CORRELATION BETWEEN ASPERGILLOSIS AND RENAL FUNCTION PROFILE ANALYSIS IN BROILERS OF DIYALA PROVINCE – IRAQ

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ABSTRACT

Current study designed for Isolation of *aspergillus spp*. from feed and evaluation of their pathological effects on kidney function. Fifty commercial poultry feed samples contain pellets, soybean and yellow grain were collected. Fifty grains from each sample were sterilized by 2% sodium hypochlorite solution. Dried grains were placed on Sabouraud dextrose agar medium to obtain pure culture. Four groups of broilers were exposed to aspergillus positive feed for 15, 30 and 45 days. Then blood samples were collected from wing vein to Blood urea and creatinine and kidney was taken for histopathology. Pellets were contaminated with *A. flavus* (20%), *A. niger* (14%), *A. fumigatus* (4%), *A. ochraceus* (2%), *A. terreus* (8%). soybeans were contaminated with *A. fumigatus* (4%), *A. flavus* (2%),

Reduction in blood urea level (BUN), in treated group after 15 day of exposure to contaminated feed compared with control group with significant difference (p value<0.05). After 30 days of exposure, BUN was increased without significant difference (p value>0.05) compared with zero time. After 45 days of exposure, BUN continue in elevated level without significant difference (p value>0.05) compared with zero time.

There is slight increase in serum creatinine level among exposed group after 15 day of exposure to contaminated feed compared with control group with significant difference (p value<0.05). After 30 days of exposure, serum creatinine level among exposed group come closely to its level in zero time without significant difference (p value>0.05) compared with zero time. After 45 days of exposure, serum creatinine level continue in elevated level without significant difference (p value>0.05) compared with zero time. Kidney tissue showed severe damage characterized by hemorrhage with interstitial nephritis and edema especially in and around glomeruli with damage in the ascending and descending tubules. The tubular epithelium showed vacuoles in the cytoplasm with pyknotic nuclei.

In conclusion, exposure to aspergillus spp. contaminated feed consider as important problem that required monitoring of proper poultry production. *A. flavus, A.*

niger were, the most frequent contaminants, the least one was *A. ochraceus. A. flavus, A. terreus* were detected mainly in pellets. Soybean was contaminated mainly with *A. fumigatus* and *A. terreus*. Kidney tissue appear to be affected by exposure to aspergillus and mycotoxins, creatinine and blood urea affected mainly in prolong exposure.

Key words: Aspergillosis, broiler, renal function profile, Iraq.

INTRODUCTION

Aspergillosis is one of the major causes of death-rate in immunodepressed as well as immunocompetent birds. Aspergillus species is a ubiquitous saprophytic mold with a worldwide distribution and is the common opportunistic mycotic infection of respiratory tract in birds causing high morbidity and mortality (Tell, 2005 ; Ben-Ami *et al.*, 2010). Thus inducing a significant economic losses especially in poultry (Tell, 2005).

The clinical findings of acute aspergillosis is usually observed in young birds, whereas chronic aspergillosis is more frequently observed in adults (Tell, 2005 ; Charlton B. R., 2008). The clinical signs depend on the organs or systems attacked. The major site for involvement is the pulmonary system, with lesions observed in the air sacs and lungs of a wide range of bird species, which leave the hosts potentially susceptible to infections by *Aspergillus spp*. (Charlton B. R., 2008). Clinical forms of aspergillosis have already been diagnosed in chickens (Ceolin *et al.*, 2012), turkeys, geese, penguins, ostriches, rheas (Spanamberg *et al.*, 2016), and many other species (Spanamberg *et al.*, 2012).

Inhalation of *A. fumigatus* conidia can cause a spectrum of manifestations dependent upon the immunological status of the host (Ben-Ami *et al.*, 2010), in addition to physiological and anatomical predisposing factors (Leishangthem *et al.*, 2015).

Aspergillosis is non-contagious disease of avian. The disease occurs under immune compromised situations of the host or when the bird is exposed to an overwhelming number of spores. Stress is the major predisposing factor for the development of the disease (Girma *et al.*, 2016). Aspergillosis is a frequent mismanagement problem in commercial and back yard poultry. The disease primarily affects lower respiratory system (Mark *et al.*, 2008). *Aspergillus spp* have the ability to penetrate egg shell and infect the embryo. The infected embryo may die or hatch with well-developed lesion. large numbers of spores are released, If

infected eggs are broken, which cause contamination for the hatchery equipment (Girma *et al.*, 2016). Aspergillosis come in acute or chronic form. The acute form generally occurs in young birds and resulting in high morbidity and mortality. The chronic form is sporadic and it leads to lesser mortality and generally affects older birds with compromised immune system due to poor husbandry condition (Mark *et al.*, 2008). Poor sanitation in the house and food contamination enhance Aspergillus growth. Poor ventilation in conjunction with previous factors increase the possibility of invasion and infection for the respiratory system by air borne spore.

