Studies on mycosis and mycotoxicosis in cattle

[1]Hassan, A.A.; [1]Howayda, M. El Shafei; [1]Noha, H. Oraby; [1]Rasha, M.H. Sayed El Ahl and [2]Mogeda, K. Mansour

[1]Dept. of Mycology and Mycotoxins and [2] Biochemistry,
Animal Health Research Institute, Dokki, Giza, Egypt.

Abstract

The present study was conducted on forty diseased and apparently healthy cases of cattle (20 serum samples of each) at Cairo Governorate. 100 samples of milk of mastitic animals, nasal swabs, vaginal swabs and feeds samples used in breeding of diseased animals (25 of each) were collected. The mycological examination of collected samples revealed isolation of *Aspergillus* species as the predominant cause from the above samples of infected animals, where in cattle isolated from (88%, 96%, 80%, and 100%) respectively. Other genera and species of moulds and yeast were obtained at comparatively lower significant rates. Sera of diseased cattle contained significant levels of aflatoxins and zearalenon. Meanwhile, 60% of cattle had the mean levels of aflatoxins (15.20±0.01 ppb) and zearalenon in 80% cattle with the mean level of (62±0.10). The used feed samples in breeding of these animals had the amounts of AFB_1; OA; ZEAR and T_2 detected in (60%; 40%; 32%; and 36%) of feed samples, with the mean levels of (55.00±1.50; 45.00±0.30; 31.00±0.20 and 26.00±0.02 ppb), respectively. The biochemical study on sera of infected cattle fed with feed contaminated with aflatoxins and untreated with ammonium hydroxide 3% , demonstrated that the AFB_1 and zearalenon lead to a marked elevation in the serum enzymes activity of (AST, ALT and GGT, which give indication about hepatocellular damage. The levels of serum urea and creatinine were significantly high in toxicated animals as compared to apparently healthy animals. Mycotoxins in cattle induced significant decreased values in serum total protein, albumin, alpha globulin, beta globulin, and gamma globulin . The mycotoxicated animals had significant increase in serum levels malondialdehyde (MDA) and NO which caused induction of inducible nitric oxide synthase (iNOs) protein. The feeding of treated feed with ammonium hydroxide resulted in significant improvement of all previous alterations in biochemical parameters which resulted from toxicosis. The significance of the present result was fully discussed.

Key word: *Aflatoxins (AF);OA; ZEAR.; T_2; Aspergillus spp.; Biochemical parameters; Electrophoresis patterns; NO.& MD.*

Introduction

Up to date the environmental pollution is considered the essential cause of animal diseases particularly pollution of the used feed and water with fungi and their toxins as well as contamination of human food. Hence, great attention has been paid to the increased importance of fungi and their mycotoxins, which are serious fungal metabolites. Mycotoxins are formed by certain fungal species, whenever environmental factors are conductive during the growth of these frequently occurring mycomycetes on food stuffs and animal feeds; the process takes place as a secondary metabolism. These natural toxins have broad spectrum effects **(Hassan, 2003).** Unlike many bacterial toxins, they are in general highly stable facing environmental influences. This also means that, when foodstuffs for human consumption are preserved or prepared by the conventional heating process, the mycotoxins are capable of surviving undamaged thus reaching the eventual consumer .These fungi and mycotoxins have serious effects upon the growth rate and health of human being and animals, as some mycotoxins had been found to be carcinogenic, tremorgenic, haemorrhagic and dermatitic **(Hassan, 1998)**. The mycotoxins of greatest

agricultural and public health significance include aflatoxins, ochratoxins, trichothecenes, fumonisins, zearalenone, and ergot alkaloids (**Hassan *et al*., 2008).** However**,** the fungi and their toxins are widely distributed through the world where they occur in soil, on plants, plants debris and similar organic subtracts. They cause significant economic losses in agriculture, morbidity and mortality in animals and immunological compromised humans, where it is capable of killing cells by causing extensive damage to cellular membrane **(Ajello and Hay, 1998** and **Mogda, Mansour *et al*., 2002).** It had wide range of biological effects, including pulmonary oedema in pigs and ruminants **(Harrison *et al*., 1990),** nephrotoxicity and liver cancer in different animal species **(Gelderblom *et al*., 1996** and **Hassan *et al*., 2004).** The International Agency for Research on Cancer has declared that mycotoxins are potentially carcinogenic to human. Aflatoxin B_1 had been statistically associated with a high incidence of liver cancer in certain areas of Transkei; South Africa; China and Egypt **(Chu and Li, 1994).**

The aim of the present work was to investigate the problem of fungal disease and mycotoxicosis in cattle and detection of the role of feed pollution as main environmental factors of animal diseases.

Materials and Methods

1. Serum and feed samples:

Forty diseased cases of cattle in farms at Cairo Governorate were investigated . From districts of diseased cases , 40 serum samples of cattle and 100 samples of milk of mastitic animals, nasal swabs from animals had respiratory manifestations , vaginal swabs of cows suffered from abortion and feeds samples) used in breeding of diseased animals(25 of each) were collected.

2. Mycotoxins standards:

Standards and immunoaffinity column of AF-B_1,B_2,G_1,G_2 and OA; ZEAR and T_2 were purchased from Sigma Chemical Company (USA).

3. Mycological examination of samples:

The collected samples of feeds, milk and swabs were subjected for isolation and identification of fungi as recommended by **Conner *et al.* (1992).**

4. Detection of mycotoxins in feed and sera of diseased cattle :

By fluerometric methods as described by **Hansen (1993)** using immune-affinity column method**.**

5. Detoxification of aflatoxicated feeds by addition of ammonium hydroxide 3% (Hassan, 1994):

The feed samples contaminated with permissible limits (over 700-1000 ppm/ Ton of feed) were treated by addition of ammonium hydroxide 3%**.** After 7 days of treatment, the treated feeds were given to group of apparently healthy cattle calves in comparison to other group given toxicated feeds without any treatment. The feeding was continued for 1 month. All the animals were tested biochemically every10 days during treatment for evaluating the influence of ammonium hydroxide on the toxicities of aflatoxins on the function of vital internal organs and patterns of serum protein electrophoresis.

