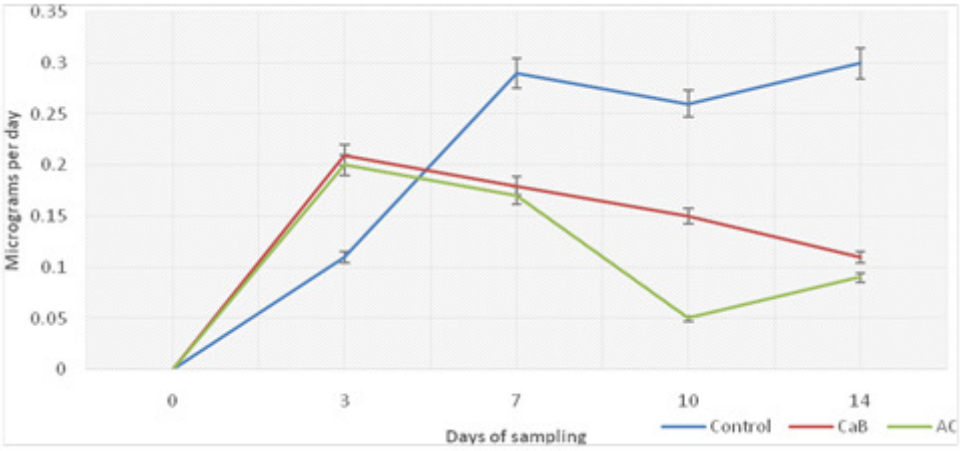

Figure 1. Aflatoxin M_1 concentration (µg l^{-1}) in milk of lactating goats over time in Uganda.

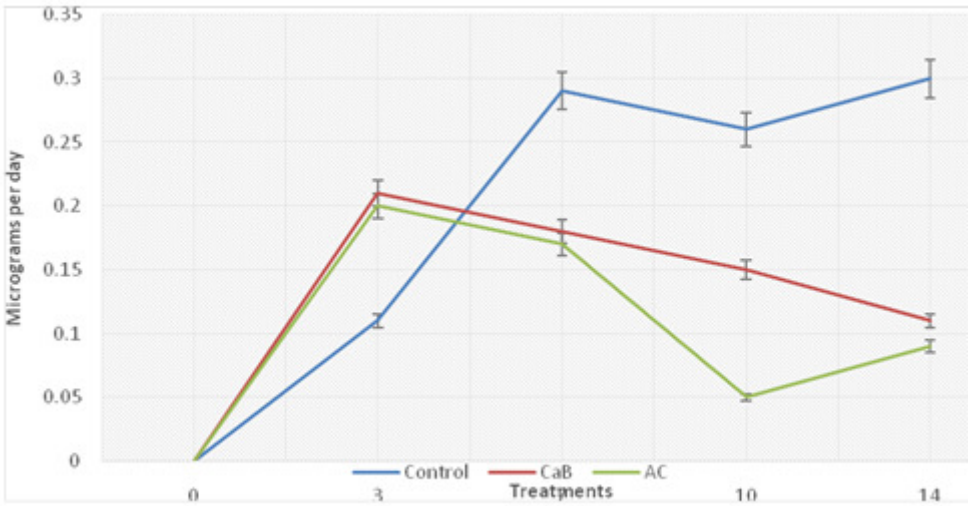

Figure 2. Aflatoxin M_1 excretion (µg day^{-1}) in milk of lactating goats at different sampling dates.

Discussion

Daily milk yield and dry matter intake

Results from the present study suggest that aflatoxins may have no effect on daily milk yield and dry matter intake, although previous studies (Applebaum *et al*., 1982; Malin, 1982) reported decline in daily milk yield and dry matter intake as a result feeding lactating animals with rations contaminated with aflatoxin B_1, The difference could be attributed to the fact that previous studies subjected lactating animals to very high doses of aflatoxin B_1 (7 to 9 mg per day) compared to the low dose (100 ppb) used in the current study.

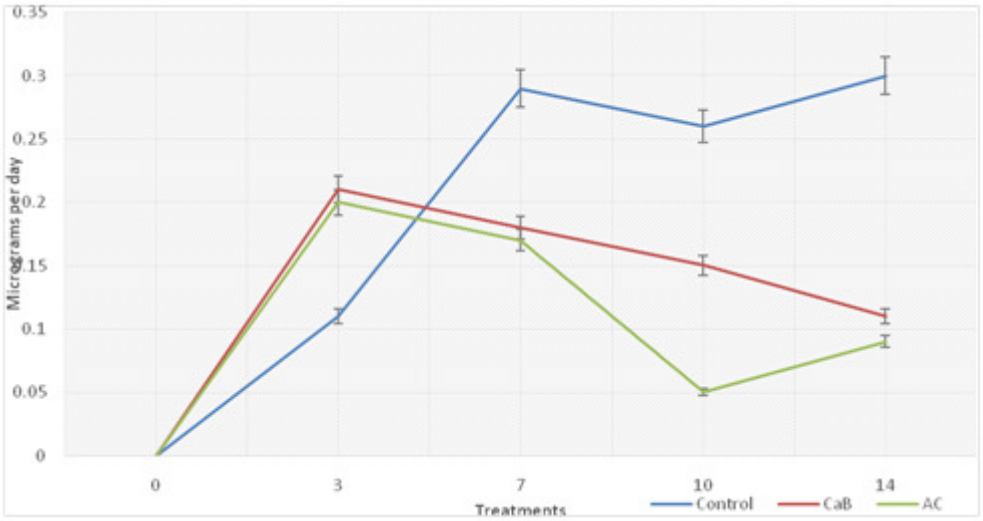

Figure 3. Carryover of Aflatoxin B_1 as Aflatoxin M_1 in milk of lactating goats.

