Full Length Research Paper

The effect of aflatoxin levels on milk production, reproduction and lameness in high production Holstein cows

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The aim of this study is to investigate the effect of aflatoxin levels in diet on milk production and composition, reproduction problems and indices and lameness in dairy cattle. To determine the levels of total aflatoxin and $B_1$, $B_2$, $G_1$ and $G_2$ in the feedstuff and $M_1$ in the milk, samples from two different dairy farms; A, low aflatoxin level (13.01 µg/kg) and B, high aflatoxin level (110.63 µg/kg), were collected and the determination of aflatoxin levels was performed by high performance liquid chromatography (HPLC). Milk yield and composition were measured during 60 days postpartum. Clinical examination and record data was used to indicate lameness and reproduction problems and indices during 50 to 100 days postpartum. The results showed that increase in the levels of aflatoxin concentration in the feedstuff was responsible for the increase in aflatoxin $M_1$ in milk samples. Also, levels of aflatoxin in diet and milk caused retained placenta which is a reproduction problem. Aflatoxin levels in feed and milk did not significantly increase reproduction indices and milk composition. However, prevalence of lameness was significantly affected by milk aflatoxin level ($P \leq 0.05$). It was concluded that there was a significant relationship between aflatoxin levels with lameness and retained placenta.

Key words: Aflatoxin, diet, milk, reproduction, lameness, dairy cattle.

INTRODUCTION

Overall, fungi decrease the quality of nutrients and cause reproductive failure by making chemical compounds called mycotoxin. Funguses produce some mycotoxins in feedstuff and about 45% of mycotoxin is spread by agricultural equipments.

Mycotoxins cause different economical damage in agriculture and veterinary. Mycotoxins have estrogenic structure therefore, they have negative effects on reproductive indices and cause decrease in growth and food intake as well as damage the main organic part of animal's body (Kabar et al., 2006; Whitlow and Halger, 2002; Zheng et al., 2005). Aflatoxin is a kind of mycotoxin mostly produced by Aspergillus flavus and Aspergillus paraziticus. Four main types of aflatoxin are determined in the animal's food and are called $B_1$, $B_2$, $G_1$ and $G_2$ according to fluorescent blue, green and movement symbols. $B_1$ aflatoxin is more active biologically than the other aflatoxins. Aflatoxin $M_1$ which is a metabolite of $B_1$ is found in milk but the other characteristics of aflatoxin are not clearly known. Potential aflatoxin poisoning depend on different factors like the type of aflatoxin, dosage, duration of effect, age, nutritional situation, metabolism and immune mechanisms of the animal's body. Aflatoxins have biological effects like decreasing reproductive ability and fertility, lameness and damage of kidney, liver and respiratory system. Growth of aflatoxin in diet and increase in the rate of this toxin from the critical range(20 in diet and 0.5µg/kg in milk) cause decrease in fertility and reproductive ability in animals (Kabar et al., 2006; Zheng et al., 2005; Ozsoy's et al., 2005; Gary et al., 2004). The determination of aflatoxin can be done using different methods like thin layer chromatography (TLC), high

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Abbreviations: HPLC, High performance liquid chromatography; TLC, thin layer chromatography; GC, gas chromatography; ELISA, enzyme linked immunosorbent assay; SCC, somatic cell count.
Table 1. The different aflatoxin levels (µg/kg) ingredient of diet in dairy farms.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Aflatoxin levels</th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>B₁</td>
<td>B₂</td>
<td>G₁</td>
<td>G₂</td>
<td>Total</td>
</tr>
<tr>
<td>Dairy Farm A</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Alfalfa</td>
<td>0.38</td>
<td>-</td>
<td>0.31</td>
<td>-</td>
<td>13.01</td>
</tr>
<tr>
<td>Corn silage</td>
<td>0.45</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>0.45</td>
</tr>
<tr>
<td>Sugar beet pulp</td>
<td>0.32</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>0.32</td>
</tr>
<tr>
<td>Concentrate</td>
<td>14.5</td>
<td>2.62</td>
<td>-</td>
<td>-</td>
<td>17.12</td>
</tr>
<tr>
<td>Dairy Farm B</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Alfalfa</td>
<td>0.7</td>
<td>-</td>
<td>0.39</td>
<td>-</td>
<td>1.09</td>
</tr>
<tr>
<td>Corn silage</td>
<td>0.36</td>
<td>0.75</td>
<td>0.94</td>
<td>-</td>
<td>2.05</td>
</tr>
<tr>
<td>Sugar beet pulp</td>
<td>0.55</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>0.55</td>
</tr>
<tr>
<td>Concentrate</td>
<td>68.2</td>
<td>9.77</td>
<td>-</td>
<td>0.57</td>
<td>78.54</td>
</tr>
</tbody>
</table>

- = Not detected.

performance liquid chromatography (HPLC), gas chromatography (GC) and immunochemical methods such as enzyme linked immunosorbent assay (ELISA). HPLC is ideal and more useful than the other methods because of its specificity (0 – 320 µg/kg) and sensitivity (Zheng et al., 2005; Kalantari et al., 1999; Tajkarimi et al., 2007).

