Review paper | DOI 10.63356/agrores.2025.029

Ensiling and presence of aflatoxin M1 in cow's milk

Aleksandra Ivetić [1]✉, Vesna Ganter[2], Bojan Stojanović[3], Marija Bajagić[4], Milivoje Ćosić[5], Slađan Stanković[1], Radmila Beskorovajni[1], Blagoje Stojković[3]

[1] *Institute for Science Application in Agriculture, Belgrade, Serbia*
[2] *Faculty of Agrobiotechnical Sciences, J. J. Strossmayer University of Osijek, Osijek, Croatia*
[3] *University of Belgrade, Faculty of Agriculture, Belgrade, Serbia*
[4] *Bijeljina University, Agriculture faculty, BIH*
[5] *Institute for Forestry, Belgrade, Serbia*

✉ farmainova.aleksandra@gmail.com

Abstract

The main goal of ensiling plants is preservation their nutritional value, as close as possible to the nutritional value they have in the form of fresh green plants. The technology of ensiling is a primary microbial process in which lactic acid bacteria are crucially important to protect energy content and prevent silo mass from the development of harmful microorganisms. The main points of risk are the selection of the correct stage of maturity of the plant for ensiling, the rapid squeezing of air inside the plant mass in the silo, and the correct covering. Changes in the silo mass occur practically as soon as the mass of plants is transferred from the field to the prepared silo object. The direction and intensity of the change depend on several factors, but mostly on those that condition the successful development of lactic acid fermentation, such as moisture in the nutrient, anaerobic environment, carbohydrate content, and temperature. These factors enable the conditions in which the desired microorganisms will dominate during the fermentation of the plant mass and obtain quality silage with high nutritional value. Contamination with undesirable microbes and chemical agents is one of the major problems in silage production. To minimize the aflatoxin contamination in crop plants, various physical, chemical, and biological methods, and breeding and genetic engineering approaches, have been used to reduce its level below the recommended one.

Key words: silage, legal regulations, aflatoxin, lactic acid bacteria, nutritional chain of humans and animals

---

INTRODUCTION

Ensiling is a microbial process of preserving fresh feed for animal production (Okoye et al., 2023). In those processes, lactic acid bacteria (LAB) play a key position in silage microorganisms and the effects of exogenous lactic acid bacteria on silage quality have been widely studied (Wang et al., 2021). Additions of LAB inoculants are frequently used to speed up the process of ensiling, prevent the growth of harmful microorganisms, and improve the silage quality of different crops. Furthermore, LAB belongs to the microorganisms that have the GRAS (generally recognized as safe) status (Fabiszewska et al., 2019). One of the most common silages in the world is made from corn. Corn (*Zea mays L.*) silage is a major forage source for ruminants in climates where corn is moderate to well adapted, consisting of the high-energy, commonly used for growing and finishing beef cattle as supplemental energy for cow and calf production, for growing dairy heifers, and for lactating dairy cows, often in combination with a complementary high-protein forage such as alfalfa (*Medicago sativa L.*) (Allen et al., 2003).

But, most often corn is infected by mycotoxins on farms, frequently in the form of aflatoxins. Aflatoxins have a significant health, nutritional, and economic impact on the nutritional chain of humans and animals. To minimize the aflatoxin contamination in crop plants, various physical, chemical, and biological methods, and various breeding and genetic engineering approaches, have been used to minimize the toxicity of aflatoxin and reduce its level below the recommended one. Several approaches have been manifested to reduce the aflatoxin contamination in crops which include various physical, chemical, and biological methods.

Worldwide, the possibility for the contamination of the nutritional chain with mycotoxins is permanently present, with a serious impact on human and animal health. As a secondary product of mold metabolism, aflatoxins can contaminate a large number of different agricultural and food products, as well as feeds. It is estimated that about 25% of the world is contaminated with at least one mycotoxin. Due to its carcinogenic effect, a major negative impact on the health of humans and animals, as well as the great impact on economic losses, aflatoxins pose an exceptional challenge for research around the world. Mycotoxins are considered one of the most important causes of nutritional stress (Ivetić et al., 2007). The International Agency for Research on Cancer (IARC) included AFB1 (Aflatoxin – AF) in the first group by the end of the second millennium, while AFB2, AFG1, AFG2, and AFM1 were grouped in the same class in the 2002 year. There is a need for continuous monitoring of the appearance of aflatoxins in food, feeds, and AFM1 in milk, intending to protect consumers from risks associated with their proven toxicity and carcinogenic effects.

NEW TRENDS AND OPPORTUNITIES FOR ENSILING

The technology of silage preparation consists of several routine techniques but, each step brings with it several possible consequences for the quality of the obtained silage if it is not implemented correctly (Ivetić, 2017). The main points of risk are the selection of the correct stage of maturity of the plant for ensiling, the rapid squeezing of air inside the plant mass in the silo, and the correct covering. Changes in the silo mass occur practically as soon as the mass of plants is transferred from the field to the prepared silo object. What will be the course and intensity of the change depend on several factors, but mostly on those that condition the successful development of lactic acid fermentation, such as moisture in the nutrient, anaerobic environment, carbohydrate content, and temperature. These factors enable the conditions in which the desired microorganisms will dominate during the fermentation of the plant mass and obtain quality silage with high nutritional value.

