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Comparative Study of Aflatoxin M1 Biotransformation from Feed to Raw Milk in Cow, Buffalo, Sheep, and Goats in Different Areas of Baghdad Province

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A B S T R A C T

Mycotoxin aflatoxin B1 (AFB1) is a threat to food safety and human health because it is present in animal feed and is metabolized into aflatoxin M1 (AFM1), a more toxic form, during lactation. The aim of this study was to quantify AFM1 concentrations in raw milk of buffalos, cows, sheep, and goats sampled randomly from four regions within Baghdad Province, Iraq, and to compare these levels with the maximum allowable levels set forth by the European Commission (EC), the Iraqi Standard Specification (IQS), and the food and Drug Administration (FDA). The carry-over of AFB1 from feed to AFM1 in milk were also calculated for each of the studied species. A total of 200 random samples, including 50 each from cows, buffaloes, sheep, and goats, were collected from farms located in Zu'afraniya, Nahrawan, Abu Ghraib, and Fedhalia regions. AFM1 and AFB1 concentrations were determined using the enzyme-linked immunosorbent assay (ELISA). Raw milk samples from cows, sheep, buffaloes, and goats were all found to have AFM1 concentrations that were below the limits set by the EC, IQS, and FDA. Animal feed samples, on the other hand, had AFB1 concentrations of 10.08, 5.95, 4.27, and 7.10 ppb for buffaloes, cows, sheep, and goats, respectively. The observed carry-over rates ranged from 0.36% in goats to 0.78% in buffaloes to 0.66% in cows. Multiple factors, including animal species, are considered, and it is determined that a universal carry-over equation cannot be applied to all cases. Therefore, it is essential to regularly monitor AFM1 levels in milk from various animal species in order to lessen potential health risks. Furthermore, the study suggests enhancing agricultural and veterinary practices to better regulate feed quality for dairy animals.

Keywords: aflatoxin M1, aflatoxin B1, raw milk, ELISA, carry-over rate

INTRODUCTION

The foundation of an optimal diet lies in maintaining good health, as it plays a crucial role in mitigating the risk of developing various diseases in the future. A significant number of individuals throughout the globe include milk and dairy products in their daily dietary intake due to their substantial contribution as a vital source of essential nutrients for human well-being (1). Human milk consumption is typically derived from bovines; however, dairy products may also be derived from other sources such as buffalo, goats, camels, and sheep. Fresh milk is known to include a range of nutrients that have been recognized for their positive impact on human health. Moreover, due to its composition of proteins, saturated fats, vitamin C, calcium, and several other constituents, it is advised for consumption by infants in cases where the digestion of supplementary nourishment poses challenges (2).

Aflatoxins (AFs) are primarily synthesized by certain strains of *Aspergillus flavus* or *Aspergillus parasiticus* (3).

Following consumption, their elevated lipid solubility renders them readily absorbable within the gastrointestinal tract, facilitating their transit to the hepatic system (4, 5). Subsequently, these substances undergo enzymatic degradation within the liver, forming Aflatoxin M1 (AFM1) or milk Aflatoxin (6). Subsequently, the substance is introduced into a milk solution (7). The conversion of AFB1 to AFM1 in milk ingested by cattle ranges from 0.3% to 6.3%, depending on the level of feed contamination (8). Mycotoxins are bioactive compounds that are produced and persist in many food sources as a result of fungal metabolism (9–12).

According to previous research (13), AFB1 and AFM1 have been identified as substances that possess hepatotoxic and carcinogenic properties. Due to its resistance to pasteurization and the manufacturing processes of dairy products, this mycotoxin stands as the only mycotoxin subject to maximum residue limits (MRL) in milk (14). The presence of AFM1 is seen on a worldwide scale; however, its concentrations exhibit considerable variation across different locations due to factors such as milk production techniques, environmental conditions, and the kind of dairy animals involved (15). Previous studies have shown that these entities have the potential to initiate and facilitate the development of liver, lung, and colon cancer (16). Numerous investigations (18–23) have examined the prevalence of AFM1 in livestock. Certain populations, particularly infants and children who consume a high quantity of dairy products, are susceptible to the potential hazards associated with AFM1 intoxication (24). The adverse consequences of AFs often present themselves in two distinct manners: firstly, via the occurrence of poisoning, and secondly, through the induction of carcinogenic properties (25). A significant proportion of animal products have been shown to contain aflatoxin residues, as indicated by many studies (26–30). The European Commission (31) and the Iraqi Standard Specification (IQS) both stipulate a maximum allowable level of 0.05 ppb for AFM1 in milk. In contrast, the United States Food and Drug Administration (FDA) sets a slightly higher limit for AFM1 at 0.5 ppb (32). The standard for AFB1 in feed was set at 20 ppb by EC, IQS, and the FDA.

