Strategies for decreasing aflatoxin in livestock feed and milk

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ABSTRACT: Aflatoxin B1 is a fungus secondary metabolite produced by Aspergillus flavus in infected livestock feed and transferred to livestock body through consumption of the infected feed. Aflatoxin B1 is converted to aflatoxin M1 in livestock body and exits through infected milk. Both the aflatoxins are carcinogenic compounds. In this overview, preventive efforts in decreasing aflatoxin in livestock feed and milk are reviewed which are carried out either indirectly in livestock feed or directly in milk. Indirect methods include controlling aflatoxin infection in agricultural farms, livestock feed factories and the feed warehouses through some methodologies and direct methods include decreasing aflatoxin with the use of absorbents, chemicals and biological methods in milk.

Keywords: Aflatoxin, Hygiene, Livestock feed, Milk

INTRODUCTION

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). 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). Aflatoxins, a group of different species of mold mycotoxins, are readily produced during the molds growth and storage of livestock feed. Regarding that most of agricultural products used for preparing feed is predisposed to infection by molds capable of producing toxic metabolites such as aflatoxin, investigation of factors influencing their production, propagation and control is necessary. Aspergillus molds grow readily in forages, grains such as corn, barley, wheat and rice as well as oil seeds such as soy, cotton and sunflower during harvest, processing, transportation and storage (Alcroft and Carnaghan, 1962; Bryden, 2012).

Seventeen strains of aflatoxin have been found in nature which is produced by different species such as Aspergillus flavus, Aspergillus ochraceus and Aspergillus glaucus (Reddy et al., 2010). Aflatoxins B1, B2, G1 and G2 are the most important species produced naturally. Consumption of livestock feed infected by aflatoxin B1 leads to hydroxylation of B1 toxins and their conversion to M1 in milk. Figure 1 shows chemical structure of aflatoxin B1 and aflatoxin M1. Aflatoxin M1 is oxidative metabolite of aflatoxin B1 which is produced in livestock body through activities of hepatic microsomal enzymes and excreted through milk, urine and stool (Barbieri et al., 1994). Most studies have shown that aflatoxin B1 present in livestock feed is converted to aflatoxin M1 at 1 to 4% (Stoloff, 1980).

Effects of aflatoxin on human and livestock health

Aflatoxin B1 present at livestock feed causes different problems in genital, digestive and respiratory tracts through different mechanisms such as interference in metabolism of carbohydrates, fats and nucleic acids. Effects of aflatoxin B1 on livestock vary with concentration and time duration of contact with the toxin, strain and feed. High concentrations of this toxin are lethal, medial concentrations lead to chronic poisoning and continuous exposure to low concentration can result in hepatic cancer (Deshpande, 2002). Since about one
fifteenth of consumed aflatoxin B1 is introduced into milk as aflatoxin M1 and different heat treatments used in preparing various dairy products cannot reduce quantity of aflatoxin M1, there is always a probability of poisoning by this toxin when consuming infected milk. Tumorigenesis and mutagenesis capability of aflatoxin M1 is less than aflatoxin B1 (Creppy, 2002).

![Chemical Structure of Aflatoxin B1 and Aflatoxin M1](image)

**figure 1. Chemical Structure of Aflatoxin B1 and Aflatoxin M1 (Patterson and Allcroft, 1970)**

**Permitted levels of aflatoxin**

Some countries have set permitted levels of aflatoxins in food in order to control and reduce detrimental effects of these toxins. These levels are variable and depend on economic and developing status of the countries (Galvano et al., 1996). In US, Food and Drug Administration (FDA) has permitted a total amount of 20 ng/g in livestock feed and 0.5μg/kg or 50 ng/l in milk (Ellis et al., 1995). In European countries, permitted levels of aflatoxin M1 in milk, milk products and baby food are 0.005 mg/kg (Creppy, 2002). Also, different countries have set different regulations for permitted levels of aflatoxin in livestock feed. For instance, European Union (EU) has set permitted levels of aflatoxin from 0.05 to 0.5 μg/kg. Factors such as weather conditions are also effective in determining permitted levels of aflatoxin. Permitted levels of this toxin in tropical countries are higher compared to mild and cold countries (Van Egmond, 1989).