Choosing the right corn hybrid, and other plant cultivar has implications for silage's nutritional value and milk production. Optimization of harvest maturity, kernel processing, theoretical length of the cut, and cutting height will improve or maintain the nutritive value and milk production of lactating dairy cows (Ferraretto et al., 2018). Authors point out that technological advancements have been developed and made available to dairy producers and corn growers desiring to enhance the fiber and starch digestibility of whole-plant corn silage. However, inconsistency in silage quality may be also due to the lack of information on gene expression and molecular mechanisms of the microbiota involved in silage production, and modern research is focused on the production of the developing nutrient-quality animal feeds with improved LAB inoculants (Okoye et al., 2023). Because the adaptability, establishment, and development of LAB in forages during ensiling are partially unknown, the additives with LAB inoculants do not always successfully regulate silage fermentation (Cheng et al., 2022, Kobayashi et al., 2010). One of the main reasons for not establishing sufficient domination of LAB inoculants is the influence of epiphytic microflora in the ensiling plants (Ivetić et al.,2024). Classical microbiological planting techniques defined the epiphytic microbial populations associated with fresh forage, the pivotal role of lactic acid-producing bacteria in the ensiling process, and the contribution of clostridia, bacilli, yeast, and molds to the spoilage of silage (McAllister et al., 2018). Authors emphasize that polymerase chain reaction-based techniques, including length heterogeneity PCR, terminal RFLP, denaturing gradient gel electrophoresis, and automated ribosomal intergenic spacer analysis were the first molecular methods used to study silage microbial communities. Also, advancements in whole comparative genomic, metagenomic, and metatranscriptomic sequencing have or are in the process of superseding these methods, enabling microbial communities during ensiling to be defined with a degree of detail that is impossible using classical microbiology (McAllister et al., 2018).

Biotech companies have produced microbial inoculants to efficiently preserve the green mass of plants and reduce the loss of nutrients during the aerobic degradation of silage. The number of LAB colonies in such products is usually about $1x10^{11}$ cfu and only 10 g of such additives need to be added for ensiling

10 tons of green plant mass. According to (Cai et al., 1999), preservation depends on the ability of LAB to produce sufficient acids to stop the growth and other activities of undesirable microorganisms (MO) under anaerobic conditions, whereas the microbial composition of the inoculants is different. Inoculants can consist only of homofermentative or heterofermentative LAB, or they can consist of a combination of both, with or without the addition of enzymes. The main differences among them are the production of lactic acid (LA) and acetic acid (AA), which greatly affect the fermentation process and aerobic stability of silage. Lactic acid rapidly lowers the pH of the ensiled mass, but unlike AA and propionic acid (PA), it has weak fungicidal properties, (Woolford, 1984). *Lactobacillus buchneri* is the dominant species used in obligate heterofermentative LAB silage additives, and it slowly converts LA to AA and 1,2-propanediol during silo storage, improving aerobic stability, while having no effect on animal productivity (Muck et al., 2018).

Preserving forages as baled silage has increased in the last 2 decades, particularly for small and mid-sized dairy and beef producers. Coblentz and Akins (2018), point out that additional wilting (45-50%DM), coupled with the long-stemmed nature of most baled silages, acts to restrict the rate and extent of fermentation for baled silages, thereby suggesting emphasis should be continued maintaining anaerobiosis through (1) applying PE-film wraps promptly; (2) using an appropriate number of PE-film layers (6 to 8); (3) selecting a storage site free of sharp objects or other debris; and (4) monitoring wrapped bales closely for evidence of puncture, particularly by birds or vermin.

Total mixed rations (TMR) are produced by mixing forages, byproducts, concentrates, minerals, vitamins, and additives formulated for a specific concentration of nutrients into a single blend of feed. Animals consume the nutrients necessary to meet the requirements of maintenance and production (Schingoethe et al., 2017). The interest in ensiling TMR for ruminants reemerged in the last decades because it has: been a sustainable alternative to efficiently handle wet byproducts in ruminant diets, has markedly higher aerobic stability than its respective fresh TMR, and increases ruminal protein degradability due to proteolysis during storage (Bueno et al., 2020). Appropriate silage particle size is important because long particles increase eating time, (Mertens, 1997), especially in silage which is the compulsory component of TMR. In that way at the farms, competitive feeding behavior is created, with limited time for DMI (dry matter intake) at the feed bunk. That limitation in the feeding behavior is often the negative response to poor silage fermentation. The compounds that are shown to have the greatest effect on feeding behavior are lactate, acetate, propionate, butyrate, ammonia-N, and amines in silage (Grant, 2018a). Also, poorly silage fermentation is connected with an exceeding 30% loss of DM (dry matter) (Grant, 2018 b).

