Determination of aflatoxin M1 in raw milk by HPLC marker as evidence of cattle-food storage conditions from the herd suppliers of a dairy company in the city of Valledupar.

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ABSTRACT

The production of mycotoxins in human or animal food represents a potential danger for health and milk production. Colombia is a country with large agricultural and livestock activities, and these areas are seriously affected by the presence of aflatoxins. The contamination by aflatoxin M1 in milk and its derivatives varies between 40% and 60% and is given by the presence of aflatoxin B1 in food and feed commodities for consumption of such cattle, which has become a problem with global significance. To contribute to the monitoring of these mycotoxins in Colombia, a study on thirty herd providers from a dairy company was carried out in the city of Valledupar to determine the concentration of aflatoxin M1 in raw milk by HPLC as evident marker of cattle-food storage conditions. First, a health inspection report of storage area was made; then, direct seeding of food stored in PDA agar was made; afterwards, the concentration of aflatoxin M1 was determined by high pressure liquid chromatography (HPLC) in 30 different milk samples obtained from suppliers of the dairy company under study. Inspection of food storage site gave as a result that, in 100% of the herds, there could be contamination of food with clearly identified hazards. Both yeast and mycelial growth were obtained from direct seeding; mycelial growth was consistent with the producer of aflatoxin B1 fungus Aspergillus flavus. In the determination of AFM1 concentrations in raw milk, samples yielded results that found concentrations of aflatoxin M1 in the raw milk of 30 herds within the levels allowed by ICONTEC. The threshold obtained was 160.2 ng/L followed by 86.9 ng/L. These, according to this study, would be the maximum limits to which consumers would be exposed as the other values were lower. According to the results of this study it is recommended to implement the determination of aflatoxin M1 in raw milk produced in the department of Cesar, in order to verify the real situation regarding this toxin and thus exercise control and surveillance measures which ensure the welfare of the consumer population.

Key words: Aflatoxin M1, HPLC, raw milk, Aspergillus flavus.

