Short Communication

Effects of Dietary Aflatoxin B1 on Physiological Biomarkers with Special Reference to Udder Health of Lactating Goats

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ABSTRACT

The aim of present study was to determine carcinogenic metabolite aflatoxin M1 (AFM1) in milk and to investigate the effects of low doses of dietary aflatoxin B1 (AFB1) on physiological biomarkers with special reference to udder health and serum parameters of lactating goats. Thirty two lactating Beetal goats of 3–4 y age, weighing 40.91±0.285, were randomly selected, and equally divided into four groups. Group A was kept as control while animals of groups B, C, and D were individually fed daily with 30µg, 40µg and 50µg of AFB1, respectively, through naturally contaminated cotton seed cake for a 10 days period. Milk samples were tested for aflatoxin M1 (AFM1) through high performance liquid chromatography (HPLC), somatic cell count (SCC) and total viable count (TVC). Blood samples were analyzed for aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase (ALP) levels. Aflatoxin M1 (AFM1) was detected in all milk samples of the Group B, C, and D in concentration higher than 0.05 ppb. The AFB1 was excreted in milk as metabolite AFM1 @ 1.35-1.59%. Udder health and milk quality deteriorated as SCC and TVC increased. Levels of serum enzymes AST and ALT increased with ingestion of dietary AFB1. It is concluded that ingestion of very low level of AFB1 by lactating goats results in excretion of carcinogenic metabolite AFM1 in milk beyond the permissible level. Dietary AFB1 has role in sub-clinical mastitis and causes injurious effects on general health status of lactating goats.

Aflatoxins are produced by the genus Aspergillus (several species) of fungi as secondary metabolites, which contaminate plants and their products (Iqbal et al., 2010). In a study Aspergillus flavus and Aspergillus parasiticus were major isolates found in food samples. Aflatoxin B1 (AFB1) is acutely toxic (Ei-Gohary, 1995) and causes liver cancer (Etzel, 2002). The types of aflatoxin B1, G1, G2, and B2 are frequently encountered in feeds. Dairy cows receiving AFB1 contaminated diet excrete AFM1 in the milk as a metabolite of AFB1 that may also be transferred to other dairy products (Creppy, 2002). The metabolite aflatoxin M1 (AFM1) excreted in milk is carcinogenic (Firmin et al., 2011). In a study, the mould-contaminated diet significantly reduced feed intake and body weight gain in poultry (Liu et al., 2011). AFM1 attains its maximum concentration in 3 days while after 4-5 days of withdrawal of AFB1 it cannot be detected in milk (Yiannikouris and Jouany, 2002). The maximum allowable concentrations of European Communities for AFB1 in feeds and concentrates for dairy animals are 20 μg/kg and 5 μg/kg, respectively (Galvano et al., 1996) and the concentration of AFM1 in animal milk is limited to 0.050 μg/kg in the European Union (European Commission, 2003a). AFM1 cannot be destroyed by storage or processing, such as autoclaving, pasteurization (European Commission, 2006). The AFM1 excretion in milk after AFB1 ingestion depends upon various factors like species of animal, milk yield, frequency of milking (Tajkarimi et al., 2008). The milk products from contaminated milk may also have AFM1 (Haris and Staples, 1992). Blood acts as an indicator of the status of the animals exposed to toxicants and other conditions. Physiological status of an animal has impact on blood constituents. Several factors like genetic make-up, breed, age, sex, and management conditions are responsible to influence blood parameters of domestic...
animals (NseAbasi et al., 2014).

Pakistan is the home tract of high yielding beetal goats with average milk yield of 290 L/lactation of 130 days thus playing an important role in country’s economy. These goats are known as poor man cow because of high milk production. These goats are usually fed with concentrate feeds in the prevailing feeding systems in the country. Feed can be a potential source of aflatoxins if not properly handled. Moreover Pakistan’s climate can support fungal growth. In light of these facts the present study was conducted to evaluate the effects of dietary AFB1 on udder health, quality of milk and blood profile in lactating Beetal goats.

