TRADITION AND MODERNITY IN VETERINARY MEDICINE, 2022, vol. 7, No 2(13): 143–153

A REVIEW OF AFLATOXIN M1 IN RAW MILK: IMPORTANCE ON HUMAN HEALTH AND RUMINANTS

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ABSTRACT

Aflatoxin is both acutely and chronically toxic for animals and humans and can cause potentially dangerous diseases including acute toxic hepatitis, liver cirrhosis and hepatocarcinoma. AFM1 contamination in dairy products or raw milk is a global problem threatening public health in all areas of the world. Despite high consumption of dairy products in Lebanon, a few credible data are available on their contamination levels with AFM1. *A. flavus* and *A. parasiticus* were identified as the organisms responsible for the elaboration of the toxin in the feed. The term Aflatoxin now refers to group of bisfuranocoumarin metabolites isolated from strains of *A. flavus* group of fungi. The toxic material derived from the fungus *A. flavus* was given the name "Aflatoxin". The contamination of feedstuffs with mycotoxins is of increasing concern as changes on agricultural practice and probably climatic changes seem to have increased the prevalence of mycotoxins contamination. Contamination of feeds with mycotoxins accounts for significant loss in animal husbandry, as well as undesirable trade barriers for raw materials and dairy consumable products. The aim of the present review is to outline the importance of raw milk contamination with AFM1 and its importance on human health and ruminants.

Key words*:* Aflatoxins, AFM1, mycotoxins, raw milk, dairy products.

Introduction

Cow's milk is an important component of the human diets and plays an important role in nutrition, growth, development, and immunity. Milk is the most important source of calcium and phosphorus of human body and due to having essential amino acids, has an important status in supplying the body's protein needs. Studies have shown that there is a close relationship between consumption of milk and health status of people in terms of efficiency, Intelligence quotient (IQ), reducing the risk of infectious diseases, regulation of metabolic activities, decreasing blood pressure, increasing beneficial blood lipids (High-density lipoprotein), preventing from colon cancer and osteoporosis (Hjartaker et al., 2002).

Approximately 150 million households around the globe are engaged in milk production. In most developing countries, milk is produced by smallholders, and milk production contributes to household livelihoods, food security and nutrition. Milk provides relatively quick returns for small-scale producers and is an important source of cash income.

In recent decades, developing countries have increased their share in global dairy production. This growth is mostly the result of an increase in numbers of producing animals rather than a rise in productivity per head. In many developing countries, dairy productivity is constrained by poor-quality feed resources, diseases, limited access to markets and services (e.g., health, credit and training) and dairy animal's low genetic potential for milk production. Unlike developed countries, many developing countries have hot and/or humid climates that are unfavourable for dairying.

Due to a close relationship between livestock feed with health and safety of milk, various researches have been conducted on livestock feed. The researches have shown that contamination

of livestock feed with certain types of molds such as Aspergillus causes Aflatoxin production and its transfer to milk (Creppy, 2002).

The contamination of feedstuffs with mycotoxins is of increasing concern as changes on agricultural practice and probably climatic changes seem to have increased the prevalence of mycotoxins contamination. Contamination of feeds with mycotoxins accounts for significant loss in animal husbandry, as well as undesirable trade barriers for raw materials and consumable products (Wu, 2006).

The consumption of milk and dairy products is widespread in Lebanon; however, there are scarce surveys done on AFM1 content in these products (Elkak et al., 2011). The aim of the present review is to outline the importance of raw milk contamination with AFM1 and its importance on human health and ruminants.

Definition

Aflatoxins are fungal toxins produced by certain species of Aspergillus especially *parasiticus*, but rarely by *A. nominus* (Rahimi *et al.*, 2010) which may grow on several kinds of agricultural products. The major type of naturally occurring AFs have been identified: Aflatoxin B1 (AFB1), Aflatoxin B2 (AFB2), Aflatoxin G1 (AFG1), Aflatoxin G2 (AFG2), Aflatoxin M1 (AFM1) and Aflatoxin M2 (AFM2). AFB1 represents the highest degree of toxicity followed by AFM1, AFG1, AFB2 and AFG2 (Gourama and Bullerman, 1995). AFB1 is considered to be the most hepatocarcinogen, teratogen and mutagen of this group of mycotoxins. AFM1, the hydroxylated metabolites of AFB1, may be found in milk, milk products and meat of dairy cattle and mammals that have ingested the feedstuffs contaminated with AFB1 (Creppy, 2002). AFM1 can cause serious human disease, especially primary liver cancer, DNA damage and acute toxicity and carcinogenicity comparable with that of the parent molecule (figure 1). Therefore, it is now classified by the International agency for research on cancer (IARC) as a group 1 human carcinogen (IARC, 2002). Among human foods of animal origin, the rate of feed-to-tissue transfer of aflatoxins is the highest for milk. Milk has the greatest demonstrated potential for introducing AFM1 into the human diet and exposure to AFM1 through milk products is a serious problem for public health (Ruangwises et al., 2010). Exposure of children, including infants to AFM1 is of concern, because they have potentially greater vulnerability and sensitivity than adults and their capacity of biotransformation of carcinogen is generally slower than adults (Lopez et al., 2003); and, as result, occurrence of aflatoxins in human foods is strictly regulated to very low concentrations in developed countries. Thus, in these countries, the drive to abate Aflatoxin contamination is due to loss in crop value resulting from stringent government regulations on crop intended for human consumption, maximum permitted Aflatoxin levels range from 2 ng/g in the European Union to 20 ng/g in the United States. Aflatoxins are readily transferred from feed to milk resulting in similarly stringent regulations on feed intended for dairies (Van Egmond, 2004; Wu, 2004). Maximum permissible levels of aflatoxins in milk are 0.05 ng/g in the European Union and 0.5ng/g in the United States.