This study aimed to assess the concentration of AFM1 in raw milk of buffaloes, cows, sheep, and goats sampled from various regions of Baghdad Province, Iraq and compare the findings with the limits set by the EC, IQS, and FDA. The study also examined the AFB1 carry-over from feed to AFM1 in milk.

MATERIALS AND METHODS

Ethical Approval

All procedures in this study were reviewed and approved by the local Animal Care and Use Committee (Approval No. P.G. 1261) at College of Veterinary Medicine, University of Baghdad.

Sample Collection

The study was conducted in July and August 2022, during the dry season. Farms in several Baghdad Province were randomly sampled. A total of 200 raw milk samples from farms located in four areas of Baghdad (Zu'afraniya, Nahrawan, Abu Ghraib, and Fedhalia) were sampled at random. Animals represented by the samples included buffalo (50), cows (50), goats (50), and sheep (50). Simultaneously, sterile feed samples (about 50-100 g) were collected from the same farms (13 feed samples from Zu'afraniya, Nahrawan, 11 feed samples from Abu Ghraib, and 8 feed samples from Fedhalia) with the use of gloves and plastic bags. Samples of milk and feed were kept in ice-cold boxes while being transported to the lab. The samples were then stored at -20 °C until analysis.

Analytical Methods

The Enzyme-Linked Immunosorbent Assay (ELISA) tests were conducted at the Serology Unit of the Central Veterinary Laboratory and Research, which is part of the Ministry of Agriculture in Baghdad, Iraq. The protocols for sample preparation and analysis were based on the guidelines provided by Helica Biosystems Inc. (USA) and NEOGEN (USA), the manufacturers of the ELISA kits used for milk and feed samples, respectively. The carry-over percentage of AFB1 to AFM1 was calculated using the following formula:

$$\text{Carry} - \text{over}\ (\%) = \frac{\text{AFM1 in milk (ppb)}}{\text{AFB1 in feed (ppb)}} \times 100$$

Statistical Analysis

Data were analyzed using SAS program (2010). Multivariate analysis of variance (MANOVA) was used. MANOVA is an extension of the univariate analysis of variance (ANOVA). In an ANOVA, we examine for statistical differences on one continuous dependent variable by an independent grouping variable. The MANOVA extends this analysis by taking into account multiple continuous dependent variables.

RESULTS

The results of AFB1 in feed, AFM1 in milk, and carry-over rates are shown in Table 1. Significant variations ($P < 0.05$) were observed in the concentration of AFB1 in feed among the four species (buffaloes, cows, sheep, and goats). The data indicates that buffaloes feed had the highest mean value of AFB1 at 10.1±0.59 ppb, followed by goats, cows, and sheep with mean values of 7.10±0.59, 5.95±0.58, and 4.27±0.58 ppb, respectively. However, all AFB1 levels were well below the regulatory limits of 20 ppb set by the EC, IQS, and FDA, indicating that the feeds were relatively safe in terms of aflatoxin content.

Significant variations in the concentration of AFM1 in raw milk were observed among the animal species ($P < 0.05$), with sheep (0.012±0.001 ppb) and goats

(0.011±0.001 ppb) exhibiting the highest levels. Despite these differences, all AFM1 concentrations remained below the regulatory limits set by the EC, IQS, and FDA of 0.05 ppb, suggesting that the milk, while contaminated, does not exceed safety thresholds.

The carry-over rates of AFB1 from feed to AFM1 in raw milk were significantly different among species, with sheep and goats showing higher rates than cows and buffaloes. There were significant differences ($P<0.05$) between the results for cows had significantly higher carry-over rate compared to buffaloes. These differences underscore the varying susceptibility of these species to aflatoxin contamination and transfer.