6. Biochemical investigations of sera of diseased cattle :

Blood samples were collected in small labeled dry and clean vials without anticoagulant in centrifuge tube, allowed to clot and then centrifuged at 3000 rpm for 15 minutes for separation of serum which were used to assay the biochemical parameters as aspartate aminotransferase (AST) and alanine aminotransferase (ALT) activities according to **Reitman and Frankel (1957**), serum urea according to **Wybenga *et al.* (1971**), serum creatinine level according to **Henry (1974)**. Estimation of serum total protein and electrophoretic pattern were carried out after **Davis (1964) and Sonnenwirth and Jareet (1980),** respectively. Serum lipid peroxide levels of malondialdehyde (MDA) were determined according to the method described by **Yoshioka *et al.*** (**1979**). Blood GSH concentration was measured using the method described by **Beutler *et al.* (1963).**

Serum NO levels of the cattle were measured by enzymatic Greiss reaction (**Burgner *et al*, 1999**). Estimation of vitamins E; A and C were performed according to **Henry (1974).**

7. Statistical Analysis:

The obtained date were computerized and analyzed for significance, Calculation of standard error and variance according to (**SPSS 14, 2006**).

Results and Discussion:

The past three decades have witnessed dramatic change in man's and animal's environment and their immune defenses. Consequently, the fungi have assumed a major role in respiratory and systemic infectious diseases. In the present study the most common isolated fungi from mastitic milk samples of cattle were *Aspergillus* sp. (88%), *Fusarium* sp. (40%), *Pencillium* sp.(8%), *Cladosporium* sp.(8%), *Alternaria* sp. (4%), *C.albicans* (60%), *C. tropicalis* (24%) and *Rhodotorula rubra*(16%). The percentage of moulds in raw milk samples varies depending on geographical location and season of the year. The specific qualities of climate, vegetation and land are the important factors affecting the prevalence of moulds in connection with a certain geographical location. Mould spores can disperse in the air with the wind or in combination of wind and rain (**Mikulec *et al.,* 2005**). The most frequent organisms implicated in mycotic mastitis were yeasts, especially of the genus *Candida spp.* are always present on the skin of the udder and teats, and could have entered the teat canal either due to inappropriate use of instruments or due to contaminated antibiotic preparations used for infusion **(Timoney *et al*., 1988** and **Hassan *et al*., 2010).**

With reference to Table 1, it is obvious that the most common isolated moulds from cows' nasal swabs were *Aspergillus s*p. (96%), *Fusarium* sp. (64%), *Mucor* sp.(8%), *Cladosporium* sp.(16%), *Alternaria* sp. (4%), *C.albicans* (24%), *C. tropicalis* (68%) and *Rhodotorula rubra*(12%). The present results coincide to some extent with that of` **Nilsson and Persson (1984); Maity and Deb (1993); Mahendra *et al*., (1998**) and **Hussein** (**2004**). Breeding factors such as animal housing, feeding on moldy hay and ventilation system or environmental factors such as temperature, wind and dew increase the odds of contracting the infection (**Chihaya *et al*.**, **1991** and **Moubasher, 1995).**

Fungi can produce reproductive failure in animals either by direct infection of the genital system or by producing toxin metabolites (mycotoxins), which are subsequently ingested and absorbed. Moreover, mycotic abortion is the most important consequence of fungal infection of the genital tract (**Garoussi *et al.,* 2007**).

From the obtained results in (Table 1), a total of 20 fungal species related to 8 genera were isolated from 25 samples of vaginal swabs collected from cases of cows suffered from abortion. The main isolated genera of fungi were *Aspergillus s*pp. (80%), *Fusarium* spp. (16%), *Pencillium* spp.(32%), *Cladosporium* spp. (12%), *Alternaria* spp. (8%), *C.albicans* (40%), *C. tropicalis* (32%) and *Rhodotorula rubra*(3%). The results concur to some extent with the findings of **Panangala *et al*., (1978); Patgiri and Uppal (1983)** and **Abdel-Rahman and Ibrahim** (**2007**) who isolated species of *Alternaria, Aspergillus*, and *Cladosporium*, *Candida, Fusarium* and *Pencillium* from vaginal swabs of repeat breeder cows. The predominance of dark-colored fungi may be explained by their higher resistance to dissication, strong light and ionizing radiation than non-pigmented forms **(Mirchink *et al*., 1969).** On the contrary, *A. fumigatus* has been reported by **Knudtson and Kirkbride** (**1992**) as the most common cause of mycotic placentitis. *Candida albicans* and *C. tropicalis,* were the most frequent yeast species obtained from cows' vaginal swab samples as yeasts have been implicated as causes of bovine reproductive problems, including abortion in cows and infertility in bulls **(Chengappa *et al*.**,1**984** and **Morsy, 2007**).

It is clear that the most common isolated mould from cows' feed were *Aspergillus* spp. (100%), *Fusarium* spp. (24%), *Mucor* spp. (28%), *Pencillium* spp.(52%), *Cladosporium*

spp.(8%), *Alternaria* spp. (28%), *C.albicans* (80%), *C. tropicalis* (40%) and *Rhodotorula rubra*(32%). These findings were in agreement with the results of **Arakawa** (**2007**) and **Hassan *et al*., (2008)**. Also, it has been estimated that 25% of the world's crop production is contaminated with mycotoxins. **Pusterla *et al.*** (**1996**) suggested that the primary infection was attributed to inhalation of spores originating from moldy hay or soil.