Unfortunately, in developing countries, crop from small scale farmers frequently pass from field to storage to consumption with no regulatory oversight and without a test of the Aflatoxin contamination. This will lead not only to economic loss but also to a tremendous impact on human health (Wu, 2004).

In other side the Joint FAO/WHO Expert Committee on Food Additives (JECFA) has evaluated aflatoxins B and G on several occasions since 1987 (JECFA, 1999 and 2007) and has recommended that, due to their carcinogenic potential, dietary exposure to aflatoxins should be minimized

as much as possible. In this way, the 2007 report by the Panel on Contaminants of the European Food Safety Authority (EFSA) indicated that exposure to aflatoxins from any food source should be kept as low as reasonably as possible, due to their genotoxic and carcinogenic properties (EFSA, 2007). Recently, Codex Alimentarius has established maximum limits for total aflatoxins (the sum of aflatoxins B1, B2, G1 and G2) in some nuts (almonds, peanuts, hazelnuts, pistachios and Brazil nuts) intended for further processing, at 15 μg/kg in comparison to the 10 μg/kg allowed for the same ready to eat products, based on the information provided by JECFA (Codex Alimentarius, 2008).

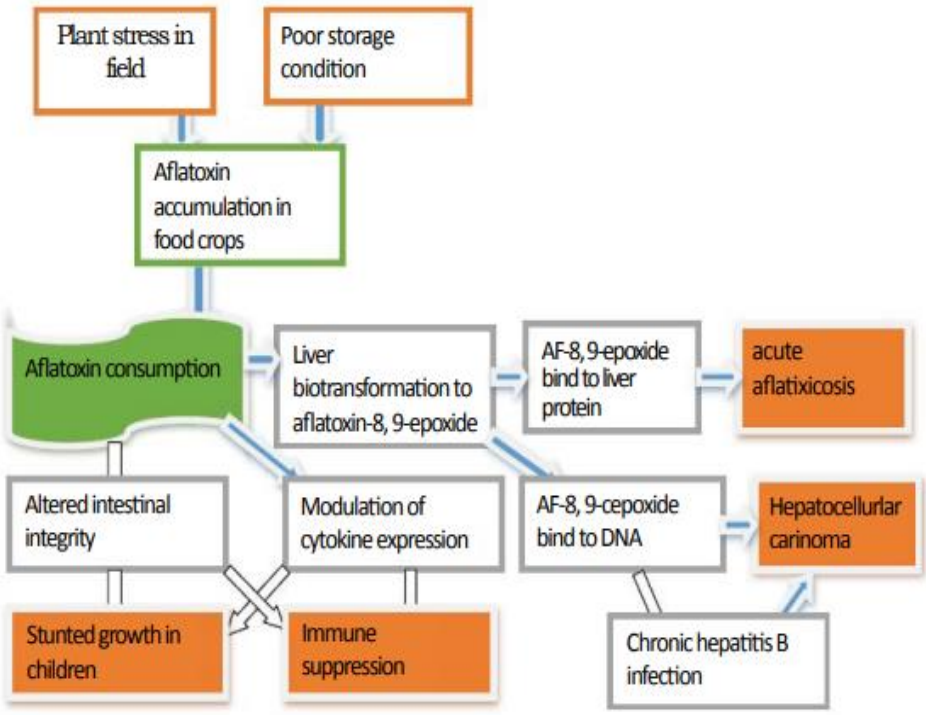

Figure 1: Aflatoxin and disease pathways in human (Wu F, Liu Y, Bhatnagar D , 2008)

