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Correlation Between Aspergillosis And Liver Function Profile Analysis In Broiler.

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ABSTRACT

Current study designed to fulfill the following aims : Isolation of the causative agents of aspergillosis from feed and evaluation of pathological effects of aspergillosis on Liver enzymes. Commercially prepared Poultry feed samples contain pellets, soybean and yellow grain (n=50) were collected from September 2016 to March 2017. Fifty grains from each maize sample were surface-sterilized by immersion in 2% sodium hypochlorite solution. The grains were dried by using sterilized filter paper and placed on SDA medium to obtain pure culture. Four groups of broiler chicken were exposed to aspergillus positive feed for 15,30 and 45 days. Then blood samples were collected from wing vein to determine Alkaline Phosphatase (ALP) , Aspartate Aminotransferase, AST and Alanine Aminotransferase (ALT). *A.niger* was isolated from 14% pellets. *A.flavus* was isolated from (20%) pellets samples and (2%) from soybean only . *A. fumigatus* was isolated from (4%) pellets and soybean samples equally. *A. ochraceus* was isolated from (2%) pellets samples only . *A.terreus* was isolated from 4(8%) pellet samples only. No significant differences (p value=0.670758),between control group and those exposed to aspergillus spp. contaminated feed for 15 days in ALT level . ALT level was decreased after 30 days and 45 days of exposure to aspergillus spp. contaminated with significant difference compared with control group (p value=0.000218), (p value=0.000318) respectively. No significant difference in AP between zero time and 15 days exposure (p value=0.097845). AP level was increased after 30 days with significant difference compared with zero time (p value=0.041707) .After 45 days of exposure, the AP level was still elevated compared with zero time without significant difference(p value=0.194352). No significant difference in AST level between control group and 15 days exposure group (p value=0.06193),30 days exposure group (p value=0.119253). AST level was decreased after 45 days of exposure , with marginal significant difference compared with control group (p value=0.05173. No significant difference in direct Bilirubin between control group and 15 days exposed group (p value=0.083805). significant difference in direct Bilirubin between control group and 30 days exposed group (p value=0.01003),45 days exposed group (p value=0.000244). No significant difference in total Bilirubin between control group , 15 days exposure (p value=0.569794), 30 days exposure (p value=0.169383) and after 45 days (p value=0.182412). A.flavus and A.niger were the most common aspergillus species isolated from contaminated feed .ALT level significantly affected by exposure to aspergillus species for 30 and 45 days .AP significantly affected by exposure to aspergillus species for 30 days .Direct Bilirubin level significantly affected by exposure to aspergillus species for 30 and 45 days

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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 liver of infected chickens also evaluation of pathological effects of aspergillosis on Liver 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) using five Petri plates for each sample (5-10 grains / each plate). 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 PDA 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 liver function enzymes Alkaline Phosphatase (ALP) determined according to (Kamiya Biomedical 2016) . Aspartate Aminotransferase, AST determined according to (Eiaab 2017) .while Alanine Aminotransferase (ALT) determined according to (cloud-clone 2016).

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 ($P \leq 0.001$), high significant ($P<0.01$) and significant at ($P< 0.05$) and non-significant at ($P> 0.05$).

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RESULTS

Isolation and Identification of *Aspergillus spp.* From Feed Samples

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,B).

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).

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,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) .

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 7cm 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,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 .

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.

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 (Figure5 .A,B). *A. terreus* 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).

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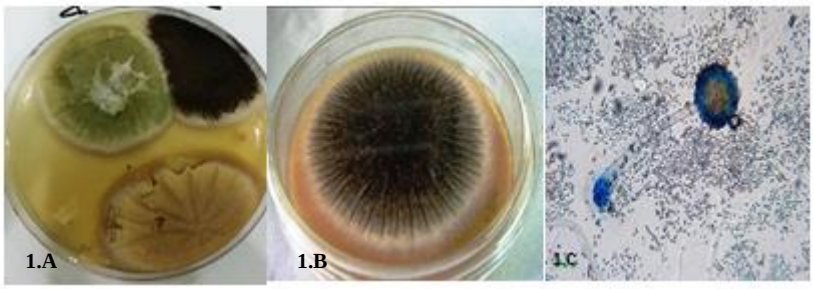

Figure (1). Growth of *A. niger* isolated from pellets feed on SDA medium at 28°C for 7 days. A) mixed culture , black colony ,upper right corner . B) pure culture .C. Microscopic appearance of A. niger isolated from pellets feed, stained with lactophenol cotton blue, showing non-branched conidiophore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