Materials and methods

Thirty two previously synchronized, lactating Beetal goats 3-4 y old having body weight 40.91 ± 0.285, were randomly selected. The goats with 6-8 weeks lactation period were randomly divided into 4 equal groups. After one week of adaptation period, group A was kept as control while group B, C, and D were fed with 200g, 267g and 333g of contaminated cotton seed cake to provide them 30µg, 40µg and 50µg of AFB1 per animal per day, respectively for 10 days. The concentration of AFB1 in experimental cotton seed cake was determined through high performance liquid chromatography (Masoero et al., 2007) with modifications. Briefly 25 g of grounded cotton seed cake were mixed with 84 ml of acetonitrile and 16 ml distilled water added with 5 g of sodium chloride. Acetic acid 70µl was added to 9 ml of the filtrate in a tube and was vortexed (Barnstead international company, M37610-33, USA). The mixture was eluted through immunoaffinity column (mycosep®, 226 aflazole + multifunctional columns, Romer labs, USA). HPLC column (Lichrospher® 100, RP-18, end capped 5µm, Germany) was used in the experiment. The concentration of AFB1 in experimental cotton seed cake was 150 µg / kg.

The milk samples were collected 24 h before the first AFB1 feeding and on days 5 and 10 of the experiment. The data were analyzed by one-way analysis of variance (ANOVA) using SPSS 18. Differences among mean values of experimental groups were tested using least significant difference (LSD) 95% confidence intervals. Paired t-test was used for the comparison of before and after treatment data of the same group.

Results and discussion

Different feedstuffs may become contaminated during improper storage and also during growing in the field (Richard et al., 2009). Cotton seed cake consists of certain components like protein and lipids that favor the growth and multiplication of fungi. These components act as nutrient source for fungi (Jones and King, 1990). As cotton seed cake usually contains high concentration of aflatoxins, so it was used as a source of dietary AFB1 to experimental animals in the study. Milk samples from all experimental goats were tested before AFB1 feeding and were found negative for AFM1 (Table I). AFM1 was detected in the milk of AFB1 treated groups, which indicates the metabolism of AFB1 into AFM1 in goats’ body. AFM1 concentration in milk ranged from 0.210 to 1.073 ppb. AFM1 concentration showed linear increase with the dose of AFB1 (Table II). It was noted that even lowest dose (30µg/animal) of AFB1 used in this study resulted in excretion of AFM1 in milk beyond permissible level. Increase in AFM1 excretion in Greek indigenous goats’ milk with increasing the amount of AFB1 administered has been reported by earlier researchers (Kourousekos et al., 2012).

SCC of milk increased in AFB1 treated groups B, C and D (Table II). The animals remained uninfected throughout the experimental period, therefore the increase in SCC cannot be attributed to clinical mastitis, which causes increase in SCC (Applebaum et al., 1982). The findings are not in agreement with earlier study, reported no changes in goats SCC when administered with pure AFB1 orally (Jones and King, 1990). However the results of current study agree with previous research in which high score of California mastitis test was observed in cows after AFB1 intake (Bansal et al., 2005). In one study differences in results were recorded regarding milk quality when cows were administered with same amounts of pure and impure
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AFB1 (Brown et al., 1981). This increase of SCC can be attributed to sub-clinical mastitis in experimental animals as AFB1 causes immunosuppression (Applebaum et al., 1982).

TVC of all treated groups significantly increased after AFB1 consumption (Tables I, II). After feeding AFB1, the maximum TVC recorded in one goat was 6.0 × 10⁴ cfu/ml of milk (Table II). These findings are contrary to the results previously described as after administration of AFB1 in pure form to lactating goats caused no changes in the TVC of milk (Applebaum et al., 1982). In present study TVC was positively correlated to SCC of the milk, while similar findings have been reported by earlier researchers (Cheng et al., 2002). The increase in TVC of treated groups may be attributed to the immunosuppressive effects of AFB1, as significant decrease in humoral and cell mediated immune response occurred in New Zealand white rabbits subjected to different levels of mycotoxins (Georgios et al., 2012).

Results showed significant increase in AST level of groups B, C and D, while remained statistically unchanged in control group (Tables I, II). ALP remained statistically unchanged in groups A, B and D, while increased in group C. The enzyme ALT level significantly increased in treated groups B and D only. The findings are in agreement with those described by earlier researchers, who administered AFB1 in 3 different levels to ewes and noted significant effect on AST and ALT levels (McDougal et al., 2007). Regarding ALP he reported decrease in its level with AFB1 administration, while in this study, present doses of AFB1 exerted no significant effect on ALP level in goats. After aflatoxin administration, increased AST activity in goats was recorded in a previous study (Prabu et al., 2013). In the present study, the level of AST was dose dependent so it can be assumed that AFB1 has significant effect on hepatocytes. The increase in AST and ALT can be attributed to the cytotoxic effect of AFB1 on liver cells. It has been described that AFB1 caused oxidative damage through lipid peroxidation induction in rats (Battacone et al., 2003).

