



## Aflatoxin Levels in Roughage, Concentrates, Compound Feed and Milk Samples from Dairy Farms in Erzurum Province\*

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**Abstract:** Aflatoxin in roughage, concentrates and compound feed from dairy farms located in Erzurum province, and the presence of Aflatoxin M<sub>1</sub> (AFLM<sub>1</sub>) in the milk of animals fed with these feeds were determined in four different seasons. The mean level of Aflatoxin M<sub>1</sub> detected in milk samples was 0.03 ppb. Aflatoxin M<sub>1</sub> levels in the milk samples taken from the holdings were lower in autumn and summer (0.02 ppb) compared to winter and spring (0.04 ppb). The total aflatoxin levels in feed samples were higher in spring and summer, compared to autumn and winter. Furthermore, Aflatoxin B<sub>1</sub> (AFLB<sub>1</sub>) levels in roughage, concentrate and compound feed were significantly higher in spring and summer, compared to autumn and winter (P<0.01). The results obtained in this study demonstrated that Aflatoxin B<sub>1</sub> received with compound feed by cattle led to a carry-over of Aflatoxin M<sub>1</sub> into milk at a level of 1.219±0.4139%. The mycotoxin levels detected were below the maximum residue limits laid down in the Turkish Food Codex.

**Key words:** Compound feed, Concentrate, Milk, Mycotoxin, Roughage.

## Erzurum Bölgesinde Süt Sığırı İşletmelerinden Alınan Kaba, Konsantre, Karma Yem ve Süt Örneklerinde Aflatoksin Düzeyleri

**Özet:** Erzurum bölgesinde bulunan süt sığırı işletmelerinden kaba, konsantre ve karma yemde aflatoksin ve bu yemlerle beslenen hayvanların sütünde Aflatoksin M<sub>1</sub> (AFLM<sub>1</sub>) varlığı dört farklı dönemde tespit edildi. Süt örneklerinde tespit edilen ortalama aflatoksin düzeyi 0.03 ppb'dir. İşletmelerden alınan süt örneklerindeki aflatoksin düzeyleri kış ve ilkbaharla (0.04 ppb) mukayese edildiğinde sonbahar ve yazın (0.02 ppb) düşük bulundu. Yem örneklerindeki total aflatoksin düzeyleri kış ve sonbahar ile mukayese edildiğinde ilkbahar ve yazın daha yüksek bulundu. Bundan başka, kaba, konsantre ve karma yemlerdeki Aflatoksin B<sub>1</sub> (AFLB<sub>1</sub>) düzeyleri sonbahar ve kış ile mukayese edildiğinde ilkbahar ve yazın önemli derecede yüksek bulunmuştur (P<0.01). Bu çalışmada elde edilen sonuçlar karma yemde aflatoksin B<sub>1</sub> e maruz kalan süt sığırlarının sütüne % 1.219±0.4139 oranında geçtiği tespit edildi. Tespit edilen mikotoksin düzeylerinin Türk Gıda Kodeksi tarafından belirtilen sınırların altında olduğu tespit edildi.

**Anahtar kelimeler:** Kaba, Karma, Konsantre yem, Mikotoksin, Süt.

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## INTRODUCTION

In the past decades, aflatoxins giving their negative impact on animal health and animal health economics have drawn great attention, and the potential risk of their residues pose for public health (Kaya, 2007). Apart from the significance they bear for animal nutrition, feed stuffs also constitute a favourable medium for the growth and reproduction of microorganisms. Under inappropriate growth, harvesting, processing and storage conditions, the feed may be contaminated with microorganisms. The microbial contamination of feed may result in the spoilage and loss of nutritional value within a short period of time, and may also damage the health of animals. As a result of their metabolism, some fungi produce and release into the environment they reproduce, toxic substances referred to as "mycotoxins" (Steyn and Stander, 1999).

