Content is avaliable at SCOPUS

Meat Technology — Special Issue 66/3

www.meatcon.rs ▪ www.journalmeattechnology.com

UDK: 664.9

ID: 179608585

https://doi.org/10.18485/meattech.2025.66.3.54

Review Paper

Aflatoxin M1 in donkey milk from indigenous breeds in the Zasavica natural reserve

Dragana Ljubojević Pelić[1], Sandra Jakšić[1], Nenad Popov[1], Suzana Vidaković Knežević[1], Tomislav Kačarević[1], Miloš Pelić[1] and Milica Živkov Baloš[1]*

[1] Scientific Veterinary Institute „Novi Sad", Rumenački put 20, 21000 Novi Sad, Serbia

ARTICLE INFO

Keywords:
Aflatoxin M1
Autochthonous donkey breeds
Food safety
Organic farming
Sustainable production

ABSTRACT

Donkey milk is considered highly valued for its hypoallergenic properties and distinctive nutritional benefits. However, there is limited information on its potential contaminants, such as aflatoxin M1 (AFM1). This study aimed to thoroughly assess the safety of milk from Balkan and Banat donkey breeds raised under extensive organic (free-range) conditions, focusing on a significant concern - AFM1 concentrations across different seasons. Monthly samples were collected over the course of a year, resulting in a total of thirty-six pooled samples. AFM1 levels were quantified using a validated enzyme-linked immunosorbent assay (ELISA), with results showing concentration of AFM1 in donkey milk remained below the detection limit throughout the year.". This study underscores the need for additional research on both the nutritional and safety aspects of donkey milk, particularly in relation to seasonal variations, potential presence of other environmental toxins, and differences among donkey breeds.

1. Introduction

Donkey milk is highly valued for its unique nutritional profile (*Živkov Baloš et al*., 2023; 2024), especially its similarity to human milk and hypoallergenic properties, making it a suitable alternative for individuals with cow's milk allergies, including infants (*Martini et al.*, 2021). The nutritional composition of donkey milk, rich in proteins, amino acids, fatty acids, lactose, and essential minerals, supports its potential as a beneficial food source (*Živkov Baloš et al*., 2023). Given its growing use in high-sensitive population., concerns about contaminants, such as aflatoxin M1 (AFM1), warrant further investigation into its safety for human consumption.

Aflatoxins, primarily produced by *Aspergillus flavus* and *Aspergillus parasiticus*, are mycotoxins with carcinogenic, mutagenic, and immunosuppressive properties (*Gizachew et al*., 2019). AFM1, a metabolite of aflatoxin B1 (AFB1) found in contaminated feed, poses significant health risks, particularly to vulnerable populations like infants. Classified as a human carcinogen by the International Agency for Research on Cancer (*IARC,* 2012), AFM1 can also impair growth in children and cause immune-related issues (*Tesfamariam et al*., 2020). While AFM1 contamination in commonly consumed milk, such as cow and goat milk, has been well-documented (*Ljubojević Pelić et al*., 2024), limited studies focus on its presence in donkey milk, despite growing

***Corresponding author:** Dragana Ljubojević Pelić, *dragana@niv.ns.ac.rs*

Paper received May 20th 2025. Paper accepted May 27th 2025.
The paper was presented at the 63rd International Meat Industry Conference "Food for Thought: Innovations in Food and Nutrition" – Zlatibor, October 05th-08th 2025.
Published by Institute of Meat Hygiene and Technology – Belgrade, Serbia.

interest in the latter due to its nutritional and hypoallergenic qualities.

The Balkan and Banat donkey breeds, known for their adaptability and high milk quality, are reared in the Zasavica Natural Reserve, providing an ideal setting for studying both the nutritional value and safety of their milk. Although research on donkey milk contamination is limited compared to other milk types, the growing demand especially among children underscores the importance of continuous monitoring to ensure it remains safe and free from contaminants. Understanding how environmental pollution may affect contamination levels is crucial. The present study aims to assess AFM1 concentrations in milk from Balkan and Banat donkeys over different seasons, addressing the need for further research on potential contaminants and their impact on milk safety and quality. This study is the first to examine AFM1 levels in the Balkan and Banat donkey breeds, contributing significantly to the knowledge base and the conservation of indigenous breeds.

