INFLUENCE OF AFLATOXIN PRESENT IN FORAGES
AND CONCENTRATED FEEDINGSTUFFS ON MILK
AND SOME SERUM BIOCHEMICAL PARAMETERS IN GOATS

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Abstract

Eight private farms as groups were used in the study. Each group contained randomly selected ten goats. These animals were fed forage and concentrated feed. Serum glucose, total protein, albumin, globulin, cholesterol, and triglyceride levels, and ALP (alkaline phosphatase), ALT (alanine-amino transferase), AST (aspartate amino transferase), and LDH (lactate dehydrogenase) activities were analysed. There were no correlations between glucose, ALP, AST, GGT and feed total aflatoxin (AF) concentrations. There were positive correlations between feed AF and LDH activities (P<0.01), between feed AF and milk aflatoxin M1 (AFM1) (P<0.01). On the other hand, there was a negative correlation (P<0.01) between feed AF and total protein levels were also present. There was negative correlation between ALT concentration (P<0.05) and AF in feed. There was negative correlation between concentrations of albumin and globulin (P<0.01) and positive correlation between triglyceride concentration (P<0.05) and AF level in feed. It was noticed that a marked increase in the level of AFM1 in milk due to an increase in total aflatoxin levels in feeds (P<0.01).

Key words: goat, aflatoxin, feed, milk, biochemical parameters.

Aflatoxins are a family of fungal toxins produced mainly by two Aspergillus species (Aspergillus flavus and Aspergillus parasiticus), which are especially abundant in areas of the world with hot and humid climates. Aflatoxins can be produced under conditions of 85% of relative humidity (2, 6, 9). The toxins are potent hepatotoxins, hepatocarcinogens, and teratogens. They have been found to be the cause of impaired protein formation, coagulation, weight gains, and immunity (6, 7, 23).

Aflatoxicosis in animals is usually manifested by pathologic changes in the liver. Swine, cattle, and poultry are the domestic species of greatest economic concern in terms of aflatoxicosis (23). Ruminants are comparatively resistant to aflatoxicosis, but the presence of aflatoxins in milk of dairy cows is closely monitored for humans (9).

Aflatoxin M1 is a metabolite of aflatoxin B1 in humans and animals. In cattle, milk production is affected, but of a greater significance is that the aflatoxins in feeds can be rather easily converted to toxic metabolites in milk, with even small amounts being readily detectable (23). Aflatoxin B1 (AFB1) present in feeds passes to milk at the rate of 0.18% in dairy cows and 0.1% in sheep (27).

The aim of this study was to examine the influence of aflatoxin levels in forages and concentrated feedingstuffs on some biochemical parameters in the serum of goats. Moreover, the relationship between aflatoxin levels in feed and milk were investigated.

Material and Methods

The study was performed in eight private farms treated as individual groups. Ten goats were selected randomly from each farm. Each animal group was born and raised at their farm. The animals were of a native goat breed and between 36 and 48 months of age. They were fed routinely ad libitum both forage and concentrated (barley+wheat+soybean meal) feed. No supplementary feed was added to the diet. The feed used routinely for nutrition of goats at each of the private farms was collected for aflatoxin analyses.

Blood and milk samples were collected about 4 h after feeding. The blood was collected by routine methods. Serum glucose, total protein, albumin, globulin, cholesterol, triglyceride levels, and ALP, ALT, AST, GGT, and LDH activities were estimated by auto analyser (Hitachi Modular (PP), Japan) with commercial kits.

Samples of milk, forage, and feed concentrates were collected by routine methods. Aflatoxin content in feed (RIDASCREEN Aflatoxin Total; r-biopharm, Art. No.4701) and milk (RIDASCREEN Aflatoxin M1 -
AFM1; r-biopharm, Art. No: R1101) were analysed. All results were evaluated using the ELISA (R-Biopharm).

Data analysis was conducted using PROC GLM for analysis of variance and means were compared using the Duncan’s New Multiple Range Test. The mean values are reported with standard deviations. In addition PROC CORR was used to estimate the Pearson correlation coefficients. In all statistical tests, P<0.05 was regarded as significant. SAS Software (25) was used for all data analysis.

