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USING ELISA METHOD TO STUDY THE AMOUNT OF AFLATOXIN IN SOYBEAN MEALS AND LIVER SAMPLES OF POULTRY IN FOUR TOWNS IN NORTHERN IRAN

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ABSTRACT
Most human and animal foods are susceptible to fungal growth and reproduction. Mycotoxins are considered to be fungal toxins, the most important of which are the aflatoxins. There are different types of aflatoxins and the most toxic of which is referred to as aflatoxin B_1. The most significant symptoms and indicators of aflatoxins in poultry are regarded as a reduction in growth, an increase in food conversion rate (FCR), decrease in body weight, decrease in production and weakened immune system. Moreover, the consumption of animal food products by humans can transfer these toxins from poultry to humans and hence, they can potentially threat human health. In the present study, ELISA method was used to examine and measure the amount of available aflatoxins in soybean foods and in livers of broilers in west Mazandaran (in the twons of Kalardasht, Chalous, Salmanshahr and tonikabon). For this purpose, in the summer months, 400 samples of soybean foods (100 samples from each of the above-mentioned towns) and 80 samples of the livers of broilers (20 from each town) were collected. These samples were evaluated and analyzed with respect B_1 aflatoxins. When the samples were prepared, the competitive ELISA method was used to evaluate the samples with regard to B_1. According to the International Commission and FAO/WHO standards, the highest amount of mycotoxins in foods should be 12 microgram. The results from the experiments reveal that the highest amount of B_1 in soybean food is related to the month of September in the town of Salmanshahr. The highest amount of aflatoxin contamination in the livers of broilers was related to the towns of Salmanshahr and Tonikabon. The obtained results were consistent with the food produced which was produced from poultry.

KEYWORDS: Aflatoxin B_1, Broiler chicks, ELISA, Liver mycotoxin, Soybean food.

INTRODUCTION

Food products containing proteins meet the major nutritional needs of humans and hence are regarded as the major supplies of food. In sites where there is a shortage of natural pastures and they are also limited with respect to artificial pastures, thus, the development of the industry of breeding poultry is considered to be an undeniable necessity. The demand for the consumption of poultry is increasing which is due to the achievements of the medial science and heath advices. Moreover, the production of poultry is less costly than the production of red meat. The breeding of poultry in Iran throughout the last half century has undergone significant evolutions and has changed into an industry which together with its related industries constitutes the widest economic activities of the country. Following the oil industry, the industry of breeding poultry has the second highest amount of investment. Improving the efficiency and the potential return in different economic sections to produce high-quality and low-cost products will lead to a competition among world markets. Nutrition is the most significant issue in the economy of the poultry industry. In other words, the production of foods which are balanced in energy and nutrients for different age groups and locations, supplying appropriate and healthy foods by the factories, healthy keeping and maintenance of food and food management are regarded as the fundamental principles for reducing loss and improving food conversion coefficient.

The importance of providing high-quality and healthy food has always been underscored by different authorities in breeding poultry. Several research studies have revealed that the lack of control on supplying and distributing food and the use of non-standard ingredients and compounds have caused a 7 to 10 % increase in food waste (Shahvali and Nematzadeh, 2008). Anti-nutritional food combinations and many poisons in grains, legume and other plants are used to feed poultry. According to some annual estimates, more than 25% of the entire grains produced around the world are exposed to fungal contamination. Some of the different varieties of aspergillus can produce aflatoxin under normal conditions (Riazipour *et al.*, 2010). Aflatoxins are considered to be particular chemical compounds which are produced on many substances. They are, indeed, produced under typical conditions through a series of continuous enzymatic reactions by a variety of different aspergillus and pencillium fungi. Eighteen different types of aflatoxins have already been identified but only **B_1**‘**B_2** ‘**G_1** and **G_2** types of them are considered as the contaminants of food and food sources. Among these mentioned types of aflatoxins, B_1 is regarded to have the highest amount of poison and toxicity. These

toxins attenuate and weaken the blood immune system in the cells birds and hence make them more sensitive and vulnerable to the infections (Mokhtarian and Mohsenzadeh, 2004). The harmful effects of aflatoxins on birds depend on two factors: the amount of poison and the length of time the birds are exposed to toxins. The vulnerability and sensitivity of different bids to aflatoxins is different. That is, their vulnerability to these toxins can differ with regard to the variety of the bird, age of the bird and the gender of the bird. A majority of the foods which are consumed by animals and humans are a potential cultivation milieu for fungi and toxins.