In the present study, the current data in table (2) showed that, sera of twenty cases of diseased cattle that suffered from loss of weight gain, low productivity, diarrhea, disturbance in fertility and sudden mortality of some cases, contained significant levels of aflatoxins and ZEAR. Meanwhile, 60% of cattle had mean levels of aflatoxins (15.20±0.01 ppb) and zearalenon detected in 80% of cattle with the mean level of (62±0.1), respectively((Table, 2). Mycotoxins in cattle sera in Egypt in association with symptoms of toxicities were previously reported by **Hassan (1994).** The effects of mycotoxins in human and animals varied from carcinogenic; nephrotoxic and immunosuppressive health effects (**Morriss, *et al*., 1997** and **Hassan *et al*., 2009)**. Although the main route of human exposure to mycotoxins has been identified as the direct ingestion of contaminated cereals, grains and food of animal origin **(Morriss, *et al*, 1997)**, there are many studies about whether or not the ingestion of meat, milk, and eggs originating from mycotoxin-exposed food-production animals is a significant pathway for mycotoxins among humans **(Wafia and Hassan, 2000** and **Hassan *et al*., 2003).** Feed samples were examined for fungal contamination and detection of mycotoxins . They were subjected for detection of aflatoxins , the results revealed that the amounts of AFB_1; OA; ZEAR. and T_2 were detected in (60%; 40%; 32%; and 36%) with the mean levels of (55.00±1.50, 45.00±0.30 and 31.00±0.20 and 26.00±0.02 ppb), respectively (Table, 3). The significant levels of mycotoxins in the present feed samples and serum of diseased animals gave a large possibility that mycotoxins were responsible for disease outbreaks in animals**.** The Food and drug administration has established recommended maximum levels for aflatoxins in animal feed are 20 µg/kg of feed **(FDA, 1994).** The permissible limits of aflatoxin for large ruminants varied between 700-1000 ppm/ Ton of feed this limit will cause loss of weight gain, high food consumption and low feed efficiency **(Keyle *et al*., 1970).** Whereas, **t**he O.A produced significant pathological changes in each pregnant animal at the levels of 2.5 ppm of animal body weight for 5 days when given orally **(Nesheim,1971)** . On the other hand the trichothecenes mycotoxins (T_2) produced such changes at doses high as 10 ppm **(Epply and Baily, 1993)** .

The detected levels of mycotoxins were significantly over the permissible limits in feeds as a result of continous feeding of toxiocated feed. The same findings were detected by many authors as **(Hassan *et al*., 2002)**, in association with significant high mycotoxin levels in feed and sera of diseased animals.

The detoxification of toxic feed become a critical demand and ammonium hydroxide was the most safe chemical compound used for this purpose **.**

In this study, we demonstrated that the AFB_1 and zearalenon in serum of cattle lead to a marked elevation in the serum enzymes activities of (AST, ALT and GGT) table (4) which indicated the hepatocellular damage, as previously reported **(Orsi *et al*., 2007** and **Nayak and Sashidhar, 2010)**. This elevation could potentially be attributed to hepatic degeneration and subsequent leakage of enzymes into circulation after rupture of the plasma membrane and cellular damage **(Lyuch *et al*., 1971 and Afzali and Devegowda, 1999)**. Such hepatic toxic effect of aflatoxin is attributed to its active metabolite in liver as epoxide, **Netke *et al*., (1997)** which covalently bind to DNA and may affect structural and enzymatic protein function **(Cullen and Newbern, 1994).** Moreover the elevation of serum GGT activity suggested hepatic necrosis, thickness of bile duct and intrahepatic cholestasis (**Kaneko *et al*., 1997).**The levels of serum urea and creatinine

were significantly high in aflatoxicated groups as compared to healthy and that group of animals fed on the treated feed with ammonium hydroxide. This increase in concentrations in toxicated animals might be due to nephrotoxic action, which causes renal impairment by destruction of epithelial cells of proximal and distal convoluted tubules and alterations in tubular function **(Nashwa *et al*, 2008).** Microbial protein synthesis in the rumen is inhibited by mycotoxins and more free ammonia remains in the rumen, then absorbed into the blood, and is metabolized to urea, resulting in elevated blood urea concentrations and reduced hepatic fractional protein synthesis rates **(Chowdhury and Smith , 2005** and **Danicke *et al*., 2005 and 2006).**

Mycotoxicosis in cattle and buffaloes induced significantly decreased values in serum total protein, albumin, alpha globulin, beta globulin, and gamma globulin (Table, 5). Decrease in serum globulin in mycotoxicated animals might be due to the adverse effect of mycotoxins on synthesis of total proteins and globulin. These results agree with **Osuna and Edds (1982)** in pigs; **Hassan and mogda (2003)** in quails and **Madheswaran *et al*., (2004)** in chickens. Mycotoxins cause inhibition of DNA and protein synthesis as well as immunosuppression due to the inflammation and cirrhosis of liver and kidneys **(Harvey *et al*., 1991 and Salem *et al*., 2007).** At the same table (5) the globulin component showed drop in a1, a2, b1 and g1 globulin in all the diseased animals, while an increase of g2 globulin was recorded in healthy animals. These results coincided with the tune of total proteins and albumin.This may be attributed to that AFB_1 causes hepatotoxic, nephrosis and liver and kidneys hemorrhages **(Tietz, 1996*)***. In addition, mycotoxins has immunosuppressive effect inhibit nearly cellular and humeral immunologic reaction **(Tietz, 1996 and Hassan *et al*., 1997).** The decrease in serum level of globulin might be due to the adverse effect of mycotoxins on synthesis of total proteins and globulin **(Agag, 2004; Awaad *et al*., 2011 and Hassan *et al*., 2011)**

This study showed that the mycotoxicated animals had significant increase in serum level of malondialdehyde (MDA). Mycotoxins particularly AFB_1 are well known to generate free radicals, disturbing the antioxidant status and ultimately leading to oxidative stress and carcinogenesis **(Rastogi *et al*., 2007).** Lipid peroxidation plays an important role in carcinogenesis (**Banakar *et al*., 2004**) and may lead to the formation of several toxic products, such as malondialdehyde (MDE) and 4-hydroxynonenal. Also, AFB_1 epoxide is a very reactive and unstable metabolite of AFB_1 that will bind to cellular macromolecules like DNA, RNA, lipids and proteins, leading to lipid peroxidation and cellular injury. **(Stresser *et al*., 1994**). The hepatic damage is associated with an increase in the tissue malondialdehyde (MDA) level, an indirect index of lipid peroxidation (**Shyamal *et al*., 2010*)***

