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Research Article/ Araştırma Makalesi

Determination of Aflatoxin M1 Levels in Some Honamli Goat Herds in Burdur Province

Burdur İlindeki Bazı Honamlı Irkı Keçi Sürülerinde Aflatoksin M1 Düzeylerinin Belirlenmesi

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Abstract: In this study, aflatoxin M1 (AFM1) levels were determined from the milk serum of 90 lactating Honamli goats aged 2-6 years in 12 herds breeding Honamli goats in Burdur province in spring and April in 2021. A statistically significant difference was found between flock 1 and flock 4-12 and between flock 3 and flock 9,11 ($p<0.05$). In addition, the mean AFM1 concentration was determined as 29.79 ng/L in 12 farms of Honamli breed goat breeding in Burdur province. The minimum AFM1 concentration was 7.99 ng/L and the maximum AFM1 concentration was 46.28 ng/L in 12 flocks. As a result, AFM1 was detected in the milk samples of all 12 flocks in Burdur city evaluated in the present study. AFM1 levels were determined at an acceptable level according to the Turkish Food Codex. However, while the presence of AFM1 in the entire flocks is acceptable according to the Turkish Food Codex, it suggests that the storage conditions of forage crops are not sufficient. The concentration of aflatoxin in plants in pastures should be investigated. Natural toxin binders, which have been actively used in animal nutrition recently, should also be used in goat breeding.

Keywords: Aflatoxin M1, Aflatoxicosis, Honamli Goat, Milk.

Öz: Bu çalışmada 2021 yılında bahar mevsiminde ve Nisan ayında Burdur ilinde Honamlı ırkı keçi yetiştiriciliği yapan 12 sürüde bulunan ve yaşları 2-6 yaş arasında değişen 90 adet laktasyondaki Honamlı ırkı keçilerin süt serumlarından aflatoksin M1 (AFM1) seviyeleri belirlenmiştir. Bulgularda sürü 1 ile sürü 4-12 arasında ve sürü 3, 9 ve 11 arasında istatistiksel olarak anlamlı farklılık ($p<0.05$) bulunmuştur. Ayrıca, Burdur iline bağlı Honamlı ırkı keçi yetiştiriciliği yapılan 12 işletmede ortalama AFM1 konsantrasyonu 29.79 ng/L olarak belirlenmiştir. 12 sürüde minimum AFM1 konsantrasyonu 7.99 ng/L maksimum AFM1 konsantrasyonu 46.28 ng/L olarak belirlenmiştir. Sonuç olarak bu çalışmada Burdur ilinde çalışmaya dahil edilen 12 sürünün tamamının süt numunelerinde AFM1 tespit edilmiştir. AFM1 düzeylerinin Türk Gıda Kodeksine göre kabul edilebilir düzeyde belirlenmiştir. Ancak Türk Gıda Kodeksine göre kabul edilebilir olsa da sürünün tamamında AFM1 bulunması yemlerin depolama şartlarının uygun olmadığını göstermektedir. Meralardaki bitkilerde bulunan aflatoksin yoğunluğu araştırılmalıdır. Hayvan beslemede son dönemlerde aktif bir şekilde kullanılan doğal toksin bağlayıcıların keçi yetiştiriciliğinde de kullanılması gerekmektedir.

Anahtar Kelimeler: Aflatoksin M1, Aflatoksikoz, Honamlı Keçisi, Süt.

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Introduction

Aflatoxins have both toxigenic and teratrogenic effects for humans and animals. Aflatoxides are naturally produced by these three species as a result of cantamine of plants and their products with *Aspergillus* (*A*) (*A. flavus*, *A. parasiticus* and *A. nomius*) species. These toxins display immunosuppressive, mutagenic, teratrogenic and carcinogenic effects as well as having acute toxic effects. The main target organ where it has toxigenic and carciogenic effects is the liver (Betina, 1989; Kaya, 2002).

A. flavus produces only Aflatoxin B1 (AFB1) and Aflatoxin B2 (AFB2). *A. parasiticus* produces AFB1, AFB2, Aflatoxin G1 (AFG1) and Aflatoxin G2 (AFG2). In particular, the toxins

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produced by *A. flavus* and *A. Parasiticus* have a great significance causing disorders in human and animals (Özkaya and Temiz, 2003; Akdemir et al., 2004).