Contamination with undesirable microbes and chemical agents is one of the major problems in silage production. The presence of yeasts and molds can negatively affect silage's nutritional value (NV) because they produce toxic compounds that are harmful to ruminants (Alonso et al., 2013). These microbes can proliferate massively once the silo is opened due to the presence of oxygen (Paradhipta et al., 2020). As a result, increasing yeast and mold populations decrease aerobic stability and reduce silage shelf life (Wilkinson et al., 2012). Consuming the contaminated silage, ruminants are often exposed to mycotoxins, primary such as aflatoxins, trichothecenes, ochratoxin A, fumonisins, zearalenone, and many others. Mycotoxins in silage can be minimized by preventing fungal growth before and after ensiling, with proper silage management to reduce mycotoxin contamination of feeds, and certain mold-inhibiting chemical additives or microbial inoculants can also reduce the contamination levels (Ogunade et al., 2018). Apart from mycotoxins, microbial hazards include *Clostridium botulinum* (associated with cattle botulism)*, Bacillus cereus, Listeria monocytogenes, Shiga toxin-producing Escherichia coli, Mycobacterium bovis,* and various mold species (Driehuis et al., 2018). Nevertheless, LAB (*L. brevis* and *L. buchneri*) is capable of producing acetate, proteinaceous compounds, peptides, and hydrogen peroxide which exert antifungal activity (Kleinschmit et al., 2005). The ability of LAB to release antifungal substances varies among strains (Schnürer and Magnusson, 2005). The mixture of homo-and heterofermentative strains improves silage quality by increasing nutrient digestibility and reducing yeast contamination. The use of starter cultures of LAB strains characterized by the ability to lower the level of pathogenic microorganisms may be an optimal method of forage preservation concerning the most hazardous species, like *E. coli*, *Salmonella* spp., or *L. monocytogenes* (Fabiszewska et al., 2019). In addition to biotic factors that can affect silage quality, it is important to mention ambient temperature (due to the recent negative climate changes) is a factor that influences all stages of silage making from production in the field to utilization at the feed bunk (Bernardes et al., 2018).

Silage management practice should include aspects of workers' safety. Silage-related injury knows no age boundary as workers and bystanders of all ages have been killed in silage accidents, (Bolsen, 2018). The risk of an accident can be dramatically reduced by correctly sizing the bunker silos and drive-over piles (Holmes and Bolsen, 2009). Every farm, feedlot, dairy, and silage contractor should have safety policies and procedures for their silage program, and they should schedule regular meetings with all their employees to discuss safety (Bolsen, 2018).

AFLATOXINS

Mycotoxins are secondary products of mold metabolism, which are synthesized by a series of reactions catalyzed by enzymes from many biochemically simple intermediate products of primary metabolism (Kos, 2015). Mycotoxins are produced by toxic strains of fungi present in food for humans and animals, (Chhaya et al., 2023). All over the world, in the nutritional chain exist the constant threat of mycotoxin contamination, with a serious impact on human and animal health (Ivetić and Ćosić, 2021). The presence of a persistent risk from mycotoxins and their undesirable presence in food and feed influenced many countries that have adopted a policy of rigorous controls. The global problems of mycotoxicosis are causing a health hazard, and the toxin-contaminated products losing economic value in the global food market. Therefore, mandatory for food industries to perform analysis on potentially contaminated commodities before the trade (Schincaglia et al., 2023). Various physical, chemical, biological, and nano-particles based approaches are used for minimizing and management of aflatoxin in food crops. However, researchers are also progressing in the development of fungal-resistant varieties through breeding and genetic engineering approaches but their outcome is still a major concern. Hence a combined approach of using resistant varieties along with recommended pre- and post-harvest practices should be followed by farmers and food industries to minimize and degrade the aflatoxin content in food crops and their derived products.

Molds that produce toxic metabolites - aflatoxins during growth are *Aspergillus flavus, Aspergillus parasiticus* (Kihal et al., 2023), *A. nominius* (Buzás et al., 2023), *A. minisclerotigenes, A. korhogoensis, A. aflatoxiformans, A. texensis, A. novoparasiticus* and *A. arachidicola* (Giacometti, 2023). The four major aflatoxins AFB1, AFB2, AFG1, and AFG2 are commonly found in a wide range of food commodities, AFB1 and AFB2 are produced by *A. flavus* while AFG1 and AFG2 are produced by *A. parasiticus*, (Kumar et al., 2021). Authors reported that AFB1 constitutes the most harmful type of aflatoxins and is a potent hepato-carcinogenic, mutagenic, and teratogenic, and it suppresses the immune system. The synthesis of AFM1 occurs in the mammalian organism after the intake of contaminated feed with AFB1. Aflatoxins affect the quality of milk because cows metabolize AFB1 to form the monohydroxide derivative aflatoxin M1 (AFM1), which is secreted into cow's milk and is highly resistant to thermal treatments such as pasteurization and freezing (Alvarado et al., 2017). AFM1 is stable in raw milk and prepared dairy products. They mostly undergo undamaged pasteurization processes, cheese production, yogurt, sour cream, and butter. During cheese processing, AFM1 with casein is linked to a specific complex that affects a higher concentration of this mycotoxin in cheese than in whey. However, contaminated whey with AFM1 is often used to feed young animals (Chavarría et al., 2017). The presence of mycotoxins in cow's milk and dairy products is one of the most serious problems in producing a health-safe diet, as milk is a key source of nutrients for humans. The effect of aflatoxin on the organism of domestic animals depends on genetic, physiological, and external factors (Ivetić et al., 2023). The biggest impact on cow's milk contamination is AFB1 with AFM1. Many authors report that the diet of cows with food containing AFB1, in milk toxin AFM-1 will appear in milk after 12-24 h, after consuming a contaminated diet, and will disappear from milk after 3-5 days (Ivetić et al., 2007). Animal feed contaminated with aflatoxins can cause different acute and chronic diseases in animals: refusal of food, weight loss, decrease in immunity, cancer, decreased reproductive capacity decreased production, and death. Secondary aflatoxicosis tends to introduce into the body a smaller amount of aflatoxin for a very long period, compared to those that lead to obvious signs of poisoning and a change in the immune system (Ivetić et al., 2023). However, in this way, altered immune function makes the animal more susceptible to other infectious diseases. Aflatoxins suppress the immune system of humans and animals by acting on the cells responsible for boosting immunity (Kumar et al., 2021). Currently, consumers are continuously exposed to low doses of AFM1 (Buzás et al., 2023).