The present study shows that serum NO level significantly increased in mycotoxicated cattle and buffaloes. It has been reported that elevated levels of lipid peroxidation stimulates host cells, mainly monocytes/ macrophages, to produce and release NO by induction of inducible nitric oxide synthase (INOS) protein, resulting in cytotoxicity and DNA damage **(Raso *et al*., 2001).**

Our finding of low serum retinol, and a-tocopherol, in toxicated cattle compared to apparently healthy and treated animals (table, 6) was in agreements with previous studies by **Clarke *et al*., 1984 and Vatassery *et al*., 1986.** Aflatoxin causes oxidative stress by inducing the epoxide reactive ring, reducing the antioxidant defense systems of cells via depleting non enzymatic antioxidant system (vitamins and glutathione) and/or increasing susceptibility of cells to oxidative attack by altering the membrane integrity and fatty acid composition **(Lee *et al*., 2009; lim *et al*., 2004 and Lee and Jacobs, 2005).**

This study, reported the dangerous effects of fungal diseases and their mycotoxins and its pollution of animal feed which resulted significant losses in animal health which is an important contributor to the country's economy in

the form of meat, milk, wool and leather, with respect to the effects of environmental factors. Therefore, frequent testing program of the animal feeds and their environment for fungi and mycotoxin contamination is a critical demand to secure the animal and human health.

Table (1). Prevalence of fungal species in clinical samples of cattle.

Fungal isolates	Milk of mastitic animals (25)		Nasal swabs (25)		Vaginal swabs (25)		Feeds (25)	
	No.of +ve	%	No. of +ve	%	No. of +ve	%	No. of +ve	%
Aspergillus sp.:	22	88	24	96	20	80	25	100
1-A. candidus.	6	24	1	4	0	0	15	60
2-A. clavatus.	2	8	2	8	2	8	1	4
3-A .flavus.	20	80	20	80	13	52	18	72
4-A. fumigatus.	21	84	16	64	7	28	5	20
5-A. niger.	10	40	20	8	23	92	12	48
6-A. ochraceus.	0	0	0	0	5	20	5	40
Fusarium sp.:	10	40	16	64	4	16	6	24
1-F. oxysporum.	10	40	1	4	0	0	6	24
2.F.verticillioides.	2	20	2	8	4	16	1	4
Mucor species.	0	0	4	8	0	0	7	28
Penicillium sp.:	2	8	0	0	8	32	13	52
1-P. citrinum.	1	4	3	12	0	0	0	0
2-P. duclauxii.	0	0	0	0	3	12	2	8
3-P. funiculosum.	0	0	10	40	2	8	0	0
4-P. islandicum.	0	0	1	4	2	8	0	0
5-P. oxalicum.	1	4	0	0	4	16	11	44
6.P.purpurogenum.	0	0	0	0	0	0	2	8
Cladosporium species.	2	8	4	16	3	12	2	8
Alternaria alternate.	1	4	1	4	2	8	7	28
Candida albicans.	15	60	6	24	10	40	20	80
Candida tropicalis.	6	24	17	68	8	32	10	40
Rhodotorula rubra.	4	16	3	12	3	3	8	32

- 25 samples of each were examined.

Table (2). Determination of mycotxoins in serum of cattle.

Animal	Total No.	Aflatoxin B_1			Zearalenone		
		Positive cases	%	Mean levels ppb	Positive cases	%	Mean levels ppb
Apparently healthy cattle	20	2	10	2.50±0.01	1	5	5.00±0.00
Diseased cattle	20	12	60	15.20±0.01	16	80	62.00±0.10

-ppb : Part per billion**.**

Table (3). Mycotoxins in feed consumed by cattle**.**

Mycotoxin in feeds	Aflatoxin B_1			Ochratoxin A			Zearalenon			T_2			Fumonisin BI		
	Positive		Mean of levels ppb	Positive		Mean of levels ppb	Positive		Mean of levels ppb	Positive		Mean of levels ppb	Positive		Mean of levels ppb
	No.	%		No.	%		No.	%		No.	%		No.	%	
Animal feeds	15	60	55±1.50	10	40	45±0.30	8	32	31±0.20	12	36	26±0.02	11	44	**40±2.1**

-Fifty five samples of feed were examined. - ppb: part per billion.

Table (4) . Changes in biochemical parameters of cattle sera due to mycotoxicosis.

Chemical tests	Apparently healthy cattle	Diseased cattle Groups	Treated cattle Groups
AST (ul/ml)	49.11±4.26	90.45 ± 7.21***	63.33±3.20*
ALT (ul/ml)	30.67± 3.84	51.34± 3.29***	43.33±3.80*
Ggt (ul/ml)	22.64 ± 1.15	64.18±4.49***	49.90±3.54*
Urea (mg/dl)	43.48±1.67	58.86±3.79**	48.65±1.60
Creatinine(mg/dl)	0.94±0.05	1.14±0.06*	0.96±0.96

Table (5). Patterns of protein electrophoresis in diseased cattle.

Patterns of protein	Apparently healthy cattle	Diseased cattle Groups	Treated cattle Groups
T.protein(g/dl)	6.16 ± 0.09	5.42 ±0.16***	6.04±0.21
Albumin(g/dl)	2.38 ±0.04	1.98 ±0.08***	2.26±0.07
Alpha 1(g/dl)	0.82 ±0.02	0.63 ±0.01***	0.72±0.03*
Alpha 2(g/dl)	0.35 ±0.03	0.29 ±0.01	0.34±0.05
T-alph(g/dl)	1.17 ±0.04	0.92 ±0.01***	1.06±0.03*
Beta 1(g/dl)	0.66 ±0.02	0.56 ±0.01***	0.66±0.03
Beta 2(g/dl)	0.40 ±0.02	0.438 ±0.02	0.48±0.06
T.beta(g/dl)	1.06 ±0.01	0.998 ±0.02*	1.14±0.05
Gamma 1(g/dl)	1.26 ±0.03	1.19 ±0.02**	1.29±0.13
Gamma 2(g/dl)	0.29 ±0.01	0.33 ±0.02	0.29±0.02
T.Gamma(g/dl)	1.55 ±0.04	1.52 ±0.05	1.58±0.13
T.globulin(g/dl)	3.78 ±0.05	3.43±0.09**	3.78±0.12
A/G	0.62 ±0.01	0.57±0.01***	0.59±0.008*

*Results are expressed as means ± SEM (n =15), student 't' test

Table (6). Alteration of Levels of antioxidant in toxicated cattle.