History

Aflatoxins were discovered in 1960 when more than 100,000 young turkeys, ducklings and pheasants died in England over the course of a few months from an apparently new disease that was termed "Turkey-X disease". After a careful survey of the outbreaks, the disease was found to be associated with the Brazilian groundnut meal An intensive study of groundnut meal revealed its toxic nature as it produced typical symptoms of Turkey-X disease when consumed A study on the nature of the toxin suggested its origin from the fungus *Aspergillus flavus*. Thus, the toxin was named "Aflatoxin" by virtue of its origin from *A. flavus*. Research on aflatoxins led to a "golden age" of mycotoxins research during which several new mycotoxins were discovered. Among all mycotoxins and polypeptides compounds synthesized by fungal species, Aflatoxins (the most potent hepatotoxic and carcinogenic metabolites) continue to receive major attention and are most intensely studied (Negash D., 2018).

Factors influencing the development of Aflatoxin production

The factors implicated in the growth of the fungus belonging to the Aspergillus genus in foods are those relating to the environment in which they develop (pH, composition of the food or water activity) or extrinsic factors: ambient humidity, storage temperature and microbial competition (Zinedine and Mañes, 2009).

The formation of aflatoxins is influenced by physical, chemical and biological factors. The physical factors include temperature and moisture. The chemical factors include the composition of the air and the nature of the substrate, biological factors are those associated with host species (Hesseltine, 1983). The molds grow and produce toxins under conducive conditions, which involve adequate substrate (carbohydrates), moisture in the substrate (=13%), relative humidity (=70%), adequate temperature and oxygen. Fungal growth and Aflatoxin contamination are the consequence of interactions among the fungus, the host and the environment (Verma, 2007). Specific nutrients, such as minerals (especially zinc), minerals, fatty acids, amino acids and energy source (preferably in the form of starch) are required for Aflatoxins formation and energy source (Wyatt, 1991). Large yield of Aflatoxins are associated with the high carbohydrate concentrations, such as wheat rice and to lesser extent in oil seeds such as cottonseed, soybean and peanuts (Dienner and Davis, 1968). The limiting temperatures for the production of Aflatoxins by *A. flavus* and *A. parasiticus* are reported as 12^{O}C to 41^{O}C, with optimum production occurring between 25 and 32^{o}C (Lillehoj, 1983). Synthesis of Aflatoxins in feed are increase at temperature above 27^{0}C (80F), humidity levels greater than 62% and moisture levels in the feed above 14% (Royes and Yanong, 2002). Therefore, crops grown under warm and moist weather in tropical and subtropical countries are especially more prone to Aflatoxin contamination than those in temperate zones.

Water stress, high temperature stress and insect damage of host plant are the other factors, which favour mold infestation and toxin production. Specific crop growth stages, poor fertility, high crop densities, weed competition have been associated with increased mold growth and toxin production (Verma, 2007). The moisture content of the substrate is a main factor regulating the fungal growth and toxin formation. A moisture content of 18% for starchy cereal grains and 9 to 105 for oil-rich nuts and seeds has been established for maximum production of the toxin (WHO, 1979).

Aflatoxins: mechanism of action

Aflatoxins are the most intensively studied mycotoxins in dairy cattle as the excretion of Aflatoxin M1 in dairy milk is of public health concern. Following ingestion of Aflatoxin-contaminated feeds, a part of the ingested Aflatoxin B1 is degraded in the rumen, resulting in the formation of aflatoxicol. The remaining fraction is absorbed in the digestive tract by passive diffusion and is hydroxylated in the liver to Aflatoxin M1 (Kuilman *et al.*, 1998). Aflatoxin M1 is either conjugated to glucuronic acid and subsequently excreted via bile, or enters the systemic circulation. Circulating Aflatoxin M1 can be excreted in the urine or appear in milk. After absorption, the highest concentration of the toxin is found in the liver. Aflatoxin B1 is metabolized by microsomal enzymes to different metabolites through hydroxylation, demethylation and epoxidation. The hydroxylation of AFB1produces AFM1 and AFQ1. Hydration of AFB1 results in the formation of AFB2 which is rapidly formed in avian species AFP1 results from O-demethylation while AFB1 epoxide is formed by epoxidation and the 2, 3-double bond. Aflatoxicol is the only metabolite of AFB1 produced by a soluble cytoplasmic reductase enzyme system. AFM1 could be detected in milk 12-24 hours after

the first AFB1 ingestion, reaching a high level after few days. When the intake of AFB1 is finished, the AFM1 in the milk decrease to undetectable levels after 72 hours (Gimeno, 2004; Ozdemir, 2007).

