

The Relationship between Lameness, Fertility and Aflatoxin in a Dairy Cattle Herd

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Abstract: This study was carried out to determine the relationship between aflatoxins taken with feed, laminitis, lameness and impaired fertility. Lesions were identified in the claw and hock region, causing lameness in 45 cattle in an establishment of 300 Holstein dairy cattle. Of these lame cattle, 27 had cystic ovaries and 10 had cystic ovaries together with clinical metritis. The increase in lameness and fertility problems occurring in this herd, living under the same management and feeding conditions, was determined to start with the change in feed concentrates and, in order to investigate the relationship with mycotoxins, Aflatoxin B₁ (AFB₁) analysis was carried out in the feed and Aflatoxin M₁ (AFM₁) analysis was carried out in the milk of cattle both with and without illness. While a value above normal was determined in AFB₁ in feed concentrate, hay and clover, when compared to the milk of normal cattle, high values of AFM₁ were determined in the milk of lame cattle with cystic ovaries. It was concluded that aflatoxins taken with feed cause lameness (subclinical laminitis) and impaired fertility (cystic ovaries).

Key Words: Cow, laminitis, lameness, fertility, aflatoxin

Bir Sütçü Sığır Sürüsünde Topallıklar, Fertilité Bozuklukları ve Aflatoksin Arasındaki İlişki

Özet: Bu çalışma, yemlerle alınan aflatoksinler, laminitis, topallıklar ve fertilité bozuklukları arasındaki ilişkiyi araştırmak amacıyla gerçekleştirildi. Üçyüz Holstein sütçü sığırı bulunan bir işletmede 45 sığırdaki topallığa neden olan tırnak ve tarsal eklem bölgesinde lezyonlar belirlendi. Topallık görülen sığırların 27'sinde ovaryum kisti, 10'unda da ovaryum kisti ve klinik metritis birlikteydi. Sürekli aynı bakım ve beslenme koşullarında bulunan bu sürüde oluşan topallık ve fertilité problemlerindeki artışın, konsantre yem değişimi ile birlikte başladığı belirlendi ve mikotoksinlerle olan ilişkisini araştırmak amacıyla yemlerde Aflatoksinin B₁ (AFB₁), hastalısız ve hastalık bulunan sığırların sütlerinde de Aflatoksin M₁ (AFM₁) analizleri yapıldı. Konsantre yem, ot ve yoncada normal değerlerin üstünde AFB₁ tespit edilirken, normal sığır sütleri ile karşılaştırıldığında, topallık ve ovaryum kisti bulunan sığırların sütlerinde yüksek değerlerde AFM₁ belirlendi. Yemlerle alınan aflatoksinlerin, topallıklara (subklinik laminitis) ve fertilité bozukluklarına (ovaryum kisti) neden olduğu sonucuna varıldı.

Anahtar Sözcükler: Sığır, laminitis, topallık, fertilité, aflatoksin

Introduction

Lameness in dairy cattle causes large financial losses owing to a drop in milk production, impaired reproductive performance, exclusion from the herd and administration of treatment and control methods (1,2).

Laminitis is the most important reason for lameness in dairy cattle herds and has a multi-factorial etiology (3,4). The most important etiological factors in the formation of laminitis are feeding, systemic diseases, management and the calving period (5-7). In particular, excessive amounts

of carbohydrates and proteins taken during the peri-partum (around calving) period, inadequate quality feed consumption (8), and mycotoxins and nitrites taken with feed (6) play a part in the formation of laminitis. Mycotoxins cause insufficiency in reproductive performance and immune functions and a decrease in feed consumption (9).

The disease of cystic ovaries is one of the largest reproductive problems in dairy cattle (10). Cystic ovaries develop in the early post-partum period. Body condition, increase in milk production, calving season, number of lactations and lameness in the pre-partum period may cause an increase in cystic ovaries (11). Melendez et al. (12), reported the possibility of cystic ovaries developing in the first 30 days after calving to be 2.63 times higher in lame cattle than in healthy ones.

This study aimed to investigate the relationship between lameness and impaired fertility, which causes significant problems in a dairy cattle herd, and their link to aflatoxins taken with feed.