Permission for a trial on the animals was obtained from the private farm owners by the consent of a letter.

**Results**

The AF levels of feed and milk samples are shown in Tables 1 and 2. Mean serum glucose, total protein, albumin, globulin, cholesterol, and triglyceride levels, and ALP, ALT, AST, GGT, LDH activities are presented in Table 3. Correlations coefficient of combination of both feed and milk AFM1, and some serum biochemical parameters are shown in Table 4.

Total aflatoxin levels of feed in group 8 were found higher than those in other groups. Total aflatoxin levels of all concentrated feed samples were higher than those of forage feed samples.

Aflatoxin levels in milk were at a maximum in group 4 and at a minimum in group 2. They were higher than the tolerance limits in groups 4 and 6.

There were differences between the groups in all parameters. Glucose levels were at a maximum in group 5, and at a minimum in group 6. Total protein levels were at a maximum in group 2 and at a minimum in group 5. Albumin levels were at a maximum in group 2 and at a minimum in group 7. Globulin levels were at a maximum in group 1 and at a minimum in group 5. Cholesterol levels were at a maximum in group 3 and at a minimum in group 1. Triglyceride levels were at a maximum in group 5 and at a minimum in group 2. ALP activities were at a maximum in group 1 and at a minimum in group 8. ALT activities were at a maximum in group 5 and at a minimum in group 8. There were no differences between the groups except for the groups 5 and 8. AST activities were at a maximum in group 3 and at a minimum in group 8. GGT activities were at a maximum in group 1 and at a minimum in group 5. GGT activities in group 1 were higher than in the other groups (P<0.05). LDH activities were at a maximum in group 4 and at a minimum in group 1. LDH activities in group 4 were higher statistically than the other groups (P<0.05).

There were no correlations between feed AF and glucose, cholesterol concentrations, ALP, AST, and GGT activities. There were positive correlations between feed AF and LDH activities (P<0.01), triglyceride concentration (P<0.05), and AFM1 (P<0.01). Furthermore, there were negative correlations between feed AF and ALT activities (P<0.05), total protein, albumin, and globulin concentrations (P<0.01), which can be seen in Table 4.

**Table 1**

<table>
<thead>
<tr>
<th>Feed</th>
<th>Group 1</th>
<th>Group 2</th>
<th>Group 3</th>
<th>Group 4</th>
<th>Group 5</th>
<th>Group 6</th>
<th>Group 7</th>
<th>Group 8</th>
</tr>
</thead>
<tbody>
<tr>
<td>Concentrated</td>
<td>32</td>
<td>20</td>
<td>360</td>
<td>420</td>
<td>370</td>
<td>450</td>
<td>440</td>
<td>470</td>
</tr>
<tr>
<td>Forage</td>
<td>50</td>
<td>15</td>
<td>30</td>
<td>400</td>
<td>77</td>
<td>57</td>
<td>63</td>
<td>70</td>
</tr>
</tbody>
</table>

**Table 2**

Mean aflatoxin M1 levels in goat milk (ppt)

<table>
<thead>
<tr>
<th>Group 1</th>
<th>Group 2</th>
<th>Group 3</th>
<th>Group 4</th>
<th>Group 5</th>
<th>Group 6</th>
<th>Group 7</th>
<th>Group 8</th>
</tr>
</thead>
<tbody>
<tr>
<td>20.86±2.91c</td>
<td>19.00±4.24c</td>
<td>28.86±6.09c</td>
<td>75.57±65.52c</td>
<td>29.14±10.48c</td>
<td>63.57±44.13c</td>
<td>36.43±18.26c</td>
<td>26.00±4.93c</td>
</tr>
</tbody>
</table>

± SD
a, b, c: statistically significant differences at P≤0.05.

