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RESEARCH ARTICLE

Detection of the Main Mycotoxins in Feed of Horses in Al-Zawra'a Park and Study their Effects on Hematological Feature

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ABSTRACT

The aim of the current study is to detect fungi and mycotoxins presence in horse's feed and to determine the effect of these mycotoxins on haematological feature in these animals. Twenty five samples of horse's grains were obtained from Al-Zawra'a Park in Baghdad during the period of November 2016 to April 2017 for isolation of fungi, by using Sabouraud dextrose Agar and incubated at 28±2 ºC for (3-7) days. Then, detection of mycotoxin by Elisa in two seasons, winter and summer. Blood samples were collected from 20 horses in Al-Zawraa Park in Baghdad for determination of Red blood cell count, Hemoglobin concentration, packed cell volume determination, Total leukocyte count and differential leukocyte count for lymphocytes and neutrophils by using device for haematological analysis. The present study reported 92% of fungal contamination in the grains of horses that collected from Al-Zawra'a Park. and demonstrated highly significant variation between seasons with ($P<0.01$) by expression the mycotoxin contaminated feed of horse is very common in Summer than in Winter particularly with Ochratoxin A. Heamatologically, the study showed highly significant difference in 20% of horses suffering from anemia (6.38 ± 0.72, 8.25 ± 0.59, 24.70 ± 2.07 for RBCs,Hb and PCV respectively) and 100% suffering from leukopenia with ($P<0.01$). In conclusion, routine inspection of horse feeds should be done for fungal growth. Fungi identification procedures and tools in addition to valid testing can be used to detect the potential of ochratoxin A, besides, monitoring of the presence of fungi and mycotoxins in foods of animal is very important as considerations of their hazards for health.

Keywords: Mycotoxins, horses feed, Hematological paramaters, Ochratoxin A, Iraq.

INTRODUCTION

Mycotoxins are considered to be secondary metabolites that are structurally have diverse toxic ability and usually produced by a wide range of moulds which infesting different agricultural commodities [1, 2]. It was found that moulds that contaminate food grains not only cause spoilage, but also they can turn them to be toxic as a result of mycotoxins elaboration that are known to be important bio-agents and are responsible for variety hazards of man and animal health [3,4].Mycotoxins, as it is known, are present in environments where they are developed from. Such environments are feed, cereals and food that are based on grain. It was found that high substrate humidity which is usually associated with the processing, ambient temperature, type of food and production or storage are considered to be the main environmental factors that contribute to the mycotoxins occurrence [5,6].Bacelar *et al*., (2016)[7] mentioned that the most significant mycotoxins in food industry and agriculture are from the six classes that are widely spread in human food and animal feed. These classes are: ochratoxins (OTs), aflatoxins (AFs), zearalenone (ZEN), T2 toxin, deoxynivalenol (DON) fumonisins (FBs) and Patulin .These mycotoxins have various effects that are rendered them to be hepatotoxic, carcinogenic, neurotoxic, nephrotoxic, and teratogenic. Besides, in according to the type of mycotoxin involved, they can impair immune system [8,9,10].

Furthermore, different mycotoxins or more than one mycotoxin can contaminate the typical feed of animal that usually formed from several sources. These mixed feeds may possess all the mycotoxins that found in several individual ingredients [11].liver physiological, biochemical and haematological changes, in addition to animals growth depression may be induced by the consumption of diet that is contaminated with multiple mycotoxins [12].Many studies had demonstrated that swine, cattle and poultry feeds had been extensively screened for mycotoxins [13]. On the other hand, few researches had been focused on mycotoxins in the foods of horse because of the rare interest of the productivity of such animals. So, this research was performed to detect fungi and mycotoxins presence in horses feed and to determine the effect of these mycotoxins on haematological features in these animals.

MATERIALS AND METHODS

Source of samples

The samples of horses' grains had been collected from Al-Zawraa Park in Baghdad during the period of November 2016 to April 2017. Twenty five samples with 150 gram were taken and each one was divided into 3 parts, so the total cultivated samples were 75. Each sample subjected to surface sterilization by 2% sodium hypochloride for isolation of fungi by using Sabouraud dextrose Agar (BDH England) then incubated at 28±2 ºC for (3-7) days according to [14].

