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Identification of M1 Aflatoxin in Milk of the Collector Tank

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Abstract: The Aflatoxins (AF) they are the group of more studied mycotoxins, they are classified in aflatoxins B1, B2, G1 and G2; metabolized to aflatoxins M1 and M2, also called secondary metabolites of aflatoxins, they are harmful in quantities appearances - $\mu g\ kg^{-1}$ or parts for trillion, pb. They are in the milk in form of metabolic residual. The aflatoxins doesn't have flavor, scent, they are fluorescent under the ultraviolet light and they are resistant to high temperatures, to more than 320° C, so the cooking process or pasteurization doesn't exterminate them. Aflatoxins can be detected without the presence of the producing mushroom, since this inactivate can be had by chemical processes or for alteration of environmental factors, but I didn't seize to the aflatoxin or mycotoxin that it can remain in the sustrate. Mainly the Aflatoxin B1 (AFB1), it is directly related with the Aflatoxin M1 (AFM1), since it is the one derived 4-hidroxi of AFB1 and excreted in the milk during the nursing process in form of secondary metabolites, in the females cows that AFB1 consumes in the diet. Between the 0.3 and 6.2% of the present AFB1 in the food, it is biotransformation to AFM1 and excreted in the milk. For what a lineal relationship exists among the levels of AFB1 in polluted food and the content of AFM1 in the milk. Therefore the contamination of the milk for toxic metabolites, aflatoxin M1 takes place during the concentration of sharp or chronic consumption of polluted food with aflatoxin B1. The objective of this research was to identify the Aflatoxin presence M1 (AFM1) in milk of collection tank in a stable of the State of Hidalgo, Mexico. They took five samples directly of the collection tank with interval of one week, which were analyzed by means of the method of chromatography of liquids (AOAC) for the identification of AFM1. At the same time, he/she was carried out a multiple toxicological analysis of micotoxinas for chromatography in fine layer from the proportionate diet to the animals. The results obtained by means of AOAC were 225 parts for trillion (ppb), Non detectable (N/C), 332ppb, 167ppb and N/C. The obtained results of the food, they went negative to AFB1. These results indicate that the presence of AFM1 in milk, is previously related with the consumption of polluted food with AFB1, that which presents a problem of public health for the consumers; a solution alternative is to change the polluted food with AFB1 for one that is not.

Key words: Mycotoxin, aflatoxin in milk, AFM1, AFB1, toxicological analysis, AOAC

INTRODUCTION

The micotoxinas is metabolites secondary fungics, taken place by species of mushrooms toxigenics, at least under certain conditions that they appear in the forages, cereals and all kinds of foods (Bars and Bars, 1998), able to unchain squares of sharp and/or chronic intoxication, because they are carcinogenic, mutagenics, teratogenics and estrogenics (Pittet, 1998) they are present in the field, during the sowing, development and it harvests, as well as to the moment of the storage of foods. Also, the climate influences in the development of mushrooms

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producing of mycotoxins, mainly of the gender Aspegillus (Humphryes, 1990; Pittet, 1998; Lucas, 2001), low temperatures grow from 25 to 35°C, with a relative humidity between 65 and 80% (Lucas, 2001).

They have been discovered more than 3500 micotoxinas with different toxicity levels. However, the Aflatoxins (AF) they are the most frequent and harmful in quantities appearances (μg Kg^{-1} or parts for trillion). they are the group of more studied mycotoxins, they are classified in aflatoxins B1, B2, G1 and G2, (Hamphryes, 1990), metabolized to aflatoxins M1 and M2, calls also secondary metabolites. They are in the milk in form of metabolic residual. The aflatoxins doesn't have flavor, scent, they are fluorescent under the ultraviolet light and they are resistant to high temperatures, more than 320°C without fragmenting; to boil, to sew, to ferment or to pasteurize the foods, it doesn't exterminate them (Carvajal, 1999; Early, 2000).