Current study designed to fulfill the following aims:

Isolation of the causative agents of aspergillosis from feed and evaluation of gross and histopathological changes in kidney of infected chickens also evaluation of pathological effects of aspergillosis on kidney enzymes.

MATERIALS AND METHODS

Sample collection

Poultry feed samples (n=50) comprising of commercially prepared feed were collected over a period from September 2016 to March 2017. Samples were collected at regular intervals evenly spread over the study period. The samples of poultry feeds were collected from feeds present at the farms contain pellets, soybean and yellow grain. For each sample, 3 kg feed was collected from each of the three different areas of a lot. After thorough mixing a composite sample of 1kg was kept in a polythene bag and stored in refrigerator prior to inoculation onto culture media every week.

Methods

Isolation of Fungi

Fifty grains from each maize sample were surface-sterilized by immersion in 2% sodium hypochlorite solution in 250 ml conical flask for one minute, and then washed three times using sterilized Distilled water. The grains were dried by using sterilized filter paper and placed on Malachite green agar 2.5 and SDA medium containing chloramphenicol (50 mg L^{-1}) using five Petri plates for each sample (5-10 grains each $plate^{-1}$). After incubation at 25 °C for seven days, the fungi were isolated and sub-cultured to obtain pure culture. All fungi were identified by morphological characteristics on SDA (Domsch *et al.*, 2007).

Direct Examination of Aspergillus

Specimen was placed on a microscopic slide, a cover slip added and warmed over a small flame just before boiling. The slide was examined under the low power and high dry objectives to detect fungi and their septate hyphae (McClenny, 2005).

Culturing of Aspergillus spps.

Sabouraud Dextrose Agar (SDA) supplemented with 0.04 mg ml^{-1} chloramphenicol to inhibit the growth of bacteria, then incubated at 37 °C and examined for (McClenny, 2005).

After seven days of incubation, plate was observed for macroscopic characteristics such as colony diameter, exudates, colony reverse and the isolates were identified to the species level on the basis of microscopic characteristics (Diba *et al.*, 2007). For microscopic characteristics slides were stained with lactophenol cotton blue (Fischer and Lierz, 2015) with using adhesive tape preparation in which a small piece of transparent-adhesive tape was touched to the surface of the suspected colony, and then adhered to the surface of a microscopic slide (McClenny, 2005). Photographs were taken with digital microscope camera.

A morphological examination of species was first made with naked eye and at low magnification power of microscope after that detailed examination was done according to (McClenny, 2005) by measuring the dimensions of the microscopic structures, photographing the microscopic structures and using relevant literature (Diba *et al.*, 2007).

Scotch Tape Preparation

A drop of lacto phenol cotton blue was put on the clean microscope slide. The ends of a transparent adhesive tape were hold between the thumb and index finger, the center adhesive side of the tape was pushed gently to touch the surface of the colony to collect spores and spread on to the drop on the microscopic slide (Harris, 2000).

Slide Culture Technique

A small block of SDA was cut either by a sterile scalpel blade or by heated and sterile mouth-less tube, then removed by a sterile loop to the surface of clean sterilized slide and put in a sterile petri dish containing V-shaped glass tube that served as support or bed for microscopic slide. Round piece of filter paper was placed under the V-shaped glass tube. The sides of agar block was inoculated with

fungus to be cultured. A sterile cover slip was applied on to the surface of the agar block and few drops of sterile D.W was added to the bottom of plate before incubation to give enough moisture for fungal growth and prevents agar block from drying out. The plates were incubated at 25ºC for seven days. The slide was examined under microscope, to observe hyphae (Carter and Cole Jr, 2012).

Biochemical Assays for detection of renal function Serum creatinine and Urea were assayed using ELISA.

Serum creatinine: Test procedure according to Designs, 2017:

1. A 25 µl of water was Pipetted into the blank wells.
2. A 25 µl of Assay diluent was Pipetted to all wells used.
3. A 25 µl of standards was pipetted into the bottom of the appropriate wells.
4. A25 µl of samples was pipetted into the bottom of the appropriate wells.
5. A 100 µl of creatinine detection reagent was Pipetted into each well.
7. The plate was incubated at room temperature.
8. Reading of optical density at 490 nm after 1 minute.

