ORIGINAL ARTICLE

A survey of Fungal Infection and Measuring Aflatoxine (B, G) by B.F Method in the Fish Meal Produced in Gilan Province, Iran

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ABSTRACT

A survey of fish meal produced in Gilan from the viewpoint of fungal infection and possibility of Aflatoxine existence. Our target in this research is recognizing the fungal infection and the existence of Aflatoxine in the fish meal produced in Gilan province. This study was accomplished on 75 samples of fish meal from 7 manufacturing factories of this product in Gilan province from May through November of 2000.the reason for the present survey was studying fungal infection in the exclusive environment of fungi and measuring Aflatoxine by the thin layer chromatography method. Different fungi such as *Penicillium, Aspergillus, Cladosporium, Fusarium, Fermentative, Psylomyces, Mucor and Rhizopous* were identified, and the highest infection rate in the fish meal was due to Penicillium, where as the lowest was due to Rhizopous. Also, the infection rate of Aflatoxine was reported underdetermined in the survey in terms of (B) and (G) types, in samples collected from the fish meal of Gilan province. The results of study showed that one of the fungi producing Aflatoxine, Aspergillus flavous, was present in samples of fish meal, but no toxine was produced because of suitable condition like moisture, temperature, appropriate time for the appearance of Aflatoxine.
Keywords: fungal, infection, aflatoxine, foodstuffs microbiology

INTRODUCTION

Fish meal is one of the most valuable and consumable food ingredients with animal origin in nourishing animals, birds and fishes that in addition to considerable protein and stable amino acids, it has sufficient amount of energy, minerals and vitamins A,D, E and group of B specially B12. These elements accelerate growing frequently and are considered as suitable animal protein factors (A.P.F). So the fish meal has great food value and due to containing sufficient amount of U.G.F, it has found great value and importance in nourishing animals. Animal protein sources are also necessary foodstuffs for human beings and they have a main protein in nourishing human beings. Nourishing needs specially in developing countries, and the possibility of supplying a part of these needs by sea sources confront many problems; such as raising of after fishing wastes, lack of knowledge about many edible and non-edible marines, non availability of advanced technology and improper maintenance, producing and suitable supplying methods. Fungi are microorganisms that their spores due to wide expansion in nature, cause contamination in food products.

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Mycotoxins are metabolical secondary compounds of moulds and have toxic features which cause acute, low-acute and chronic toxication, sometimes leading to cancer, in sensitive living creature. Some of them are able to create mutation in sensitive living creature and some others are teratogenic and cause transformation in growing fetus. The toxication created due to swallowing foodstuffs containing fungal poisons is called "Mycotoxicosis". Some of moulds that are able to produce Mycotoxin and are very important are Aspergillus, Penicillium and Fusarium. Some species of Aspergillus and Penicillium are able to produce Aflatoxine. Aflatoxines in view of carcinogenesis are the strongest compounds. B1 is the strongest Aflatoxine.

Most of raw foodstuffs in some stages of storage, transfer or producing, are exposed to deterioration by different degrees. This infection causes disorder to human health and reduction of usable foodstuffs by decreasing animal protein sources directly or indirectly. Therefore, considering the special importance of food ration in animals, this need for evaluation of the health of this product in view of fungal infection and the possibility of Aflatoxine existence, is completely felt. The research was conducted based on culture standard method.

METHODOLOGY

This research was conducted in Gilan province, Iran, in 2008. 75 samples of fish meal were collected from