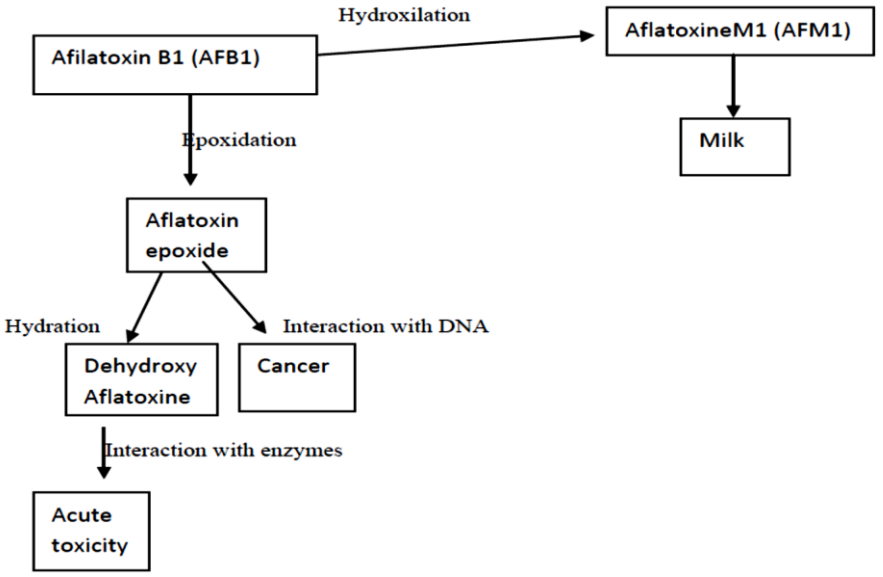

Figure 2: Some metabolic products from AFB1 (Gimeno, 2004; Ozdemir, 2007).

Aflatoxins: diagnosis

A number of methods for determination of AFM1 have been developed which can be classified as two main groups: The chromatographic methods and immunochemical methods. As a general rule, aflatoxins are low molecular weight compounds, which pose significant UV absorption and fluorescence properties. For this reason, liquid chromatographic techniques have predominated in their analysis initially TLC (Kamkar, 2005), and consequently HPLC (Rastogi, 2004).

The Immunochemical methods are used for rapid screening of aflatoxins in various samples. These techniques are based on using specific antibodies with good sensitivity. A number of immunochemical approaches enzyme-immunosorbent assay (ELISA), immunoaffinity column assays (ICA), radioimmunoassay (RIA), have been developed for the determination of AFM1 in milk. In other hand, ELISA tests however suffer from the disadvantage of false positive results, and on occasion, unacceptable quantification accuracy, therefore confirmatory analysis are required. ELISA kit is also not feasible for on-site detection because of long incubation time and numerous washing steps.

Aflatoxins: symptoms in ruminants

In Calves:

The LD50 dosage of AFB1 in calves has been estimated to be 0.5 to 1.5 mg/kg. Affected calves had anorexia, depression, and jaundice, photosensitization of pigmented skin, submandibular edema, severe keratoconjunctivitis and diarrhea with dysentery. Collapse and death followed.

In beef dairy cattle:

The signs most commonly reported with acute toxicosis in cattle include anorexia, depression, dramatic drop in milk production, weight loss, lethargy, ascitis, icterus, tenesmus, abdominal pain (animals may stretch or kick at their abdomen), bloody diarrhea, abortion, hepatoencephalopathy, photosensitization and bleeding. Hair loss are also observed in chronically exposed dairy cattle (Guthrie, 1979). Other signs associated with acute aflatoxicosis include blindness, walking in circles, ear twitching, frothy at the mouth, keratoconjunctivitis and rectal prolapse (Radostits *et al.*, 2000). High Aflatoxin levels (4 ppm) can cause milk production to drop within one week while, lower levels (0.4 ppm) can cause production drop in 3 to 4 weeks (Hutjens, 1983).

In additions, chronic aflatoxicosis may impair reproductive efficiency including abnormal estrous cycle (too short and too long) and abortions, induce immunosuppression and increase susceptibility to disease (Cassel *et al.*, 1988). The immunotoxic effect of AFB1 was expressed via the cell-mediated immune system (Raisbeck et al., 1991). Hepatic damage is a constant finding in acute aflatoxicosis. Lesions include fatty degeneration, megalocytosis and single-cell necrosis with increasing fibrosis, biliary proliferation and veno-occlusive lesions as the disease progresses (Burnside et al., 1957). Another character of Aflatoxin exposure in dairy cattle is the conversion to AFM1 in milk (Price *et al.*, 1985). Experiments have shown that milk will be free of Aflatoxin after 96 hours of feeding non-contaminated feed. The level of Aflatoxin in the feed and milk at the starting point will influence clearance time (Hutjens, 1983).