Blood Urea: Test procedure (Cell Biolabs, 2017):

a) A10 µl of the diluted urea standards or samples was added to the 96-well microtiter plate wells.

b) The Urease enzyme was reconstituted prior to use at 4 mg mL^{-1} in the Ammonia Reagent solution and mixed thoroughly until dissolved (eg. For a 10 mL solution or 100 assays, add 40 mg of Urease to 10 mL Ammonia Reagent.

c) A 100 µl of the Urease/Ammonia Reagent mixture was added to each well using a multichannel pipette. Then mixed thoroughly and carefully so as not to create foaming in the well.

d) The plate was incubated for 10 minutes at 37 ºC.

e) A 100 µl of the developing reagent was added to each well using a multichannel pipette and the solution was mixed thoroughly and carefully so as not to create foaming in the well, then incubate d for 30 minutes at 37 ºC.

f) Reading the plate at 580-630 nm.

Histopathology

kidney was removed from affected birds and but in 10% buffered formalin then embedded within paraffin wax and cut in 4 µm sections for staining with Haematoxylin and eosin.

Statistical analysis

All analysis was performed using the statistical package (SPSS) version thirteen. The data were expressed as mean ± standard deviation; percentage. T- test, person chi square, person correlation coefficient were used to analyze the data. Results were determined as very high significant at (P<0.001), high significant (P<0.01) and significant at (P< 0.05) and non-significant at (P> 0.05).

RESULTS AND DISCUSSION

From the three types of feed under investigation as shown in table 1, *A. niger* was isolated from 7 pellet samples only (14%). Macroscopic appearance of colonies on SDA, initially is white, becoming black later on giving "salt and pepper appearance" which results from darkly pigmented conidia borne in large numbers on conidiophores and reverse turning pale yellow shown in figure (1. A and B).

Table 1. Frequency of Aspergillus spp. isolated From Feed

Aspergillus spp. isolates		Feed samples			Total	χ2	P value	R	P value
		pellet	Yellow grain	soybean					
A.niger	negative	37(74%)	3(6%)	3(6%)	43(86%)	1.110	>0.05	-0.140	>0.05
	positive	7 (14%)	0(0%)	0(0%)	7 (14%)				
	Total	44(88%)	3(6%)	3(6%)	50(100%)				
A.flavus	negative	34(68%)	3(6%)	2(4%)	39(78%)	1.084	>0.05	.002	>0.05
	positive	10(20%)	0(0%)	1(2%)	11(22%)				
	Total	44(88%)	3(6%)	3(6%)	50(100%)				
A. fumigatus	negative	42(84%)	3(6%)	1(2%)	46(92%)	15.003	0.001	0.467	0.001
	positive	2(4%)	0(0%)	2(4%)	4(8%)				
	Total	44(88%)	3(6%)	3(6%)	50(100%)				
A. ochraceus	negative	43(86%)	3(6%)	3(6%)	49(98%)	0.139	>0.05	-0.050	>0.05
	positive	1(2%)	0(0%)	0(0%)	1(2%)				
	Total	44(88%)	3(6%)	3(6%)	50(100%)				
A.terreus	negative	40 (80%)	3(6%)	3(6%)	46(92%)	0.593	>0.05	-0.103	>0.05
	positive	4(8%)	0(0%)	0(0%)	4(8%)				
	Total	44(88%)	3(6%)	3(6%)	50(100%)				

Many slides were prepared from pure culture of *A. niger* and examined after staining with lactophenol cotton blue. The microscopic characters were non-branched conidiophore with bulb end carries conidia like sun rays as shown in figure (1. C).

