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The effect of *Aspergillus* fungus on the diet of broilers in Ahwaz

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ABSTRACT

Contamination of food and livestock feed to common poisonous fungus in the air and environment is important. Contaminated animal feed to aflatoxin producing fungus leads to the disturbance in the cycle of animal health, milk and consumers of animal products. In this study, all isolates of *Aspergillus* on food have been studied with the aim of identifying and toxin-producing. *Aspergillus* are a group of fungus that have replication power and abundant growth, and are known as a part of fungal flora of most of places, and among Aspergillus, a number of species have capability of toxin-producing and can create disease in humans and animals. In this study, 180 samples of poultry in the city of Ahvaz were prepared and after transfer to the laboratory and culture, purification was performed, then the identification of isolates using the valid keys of mycology was done and extracting toxin of species on the context of corn was also performed, also determining toxin-producing of species was done in two ways of coconut agar and TLC. The results showed in this study several species of Aspergillus, including species of f*lavus A. A.parasitichus, A.ochraceus, A.niger* were identified. Species *flavus* with 52 isolates (0.028) allocated the most frequency and species *A.ochraceus* with 9 isolates (4.98) had the lowest frequency. Informing toxin-producing of the fungus on poultry diet and how toxin-producing of this fungus can be helpful in the management and prevention of infection by the fungus.

KEY WORDS: POULTRY, ASPERGILLUS, DIET

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INTRODUCTION

Fungus are present in abundant in the air, soil and our environment, so in the presence of humidity and the temperature, growth and reproduction of fungi are escalated (Ersali et al., 2008). Some of the toxins in the environment are fungal toxins that aflatoxins are considered the most important of them (Aghababaei et al., 2012). Aflatoxins are among the most important mycotoxins produced by some Aspergillus species *(Aspergillus flavus, Aspergillus parasiticus*) and Penicillium (*Penicillium Pvbrvlvm*) (Jamali Emam Ghedis and Moeini., 2010).

Aspergillus is fungal infectious disease of young poultry and chronic disease of older poultry that occurs with breathing difficulties. Fumigatus is the cause of *Aspergillus* fungus that remains in the nature by producing spore in nature. Fungus grows and reproduces rapidly in temperature 37 degrees and proper humidity but the increase of humidity causes the stop of growth. Considering its growth speed, it eliminates one-day chicks earlier that have lower resistance. Disease course varies depending on the age and power of poultry that in young poultry in acute form takes one week and in old poultry in chronic form takes a few weeks. The existence of some fungi in feed for livestock and poultry is natural and if they not have uncontrolled growth, they not have health risks but some fungi that are called fungus of toxin-producing have particular importance.

Mycotoxins are secondary metabolites produced by some filamentous fungi that infect agricultural products. They are toxic for humans and animals and causing a significant reduction in product returns and economic losses. There are widespread reports of outbreak of fungal damages in different countries. The metabolites are produced by different species under special conditions in terms of temperature, humidity and oxygen and are not essential for cellular activities. Consumption of foods contaminated with mycotoxins has been associated with a number of human poisoning, and even poisoning with mycotoxins can sometimes cause death (Aghababaei et al., 2012).

Peanuts, pistachios, wheat, rice, corn, almonds and figs are the main hosts of the fungus and are known as the most perfect natural environment for the growth of aflatoxin-producing fungi in the world, yet, several types of aflatoxin have been detected that among them B1, B2, G1, G have the utmost importance in the world. Among the different types of aflatoxin known, aflatoxin B1 by the International Agency for carcinogens is placed in Group A, research on cancer and in the meantime the toxicity and carcinogenicity of aflatoxin B1 is reported over other types, contamination of feed for livestock and poultry to fungus, followed by it aflatoxins, in addition to economic losses jeopardizes consumers of food with animal origin. Therefore, tracking and evaluation of aflatoxins in food and animal feed and comparison it with standard values in order to knowledge, proposals and measures to prevent Aflatoxicosis in animals and humans is necessary. Several studies are conducted on investigating the presence of aflatoxins in feed in Iran and many countries (Kan and Meijer, 2007).