Aflatoxin contamination in crops is a global threat that compromises the safety of food, and feed, and also influences the agricultural economy and crop-dependent small-scale industries (Kumar et al., 2021). Crops can be contaminated during the process of harvesting, storing, and transporting by the fungi which leads to the production of several mycotoxins. Mycotoxins are produced by certain fungi as secondary metabolites and aflatoxin is one of them. Aflatoxin contamination in crops caused a serious threat to production, the food market, health, and economics.

All participants in the production and food chain such as farmers, grain producers, distributors, crop processors, farmers, and consumers have consequently losses (Ivetić et al., 2022). Direct effects include increased veterinary care costs, reduced livestock production, and continued endangered food safety for humans and animal feed (Ivetić, 2007b).

Legal regulations

Both toxins, AFB1 and AFM1 are carcinogenic. However, AFM1 is the most toxic secondary metabolite secreted in milk and is classified as group 1 carcinogenic by the International Agency for Research on Cancer (IARC, 2002). As such, AFM1 poses a global health risk to food safety for humans and animals. Regulation (EC) No 1881/2006 sets a maximum limit of 0.05 μg/kg for AFM1 in raw milk, heat-treated milk, and milk for the production of milk-based products (European Commission, directive EC/1881/2006, 2002/32/EC). The same limit was valid in Serbia from 2011 (Official Gazette of RS, 28/2011) until the end of February 2013.

However, after the appearance of contamination of milk with AFM1 in Serbia at the end of February 2013, the Government of Serbia established a new maximum level of 0.25 μg/kg of milk. The permissible level of aflatoxin (AFM1) in milk in Serbia, is five times higher than in the European Union and may be reduced at the end of 2024, according to the Regulation amending the Regulation on maximum concentrations of certain contaminants in food (Official Gazette of the Republic of Serbia, No. 110/2023). The amendment of the Regulation on maximum concentrations of certain contaminants in foodstuffs (Official Gazette of the Republic of Serbia, No. 81/2019, 126/2020, 90/2021, 118/2021, 127/2022, 110/2023,) provides for the lowering of the permitted levels of this substance from 1 December 2023, Table 1.With the new amendment to the Rulebook on maximum concentrations of certain contaminants in foodstuffs ("Official Gazette of the Republic of Serbia", No. 73/2024), the same limit value of aflatoxins in raw milk of 0.25 micrograms per liter will be maintained in the next year, i.e. until December 1, 2025.

Table 1. Permitted levels of aflatoxin contamination in foodstuffs, Regulation on maximum concentrations of certain contaminants in foodstuffs (Official Gazette of the Republic of Serbia, No. 81/2019, 126/2020, 90/2021, 118/2021, 127/2022 and 110/2023*)

No	Food		Maximum permissible concentration (μg/kg)
			M_1
2.1.13.	Raw milk, thermally processed milk, and milk for the production of dairy products	Until 30 November 2023. *Extend to ▼ Until 30 November 2024. *Extend to ▼ Until 30 November 2025.	0,25
		As of December 1, 2023. *Extend to ▼ As of December 1, 2024. *Extend to ▼ As of December 1, 2025.	0,050

Regulation on the quality of feed (Official Gazette of the Republic of Serbia, No. 4 /2010, 113 /2012, 27 /2014, 25 / 2015, 39 /2016, 54 /2017), more closely prescribes the conditions regarding the quality of feed. The limits of the levels of AFB1 in feedstuffs, complementary and complete mixtures for animals, are prescribed in Article 99 of this Regulation and are shown in Table 2. Feed, for this Regulation, means products of plant, animal, and mineral origin, produced naturally or industrially, which are used for the nutrition and production of premixes and mixtures.