Detection of mycotoxins in the samples of horses' grains

The other parts of sample were used for detection of mycotoxin by Elisa technique in Veterinary Directorate - Central Veterinary Laboratories and researches Department- Public health and food safety Laboratory in two seasons, winter and summer.

Hematological parameters analysis

Blood samples were collected aseptically from jugular vein of 20 horses in Al-Zawra'a Park in Baghdad and using EDTA-contained tubes for determination of haematological parameters.Red blood cell count, packed cell volume, Hemoglobin concentration, Total leukocyte count and differential leukocyte count for lymphocytes and neutrophils were determined by using device for haematological analysis called Human Count 60 VET(Germany).

Statistical Analysis

The Statistical Analysis System- SAS [15] program was used to effect of difference factors in study parameters. Chi-square test was used to significant compare between percentage and T-test was used to significant compare between means in this study.

RESULTS

Results of fungal isolation

The present study reported high percentage of fungal contamination in the grains of horses that collected from Al-Zawraa Park in Baghdad which reached to 92% represented by isolation of 69 fungal isolates from the total cultivated samples were 75. These isolates were classified into 6 types of important molds as shown in table (1).

Detection of mycotoxins in the samples of horses' grains

All samples of commercial horse feed were analysed quantitatively by Elisa technique for detection three different mycotoxins which are Aflatoxin B1, Ochratoxin A and T2 toxin in two seasons, winter and summer. The results demonstrated highly significant variation between seasons with ($P<0.01$) by expression the mycotoxin contaminated feed of horse is very common in Summer than in Winter (Table 2) particularly with Ochratoxin A, While the levels of other two mycotoxin were lower than the critical or even toxic level.

Hematological parameters analysis

Twenty horses were taken to detect the effect of mycotoxins on their haematological feature. These animals were divided according to the sex as in table 3.The current study expressed highly significant difference ($P<0.01$) in 20% of horses suffering from anemia (table 4) through detection the number of circulating erythrocytes, packed cell volume and hemoglobin concentration which revealed to decrease significantly($P < 0.05$) when compared with control animals(table 5).On the other hand, There was a marked significant reduction in total leukocytes count ($P<0.01$)which was observed in all animals that fed on contaminated grains with 100% (table 6), Whereas there was a significant changes observed between differential leukocyte count in neutrophil and lymphocyte which appeared as increase in neutrophil and a decrease in lymphocyte counts ($P<0.05$)when compared with control group (table 7).

DISCUSSION

The main two fungi groups of the major concern in the equine world are field fungus *Fusarium*, that has the ability to produces zearalenone, fumonisin and T2, and the storage fungus *Aspergillus*, that is responsible for aflatoxin and ochratoxin production [16]. Aflatoxicosis with its deleterious toxicological effects in horses had been focused on in many studies according to their risk [17, 18]. While [7] highlighted on the Equine leukoencephalomalacia (ELEM) which is demolished neurologic system of equidae that is attributed to contaminated corn consumption with one or more types.According to our knowledge, there is little or no studies were carried out to detect the impact of food-contaminated mycotoxins on the health and particularly haematological features in Iraqi horses in Baghdad. So the current study was performed to detect 3 types of mycotoxins in feed of horses but the result express only Ochratoxin A (OTA) has significant value when compared with limited value (2ppb) in the summer season whereas Aflatoxin B1 and T2 toxin expressed below the limited level (20ppb and 150ppb) respectively inspite of the Aflatoxin showed in near with limited value. This result may be attributed to environmental conditions efficacious and nutritional

necessities for growth and production of OTA which in constant with [19] how investigated the impact of temperature and relative humidity on production of ochratoxin A and found that the relative humidity has less significant role than temperature in OTA production. which elucidate the finding of the present study about OTA production in summer increase significantly. Morever, Ali *et al*., (2013)[20] studied the effect of varies degrees of temperature on OTA production in common cereals by Aspergillus spp. and the results showed the production is temperature dependent and 30ºC was the most favorable temperature to produce significant quantity of OTA as compared to other temperatures.