INTRODUCTION

Polluted milk and dairy products with aflatoxin M1 varies between 40% and 60% and is given by the presence of aflatoxin B1 in food and raw materials used for the manufacture of feed which are intended for consumption by cattle, what has become an issue with sufficient global significance. There is a lot of evidence indicating that chronic exposure to these toxins induces the production of cancer cells, making it a public health complications, especially when it says that 20 to 50% of all cancers are related to factors diet. This toxin along with hepatitis B are considered as risk factors of HCC in China and North Africa, an estimated 250,000 deaths annually. According to FAO (Food and Agriculture Organization 2001), over 25% of world production of cereals and raw materials for human and animal consumption, are contaminated with some kind of toxin of fungal origin and between 25 and 40% of them cereals are contaminated with various types of mycotoxins [1]. In the United States has been estimated that economic losses caused by mycotoxins such as aflatoxin and fumonisin, are approximately 932 million dollars each year (Bhatnagar et al, 2003). The production of mycotoxins in human food or animal is a potential hazard to health and production. Colombia is a country with extensive agriculture and livestock, and these areas are seriously affected by the presence of aflatoxins. For example in cattle, an animal patient is a malnourished animal, usually under weight and height, low milk production and lack of appetite, low reproductive capacity while naming the difficulty of milk
exports in those countries demanding in terms of maximum [2]. It is interesting that the evidence to quantify aflatoxin in Colombia do not become routine for humans, while these tests are obtained with relative ease for food control animals without neglecting that this country lacks a serious and ongoing program monitoring of contamination by fungi and mycotoxins in raw materials. Colombia has been reported the presence of AFB1 in foods of both animal and human consumption, with an incidence of 29. % Contamination in food for human consumption was found a lower incidence of 8.9% the previous higher levels than those found in animal feed [3]. The ceiling is AFM1 400ng / L (voluntary standard recommended by the Colombian Institute of Technical Standards "ICONTEC). It is based on studies in other countries and regulations established by external agencies as there are no studies of incidence and contamination levels of aflatoxin that such limits to establish a more consistent approach to the local situation. As Valledupar no figures for economic loss, or rejection by the processing companies have not implemented for aflatoxin analysis in laboratories both for raw milk as food for livestock consumption, the lots are released be sold without control, this particular behavior becomes a limiting factor for future exports [4]. For the experiment, few studies on the presence of aflatoxins have been carried out in the country, making it difficult to determine the role of mycotoxins in terms of public health concerns, since there are no data on its occurrence natural. For that reason we must conduct monitoring to determine not only the incidence of mycotoxins but also the level of contamination in stored food for the consumption of beef, for dairy firms to ensure optimum quality of products sold analytical techniques using highly reliable and sensitive issues such as the High Resolution Liquid Chromatography. The effect it has on dairy cows consume feed contaminated with aflatoxin B1 not only reduces the yield in milk production and affect animal health but also leads to the risk of contamination of milk with aflatoxin M1. Although a very indicative stipulates that aflatoxin M1 residues in milk may occur account for 1 to 2% (1.7% on average) level of aflatoxin B1 in the diet. However, there are many factors that can interfere with or alter in some ways this relationship, without forgetting that the foods most susceptible to fungal contamination and consequent production of aflatoxins are grains and cereals (peanuts, corn, wheat, barley, oats, sorghum, rice, almonds, beans, oilseeds, cotton, sunflower, soybeans), nuts, dried fruits, milk and dairy products, herbs, spices, coffee, cocoa, feed, vegetable oils, beer, among others. Moreover, in Colombia, the lack of adequate laboratory and human and economic resources may also be a cause of the limited information on occurrence of mycotoxins. However, another factor that plays an important role is poor regulation and lack of supervision and control by agencies should conduct these activities [3]. In response to the above it is necessary to visit the dairy farms to inspect the site of storage of food, with the aim of verifying the conditions under which this is stored. Because many herds do not have a place reserved for that purpose, the food is exposed to conditions that favor the presence of aflatoxin-producing fungus, so it is necessary to potentially sensitive analysis (HPLC), which is determined AFM1 concentration in raw milk from different herds suppliers of a dairy company in Valledupar, in order to correlate the presence of the fungus Aspergillus flavus in dairy cattle feed, so that preventive measures are taken regarding the collection and storage conditions and at the same time warn companies of the responsibility that comes with free milk products that have not undergone a proper process of traceability. As the raw milk, should ensure the quality of food consumed by cattle that produces it, to really give consumers a safe and secure for your health. [5]

MATERIALS & METHODS

This research is descriptive. The population consisted of 97 herds suppliers of a dairy company in the city of Valledupar. The sample used consisted of the 30 herds of providers who agreed to participate in the study. The sampling was carried out for convenience. The analysis variables were the concentration of aflatoxin M1 in raw milk and food storage conditions for livestock consumption. The research was conducted in three phases: In the first stage data were organized in the forms of acceptance (forms filled out by the owners of herds that agreed to participate in the study) to create access routes herds, which showed a map which specifies the conventions that helped the location of the farms. Subsequently conducted an inspection of the storage in herds for which they filled out the health inspection form. In the second phase proceeded with the test sample of 250g of the food field collecting 5 points equidistant from storage and placed in ziploc bags.
these were labeled with the date and number of the herd. The samples were transported to the laboratory of food science at the University of Santander. Then processed the samples obtained by direct seeding in the PDA medium. It took 10 g sample of 250g collected in each of the points of food stored in herds to seek the fungal growth. Once growth was obtained microorganisms were replicated to PDA agar for isolation and identification. In the third stage were collected on the platform of receipt of the dairy raw milk 500ml (as required by the toxicology laboratory for HPLC analysis) of the thirty herds under study obtained a sample, was conducted in this way (a tub representative of each herd) in order to minimize costs. Subsequently refrigerated and were transported to the toxicology laboratory at the National University where they were processed by HPLC technique for determination of aflatoxin M1. The limit of quantification LOQ of the technique was from 5ng/L-1 and the limit of detection (LOD) was less than 5ng/-1 concentration. The results were reported with respect to the LOQ. The statistical handling of the results was carried out by the Microsoft Office Excel 2003.