It should be noted that as long as the parent compound AFB1 is not controlled both in food and in feed, it cannot be expected that only control of AFM1 exposure cannot be expected to lead to a significant global reduction in hepatocellular carcinoma (liver cancer) (Turna et al., 2022). Chhaya *et al.* (2023), state that in the European Union, the maximum permitted level of aflatoxin B1 in feed is 0.02 mg kg^{-1} (moisture content 12%) or 20,000 ng kg^{-1}, with a maximum allowable content in concentrated feed for dairy cows of 0.005 mg kg^{-1} (moisture content 12%) or 5000 ng kg^{-1} (Directive 2002/32/EC).

Farm productivity problems caused by toxins can be prevented (Ivetić et al., 2013). Early and rapid detection of aflatoxin M1 by applying a strict self-control strategy resulting in the application of mitigation measures can significantly reduce the concentration of aflatoxin M1 in milk.

Table 2 Limits of permitted levels of AFB1 in feed, supplementary and complete mixtures for animals, (Regulation on the quality of feed, Article 99, Official Gazette of the Republic of Serbia, No. 4 /2010, 113 /2012, 27 /2014, 25 / 2015, 39 / 2016, 54 /2017)

Undesirable substances	Products intended for feed	Maximum allowable amount expressed in mg/kg (ppm), when the moisture content of feed is calculated at 12%
Aflatoxin B1	Feeds	0,03
	Complementary and complete mixtures	0,01
	Except:	
	– mixtures (complementary and complete) for dairy cows and calves, dairy sheep and lambs, milk goats and goats, piglets and young poultry	0,005
	– mixtures (complementary and complete) for cattle (excluding dairy cows and calves), sheep (except dairy sheep and lambs), goats (except dairy goats and goats), pigs (except piglets), and poultry (except young animals).	0,02

There is a need for continuous monitoring of the appearance of aflatoxins in feeds and AFM1 in milk, to protect the risks associated with their proven toxicity and carcinogenic effects. Worldwide, the possibility of contamination of the food chain with mycotoxins is permanently present, with a serious impact on human health. Therefore, ELISA methods, as well as Liquid Chromatography methods with different detectors (HPLC-FLD, HPLC-UV light-FLD, LC-MS/MS) are developed and optimized for the implementation, (Kos et al., 2016). If the primary toxin AFB1 is not controlled in both food and feed, controlling AFM1 exposure cannot be expected to lead to a meaningful global reduction. In the long term, regular farm-to-fork aflatoxin monitoring should be an essential tool to prevent the presence of aflatoxin levels in food and feed.

CONCLUSION

The technology of silage preparation consists of several routine techniques but, each step brings with it several possible consequences for the quality of the obtained silage if it is not implemented correctly. The main points of risk are the selection of the correct stage of maturity of the plant for ensiling, the rapid squeezing of air inside the plant mass in the silo, and the correct covering. Changes in the silo mass occur practically as soon as the mass of plants is transferred from the field to the prepared silo object. What will be the course and intensity of the change depend on several factors, but mostly on those that condition the successful development of lactic acid fermentation, such as moisture in the nutrient, anaerobic environment, carbohydrate content, and temperature. These factors enable the conditions in which the desired microorganisms will dominate during the fermentation of the plant mass and obtain quality silage with high nutritional value. Crops can be contaminated during the process of harvesting, storing, and transporting by the fungi, and this leads to the production of several mycotoxins. Mycotoxins are produced by certain fungi as secondary metabolites and aflatoxin is one of them. Aflatoxin contamination in crops caused a serious threat to production, the food market, health, and economics. The presence of a persistent risk from mycotoxins and their undesirable presence in food and feed influenced many countries that have adopted a policy of rigorous controls. The global problems of mycotoxicosis are causing a health hazard, and the toxin-contaminated products losing economic value in the global food market. There is a need for continuous monitoring of the appearance of aflatoxins in feeds and AFM1 in milk, to protect the risks associated with their proven toxicity and carcinogenic effects. The global problems of mycotoxicosis are causing a health hazard, and the toxin-contaminated products losing economic value in the global food market.

ACKNOWLEDGEMENT

The paper was created as a result of research within the Contract on the realization and financing of scientific research work in 2025 between the Institute for the Application of Science in Agriculture, Belgrade and the Ministry of Science, Technological Development and Innovation of the Republic of Serbia, contract registration number: 451-03-136/2025-03/ 200045.

REFERENCES

Allen, M., Coors, J. G., & Roth, G. (2003). Silage Science and Technology. Agronomy Monograph 42. Corn silage, pp. 547-608. DOI: 10.2134/agronmonogr42.c12

Alonso, V. A., Pereyra, C. M., Keller, L. A. M., Calcero, A. M., Rosa, C. A. R., Chiacchiera, S. M., & Cavaglieri, L. R. (2013). Fungi and mycotoxins in silage: an overview. *Journal of Applied Microbiology*, 115, 637–643. DOI: 10.1111/jam.12178

Alvarado, A. M., Zamora-Sanabria, R., & Granados-Chinchilla, F. (2017). A Focus on Aflatoxins in Feedstuffs: Levels of Contamination, Prevalence, Control Strategies, and Impacts on Animal Health. Aflatoxin-Control, Analysis, Detection and Health Risks. InTech. DOI: 10.5772/intechopen.69468

Bernardes, T. F., Daniel, J. L. P., Adesogan, A. T., McAllister, T. A., Drouin, P., Nussio, L. G., Huhtanen, P., Tremblay, G. F., Bélanger, G., & Cai, Y. (2018). Silage review: Unique challenges of silages made in hot and cold regions. *Journal of Dairy Science*, 101(5), 4001-4019. DOI: 10.3168/jds.2017-13703.