These toxins make some changes in tissues and in particular, most of these changes and effects are observed in livers which result in liver disorders and ultimately lead to liver cancer. Aflatoxicosis leads to the reduction of growth and production, an increase in the calcification of bones and the time of blood coagulation and also an increase in carcinogenesis effects. Aflatoxicosis is produced in humans either directly through consuming fodds which are contaminated with toxins or indirectly through dairies and animal food products such as milk, meat and eggs. It can be argued that fungal infection and toxin resulting from it is present in the majority of food products. Some of the areas and provinces in north and south of Iran especially in the summer have relatively high temperature and moisture which creates the required conditions for the growth of toxin producing and infectious fungi on food products (Miahi *et al.*, 2007).

MATERIALS AND METHODS

Sampling

In the present study, 400 samples of soya oil cake were supplied from four towns of western Mazandaran province of Iran (Kalardasht, Chaloos, Salmanshahr and Tonekabon). Also, the amount of aflatoxins in the livers of the poultries of the mentioned towns was measured and about 20 samples were obtained from each town.

ELISA method

EIA can be implemented in two ways:

- Homogenous and smooth EIA in which testing agents or indicators are not isolated from each other.
- Non-homogenous EIA in which immunity complex and the substance which has not participated in reaction are isolated from each other (Gilbert and Anklame, 2002).

One of the most popular non-homogenous EIA methods is known as ELISA which is usually used for specifying and determining aflatoxin. In this method, a testing agent (antigen or antibody) was fixed and made firm on a solid phase such as plastic pipes, bolts or microplates. Then, a test solution containing a standard toxin and an antigen marked by enzyme or an antibody marked by enzyme was added to the environment (Gilbert and Anklame, 2002). The enzymes used for marking antibodies and antigens usually include alkaline phosphatase or peroxidase which results from horseradish. After a period of incubation, washing and lavation is used to remove free substances and the ones which did not participate in the reaction. Next, Chromogenic substrate is added to alkaline phosphatase to determine the amount of enzyme attached to the solid phase; or O-Phenylene diamine (OPD); for the enzyme of peroxidase, zino 3-D, Ethyl benzenesulphonate or Tetra-methyl-benzidine is used. Then, the obtained color results were measured and evaluated by means of spectrophotometer or by comparing them with visual standards (Gilbert and Anklame, 2002).

The competitive ELISA method was used to measure aflatoxin B_1 in the gathered samples. Antibody was pasted to the bottom of the available sinks in the kit against aflatoxin B_1. The tests and standard should be conducted in a duplicate manner. Hence, there were enough sinks for 40 tests (Gilbert and Anklame, 2002).

Statistical analysis

The obtained data and results of the experiment were fed into the Excel and SPPSS. SPSS was used to check the probability of a significant and meaningful difference between the mean values. P-value was set at 0.05. T-test was used to compare the mean values.

RESULTS AND DISCUSSION

The tests and experiments of the study revealed the magnitude of contamination of food samples in the four experimented towns in late spring and summer months of the year. The following figure illustrates the contamination of the samples in terms of percentage. As shown in the figure, the highest degree of contamination is related to Salmanshahr town.

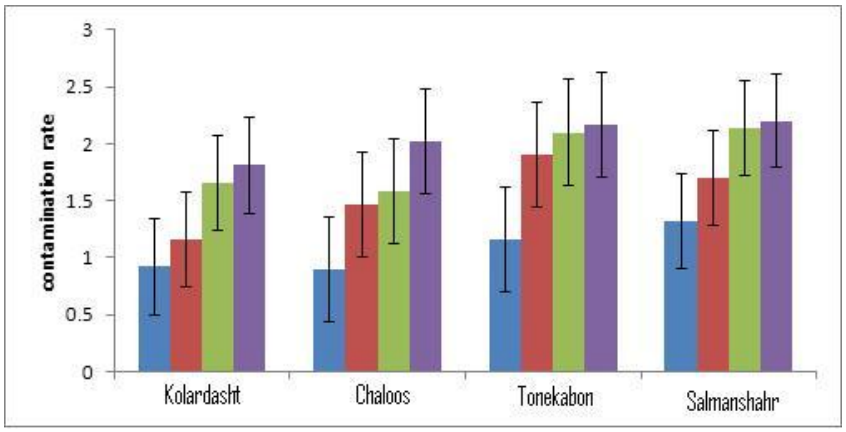

Figure 1. The percentage of soya oil food contaminated by aflatoxin in four towns in different months of summer and late spring (different colors shows different seasons)

This figure illustrates the degree of the contamination of the foods consumed by the poultry in the four experimented towns in different months. It was found that, among all the months in which experiments were done, the highest contamination of the samples were related to September month. According to the results, the contamination of the food products consumed by the poultry was similar for the two towns of Salmanshahr and Tonkabon in the summer. The highest contamination was observed in Salmanshahr town in the late summer. One likely explanation for this finding is that moisture and humidity in Salamnshar town is very high and the animals and poultry consumed foods which were contaminated with aspergillus fungi. Figure 2 depicts the percentage of aflatoxin in the chicken livers of the experimented towns.