RESULTS & DISCUSSION

After carrying out the inspection of the 30 herds the results were based on the parameters taken into account in the application form:
1. Exclusive site for storage of food.
2. The presence of factors that impair the physical state of food.
3. Presence of chemical factors that promote growth of fungi in stored food.
4. It notes the presence of mold in the food.
5. There are control measures for pests and rodents.
6. Identified hazards occur in stored food contamination.

Figure 1.1 shows that of the 30 herds, 68% do not have an exclusive site for storage of food for cattle, given that most are exposed to environmental conditions (extrinsic factors) that favor the spread and growth aflatoxin-producing fungus, 100% were found with agents that impair the physical state of food such as insects, which are part of the biological factors, acting as vectors of fungal spores or be responsible for mechanical damage allowing the growth of mold, so the presence of insects acting as an agent for dissemination of the microflora and thus contributes to the growth and multiplication of fungi. In 96% of herds were found some element that promotes the growth of fungi in stored food, such as humidity, physical - chemical factors determining the synthesis of mycotoxins as well as the outdoor temperature. In 55% of the herds showed the presence of mold in the storage area being part of the microbial flora and promoting competition among fungal strains, in a forage may be small areas of food with high moisture content which may trigger a fungal development, which can cause a general increase in moisture in the substrate and consequently a greater predisposition to fungal contamination and mycotoxin production. In 93% of herds are not kept control measures for pests and rodents, which can damage the food by encouraging the growth of fungi. In 100% of the herd is estimated this could result in contamination of food with hazards clearly identified as those listed above. These figures show that the storage conditions studied herds are deficient and
fail to meet established parameters. There are also predisposing factors such as moisture and mold contamination to facilitate the presence of aflatoxins. [6] In terms of direct seeding in agar PDA made the search for fungal growth was growth in the three assemblies.

Figure 1.1. Macroscopic fungal growth

Figure 1.2. Microscopic fungal structures

Figure 1.1 shows characteristic growth of a fungus mycelium. The reverse is pale yellow colonies (A) and the front green (B), with yellowish-white mycelium (C) and / or green and grainy texture. Figure 5.2 shows characteristic microscopic structures of fungi. Conidiophore vesicles lining the test fully with KOH 10% (A). Uniseriate conidial head, radial, spherical vesicle; Metula that occupy almost the entire surface of the gallbladder (B). Conidia globose, smooth, hyaline, pale brown (C). According to the microscopic and macroscopic features observed, the fungus isolated was Aspergillus flavus, aflatoxin B1-producing fungus which shows the inadequate conditions of sto...
aflatoxin levels were detectable in 57% of herds and 43% had undetectable levels. It should be noted that this route were found the highest and the lowest detectable level throughout the study. See Figure 1.3.

Figure 1.3. Detection levels of aflatoxin M1 per herd.

In the route three herd number 23 had a concentration of 19.8 ng/L of AFM1, while in the herd 14 concentration was not detectable. Herds 28 and 29 belonging to the route number 4 had no detectable limits for aflatoxin M1. By analyzing the concentration levels per route can be seen that track two is the more levels of concentrations of AFM1 has an average concentration 20.7 ng/L, followed by Route 3 to a concentration of 9.9 ng/L and Route 1 with an average concentration of 4.7%. This relates to the herd 6 who had the highest 160.2 ng/L and is part of this route, not to mention that comprises 70% of herds entering the study. On Route 4 concentration levels were undetectable.