7 factories of Gilan province. It should be stated that the samples were transferred to the laboratory in totally aseptic conditions. The method used was on the basis of culture standard and counting of fungi in foodstuffs. Fungi were maintained in 25ºC for at least 48 to 72 hours. Grown fungal colonies were prepared after purification in second passage of slide culture. After the formation of spore genesis structure, the lamella of slide culture was taken with a sterilized pincer and one drop of Methyl alcohol was dropped on the internal surface of lamella to make the fungi stable. Then, when the lamella surface got dried, one drop of colored- solution of Lakto phenol cotton blue was poured on another sterilized lamella and the first lamella was placed on it. Then fungi were studied on the basis of Mycelium structure and generating organs. The fungal culture environment was used for separating the ferment in 25 and 35ºC. In order to measure Aflatoxine, B.F method was used, in which 50gr. of fish meal sample along with 2-3gr. salt were mixed with 250ml. Methanol 55% for 1 minute, to help separating Methanol and Chloroform phases. The centrifuge pipe was filled with this solution and centrifuged for 5 minutes with a speed of 2000 turn per minute. When the centrifuging was completed, suspended solids deposited and the Methanol phase was placed at the upper part. This phase was passed through a filter paper and collected in a 50ml. scaled cylinder. Then it was placed in a 50ml. de-cantered funnel of chloroform under the ventilating machine, and after stirring the decanter funnel for 30 to 60 seconds, two phases were completely separated from each other, and the chloroform phase was placed at the lower part. The chloroform phase was passed through the filter paper containing Sodium sulphate without water, and carried to a wide-mouthed beaker. Then it was heated on the heater till its volume decreased to lower than 2ml. The solution was transferred to vials by chloroform help and dried completely (but not burned). When vials became cool by a micro liter pipette, 200 micro liter of Benzene-Acetonitrile solution was added to substances at the bottom of the vial. In order to solve all substances completely, it was stirred on the shaker for 1 minute. Then it was dotted on particular plates with the micro liter syringe or a pointer set having a special pen. Points 2, 5, and 10 micro liters of each sample and then these points were injected a standard poison with a special ruler. Then these plates were put in a special tank where the mixture of Acetone+ chloroform (90:10) was up to with 1 centimeter height. This action was done in darkness. After about 30 minutes, the solution came up on the plate to a sufficient extent, and carried Aflatoxine with itself. Other materials did not solve in this solvent and remained in the injection point. For deleting obtrusive spectrums, it was put in a pure Ether tank, then the development action was done. The plate was taken out of the tank, and placed under UV lamp for observing the created spectrum. Due to fluorescence property, Aflatoxines were seen in blue-green colors depending on their types (B, G), under UV radiation light.

The amount of Aflatoxine PPb in the sample is calculated as follows:

$$\text{Concentration on PPb basis} = \frac{S \times Y \times Z}{X \times W}$$

Y= Aflatoxine concentration in standard solution
S= standard point similar to the sample in view of color
X= sample point similar to the standard point in view of color
W= sample weight
Z= volume of the added solvent

RESULTS

According to studies accomplished on 75 samples of fish meal in Gilan province, the fungi isolated from the fish meal are: *Penicillium 53.3%, Aspergillus 16%, Cladosporium 13.3%, Fusarium 12%, Fermentative 10.7%, Psylomyces 9.3%, Mucor 8% and Rhizopous 4%.*

The rate of fungal infection in the collected samples of fish meal produced in Gilan province has been shown in chart (1).

Chart 1: Rate of fungal infection in collected samples of fish meal produced in Gilan province on the basis of fungi type

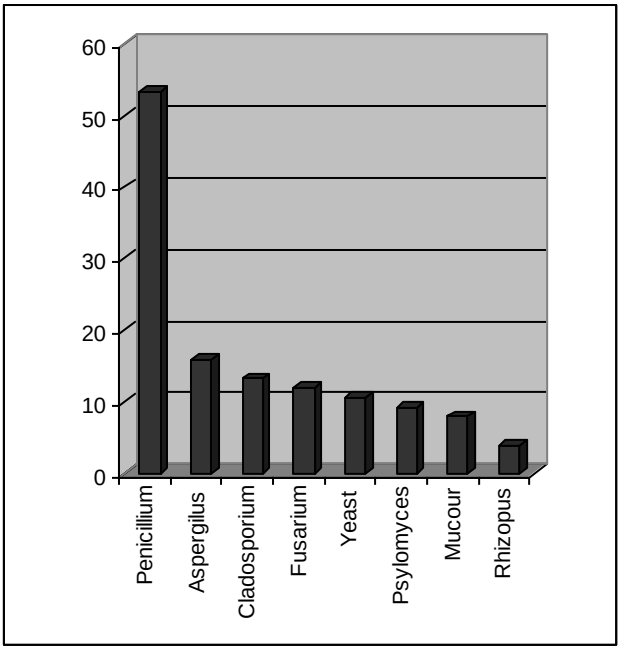


Chart (2): Rate of fungal infection in collected samples of fish meal produced in Gilan province on the basis of moulds producing Mycotoxin