**Table 4**

Correlation coefficients of forages, concentrated feed-stuffs and some serum biochemical parameters

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Feed</th>
<th>Glucose</th>
<th>Total protein</th>
<th>Albumin</th>
<th>Globulin</th>
<th>Cholesterol</th>
<th>Triglycerides</th>
<th>ALP</th>
<th>ALT</th>
<th>AST</th>
<th>GGT</th>
<th>LDH</th>
<th>AFM1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Combination of both</td>
<td>-0.05</td>
<td>-0.67&quot;&quot;&quot;&quot;</td>
<td>-0.50&quot;&quot;&quot;&quot;</td>
<td>-0.54&quot;&quot;&quot;&quot;</td>
<td>0.18</td>
<td>0.52&quot;&quot;&quot;&quot;</td>
<td>-0.01</td>
<td>-0.26&quot;&quot;&quot;&quot;</td>
<td>-0.03</td>
<td>-0.19</td>
<td>0.67&quot;&quot;&quot;&quot;</td>
<td>0.47&quot;&quot;&quot;&quot;</td>
<td></td>
</tr>
</tbody>
</table>

**P<0.01, *P<0.05**
<table>
<thead>
<tr>
<th>Parameter</th>
<th>Group 1</th>
<th>Group 2</th>
<th>Group 3</th>
<th>Group 4</th>
<th>Group 5</th>
<th>Group 6</th>
<th>Group 7</th>
<th>Group 8</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose (mg/dL)</td>
<td>19.42±3.69</td>
<td>17.29±4.15</td>
<td>27.43±5.62</td>
<td>20.86±5.73</td>
<td>49.14±7.15</td>
<td>14.71±3.25</td>
<td>21.00±3.87</td>
<td>18.29±3.86</td>
</tr>
<tr>
<td></td>
<td>a, b, c, d</td>
<td></td>
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<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Total protein (g/dL)</td>
<td>9.01±0.34</td>
<td>9.21±0.59</td>
<td>7.01±0.54</td>
<td>7.01±0.58</td>
<td>6.73±0.73</td>
<td>7.14±0.40</td>
<td>7.04±0.53</td>
<td>7.61±0.42</td>
</tr>
<tr>
<td></td>
<td>a</td>
<td>a</td>
<td>bc</td>
<td>bc</td>
<td>c</td>
<td>a</td>
<td>bc</td>
<td>b</td>
</tr>
<tr>
<td>Albumin (g/dL)</td>
<td>2.93±0.46</td>
<td>3.54±0.30</td>
<td>2.84±0.5</td>
<td>2.70±0.21</td>
<td>2.91±0.33</td>
<td>2.90±0.21</td>
<td>2.51±0.26</td>
<td>2.71±0.18</td>
</tr>
<tr>
<td></td>
<td>b</td>
<td>a</td>
<td>bc</td>
<td>bc</td>
<td>b</td>
<td>a</td>
<td>bc</td>
<td>b</td>
</tr>
<tr>
<td>Globulin (g/dL)</td>
<td>6.14±0.69</td>
<td>5.71±0.49</td>
<td>4.21±0.53</td>
<td>4.28±0.56</td>
<td>3.59±0.36</td>
<td>4.43±0.48</td>
<td>4.53±0.53</td>
<td>4.90±0.47</td>
</tr>
<tr>
<td></td>
<td>a</td>
<td>b</td>
<td>c</td>
<td>c</td>
<td>b</td>
<td>bc</td>
<td>bc</td>
<td>b</td>
</tr>
<tr>
<td>Cholesterol (mg/dL)</td>
<td>53.86±6.15</td>
<td>69.57±10.75</td>
<td>73.14±17.68</td>
<td>65.85±9.63</td>
<td>55.00±18.56</td>
<td>72.14±12.14</td>
<td>72.43±14.06</td>
<td>69.43±9.93</td>
</tr>
<tr>
<td></td>
<td>c</td>
<td>ab</td>
<td>a</td>
<td>ab</td>
<td>bc</td>
<td>a</td>
<td>a</td>
<td>ab</td>
</tr>
<tr>
<td>Triglycerides (mg/dL)</td>
<td>7.14±3.02</td>
<td>5.86±2.19</td>
<td>13.29±3.25</td>
<td>14.00±5.72</td>
<td>16.00±3.96</td>
<td>11.86±3.76</td>
<td>15.71±4.92</td>
<td>14.43±3.26</td>
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<tr>
<td>ALP (IU/L)</td>
<td>397.00±578.39</td>
<td>172.71±124.94</td>
<td>307.43±359.49</td>
<td>369.29±319.72</td>
<td>158.14±138.13</td>
<td>115.71±114.48</td>
<td>197.57±338.22</td>
<td>74.14±43.15</td>
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<tr>
<td></td>
<td>ab</td>
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<td>a</td>
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<td>a</td>
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<tr>
<td>ALT (IU/L)</td>
<td>19.71±7.72</td>
<td>19.57±7.80</td>
<td>15.43±2.23</td>
<td>16.71±4.61</td>
<td>21.86±3.76</td>
<td>18.43±5.68</td>
<td>15.57±3.74</td>
<td>13.71±3.55</td>
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<td></td>
<td>ab</td>
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<td>ab</td>
</tr>
<tr>
<td>AST (IU/L)</td>
<td>99.29±9.12</td>
<td>120.00±15.60</td>
<td>120.14±33.63</td>
<td>119.57±27.42</td>
<td>113.43±15.68</td>
<td>107.43±21.56</td>
<td>99.86±26.03</td>
<td>82.86±13.61</td>
</tr>
<tr>
<td></td>
<td>ab</td>
<td>a</td>
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<td>a</td>
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<td>a</td>
</tr>
<tr>
<td>GGT (IU/L)</td>
<td>57.71±21.07</td>
<td>42.29±11.01</td>
<td>36.14±15.25</td>
<td>41.74±6.92</td>
<td>31.57±16.32</td>
<td>38.86±9.41</td>
<td>38.71±10.21</td>
<td>42.00±13.05</td>
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<td></td>
<td>a</td>
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<td>b</td>
</tr>
<tr>
<td>LDH (IU/L)</td>
<td>343.29±41.55</td>
<td>410.86±23.96</td>
<td>731.86±100.68</td>
<td>812.43±142.05</td>
<td>767.57±126.36</td>
<td>586.86±60.92</td>
<td>662.57±88.95</td>
<td>580.71±97.88</td>
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<tr>
<td></td>
<td>d</td>
<td>d</td>
<td>ab</td>
<td>a</td>
<td>ab</td>
<td>c</td>
<td>bc</td>
<td>c</td>
</tr>
</tbody>
</table>