Antioxidants tests	Apparently healthy cattle	Diseased cattle Groups	Treated cattle Groups
Malondialdehyde (MDA)(nmol/ml)	1.94±0.37	4.18±0.39***	2.14±0.35**
GSH(Glutathion)mmol/l)	12.40±6.80	18.20±5.70**	16.33±1.74
Nitric oxide(N O) (µmol/L)	35.17 ± 2.40	55.84 ± 6.50 **	48.01±4.21*
Vitamin A(ug/dl)	33.25± 2.44	28.67 ± 2.51	30.00±1.54
Vitamin E(ug/ml)	14.54 ±3.58	12.91±2.63	13.68±1.65

*Results are expressed as means ± SEM (n =15), student 't' test.

References:

Abdel-Rahman, A.A. and Ibrahim, D.R. (2007). Studies on microbial infection associated repeat breeder in buffaloes and cows and its sensitivity to different antibiotics in vitro. Assiut Vet.Med. J., 53 (112). 296-311.

Afzali, N. and Devegowda, G. (1999). Ability of modified mannanoligosaccharide to counteract aflatoxicosis in broiler breeder hens. Poult. Sci. 78:228.

Agag M(2004). mycotoxins in foods and feeds 1-aflatoxins Ass. Univ. Bull. Environ. Res. Vol. 7 No. 1, March 2004.

Ajello, L. , R.J. Hay (1998). Medical Mycology, Vol. 4, 9th Ed. Co-Publiched in the USA, Oxford University Press, Inc, New York, London, Sydney, Auckland.

Arakawa, K. (2007). Studies on infectious and toxic metabolite activities of saprophytic fungi in domestic animal breeding environment. Bulletin of Nippon Veterinary and Life Science University, 56: 75-77.

Awaad, M.H.H.; Atta,A.; Wafaa, A.Abdel Ghany; Elmenawy, M.; Ahmed, K.; Hassan, A.A.; Nada, A.A. and Abdelalem (2011). Effect of a Specific Combination of Mannan-Oligosaccharides and β-Glucans Extracted from Yeast Cells Wall on the Health Status and Growth Performance of Ochratoxicated Broiler Chickens. J. of American Science, 2011,7 (3), 82-96.

Banakar, MC; Paramasivan, SK. and ChattopadhyaY MB. (2004). 1alpha, 25-dihydroxyvitamin D3 prevents DNA damage and restores antioxidant enzymes in rat hepatocarcinogenesis induced by diethylnitrosamine and promoted by phenobarbital. World J Gastroenterol; 10:1268-75.

Beutler, E.; Dubon, O .and Kelly, BM (1963). Improved method for the determination of blood glutathione. J Lab Clin Med 61: 882-888.

Burgner, D.; Rockett, K and Kwiatkowski, D (1999). Nitric oxide and infectious diseases. Arch Dis Child, 81, 185–188.

Chengap pa, M. M.; Maddux, R. L.; Greer S. C.; Pincus D. H., and
Geist L. L.(1984). Isolation and Identification of Yeasts and Yeastlike Organisms from Clinical Veterinary Sources. Journal of clinical microbiology, 19 (3). 427-428.

Chihaya,Y.; Furusawa, Y.; Okada, H.; Matsukawa, K. and Matsui,Y. (1991). Pathological studies on systemic mycoses in calves. Journal of Veterinary Medical Science, 53(6). 1051-1058.

Chowdhury, S. R., and T. K. Smith (2005). Effects of feeding grains naturally contaminated with Fusarium mycotoxins on hepatic fractional protein synthesis rates of laying hens and turkeys.Poult.Sci. 84:1671–1674.

Chu, F. S. and Li, G. Y. (1994). Simultaneous occurrence of fumonisin B9 and other mycotoxins in moldy corn collected from the people's of republic of China in regions with high incidence of oesophageal cancer. Appl. Environ.Microbiol., 60, 847-852.

Clarke, E.D., Wardman P, and Wilson I. (1984). Cis-trans isomerization of the (5-nitro -2-furyl)acrylamide, AF-2, initiated by ascor-

bate, glutathione, Fe (II) and OH-Biochem Pharmacol. Jan 1;33(1).83-7.

Conner, D.E.; Samson, R.A.; Hoching, A.D.; Pitt, J.I. and King, A.D. (1992). Evaluation of methods for the selective enumeration of Fusarium species in feed stuffs. Modern method in food mycology. Development in Food Sci., 31, 229 – 302.

Cullen, J.M. and Newbern, P.M. (1994). The toxicity of aflatoxin human health, veterinary and agricultural significance. 2nd Ed., Academic Press, Philadelphia.

Danicke, S., K. Matthaus, P. Lebzien, H. Valenta, K. Stemme, K.-H. Ueberschar, E. Razzazi-Fazeli, J. Bohm, and Flachowsky (2005). Effects of Fusarium toxin-contaminated wheat grain on nutrient turnover, microbial protein synthesis and metabolism of deoxynivalenol and zearalenone in the rumen of dairy cows. J. Anim. Physiol. Anim. Nutr. (Berl.) 89:303–315.

Danicke, S., T. Goyarts, S. Doll, N. Grove, M. Spolders, and Flachowsky, G. (2006). Effects of the Fusarium toxin deoxynivalenol on tissue protein synthesis in pigs. Toxicol. Lett. 165:297–311.

Davis, B. (1964). Disk electrophoresis. II Method and application to human serum protein. Ann. N.Y. Acad. Sci., 929: 404-427.

