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Effect of the use of mycotoxin-adsorbing agents in cow's food, on the reduction of Aflatoxin M in milk

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Abstract
Aflatoxins are a major threat to human health because it causes mutations in DNA and weakens the immune response. The body is inserted through the food of animal origin (milk and dairy products) and plant origin and inhalation of contaminated air. There are 4 types of aflatoxins (B-1, B-2, G-1, G-2), of which the most dangerous is B-1, which is metabolized in the liver to the carcinogen metabolite M-1. Aflatoxins are heat stable and are not eliminated by pasteurization. Because of these characteristics, regular checkups are necessary in order to prevent contamination of raw materials and food.
The specific objective of the analysis was to confirm the usefulness of the mycotoxin absorbing agents, as food supplements in dairy cows, in minimization of aflatoxin levels in raw milk.
For that purpose, all bulk tank milk from different lines in Pelagonia region were tested using ELISA method. When the first test showed data suggesting the presence of contamination with aflatoxins, the second check for individual farmers was done and with the proven contamination, the addition of Mycosorb, as a mycotoxin absorbing agents, to the feed was recommended to reduce the amount of aflatoxins.
The data were collected for 3 consecutive years, from Pelagonia region. The contamination of the bulk tank milk during the years was distributed unequally, with the greater predominance of the autumn and winter, when more corn silage was used as an animal feed. The maximum contamination was observed during 2013 (November) of 1.03 $\mu g.kg^{-1}$, and for the rest of the time, the highest contamination level for specific sectors was around 0.6$\mu g.kg^{-1}$. After the use of Mycosorb, the level of Aflatoxin M in the milk was reduced below the acceptable limit of 0.05 $\mu g.kg^{-1}$.
According to the results obtained during this study, we can conclude that the use of Mycosorb can significantly help in regulation of the level of aflatoxin M1 in raw milk without impairing the quality and safety of milk as a raw material in the dairy industry.

Key words: aflatoxins, milk and dairy products, Mycosorb

Introduction

Human health begins with the good nutrition. The diet can protect the human body against illnesses throughout the life, but food can detriment human health too. Therefore, the challenge for food producers as well as legislators, responsible for the proper protection of consumers is the production of safe food.

Mycotoxins are prevalent in many countries, especially in tropical and subtropical areas, and are a potentially major problem for food safety. In these areas, the conditions, such as temperature, and moisture, are optimal for the development of molds, which are producers of mycotoxins. Thus, mycotoxins can be found in agricultural products, in animal feed, and therefore in meat and meat products and in milk and milk products.

Mycotoxins are compounds with low molecular weight that are synthesized during the metabolism of filamentous fungi.

Mycotoxins are natural contaminants in raw materials, food, and gases. Mycotoxin-producing molds are very common and grow under a very wide range of environmental conditions. They appear in agricultural products around the world (Bennet & Klich, 2003). Many mycotoxins can be toxic to vertebrates and other animal groups, and even in low concentrations, some of which may cause autoimmune diseases, have allergic properties, others are teratogenic, carcinogenic, mutagenic (Bennet & Klich, 2003; Council for Agricultural Science and Technology, 2003).

Most mycotoxins are relatively thermostable within conventional food processing with a temperature range (80-121°C), so they have little or no destruction under cooking conditions, such as cooking and frying, even after pasteurization (Milicevic et al., 2010).

At present, more than 400 mycotoxins are known. Scientists focus mainly on those mycotoxins that have been toxic/carcinogenic to animals and humans and are the most important in agriculture and the food industry. Even today, the contamination of food with mycotoxins remains a worldwide problem. According to the United Nations Food and Agriculture Organization (FAO), it is estimated that about 25% of the world's crops are significantly contaminated with mycotoxins. The presence of mycotoxins in food is different from country to country and depends on different types of seeds, agricultural practices and climatic conditions (Bryden, 2007). When applying good agricultural practice, mycotoxins are an inevitable food contaminant. The transfer of different seeds internationally contributes to the mycotoxins becoming a worldwide problem of food contamination.

Milk is one of the basic foodstuffs, which is used in everyday life, especially in children. His biochemical complex, as well as the amount of protein, is ideal for satisfying human needs for amino acids. Studies show that there is a close relationship between milk consumption and people's health in terms of efficiency, IQ, reducing the risk of infectious diseases, regulating metabolic activities, lowering blood pressure, increasing useful lipid levels (high-density lipoprotein), prevents bowel cancer and osteoporosis (Hjartaker et al., 2002). This is also the reason for the growing use of milk and dairy products in people's daily diet.

Due to the close relationship between feeding to the health and safety of milk, various researches have been carried out on the feed. Studies have shown that feed contamination with certain types of molds such as Aspergillus causes the production of aflatoxin that is transmitted to milk (Creppy, 2002).

The aim of the study

The aim of our study was to determine the effect of the addition of mycotoxin-adsorbing agents in cow’s feed, on the reduction of Aflatoxin M in milk produced from the same cows.