There were no significant differences ($P>0.05$) between the AFB1 values in feed from the four areas around Baghdad province (Fedeliah, Abu-Ghraib, AL-Nahrawan and AL-Zu'afraniya) as shown in Figure 1. All regions had feed AFB1 levels below the permissible 20 ppb limit.

Table 1. Concentrations of AFB1 in feed, AFM1 in milk, and carry-over rates of AFB1 from feed to AFM1 in milk for buffaloes, cows, sheep, and goats

Animal Species	AFB1 in Feed (ppb)	AFM1 in Milk (ppb)	Carry-over Rate (%)
Buffaloes	10.1 ± 0.59 [a]	0.002 ± 0.0001 [c]	0.17 ± 0.08 [c]
Cows	5.95 ± 0.58 [b]	0.007 ± 0.001 [b]	0.36 ± 0.08 [b]
Sheep	4.27 ± 0.58 [c]	0.012 ± 0.001 [a]	0.78 ± 0.08 [a]
Goats	7.10 ± 0.59 [b]	0.011 ± 0.001 [a]	0.66 ± 0.08 [a]

Means with a different small letter in the same column are significantly different ($P \leq 0.05$)

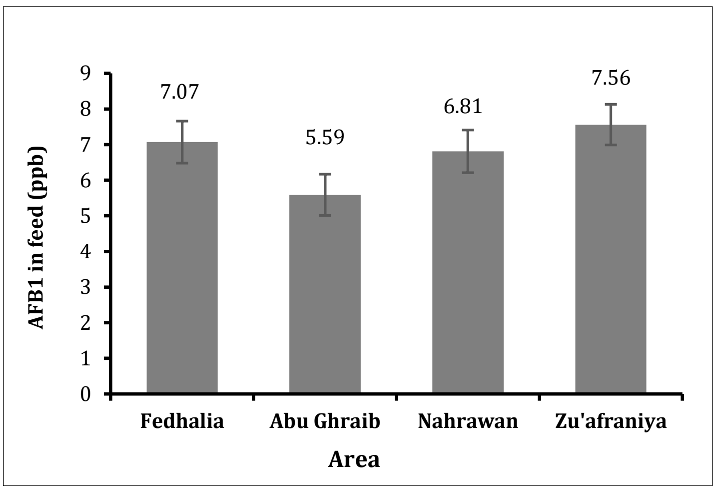

Figure 1. Concentration of AFB1 in feed of different areas Fedeliah, Abu-Ghraib, AL-Nahrawan and AL-Zuafarania of Baghdad province (ppb)

DISCUSSION

According to the Food and Agriculture Organization (FAO), more than 25% of global cereal output and raw materials intended for human and animal use are found to be contaminated with various types of fungal toxins (33). Aflatoxins are the most prevalent and highly poisonous mycotoxins found in dietary sources (34). The impact of mycotoxin toxicity may be seen in its impact on the health and productivity of crops, people, and animals. These consequences, in turn, can have implications for the economic value connected with human activities and the production of agricultural and livestock goods (35). This study represents the inaugural investigation in Iraq that analyzes the presence of AFB1 residue in the unpasteurized milk of various domesticated animals, including cows, buffaloes, sheep, and goats. The findings of this study reveal distinct levels of AFM1 in milk, which vary among different animal species. However, it is noteworthy that all identified levels fall within the acceptable thresholds established by European, American, and Iraqi regulatory standards. Nevertheless, it is crucial to acknowledge that these concentrations, while deemed permissible, do not guarantee absolute safety. The potential for increased AFM1 levels exists under specific circumstances, posing a persistent risk and warranting ongoing concern.