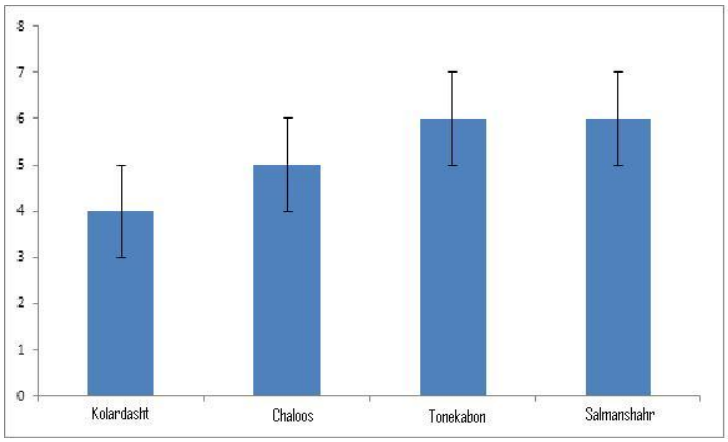

Figure 2. The percentage of broiler livers contaminated with aflatoxin in four studied towns

The results of examining the amount of aflatoxin in chicken livers revealed that the highest degree of infection was attributed to Slamnashahr and Tonkabon towns. That is, 6% of the livers of the slaughtered chickens were infected with aflatoxin in these towns. In the other investigated towns i.e. Chaloos and Kalardasht, the percentage of infection with alfatoxin were 4 and 5 % respectively. According to the view of the Council of Agricultural Engineering and Natural Resources, the reaming amount of aflatoxin in chicken rivers should be less than 20 ppb. This amount is consistent with the measures of aflatoxin in chicken livers based on poultry foods.

In the study done by Miahi *et al.*, (2008) in the slaughterhouses of Ahvaz (a southern city), researchers found that 5.37 % of the livers, 5.22% of the breast and thigh muscles of the slaughtered chickens were infected by aflatoxin. It was found that Liver and breast muscles respectively had the highest and lowest amounts of infection to B_1 and M_1aflatoxins. The statistical analysis of the results revealed that the average remaining amounts of B_1 and M_1 aflatoxins

in liver were significantly higher than those in the breast and thigh muscles of the slaughtered chickens. In general, young animals and chickens are more vulnerable to the harmful effects of these combinations and substances which are indicated by symptoms such as digestive disorders, anemia, paleness, reduction in food consumption and efficiency. Affliction to cancer by aflatoxin has been extensively studied and studies indicate that liver is main organ which is attacked in many varieties of animals.

The results of the present study show that B_1, M_1 and G_2 can cause different kinds of cancer in different verities of animals. The animals which are affected by aflatoxin have reduced appetite for food and the impacts of the toxin can be easily observed. However, in calves and cows, clinical symptoms emerge only when they receive 150 to 600 PPB amounts of aflatoxin. In the extreme form of illness, symptoms such as lethargy, phlegm, abdominal pain, diarrhea, rectal prolapse and finally death are observed. However, in the chronic form of the illness, in addition to liver lesions, reduction in growth, reduction in food consumption efficiency, renal lesions and disorders in the metabolism of proteins and fats can be observed. Aflatoxins are capable of creating acute kidney injuries, cirrhosis and liver tumors in different verities of animals. For example, B_1 aflatoxin can be carcinogenic in many verities of animals including mammals, birds, fish and rodents. According to research studies, the LD of 50 toxins for the majority of verities 5-10 MG/KG has been reported. Aflatoxins are metabolized in the livers of animals and the high concentration and density of them can cause liver diseases and finally bring about death for animals. However, low concentrations and densities of aflatoxins can lead to effects such as birth defects, breeding of the muscles and reduction of activities of the immunity system.