The objectives of the present study are to evaluate the relationship between type (B₁, B₂, G₁, and G₂) and level of dietary aflatoxin with milk aflatoxin and their effects on reproductive problems and indices, lameness, milk yield and composition in dairy cattle.

MATERIALS AND METHODS

Animals

This study was performed using two Holsteins dairy cattle farms (A and B) in Esfahan province in Iran. Management and environment conditions in each farm were the same. Calves were separated from their mothers and cared for in a special compartment.

Study design

Our selection of these two farms was based on dietary aflatoxin level in high milk production cattle: A: low aflatoxin level (13.01µg/kg) and B: high aflatoxin level (110.63µg/kg). The farms of A and B were with the capacity of 405 and 203 Holstein dairy cattle, respectively. The herds were kept under the same feeding and management conditions.

At the two farms, postpartum examination such as reproduction problems (retained placental, still birth, uterine infectious and irregular estrus), reproduction indices (days open, inability to conceive, increase in number of inseminations, lengthening of the period between calving and conception) and clinical lameness was carried out during 50 to 100 days by resident veterinary surgeons at the farm. Milk yield and composition were measured during 60 day postpartum.

Determination of aflatoxin in feed and milk

The amount of different aflatoxin (B₁, B₂, G₁ and G₂) in diet, separately in each feed (corn silage, Alfalfa hay, sugar beet pulp and concentrate) and milk (M₁) were determined by HPLC system (Perkin Elmer model) according to methods of Charoenpornsook and Kavisarasai (2006) and Rosi et al. (2007), respectively.

Statistical analysis

The data from each farm were analyzed by spearman ranked correlation coefficient by using software statistical package for the social sciences (SPSS) (Ver. 12.0).

RESULTS

The levels of aflatoxin in diet ingredients of the dairy farms are shown in Table 1. As anticipated, the level of feed aflatoxin in herd B was evidently greater than herd A (110.63 Vs. 13.01 µg/kg) which is more than five times the standard level (20 µg/kg). Aflatoxin G₂ was not found in feedstuff from farm A.

Table 2 shows the relationship between aflatoxin of diet with milk in dairy farms A and B. There was a significant relationship (p < 0.01) between different kinds of aflatoxin (B₁, B₂, G₁ and G₂) and total aflatoxin levels in the ratio with milk aflatoxin (M₁).

The results showed that there was no relationship between aflatoxin of diet with milk in dairy farms, but a significant relationship (p ≤ 0.05) between aflatoxin M₁, G₁ and G₂ with retained placental in dairy farm B was observed.

The relationship between aflatoxin level in diet and milk with reproduction indices in dairy farm A and B were evaluated. Average of calving to first service, calving to conception interval and number of service per conception were 57.9, 110.5 day and 2.1 time in farm A, whereas, they were 76.3 139.8 day and 2.9 time in farm B. Poor relationship were observed between aflatoxin level with average of calving to first service, number of service per conception and calving to conception but, this relationship was not significant.

According to Table 3, there was a significant relationship
Table 2. The rank correlation between diet aflatoxin and milk in dairy farms.

<table>
<thead>
<tr>
<th>Aflatoxin M&lt;sub&gt;t&lt;/sub&gt;</th>
<th>Aflatoxin levels</th>
<th>B&lt;sub&gt;1&lt;/sub&gt;</th>
<th>B&lt;sub&gt;2&lt;/sub&gt;</th>
<th>G&lt;sub&gt;1&lt;/sub&gt;</th>
<th>G&lt;sub&gt;2&lt;/sub&gt;</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Farm A</td>
<td></td>
<td>0.986**</td>
<td>0.958**</td>
<td>0.969**</td>
<td>-</td>
<td>0.984**</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.909**</td>
<td>0.919**</td>
<td>0.929**</td>
<td>0.930**</td>
<td>0.905**</td>
</tr>
</tbody>
</table>

** Significant at p ≤ 0.01; - = not detected.

Table 3. The rank correlation between aflatoxin and lameness in dairy farms.

<table>
<thead>
<tr>
<th>Lameness</th>
<th>Aflatoxin levels</th>
<th>M&lt;sub&gt;t&lt;/sub&gt;</th>
<th>B&lt;sub&gt;1&lt;/sub&gt;</th>
<th>B&lt;sub&gt;2&lt;/sub&gt;</th>
<th>G&lt;sub&gt;1&lt;/sub&gt;</th>
<th>G&lt;sub&gt;2&lt;/sub&gt;</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Herd A</td>
<td></td>
<td>0.022</td>
<td>0.112</td>
<td>-</td>
<td>-0.011</td>
<td>-0.011</td>
<td>-0.112</td>
</tr>
<tr>
<td>Herd B</td>
<td></td>
<td>0.432</td>
<td>0.425</td>
<td>0.323</td>
<td>0.389</td>
<td>-</td>
<td>0.425</td>
</tr>
</tbody>
</table>

* Significant at p ≤ 0.05; - = not detected.