Bolsen, K. K. (2018). Silage review: Safety considerations during silage making and feeding. *Journal of Dairy Science*, 101(5), 4122-4131. DOI: 10.3168/jds.2017-13738.

Bueno, A. V. I., Lazzari, G., Jobim, C. C., & Daniel, J. L. P. (2020). Ensiling Total Mixed Ration for Ruminants: A Review. *Agronomy*, 10, 879. DOI: 10.3390/agronomy10060879:

Buzás, H., Szabó-Sárvári, L. C., Szabó, K., Nagy-Kovács, K., Bukovics, S., Süle, J., Szafner, G., Hucker, A., Kocsis, R., & Kovács, A. J. (2023). Aflatoxin M1 detection in raw milk and drinking milk in Hungary by ELISA − A one-year survey. *Journal of Food Composition and Analysis*, 121. DOI: 10.1016/j.jfca.2023.105368

Cai Yimin, Benno, Y., Ogawa, M., & Kumai, S. (1999). Effect of Applying Lactic Acid Bacteria Isolated from Forage Crops on Fermentation Characteristics and Aerobic Deterioration of Silage. *Journal of Diary Science*, 82, 52-526. DOI: 10.3168/jds.S0022-0302(99)75263-X

Chavarría, G., Molina, A., Leiva, A., Méndez, G., Wong-González, E., Cortés-Muñoz, M., Rodríguez, C., Granados-Chinchilla, F. (2017). Distribution, stability, and protein interactions of Aflatoxin M1 in fresh cheese. *Food Control*, 73 (Part B), 581-586. DOI: 10.1016/j.foodcont.2016.09.005

Cheng, Q., Li, M., Fan, X., Chen, Y., Sun, H., Xie, Y., Zheng, Y., Chen, C., & Li, P. (2022). Effects of epiphytic and exogenous lactic acid bacteria on fermentation quality and microbial community compositions of paper mulberry silage. *Front Microbiology*, 13, 973500. DOI: 10.3389/fmicb.2022.973500

Chhaya, R. S., Membré, J. M., Nag, R., & Cummins, E. (2023). Farm-to-fork risk assessment of aflatoxin M1 in milk under climate change scenarios – A comparative study of France and Ireland. *Food Control*, 149, 109713. DOI: 10.1016/j.foodcont.2023.109713;

Coblentz, W. K., & Akins, M. S. (2018). Silage review: Recent advances and future technologies for baled silages. *Journal of Dairy Science*, 101(5), 4075-4092. DOI: 10.3168/jds.2017-13708

Ćosić, M., & Ivetić, A., (2022). Govedarska proizvodnja i pripremanje silaže. Univerzitet „Bijeljina", Poljoprivredni fakultet. ISBN: 978-99976-956-4-2

De Ondarza Mary Beth, D. O. M., Hristov, A. N., & Tricarico, J. M. (2023). A global dataset of enteric methane mitigation experiments with lactating and non-lactating dairy cows conducted from 1963 to 2022. *Data in Brief*, 49, 109459, DOI: 10.1016/j.dib.2023.109459

Dupraz, P. (2021). Policies for the ecological transition of agriculture: the livestock issue. *Review of Agricultural, Food and Environmental Studies*, 101, 529–538.

Driehuis, F., Wilkinson, J. M., Jiang, Y., Ogunade, I., & Adesogan, A. T. (2018). Silage review: Animal and human health risks from silage. *Journal of Dairy Science*, 101(5), 4093-4110. DOI: 10.3168/jds.2017-13836

European Commission DIRECTIVE 2002/32/EC OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL of 7 May 2002 on undesirable substances in animal feed (2006). https://eur-lex.europa.eu/legal-content/EN/ALL/?uri=COM:1999:0654:FIN

Fabiszewska, A. U., Zielińska, K. J., & Wróbel, B. (2019). Trends in designing microbial silage quality by biotechnological methods using lactic acid bacteria inoculants: a minireview. World Journal of Microbiology and Biotechnology, 35(5), 76. DOI: 10.1007/s11274-019-2649-2

Ferraretto, L. F., Shaver, R. D., & Luck, B. D. (2018). Silage review: Recent advances and future technologies for whole-plant and fractionated corn silage harvesting. *Journal of Dairy Science*, 101(5), 3937-3951. DOI: 10.3168/jds.2017-13728

Giacometti, F., Tomasello, F., Savini, F., Indio, V., Bacak, A., Canever, A., Bonilauri, P., De Cesare, A., & Serraino, A. (2023). Impact of the implementation of tailored management strategies to reduce the occurrence of aflatoxin M1 in milk-supply chain in Italy. *Food Control*, 149, 109720. DOI: 10.1016/j.foodcont.2023.109720

Grant, R. J., & Ferraretto, L. F. (2018a). Silage review: Silage feeding management: Silage characteristics and dairy cow feeding behavior. *Journal of Dairy Science*, 101 (5), 4111-412. DOI: 10.3168/jds.2017-13729.