Clinical signs of aflatoxin toxicity include: autopsy lesions, autopsy injuries, histopathological lesions in tissue, as well as the effects created on production indices of poultry flocks in cases of experimental and natural occurrence of Aflatoxicosis in broiler chickens in worldwide is reported (Kanungo et al., 2011). The main body attacked by the aflatoxin is liver and in human causes severe disorders of the liver occur. In animals also it causes problems in the gut, preventing immune function, reducing reproduction, increasing feed conversion efficiency, reducing the production of milk and egg, anemia, jaundice, reducing growth. Considering the importance of toxin-producing spices on food and diets of poultry in order to recognize species of toxin-producing and how their toxin-producing seems necessary.

MATERIALS AND METHODS

In order to identify isolates of *Aspergillus* fungus in poultry in Ahvaz during 2015-2016, the sampling was done in 4 active poultry farms in different parts of the city of Ahvaz. Samples taken were formed of various materials such as all diets used by poultry including: starter, middle-feeding and post-feeding, corn and soybeans, 150 samples were separated and placed inside plastic bags in the refrigerator at a temperature 4 ° C. Then the potato dextrose agar medium for culturing and isolation of fungi from sample was used. Samples were placed for 24 to 48 hours in incubation at 25 ° C then for purifying fungus, new colonies grown were cultivated.

A. Purification of fungus

In order to purify a fungal isolates, single spore method was used. So that by Anas fine needle, a small amount of Fungi spore was transferred to tube containing distilled water and then by Lam Thomas, its dilution was calculated. After preparing a suitable dilution, diameters of the suspension was transferred to medium of water agar 2% and by passing 12 to 24 hours, the colonies grown of fungus on medium (PDA) that already poured and cooled in the three spots was transferred and for 5 to 7 days at 25 ° C were reared (Lanyasunya et al., 2005).

B. Identification of fungus

To identify fungus, different culture media was used, including the culture of potato, dextrose, agar (PDA) is

known a public culture medium and used for the cultivation and primary isolation of isolates, as well as to identify the isolates, specific culture mediums such as CY20S (Czapek Yeast Extract Agar with20 sucrose) were used.

C. Preparing context of corn

Value of 100 grams of corn separately was poured in separate Erlenmeyer and the ratio of 100 to 27 was added to each of the contexts of distilled water and in temperature of 121 ° C. and 1.5 atmospheric pressure for 15 minutes was sterilized in an autoclave and 24 hours later was autoclaved again to all the external factors to be eliminated.

C. Inoculum of fungus to contexts of culture

After purification fungus, the fungus was developed on PDA medium and then spore suspension was formed in distilled water and 10 cc of this suspension in sterile conditions under the hood of Laminar was added to context and flasks with treatments were placed at room temperature and the conditions 24 hours of light and darkness.

D. Extraction of toxin

In order to extract Mycotoxins, first the contents of the flasks for 24 hours at 73 ° C were placed and then milled samples contaminated seeds acetonitrile - methanol was added and then was placed for 3 hours on a shaker. The resulting mixture was smooth with Whatman filter paper and extract was collected in clean Falcons and extract was passed from purification column containing a cationic resin and alumina-Carbon mix. For this purpose, the beginning and the end of the column with a layer of glass wool is blocked and a gram of mixture of Almunia - carbon is added on it and again a layer of glass wool blocked is placed on it and then cation resin that already for 1 hour is placed in distilled water is poured in column and then extract of samples were passed through the column.