Materials and Methods

Animals

This study was carried out in a government reproduction farm (Tahirova, Çanakkale, Turkey) with a capacity of 300 Holstein dairy cattle. Since 7 of the cattle were removed from the herd due to untreatable lameness, 293 cattle were present in the herd by the end of the study. The average amount of daily feed concentrate intake in this establishment was 9-10 kg per cow. The cattle were consuming 15-20 kg of maize silage every day. Hay and clover were given ad libitum. Feed concentrate was distributed by feed distributing tractors twice a day so that each animal received 4.5-5 kg. Feeding was not done according to the milk yield of the cattle. Feed concentrates used in this establishment were purchased from commercial feed factories every October and used throughout 1 year. Uneaten feed was gathered and disposed of. The cattle spent most of the day in concrete - based compartments with 15 cm thick straw bedding. These animals were milked 3 times a day. Average daily milk production before the disease period was 6200 kg.

Following calving, calves were separated from their mothers and cared for in special compartments. Intra-

uterine antibiotic tablet administration was routinely used in all animals to prevent uterus infections following parturition, using a Trimetoprim+sulphametaxazol combination (Atavetrin bolus®; Doğu İlaç). When necessary, the administration was repeated 1 week later. A post-partum examination was carried out on days 30-50. Cystic ovaries and uterus infections were diagnosed, treated and recorded by resident veterinary surgeons at the establishment. Pregnancy was diagnosed by rectum palpation on days 42-49 following artificial insemination.

Study design

Owing to lameness (claw and hock lesions), impaired fertility (cystic ovaries, uterine infection, inability to conceive, increase in number of inseminations, lengthening of the period between calving and conception) and a drop in milk production in a herd kept under the same feeding and management conditions, the establishment managers requested an investigation. Four visits were paid at monthly intervals. During these visits, the factory where feed concentrates were prepared was assessed and meetings were held with officials. Feed stores of the farm were also visited and the quality of the self-produced maize silage, hay and dry clover was examined.

The types of lesions were identified in lame cattle and reasons and treatment options were investigated. In animals reported by the resident veterinary surgeon to have impaired fertility, cystic ovaries and uterine infections were diagnosed by rectum palpation and using a 5 mHz linear ultrasound probe. GnRh (Receptal®; Intervet), LH (Iliren®; Topkim) and Progesterone (Crestar®; Intertvet) were used to treat cystic ovaries and intra-uterine antibiotics (Metrijet®; Intervet) and povidone iodine (Latogen®; Schering-Plough) were used for the treatment of metritis. A program was developed for the treatment of foot diseases and the resident veterinary surgeons were instructed over the telephone when necessary.

Analysis

All feed was chemically analyzed for dry matter, crude protein, crude fiber, total fat and ash (13) in the laboratory of the Department of Animal Nutrition and Nutritional Diseases, Faculty of Veterinary Medicine, Istanbul University (Table 1).

Table 1. Analysis of nutritional values of cattle feed used (% DM) (13).

	Maize silage	Hay	Clover	Feed concentrate
Dry matter	25.85	90.38	92.67	90.37
Crude ash	8.00	6.78	6.06	3.09
Crude protein	8.51	9.85	8.92	18.25
Crude fat	3.82	3.43	2.41	3.24
Crude fiber	29.21	31.99	26.99	9.39

AFB₁ analysis in feed and AFM₁ analysis in milk were carried out using the RIDASCREEN[®] aflatoxin test (14). AFB₁ analysis was performed in feed concentrate, maize silage, hay and clover fed to the animals and AFM₁ was determined in milk samples obtained from disease-free cattle, those with lameness only or those having cystic ovaries together with lameness.

According to the Turkish Nutrition Codex, the maximum rate of AFB₁ in grains has been determined as 2 ppb and the maximum rate of AFM₁ in milk as 0.05 ppb (15).

Statistical analysis

The results are presented as means with their standard errors. All data were analyzed using variance analysis, after testing the homogeneity of variance between treatments using one-way ANOVA. The treatment means were compared using Duncan's multiple range test in SPSS (16).

Results

According to the records of the resident veterinary surgeons, claw-related lameness occurred in the post-partum 30-45 day-period and lesions in the hock region developed between 30 days before parturition and 30 days post-partum.

In the records of the resident veterinary surgeons, in the period immediately prior to the extensive appearance of lesions, lameness was at a rate of 2% and cystic ovaries and metritis lesions were 3%.