(p ≤ 0.05) between milk aflatoxin level and lameness in dairy farm B so that, farm B had high level of aflatoxin milk and diet involved in lameness, and farm A had a low level of aflatoxin milk and diet which involved slight lameness.

The relationship between aflatoxin and milk yield and composition in dairy farms A and B were considerable. In both farms, days of milk production were up to 100 days and so, there was no significant relationship between diet and milk aflatoxin levels with milk production, somatic cell count, milk protein and fat.

DISCUSSION

In this study, we selected two dairy farms with low or high levels of dietary aflatoxin so that the standard level became 20µg/kg (Herrman, 2002). The contamination of M<sub>t</sub> aflatoxin in milk was indicated in five provinces in Iran by Tajkarimi et al. (2007). The average aflatoxin contamination was 0.039µg/kg in these provinces. The aflatoxin level was 0.05 µg/kg in 37 of 98 samples, but the average of contamination were 0.076, 0.008, 0.044, 0.052 and 0.061 µg/kg in Gorgan, Hamadan, Rafsanjan, Shiraz and Tehran (cities in Iran), respectively.

The results of this study showed that the highest level of aflatoxin was detected in dietary concentrate that was related to B<sub>1</sub> and it was in agreement with the findings of Ozsoy's et al. (2005). This increase in aflatoxin level may be dependent on bad storage of feed in farms and feed factories. However, it could depend on poor management and storage condition that caused increase in aflatoxin level in dairy farm B (Kabar et al., 2006).

The results showed significant relationships between milk and dietary aflatoxin (Table 2). The increase in the level of aflatoxin of the feedstuff was responsible for increase in aflatoxin M<sub>t</sub> in the milk samples. The source of aflatoxin in milk is dietary aflatoxin which is a metabolite of aflatoxin B<sub>1</sub>, while almost 2.2% aflatoxin B<sub>1</sub> in diet changed to aflatoxin M<sub>t</sub> (Ozsoy's et al., 2005; Lanyasuna et al., 2005).

In both farms, there was no statically significant relationship between aflatoxin in diet and milk with retained placental, still birth, uterine infectious and irregular estrus but there was statistically significant relationship between aflatoxin with retained placental in dairy farm B. The results indicated that aflatoxin resulted in reproduction problems and retained placental in the dairy farm B, because it has high aflatoxin level. It may be due to pain of lameness, negative energy balance, impairment of hormone and inflammation of resulted toxins in the placenta (Ozsoy's et al., 2005; Mark and Green, 1992). On the other hand, other factors such as dystocia, twin birth, immature birth, infections, microbial agents and hormonal factor were not observed (Youngquist et al., 2007).

However, research showed that aflatoxin (particular aflatoxin B) has deleterious effect on gonads, embryonic loss, change of uterine and ovary size, estrus cycle disorder and decrease of pregnancy rate and birth (Iben et al., 1994).

The results indicated that reproduction indices such as service per conception, calving to first service and calving to conception did not significantly tend to increase in farms but, difference in reproduction indices could be due to reproduction problems, negative energy balance, lameness and pain as well as hormonal insufficiency. Also, aflatoxin effect on days open was a reproductive problem and increasing number of insemination and calving to first insemination resulted in increase days open
(Iben et al., 1994; Ozsoy’s et al., 2005; Youngquist et al., 2007).

There was a significant relationship between aflatoxin and lameness (Table 3). It could be due to the toxin of aflatoxin and its effect on sensitive lamina hoof, unsuitable place straw yards, nutrition and poor management. Also, comparisons between normal cattle and lame cattle showed a slightly longer interval between calving to first insemination and conception in lame cattle. This difference may be related to the pain caused by lameness associated with aflatoxins, hormonal imbalance and nutrition imbalance (Ozsoy’s et al., 2005; Sood and Nanda, 2006).

There was no significant relationship between diet and milk aflatoxin levels with milk production, somatic cell count (SCC), milk protein and fat in the dairy farms. Researchers indicated that dietary aflatoxin had no influence on fat, protein, lactose and SNF milk and these cows had milk aflatoxin levels with milk production, somatic cell count (SCC), milk protein and fat in the dairy farms. Researchers indicated that dietary aflatoxin had no influence on fat, protein, lactose and SNF milk and these cows had low daily milk production (Diaz and Duarte, 2002). However, other researchers indicated that decreases in milk production are related to decrease in dry matter intake from feed contamination to mycotoxin and aflatoxin. However, feed consumption was affected by exposure time of diet with aflatoxin, age of animal and aflatoxin level in diet. Dietary aflatoxin could have effect on protozoa population and rumen flora, and cause decrease in cellulose utilization, volatile fatty acid and ammonium production and also, it leads to increase inactivity of alkaline phosphates enzyme in the rumen. These factors influences feed consumption and milk yield and composition (Diaz et al., 2004; Lanyasuna et al., 2005; Robens and Richard, 1992). In conclusion, the current study shows that dietary and milk aflatoxin levels had influences on lameness and retained placenta.

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