2. Materials and methods

The study was conducted at the Zasavica Nature Reserve, which houses approximately 300 milking donkeys, making it the largest such farm in Southeastern Europe. The study area and the conditions for keeping donkeys are detailed in our previous researches (*Živkov Baloš et al*., 2024; *Ljubojević Pelić et al*., 2025). Briefly, the reserve is home to donkeys, horses, Serbian Podolian cattle, Mangalitsa pigs, and various bird species. Animals were raised in an extensive production system, grazing for nine months a year and drinking water from springs within the reserve. The pastures consist of meadow grass and are not fertilized due to the high number of animals. During winter, donkeys are kept indoors and fed a grain-based diet, mainly corn and triticale.

Milk samples were collected monthly from July 2023 to June 2024, with three pooled samples taken each month (nine per season) from the milk of all donkeys milked. Only milk from clinically healthy donkeys was sampled. Samples were aseptically collected in food-safe containers and transported to the laboratory in refrigerated containers (4°C). Aflatoxin M1 levels were determined using the enzyme-linked immunosorbent assay (ELISA) method, following the manufacturer's instructions (Neogen). Limit of detection of the test was 0.005 µg/kg.

3. Results and discussion

The results of the analysis of pooled donkey milk samples as well as the results of previous studies are presented in Table 1. The results of the examination of aflatoxin M1 presence in donkey milk showed that throughout the entire study period, across all four seasons, its concentration was < 0.005 µg/kg (Table 1).

Donkey milk is highly valued for its hypoallergenic properties and nutritional benefits, making it essential to investigate potential contamination with aflatoxin AFM1. AFM1 is a metabolite of AFB1 that is excreted into milk when lactating animals consume AFB1-contaminated feed (*Zentai et al*., 2023). The liver metabolizes AFB1 into AFM1, which represents the primary contamination pathway. While donkeys, like other mammals, can consume contaminated feed, their metabolic conversion of AFB1 to AFM1 and the transfer rate of aflatoxins into milk may differ from that of ruminants (*Tozzi et al*., 2016). This may be due to lower transfer rate, as suggested in limited data.

Research on AFM1 in donkey milk is limited, with most studies showing low or undetectable levels, consistent with our findings and studies in Greece (*Malissiova and Manouras*, 2017), Germany (*Gross et al.*, 2019), Italy (*Cammilleri et al*., 2019), Greece and Cyprus (*Malissiova et al*., 2016), and China (*Xiong et al*., 2022). This low presence of AFM1 may be linked to the specific feed used in donkey farming, particularly the primary diet of grass and hay (*Altafini et al*., 2020), which reduces AFM1 detection compared to milk from other species. In our study, donkeys grazed for nine months with no additional feed supplements, and only corn and triticale were provided during winter. Additionally, the low casein content in donkey milk compared to cow's milk (*Živkov Baloš et al*., 2023) may contribute to the reduced AFM1 presence, as aflatoxins have a high affinity for casein (Indyk et al., 2021). In animals exposed to mycotoxins, there is often a correlation between AFM1 levels and casein percentage, which may be another reason for the lower AFM1 levels in donkey milk (*Ljubojević Pelić et al*., 2024).

This study highlights the importance of monitoring AFM1 in donkey milk, especially with the increasing demand for this milk due to its health benefits. Further research is needed to explore seasonal and environmental factors affecting AFM1 levels and to investigate the specific physiological mechanisms in donkeys to ensure the safety and quality of donkey milk.

Table 1. Aflatoxin M1 (AFM1) in donkey milk samples

Donkey breed	Country	Analytical method	Number of positive samples/ number of samples	Concentration/ range	Reference
The Balkan and Banat	Serbia	ELISA	0/36	< 0.005 µg/kg	
	Serbia	ELISA	3/5 (60%)	Mean: 0.02 ± 0.02/ range: 0.005–0.035 µg/kg	Kos et al., 2014
	China (Xinjiang)	ELISA	0/70	< 0.005 µg/kg	Xiong et al., 2022
Indigenous Greek Donkey and Cypriot Donkey	Greece and Cyprus	ELISA	0/90 (79 from 6 Greek farms and 11 from a Cypriot farm)		Malissiova et al., 2016
	Greece	ELISA	5 /36 (13.9 %)	0.006–0.03 µg/kg	Malissiova and Manouras, 2017
Martina Franca, Amiata, Sant' Andrea, San Domenico, Argentato di Sologno, Sardo, Ragusano, Poitou	Italy	HPLC-FLD	1/63	1 sample between LOD - and LOQ (0.004 µg/kg)	Altafini et al., 2020
	Italy (Sicily)	ELISA	0/84	< 0.007 µg/kg	Cammilleri et al., 2019
	Croatia	ELISA	14/14	Mean: 0.005 ± 0.001/ range: 0.003–0.01 µg/kg	Bilandžić et al., 2014
Baudet du Poitou	Germany	EIA	0/6	< 0.005 µg/kg	Gross et al., 2019