Conclusions

In conclusions, ingestion of very low level of AFB1 by lactating goats results in excretion of carcinogetic metabolite AFM1 in milk beyond permissible level. Dietary AFB1 has role in sub-clinical mastitis and causes injurious effects on general health status of lactating goats.

Table I.- SSC (cells/ml), TVC (cfu/ml), AST, ALT, and ALP (units/liter), and AFM1 (µg/kg) levels of goats before AFB1 administration (Mean±S.E).

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Group A</th>
<th>Group B</th>
<th>Group C</th>
<th>Group D</th>
</tr>
</thead>
<tbody>
<tr>
<td>SSC</td>
<td>1.5×10⁶±2.31×10⁵a</td>
<td>2.43×10⁶±1.47×10⁶b</td>
<td>2.0×10⁶±2.98×10⁶ab</td>
<td>8.75×10⁴±1.25×10⁵cd</td>
</tr>
<tr>
<td>TVC</td>
<td>2.5×10⁴±1.63×10⁴a</td>
<td>2.18×10⁴±1.87×10⁴a</td>
<td>2.06×10⁴±2.39×10⁴a</td>
<td>2.25×10⁴±2.83×10⁴a</td>
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<td>AST</td>
<td>77.75±2.46a</td>
<td>93.13±10.75b</td>
<td>73.50±1.33a</td>
<td>92.50±2.62b</td>
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<tr>
<td>ALT</td>
<td>13.50±0.73a</td>
<td>15.25±0.881a</td>
<td>18.63±0.865b</td>
<td>20.50±0.567b</td>
</tr>
<tr>
<td>ALP</td>
<td>60.50±6.907a</td>
<td>187.25±24.22b</td>
<td>99.38±4.37a</td>
<td>167.38±13.79b</td>
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<tr>
<td>AFM1 residues</td>
<td>n.d</td>
<td>n.d</td>
<td>n.d</td>
<td>n.d</td>
</tr>
</tbody>
</table>

*Means in each row having different superscript are significantly different at p≤0.05. AFM1, aflatoxin M1; AFB1, aflatoxin B1; SCC, somatic cell count; TVC, total viable count; S.E, standard error; cfu, colony forming unit; n.d, not detected; AST, aspartate aminotransferase; ALT, alanine aminotransferase; ALP, alkaline phosphatase.

Table II.- SSC (cells/ml), TVC (cfu/ml), AST, ALT, and ALP (units/liter), and AFM1 (µg/kg) levels of goats after AFB1 administration (Mean±S.E).

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control</th>
<th>30 µg</th>
<th>40 µg</th>
<th>50 µg</th>
</tr>
</thead>
<tbody>
<tr>
<td>SSC</td>
<td>1.44×10⁶±1.75×10⁶a</td>
<td>4.13×10⁶±4.09×10⁶b</td>
<td>3.63×10⁶±4.60×10⁶bc</td>
<td>1.38×10⁶±1.56×10⁶a</td>
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<tr>
<td>TVC</td>
<td>2.4×10⁴±1.9×10⁴a</td>
<td>4.3×10⁴±1.6×10⁴bcd</td>
<td>4.8×10⁴±3.3×10⁴b</td>
<td>3.6×10⁴±3.3×10⁴bcd</td>
</tr>
<tr>
<td>AST</td>
<td>79±2.21a</td>
<td>110±5.38abd</td>
<td>104±2.01b</td>
<td>126±9.45abd</td>
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<tr>
<td>ALT</td>
<td>14±0.32a</td>
<td>20±1.11b</td>
<td>21±1.10b</td>
<td>28±1.25a</td>
</tr>
<tr>
<td>ALP</td>
<td>62±7a</td>
<td>223±22b</td>
<td>147±4ed</td>
<td>175±16ed</td>
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<td>AFM1 residues</td>
<td>0a</td>
<td>0.4049±0.1013b</td>
<td>0.5801±0.0846bcd</td>
<td>0.7960±0.0871cd</td>
</tr>
</tbody>
</table>

*Means in each row having different superscript are significantly different at p≤0.05. AFM1, aflatoxin M1; AFB1, aflatoxin B1; SCC, somatic cell count; TVC, total viable count; S.E, standard error; cfu, colony forming unit; n.d, not detected; AST, aspartate aminotransferase; ALT, alanine aminotransferase; ALP, alkaline phosphatase.
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Statement of conflict of interest

The authors have no affiliations with any organization which may influence the results of the study.

References