The two compounds of mycotoxins, firstly, which were observed to give blue fluorescence under ultraviolet (UV) light were named as AFLB<sub>1</sub> (Aflatoxin B<sub>1</sub>) and AFLB<sub>2</sub> (Aflatoxin B<sub>2</sub>), and the other two compounds determined to give yellowish green fluorescence were named as AFLG<sub>1</sub> and AFLG<sub>2</sub>. Later, it was ascertained that a derivative of these toxins was found in the milk of dairy animals fed with aflatoxin-contaminated feed. Owing to its presence in milk, this toxin was named as the "milk toxin", in short Aflatoxin M. Research on Aflatoxin M has shown that this metabolite is a 4-hydroxy derivative of AFLB<sub>1</sub> and AFLB<sub>2</sub>, thus, two compounds, referred to as AFLM<sub>1</sub> and AFLM<sub>2</sub> have been isolated (Wood 1991; Van Egmond 1994). Based on these data, the maximum limit of AFLM<sub>1</sub> allowed in milk has been set as either 0.05 or 0.5 ppb in several countries (Arbillaga et al., 2007). In Turkey, the *Communiqué No. 2009/22 Amending the Communiqué No. 2008/26 on the Maximum Limits of Contaminants in Food Substances*, which was published in the Official Gazette in 2009 lays down the maximum limits of aflatoxin allowed in various food products. Accordingly, the maximum

limit of aflatoxin has been set as 5.0 µg/kg for nuts, 8.0 µg/kg for groundnuts, 2.0 µg/kg for cereals, and 0.050 µg/kg (AFLM<sub>1</sub>) for milk. The most recent legal arrangement regulating this issue is the *Communiqué No. 2009/22* published in 2009, which is still in force (Anonymous, 2009).

It has been reported that, across the globe, around 4.5 million people are exposed to uncontrollably increasing chronic aflatoxicosis (Williams et al., 2004). Researches conducted suggest that the exposure to AFLB<sub>1</sub> and the development of primary hepatocellular carcinoma, which is the seventh most common type of cancer in the world, are associated with each other or that the exposure to Aflatoxin B<sub>1</sub> is the most influential factor on the development of this type of cancer (Vidyasagar et al., 1997).

Therefore, this study was conducted to determine, for different seasons, the mycotoxins (total aflatoxin and aflatoxin B<sub>1</sub>) found in roughage and concentrate feed stored at dairy farms in Erzurum province, and the presence of AFLM<sub>1</sub> in the milk of dairy cattle fed on a mixed ration of concentrate and roughage feed.

## MATERIALS and METHODS

### Collection of Feed and Milk Samples

This study was conducted in 11 farms. It was considered an example of each holding. Feed and milk samples were taken separately an example from each holding in spring (April), summer (July), autumn (October) and winter (December) in 2011 years. Samples of roughage, concentrate and mixed feed ration (roughage + concentrate feed provided to the animals in feed troughs) were collected into sterile plastic sample bags (in volumes of approximately 50 g). Ten-ml milk samples from milk tanks were placed into sterile sample tubes and transferred to the laboratory under cold chain conditions. In order to determine the total aflatoxin and AFLB<sub>1</sub> levels, the feed samples were stored in a deep freezer until analysed.

### Kits Used in the Analysis of the Feed Samples

All samples (milk and feed) were analysed using the ELISA method. The test kits used in the analyses were the Aflatoxin Low Matrix Detection Kit (Helica Biosystem INC; Cat. No: 981AF01LM) for total aflatoxin, the Aflatoxin B<sub>1</sub> Detection Elisa Kit (Helica Biosystem INC; Cat. No: 941BAFL01B1) for AFLB<sub>1</sub>, and the Aflatoxin M<sub>1</sub> Assay Elisa Kit (Helica Biosystem INC; Cat. No: 961AFLM01M) for AFLM<sub>1</sub>. The protocols described by the manufacturer (Helica Biosystem INC) were applied to determine the aflatoxin levels in the samples collected.