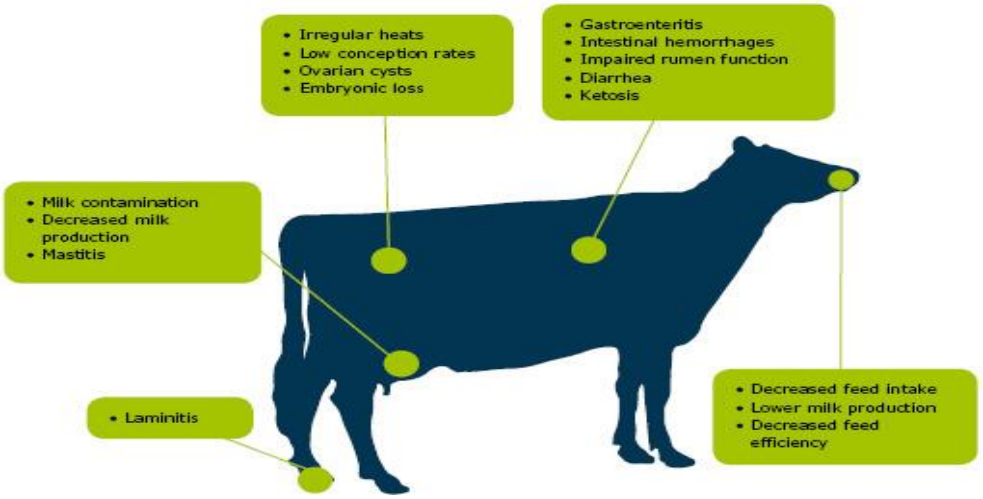

Figure 3: Aflatoxins and symptoms in ruminants (Fehr and Delag, 1970; Bodine and Mertins, 1983)

Prevention

Chemical inhibitors include one or a series of organic acids such as propionic, sorbic, benzoic and acetic acids, organic acid slats such as calcium propionate, potassium sorbate and solid or liquid copper sulphate. Also, with respect to FDA standards, use of ammonia for neutralizing Aflatoxin in livestock feed has been permitted in US. Use of different microorganisms such as *Lactobacillus pentosus* and *Lactobacillus brevis* is another way of reducing Aflatoxin in livestock feed. Mechanism of Aflatoxin B1 removal by lactic acid bacteria is not through metabolic degradation, but through binding this toxin to cellular wall of the bacteria.

Conclusion

High levels of AFM1 in milk and other dairy products are considered undesirable because it has toxic, teratogenic and carcinogenic properties. Aflatoxins are produced on livestock feed in appropriate moisture and temperature conditions for mold growth. Consumption of feed infected by Aflatoxins leads to different problems in reproductive, digestive and respiratory tracts of livestock causing infected milk production. In addition, the consumption of contaminated milk by human incurs major hygienic and pharmaceutical costs to society. Therefore, in order to prevent from introduction of Aflatoxin M1 into food industry cycle, its precursor namely Aflatoxin B1 should be controlled. To obtain this, meeting hygienic conditions, appropriate storage and control of livestock feed at all stages of production. Besides, milk products have to be controlled continuously by accurate and reliable analytical techniques for presence of AFM1 contamination.

Upcoming study in Lebanon

A study will be conducted to identify the level and types of Aflatoxin in cow raw milk specially Aflatoxin M1 produced in three different dairy regions in Bekaa Valley, Lebanon characterized by different farming types and by different microclimates over one year (4 season). Moreover, this study will be conducted under the usual and normal conditions of herds' management rather than under defined and controlled experimental conditions. Another important factor affecting the outcome of the study is the feeding guides that vary between different farmers, regions, and seasons. Some farmers follow the full TMR feeding system while many others rely on the availability of hay, forages, vegetables, and some green herbs. During winter some farmers introduce fermented corn silage to their ration which could be very dangerous if not monitored correctly. On another hand the large scale farms use it continuously with preventive measures. Small scale farms get feed from mills therefore the quality and storage time of grains is unknown leading to the possible contamination of feed. Weather is different between regions , for example winter is wet and rainy in west bekaa while it is dull and dry in north Bekaa and balanced in the cneter of the valley. The storage of feed at the farms, access of rodents and insects to storage areas plays a major role in mold formation as well. Panariti (2001) found relatively higher levels of AFM1 when cows have a diet composed mainly of stored feedstuffs rather than when the cows were at pasture. Diaz & Espitia (2006) reports that batches of contaminated milk were produced at farms using feed supplements such as corn by-products or cottonseed meal, as opposed to farms where the cows were only grazing and did not received supplemental feeds. Thirumala-Devi *et al.* (2002) analyzed milk from rural and peri-urban areas in India and found that most of the milk samples that contained high AFM1 concentrations were obtained from peri-urban areas where cows were fed with cotton cake, groundnut cake, rice bran and straw. A study published for Bognano *et al.* (2006) revealed that the contamination of samples obtained from stabulated ewes fedwith compound feed was higher than that from grazing ewes. Four studies (Ghanem & Orfi, 2009; Hassan & Kassaify, 2014; Rahimi *et al.*, 2010; Srivastava *et al.*, 2001) found a high level of AFM1 in cow milk compared with that from other animals (e.g. water buffalos, camels, sheep and goats). All these authors postulate that these low levels could be related to the fact that these species are mainly fed by grazing.