Material and Methods

Experimental design

Examinations were done during 3 year period (from March 2013 to March 2016) on 5 groups of cow's milk from the tanks from each collection line of the dairy plant. Primarily, the quantity of aflatoxin M1 per tank was tested.

According to the preliminary obtained results per tank, more detailed examination through a software program that confirms or removes the suspicion of existence aflatoxins was conducted. If this program, proved that in a certain bulk tank, the presence of aflatoxins really occurs, then additional tests were done to discover from which segment of the tank infected milk comes, which is possible because each milk tank is divided into three segments in which each of them transports milk from distinct regions, separate villages, each of which is marked with its own recognition code and within which each farmer has its own identification number.

Additionally, the presence of aflatoxin M1 in milk per farmer, as well as the presence of aflatoxin B1 in animal feed was examined.

Analysis

Sample preparation

Samples were prepared according to the rules of the commission regulation (EC) No 401/2006, where minimum 3-5 samples per cistern with minimum 1-liter milk were taken.

The lots were thoroughly mixed by mechanical means immediately prior to sampling. In this case, a homogeneous distribution of aflatoxin M1 is assumed within a given lot.

Aflatoxin detection

The presence of aflatoxins in milk and feed was determined by the enzyme-immunosorbent method or ELISA performed on microtiter plates. ELISA is a quantitative test that uses an antibody to change the color to identify the desired substance. ELISA is a popular type of analytical biochemistry test using an enzyme immunoassay (EIA) to detect the presence of a substance, usually an antigen, in a liquid sample.

Mycotoxin-binding additives

All farmers with the elevated concentration of aflatoxin M1 in milk (more than 0.05μg.kg^{-1}) were advised to use mycotoxin-adsorbing agents (Mycosorb®) according to the feeding route prescribed by the producer, and after a period of at least five days, its effect on the amount of excreted aflatoxin in the milk, was investigated. Feeding route for Mycosorb was 30g/cow/day for the first 2 weeks followed by 10g/cow/day as maintenance for the next period while the farmers are using feed susceptible to molds.

Results and Discussion

Aflatoxin detection

From the obtained results of the percentage of bulk tank milk suspicious and confirmed for being contaminated with aflatoxins, it is obvious that every year the number of potentially contaminated cisterns is decreasing, starting from a total of 34 potentially contaminated tanks in 2013, which was 11% of the total number of cisterns arrived during this period of ten months followed by 33 tanks in 2014, i.e. 9%, then totally 30 tanks in 2015, representing 8%, to only 3 tanks in the first three months of 2016, which was about 3% of the total received cisterns in the dairy plant for this time of year (Fig. 1).

During the examination period, we found that the potentially contaminated milk with the Aflatoxin M1 in bulk tanks are more frequently present during the winter period, opposing to the summer periods, where we almost didn't have any contaminated tank (Fig. 2). The reason for this type of contamination distribution is due to the type of feed the cows are using within the different seasons. Namely, during the winter period, milking cows are fed on silage and stored food that is susceptible to contamination, compared to the summer months where they are fed more fresh foods.

Mycotoxin-binding additives

After confirmation of the contamination, and using of the Mycosorb, as a feed additive, for at least five days, the second examination was performed. Obtained results are presented in Fig. 3, as a representative of the examinations during 2013. From the results it can be determined that the highest contamination of aflatoxin M1 in the milk was in November where the amount of aflatoxins was 1.003μg.kg^{-1}, (which is well above the permitted limit of 0.05 μg.kg^{-1}) which, after treatment with Mycosorb, returned below the allowed limit of aflatoxins, while the highest contamination with aflatoxins after treatment with Mycosorb was found in October (0.016μg.kg^{-1}), which is still well below the permitted limit. The similar results with Mycosorb treatment were found during the next years as well.

Conclusions

From the results obtained during the period of examination, it can be concluded that the more restrictive regulations of the dairy plant, were followed by reduction of the number of contaminated milk cisterns received in the dairy plant.

The most susceptible period for aflatoxin contamination of the milk is winter period due to the nature of feed that is used.

Addition of the mycotoxin-adsorbing agents (Mycosorb®) to the cow's feed resulted in significant reduction of the concentration of the aflatoxin M in the milk, which was well below the permitted limit.