Table 2. Effect of supplementing calcium bentonite or activated charcoal on milk composition of goats fed aflatoxin B_1 contaminated ration

Parameter	Treatment	Days of sampling		
		0	7	14
Fat (%)	T_1 (control)	3.54	3.55	3.57
	T_2 (CaB)	3.62	3.65	3.60
	T_3 (AC)	3.60	3.62	3.65
	Statistical significance	p>0.05	p>0.05	p>0.05
Solids Not Fat (SNF) (%)	T_1 (control)	10.34	10.33	10.31
	T_2 (CaB)	10.26	10.23	10.28
	T_3 (AC)	10.28	10.26	10.23
	Statistical significance	p>0.05	p>0.05	p>0.05
Protein (%)	T_1 (control)	4.07	4.06	4.05
	T_2 (CaB)	4.06	4.09	4.07
	T_3 (AC)	4.05	4.03	4.08
	Statistical significance	p>0.05	p>0.05	p>0.05
Ash (%)	T_1 (control)	0.83	0.86	0.83
	T_2 (CaB)	0.82	0.83	0.82
	T_3 (AC)	0.87	0.85	0.84
	Statistical significance	p>0.05	p>0.05	p>0.05

The present study was primarily designed to examine the effect of adsorbents on carryover pattern of AFB_1 to AFM_1 hence the dose level was fixed at 100 ppb, which did not affect milk yield, or DMI. However, it should be noted that direct effects of aflatoxins are expressed at high contamination levels or over long periods of exposure (LFRA, 2015). Since the goats were exposed for 14 days and at levels below the known lethal dose of 0.5 -10 mg kg^{-1} body weight, the effects on performance and milk yield were not realised, but long term exposure may be a problem.

Aflatoxin M_1 and carryover

Aflatoxin M_1, concentration on 3rd day was greater (P>0.05) in the adsorbent groups (T_2 and T_3) than in the control (T_1). Aflatoxins are metabolised by the hepatic mixed function oxidases, to a group of hydroxylated derivatives, which are species specific and are excreted through faeces, urine and milk of lactating animals. Aflatoxin M_1 is secreted in the milk principally with the casein fraction (Allcroft and Carnaghan, 1963). The AFM_1 blood concentration is dependent upon the amount of AFB_1 destroyed in the rumen, AFB_1 absorbed from the intestinal tract, the AFB_1 liver conversion to AFM_1 and AFM_1 excretion in urine, bile and milk (Dhanasekaran *et al*., 2011).

The discrepancy in AFM_1 content of milk of animals receiving AFB_1 in their feed at the same level (100 ppb) (Fig. 1) could be due to differences in the mixed function oxidases ability to metabolize AFB_1 not only to AFM_1, but to other several hydroxylated derivatives (Steiner *et al.,* 1990; Veldman *et al.,* 1992). Therefore, conversion of AFB_1 to AFM_1 by the mixed function oxidases system might be the cause of variation on AFM_1 concentration in milk.

By day 7, AFM_1 the concentration of milk in the adsorbent groups (T_2 and T_3) declined, whereas the reverse was the case with the T_1 (control) group (Fig. 2). The decline in AFM_1 concentration in milk of the T_2 and T_3 compared to T_1, could be attributed to the fact that adsorbent formed a complex with AFB_1 which prevented the absorption of AFB_1 from the GI tract and thus, reduced the bio-availability of AFB_1 (Ramos *et al*., 1996). In the present study, the reduction of AFM_1 residue in milk was more in the charcoal group (T_3), than in bentonite group (T2). The reduction is comparable with that of Smith *et al*. (1994).

Carryover of AFM_1 from feed to milk ranged between 0.11 and 0.3 in the control (T_1) group, which received 100 ppb aflatoxin B_1 for a period of 14 days without any added adsorbent. These values are in agreement with those of earlier studies (Veldman *et al*., 1992; Chopra *et al*., 1999). With the action point of aflatoxin levels in milk of 0.5 ppb set by the Food and Drug Administration of the United States (Pennington, 2009), the results of this study indicated that use of Calcium Bentonite and activated charcoal can reduce the AFM_1 in milk to acceptable levels while those of the control were above the acceptable levels.

Milk composition

The chemical composition of milk in the three experimental treatments was not different (P>0.05). This concurs with the finding of Smith *et al*. (1994), where there was no observed effect of aflatoxin or adsorbent on chemical composition of milk in dairy goats fed 100 ppb of AFB_1 with 1 or 2% HSCAS.

Conclusion

Supplementation of calcium bentonite or activated charcoal to lactating goats results in significant reduction in Aflatoxin M_1 content of milk and carryover of aflatoxin from feed to milk without changing the composition of milk. Thus, this approach could be utilised to detoxify milk of animals fed with aflatoxin contaminated feed to prevent the adverse effects of aflatoxin M_1 on humans consuming contaminated milk.

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