Epply, R.M. and Baily, WJ.(1993). 12,13, EpoxyTrichocethine, as the propaple mycotoxins responsible for stachybotryotoxicosis. Science, 181:758-760.

F.D.A., Food and Drug Administration (1994). Action levels for poisonous or deleterious substances in human food and animal feed. Washington, Department of Health and Human Services, 1125-126.

Garoussi, M.T; Khosrave, A.R. and Havareshti, P. (2007). Mycoflora of cervicovaginal fluids in dairy cows with or without reproductive disorders. Mycopathologia ,164:97–100.

Gelderblom, W. C. A.; Snyman, S. D.; Abel, S.; Lebepe, M. S.; smuts, C. M.; Van der Westhuizen, L.; Marasas, W. F. O.; Victor, T. C.; Knasuner, S. and Huber, W. (1996). In hepatotoxicity and carcinogenicity of the Fumonisins in food. PP. 279-296, Plenum Press, New York.

Hansen TJ. (1993). Quantitative testing for mycotoxins. Am, Assoc, Cereal Chemist. Inc., 38 (5). 5.

Harrison, L. R.; Colvin, B. M.; Greene, J. T.; Newman, L. E. and Cole, J. R. (1990). Pulmonary oedema and hydrothorax in swine produced by fumonisin B9 a toxin metabolite of fusarium moniliforme. J.Vet. Diag. Invest., 2, p8217-221.

Harvey, R.B.; Kubena, L.F.; Phillips, T.D.; Carrier,D.E.; Elisade, M.H. and Huff, W.E. (1991). Dimention of aflatoxin toxicity to growing lambs by Dietary supplementation with hydrated alumino silicate. Am. J. Vet. Res., 52(1). 152 –156.

Hassan, A.A. (1994). Detection and control of ochratoxin in food and food-stuffs. Thesis, Ph.D. Fac. Vet. Med., Cairo University.

Hassan , A. A. (1998). Mycosis in turkeys. 5th Scientific Congress proceeding, Fac. of Vet. Med., Cairo University, Vet. Med. J. Giza, 46 .(4B). 857-865.

Hassan , A.A. (2003). Detection of some mycotoxins and mycotoxins .producing fungi in both macro- and microenvironment of diseased animals. 7th Sci. Cong. Egyptian Society for Cattle Diseases, pp.112 –119, 7-9, Assiut , Egypt.

Hassan, A.A. and Mogda, KM. (2003). New trial of use of molasses and garlic extract for combating mycotoxicosis. Kafr El-Shikh Vet. Med. J. 1: 653-680.

Hassan, A.A.; M. Hussain; M.H. El-azzawy and A.E. Saad (1997). Immunosuppression effect of aflatoxins in chickens. 23" Arab Vet. Med. Congress, J. Egypt. Vet. Med. Ass., 57 (1). 917-931.

Hassan, A.A.; Koratum, K.M. and Amal, I.Y., El-khawaga (2002). Effect of selenium in broiler chicken fed a diet containing F.moniliforme culture material supplied known level of Fumonisin B9. Egypt. J. Comp. Path. and Clinical Path. , 15 (1). 98-110.

Hassan, A. A.; A.M.Montassser and K.M. Koratum (2003). Influence of Aflatoxin and

Zearalenone On Biochemical Assay and Immune Response on Cattle Naturally Infected With Brucellosis and Experimentally Vaccinated Guinea Pigs With S19.Egypt J. Agric. Res., 81(2),. 547.

Hassan, A.A.; Ragheb, R.R. and Rahmy, Nariman, A. (2004). Pathological changes in cows spontaneously fed on some mycotoxins. Egypt. J. Comp. Path. & Clinic. Path., 17 (1). 282- 293.

Hassan, A, A.; Rashid M.A. and Koratum Kh. M. (2008). Measurement of mycotoxins in feeds and sera of cattle and sheep and evaluation of its effect on some fertility related hormones in male rats. Egypt. J. Comp. Path. & Clinic. Path., 21:340-358.

Hassan, A. A.; Wael M. Tawakkol; Abdel aziz A. El maaz and Howayda M. El Shafei. (2009). The hepatoprotective effect of dimethyl 4,4- dimethoxy 5,6,5,6- dimethylene dioxy-biphenyl – dicarbxylate (D.D.B.) against liver injury induced by aflatoxin B_1 in rates . Egypt. J. Appl. Sciences , Vol. 24 No. (9), 86-100.

Hassan,A.A.; Wael M. Tawakkol; Elbrawy A.M. (2010). The hepatoprotective effect of dimethyl 4,4- dimethoxy 5,6,5,6-dimethylene dioxy-biphenyl - dicarbxylate (D.D.B.) against liver injury induced by aflatoxin B_1 in rates . Int. J. Life Sciences, Vol. 7 No. (3) (148-153).

Hassan,A.A.;Nahed,M.El Mokhtar and Samira,A.M. Snousi(2011). Studies on acute mycotoxicosis in turkey. J. of New York Science J.,4 (5), 82-91.

Henry, R.J. (1974). "Clinical chemistry, principles and techniques." 2nd Ed., Harport and Rowhogerstown, M.D. 862.

Hussein, D.A. (2004). Mycological studies on cow pneumonia in the Mansoura Governorate. Assiut Vet. Med. J., 50(103). 75-79.

Kaneko, J. J.; Harvey, J. W. and Bruss, M. (1997). Clinical biochemistry of domestic animals. academic press. California, USA.

Keyle, A.C., A.N. Booth, M.S.Masri, M.R. Gumbmann and W.E., Gagne(1970). Chronic effect of aflatoxin in farm animals feeding studies. Proceedings of the Ist. U.S-Japan Conference on toxic microbiology US. Dep. Of Interior . Washington, pp. 72-75.

Knudtson, W.U. and Kirkbride, C.A. (1992). Fungi associated with bovine abortion in the northern plains states (USA). J Vet Diagn Invest 4:181-185.