The highest concentration level found in milk samples was the herd 6 with 160.2 ng/L and the lowest detectable level was the herd of 18 with 10.9 ng/L being the two herds members of the route number 2. These levels are allowed in Colombia Colombian ICONTEC standard which states that AFM1 concentration in the milk must be below 400 ng/L, but for the European Union standard is not permitted levels found in the herd 6 and in herd 22 (160 ng/L and 86.9 ng/L respectively) as the concentration must be less than 50 ng/L, which limits future exports. In this study, all samples had aflatoxin M1, however, 53% had no detectable limits of HPLC equipment where the LOQ limit is equal to or greater than 5.1 ng/L. 47% of samples had detectable levels of aflatoxins. It must be remembered that the way the sample was collected (platform receipt) concentrations are diluted as in the basins is collected milk milk of various animals. Comparing this study with one conducted in 2008 at the University of Santander Headquarters Valledupar by Bacca and collaborators in pasteurized milk of two brands can be seen that there is a greater concentration in raw milk being the minimum level of 6.1 ng/L and maximum 22.7 ng/L for pasteurized milk while in the present study, concentrations with a minimum of 10.9 ng/L and a maximum level of 160.2 ng/L increase 7 times the concentration of aflatoxin M1 in raw milk. This comparison may be related to a study conducted in the province of Mersin Turkey which aimed to investigate AFM1 levels in raw milk and UHT milk (53 samples of raw milk and UHT milk 45) the method used was HPLC. Positivity was detected in 46 (86.7%) samples of raw milk and 33 (73.3%) UHT milk with levels ranging from 21 ng/L to 86 ng/L in raw milk and 10 ng/L to 39 ng/L in UHT milk. Toxin levels were above the official limit value reasonable (> 50 ng/L) recommended by the Rules of Turkish Food Codex. The results of this study may also be related to the research carried out in the science faculty at the Pontifical Javeriana University in 2009 with respect to the
detection of AFM1 in fresh cheese sold in Yopal Casanare, which concluded that those herds that were positive for AFM1 and supplying milk to cheese suppliers did not carry out proper food storage process of dairy cattle, which leads to produce and sell cheese that can endanger the health of the population. From the standpoint of economic and trade the free trade agreement (FTA) between Colombia and the United States plays a key role in the agricultural sector, while the FTA will eliminate trade barriers to ensure the free passage of goods and services among participating nations and thereby seeks to sanitary measures relating to prevention and control of diseases of plants and animals, are implemented in ways that do not constitute a means of discrimination against exports and simultaneously meet with the minimum requirements for the marketing of domestic products to other countries so as in the case of the dairy sector will be required sanitary standards which must be within the parameters required by the European Union where the maximum level for aflatoxin M1 of 0.05 mg / L for these products to enter world markets with special measures that put them on equal terms against other countries that protect this sector. This condition remains a limitation of the country's dairy companies as they will be forced to compete directly with large multinational companies and necessarily need to modernize its infrastructure, improve quality and efficiency [7] In the tables we can see that where ranks the highest concentration of aflatoxin M1 was in the herd 6 of Route 2 with a value of 160.2ng / L, in this herd there was no growth of the fungus Aspergillus flavus, as in 9 other herds (2, 10, 19, 11, 18, 15, 5, 20, 30) in which concentrations were detected by the test, relating to the theory which states that the fungus does not ensure that the toxin is present and this, in turn, can be present without being the fungus, unlike bacterial toxins such as botulinum toxin, which are proteins and, consequently, are deactivated by heat over 60 ° C, the fungal toxins are very resistant, supporting more than 200 ° C of temperature, so sometimes it is commonly found when the fungus is gone. [8] In herds 21, 22, 23 and 24 had growth of Aspergillus flavus thereby demonstrating that the storage conditions were necessary for development. Across the board the optimal conditions for fungal growth and proliferation are: an aw higher than 0.75, a temperature above 20 ° C and orienting a substrate moisture of 14% or more. In the herd can be found field mushrooms are invading the seeds while still not been harvested the crop. Storage fungi are invading food during it, and usually do not present any serious problems before harvest but after it, these include species of Aspergillus and Penicillium. [9] The ubiquity of Aspergillus is due to its ability to grow at different temperatures on substrates with different moisture content. The colonization of the food during storage by Aspergillus occurs explosively when the relative humidity inside the food rises above 70%, without triggering the phenomenon still budding. If a food with a moisture content of 15% is not affected by Aspergillus for a year is because the storage temperature was below 5-10 ° C. Studied herds naturally handle high temperatures and general climate of Valledupar, with little annual temperature variation, with a mean of 28.4 ° C with maximum and minimum of 22 ° C and 34 ° C respectively, indicating that temperatures are conducive to the development of this fungus. In herds where he was granted asylum A. flavus, concentrations of aflatoxin M1, however can not be attributed to the presence of the fungus as they should be used for determining the feed concentrations of aflatoxin B1 and according to the ration and concentration ingested by the animal to determine the concentration of aflatoxin excreted in milk, which was not the objective of this research. In the herd 21 in which food was stored forage based on a mixture of leaves and corn stalks, grass cutting, spikes and leaves of rice and sorghum leaves. Compared with the study conducted in Mexico there is a similarity because the maize, sorghum and rice are likely substrates for aflatoxin production due to its composition, while soy is a poor substrate for the production of these mycotoxins although we are given all the circumstances, it is excellent for the growth of Aspergillus but the production of aflatoxin in it is low. [6] Another comparison that can be set to the same study is the fact that in Mexico under Aflatoxin M1 concentrations were found in milk from cattle that were fed freshly cut grass. In the present investigation was detectable aflatoxin levels were found in 46.7% of the samples analyzed, taking into account that the food given to cattle at the time of sampling was not only stored and fresh grass for the summer season, likely could influence the occurrence of AF in this large percentage of the samples. On the other side facing the concentrations of aflatoxin M1 found in this study with those found in research conducted in Tizayuca Hidalgo can be considered that the presence of AFM1 in milk is linked to the time of year, time consumption and mishandling of food in storage conditions, as evidenced by the results of inspection reports filled out in this investigation. The presence of A.
flavus in feed intended for livestock consumption and concentrations of aflatoxin M1 found in 100% of the herds, may be related to inadequate storage conditions revealed by the inspection report, as in this, both physical factors were identified - chemical and biological underpinning the growth of aflatoxin producing fungi and the synthesis thereof.