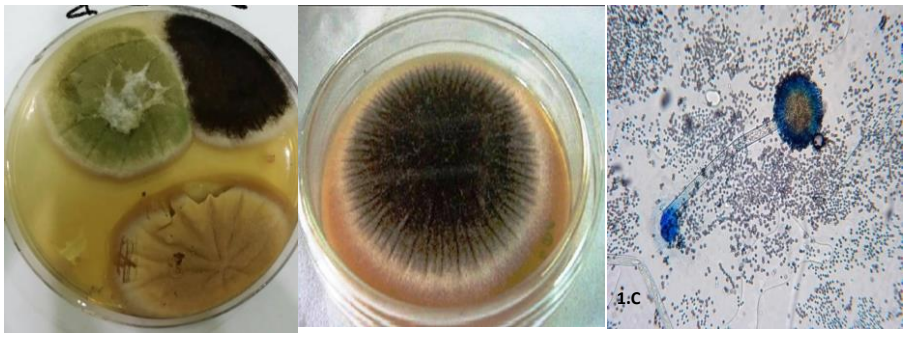

Figure 1. Growth of *A. niger* isolated from pellets feed on SDA medium at 28 °C for 7 days: A) mixed culture, black colony, upper right corner, B) pure culture, C) Microscopic appearance of *A. niger* isolated from pellets feed, stained with lactophenol cotton blue, showing non-branched conidiophore with bulb end carries conidia like sun rays (40 X)

A. flavus was isolated from 10 (20%) pellet samples and 1(2%) from soybean only. The samples cultured on SDA and then sub cultured on Czapek Solution Agar, the culture characters were determined according to (Hoekstra *et al.*, 1984 ; Williams-Woodward, 2001 ; Watanabe, 2010). The diameter of colonies was 3-5 cm after one week of incubation at 28 °C, topography flat, texture floccose to granular, the isolate was grown with green color on PDA but it was yellow – green on czapek solution agar and the color becoming dark yellow–green, reverse creamy in color, (Figure 2. A and B).

Many slides were prepared from pure culture of *A. flavus* and examined after staining with lactophenol cotton blue. The microscopic characters were conidia round to elliptical 3-6 µm smooth or finely roughened, conidiophore roughened stalk and vesicles were relative globose in shape, these characters were identical to those which mentioned in the key by (Williams-Woodward, 2001 ; Watanabe, 2010) as in figure (2. C).

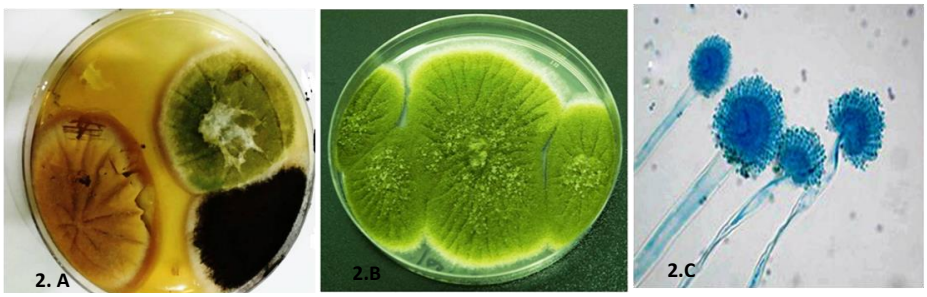

Figure 2. Growth of *A. flavus* isolated from pellets feed on SDA medium at 28 °C for 7 days. A) mixed culture. B) pure culture. C) Microscopic appearance of *A. flavus* isolated from pellets feed, stained with lactophenol cotton blue, showing conidial head (40 X)

A. fumigatus was isolated from 2 (4%) pellet and soybean samples equally. Upon culturing on SDA, colonies of *A. fumigatus* appear fast grower; the colony

size can reach 7 cm within a week when grown on SDA at 37 °C, the colony seems powdery, the color at the first seems to be white then turning to dark greenish and changed to gray, reversed side of the colonies appeared pale yellow to tan (Figure 3. A and B). Microscopic examination of *A. fumigatus* as shown in figure (3. C) appeared conical-shaped terminal vesicles, uniseriate row of phialides on the upper two thirds of the vesicle. Conidiophore stipes are short, phialides arrange uniseriate upper vesicle conidia and parallel to axis of conidophore, produced in chains of spore basipetally from phialides, The chains of spore are borne directly in the absence of metulae and represented by septet and branching hyphae.

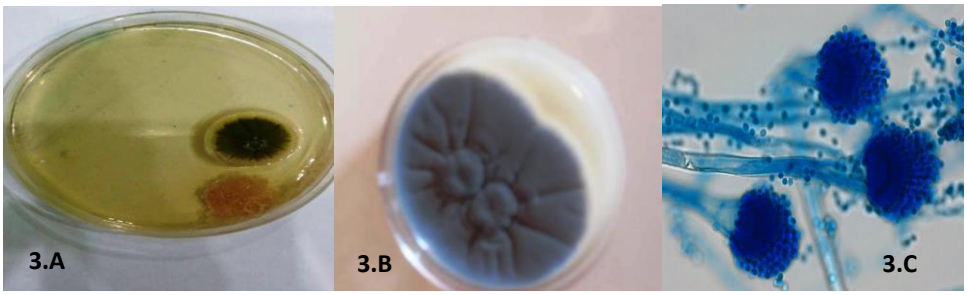

Figure 3. Growth of *A. fumigatus* isolated from pellets and soybean containing feed on SDA medium at 28 °C for 7 days. A) Mixed culture. B) pure culture. C) Microscopic appearance of *A. fumigatus* isolated from pellets feed, stained with lactophenol cotton blue, showing uniseriate row of phialides on the upper two thirds of the vesicle. (40 X)