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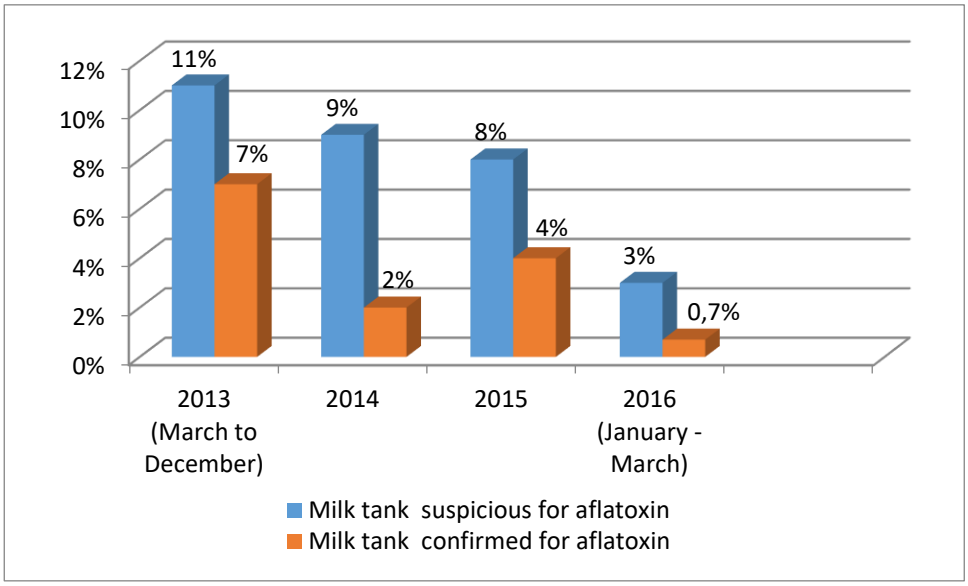

Figure 1: Percentage of suspicious and true positive bulk tank milk for aflatoxin M1.

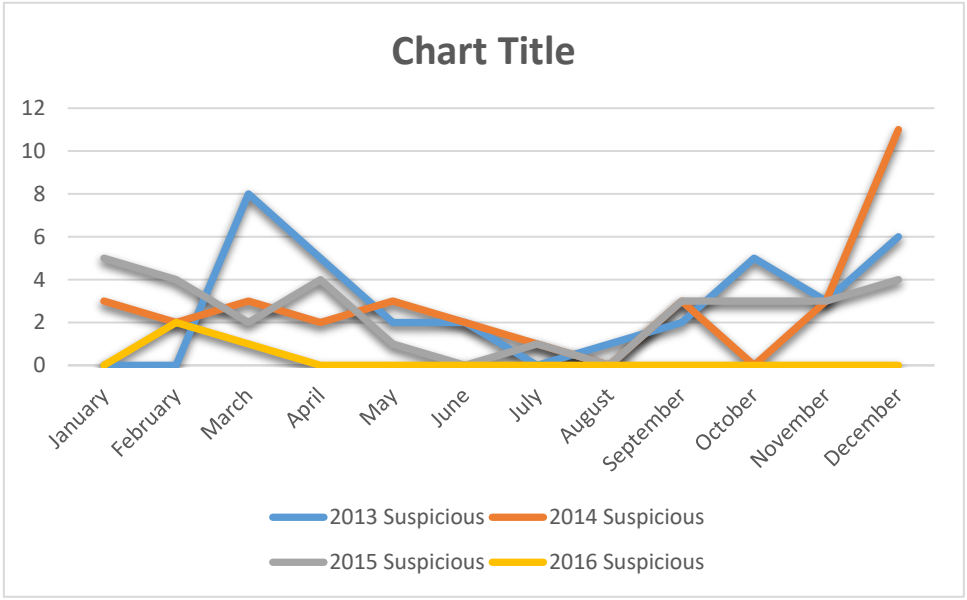

Figure 2: Distribution of potentially contaminated bulk tanks within the 3 year period**.**

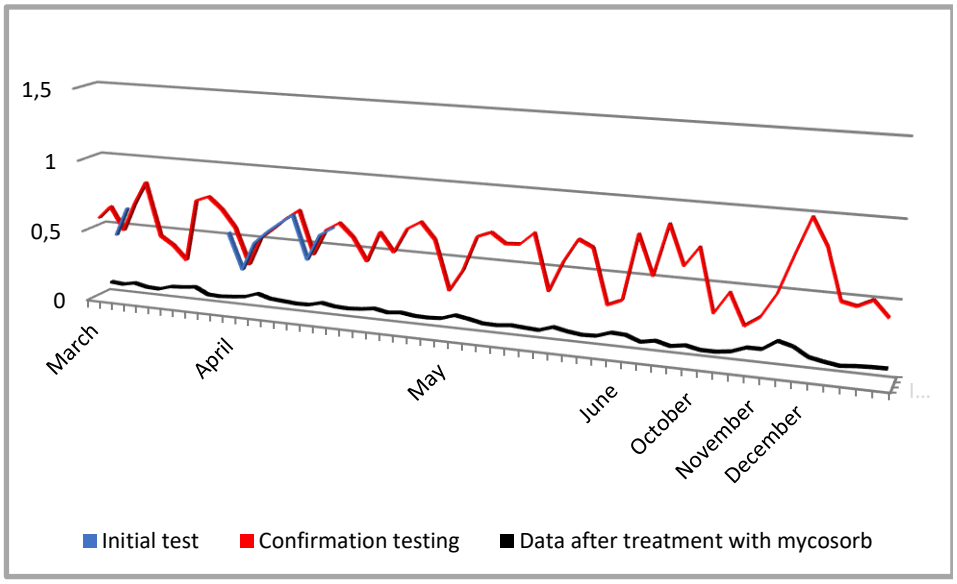

Figure 3: Presence of Aflatoxins before and after treatment with Mycosorb® - 2013.