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Studies on Mycosis and Mycotoxicosis in Cattle

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ABSTRACT
The present study was conducted on forty diseased and apparently healthy cases of cattle (20 of each) at Cairo Governorate. From districts of diseased cases, 40 serum samples of these cattle and 100 samples of milk of mastitic animals, nasal swab, vaginal swabs and feeds samples which used in breeding of diseased animals (25 0f each) were collected. The mycological examination of collected samples revealed isolation of Aspergillus species in predominant rates from the above samples of infected animals, where in cattle recovered from (88%, 96%, 80%, and 100%) respectively. Whereas, other genera and species of moulds and yeast were recovered in a comparatively lower significant rates. Sera of cases of diseased cattle contained significant levels of aflatoxins and zearalenon. Meanwhile, 60% of cattle had the mean levels of aflatoxins (15.20±0.4 ppb) and zearalenon in 80% in cattle with the mean level of (62±0.3). When, the used feed samples in breeding of these animals were had the amounts of AFB1; OA; ZEAR.; T2; and FB1 were detected in (60%; 40%; 32%; and 44%) of feed samples, with the mean levels of (55.0±1.50, 45.0±0.30 and 31.0±0.2 and 40.0±2.1 ppb), respectively. The biochemical study on sera of the infected and those fed treated toxic feed with ammonium hydroxide 3% , demonstrated that the AFB1 and zearalenon in serum of cattle lead to a marked elevation in the serum enzymes activities of (AST, ALT and GGT, which is indicate of hepatocellular damage. The levels of serum urea and creatinine were significantly high in toxicated animals as compared to apparently healthy animals. Mycotoxins in cattle inducing significantly decreased values in serum total protein, albumin, alpha globulin, beta globulin, and gamma globulin . The mycotoxicated animals had significant increase in serum level of malondialdehyde (MDA) and serum NO level which caused induction of inducible nitric oxide synthase (iNOS) protein, resulting in cytotoxicity and DNA damage. The feeding of treated toxic feed with ammonium hydroxid resulted a significant improvement of al previous altered biochemical parameters which resulted from toxicosis. The significance of the present result was fully discussed.
Key word: *Aflatoxins, zear., T2, Aspergillus sp., Biochemical parameters, Electrophoresis patterns, NO., MD.*

INTRODUCTION

Up to date the environmental pollution is considered the essential cause of animal diseases particularly pollution with fungi and their toxins of the used feed and water 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. This is in turn will contribute to the production of high quality food material for human consumption and the profitability of agricultural industry. 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 a broad spectrum effects [1-6]**.** Unlike many bacterial toxins, they are in general highly stable in face of 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 dermatitis[7,-9]. The mycotoxins of greatest agricultural and public health significance include aflatoxins, ochratoxins, trichothecenes, fumonisins, zearalenone, and ergot alkaloids [10-12]**.** 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 [13,14,5]. On the other hand, epidemiological studies associated with mycotoxins had a wide range of biological effects, including pulmonary oedema in pigs and ruminants (15Harrison et al., 1990), nephrotoxicity and liver cancer in different animal species [16, 3,6, 9]. The International Agency for Research on Cancer has declared that mycotoxins are potentially carcinogenic to human. Aflatoxin B1 had been statistically associated with a high

incidence of liver cancer in certain areas of Transkei, South Africa; in China and Egypt [17, 6]. All the previous literatures recorded that the pollution affect upon the growth rate and health of human being and animals including aneamia , stunted growth , carcinogenic, tremorgenic, haemorrhagic, dermatitic, pulmonary edema, immunosuppressive and hormonal effects [3,5,6, 18]. The aim of the present work was to investigate the problem of fungal and mycotoxicosis in cattle and detection the role of feed pollution as main environmental factors of animal diseases.

MATERIAL AND METHODS

Material

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 swab, vaginal swabs and feeds samples) which used in breeding of diseased animals(25 0f each) were collected. .

Mycotoxins standards

Standerds and immunoaffinity column of AFB_1, B_2, G_1 and $G_{2\ and}$ OA, ZER, T-2 AND FB1 were purchased from Sigma Chemical Company (USA).

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. [19].

Detection of mycotoxins in feed and sera of diseased cattle :

Detection of mycotoxins in serum of cattle and feed stuffs by fluerometric methods as described by Hansen [20] using immune-affinity column method**.**

Detoxification of aflatoxicated feeds by addition of ammonium hydroxid 3% : The feed samples that contaminated over permissible limits(over 700-1000 ppm/ ten of feed) were treated by addition of ammonium hydroxid 3%**.** After 7 dayes 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 continues for I month. All the animals were tested biochemically every 10 dayes during treatment for evaluation the influence of ammonium hydrox**id** on the toxicities of aflatoxins on the function of vital internal organs and patterns of serum protein electrophoresis.