AFM1 is a toxic metabolite, and as a result of the intake of AFB1 contaminated foods by lactating animals such as cattle, sheep and goats, this metabolite is converted into a hydroxylated form called Aflatoxin M1 (AFM1), which is a cytotoxic and genotoxic substance. AFB1 is biotransformed into AFM1 by hepatic microsomal cytochrome P450. AFM1 has ten-times lower carcinogenic potential than AFB1. AFM1 can be detected in milk 12-24 hours after ingestion of AFB1. It reaches a high level a few days after detection in milk. When AFB1 uptake is stopped, the concentration of AFM1 in milk falls below to detection level after 72 hours (Van Egmond, 1989; Bbosa et al., 2013). Although it is suggested that the ratio between ingested AFB1 and excreted AFM1 is 1-3 %, there are studies reporting that it increases as much as 6 %. However, this excretion differs in each animal (nutrition, milking time, etc.). AFM1, a metabolite of AFB1, which has cytotoxic and genotoxic properties in animals, is an extremely important toxin (Martins and Martins, 2000; Özdemir, 2007). Burdur city is one of the provinces where Honamli goat breeding is done intensively. In this study, it was aimed to determine the AFM1 levels in Honamli goats in Burdur province.

Materials and Methods

Ethical Considerations

Since milk sample was used in this study, it is not necessary to obtain an ethics committee approval in accordance with the Regulation on Working Procedures and Principles of Animal Experiments Ethics Committees. However, since AFM1 levels will be determined in Honamli goats in Burdur province, this study was carried out with the knowledge of the Ministry of Agriculture and Forestry.

Study Design and Sampling

In this study, it was aimed to determine the levels of AFM1 from the milk serum of 90 lactating Honamlı goats aged 2-6 years in 12 herds breeding Honamlı goats in Burdur province. All herds included in the study is fed with ground barley and grazed in the bushes. For this purpose, fifteen milliliters (mL) of milk samples were taken from each goat into falcon tubes in spring and April in 2021. The serum of the samples were extracted by centrifugation (3500 rpm/10 min). AFM1 levels from the extracted sera were determined by using commercial ELISA assay kit (ELABSCIENCE KA36762ETS E0099Go) according to the manufacturer's instructions.

Data Analysis

IBM SPSS 22.0 for Windows package program was used for statistical analysis of study data. The normal distribution of the groups in the analyzes was evaluated by using the Shapiro-Wilk test. Due to the normal distribution of the data, comparisons among the groups were carried out by using one-way analysis of variance test. The Bonferroni test was used as a multiple comparisons. The statistical significant was considered as $p<0.05$.

Results

A statistically significant difference ($p<0.05$) was found between flock 1 and flock 4-12 and between flocks 3, 9, and 11. In addition, the mean AFM1 concentration was determined as 29.79 ng/L in 12 farms of Honamlı breed goat breeding in Burdur province. The minimum AFM1 concentration was 7.99 ng/L and the maximum AFM1 concentration was 46.28 ng/L. As a result, AFM1 was detected in the milk samples of all 12 herds included in the Burdur study in this study.

Discussion

Mycotoxins; are toxic metabolites produced by fungi. They are generally formed during growth or and storage of feed and feed raw materials under unsuitable conditions (Van Egmond,

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1989). Mycotoxins cause poisoning called mycotoxicosis in humans and animals (Guntekin et al., 2016; Türel and Calapoğlu, 2017). For toxin formation, appropriate environmental conditions are needed.

In this context, optimal conditions for mycotoxins; the humidity rises above 50-60 %, the ambient temperature is between 25-38 °C, the pH is near 6.0, and the low levels of oxygen concentration (Navarro and Zettler, 2001; Özkaya and Temiz, 2003; Çankırı and Uyarlar, 2013; Whitlow et al., 2010). Anorexia, weight loss, icterus, nervous symptoms, and finally death occur in acute aflatoxicosis cases in animals. Autopsy shows decreased color and lipidosis in the liver. Hemorrhage in the kidneys and fluid collection in the body cavities can be also observed. On the other hand, teratrogenicity and hepatotoxicity are the main findings in chronic toxications (Pohland 1993).

Table 1. AFM1 levels by flocks.