The number of diseased cattle present during the first visit was 45 of the 300 (15%). The distribution of lesions was as follows: Rusterholz ulcer in 12 (26.2%), heel and sole erosion in 10 (22.2%), white line separation and infection in 7 (15%), interdigital necrobacillosis in 2 (4.4%) and swelling in the hock region in 14 (31%).

Of the lesions identified in the claws, 29 (93.5%) were in the hindlimbs and 2 (6.5%) in the forelimbs. Of the lesions causing lameness and identified in the hock region, 9 (64.2%) were unilateral and 5 (35.8%) were bilateral. There were bilateral hock skin abrasions and hair loss in 15 cattle that were not included in the evaluation due to lack of lameness.

While lameness alone was seen in 8 animals, in 27 animals it accompanied cystic ovaries and in 10 cattle it was seen together with cystic ovaries and uterus infection. Seven cattle, with pododermatitis profunda and septic inflammation of the hock, were removed from the herd.

The interval of inseminations was 35-110 days. Due to widespread cystic ovaries and metritis in the herd, the first insemination, which is performed at 35-56 days in normal cows, was as late as 180 days in diseased cows and conception was as late as 270 days.

Milk production was decreased at the first visit when the disease was most severe and dropped to 3600-3800 kg/day. Since records of milk yield for each cow were not kept at the farm, drop in milk yield could not be established in lame and healthy cattle. However, according to the information obtained from the resident veterinary surgeons, 20 severely lame cows had not been milked. There was a decrease in feed consumption during this period; however, the amount of feed consumed by diseased cattle was not recorded. The feed that was not consumed was disposed of.

The problems in the herd were seen to begin approximately 2.5-3 months after the change in feed concentrate. The first visit by the authors was in the fifth month of the feed concentrate change. While lesions were identified in 45 cattle during this visit, no lesions were encountered in other cattle during subsequent visits. Upon the authors' recommendation, feed concentrate

was changed 20 days after the first visit and the cattle were fed a new feed combination. During each visit, the treatment was seen to be giving positive results. On the fourth visit, fertility problems were seen to have completely disappeared and the treatment of 5 lame cows was continuing.

According to the information obtained from factory officials and the resident veterinary surgeons, mould had formed in a large portion of the corn, due to rainwater entering through the roof of the warehouse where the corn used in the feed concentrate was stored, and this was used in the production of feed concentrate. Since this incident had taken place 4 months before the authors' first visit, the authors were unable to check the feed. Feed for the farm was purchased annually in the October of each year, sufficient for a year's consumption. The diseases were determined to be extensive in December-January.

Aflatoxin analysis

In the analysis of feed, aflatoxin could not be detected in corn silage; values found in other feed matter are shown in Table 2.

AFM₁ values in milk were significantly higher ($P < 0.05$) in lame cattle (0.2318 ± 2.4) and cattle with lameness together with cystic ovaries (0.2577 ± 1.9) compared to normal cattle (0.0992 ± 2.2). There was no statistical difference between lame cattle and lame cattle with cystic ovaries (Table 3).

Discussion

When the annual lameness rate in a herd exceeds 10%, subclinical laminitis should be suspected. Yellow waxy discoloration at the base of the claw, white line

disease, sole ulcer, double sole, sole and heel erosion, interdigital dermatitis and sole hemorrhage lesions are related to subclinical laminitis (4,6,17). Sole lesions occurring due to subclinical laminitis are frequently seen in the post-partum period and are widespread during post-partum 36-70 days (18). In our study, lameness and fertility problems were mostly seen in the first 30-45 days post-partum. The period during which both claw and hock region lesions were mostly observed was the early post-partum period. Lesions of cystic ovaries in particular were also seen frequently during this period. In the authors' opinion, the hormonal changes in this period and the toxins (Aflatoxin, bacteria toxins) could affect the very sensitive laminae and cause subclinical laminitis and cystic ovaries.

Smilie et al. (19) reported 36.2% yellow waxy discoloration, 75.2% white line disease and 73.9% heel erosion in different herds; while Bargai and Levin (17) reported 14.7% sole hemorrhage, and 15.4% white line separation in different herds. Both studies express the extent of subclinical laminitis lesions.