4. Conclusion

The global consumption of donkey milk is expected to rise, particularly in Europe, due to the increasing prevalence of cow's milk allergies. Our study found that milk from Balkan and Banat breed donkeys is safe in terms of AFM1 contamination, with levels consistently below the detection limit. However, the potential presence of AFM1 and other toxic substances in donkey milk poses a food safety risk, highlighting the need for comprehensive monitoring and control measures.

Future research should focus on larger-scale studies, risk assessment, and developing preventive measures to ensure the safety and quality of donkey milk as it becomes an increasingly popular alternative milk source.

Disclosure Statement: No potential conflict of interest was reported by the authors.

Funding: This research was supported by the Science Fund of the Republic of Serbia, grant no. 3535, Project Health Protection and Biodiversity Conservation of Domestic Donkeys in the Special Nature Reserve "Zasavica" — PROTECTDonkey, and by the Ministry of Science, Technological Development and Innovation of Republic of Serbia by the Contract of Implementation and Funding of Research Work of NIV-NS in 2025, Contract No: 451-03-136/2025-03/200031.

Acknowledgements: The authors are grateful to Slobodan Simić and Nikola Nilić (Special Nature Reserve "Zasavica", Serbia) for providing technical support.

References

Altafini, A., Tassinari, M., Guerrini, A., & Roncada, P. (2020). Occurrence of aflatoxin M1 (AFM1) in donkey milk collected in northern Italy. *Veterinary Sciences*, 7(4), 176. https://doi.org/10.3390/vetsci7040176.

Bilandžić, N., Božić, Đ., Đokić, M., Sedak, M., Kolanović, B. S., Varenina, I., & Cvetnić, Ž. (2014). Assessment of aflatoxin M1 contamination in the milk of four dairy species in Croatia. *Food Control*, 43, 18–21. https://doi.org/10.1016/j.foodcont.2014.02.044.

Cammilleri, G., Graci, S., Collura, R., Buscemi, M. D., Vella, A., Macaluso, A., ... & Ferrantelli, V. (2019). Aflatoxin M 1 in cow, sheep, and donkey milk produced in Sicily, Southern Italy. *Mycotoxin Research*, 35, 47–53. https://doi.org/10.1007/s12550-018-0329-y.

Gizachew, D., Chang, C. H., Szonyi, B., De La Torre, S., & Ting, W. T. E. (2019). Aflatoxin B1 (AFB1) production by Aspergillus flavus and Aspergillus parasiticus on ground Nyjer seeds: The effect of water activity and temperature. International journal of food microbiology, 296, 8–13. https://doi.org/10.1016/j.ijfoodmicro.2019.02.017.

Gross, M., Ploetz, C. P., & Gottschalk, C. (2019). Immunochemical detection of mycotoxins in donkey milk. *Mycotoxin Research*, 35, 83–87. https://doi.org/10.1007/s12550-018-0333-2.

IARC Working Group on the Evaluation of Carcinogenic Risks to Humans, (2012). A review of human carcinogens: Part B: Biological agents. World Health Organisation.

Indyk, H. E., Wood, J. E., & Gill, B. D. (2021). Aflatoxin M1 binding to bovine α-and κ-caseins demonstrated by surface plasmon resonance. *International Dairy Journal*, 121, 105119. https://doi.org/10.1016/j.idairyj.2021.105119.

Kos, J., Lević, J., Đuragić, O., Kokić, B., & Miladinović, I. (2014). Occurrence and estimation of aflatoxin M1 exposure in milk in Serbia. *Food Control*, 38, 41–46. https://doi.org/10.1016/j.foodcont.2013.09.060.