### Statistical Analysis

The SPSS v19 statistical software (SPSS 2010)

was used for the analytical and descriptive analyses performed in this study.

### RESULTS

The assessment of the impact of seasons on the level of total aflatoxin in roughage demonstrated that the lowest level was determined in autumn, followed by summer and winter, with the highest level found in spring ( $P<0.01$ ). The results obtained showed that the total aflatoxin levels detected in the concentrate and compound feed in autumn and winter were lower than those determined in spring and summer ( $P<0.01$ ). The mean level of total aflatoxin was 6.692 ppb in roughage, 6.013 ppb in concentrate feed and 7.835 ppb in compound feed (Table 1).

**Table 1.** The levels of total aflatoxin and AFLB<sub>1</sub> in roughage, concentrate and compound feed with respect to season (ppb).

**Tablo 1.** Mevsimlere bağlı olarak kaba, konsantre ve karma yemlerde AFLB<sub>1</sub> ve total aflatoksin düzeyleri (ppb).

Seasons	Spring	Summer	Autumn	Winter	SEM	P
n	11	11	11	11		
RFTA	7.955 <sup>a</sup>	6.825 <sup>b</sup>	5.912 <sup>c</sup>	6.076 <sup>b</sup>	0.1930	**
Con.FTA	6.757 <sup>a</sup>	6.693 <sup>a</sup>	5.197 <sup>b</sup>	5.405 <sup>b</sup>	0.2200	**
Com.FTA	9.187 <sup>a</sup>	8.811 <sup>a</sup>	6.075 <sup>b</sup>	7.267 <sup>b</sup>	0.2630	**
RF AFLB <sub>1</sub>	4.539 <sup>a</sup>	4.429 <sup>a</sup>	1.244 <sup>b</sup>	1.240 <sup>b</sup>	0.0280	**
Con.F AFLB <sub>1</sub>	2.904 <sup>a</sup>	2.852 <sup>a</sup>	1.455 <sup>b</sup>	1.487 <sup>b</sup>	0.0930	**
Com.FAFLB <sub>1</sub>	3.835 <sup>a</sup>	3.577 <sup>b</sup>	1.622 <sup>d</sup>	2.488 <sup>c</sup>	0.0650	**
M AFLM <sub>1</sub>	0.04 <sup>a</sup>	0.02 <sup>b</sup>	0.02 <sup>b</sup>	0.04 <sup>b</sup>	0.00	**

\*\* :  $P<0.01$ ; a, b, c: Differences between the mean values shown with different superscripts in the same column were statistically significant. RFTA: Roughage Feed Total Aflatoxin; Con.FTA: Concentrate Feed Total Aflatoxin; Com.FTA: Compound Feed Total Aflatoxin; RF AFLB<sub>1</sub>: Roughage Feed AFLB<sub>1</sub>; Con.F AFLB<sub>1</sub>: Concentrate Feed AFLB<sub>1</sub>; Com.F AFLB<sub>1</sub>: Compound Feed AFLB<sub>1</sub>; M AFLM<sub>1</sub>: Milk AFLM<sub>1</sub>

The effect of season on the AFLB<sub>1</sub> levels of all of the feed types sampled from the holdings was found significant ( $P<0.01$ ). The AFLB<sub>1</sub> level in roughage that the levels detected in autumn and winter were lower than those determined in spring and summer. Similarly, the AFLB<sub>1</sub> levels found in the

concentrate feed in autumn and winter were also lower than those detected in spring and summer. The compound feed included the lowest AFLB<sub>1</sub> level in autumn, followed by winter, spring and summer ( $P<0.01$ ). Furthermore, the season on the AFLM<sub>1</sub> levels detected in milk samples was determined as

significant ( $P<0.01$ ) and the mean AFLM<sub>1</sub> level in milk was found to be 0.04 ppb in spring and winter and 0.02 ppb in summer and autumn. The differences between the seasons in terms of AFLM<sub>1</sub> levels in milk were detected to be highly significant ( $P<0.01$ ) (Table 1).