In our study, the fact that the lameness rate in the herd exceeded 15% and that 10% of these lesions were claw sole lesions suggests the presence and extent of subclinical laminitis. In this herd, if lesions such as sole hemorrhage and yellow waxy sole and double sole, which are indicators of subclinical laminitis but which do not cause lameness, had been evaluated as well as clinically lame cattle, subclinical laminitis lesions could have been identified at a higher rate and more realistically.

The conception rate is rather low during the period of lameness in lame cattle compared to healthy cattle. As reported in our study, this could be related to pain, negative energy balance and hormonal insufficiency (1).

Table 2. Analysis values of Aflatoxin B₁ in feed, ppb.

Feed	Aflatoxin B ₁ (ppb)
Maize silage	-
Hay	4.1
Clover	2.7
Feed concentrate	5.4

Table 3. Aflatoxin M₁ values in milk, ppb (n = number of milk samples).

Animals	n	Aflatoxin M ₁ x Sx
Normal cattle	30	0.0992 ± 2.2 ^b
Lame cattle	8	0.2318 ± 2.4 ^a
Lame cattle with ovarian cysts	27	0.2577 ± 1.9 ^a

^{a,b} Differences between groups with different letters in the same column are significant ($P < 0.05$).

Lucey et al. (20) has reported the first insemination time as 75 days and the conception interval as 100 days, while Hernandez et al. (1) reported the latter to be 140 days. A comparison between normal cattle and lame cattle was not performed in our study. The calving and insemination interval was 35-110 days in normal cows in the study herd. However, determining a slightly longer interval between calving and first insemination and between calving and conception in lame cattle was significant. In the authors' opinion, this difference may be related to the pain caused by lameness possibly associated with aflatoxins, hormonal imbalance and nutrition imbalance Livesey et al. (8) have reported that hock lesions have the lowest incidence in straw yards, while Singh et al. (21) have reported that dairy cattle prefer straw in yards. If it is considered that the cattle in the establishment spent most of the day in compartments in the straw yard, the fact that the number of hock lesions in the authors' findings was rather high is contrary to reports that lesions are lower in straw yards. However, the authors think that with respect to the presence of high levels of AFB₁ taken with feed an increase in susceptibility to injury may explain the increase in hock lesions. Although the cattle in this establishment were previously kept under similar management and feeding conditions and housed in compartments with plentiful straw bedding, the small number of hock lesions supports this idea.

It has been reported that mycotoxins in fungi-damaged feed may be involved in the etiology of laminitis (6). Aflatoxins, which are one of the most important mycotoxins, cause inadequate growth and reproduction, increased susceptibility to injury, decreased feed intake and an impaired cellular and humoral immune system (22). AFM₁ is a metabolite of AFB₁ taken with feed by dairy cattle and is seen in milk and dairy products (14).

Patterson et al. (23) stated that approximately 2.2% of the AFB₁ taken with feed converts into AFM₁.

Aflatoxin rates determined both in feed and in milk in this study are well above normal values. It can be seen that these rates do not produce the lesions seen in chronic aflatoxicosis, such as a decrease in feed intake, liver or kidney malfunctions or, in advanced cases, death (22). While it is not clear how these toxin levels would produce laminitis, it may be assumed to occur similar to the pathophysiology of toxins causing laminitis. The fact that there was no lameness or reproduction system disease in cows consuming the same feed can be explained by some of the cows coincidentally consuming more feed containing aflatoxin. Lower quantities of toxins may cause decreases in feed consumption and milk yield. This would explain the drop in milk production in the herd.

According to the information given by resident veterinary surgeons, the appearance of these problems coincided with the starting of feed concentrates and there was an 2.5-3 month period between feed change and the appearance of lameness. Although minimum and maximum values of AFB₁ are known, toxin levels able to cause laminitis and similar lesions have not been identified. Aflatoxin levels in feed and milk were above the minimum values. However, it is possible that these values were higher in the initial stages of intake of concentrated feed, which was reported to have been kept under inappropriate storage conditions in feed factories and fed to the animals before the onset of the diseases. The fact that onset of these diseases was 2.5-3 months after feed intake and in the parturition period may suggest that nutritional, hormonal and toxic changes in this period may act as a trigger by increasing susceptibility to injury.

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