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 *Asgrellus flavus* and *Aspergillus 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 *Aspergillus flavus* and *Aspergillus 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 (\( B_1, G_1, B_2, G_2 \)).

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G2) were identified. Among all aflatoxins, B1 (AFB1) 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 AFB1 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, 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 (WHO, 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 AFB1 excrete aflatoxin metabolite, aflatoxin M1 (AFM1) in milk in concentrations related to feed aflatoxin (Veldman et al., 1992; Chopra et al., 1999). Upon ingestion by ruminants, AFB1 is partially destroyed in the rumen, whereas the absorbed AFB1 rapidly undergoes metabolic processes in the liver to various secondary metabolites (Kuilmans et al., 1998; Kuilmans et al., 2000; Kensler et al., 2011). Aflatoxin M1 (AFM1), a possible human carcinogen (IARC, 2002), is the major oxidized metabolite of AFB1 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 AFM1 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 Muduli, 1992). This exposes humans to high levels of Aflatoxin ingestion and related health hazards, 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 AFM1 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 AFM1 in milk (Galvano et al., 1996). Earlier, Smith et al. (1994) reported reduction of AFM1 excretion in dairy goats using HSCAS.

This present study was designed to investigate the effect of supplementation
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of calcium bentonite (CaB) or AC on aflatoxin carryover in milk, of lactating goats fed $\text{AFB}_1$ 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<sub>1</sub> in milk

Supplementing lactating goats fed on aflatoxin B<sub>1</sub> contaminated ration, with nutritionally inert adsorbents (calcium bentonite or activated charcoal) reduced the concentration of aflatoxin M<sub>1</sub> in milk (Table 1). On the 14<sup>th</sup> day, the aflatoxin M<sub>1</sub> concentration was significantly (p<0.05) higher in T<sub>1</sub> compared to T<sub>2</sub> and T<sub>3</sub>. Also, the change in AFM<sub>1</sub> concentration was significantly higher (P<0.05) and positive in T<sub>1</sub> (225) than in T<sub>2</sub> (-44) and T<sub>3</sub> (-50). Although the difference in the change between T<sub>2</sub> and T<sub>3</sub> was not significant, the lowest change in aflatoxin M<sub>1</sub> concentration was observed in T<sub>3</sub>. The total AFM<sub>1</sub> excretion in milk followed the same trend. The aflatoxin M<sub>1</sub> excretion was significantly (p<0.05) higher in T<sub>1</sub> compared to T<sub>2</sub> and T<sub>3</sub>. Still, the change in AFM<sub>1</sub> excretion was significantly higher (P<0.05) in T<sub>1</sub> (223) in comparison to T<sub>2</sub> (-48) and T<sub>3</sub> (-58.6). The AFM<sub>1</sub> content and total excretion in milk in T<sub>1</sub> continued to increase with time; whereas, the same declined in the adsorbent fed groups (Figs. 1 and 2).

Carryover of aflatoxin into milk (B<sub>1</sub> to M<sub>1</sub>)

The carryover of AFM<sub>1</sub> in milk in T<sub>1</sub> 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<sub>1</sub> (172.7) than at T<sub>2</sub> (-47.6) and T<sub>3</sub> (-57.1).

Composition of milk

The milk samples collected on 0, 7 and 14<sup>th</sup> day were not significantly (P>0.05) different for fat, SNF, protein and ash between T<sub>1</sub>, T<sub>2</sub> and T<sub>3</sub> (Table 2).

### Table 1. Effect of supplementing calcium bentonite and activated charcoal on aflatoxin M<sub>1</sub> concentration, excretion and carryover in milk of lactating goats fed aflatoxin B<sub>1</sub> contaminated ration in Uganda

<table>
<thead>
<tr>
<th>Parameter:</th>
<th>Treatment</th>
<th>3&lt;sup&gt;rd&lt;/sup&gt; day</th>
<th>14&lt;sup&gt;th&lt;/sup&gt; day</th>
<th>Change (%) from 3&lt;sup&gt;rd&lt;/sup&gt; to 14&lt;sup&gt;th&lt;/sup&gt; day</th>
</tr>
</thead>
<tbody>
<tr>
<td>AFM&lt;sub&gt;1&lt;/sub&gt; concentration (µg/l)</td>
<td>T&lt;sub&gt;1&lt;/sub&gt; (control)</td>
<td>0.12</td>
<td>0.39&lt;sup&gt;a&lt;/sup&gt;</td>
<td>225&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>T&lt;sub&gt;2&lt;/sub&gt; (CaB)</td>
<td>0.19</td>
<td>0.11&lt;sup&gt;b&lt;/sup&gt;</td>
<td>-44&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>T&lt;sub&gt;3&lt;/sub&gt; (AC)</td>
<td>0.18</td>
<td>0.09&lt;sup&gt;b&lt;/sup&gt;</td>
<td>-50&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>AFM&lt;sub&gt;1&lt;/sub&gt; excretion (µg per day)</td>
<td>T&lt;sub&gt;1&lt;/sub&gt; (control)</td>
<td>0.18</td>
<td>0.6</td>
<td>223&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>T&lt;sub&gt;2&lt;/sub&gt; (CaB)</td>
<td>0.27</td>
<td>0.14</td>
<td>-48.1&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>T&lt;sub&gt;3&lt;/sub&gt; (AC)</td>
<td>0.29</td>
<td>0.12</td>
<td>-58.6&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Carryover (%)</td>
<td>T&lt;sub&gt;1&lt;/sub&gt; (control)</td>
<td>0.11</td>
<td>0.30</td>
<td>172.7&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>T&lt;sub&gt;2&lt;/sub&gt; (CaB)</td>
<td>0.21</td>
<td>0.11</td>
<td>-47.6&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>T&lt;sub&gt;3&lt;/sub&gt; (AC)</td>
<td>0.21</td>
<td>0.09</td>
<td>-57.1&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a</sup>Values with different letters in a column differ significantly (P<0.05). (T<sub>1</sub>, Control, T<sub>2</sub>, Calcium Bentonite, T<sub>3</sub>, Activated Charcoal, AFM<sub>1</sub> = Aflatoxin M<sub>1</sub>)
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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