**Losses due to aflatoxin infection**

In addition to financial losses and economic damage to agricultural and animal husbandry industries, losses due to aflatoxin contamination of foods include major pharmaceutical and health costs to treat food poisoning. Based on Food and Agriculture Organization (FAO) reports, annually, about 20% of the foods produced in the world are contaminated by mycotoxins; in which aflatoxins have a greater share than the others. Prevalence of cancer and livestock disease in farms, weakening of livestock immune system, reduction in milk production and productivity are a few examples of damages to food and livestock industry. Considering huge economic losses and public health protection, prevention and neutralization of the toxins in livestock feed and food products of animal origin such as milk is essential (Miller and Casman, 2004; Miličević et al., 2010).

**Indirect methods of aflatoxin reduction in livestock feed**

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 (Cleveland et al., 2003). Livestock feed is mostly include corn, cotton seed and canola, dietary supplements, wheat bran, dried bread, fat powder and alfalfa. Given that a significant percentage of the feed composition is derived from crops, health consideration during planting, harvesting and storage of crops are factors affecting grain quality. Drought, rainfall, infection by insects and high moisture during flowering can be referred as major causes of aflatoxin occurrence in farms. Adherence to appropriate irrigation programs, planting varieties resistant to moisture and molds, weed control, insecticides application, harvesting at appropriate time, crop rotation in order to reduce the risk of pathogen transfer from current farming year to the next year and fertilizing soil are useful ways of preventing pre-harvest aflatoxin infection. In addition, appropriate storage of crops which includes placing crops on clean and dry surfaces, protecting crops from moisture, heat, insects and use of fungicides are effective ways of reducing the infection (Wu et al., 2008). Adherence to hygienic conditions in factories and livestock feed warehouses both in traditional and industrial levels is another important way of controlling and reducing aflatoxin infection.

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). For example, it has been shown that the amount of aflatoxin in milk produced in autumn and winter is higher compared to spring and summer (Panariti, 2001). This is because in cold seasons, feeding livestock on fresh forages is not possible due to unfavorable weather conditions and farmers have to
use stored forages. Regarding that warehouse improper temperature and moisture conditions favor mold growth; therefore, it is necessary to improve storage conditions of livestock feed (Panariti, 2001). Results in some countries have shown that meeting safety conditions of livestock feed has led to decrease aflatoxin infection (Akande, 2006).

In addition, protecting feed from infection sources, inhibition of microorganisms’ propagation in feed, alleviating infection and inhibition of reinfestation are regarded as principles of controlling infection in industrial livestock feed factories. To obtain these ends, continuous and careful monitoring of different procedures of storing raw materials and production, controlling moisture content and temperature by ventilation systems, use of clean and hygienic facilities such as mills and mixers and avoiding from broken or damaged grains when preparing feed are necessary (Degirmencioglu et al., 2005).

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 (Alexander et al., 2001; Dakovic et al., 2008). Use of aflatoxin absorbents in infected feed is a promising way of reducing this infection in livestock feed. Through binding to absorbents, aflatoxins present in feed inhibits from toxic reactions in livestock body as well as from absorption into digestive tract. Properties of the absorbents when choosing them to hinder the aflatoxin infection should include capability of toxicity inhibition in livestock feed, not having detrimental effects on livestock health and being economic (Dakovic et al., 2008). Studies on application of aflatoxin absorbents have been conducted for years. Some aflatoxin absorbents in infected feed include active carbon, aluminio (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 cholesteryamine and polyvinyl pyrolidone 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; Dakovic et al., 2008).