Grant, R. J., & Adesogan, A. T. (2018b). Journal of Dairy Science Silage Special Issue: Introduction. *Journal of Dairy Science*, 101(5), 3935-3936. DOI: 10.3168/jds.2018-14630.

Holmes, B. J., & Bolsen, K. K. (2009). What's new in silage management? Proceedings of 15th International Silage Conf., Madison, pp. 61–76. https://www.asi.k-state.edu/doc/beef/silagemgmtnew.pdf

IARC, International Agency for Research on Cancer, (2002). Some traditional herbal medicines, some mycotoxins, naphthalene and styrene. Monographs on the Evaluation of Carcinogenic Risks to Humans. 82, Lyon, France (2002), pp. 171-249. ISBN-13978-92-832-1282-9; ISBN 13978-92-832-1582-0

Ivetić, A., Stojanović, B., Petrujkić B., Beskorovajni R., Maslovarić M., Ćosić, M., & Radulović, S., (2024). The influence of the *Lactobacillus buchneri*, *Lactobacillus plantarum*, and *Enterococcus faecium* on the nutritional value and health safety of the different maize hybrid silages. *Veterinarski Arhiv*, 94(4), 339-352. DOI: 10.24099/vet.arhiv.2486.

Ivetić, A. (2017). Uticaj mikrobioloških inokulanata na hranljivu vrednosti i aerobnu stabilnost silaže kukuruza i senaže lucerke. Univezitet u Beogradu, Poljoprivredni fakultet, Doktorska disertacija.

Ivetić, A., & Ćosić, M. (2021a). Aerobic stability of silage. Scientific conference with International Participation "Village and Agriculture". Bijeljina, Republika Srpska. Proceedings, 125-142. ISBN 978-99976-956-1-1

Ivetić, A., Ćosić, M., Radulović, S., Cvijanović, G., Stojanović, B., Davidović, V., Jandrić, M. (2022). Application of biological waste of the food industry for animal feeding. Book of Abstracts, International Scientific Conference "Village and Agriculture", Biljeljina, BIH. ISBN 978-99976-956-7-3

Ivetić, A., Đorđević, N., Radin, D., Stojić, P., Grubić, G., & Stojanović, B. (2013). Značaj aerobne stabilnosti silirane stočne hrane. *Zbornik naučnih radova Instituta PKB Agroekonomik*, 19(3-4), 47-60.

Ivetić, A., Grubić, G., & Tončić, T. (2007a). Aflatoksin. Stručna Konferencija Saveza zdravstvenih radnika Vojvodine. Tara, 19-22. april, 2007; Zbomik radova, p. 8.

Ivetić, A., Grubić, G., Adamovié, M., & Tončić, T. (2007b). Uloga zeolita u dobijanju zdravstveno bezbedne hrane. Majski susreti zdravstvenih radnika Republike Srbije, sa medunarodnim učešćem. Savez zdravstvenih radnika Republike Srbije, Zlatibor, 9-13. maj 2007, Zbornik radova, p. 407.

Ivetić, A., Jovanović, R., Radulović, S., Stojanović, B., Ćosić, M., Davidović, V., & Bajagić, M. (2023). Uticaj aflatoksina na zdravstvenu bezbednost i kvalitet mleka. 34 Conference Veterinarian of Serbia. Zlatibor 7-10 September 2023.

Kihal, A., Rodrigez-Prado, M., & Calsamiglia, S. (2023). A network meta-analysis on the efficacy of different mycotoxin binders to reduce aflatoxin M1 in milk after aflatoxin B1 challenge in dairy cows. *Journal of Dairy Science*, 108 (8), 5378-5387. DOI: 10.3168/jds.2022-23028

Kleinschmit, D. H., Schmidt, R. J., & Kung, L., Jr. (2005). The effects of various antifungal additives on the fermentation and aerobic stability of corn silage. *Journal of Dairy Science*, 88, 2130-2139. DOI: 10.3168/jds.S0022-0302(05)72889-7

Kobayashi, H., Cai, Y., Uegaki, R., Shimizu, M., Nakajima, M., Kanaya, C., Okajima, T., & Takada, R. (2010). Microorganism composition of high moisture Italian ryegrass (*Lolium multiflorum* Lam.) and its fermentation characteristics of silage inoculated with lactic acid bacteria. *Japanese Journal of Grassland Science*, 56(1), 39-46. DOI: 10.14941/grass.56.39

Kos, J. (2015). Aflatoksini: analiza pojave, procena rizika i optimizacija metodologije određivanja u kukuruzu i mleku. Doktorska disertacija, Tehnološki fakultet, Univerzitet u Novom Sadu.