A. ochraceus was isolated from 1 (2%) pellet samples only. On SDA, Colony diameter after 7 days of incubation at 25 ˚C; 40-50 mm. Colonies were orange or cinnamon on SDA with colorless mycelia, lacked exudates and soluble pigments. Reverse colour was yellow (Figure 4. A). Microscopic examination of *A. ochraceus* as shown in figure (4. B) shows distinct globose conidial head.

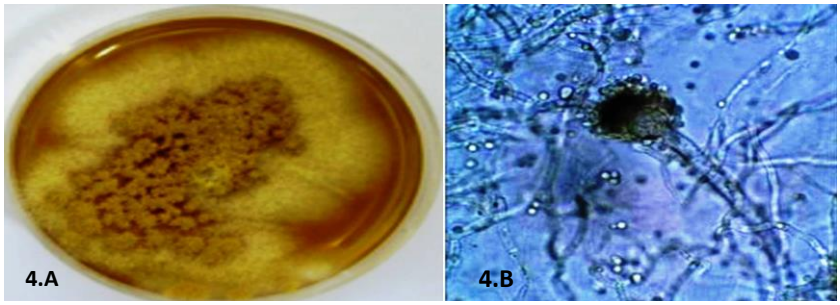

Figure 4. A. Pure culture growth of *A. ochraceus* isolated from pellets feed on SDA medium at 28 °C for 7 days. B) Microscopic appearance of *A. ochraceus* isolated from pellets feed, stained with lactophenol cotton blue, showing distinct globose conidial head. (40 X)

A. terreus was isolated from 4 (8%) pellet samples only. On SDA, Colony diameter after 7 days of incubation at 25 ˚C; 40-50 mm (Figure 5. A and B). *A. terre-*

us is brownish in colour and gets darker as it ages on culture media. Microscopically. *A. terreus* has conidial heads that are compact, biseriate and densely columnar. Conidiophores of *A.terreus* are smooth and hyaline. The conidia of *A.terreus* are small, globose-shaped, smooth-walled, and can vary from light yellow to hyaline (Figure 5. C).

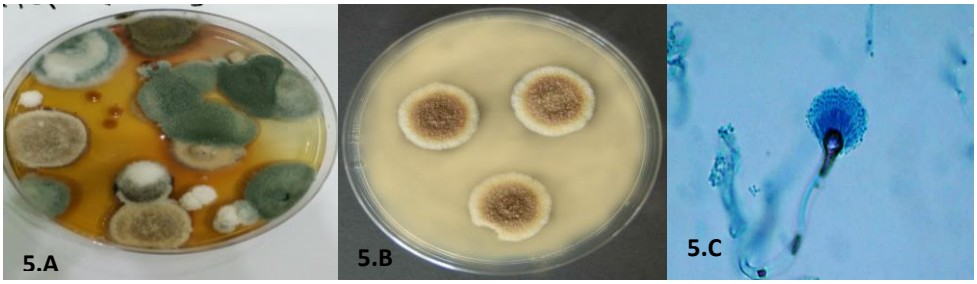

Figure 5. growth of *A. terreus* isolated from pellets and soybean containing feed on SDA medium at 28 °C for 7 days: A) Mixed culture, B) pure culture, C) Microscopic appearance of *A. terreus* isolated from pellets feed, stained with lactophenol cotton blue, showing small, globose-shaped, smooth-walled, and vary from light yellow to hyaline (40 X)

Fungal contamination of animal feed, with the consequent mycotoxins production, is one of the main impendence to human and animal health (Greco *et al.*, 2014). In our study, The isolation rate of *A.niger* was lower than ported by (Accensi *et al.*, 2004) who found that *A. niger* var. *niger* was detected in 23% of the feed samples. In other study (Saleemi *et al.*, 2010), reported that *A. niger* isolated from (37.74%) poultry feed samples in Pakistan. while in Slovakia (Labuda and Tancinova, 2006) reported that *A. niger* isolated from (7%) poultry feed samples. In Egypt (Gouda, 2015), recovered *A.niger* from 2.7% of poultry feed samples.