#### DISCUSSION and CONCLUSION

In the past 40 years, the understanding of the significance of mycotoxins has led to multiple studies having been conducted on the mycotoxin contamination of plant products, animal feed, milk and milk products and poultry products (Demirer et al., 1979; Karakaya and Atasever, 2010).

The assessment of the effect of seasons on the mycotoxin contamination levels of roughage revealed that AFLB<sub>1</sub> levels in roughage were lower in autumn and winter, compared to summer and spring. Similarly, AFLB<sub>1</sub> levels in concentrate feed were also observed to be lower in autumn and winter, when compared to spring and summer. In compound feed, AFLB<sub>1</sub> level was the lowest in autumn, followed by winter, spring and summer ( $P<0.01$ ). Given the seasonal pattern of Erzurum province and the harvest of particularly the feed crops used as roughage in autumn and the adequate drying of these crops prior to storage, it is considered that the season presenting with the lowest risk of mycotoxin contamination at storage is autumn. During the winter season, owing to the storage period after harvest not being very long and the temperature being low, conditions were not favourable for fungal growth, thus marked levels of mycotoxin contamination were not detected in all the feed types. In Erzurum province, the winter season begins shortly after harvest and lasts for a relatively longer period, compared to the other geographical regions of Turkey. Thus, the coming of spring in this province is observed at a later period of the year. In the present study, the assessment of the impact of seasons on the mycotoxin contamination levels of roughage and concentrate feed demonstrated that AFLB<sub>1</sub> levels were lower in

autumn and winter, in comparison to spring and summer. The level of AFLB<sub>1</sub> in compound feed was the lowest in autumn, followed by winter, spring and summer ( $P<0.01$ ). AFLB<sub>1</sub> determined at higher levels in compound feed during spring and summer, compared to autumn and winter, was attributed to higher weather temperatures and humidity rates as well as to the poor hygiene of feed troughs.

Demirer et al. (1979), upon performing aflatoxin analyses in compound feed and feed stuff samples ( $n=92$ ) reported that the presence of AFLB<sub>1</sub> was detected in only one sample at a level of 30 ppb. Shreeve and Patterson (1977), determined AFLB<sub>1</sub> at a mean level of 0.05 ppm in barley used as a feed stuff. Shotwell et al. (1969a,b), found 3-19 ppb AFLB<sub>1</sub> in 35 out of 1311 maize samples and 7-10 ppb AFLB<sub>1</sub> in 2 out of 866 soybean samples. In a study conducted by Karakaya and Atasever (2010), in Erzurum province, AFLB<sub>1</sub> was reported in 3 out of 72 feed samples (4.16%), and it was indicated that the AFLB<sub>1</sub> levels found in the remaining samples were below the maximum residue limit laid down in the national legislation. In the present study, the assessment of the AFLB<sub>1</sub> levels detected in all roughage, concentrate and compound feed samples taken from the holdings included in the present study were below the maximum limit set for AFLB<sub>1</sub> in the Turkish Food Codex (5 ppb). The findings reported by some researchers (Shreeve and Patterson 1977; Demirer et al., 1979; Karakaya and Atasever, 2010) for Aflatoxin B<sub>1</sub> levels were similar to the results obtained from the present study.

The World Health Organization (WHO) and the United Nations Food and Agriculture Organization (FAO) have set the maximum level of AFLB<sub>1</sub> that may be allowed in milk as 0.05 ppb (Ruiqian et al., 2004). In Turkey, similarly, the maximum limit of AFLB<sub>1</sub> in milk has been laid down as 0.05 ppb in the national legislation (Anonymous, 2009).