a, b, c, d: means in the same rows with different letters are statistically different (P<0.05); ±SD
Discussion

Aflatoxin risk assessment presented by the 49th (1997), and 56th (2000) the Joint Expert Committee on Food Additives (JECFA), indicated that the level of 0.5 ppb as being adequate to protect public health (30). The JECFA noted that no significant public health gain would be achieved by setting a level lower than 0.5 ppb for aflatoxin M1 in milk. JECFA compared the predicted risk reduction under worst case assumptions (life time exposure to all milk at the highest level) at maximum levels of 0.05 and 0.5 ppb, and concluded that the additional risks were insignificant (2, 19).

Aflatoxins remain as a threat to the health of livestock as well as humans, by their continuing intermittent occurrence in both feeds and foods. The level of AFB1 contamination in broiler feed is lower than the legal levels (10–100 ppb) for animal feedstuffs in Asian and African countries. The regulatory level for AFB1 in foods adopted in many countries is 5 ppb posed on risk assessments (6, 11, 23, 29). In the present study, it was observed that total aflatoxin in feed given to group 8 were found higher than those in other feeds. The total aflatoxin levels in all concentrated feed samples were higher than those in forage samples. In feed of all groups, they were higher than reference levels except for groups 1 and 2 (Table 1).

Aflatoxin M1 is the principal aflatoxin residue in meat and milk. FDA limit of aflatoxin M1 in milk is 0.5 ppb (6, 11, 16, 23, 29). The milk AFM1 concentration significantly depended on by the AFB1 dose (4, 5). In this study, aflatoxin levels in milk were at a maximum in group 4 and at a minimum in group 2. They were higher than the tolerance limit in groups 4 and 6. There was seen important increase in the level of AFM1 in milk due to increasing total aflatoxin levels in combination of feeds (P<0.01) (Table 2).