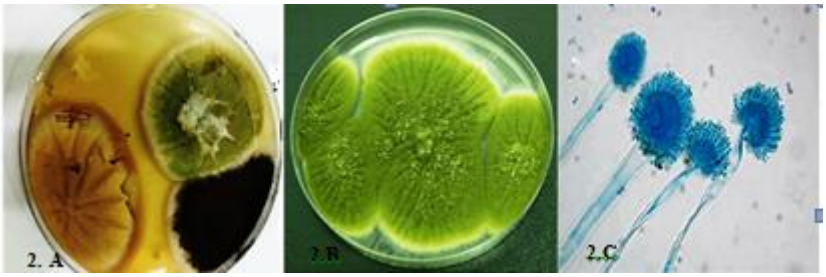

Figure (2).Growth of A. flavus isolated from pellets feed on SDA medium at 28°C for 7 days. A) mixed culture . B) pure culture .c. Microscopic appearance of A. flavus isolated from pellets feed, stained with lactophenol cotton blue, showing conidial head (40 X).

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).

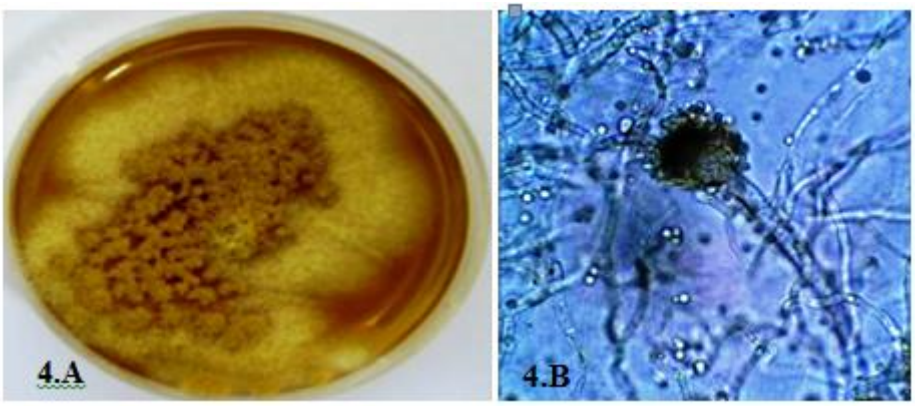

Figure (4). A. Pure culture growth of A. ochraceus isolated from pellets feed on SDA medium at 28°C for 7 days. B). Microscopic appearance of A. ochraceus isolated from pellets feed, stained with lactophenol cotton blue, showing distinct globose conidial head (40 X) .

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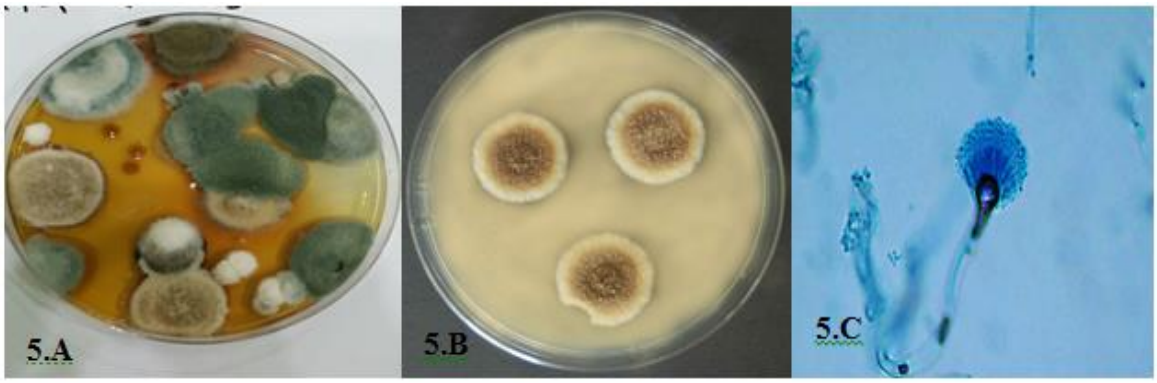

Figure (5): growth of A. terreus isolated from pellets and soybean containing feed on SDA medium at 28°C for 7 days. A) Mixed culture. B) pure culture .C. Microscopic appearance of A. terreus isolated from pellets feed, stained with lactophenol cotton blue, showing small, globose-shaped, smooth-walled,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

As shown in table(2), Alanine Aminotransferase (ALT) level among control group slightly elevated (5.2160±0.87871) U/L compared with those exposed to aspergillus spp. contaminated feed for 15 days(4.8933 ± 0.79400),without significant difference (p value=0.670758). ALT level was obviously decreased after 30 days of exposure to aspergillus spp. contaminated , (1.9100±0.81462) with significant difference compared with control group (p value=0.000218). ALT level was obviously decreased after 45 days of exposure to aspergillus spp. contaminated , (1.4075± .84968) with significant difference compared with control group (p value=0.000318).