It was determined that the carry-over rate of Aflatoxin B<sub>1</sub> in feed into cows' milk in the form of

$$\text{AFLM}_1 \text{ was } 1.219 \pm 0.04139\% \left[ \left( \frac{\text{AFLM}_1}{\text{AFLB}_1} \right) * 100 \right].$$

In a two-year study conducted in Russia by Tutelyan et al. (1989) for the investigation of the presence of AFLM<sub>1</sub> in milk and milk products (n=250), it was ascertained that the levels of this mycotoxin did not exceed 0.05 µg/L in any of the samples. In another study carried out in Germany, of the pasteurized milk samples collected from the market and analysed for AFLM<sub>1</sub> (n=473) only 19 presented with a contamination level exceeding 0.05 µg/L (Heeschen et al., 1990). In a study conducted in the USA, AFLM<sub>1</sub> was detected in any of the 182 milk and milk product samples analysed for the presence of AFLM<sub>1</sub> (Anonymous, 1992). In Turkey, several studies have been performed to investigate AFLM<sub>1</sub> levels in milk (Demirer et al., 1979; Dağoğlu et al., 1995; Sarımehtemoğlu et al., 2000; Bakırcı, 2001; Özkaya et al., 2002; Mavuş, 2003; Akdemir and Altıntaş, 2004; Gurbay et al., 2005; Karakaya and Atasever, 2010). Demirer et al. (1979) reported that no detectable levels of AFLM<sub>1</sub> were encountered in any of the 150 raw milk samples analysed. In another study, in which 90 milk samples were taken from the Faculty of Agriculture of Yüzüncü Yıl University, 79 samples were determined to contain AFLM<sub>1</sub> and in 35 of these samples the mycotoxin level was found to be above the maximum limit (0.05 ppb) (Bakırcı, 2001). In an investigation performed on 360 raw milk samples taken from various provinces of Turkey, 159 of the samples (44.3%) contained AFLM<sub>1</sub> at a maximum level of 1.4 µg/L and 48 of these (13.3%) were contaminated with the levels exceeding the maximum limit allowed in Turkey (Özkaya et al., 2002). Out of 85 pasteurized milk samples, 75 (88.23%) were determined to contain AFL and in 48 of these samples (64%) the aflatoxin levels were above the maximum limit set in Turkey (50 ng/kg<sup>-1</sup>) (Sarımehtemoğlu et al., 2000). In an investigation conducted in 2003 in Kayseri province, out of 90 milk samples analysed, 15 were detected to be contaminated with AFLM<sub>1</sub> levels above the maximum limit set in the Turkish Food Codex (50ppb) (Mavuş, 2003). In another research

conducted in Ankara province, 48 raw milk samples were analysed for AFLM<sub>1</sub> and 70.83% of the samples were determined to contain AFLM<sub>1</sub>, 33.3% of which were contaminated with the levels exceeding the maximum limit allowed in Turkey (0.05 ppb) (Akdemir and Altıntaş, 2004). In an investigation carried out in the same region, only 1 sample was detected to contain an AFLM<sub>1</sub> level above the maximum limit laid down in the Turkish Food Codex (Gurbay et al., 2005). Another study reported that none of the 72 milk samples analysed contained AFLM<sub>1</sub> levels exceeding the legal maximum limit (Karakaya and Atasever, 2010). The results obtained herein demonstrated the presence of AFLM<sub>1</sub> in all the milk samples analysed, but within the legally allowed limits. The general mean value of AFLM<sub>1</sub> was calculated as 0.003 ppb. The results of the present study differed from the findings reported by some researchers (Dağoğlu et al., 1995; Sarımehtemoğlu et al., 2000; Bakırcı 2001; Özkaya et al., 2002; Mavuş, 2003; Akdemir and Altıntaş, 2004) and displayed similarity to the results reported by others (Demirer et al., 1979; Tutelyan et al., 1989; Heeschen et al., 1990; Gurbay et al., 2005). Furthermore, the results of the present study were observed to be in parallel with another study conducted by Karakaya and Atasever (2010) in the same region. Multiple studies have demonstrated Aflatoxin M<sub>1</sub> levels in milk to vary with season (Van Egmond, 1989; Wood, 1991; Bakırcı, 2001; Özkaya et al., 2002; Akdemir and Altıntaş, 2004; Kamkar, 2005). Due to the concentrate feed either being added at lower levels or not being incorporated into the feed ration of dairy animals in summer (Wood, 1991; Sarımehtemoğlu et al., 2003) and in view of dairy cattle consuming greater amounts of compound feed in winter and spring compared to summer and autumn, it has been reported that higher levels of AFLM<sub>1</sub> are indicated in milk during the cold seasons, compared to the warm seasons. The comparative assessment of seasons for mycotoxin contamination revealed that the highest level of contamination was observed in spring with