In current study, the milk samples collected from multiple animal species, including sheep, goats, cows, and buffalo, showed varying levels and percentages of contamination with AFM1. The study revealed that the mean value of AFM1 was greatest in sheep milk (0.012±0.001 ppb) and goat milk (0.011±0.001 ppb), followed by cow's milk (0.007±0.001 ppb) and buffalo's milk (0.002±0.0001 ppb). Several countries have established acceptable limits for AFM1 in milk and its derivatives to minimize potential harm to humans. The

European Union has established a regulatory threshold of 0.05 ppb for AFM1 in liquid milk and dried or processed milk products (European Commission Regulation, 2006). Similarly, IQS (5144/2022) sets the limit at 0.05 ppb, while the US FDA regulates AFM1 at 0.5 ppb in the United States. In relation to the potential health risks posed to consumers, it is noteworthy that none of the milk samples analyzed exceeded the acceptable thresholds outlined by both the Iraqi standard and the European Commission standards, which have set the limit at 0.05 ppb.

The findings of current study suggest that the utilization of stored forages by farmers during colder seasons may be attributable to the impediments imposed by adverse weather conditions on the provision of fresh forages to cattle. These outcomes underscore the significance of the timely and appropriate storage of forages, especially in regions with harsh weather conditions, to ensure the availability of adequate feed for cattle. The presence of inadequate temperature and moisture conditions in the warehouse creates a favorable environment for mold development. Consequently, it becomes imperative to enhance the storage conditions of livestock feed to mitigate this issue (36). Levels of AFs in milk tend to be higher in the fall and winter than in the spring and summer, according to prior reports (36).

Carry-over has been the subject of extensive research in numerous animal species. In this study, the carry-over of AFB1 from feed to raw milk in four different animal species: buffalo, cows, sheep, and goats were calculated. The results showed that neither sheep nor goats had significantly different carry-overs from one another ($P>0.05$), with both species showing higher values than cows and buffalos (0.36±0.08 and 0.17±0.08, respectively). When comparing the outcomes for cows and buffaloes, however, there were statistically significant differences ($P<0.05$). These results suggest that sheep and goats are more susceptible to AFB1 in feed being transferred to AFM1 in raw milk than cows and buffaloes. Assuming a constant intake of AFs, the model predicts that the concentration of AFM1 in milk will be unaffected by the concentration of AFB1 in the feed. Because grazing seasons allow animals to consume feed with a higher proportion of fresh product and a lower proportion of stored raw materials, the summer months of current study may also affect the feeding procedure. Values between 0.26% and 0.33% have been found across several studies of transfer into sheep milk (37).

Previous studies have shown carry-over rates in goats ranging from 0.018% to 3.1% and 0.11% to 0.3% (38, 39). Buffalos were found to have carry-over values of 0.2%, 2.13%, 3.13%, and 4.14–5.06% in previous studies (40-43).

In current study, it was observed that the carryover of AFM1 in buffaloes and cows was comparatively lower than in sheep and goats. This finding suggests that buffaloes and cows exhibit a higher level of activity in terms of detoxification of mycotoxins, which can be attributed to species-specific differences (17). The particle size of the feed may also play a role in this context. Specifically, sheep and goats consume feed with smaller particles than buffaloes and cows. This characteristic facilitates quicker digestion in the rumen and reduces the degradation of mycotoxins (44). The variation in fodder sources, their composition, the specific dietary mixtures and feed types consumed by each animal, and the level of exposure to mycotoxins present in the diet, may contribute to these observed differences (45).

Animal feed crops are susceptible to aflatoxin contamination during cultivation, and this contamination is influenced by seasonal changes. Additionally, seasonal variations may have an impact on the feeding process. During times conducive to grazing, animals have the opportunity to ingest feed that has a reduced amount of stored raw materials and a higher proportion of fresh food. Aflatoxin contamination was found to be more frequent in stored items. (46, 47). Under unaltered circumstances, a rise in aflatoxin contamination of livestock feed leads to an elevated consumption of aflatoxin and, in terms of quantity, a greater excretion of aflatoxin in milk. The level of contamination is contingent upon the specific kind of feed used. Aflatoxin contamination in milk has been linked to the use of more complex feeds (46). Additionally, particular raw materials, such as cottonseed, have been hypothesized to be more susceptible to aflatoxin contamination (46).

Several factors influence the amount of milk an animal produces. These include the animal's age, species, breed, time of day, metabolic status, rumen microflora, microsomal mixed-function oxidase (MFO) activity, and liver biotransformation capacity. Moreover, the blood-milk barrier, the rate of aflatoxin intake and digestion, the interaction of toxins in the animal, and the health of the udder and the integrity of the alveolar (milk-producing) cell membranes of the udder have all been reported (46, 48-53).