Kos, J., Janić Hajnal, E., Jajić, I., Krstović, S., Mastilović, J., Šarić, B., & Jovanov, P. (2016). Comparison of ELISA, HPLC-FLD and HPLC-MS/MS Methods for Determination of Aflatoxin M1 in Natural Contaminated Milk Samples. *Acta Chimica Slovenica*, 63(4), 747-756. DOI: 10.17344/acsi.2016.2451

Kumar, A., Pathak, H., Bhadauria, S., & Sudan J. (2021). Aflatoxin contamination in food crops: causes, detection, and management: a review. *Food Production, Processing and Nutrition*, 3, 17. DOI: 10.1186/s43014-021-00064-y

McAllister T. A., Dunière L., Drouin P., Xu S., Wang Y., Munns K., & Zaheer R. (2018). Silage review: Using molecular approaches to define the microbial ecology of silage. *Journal of Dairy Science*, 101(5), 4060-4074, DOI: 10.3168/jds.2017-13704

Mertens, D. R. (1997). Creating a system for meeting the fiber requirement of dairy cows. *Journal of Dairy Science*, 80, 463-1481. DOI: 10.3168/jds.S0022-0302(97)76075-2

Muck, R. E., Nadeau, E. M. G., McAllister, T. A., Contreras-Govea, F. E., Santos, M. C., & Kung, L. Jr. (2018). Silage review. Recent advances and future uses of silage additives. *Journal of Dairy Science*, 101(5), 3980-4000. DOI: 10.3168/jds.2017-13839.

Ogunade, I. M., Martinez-Tuppia, C., Queiroz, O. C. M., Jiang, Y., Drouin, P., Wu, F., Vyas, D., & Adesogan, A. T. (2018). Silage review: Mycotoxins in silage: Occurrence, effects, prevention, and mitigation. *Journal of Dairy Science*, 101(5), 4034-4059. DOI: 10.3168/jds.2017-13788

Okoye, C. O., Wang, Y., Gao, L., Wu, Y., Xia, Li, Sun, J., & Jiang, J. (2023). The performance of lactic acid bacteria in silage production: A review of modern biotechnology for silage improvement. *Microbiological Research*, 266, 127212. DOI: 10.1016/j.micres.2022.127212

Paradhipta, D. H. V., Lee, S. S., Kang, B., Joo, Y. H., Lee, H. J., Lee, Y., Kim, J., & Kim, S. C (2020). Dual-Purpose Inoculants and Their Effects on Corn Silage. *Microorganisms*, 8. DOI: 10.3390/microorganisms8050765

Pravilnik o kvalitetu hrane za životinje („Službeni glasnik RS", br. 4/10, 113/12, 27/14, 25/15, 39/16 i 54/17)

Pravilnik o maksimalnim koncentracijama određenih kontaminenata u hrane, ((Sl. glasnik RS br. 81/19, 126/20, 90/21, 118/21, 127/22, 110/23)

Pravilnik o maksimalnim koncentracije određenih kontaminenata u hrani, (Službeni glasnik RS, br. 28/2011, 81/2019, 126/2020, 90/2021, 118/2021 i 127/2022)

Regulation on maximum concentrations of certain contaminants in foodstuffs - Amendment (Official Gazette of the Republic of Serbia, No. 81/2019, 126/2020, 90/2021, 118/2021, 127/2022, 110/2023

Rulebook on maximum concentrations of certain contaminants in foodstuffs – Amendment, (Official Gazette of the Republic of Serbia", No. 73/2024)

Schincaglia, A., Aspromonte, J., Franchina, F.A., Chenet, T., Pasti, L., Cavazzini, A., Purcaro, G., & Beccaria M. (2023). Current Developments of Analytical Methodologies for Aflatoxins' Determination in Food during the Last Decade (2013-2022), with a Particular Focus on Nuts and Nut Products. *Foods*, 12(3), 527. DOI: 10.3390/foods12030527

Schingoethe, D. J. (2017). A 100-Year Review: Total mixed ration feeding of dairy cows. *Journal of Dairy Science*, 100, 10143-10150. DOI: 10.3168/jds.2017-12967

Schnürer, J., & Magnusson, J. (2005). Antifungal lactic acid bacteria as biopreservatives. *Trends in Food Science & Technology*, 16(1-3), 70-78. DOI: 10.1016/j.tifs.2004.02.014

Turna, N. S., Havelaar, A., Adesogan, A., & Wu, F. (2022). Aflatoxin M1 in milk does not contribute substantially to global liver cancer incidence. *American Journal of Clinical Nutrition*, 115(6), 1473-1480. DOI: 10.1093/ajcn/nqac033

Wang, X., Cao, X., Liu, H., Guo, L., Lin, Y., Liu, X., Xiong, Y., Ni, K., & Yang, F. (2021). Effects of Lactic Acid Bacteria on Microbial Metabolic Functions of Paper Mulberry Silage: A BIOLOG ECO Microplates Approach. *Frontiers in Microbiol*ogy, 12, 689174. DOI: 10.3389/fmicb.2021.689174.

Wilkinson, J. M., & Davies, D. R. (2012). The aerobic stability of silage: Key findings and recent developments. *Grass and Forage Science*, 68, 1–19. DOI: 10.1111/j.1365-2494.2012.00891.x

Woolford, M.K. (1984) The Silage Fermentation. Marcel Decker, New York. ISBN 100824770390