Milk contaminated with aflatoxins is produced mostly from use of infected feed. Therefore, reducing aflatoxin contamination indirectly via control of livestock feed hygiene is possible. To achieve the aim, principles and health considerations during farming and crop production in farms

and livestock feed factories, storage of livestock feed in traditional and industrial warehouses is necessary (Rasic et al., 1991)

Controlling mold growth and aflatoxin formation in traditional farms and warehouses is highly important. In this regard, several studies have been carried out on quality of livestock feed and the amount of aflatoxin in produced milk (creppy, 2002).

In addition, protecting feed from infection sources, inhibition of microorganisms' propagation in feed, alleviating infection and inhibition of reinfection are regarded as principles of controlling infection in industrial livestock feed factories.

Moreover, absorbents, chemicals, microorganisms and ionizing rays can be used to prevent mold growth and development of the molds when initial infection has been occurred (Sinha, 1998). Several researches have been conducted on using absorbents in infected livestock feed (Dakovic *et al*., 2008; Alexander *et al.*, 2001). Through binding to absorbents, aflatoxins present in feed inhibits from toxic reactions in livestock body as well as from absorption into digestive tract.

Some aflatoxin absorbents in infected feed include active carbon, alumino (clay, bentonite, montmorillonite, zeolite and phyllosilicates), complex carbohydrates (cellulose and polysaccharides present at cellular wall of yeasts and bacteria such as glucomannans, peptidoglycans), synthetic polymers such as cholestyramine and polyvinyl pyrrolidone and its derivatives. Although this method leads to good results in the laboratory conditions, the use of these substances in livestock body is different and requires time-consuming and various experiments. Livestock species, age and genus influence results of the experiments (Alexander et al., 2001).

The quantity of Aflatoxin was determined according to Enzyme-linked Immuno Sorbent Assay (ELISA) by using the RadiScreen® Aflatoxin M1 (R-biopharm, Darmstadt, Germany) test kit which is a competitive enzyme immunoassay based on antigen-antibody reaction. Special software, the RIDA®SOFT win is available to evaluate the RIDASCREEN enzyme immunoassay.

References

1. Alexander H., Stefan F., Othmar K., Hans D. (2001). *Mycotoxin detoxification of animal feed by different adsorbants.* Toxicol. Lett., 122: 179–188.
2. Bailey G.S., Price R.L., Park D.L. and Hendricks J.D. (1994). *Effect of ammoniation of aflatoxin B1-contaminated cottonseed feedstock on the aflatoxin M1 content of cows' milk and hepatocarcinogenicity in the trout bioassay.* Food and Chemical Toxicology 32, 707–715.
3. Bodine, A.B. & Mertins, D.R. (1983). *Toxicology, metabolism and physiological effects of aflatoxin in the bovine.* pp. 46–50. In: U.L. Diener; R.L. Asquith and J.W. Dickens Eds. Aflatoxin and A. flavus in corn. AAES, Auburn Univ., Alabama.
4. Bognanno M., La Fauci L., Ritieni A., Tafuri A., De Lorenzo A., Micari, P., Di Renzo L., Ciappellano S., Sarullo V. & Galvano, F. (2006). *Survey of the occurrence of Aflatoxin M1 in ovine milk by HPLC and its confirmation by MS.* Molecular Nutrition & Food Research, 50(3), 300–305.
5. Burnside, J.E.; Sipple, W.L. Forgacs, J, Carll, W.T, Atwood, M.B. & Doll, E.R. (1957). *A disease of swine and cattle caused by eating moldy corn. II. Experimental production with pure cultures of molds.* Am. J. Vet. Res, Vol.18, p. 817.
6. Cassel, E.K.; Barao, S.M. and Carmal, D.K. (1988). *Aflatoxicosis and ruminants. Texas Vet. Med. Diagnostic lab, Tesas College.* The national dairy database (1992) NDB, Health, Test, and Hf100200.
7. CCMMP8 *Codex Committee on Milk and Milk Products* 04/02/2008 – 08/02/2008 | Queenstown, New Zealand.