As shown in table(2),the mean level of Alkaline Phosphatase (AP) was (846.6020±114.20967) U/L among control group compared with those exposed to aspergillus spp. contaminated feed for 15 days(1224.1833 ± 399.09108) without significant difference (p value=0.097845). AP level was obviously increased after 30 days of exposure to aspergillus spp. contaminated , (1399.5433±481.72331) with significant difference compared with control group (p value=0.041707) .After 45 days of exposure the AP level was still elevated (1145.5900±455.66121) compared with zero time without significant difference(p value=0.194352).

Aspartate Transaminase (AST) U/L level among control group slightly elevated (291.9220± 101.17846) U/L compared with those exposed to aspergillus spp. contaminated feed for 15 days(192.6900 ± 19.31885),without significant difference (p value=0.06193). AST level was obviously decreased after 30 days of exposure to aspergillus spp. contaminated , (182.2133 ±7.17080) without significant difference compared with control group (p value=0.119253). AST level was obviously decreased after 45 days of exposure , (170.9925 ± 13.45635) with marginal significant difference compared with control group (p value=0.05173).

As shown in table(2),the mean level of Direct Bilirubin was (0.0760 ±0.01140) µmol/L among control group compared with those exposed to aspergillus spp. contaminated feed for 15 days(0.7633 ±0.91511) without significant difference (p value=0.083805). Direct Bilirubin level was obviously increased after 30 days of exposure to aspergillus spp. , (0.1633±0.04163) with significant difference compared with control group (p value=0.01003) .After 45 days of exposure, the Direct Bilirubin level was still elevated (.2975± .05560) compared with zero time without significant difference(p value=0.000244).

The mean level of total Bilirubin was (1.6720± 1.57923) µmol/L among control group compared with those exposed to aspergillus spp. contaminated feed for 15 days(1.0300 ± 0.94557) without significant difference (p value=0.569794). total Bilirubin level was obviously decreased after 30 days of exposure to aspergillus spp. , (0.2500±0.10440) with significant difference compared with control group (p value=0.169383) .After 45 days of exposure, the total Bilirubin level was still decreased(0.5900 ± .06218)compared with zero time without significant difference(p value=0.182412).

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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	0.574	-0.140	0.331
	positive	7 (14%)	0(0%)	0(0%)	7 (14%)				
A.flavus	negative	34(68%)	3(6%)	2(4%)	39(78%)	1.084	0.582	.002	0.990
	positive	10(20%)	0(0%)	1(2%)	11(22%)				
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%)				
A. ochraceus	negative	43(86%)	3(6%)	3(6%)	49(98%)	0.139	0.933	-0.050	0.732
	positive	1(2%)	0(0%)	0(0%)	1(2%)				
A.terreus	negative	40 (80%)	3(6%)	3(6%)	46(92%)	0.593	0.743	-0.103	0.478
	positive	4(8%)	0(0%)	0(0%)	4(8%)				

Table(2). Correlation Between Aspergillosis And Liver enzyme analysis

Days after exposure		Alanine Aminotransferase (ALT) U/L	T- test P value	Alkaline Phosphatase U/L	T- test P value	Aspartate Transaminase (AST) U/L	T- test P value	Direct Bilirubin µmol/L	T- test P value	Total Bilirubin µmol/L	T- test P value
Zero Time	Minimum	4.28		698.30		182.86		0.06		0.23	
	Maximum	6.11		958.74		421.97		0.09		4.10	
	Mean± SD	5.2160±0.87871		846.6020±114.20967		291.9220± 101.17846		0.0760 ±0.01140		1.6720± 1.57923	
15 Days	Minimum	4.42		763.36		171.93		0.23		0.43	
	Maximum	5.81	0.670758	1456.85	0.097845	210.14	0.06193	1.82	0.083805	2.12	0.569794
	Mean± SD	4.8933 ± 0.79400		1224.1833 ± 399.09108		192.6900 ± 19.31885		0.7633 ±0.91511		1.0300 ± 0.94557	
30 Days	Minimum	1.05		891.73		174.34		0.13		0.18	
	Maximum	2.67	0.000218	1850.05	0.041707	188.37	0.119253	0.21	0.01003	0.37	0.169383
	Mean± SD	1.9100±0.81462		1399.5433±481.72331		182.2133 ±7.17080		0.1633±0.04163		0.2500±0.10440	
45 Days	Minimum	.69		769.76		152.04		.23		.50	
	Maximum	2.38	0.000318	1768.37	0.194352	183.77	0.05173	.36	0.000244	.64	0.182412
	Mean± SD	1.4075± .84968		1145.5900±455.66121		170.9925 ± 13.45635		.2975± .05560		.5900 ± .06218	

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DISCUSSION

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 7.3). Microscopic examination of *A. ochraceus* as shown in figure (8.4) 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).