The AF, is the mycotoxins with more carcinogenic activity (Luca, 2003), aflatoxins can be detected without the presence of the producing mushroom, since inactivate could be had by chemical processes or for alteration of environmental factors, but it is present or some other mycotoxin, since these remain in the substrate (Anton and Lizaso, 2001; Cesar, 2002). they Cause many problems, from the farmer until the consumer; drops in the production of the animals, due to the presence of illnesses inmunosupresors, until alterations that suffer the foods, or toxic foods of animal origin for the human (Diaz, 2003). Chemically, they are dihidrofurans portions or tetrahidro-furans, fused to a ring cumarinic; they are to each other a group of metabolites closely related heterocyclic. Mainly the AFB1, is directly related with the AFM1, it is the one derived 4-hidroxi of AFB1 and excreted in the milk during the nursing process in form of secondary metabolites, in the female mamals that AFB1 consumes in the diet (Luca, 2003). Between the 0.3 and 6.2% of the present AFB1 in the food of the cows, it is biotransformate to AFM1 and excreted in the milk. For what a lineal relationship exists among the levels of AFB1 in polluted food and the content of AFM1 in the milk (Diaz, 2003).

Permissible maximum levels have settled down: 20 parts for trillion (ppb) for AFB1 in foods for cows milkmaids (Diaz, 2003). In Mexico, the limit of AFM1, it should not be bigger to 0.05 μg L^{-1} (NOM-091-SSAI, 1994). The quantity of AF excreted by milk, doesn't have relationship with the production of AF (Humphryes, 1990), since it spreads to disappear of 3 or 4 days after moving away the administration of the toxic food (Battacone *et al*., 2003; Cesar, 2002). therefore the contamination of the milk for toxic metabolites of AFM1, takes place during the concentration of sharp or chronic consumption of polluted food with AFB1.

The light effects, such as alterations in the system inmunitaric, inmunosupression and reproductive disfuntion, they are difficult to recognize and to evaluate in cows milkmaids. In the case of the human, the diagnosis of a lingering ingestion of foods with certain levels of AF, is subject to diverse factors: Micotoxin type, biodisponibility, toxicity, concentration in the food, quantity of the consumed food, consumption persistence, intermittence of the ingestion, the individual's weight, physiologic state and age of the animal (Anton and Lizaso, 2001; Cesar, 2002). However, the chronic intoxication that is the most frequent form in human and animal, is due to the consumption of polluted foods during weeks and/or months, the signs that are observed are very specific: little gain of weight, smaller conversion index, decrease of the production milky and bigger susceptibility to diverse infectious illnesses (Cesar, 2002). The AF has diverse biological and pathological effects; the AFM1, is the most noxious for the human consumption, it is a possible carcinogen for the man, besides other effects. He/she has great importance for the human health, since it can act in way sinergic, aditive and antagonistic (Anton and Lizaso, 2001; Cesar, 2002). These substances are a serious problem for the inocuid of the milk, due to the effects that cause in the organism of any individual (Cesar, 2002).

The objective of this research was to identify the presence of AFM1 in milk, taken directly of the collection tank in a stable milkman of the State of Hidalgo, Mexico.

MATERIALS AND METHODS

They were carried out 5 samplings, according to the norm: NOM-091-SSA1-1994. The samples of milk were obtained directly of the tank of general gathering of the first one it milks, at the 14:00 h, with an interval of one week.

The samples were subjected to a toxicological analysis, for the method of chromatografic of liquids of high resolution, HPLC, With the use of a standard, to identify and to quantify the levels of AFM1.

Parallel to the last sampling, took a sample of the diet administered to the cluster, directly of the troughs, which was subjected to the toxicological analysis of chromatografy in fine layer.

RESULTS

In the following Table 1 the obtained results are shown.