Passed extract of first column using the second purification column includes respectively glass wool and alumina-carbon and net glass wool and then extract passed through the second purification column for solvent evaporation was transferred to oven 70 °. After drying the extract, methanol-water solution is added to it and for an hour is placed at room temperature, then methanol-water and acetonitrile-methanol solution was added and in order to evaporate, the solvent again is placed in an oven at 73 ° and finally methanol-water was added to dried extract and the extracts were stored at -20 ° C (Rahimi et al., 2011).

E. Investigating toxin-producing by T LC

After extracting the toxins, solutions of sample and aflatoxin standard on TLC of TLC aluminum plate with silica gel in a direction were dotted, after the plate was placed in TLC tank and methanol: acetonitrile (88:12) risen and the plate is washed up. Plate is dried in the air and under the lamp UV (365 nm) will be judged and blue fluorescence intensity is compared with standard fluorescence (Rahimi et al., 2008).

RESULTS AND DISCUSSION

The results of contamination of food rations including corn, soybean, starter, middle-feeding, post-feeding and context of poultry to *Aspergillus* fungus are in Table 1. The results showed in this study several species of *Aspergillus,* including species *A. flavus A. parasitichus, A.ochraceus, A.niger* were identified. Species of *flavus* with 52 isolates (0.028) allocated the most frequency and the species *A.ochraceus* with 9 isolates (4.98) had the lowest frequency. The presence of fungi such as *A.flavus* with the most frequency among *Aspergillus* species can be important in terms of toxin-producing; this fungus can produce aflatoxin B1 that has the highest toxicity than other Aflatoxin produced by Aspergillus. As well as there are species of other toxin-producing on diet of poultry that including them can be noted to *Aspergillus*

Table 1. The sample and the number of food rations contaminated with Aspergillus fungus

number and percent of sample				Total sample	Sample
A. ochraceus	*A. Niger.*	*A. Parasitichu.*	*A Flavus.*		
-	20	20	20	30	Corn
-	7	5	12	30	Soybean
-	-	-	8	30	Starter
4	-	-	5	30	Middle-feeding
-	5	-	3	30	Post-feeding
5	10	6	4	30	Context
9	42	31	52	30	Total
4.98	22.25	0.018	0.028	30	Percent of contamination

parasiticus fungi. The species has the ability to produce each 4 kinds of aflatoxin B1, B2, G1, G2, and in this sense can be considered a dangerous toxin-producing fungus.

Ochratoxin A is one of mycotoxins produced by the fungi *Aspergillus* and *Penicillium*. Spore of this fungus are widely dispersed in the environment and a lot of food, especially cereals are infected these fungus (Thompson and Henke. 2000) hence the toxin-producing spices in poultry as a pathogen agent could jeopardize poultry health. Since the existence of the toxin-producing spices on the dietary food for poultry and food can be dangerous, so the availability of toxin-producing agents and how toxin-producing is very important, *Aspergillus* is the cause of Aflatoxicosis in poultry as well as carcinogenic in human (Kanungo et al., 2011).

Brugfer et al (2003) in Australia on wheat and wheat flour used in animal feed of farms studied isolated and identified molds of *Aspergillus*, Cladosporydyom and *Penicillium*, the type of fungi identified in this study confirms the results of previous studies. Among the feed of livestock, corn silage and concentrate, respectively, had the highest number of fungal colonies. In a study that was done in Mazandaran, corn has the highest percentage to fungus *Aspergillus flavus* (Rahimi et al., 2008). This study also has identified corn silage as a feed contaminated with Aspergillus flavus that hence, confirms previous studies, but in this study, the most frequency of this type of fungus has been observed in straw and this could be due to difference in preparation and storage conditions of animal feeds in different farms and cities.

CONCLUSION

In our country, according to the climatic variation, kinds of toxin-producing strains of aspergillus can contaminate food and cause huge physical and financial losses. Also, due to diverse conditions of weather, there is the possibility of a wide range of fungi pruducing mycotoxins with toxins relevant in the environment, and it should be done sanitation and prevention principles as well as compliance with international standards for the maintenance and storage of goods and food rations.

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