Changes in the plasma-milk barrier and a striking rise in the consumption of concentrated feeds have been linked to an increased carry-over rate in high-yielding cows (54). Several other variables that have an impact on carry-over include meteorological conditions, the specific geographical positioning of the farm, and variations in animal feeding methodologies (46-53).

Based on the current research findings, it is imperative to conduct a comprehensive survey on the occurrence and concentrations of aflatoxins in milk and feedstuffs across all seasons of the year in the four areas of Baghdad. The results of our study reveal a concerning trend. Regular monitoring of AFM1 levels in the milk of buffaloes, cows, sheep, and goats is essential in order to mitigate the potential health risks associated with AFM1 contamination. Efforts should be made to enhance agricultural and veterinary procedures in order to effectively manage the feeding of dairy sheep, goats, cows, and buffaloes.

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Conflict of interest

The authors declare no conflict of interest.

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دراسة مقارنة حول مدى انتقال السموم الفطرية AFB1 من العلف إلى الحليب AFM1في الأبقار والجاموس والأغنام والماعز في مناطق مختلفة من محافظة بغداد

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الخلاصة

السمّ الفطري الأفلاتوكسين B1 الموجود في أعلاف الحيوانات يشكل تهديداً لسلامة الغذاء وصحة الإنسان، لأنه يتحول إلى الأفلاتوكسين M1 وهو أكثر سمية خلال فترة إنتاج الحليب. هدفت هذه الدراسة إلى قياس تركيزات الأفلاتوكسين M1 في الحليب الخام للجاموس والأبقار والأغنام والماعز المجمّعة عشوائياً من أربع مناطق في محافظة بغداد، العراق، ومقارنة هذه المستويات مع المستويات القصوى المسموح بها من قبل المفوضية الأوروبية والمواصفة القياسية العراقية وإدارة الغذاء والدواء الأمريكية. كما حُسب معدل انتقال الأفلاتوكسين B1 من الأعلاف إلى الأفلاتوكسين M1 في الحليب بالنسبة لكل نوع من أنواع الحيوانات المدروسة. تم جمع ما مجموعه ٢٠٠ عيّنة عشوائية، ٥٠ عيّنة من كل من الأبقار والجاموس والأغنام والماعز، من مزارع موجودة في مناطق الزعفرانية والنهروان وأبو غريب والفضيلية. تم تحديد تركيزات الأفلاتوكسين M1 و B1 باستخدام اختبار المناعي المرتبط بالإنزيم. وُجد أن عيّنات الحليب الخام من الأبقار والأغنام والجاموس والماعز جميعها كانت تحتوي على تركيزات من الأفلاتوكسين M1 أقل من الحدود المسموح بها من قبل المفوضية الأوروبية والمواصفة القياسية العراقية وإدارة الغذاء والدواء الأمريكية. من ناحية أخرى، كانت عيّنات الأعلاف تحتوي على تركيزات من الأفلاتوكسين B1 بلغت ١٠,١ و ٥,٩٥ و ٤,٢٧ و ٧,١٠ أجزاء في البليون للجاموس والأبقار والأغنام والماعز على التوالي. تراوحت معدلات الانتقال الملاحظة من ٠,٣٦٪ في الماعز إلى ٠,٧٨٪ في الجاموس و ٠,٦٦٪ في الأبقار. هناك عوامل متعددة تؤثر بما في ذلك نوع الحيوان، ولا يمكن تطبيق معادلة انتقال عالمية على جميع الحالات. لذلك من الضروري رصد مستويات الأفلاتوكسين M1 بانتظام في الحليب من مختلف أنواع الحيوانات من أجل الحد من المخاطر الصحية المحتملة. علاوة على ذلك، تقترح الدراسة تحسين الممارسات الزراعية والبيطرية للسيطرة بشكل أفضل على جودة الأعلاف لحيوانات الألبان.

الكلمات المفاحية: الأفلاتوكسين M1، الأفلاتوكسين B1، الحليب الخام، الاختبار المناعي المرتبط بالإنزيم، معدل الانتقال