The isolation rate of *A. flavus* in current study (22%) come closely to (Saleemi *et al.*, 2010) who reported (22.64%) of feed samples in Pakistan. In Slovakia (Labuda and Tancinova, 2006) reported that *A. flavus isolated* from (30%) feed samples. In Nigeria, *A. flavus* was isolated from (91.8%) of commercial poultry feed (Ezekiel *et al.*, 2014). In Egypt (Gouda, 2015), recovered *A. flavus* from 56% of poultry feed samples. In Iran (Ghaemmaghami *et al.*, 2016) reported that *A. flavus* was the predominant species which isolated from 64.3% of feed samples, mainly from corn (46.6%), soybean meal (72.7%) and feed before and after pelleting (75%).

The isolation rate of *A. fumigatus* in current study (8%) come in line with (Labuda and Tancinova, 2006) in Slovakia and higher than (Saleemi *et al.*, 2010) who reported a contamination of (3.77%) feed samples in Pakistan with fungus. In

Iran (Ghaemmaghami *et al.*, 2016) reported that *A. fumigatus* was isolated from 10.7% of feed samples, mainly from corn (7%), soybean meal (23.3 %) and feed before pelleting (9%).

In current study, *A. ochraceus* was isolated from 1 (2%) pellet samples only. On SDA, Colony diameter after 7 days of incubation at 25 ˚C; 40-50 mm. Colonies were orange or cinnamon on SDA with colorless mycelia, lacked exudates and soluble pigments. Reverse colour was yellow (Figure 7 and 3). Microscopic examination of *A. ochraceus* as shown in figure (8 and 4) shows distinct globose conidial head. The isolation rate come closely to (Accensi *et al.*, 2004), who reported that *A. ochraceus* was present in 7.3% of processed poultry feed.

The isolation rate in current study was lower than that reported by (Shareef, 2010), who found that *A. ochraceus* was isolated from 73% of wheat samples, 69% of soya beans samples, while corn samples were in the third order with the lowest percentage of contamination (52%). In Pakistan Saleemi *et al.*, 2010 reported that *A.ochraceus* was isolated from 7.56% of poultry feeds, which is obviously higher than current study. In Egypt, Gouda, 2015, recovered *A.ochraceus* from 2.2% of poultry feed samples. In Iran, Ghaemmaghami *et al.*, 2016 reported that *A. ochraceus* was isolated from 2.4% of poultry feed samples, mainly from corn (6.7%). The isolation rate of *A. terreus* come in line with other studies in Slovakia (Labuda and Tancinova, 2006) in which the fungus was isolated from 8% of poultry mixed feed samples.

In current study, the contamination of poultry feed by Aspergillus species come in consistent with other studies in Brazil (Oliveira *et al.*, 2006 ; Rosa *et al.*, 2006), Slovakia (Labuda and Tancinova, 2006), Spain (Accensi *et al.*, 2004), Pakistan (Saleemi *et al.*, 2010 ; Anjum, 2012), Nigeria (Ariyo, 2013), Argentina (Greco *et al.*, 2014), Egypt (Gouda, 2015), and Iran (Ghaemmaghami *et al.*, 2016), they recorded that the most dominant species isolated from poultry feed samples belonged to the genus Aspergillus in which 33.33% -77% of samples were contaminated with this fungus. The toxigenic fungal contamination of the raw materials occurs during the pre-harvest and/or the postharvest periods, they are exposed during production, processing, transportation, and storage (31). Current result are in accordance with (Accensi *et al.*, 2004 ; Azarakhsh *et al.*, 2011 ; Ghaemmaghami *et al.*, 2016) confirmed that *A. flavus* was the predominant *aspergillus* species isolated from poultry feeds and *A. niger* was the second prevalent species followed by *A. fumigatus and A. terreus.* While the last one was *A. ochraceus.*

The thermo-resistant of *A. flavus* causes of abundantly in poultry feed and the presence in pellet feed may be an indicative of their predominance potentially to produce of afla toxins (Azarakhsh *et al.*, 2011).