Lee DH, and Jacobs DR Jr. (2005). Association between serum gamma-glutamyltransferase and C-reactive protein. Atherosclerosis 178:327-330.

Lee, J. H.; Lee, B. K. and Hong , S. K. (2009). Comparison of chemical compositions and antimicrobial activities of essential oils from three conifer trees. J. Microb. Biotechnol., 19 (4). 391-396.

Lim JS; Chun BY; Kam S;Jacobs DR Jr, and Lee DH. (2004).
Is serum gamma-glutamyltransferase inversely associated with serum antioxidants as a marker of oxidative stress? Free Radic Biol Med 37:1018-1023.

Lyuch, M.S.; Shalkop,W.T.; Jacoby, N.M.; Smith, D.F. and Miller, R.W. (1971). Responses of dairy calves to oral dose of aflatoxin. J. Dairy Sci., 54:1688.

Madheswaran, R.; Balachandran, C. and Murali Manohar, B(2004). Influence of dietary culture material containing aflatoxin and T_2 toxin on certain serum biochemical constituents in Japanese quail. Mycopathologia. 158 (3).337-41.

Mahendra Pal, Lee; Chanqwood, Pal, M. and Lee, Cw. (1998). Spontaneous pneumonia in a goat due to Candida albicans. Korean J. of Vet. Clinical Medicine, 16(2).467-468.

Maity, B. and Deb, P. (1993). Incidence and histopathology of bovine pneumonia due to fungal infection. Indian Journal of Animal Health, 32(2). 109-112.

Mikulec, S.D.; Stojanović, L. and Jovanović, L. (2005). Moulds associated with milk depending on Macroclimate and geographical location. Applied Ecology and Environmental Research, 3(2). 61-65. http://www. ecology.kee.hu.

Mirchink,T.; G. Kashkina,G. B. and Abaturov, Y.D. (1969). Stability of pigmented fungi stemphylium botryosum wallr. and

cladosporium cladosporioides (fries) devries to gamma-radiation, Army Foreign Science and Technology Center Washington D C, pp.:12.

Mogda, K. Mansour; Hassan, A.A. and Rashed M.A. (2002). The fungi recorded in imported feed samples with reference to control of T-2 toxicosis by antioxidant substances in chicks. Vet. Med. J., Giza, 50 (4). 485-499.

Morriss, C. M., D. R. Ledoux, J. Broomhead, A. Bermudez, G. E. Rottinghaus, and A. Logan, (1997). Effects of pelleting on the toxicity of moniliformin in ducklings. Poultry Sci. 76(Suppl. 1).15.

Morsy, A. A. A. (2007). Mycotic causes of infertility in farm animals. Ph.D. Thesis, Fac. Vet. Med., Assiut University, Egypt.

Moubasher, A.H. (1995). Soil fungi are an active patner of our ecosystem. Their biodiversity and activities should be appreciated. Qatar Univ. Sci. J., 15:239-247.

Nashwa, A.Abu-Aita; Mogda K.Mansour, Samer,M.Moneir and A.A. Nada (2008). Influence of the probiotic pediococcus acidilactici on chromosomal aberrations, clininicopathological alterations and immunological changes in aflatoxicated rabbits. Vet. Med. J., Giza. Vol. 56(1).

Nayak, S. and Sashidhar, RB. (2010). Metabolic intervention of aflatoxin B_1 toxicity by curcumin. J Ethnopharmacol. 17;127(3).641-644.

Nesheim, S. (1971). Ochratoxin : occurrence, production, analysis and toxicity. 85 th. ANNM. Meeting AOAC. Washington, D.C.A. pp,23.

Netke SP, Roomi MW, Tsao C, and Niedzwiecki A(1997). Ascorbic acid protects guinea pigs from acute aflatoxin toxicity. Toxicol Appl Pharmacol.; 143(2).429-35.

Nilsson, S. and Persson, N. (1984). Occurrence of a disease in a dairy herd manifested by fever, dyspnoea and abortion. Svensk-Veterinartidning, 36(8/9). 415-416.

Orsi, RB, Oliveira CA, Dilkin P, Xavier JG, Direito GM and Corrêa B. (2007). Effects of oral administration of aflatoxin B_1 and fumonisin B_1 in rabbits (Oryctolagus cuniculus). Chem Biol Interact. 15; 170(3).201-208.

Osuna, O. and Edds, G.T. (1982). Toxicology of aflatoxin B_1, warfarin, and cadmium in young pigs: clinical chemistry and blood coagulation. Am J Vet Res. 43(8).1387-94.

Panangala; V.S.; Fish, N.A. and Barnum, D.A. (1978). Microflora of the cervicovaginal mucus of repeat breeder cows. Canadian Veterinary Journal, 19 (4). 83-89.

Patgiri, G.P. and Uppal, P.K. (1983). Microflora of bovine female genital tract affected with various reproductive disorders. Indian Journal of Microbiology, Immunology and Infectious Diseases,4 (1). 19-22.

Pusterla, N.; Ossent, P.; Braun, U. (1996). A case of acute disseminated mycotic pneumonia in a cow. Schweizer-Archiv-fur-Tierheilkunde. , 138(4). 189-194

Raso, GM.; Meli, R and Di, Carlo G, (2001). Inhibition of inducible nitric oxide synthase and cyclooxygenase-2 expression by flavonoidsin macrophage J774A.1. Life Sci;68:921 -31.

Rastogi S, Shukla Y, Paul BN, Chowdhuri DK, Khanna SK, Das M. (2007). Protective effect of Ocimum sanctum on 3-methylcholanthrene, 7, 12-dimethylbenz(a) anthracene and aflatoxin B1 induced skin tumorigenesis in mice. Toxicol Appl Pharmacol. 1; 224(3).228-40.

Reitman, S. and Frankel, S. (1957). "Acolorimetric determination of serum glutamic oxaloacetic acid and glutamic pyruvic transaminase." Am. J. Clin. Path., 28: 56- 58.

Salem, R.M.; Mogda, K.; Mansour, M.A, Rashid and Koratum, K.M.