Ljubojević Pelić, D., Lazić, S., & Živkov Baloš, M. (2024). Chemical contaminants in donkey milk: A review of literature on sources, routes and pathways of contamination, regulatory framework, health risks, and preventive measures. *Heliyon*, 10, 21, e39999. https://doi.org/10.1016/j.heliyon.2024.e39999

Ljubojević Pelić, D., Popov, N., Gardić, E., Vidaković Knežević, S., Žekić, M., Gajdov, V., & Živkov Baloš, M. (2025). Seasonal Variation in Essential Minerals, Trace Elements, and Potentially Toxic Elements in Donkey Milk from Banat and Balkan Breeds in the Zasavica Nature Reserve. *Animals*, 15(6), 791. https://doi.org/10.3390/ani15060791

Malissiova, E., & Manouras, A. (2017). Monitoring aflatoxin M1 levels in donkey milk produced in Greece, intended for human consumption. *World Mycotoxin Journal*, 10(2), 203-206. https://doi.org/10.3920/WMJ2017.2169.

Malissiova, E., Arsenos, G., Papademas, P., Fletouris, D., Manouras, A., Aspri, M., ... & Arvanitoyannis, I. S. (2016). Assessment of donkey milk chemical, microbiological and sensory attributes in Greece and Cyprus. *International Journal of Dairy Technology*, 69(1), 143–146. https://doi.org/10.1111/1471-0307.12245.

Martini, M., Altomonte, I., Tricò, D., Lapenta, R., & Salari, F. (2021). Current knowledge on functionality and potential therapeutic uses of donkey milk. *Animals*, 11(5), 1382. https://doi.org/10.3390/ani11051382.

Tesfamariam, K., De Boevre, M., Kolsteren, P., Belachew, T., Mesfin, A., De Saeger, S., & Lachat, C. (2020). Dietary mycotoxins exposure and child growth, immune system, morbidity, and mortality: a systematic literature review. *Critical Reviews in Food Science and Nutrition*, 60(19), 3321–3341. https://doi.org/10.1080/10408398.2019.1685455.

Tozzi, B., Liponi, G. B., Meucci, V., Casini, L., Dall'Asta, C., Intorre, L., & Gatta, D. (2016). Aflatoxins M1 and M2 in the milk of donkeys fed with naturally contaminated diet. *Dairy Science & Technology*, 96, 513–523. https://doi.org/10.1007/s13594-016-0285-2.

Xiong, J., Chen, F., Zhang, J., Ao, W., Zhou, X., Yang, H., ... & Qiu, Y. (2022). Occurrence of aflatoxin M1 in three types of milk from Xinjiang, China, and the risk of exposure for milk consumers in different age-sex groups. *Foods*, 11(23), 3922. https://doi.org/10.3390/foods11233922.

Zentai, A., Jóźwiak, Á., Süth, M., & Farkas, Z. (2023). Carry-over of aflatoxin B1 from feed to cow milk—a review. *Toxins*, 15(3), 195. https://doi.org/10.3390/toxins15030195.

Živkov Baloš, M., Ljubojević Pelić, D., Jakšić, S., & Lazić, S. (2023). Donkey milk: an overview of its chemical composition and main nutritional properties or human health benefit properties. *Journal of Equine Veterinary Science*, 121, 104225. https://doi.org/10.1016/j.jevs.2023.104225

Živkov Baloš, M., Popov, N., Vidaković Knežević, S., Savić, S., Gajdov, V., Jakšić, S., & Ljubojević Pelić, D. (2024). Nutritional quality of donkey milk during the lactation. *Biotechnology in Animal Husbandry*, 40(2), 155-168. https://doi.org/10.2298/BAH2402155Z

Authors info

Dragana Ljubojević Pelić, https://orcid.org/0000-0002-7833-7696
Sandra Jakšić, https://orcid.org/0000-0002-0799-1797
Nenad Popov, https://orcid.org/0000-0002-3102-4334
Suzana Vidaković Knežević, https://orcid.org/0000-0001-7711-3917
Tomislav Kačarević, https://orcid.org/0009-0005-2455-3077
Miloš Pelić, https://orcid.org/0000-0002-9055-0625
Milica Živkov Baloš, https://orcid.org/0000-0002-4266-1232