The sample 1, it shows a quantity of AFM1 for up of the level allowed in Mexico, <.050ppb = corresponding .225ppb; the sample 2, an increase of AFM1, >20ppb,

Table 1: Results of AFMI

Simple	AFM1/ppb
1	.225
2	N/C >
3	.332
4	.167
5	N/C<

N/C: Non detectable, >, <: Bigger or smaller than the standard detectable

was observed that could not be detected by HPLC. The sample 3, it lowered in comparison with the sample 2, but the levels of AFM1 are above the opposing one in the sample 1,> .225ppb = .332ppb. In the case of the sample 4, a drop was observed in the levels of AFM1, 0.167ppb, in comparison with the previous samples; however these levels didn't diminish the permissible ones in Mexico. The sample 5, reflective the existence of AFM1 in levels non detectables, <.050ppb.

DISCUSSION

The results of AFM1 obtained in the first four samples, surpass the level allowed in Mexico and in the European Union: <.050ppb (Battacone *et al*., 2003). In the 2003 (Gimeno, 2003) indicated that the allowed maximum concentration of AFM1 in the raw milk dedicated to consumption human is of 0.05ppb. When the level of AFM1 is high, it is due to the ingest of AFB1 by means of the food (Anton and Lizaso, 2001; Battacone *et al*., 2003; Cesar, 2002; Garcia, 2002; Carlson *et al*., 2002).

The influence of the stations of the year on the concentration of AFM1 in milk, is related with the consumption of AFB1. Also, he/she is also related with the crop time (Quezada, 2002). Later on, in the one stored you can increase the contamination for aspergillus and therefore with the micotoxin,

Taking into account these factors, in this study was carried out an analysis to the sample 5, coming from the proportionate food to the livestock, Multiple analysis of Mycotoxins "Stoloff", which showed negative results to AFB1, this indicates that the consumption of polluted food with AFB1 was minimum or null during it finishes it week of sampling, because the food was retired of the diet of the animals, in one period of 3 to 4 days before (Battacone *et al*., 2003; Cesar, 2002), that which indicates that the ingest of polluted food and the excretion of AFM1 in milk are intimately related. To the beginning of the study it was in the sample 1 0.225ppb, in the sample 2 >20ppb non detectable; diminishing lightly in the sample 3 0.332 ppb; however, it presents bigger level that the sample 1, what indicates that the consumption of AFB1 was present in a chronic way, this agrees with that indicated by Gimeno (2003) where the administration of AFB1 in the food in big concentrations, for up of 433ppb, they indicate concentrations of AFM1 in big quantities 1.05-10.58 ppb, in later 6 h to the consumption, persisting during 62 h after having administered the micotoxin. In the same way, when the standard detectables is surpassed, due to the high concentration AFM1 excreted in milk, indicates that the consumption of I think polluted, for up of the permissible levels for bovine, 20-25ppb of AFB1 in food (Gimeno, 2001; Carlson *et al*., 2002).

Anton and Lizaso (2001) they found results non superiors to 0.040ppb in milk, coinciding with the result of the I finish sampling carried out in this study, which were concentrations below the standard. It is considered unnecessary the determination of AFB1 in the food, since AFM1 never surpassed the permissible levels. However, (Cesar, 2002) it determines that the diagnosis of AFB1 should be carried out in foods, as well as its quantification, independently of the quantity of opposing AFM1 in the milk, if the problem, the food is diagnosed it should be moved away immediately.

CONCLUSION

The existence of AFM1 found in milk, is related with the chronic consumption and without control of the polluted food with AFB1 given to the cluster. The presence of AFB1 in foods for animal consumption represents a serious problem of public health, since besides affecting the agricultural production, it affects the human health, due to the direct or indirect consumption of the metabolites of AFB1 expressed as AFM1. The aflatoxins presence in the milk is also related to the time of the year, time of consumption and to the wrong handling of the food; however, the tendency of decrease of AFM1 in the last sample, reflective that the change of foods contaminated by non polluted foods of AFB1 is the most correct method for the decrease of AFM1 in milk.

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