8. Codex Alimentarius (2008). *Codex General Standard for contaminants and toxins in food and feed.Codex* Stan 193–1995. p. 9.
9. Creppy EE. (2002). *Update of survey, regulation and toxic effects of mycotoxins in Europe*. Toxicol. Lett. 127: 19–28.
10. Dakovic A., Matijeasevic S., Rottinghaus G.E., Ledoux D.R., Bukeraitis P., Sekulic Z. (2008). *Aflatoxin B1 adsorption by natural and copper modified montmorillonite, Colloids and Surf, B*., 66: 20–25.
11. Diaz, G. J. & Espitia, E. (2006). *Occurrence of aflatoxin M1 in retail milk samples from Bogota, Colombia.* Food Additives and Contaminants, 23(8), 811–815.
12. Diener, U.L. & Davis, N.D. (1968): *Effect of environment on aflatoxin production in peanuts.* Tropical science, Vol.10, pp. 22–25.
13. EFSA (2007). *The European Food Safety Authority, opinion of the scientific panel on contaminants in the food chain related to the potential increase of consumer health risk by a possible increase of the existing maximum levels for aflatoxins in almonds, hazelnuts and pistachios and derived products.* Question number: EFSA-Q-2006-174.
14. Elkak A., Mohamad A., Oula E. (2011). *A survey on the occurrence of aflatoxin M1 in raw and processed milk samples marketed in Lebanon*. Food Control, 22 (12):1856–1858.
15. Fehr, P.M. & Delage, J. (1970). *Effect de l'Aflatoxine sur les fermentations du rumen.* Can. Nutr. Diet, Vol.5, pp. 59–61.
16. Ghanem, I. & Orfi, M. (2009). *Aflatoxin M1 in raw, pasteurized and powdered milk available in the Syrian market.* Food Control, 20(6), 603–605.
17. Gimeno A. (2004). *Aflatoxina M1 no leite. Riscos para a saúde pública, prevenção e controlo. Alimentação Animal (Revista de la Associação Portuguesa dos Industriais de Alimentos Compostos para Animais* (IACA)), 49:32–44.
18. Gourama H, Bullerman L. (1995). *Aspergillus flavus and Aspergillus parasiticus: Aflatoxigenic Fungi of Concern in Foods and Feeds: a Review*. J Food Prot; 58: 1395–04.
19. Guthrie, L.D. (1979). *Effect of aflatoxin in corn on production and reproduction in dairy cattle*. J. Dairy Sci, Vol.62, p. 134.
20. Hesseltine, C.W. (1983). *Introduction, definition and history of mycotoxins of importance to animal production. In: Interactions of mycotoxins in animal production*. National Academy of Science, Washington, DC.
21. Hjartaker A, Lagious A, Slimani N, Lund E, Chirlaque MD, Vasilopoulou E. (2002). *Prospective investigation into cancer and nutrition (EPIC) cohort.* Public Health Nutr. 5: 1259–1271.
22. Hutjens, M. (1983). *Aflatoxin contaminated feed and dairy. In: J. Jacobsen, Aflatoxin 83.* Texas Vet. Med. Diagnostic Lab., Texas collage.
23. IARC, (2002). *Monographs on the evaluation of carcinogenic risks to human. Some traditional herbal medicine, some mycotoxins, naphthalene and styrene*; No. 82. IARC, Lyon, France.
24. JECFA (1999). *Forty-ninth meeting of the Joint FAO/WHO Expert Committee on Food Additives.* WHO Technical Report Series, No. 884.
25. JECFA (2007). *Sixty-eighth report of the Joint FAO/WHO Expert Committee on Food Additives.* WHO TechnicalReport Series, No. 947.
26. Joint FAO/WHO Expert Committee on Food Additives (53rd: 1999: Rome, Italy) & World Health Organization. (2000). *Evaluation of certain food additives and contaminants: fifty-third report of the Joint FAO/WHO Expert Committee on Food Additives. World Health Organization.*

27. Kamkar A. (2005). *A study on the occurrence of aflatoxin M1 in raw milk produced in Sarab city of Iran*. Food Control 16: 593–599.

28. Kuilman, M. E. M., R. F. M. Maas, D. J. Judah and J. Gremmels. (1998). *Bovine hepatic metabolism of aflatoxin B1*. J. Agric. Food. Chem. 46:2707–2713.

29. Lillehoj, E.B. (1983). *Effect of environmental and cultural factors of aflatoxin contamination of developing corn kernels.* In: V.L. Diener, R.L. Asquith and J.W. Dickens (Eds). Aflatoxin and A. flavus in corn. Southern Coop Serv. Bull. 279, Craftmaster, Opelika, Ala, 112 p.

30. Lopez C.E., Ramos L.L., Ramadán S.S. and Bulaciol C. (2003). *Presence of aflatoxin M1 in milk for human consumption*.