In the summer months when the temperature and moisture are high and in extremely cold areas where the temperature difference between day and night reaches plus and minus eight, poor nutrition starts which is due to disorders of metabolism; these conditions enhance the risk of animal death above the average level. Since animals are accustomed to these conditions, their excrement becomes hard and stiff or diarrheal which can result in abortion, reduction in egg-laying or milk and the animals are infected by toxins which are produced by fungi. Sequentially, their nutrition becomes weak and some disorders in the metabolism and reproduction of animals are created and their organs are hurt. Kidney, pancreas and lymph nodes are injured. Their alimentary canal is necrotized and the mouths of the animals are injured. The laying of egg and milk production are reduced and the infection is permeated. Aflatoxicosis in poultry results in major economic losses.

The clinical symptoms of Aflatoxicosis include anorexia, reduction in weight, and reduction in laying egg, bleeding, loss in fetuses, and an increase in the sensitivity against stress-causing and microbial factors. Aflatoxicosis mainly appears in broiler chickens in chronic forms and causes a delay in growth, reduction in body weight, increase in food conversion coefficient, paralysis and lameness. Intoxication and poisoning in egg-laying chickens and mother hens can always be indicated by symptoms such as reduced production, reduced size and weight of the eggs and reduction consumption of food. Also, intoxication of mother hens can be indicated by a reduction in the hatching of eggs. When chickens are intoxicated by aflatoxin, the liver becomes inflated and pale and kidney looks inflated and congested. As a result of intoxication, thymus and bleeding can be seen on the muscles of thigh. Furthermore, the amount of cholesterol, glucose, calcium, phosphor and blood iron will be reduced. Dietary intake and consumption of food which infected with mycotoxins (toxins of fungi) in poultry and birds lead to hemorrhagic syndrome and affects the process of hematopoietic; this syndrome develops as hemorrhagic lesions occur in important organs and muscles. Egg yolk and skin color are affected by Hypo carotenoids.

The consumption of rations infected by these toxins in animals leads to a decline in their range of growth, disorders in nervous systems, irritation of digestive system and bleeding. Also, infertility has been reported in some animals. Low food consumption rate in the animals infected with these toxins has been reported to weaken the immunity system. It has been demonstrated that there are toxin-causing fusariums in corn seeds in different provinces of Iran. At the present time, poultry and animal rations in Iran are prone to be seriously affected and infected by these toxins. Recent studies indicate that available nutrients in rations include proteins, fats, rare elements, additives such as antibiotics and preservatives reduce the toxic effects of aflatoxins. At the moment, the majority of food materials consumed by the poultry and animals in our country (Iran), especially those such as corn, barley and fish meal, etc., which is imported from ports are infected by (mycotoxins) while they are transported and imported into the country. The significant point in this regard is that major ports of the country are all located in hot, humid and tropical areas which multiply the

problems. While using the food products, one should notice that those materials which are known or are highly likely to be infected by toxins should not be used. Using materials in the production and distribution stages of food for preventing and hindering the growth of fungi by absorbing their resulting toxins in primary ingredients and the grains are considered as essential preventive steps. If mycotoxicosis is likely and probable to be present, it is recommended that the herds and flocks should not be given grains for six hours on three consecutive days. If the respiratory symptoms and losses tend to diminish, it can be assumed that the presence of mycotoxicosis in the herd is considered to be certain. In some methods of controlling mycotoxicosis in broiler flocks, when the broilers reach the 14-day stage, grains are not given to them for a couple of hours. Also, certain materials are added to the grain which absorbs mycotoxicosis to them. Hence, the grain is free from mycotoxicosis. Since the required conditions for the growth and outbreak of molds are present in northern areas of Iran where temperature and moisture are remarkably high, it should be considered that the probability of the presence of fungi and the resulting toxins (aflatoxins) in the foods consumed by birds and poultry especially soya oil food is remarkably high.

Appropriate plans and managing measures should be taken in the transporting, storing and maintaining stages to prevent the excessive increase of aflatoxins in food materials of poultry and birds. In a study Taj Karimi (2011) investigated the presence of aflatoxins in the milks of cows in west Mazandaran (a northern province in Iran), researchers found that the degree of aflatoxins in the milk of the cows is beyond the acceptable limit and the amount of aflatoxins is different in different areas of the province.

CONCLUSION

The results of the present study indicated that the highest level of aflatoxins is related to September month. Based on the findings of the study, the highest infection of soya food to aflatoxin was related to the samples of the town of Slamanshahr. This finding can be explained by the high humidity of this town during the summer months, especially in September. Furthermore, the investigation of the aflatoxin level in the livers of broilers revealed that the highest amount of aflatoxin in the reared chickens were related to the ones in the towns of Salmanshahr and Tonkabon where 6 % of the sampled livers were found to be infected by aflatoxins.

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