On the other hand, Hashem *et al*., (2015)[21] reported the formation of OTA from *Aspergillus carbonarius* had relationship with nitrogen, carbon sources and water activity. Dhanasekaran *et al.,* (2011)[22] pointed to the maximum temperatures for Aflatoxin production have been reported to be 40-42^{0}C that may be explain the finding of this study in which the Aflatoxin B1appear lower than the embarrassing level, Also, it was found that when Aflatoxin B1 mixed with OTA in feed contamination, OTA prevents the major impact of Aflatoxin then decrease the chance diagnosis of aflatoxicosis in feed.Concerning to the sex of animal, the present study showed that the dealing with 60% of animals were mares. This subject has major importance because it was found that teratogenicity caused by natural occurrence of OTA and it's transferring through horse placenta as firstly discovered by [23] who mentioned that the more sensitive animals were monogastric species. This return to the OTA kinetic which vary between horses and ruminant in which the OTA degraded in rumen by enzymes while in monogastric like horse, OTA is absorbed from the gastrointestinal tract without, or with little, prior degradation as mentioned by [24, 25].

The current study aimed to investigate the effect of OTA on haematological feature in horses and the results showed 20% of animals have anemia due to marked decrease in number of circulating erythrocytes, packed cell volume and hemoglobin concentration significantly. This finding is in line with [26] who found that the ochratoxin A induced Iron deficiency anemia and does not cause syndrome of hemorrhagic anemia, whereas the anemia usually caused by deficiency of nutrition that resulted from mycotoxin. While in poultry, it is documented that the OTA cause anemia as reported by [2].Regarding to leukopenia in this study was observed in all tested animals that fed on contaminated grains with 100%, this result agree with [27] who revealed that white blood cells number was decreased when mice fed on OTA due to spleen and lymph nodes necrosis which in turn, led to decrease lymphocytes proliferation and production of cytokines.In addition, feeding OTA contaminated diets may increase the severity of secondary infections in animals causing increase in neutrophil count [28] which in constant with the present result.

CONCLUSION

As a conclusion, routine inspection of horse feeds should be done for fungal growth. Fungi identification procedures and tools in addition to valid testing can be used to detect the potential of ochratoxin A, besides, monitoring of the presence of fungi and mycotoxins in foods of animal is very important as considerations of their hazards for health.

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Table 1: No. of samples that give +ve result for fungal contamination

Spp.	No.	Percentage (%)
Penicillium spp.	31	44.93
Alternaria spp.	16	23.19
A. ochraceus	10	14.49
A. flavus	8	11.59
Mucor spp.	2	2.90
Rhizopus spp.	2	2.90
Total	69	100%
Chi-Square (χ^2)		12.438 **
** (P<0.01).		

Table 2. Detection of Ochratoxin A in winter and summer season (PPb)

Season	No.	Mean ± SE
Winter	4	2.55 ± 0.19
Summer	14	19.62 ± 1.07
T-Test	---	4.217 *
* (P<0.01).		

Table 3. Division of animals according to sex.

Sex	No .	Percentage (%)
Male	8	40.00
Female	12	60.00
Total	20	100%
Chi-Square (χ^2)	---	8.250 **
** (P<0.01).		

Table 4. Distribution of cases according to Anemia

Cases of Anemia	No .	Percentage (%)
+ve	4	20.00
-ve	16	80.00
Total	20	100%
Chi-Square (χ^2)	-b--	13.00 **
** (P<0.01).		

Table 5. Effect of ochratoxin A on parameters associated with anemia

The Group	Mean ± SE		
	RBCs (x 10^{12})	Hb (g/dl)	HCT (%)
Cases	6.38 ± 0.72	8.25 ± 0.59	24.70 ± 2.07
Control	8.70 ± 0.50	13.80 ± 1.60	37.10 ± 4.20
T-Test	1.273 *	2.884 *	6.603 *
* (P<0.05).			

Table 6: No. and percentage of cases suffering changing in WBC, Lymphocyte and Neutrophil

Parameters	Total No.	Decreased No. (%)	Increased No. (%)	Chi-Square (χ^2)
WBCs	20	20 (100%)	0 (0.00%)	15.00 **
Lymphocyte	20	14 (70.00%)	6 (30.00%)	10.75 **
Neutrophil	20	3 (15.00%)	17 (85.00%)	13.63 **
** (P<0.01).				

Table 7. Compare between differential count of Lymphocytes and Neutrophill with control

The Group	Mean ± SE	
	Lymphocytes (%)	Neutrophill (%)
Cases	18.56 ± 4.17	72.08 ± 6.22
Control	61.00 ± 5.00	33.00 ± 2.94
T-Test	12.461 *	17.935 *
* (P<0.05).		