In current study, ALT level was decreased among poults exposed to aspergillus spp. contaminated feed for 15 without significant difference and for 30,45 days with significant difference. These results come in agreement with , (Stanley et al. 1993, Valdivia et al. 2001, Tedesco et al. 2004, Sawale et al. 2009, Zahoor-ul-Hassan et al. 2010, Umar et al. 2012), on the other hand(Andretta et al. 2012),reported that the serum concentration of ALT was 12% higher in broilers challenged by mycotoxins and 17% higher in those challenged by aflatoxins. While (Fernandez et al. 1994), found that ALT levels in serum did not vary in broilers fed aflatoxin contaminated feed, whereas they did in laying hens.

On the other hand (Umar et al. 2012) revealed that at the ALT values of chicks fed with mycotoxins was increased at day 10,20,30 and 40 after exposure. The altered activity of serum biochemical parameters indicate impaired function, decreased activity or degenerative changes in particular organ producing the biochemical constituents (Verma et al. 2004, Zahoor-ul-Hassan et al. 2012).ALTs, lysosomal enzymes are present in high concentration within the hepatocytes. Their detection in blood is usually considered as first sign of liver injury. The increased serum level of ALT occurs due to the leakage of this enzyme from damaged lysosomes of the hepatocytes (Umar et al. 2012).

In current study, Ap level was increased among poults exposed to aspergillus spp. contaminated feed for 15 without significant difference and continued in elevation for 30 with significant difference.No significant difference was reported after 45 days.

These results come in agreement with (Eraslan et al. 2006). (Andretta et al. 2012), reported that the concentration of alkaline phosphatase was 54% higher ($P < 0.05$) in broilers challenged by mycotoxins and 47% higher ($P < 0.05$) in broilers fed with diets containing aflatoxins.

In current study, AST level was decreased among poults exposed to aspergillus spp. contaminated feed for 15 ,30 days without significant difference and for 45 days with marginal significant difference compared with control group.

These results come in agreement with (Huff et al. 1992) ,in which aflatoxin caused a decrease in the activity of AST. Current results come in contrary to (Andretta et al. 2012) , reported that the concentration of AST was 16% higher ($P < 0.05$) in broilers challenged by mycotoxins and 14% higher in broilers challenged by aflatoxins. (Andretta et al. 2012),recorded a quadratic effect on the relationship among the concentration of aflatoxin in diets and the serum concentration of AP, γ-glutamyl transferase,ALT, and AST. The increase in the concentration of these enzymes is recognized as evidence of injury on the membrane integrity of liver cells. On the other hand,(van Rensburg et al. 2006) reported that aflatoxin-contaminated diets did not significantly affect serum AST activity. In current study, direct bilirubin was decreased after 15 days of exposure to aspergillus spp. contaminated feed without significant difference . Direct bilirubin level was increased after 30 days of exposure to aspergillus spp. with significant difference compared with control group .After 45 days of exposure, the direct bilirubin level was still elevated compared with zero time without significant difference . These results come in contrary with that reported by (Tedesco et al. 2004),mentioned that ,aflatoxin has no effects on direct bilirubin level in exposed chicks.

The level of total bilirubin was decreased with those exposed to aspergillus spp. contaminated feed for 15 days. Total Bilirubin level was decreased after 30 days of exposure to aspergillus spp. with significant difference compared with control group .After 45 days of exposure, the total bilirubin level was still decreased compared with zero time without significant difference(p value=0.182412). These results come in contrary with that reported by (Tedesco et al. 2004),mentioned that ,aflatoxin has no effects on total bilirubin level in exposed chicks. Also come in contrary with (Eraslan et al. 2006), reported that , the significant increase in total bilirubin was detected in the group received aflatoxin. This increase is an indicator of malfunction in the liver, which resulted from aflatoxin action and this increase was related to the changes located specifically in bile ducts and hepatocytes. The disturbances in total bilirubin indicate abnormality in function of hepatocytes even without significant differences between group, which reflect a dose and time dependent effect of mycotoxins and fungal contaminants. 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. liver enzymes (ALT,AST) and bilirubin affected mainly in prolong exposure .

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