31. *Negash D*. (*2018*). *A Review of Aflatoxin: Occurrence, Prevention, and Gaps in Both Food and Feed Safety*. J Appl Microb Res Vol: 1, Issu: 1 (35–43).

32. Ozdemir, M. (2007). *Determination of aflatoxin M1 levels in goat milk consumed in Kilis province.* Ankara Üniversitesi Veteriner Fakültesi Dergisi.

33. Panariti, E. (2001). *Seasonal variations of aflatoxin M1 in the farm milk in Albania.* Arh Hig Rada Toksikol, *52*, 37–41.

34. Price, R.L.; Paulson, J.H. Lough, O.E. Gingg, C. & Kurtz, A.G. (1985). *Aflatoxin conversion by dairy cattle consuming naturally contaminated whole cottonseed*. J. Fd. Prot, Vol.48, pp. 11–15.

35. Radostits, O.M.; Gay, C.C. Blood, D.C. & Hinchcliff, K.W. (2000). *Veterinary medicine*, pp.1684-1688, W.B. Saunders Co. Ltd., London.

36. Rahimi, E., Bonyadian, M., Rafei, M., Kazemeini, H.R. (2010). *Occurrence of aflatoxin M1 in raw milk of five dairy species in Ahvaz, Iran.* Food Chem Toxicol. 48(1), 129–131.

37. Raisbeck, M.F.; Rottinghaus, G.E. & Kendall, J.D. (1991). *Effects of naturally occurring mycotoxins on ruminants*. pp. 647–677.

38. Royes, J.B. & Yanong, R.P. (2002). *Molds in fish feeds and aflatoxicosis.* Copyright by the University of Florida, Institute of Agric. Sci. (UF/ IFAS).

39. Ruangwises, S.; Ruangwises, N. (2009). *Occurrence of Aflatoxin M-1 in Pasteurized Milk of the School Milk Project in Thailand*. J. Food Prot, *72*, 1761–1763.

40. S. Rastogi, P.D. Dwivedi, S.K. Khanna, M. Das. (2004). *Detection of Aflatoxin M_1 contamination in milk and infant milk products from Indian markets by ELISA*. Food Contr., 15, pp. 287–290.

41. Sinha K.K. (1998). *Detoxification of mycotoxins and food safety. In Sinha K.K., Bhatnagar D (Eds). Mycotoxins in Agriculture and Food Safety*, New York, Marcel-Dekker, pp. 381–406.

42. Srivastava, V. P., Bu-Abbas, A., Alaa-Basuny, Al-Johar, W., Al-Mufti, S. & Siddiqui, M. K. (2001). *Aflatoxin M1 contamination in commercial samples of milk and dairy products in Kuwait.* Food Additives and Contaminants, 18(11), 993–997.

43. Thirumala-Devi, K., Mayo, M. A., Hall, A. J., Craufurd, P. Q., Wheeler, T. R., Waliyar, F., Subrahmanyam, A. & Reddy, D. V. (2002). *Development and application of an indirect competitive enzyme-linked immunoassay for aflatoxin m(1) in milk and milkbased confectionery.* Journal of Agricultural and Food Chemistry, 50(4), 933–937.

44. Van Egmond H. P. (1999). *Worldwide Regulations for Mycotoxins*. Working Document Third Joint FAO/WHO/UNEP International Conference on Mycotoxins. *MYC-CONF/99/8a*, Tunis Tunisia, March, 3–61999.

45. Van Egmond HP, Jonker MA. (2004). *Worldwide regulations on aflatoxins—the situation in 2002.* J Toxicol Toxin Rev.*;* 23(2&3):273–293.

46. Verma, D. (2007). *New facts about mycotoxins control-intensive research in the field of mycotoxins deactivation gives new insights*! Biomin India.

47. WHO, World Health Organization (1979). *Environmental Health Criteria, Safety evaluation of certain food additives.* pp. 1–12.

48. Wu F, Liu Y, Bhatnagar D. (2008). *Cost-effectiveness of aflatoxin control methods: economic incentives.* Informa Healthcare. 2008; 27(3-4):203–225.

49. Wu F. (2004). *Mycotoxin risk assessment for the purpose of setting international regulatory standards*. Environ Sci Tech.*;* 38:4049–4055.

50. Wu F. (2006). *Mycotoxins reduction in BT corn: potential economic, health, and regulatory impacts.* Transgenic Res. 15 277–289. 10.1007/s11248-005-5237-1.

51. Wyatt, R.D. (1991). *Poultry*, pp. 553–578, In: Smith, J.E. and Henderson, R.S. (1991).

52. Zinedine A., Mañes J. (2009). *Occurrence and legislation of mycotoxins in food and feed from Morocco.* Food Control. 20:334–344.