Grup	AFM1 (ng/L)	
	$\bar{x}\pm sd$	*Mean (min-max)*
Flock 1(n=8)	43.69±2.19[a]	44.57 (40.02-46.28)
Flock 2 (n=8)	32.63±5.54[ab]	32.65 (26.05-40.68)
Flock 3 (n=8)	37.10±5.28[a]	37.64 (29.17-45.59)
Flock 4 (n=8)	30.67±6.74[bc]	28.36 (21.95-44.05)
Flock 5 (n=7)	29.30±9.46[bc]	30.95 (10.96-42.49)
Flock 6 (n=7)	26.47±8.11[bc]	26.47 (15.73-40.07)
Flock 7 (n=8)	26.02±3.18[bc]	26.88 (20.29-30.81)
Flock 8 (n=7)	27.42±6.12[bc]	26.63 (18.86-34.50)
Flock 9 (n=7)	23.45±3.80[b]	24.47 (15.63-27.53)
Flock 10 (n=8)	25.80±4.72[bc]	25.72 (19.06-33.68)
Flock 11 (n=7)	22.66±10.41[b]	25.23 (7.99-34.76)
Flock 12 (n=7)	29.76±9.46[bc]	27.66 (12.51-40.31)
Total (n=90)	29.79±8.52	28.47 (7.99-46.28)

There is a statistically significant difference ($p<0.05$) between rows with different lowercase superscripts.

After AFB1 is taken with feed, it is excreted as AFM1 with milk, urine and faeces (Baygeldi and Tanyıldızı, 2018). AFB1 is detected in milk as AFM1 within a 6-24 hour period following ingestion. The time to reach the highest level is 12-48 hours. When AFB1 uptake does not continue, the level of AFM1 visibility reaches its lowest levels within 72-96 hours (İşleyici et al., 2015; Baygeldi and Tanyıldızı, 2018).

The maximum acceptable AFM1 level in milk is as 50 ng/L according to the Turkish Food Codex (TFC) (Turkish Food Codex, 2002). As mentioned, AFM1 levels in Honamlı goats in Burdur province were determined below the limit.

AFM1 levels may vary bycountries, regions, seasons, geographical and climatic characteristics, rations of animals, and lactation period (Kaya Tuz et al., 2017; Karadal et al., 2018). In the present study, the differences between flock 1 and flock 4-12, flock 3 with flock 9 and flock 11 were significant ($p<0.05$), and this can be linked to the care and feeding and lactation status of the animals.

Özdemir et al. (2007) found that the mean AFM1 concentration in the milk of goats in Kilis farms in March and April was 19.23 ng/L. They reported that the minimum and maximum values of AFM1 concentration ranged from of 5.16 to 116.78 ng/L. In another study, Karadal et al. (2018) found that the concentration of AFM1 in goat milk rangin between 0.33-11.79 ng/L in

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Niğde province. In accordance with this information, the average AFM1 concentration was found to be 29.79 ng/L in April in Honamlı goats in Burdur province in the current study. In addition, the lowest AFM1 concentration was 7.99 ng/L, while the highest AFM1 concentration was 46.28 ng/L.

The ability to convert AFB1 to AFM1 excreted in milk varies between large and small ruminants. In this manner, the rate of conversion of AFB1 to AFM1 are 0.35 % and 3 % in cows (Veldman et al., 1992; Frobisch et al., 1986), 0.018 % to 3.1 % in goats (Goto and Hsieh, 1985; Nageswara Rao and Chopra, 2001; Ronchi et al., 2005), and 0.08 % and 0.33 % in sheeps (Battacone et al., 2005).

It is well known that the AFM1 concentrations in cow's milk is higher than in goat and sheep milk (Virdis et al., 2014). Compared to cattle fattening, due to pasture grazing reduces the intake of feeds contaminated with mold strains and aflatoxin exposure. The use of concentrates and feedstuffs in sheep and goats is limited due to economic reasons and milk production (Molle et al., 2008).

The AFM1 concentrations (mean±standard deviation) in goat milk were greatly varry between the contries. In this context, it was reported that the AFM1 levels were 14.5±8.4 ng/L in Italy (Virdis et al., 2014), 19±13.8 ng/L in Syria (Ghanem and Orfi, 2009), 2.0±5.0 ng/L in Pakistan (Hussain et al., 2010), 31.8±13.7 ng/L in Iran (Rahimi and Ameri, 2012), 7.6±8.94 ng/L in Croatia (Bilandzic, 2014). In this study, the AFM1 concentration was determined as 29.79±8.52 ng/L.

Conclusion

In conclusion, AFM1 was detected in the milk samples of all 12 herds included in the Burdur city in this study. AFM1 levels were determined below the maximum acceptable level according to the TFC. However, while the presence of AFM1 in the entire flocks is acceptable according to the TFC, it suggests that the storage conditions of forage crops are not sufficient. When feeding animals forage plants with poor storage conditions, caution should be taken. The concentration of aflatoxin in plants in pastures should be investigated. Natural toxin binders, which have been actively used in animal nutrition recently, should also be used in goat breeding. This study has proven that natural toxin binders, which have been actively used in animal nutrition recently, should also be used in goat breeding.

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