(2007). "Comparative study on the efficacy of salicylic acid and silicate

compounds in adsorption of aflatoxins." Egypt. J. Comp. Path. And Clin. Pathol., 20 (1). 149-164.

Shyamal S, Latha P G, Suja S R, Shine V J, Anuja G I, Sini S, Pradeep S, Shikha P. and Rajasekharan S (2010). Hepatoprotective effect of three herbal extracts on aflatoxin B_1-intoxicated rat liver.Singapore Med J 2010; 51 (4). 326-331.

Sonnenwirth, A. and Jareet, L. (1980). Garduals Clinical Laboratory Methods and Diagnosis." Vol.9,8th Ed., Mosby.

SPSS 14 (2006). "Statistical Package for Social Science, SPSS for windows Release 14.0.0, and 12 June, 2006." Standard Version, Copyright SPSS Inc., 1989-2006, All Rights Reserved, Copyright ® SPSS Inc.

Stresser, DM.; Bailey, GS.AND Williams DE.(1994). Indol-3-carbinol and beta-naphthoflavone induction of aflatoxin B_1 metabolism and cytochromes P-450 associated with bioactivation and detoxication of aflatoxin B_1in the rat.Drug Metab Dispos1994; 22: 383-391.

Tietz, N. W. (1996). Fundamentals of clinical chemistry 4th Ed., Vol 9, (Moss, D.W and Hendersson, A. R.) W. B Saunders company.

Timoney, J. F., Gillespi, J.H.; Scott, F.W.; Barlough, J.E. (1988). Microbiology and Infectious Diseases of Domestic Animals, Hagan and Bruner's: 8th ed. Ithaca, New York: Cornell Univ Pr, 1988: 418.

Vatassery, G. T., C. K. Angerhofe, R. C. Robertson and M. I. Sabri (1986). Vitamin E concentrations in different regions of the spinal cord and sciatic nerve of the rat .Journal of Neurochemistry,11,1419-1424

Wafia, H. Abdallah and Hassan, A.A. (2000). Sanitary status of some ready to eat meat meals in Ciaro and Giza Governorates.J. Egypt. Vet. Med. Ass., 60 (7). 95-104.

Wybenga, D.R.; Digigorgio, J. and Piliggi, V.J. (1971). "Automated method for urea measurement in serum." Clin. Chem., 97: 891 - 895.

Yoshioka, T.; Kawada, K.; Shimada, T. and Mori, M. (1979). Lipid peroxidation in maternal and cord blood and protective mechanism against actived oxygen toxicity in the blood. Am J Obstet Gynecol, 135, 372-376.

دراسات عن الامراض الفطرية والتسمم الفطرى فى الابقار.

ا.د/ عاطف عبد العزيز حسن [1] ، د/هويدا محمد السيد الشافعى[1] ، د/نهى حسن عرابى[1]، د/ رشا محمود حمزة سيد الاهل[1] و ا.د/ مجدة كامل منصور[2].

اقسام [1] الفطريات والسموم الفطرية و[2] الكيمياء- معهد بحوث صحة الحيوان - الدقى– مركز البحوث الزراعية – الجيزة – مصر .

الملخص العربى

تم إجراء هذة الدراسة على 40 حالة من الابقار المريضة و السليمة ظاهريا (20عينة سيرم من كل) في محافظة القاهرة . ، كما تم تجميع عدد 100 عينة من البان الحيوانات المريضة بالتهاب الضرع و مسحات أنفية و مسحات مهبلية والعلائق المستخدمة لتغذية الحيوانات المريضة (25 عينة من كل نوع). وقد اوضح الفحص الفطري للعينات ،عزل فطريات الاسبرجيلس بمعدلات واضحة. حيث سجل فطر الاسبرجيلس نسبة عدوى في عينات الابقار الموضحة عالية (88%،96%،80%،100%) على الترتيب. بينما سجلت الاجناس و الانواع الاخرى من العفن و الخمائر بنسب قليلة نسبيا. أوضحت عينات السيرم المجمعة من الابقار المصابة نسب عالية من الافلاتوكسين و الزيرالينون.وقد كان مستوى الافلاتوكسين في 60%من الابقار (15.20±0.01) و كان مستوى الزيرالينون 80% من الابقار(62±0.1) على الترتيب. بينما عينات العلائق المستخدمة في تربية الحيوانات أحتوت على كميات من AFB_1; OA; ZEAR.; T_2; بنسبة (60%،40%، 32%، 36%) من العلائق بمتوسط(50.1±0.55)و) 30.0±0.45)و(2.0±0.31)و(0.0±0.26)ppbعلى الترتيب. وعند اجراء الفحص الكيميائي لعينات الامصال من الحيوانات المريضة والسليمة ظاهريا مقارنة بمجموعة من الابقار المغذاة على علائق بها الافلاتوكسين ومعالجة ب 3% هيدروكسيد الامونيوم قد أوضحت النتائج أن الافلاتوكسين B_1، و الزيرالينون يسبب ارتفاع واضح في مستوى انزيمات AST, ALT , GGTمما يتسبب في تدمير خلايا الكبد. وكانت نسب اليوريا و الكرياتنين عالية بصورة واضحة في الحيوانات المريضة بالتسمم الفطري مقارنة بالحيوانات السليمة ظاهريا والمعالجة. وأدت السموم الفطرية الى انخفاض واضح لمستويات البروتين الكلي في الدم، الزلال ، الالفا ، البيتا، الجاما جلوبيولين. وعند فحص امصال الحيوانات بالمجموعات الثلاثة لعوامل الاكسدة اوضحت النتائج وجود ارتفاع فى MDA واكسيد النيتريك NOالذى يتسبب فى تحفيز افراز الانزيم المخلق لاوكسيد النيتريك وعند اجراء التغذية على علائق سامة معالجة بهيدروكسيد الامونيوم ادت الى احداث تحسن واضح فى المؤشرات الكيميائية السابقة مقارنة بالتغيرات المرضية فى امصال الحيوانات المريضة المسممة ، وقد تمت مناقشة مستفيضة للنتائج الحالية بوضوح تام .