differences between the other seasons as well (Özkaya et al., 2002). In a study conducted in Iran, 111 raw milk samples were analysed for aflatoxin, and it was determined that in 85 of these samples AFLM<sub>1</sub> levels ranged between 0.015-0.28 µg/l. The lowest aflatoxin level was determined in August and the highest level was measured in December. Levels of AFLM<sub>1</sub> were found to be higher in January, February, April and December compared to the remaining months of the year (Kamkar, 2005). The results obtained for AFLM<sub>1</sub> levels in the present study were in parallel with those previously reported by other researchers (Wood, 1991; Bakirci, 2001; Özkaya et al., 2002; Akdemir and Altıntaş, 2004; Kamkar, 2005). The existence of a positive and linear correlation between the level of AFLB<sub>1</sub> ingested by cattle in feed and the level of AFLM<sub>1</sub> in milk has been confirmed by several researchers. The same researchers have pointed out that this correlation may vary with individual cattle, milking time and interval. Research on the carry-over of mycotoxins found in contaminated feed into milk has demonstrated that the level of carry-over of mycotoxins from feed into milk varies (Van egmond, 1989; Wood, 1991; Van Egmond, 1994; Gremmels, 2008). Hui (1992) reported that, in case of the presence of AFLB<sub>1</sub> at a level of 20 g/kg in feed, the carry-over of AFLM<sub>1</sub> into milk occurred at a level of 0.06 g/kg AFLM<sub>1</sub>. Rodricks and Stoloff (1977) indicated that the concentration of AFLB<sub>1</sub> in feed is 34-1600 times greater than the concentration of AFLM<sub>1</sub> in milk. In various researches, this rate has been reported to be 0.8-2.2% (Patterson et al., 1980; Sarımeahmetoğlu and Küplülü, 2004). In the present study, the level of the carry-over of AFLB<sub>1</sub> in compound feed into cows' milk in the form of AFLM<sub>1</sub> was determined as 1.219±0.4139%. This result is in parallel with the values previously reported by other researchers (Patterson et al., 1980; Sarımeahmetoğlu and Küplülü, 2004).

In conclusion, the assessment of the total aflatoxin and AFLB<sub>1</sub> levels indicated in feed and the AFLM<sub>1</sub> levels detected in milk demonstrated that

the AFLB<sub>1</sub> levels in both roughage and concentrate feed were within the normal limits. These favourable results were attributed to the right harvesting season having been selected, the feed having been well dried and the storage conditions (temperature, humidity, feed trough hygiene, etc.) being proper. Attention should be paid to avoiding the feeding of animals with spoilt, moulded and contaminated feed. Furthermore, such material should not be used as bedding. It may not always be possible to determine the level of mould growth macroscopically. Therefore, it is suggested that particularly during the winter and spring months, the large-scale animal holdings operating in the region should have their feed analysed for mycotoxins of major concern. The conduct of further detailed studies on the prevention of the contamination of feed with mycotoxins by taking measures at the stages of the growth, harvesting and storage of feed crops would contribute greatly to the protection of both human and animal health. It is considered that the routine analysis of milk and milk products for AFLM<sub>1</sub> is of utmost significance as these products have an important place in the nutrition of humans.

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