Furthermore, 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 sulfate (Sinha, 1998). Also, with respect to FDA standards, use of ammonia for neutralizing aflatoxin in livestock feed has been permitted in US. 95% of aflatoxin in feed has been alleviated with the aid of gaseous or liquid ammonia (Prudente and King, 2005). When applying ammonia, if conditions such as time of use, temperature and concentration are met, reduction of aflatoxin infection is carried out more effectively. Ammonia alleviates toxicity of aflatoxin B1 and converts it to non-toxic compound of aflatoxin D1 through hydrolyzation of aflatoxin B1 and its decarboxylation (Prudente and King, 2005).

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. However, some researchers have attributed removal property of aflatoxin by the aforesaid bacteria to their ability in producing radicals, thus creating conditions required for decomposition of aflatoxins. In dry conditions, aflatoxin B1 shows high resistance to detrimental effects of gamma ray (Dakovic et al., 2008).

Direct methods of aflatoxin reduction in milk

Toxin absorbents, chemical and biological methods are also used directly for reducing aflatoxin in milk (Masimango et al., 1978; Sarr et al., 1990; Applebaum and Marth, 1982a; Applebaum and Marth, 1982b; Bovo et al., 2012). Use of toxin absorbents is one of the main methods to reduce aflatoxin amount in milk. Absorbent soils such as bentonite, vermiculite, hydrated sodium calcium aluminosilicate (HSCAS) and active carbon have been known as absorbent compounds for absorbing various toxins in aqueous environments (Masimango et al., 1978; Applebaum and Marth, 1982a). For instance, bentonite has been known as an effective reducer of aflatoxin M1 in infected milk (Applebaum and Marth, 1982a). Binding capacity and stability of compounds formed between absorbent and toxin are highly variable and influenced by temperature and pH. Information about the effect of absorbents on milk constituents is scarce; however, it has been shown that these substances have slight effect on nutritional quality of milk (Ellis et al., 1995). In a study, effect of bentonite on milk protein content was not considerable and maximum reduction in protein content was only 5% (Applebaum and Marth, 1982a). It should be mentioned that with respect to acceptability of absorbents as healthy additives by international authorities and ability to separate them from milk after absorption of aflatoxin, further investigations are in progress.

In addition to absorbents, chemicals such as hydrogen peroxide used for storage of some food products have reduced aflatoxin in milk at different conditions of time and concentration (Applebaum and Marth, 1982b).
Use of hydrogen peroxide combined with other additives like riboflavin and lactoperoxidase as well as hydrogen peroxide application along with heat treatment have led to more satisfactory results in reducing aflatoxin in milk. In addition, potassium sulfite has been used for neutralizing aflatoxin M1 in milk (Applebaum and Marth, 1982b).

Biological methods as inexpensive and easy techniques of reducing aflatoxin have also been of interest. Therefore, widespread studies on identifying effective microorganisms are being conducted (Elgerbi et al., 2006; Wu et al., 2009; Bovo et al., 2012). For instance, in a study, Flavobacterium aurantiacum which is gram-negative, aerobic, bacilli like and non-sporogenous bacterium has been used for reducing aflatoxin in milk (Line et al., 1994). The bacterium can use aflatoxin present in milk as a substrate and convert it to non-toxic substances. However, formation of proteolytic and lipolytic enzymes by this microorganism leads to undesirable changes in milk flavor (Line et al., 1994).

**Sampling condition**

Sampling and preparing samples suspected to aflatoxin is very important. Inappropriate sampling may lead to incorrect detection of aflatoxin in infected livestock feed or milk. All experimental methods include derivation, purification and quantification of toxin. Liquid Chromatography (LC) and Thin Layer Chromatography (TLC) are two common laboratory methods for identification of aflatoxin in the food products. Regarding that these methods are time-consuming and that impurities may affect experimental results, new methods based on specific antibodies are used today. These methods include Immunoassay, Islet Cell Antibody (ICA) and Enzyme-Linked Immuno Sorbent Assay (ELISA) which are based on composition of aflatoxin and antibodies (Kim et al., 2000; Rodriguez Velasco et al., 2003; Manetta et al., 2005; Gurbay et al., 2006).

**CONCLUSION**

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. Consumption of infected 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 planting, growing, harvesting, producing and storing are necessary.

**REFERENCES**


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