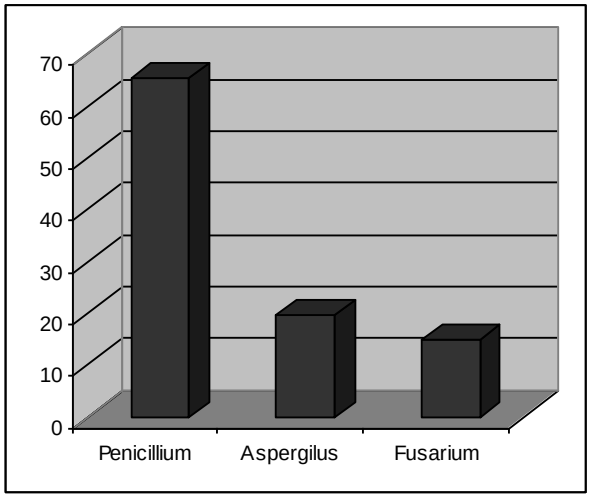
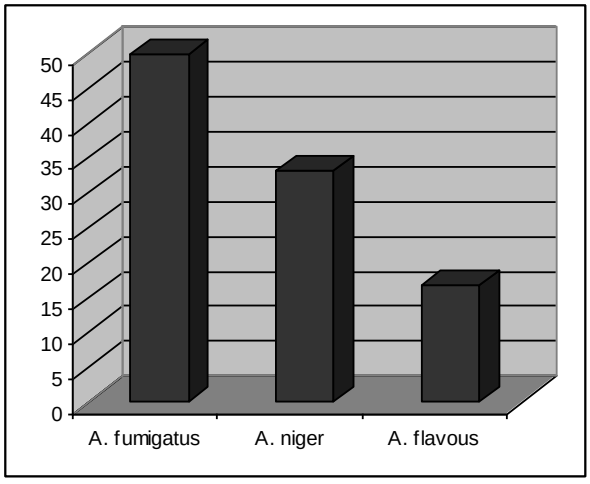


Chart (3): The rate of fungi infection and collected samples of fish meal In Gilan province on the basis of Aspergillus



Rate of infection of moulds, able to produce Mycotoxin in samples of fish meal collected from Gilan province has been shown in chart (2).

The rate of fungi infection in samples of fish meal collected from Gilan province on the basis of the type of Aspergillus has been shown in chart (3): the highest rate belongs to Fumigatus Aspergillus, then to Niger Aspergillus, and the lowest rate of infection belongs to Flavous Aspergillus.

Research findings also indicate that:

1. Standard concentration was compared with sample of fish meal. Aflatoxine peaks were not similar to peaks of fish meal sample.
2. Aflatoxine standards were compared with samples of fish meal, and no trace of Aflatoxine G, B was observed.

DISCUSSION

Measuring the amount of foodstuffs or raw materials attacked by fungi and consequently destroyed is difficult. But some peoples estimate that it includes 45% of annual production of tropical regions of developing countries. Damages of moulds in this reduction are very considerable, and it has a great share in deterioration of foodstuffs used in the world. Many of damages made on foodstuffs are due to the decrease of goods value and its deterioration as a result of mould growing. Researches showed that some of fungi including Penicillium and Aspergillus that have a main role in breaking up of protein due to their proteolytic enzymes, leading to deterioration and unpleasant smells and tastes, cause deterioration and destruction of products. Some of fungi such as Penicillium, Aspergillus, Fermentative and Rhizopous are among Lipolytic fungi that hydrolyze and oxidize the fat of foodstuffs and lead to its deterioration.

Some of fungi has a role in pathogenesis and fungal abortion in cows and sheeps and also pulmonary infection and poisoning in birds. Mucor and Rhizopous fungi are in this group. In addition, some of fermentative species such as Candida Albicans are of pathogenesis types causing disease, stop in growing and death in some animals. Fusarium fungi are separated from swimming bursa of rainbow trout in amassed-farms, and cause difficulty in swimming of fish.

In addition, some of fungi produce Mycotoxin which the most important ones are *Penicillium, Aspergillus and Fusarium.* Among *Mycotoxins, Aflatoxine, Ochratoxin, Patulin, Zerealeon and* can be pointed out among which the most important one is Aflatoxine. In researches done, all three types of the above-mentioned fungi in collected samples of fish meal were identified, but since Aflatoxine has been known as the most important and dangerous Mycotoxin, this poison was tested in samples of fish meal. About 30% of Aspergillus Flavous produces Aflatoxine that is frequently poisons B1 and B2. Other species of Aspergillus parasiticus is Aspergillus SP. Aflatoxine is produced in lower amount by *Niger, Aspergillus ruber, Aspergillus ventii, Penicillium citrinium, and Penicillium variable.* Ducklings and young trouts are among the most sensitive animals toward Aflatoxine. Aspergillus flavous is one of the identified fungi in samples of fish meal. Any how, if most of fungi producing poison do not reach to their desirable conditions, they will never produce toxin. Nevertheless, the danger of Aflatoxine or other

fungus infection is always present. The most important factors helping the production Aflatoxine poison are moisture, temperature and time. To remove toad stools from foodstuffs, some procedures such as physical and chemical elimination, inactivating by heat, radiation and microbial omission are used.

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