As shown in table 2, there is an obvious decreasing in blood urea level (BUN), in treated group after 15 day of exposure to aspergillus spp. contaminated feed (0.0700±0.01732) compared with control group (0.2180± 0.11454), with significant difference (p value<0.05) After 30 days of exposure, BUN start to increase (0.1633 ±.02082) without significant difference (p value>0.05) compared with zero time. After 45 days of exposure, BUN continue in elevated level (2.2500±.17321) without significant difference (p value>0.05) compared with zero time.

There is an obvious slight increase in serum creatinine level in treated group after 15 day of exposure to aspergillus spp. contaminated feed (18.5867±1.01614) compared with control group (18.00±0.0000), without significant difference (p value>0.05). After 30 days of exposure, serum creatinine level among exposed group come closely to its level in zero time (0.1633±.02082) without significant difference (p value> 0.05) compared with zero time, (18.00±0.0000). After 45 days of exposure, serum creatinine level continue in elevated level (20.2500±3.86221) without significant difference (p value>0.05) compared with zero time.

Table 2. Correlation between Aspergillosis and Renal Function Profile

Groups	Days after exposure	Parameters				
		Statistics	Blood Urea Nitrogen BUN (µ moI l^{-1})	T- test P value	Creatinine (µ moI l^{-1})	T- test P value
Control	Zero Time	Minimum	0.06		18.00	
		Maximum	0.36		18.00	
		Mean± SD	0.2180±0.11454		18.00±0.000000	
Treated	15 Days	Minimum	0.05	<0.05	18.00	>0.05
		Maximum	0.08		19.76	
		Mean± SD	0.0700±0 .01732		18.5867±1.01614	
	30 Days	Minimum	0.14	>0.05	18.00	ND
		Maximum	0.18		18.00	
		Mean± SD	0.1633±0.02082		18.00±0.00000	
	45 Days	Minimum	2.10	>0.05	15.00	>0.05
		Maximum	2.50		24.00	
		Mean± SD	2.2500±0.17321		20.2500±3.86221	

ND: Not detected

These results come in agreement with Huff *et al.* (1988) , Stoev *et al.* (2002) , Zahoor-ul-Hassan *et al.* (2010) and Umar *et al.* (2012), on the other hand, Andretta *et al.* (2012) reported that Mycotoxins reduced ($P < 0.05$) the concentrations of uric acid (-31%), creatine kinase (-27%), and creatinine (-23%). Also (Andretta *et al.*, 2012), mentioned that, The variables were affected ($P < 0.05$) by the presence of aflatoxins in diets, with a reduction of 20% for uric acid, 7% for creatine kinase, and 13% for creatinine compared with those of the control groups. The concentration of urea was not affected ($P > 0.05$) by the presence of mycotoxins or aflatoxins in diets. Renal toxicity may be indicated by lower levels of creatinine, as described in previous studies (Harvey *et al.*, 1993).

The decrease observed in uric acid levels can be explained by changes in the efficiency of amino acid utilization, in enzyme systems, in renal filtration, or in reabsorption rates or by unknown aspects (Andretta *et al.*, 2012). However, the level of uric acid appeared increased as evidence of injury in renal tissue, where the substance is produced (Andretta *et al.*, 2012).

Creatinine and urea values increased in a dose related manner by the administration of aflatoxins in chicks. These findings are consistent with studies reported by others in birds, who reported increased level of urea and creatinine during aflatoxicosis in layers (Zahoor-ul-Hassan *et al.*, 2012). Increased serum creatinine and urea levels indicated inflammatory or degenerative changes in the kidney (Umar *et al.*, 2012). Aflatoxin induced nephrotoxicity is assumed to be due to interference with transport function in collecting tubule cells together with diffused impairment of the proximal tubules function (Ortatatli *et al.*, 2005).

Histopathology

In current study, kidney tissue showed severe damage characterized by hemorrhage (Figure 1. A) with interstitial nephritis (Figure 1. B) and edema especially in and around glomeruli (Figure 1. C) with damage in the ascending and descending tubules (Figure 1. D). These findings come in line with Manafi *et al*, (2015), who reported that the lesions of the kidneys among T-2 exposed chickens consisted of mild to moderate congestion and hemorrhages. The tubular epithelium showed vacuoles in the cytoplasm with pyknotic nuclei.