Many researchers have reported that glucose and its metabolite concentrations were affected by aflatoxicosis (1, 12, 18, 21, 22). In this study, glucose levels were lower than normal levels in all groups (Table 3).

It was reported that serum total protein, albumin and globulin levels were decreased in aflatoxicosis (12, 17, 18, 20, 24). In the study, it was found that there was a negative correlation between total protein levels and combination of both feed AF (P<0.01). There was seen a marked decrease in the total protein level due to increasing total aflatoxin concentration in feeds (P<0.05) (Table 4).

In the present study, albumin levels decreased (P<0.01), while aflatoxin levels increased. This result is comparable with the above literature. Albumin levels in all groups were in normal ranges except for group 7 (Table 3). Kececi et al. (15) found that the AF treatment significantly decreased serum albumin.

There was a negative correlation between albumin and globulin concentrations and feed AF (P<0.01) (Table 4). It was seen that while aflatoxin levels increased, albumin and globulin levels decreased (Table 3).

Highly significant (P<0.01) differences were observed for cholesterol levels between control and toxin treated groups (14, 18). Cholesterol levels in all groups were within the normal range. It was seen that cholesterol concentration increased in parallel with increasing aflatoxin level in feed (P<0.05) (Table 3). Triglyceride levels were at a maximum in group 5 and at a minimum in group 2. It was seen that triglyceride level increased depending on increasing total aflatoxin level in combination of both feeds (P<0.01) (Table 4).

Plasma enzyme activities increased when enzyme was released to blood circulation by some reasons (necrosis, cell damage, tissue regeneration etc.). ALT, AST, GGT, and ALP are specific enzymes for diagnosis of liver diseases. The hepatic enzyme activities were increased by aflatoxins (8, 13, 23). More conclusive evidence of aflatoxin involvement in disease includes acute to chronic liver disease with concomitant increases in specific liver enzymes in the serum (23).

Many researchers found that the activity of ALP increased with AF exposure (12, 14, 17, 21, 26). Finding of the present study could be attributed to previous reports (Table 3).

AST is released into general circulation upon hepatocellular destruction and indicates that hepatic tissue damage increases after 5 d in birds given aflatoxin (7). AF treatment significantly increased the AST and ALT activities (3, 18, 20). Increases in AST and ALT levels were related to AFB1 doses directly (28). But, Kubena et al. (17) reported that feeding the combination of AF resulted in decreased activity of ALT (17). Besides, Rhona et al. (22) reported that ALP, AST, and ALT activity in cows treated experimentally with AFB1 remained relatively unchanged during the treatment.

In the present study, there was no correlation between ALP and AST activities and combination of both feed AF, but, there was negative correlation between ALT activities (P<0.05) and combination of both feed AF (Table 4). These findings are parallel with the results of other researchers.

The AF given alone caused alterations in serum values of GGT (10, 12, 18, 26). Administration of AFB1 to rats (2 mg/kg intraperitoneally) caused a significant increase in the activities of GGT (21). In our study, similarly, GGT activities in all groups were found higher than the normal range (Table 3).

Serum LDH activity is not specific for the tissues. Muscles, liver, and erythrocytes can be high activity source (13). LDH activities after receiving AFB1 were significantly increased (14). LDH activities in all groups were found higher than the normal range, except for group 1 (Table 3). There was positive correlation on LDH activities (P<0.01) and feed AF (Table 4).

There were no correlations between glucose, ALP, AST, and GGT concentration and feed AF content (Table 4). Oğuz et al. (20) reported that low AF levels in feed did not change the serum biochemistry, but significantly affected the enzyme activities in broilers.

In conclusion: increase in AFM1 level in milk and some biochemical parameters in serum altered negatively due to increasing aflatoxin level in feed.
Therefore, aflatoxin levels in feed should be under control by periodic repeating the aflatoxin analysis.

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References