CONCLUSION

Storage conditions in the herds are not adequate for this purpose, which was corroborated with data from the inspection report, according to which 100% showed clearly identified hazards of contamination of food intended for livestock consumption. Similarly, 68% do not have an exclusive site for storage of food for cattle, given that most are exposed to environmental conditions that favor the spread and growth of A. flavus. Of direct seeding in stored food PDA agar growth was obtained corroborating mycelial yeasts and inadequate food storage conditions, this procedure was isolated from the fungus Aspergillus flavus aflatoxin B1 producer, but can not relate strictly the presence of this fungus with aflatoxin M1 concentrations found in milk samples for this influence as other factors such as the aw. The found concentrations of aflatoxin M1 in raw milk of 30 herds were within the levels permitted by the Colombian technical standard ICONTEC, but it would be limiting if you want to export the product since it significantly exceeds the maximum established for that purpose. The upper limit was obtained 160.2ng / L followed by 86.9ng / L. These according to this study would be the maximum to which consumers would be exposed as the other values were lower. It is recommended from the academy to support the primary extractive sector with the development of research to carry out monitoring of food for livestock consumption, assessing the conditions of storage and concentration of aflatoxin in milk, in a larger number of herds to those used in this study, in order to get a real profile of behavior in regard to these mycotoxins in the region, before moving to technology transfer of results to the Departmental Health Secretariat and the Ministry of Agriculture and Rural Development to to take measures to control and be made aware to suppliers of milk and dairy companies, the importance of precedent in regard to these pollutants and their impact on the health of both humans and animals, as well as carry implications for production.

REFERENCES