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Treatment</th>
<th>Days of sampling</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>0</td>
</tr>
<tr>
<td>Fat (%)</td>
<td>$T_1$ (control)</td>
<td>3.54</td>
</tr>
<tr>
<td></td>
<td>$T_2$ (CaB)</td>
<td>3.62</td>
</tr>
<tr>
<td></td>
<td>$T_3$ (AC)</td>
<td>3.60</td>
</tr>
<tr>
<td>Statistical significance</td>
<td></td>
<td>$p&gt;0.05$</td>
</tr>
<tr>
<td>Solids Not Fat (SNF) (%)</td>
<td>$T_1$ (control)</td>
<td>10.34</td>
</tr>
<tr>
<td></td>
<td>$T_2$ (CaB)</td>
<td>10.26</td>
</tr>
<tr>
<td></td>
<td>$T_3$ (AC)</td>
<td>10.28</td>
</tr>
<tr>
<td>Statistical significance</td>
<td></td>
<td>$p&gt;0.05$</td>
</tr>
<tr>
<td>Protein (%)</td>
<td>$T_1$ (control)</td>
<td>4.07</td>
</tr>
<tr>
<td></td>
<td>$T_2$ (CaB)</td>
<td>4.06</td>
</tr>
<tr>
<td></td>
<td>$T_3$ (AC)</td>
<td>4.05</td>
</tr>
<tr>
<td>Statistical significance</td>
<td></td>
<td>$p&gt;0.05$</td>
</tr>
<tr>
<td>Ash (%)</td>
<td>$T_1$ (control)</td>
<td>0.83</td>
</tr>
<tr>
<td></td>
<td>$T_2$ (CaB)</td>
<td>0.82</td>
</tr>
<tr>
<td></td>
<td>$T_3$ (AC)</td>
<td>0.87</td>
</tr>
<tr>
<td>Statistical significance</td>
<td></td>
<td>$p&gt;0.05$</td>
</tr>
</tbody>
</table>
The present study was primarily designed to examine the effect of adsorbents on carryover pattern of AFB<sub>1</sub> to AFM<sub>1</sub>, 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<sup>-1</sup> body weight, the effects on performance and milk yield were not realised, but long term exposure may be a problem.

**Aflatoxin M<sub>1</sub> and carryover**

Aflatoxin M<sub>1</sub>, concentration on 3<sup>rd</sup> day was greater (P>0.05) in the adsorbent groups (T<sub>2</sub> and T<sub>3</sub>) than in the control (T<sub>1</sub>). 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<sub>1</sub> is secreted in the milk principally with the casein fraction (Allcroft and Carnaghan, 1963). The AFM<sub>1</sub> blood concentration is dependent upon the amount of AFB<sub>1</sub> destroyed in the rumen, AFB<sub>1</sub>, absorbed from the intestinal tract, the AFB<sub>1</sub> liver conversion to AFM<sub>1</sub>, and AFM<sub>1</sub>, excretion in urine, bile and milk (Dhanasekaran et al., 2011).

The discrepancy in AFM<sub>1</sub> content of milk of animals receiving AFB<sub>1</sub> 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<sub>1</sub> not only to AFM<sub>1</sub>, but to other several hydroxylated derivatives (Steiner et al., 1990; Veldman et al., 1992). Therefore, conversion of AFB<sub>1</sub> to AFM<sub>1</sub> by the mixed function oxidases system might be the cause of variation on AFM<sub>1</sub> concentration in milk.

By day 7, AFM<sub>1</sub> the concentration of milk in the adsorbent groups (T<sub>2</sub> and T<sub>3</sub>) declined, whereas the reverse was the case with the T<sub>1</sub> (control) group (Fig. 2). The decline in AFM<sub>1</sub> concentration in milk of the T<sub>3</sub> and T<sub>2</sub>, compared to T<sub>1</sub>, could be attributed to the fact that adsorbent formed a complex with AFB<sub>1</sub> which prevented the absorption of AFB<sub>1</sub> from the GI tract and thus, reduced the bioavailability of AFB<sub>1</sub> (Ramos et al., 1996).

In the present study, the reduction of AFM<sub>1</sub> residue in milk was more in the charcoal group (T<sub>3</sub>), than in bentonite group (T2). The reduction is comparable with that of Smith et al. (1994).

Carryover of AFM<sub>1</sub> from feed to milk ranged between 0.11 and 0.3 in the control (T<sub>1</sub>) group, which received 100 ppb aflatoxin B<sub>1</sub> 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<sub>1</sub> 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<sub>1</sub> 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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