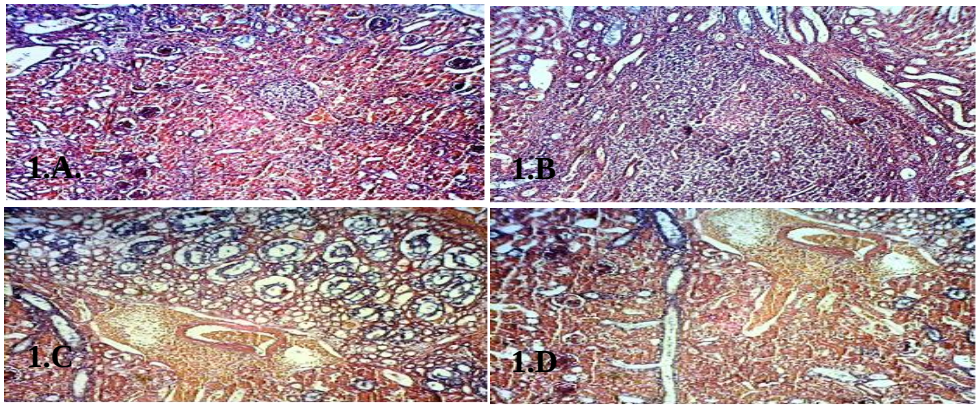

Figure 1. Shows the pathological changes of renal tissue in chicken exposed to mycotoxins: a) Hemorrhage in renal tissue, b) Obvious interstitial nephritis. c) Edema around glomeruli, d) Damage in the ascending and descending tubules

In conclusion, exposure to aspergillus spp. contaminated feed consider as important problem that required monitoring of proper poultry production. A. flavus, A. niger were, the most frequent contaminants, the least one was *A. ochraceus*. A. flavus, A. terreus were detected mainly in pellets. Soybean was contaminated mainly with *A. fumigatus* and *A.terreus*. Kidney appear to be affected by exposure to aspergillus and mycotoxins, Blood urea and creatinine affected mainly in prolong exposure.

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العلاقة بين داء الرشاشيات ووظائف كلية فروج اللحم في محافظة ديالى

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المستخلص

صممت الدراسة الحالية لعزل انوع aspergillus من العلف وتقييم تاثيراتها المرضية على وظيفة الكلية في دجاج اللحم. تم اخذ 50 عينة علف تجاري حاوية على البلت والصويا والذره الصفراء. اخذت خمسون حبة من كل عينة وعقمت باستخدام محلول القصر 2% بعد التجفيف زرعت على وسط Sabouraud dextrose agar.

اربعة مجاميع من دجاج اللحم تم تعريضها للعلف الملوث لمده 15 و 30 و45 يوما ثم جمعت عينات الدم من وريد الجناح لغرض قياس يوريا الدم والكرياتنين وتم اخذ الكلية لمعرفة التغيرات المرضية.

علف البلت كان ملوث بـ *A. flavus* (20%) , *A. niger* (14%), *A. fumigatus* (4%), *A. ochraceus* (2%), *A. terreus* (8%). وعينات الصويا كانت ملوثه ب *A. fumigatus* (4%) *A.flavus* (2%, p value < 0.05). انخفظت BUN في المجموعة المعالجة بعد 15 يوما مقارنة مع مجموعة السيطرة بعد 30 يوما ارتفعت BUN وبلا فرق معنوي واستمرت لـ 45 يوما تزداد مع عدم وجود فرق معنوي مقارنة بمجموعة السيطرة. ارتفع الكرياتنين بعد التعرض للعلف الملوث لمدة 15 يوما مع فرق واضح مقارنه بالسيطرة (p value <0.05). بعد 30 يوما من التعرض اقترب مستوى الكرياتنين من مستواه في مجموعة السيطرة في حين استمر بالارتفاع بعد 45 يوما من التعرض وبلا فرق معنوي، وتميز نسيج الكلية بوجود تدمير واضح ونزف مع التهاب منتشر ووذمة خصوصا حول الكبيبات مع وجود تدمير النبيبات الصاعدة والنازلة التي تعرضت الضهاره فيها الى تفجي ووجود الانوية المنتكسة. تستنتج هذه الدراسة ان تلوث العلف بفطر الاسبرجلس يعد مشكلة مهمة تحتاج متابعة لغرض انتاج الدواجن الجيد. اهم الملوثات كان *A. flavus, A. niger* في حين اقلها *A. ochraceus. A. flavus, A. terreus* التي شخضت في البلت. الصويا كانت ملوثة بـ *A. terreus*. و *A. fumigatus*. اظهرت الدراسه تاثر الكرياتنين وBUN بعد التعرض للعلف الملوث لمدة 15 يوما وخلل في نسيج الكلية.

الكلمات المفتاحية: داء الرشاشيات، فروج اللحم، وظائف الكلى، العراق.