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 used to assay the biochemical parameters as aspartate aminotransferase (AST) and alanine aminotransferase (ALT) activities according to Reitman and Frankel, [21], serum urea according to Wybenga *et al.* [22], serum creatinine level according to *Henry (231974),* Estimation of serum total protein and electrophoretic pattern were carried out after SonnenWirth and Jaret [24] and Davis [25], respectively. Serum lipid peroxide levels of malondialdehyde (MDA) were determined according to the method described by Yoshioka *et al.* [26]. Blood GSH concentration was measured using the method described by Buetler *et al.* [27]**.**Serum NO levels of the cattle were measured by enzymatic Greiss reaction [28]. Estimations of vitamin E, A and C were performed according to Henry [23].

Statistical Analysis

The obtained date were computerized and analyzed for significance, Calculation of standard error and variance according to [29].

RESULTS AND DISCUSSION

The past three decades have witnessed dramatic change in man's and animal's environment and his immune defenses. Consequently, the fungi have assumed as a major role in respiratory and systemic infectious diseases. The most common isolated fungi from mastitic milk samples of cattle were *Aspegillus* sp. (88%), *Fusarium* sp. (40%), *Pencilium* 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 [30]. The most frequent organisms implicated in mycotic mastitis

were yeasts, especially of the genus *Candida. 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 [31].

With reference to Table 1, it is obvious that the most common isolated moulds from cows' nasal swabs were *Aspegillus 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 [32]; Maity and Deb [33]; Mahendra et al., [34]. 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 [35**,** 36]**.**

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 [37].

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 recovered genera of fungi were *Aspegillus s*p. (80%), *Fusarium* sp. (16%), *Pencilium* sp.(32%), *Cladosporium* sp.(12%), *Alternaria* sp. (8%), *C.albicans* (40%), *C. tropicalis* (32%) and *Rhodotorula rubra*(3%). The results concur to some extent with the finding of Panangala *et al.,* [38]; Patgiri and Uppal [39]; Abdel-Rahman and Ibrahim [40] and [11] 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 [41]. On the contradictory, *A. fumigatus* has been reported by Knudtson and Kirkbride [42] as the most common cause of mycotic placentitis. *Candida albicans* and *C. tropicalis,* were the most frequent yeast species recovered from cows' vaginal swab samples as yeasts have been implicated as causes of bovine reproductive problems, including abortion in cows and infertility in bulls [43, 44].

It is clear that the most common isolated mould from cows' feed were *Aspegillus* sp. (100%), *Fusarium* sp. (24%), *Mucor* sp.(28%), *Pencilium* sp.(52%), *Cladosporium* sp.(8%), *Alternaria* sp. (28%), *C.albicans* (80%), *C. tropicalis* (40%) and *Rhodotorula rubra*(32%). These findings were in agreement with the results of Arakawa [45] and Hassan et al. [4, 5, 6,11].Also, it has been estimated that 25% of the world's crop production is contaminated with mycotoxins. Pusterla *et al.,* [46] 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 (3) showed that, sera of twenty cases of diseased cattle outbreaks which 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 the mean levels of aflatoxins (15.20±0.4 ppb) and zearalenon detected in 80% of cattle with the mean level of (62±0.3), respectively((Table, 2). Mycotoxins in sera cattle in Egypt in association with symptoms of toxicities were previously reported by Hassan [11]; Hassan et al. [2-6]. The effects of mycotoxins in human and animals were varied from carcinogenic; nephrotoxic and immunosuppressive health effects [47, 3-6]. 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 [47], there are many studies about whether the ingestion of meat, milk, and eggs originating from mycotoxin-exposed food-production animals is a significant pathway for mycotoxins among humans [48, 4, 5, 6,11]. In the feed samples were examined for fungal contamination and detection of mycotoxins. When, the used feed samples in breeding of these animals were subjected for detection of aflatoxins, the results revealed that the amounts of AFB1; OA; ZEAR.; T2; and FB1 were detected in (60%; 40%; 32%; and 44%) of feed samples, with the mean levels of (55.0±1.50, 45.0±0.30 and 31.0±0.2 and 40.0±2.1 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 the disease outbreak in animals**.** The Food and drug administration has established recommended maximum levels for aflatoxins in animal feed are 20 µg/kg of feed [49]. The permissible limits of aflatoxin for large ruminants were varied between 700-1000 ppm/ ten of feed which caused loss of weight gain, high food consumption and low feed efficiency [50]. Whereas, the O.A in each produced significant pathological changes in pregnant animal at the levels of 2.5 ppmof animal body

weight for 5 days when given orally [51] . On the other hand the trichothecenes mycotoxins (T2) was produced such change at the doses as low as 10 ppm [52].

Therefore, the detected levels of mycotoxins were significantly over the permissible limits in feeds which may resulted of toxicosis due to continous feeding of toxiocated feed. The same findings were detected by many authors as [3-6], who detected such these diseased cases in association of significant high mycotoxin levels in feed and sera of diseased animals.

Therefore, the detoxification of toxic feed become a critical demand, ammonium hydroxide was the most safe chemical compound used for this purpose [1,3,4,5].

In this study, we demonstrated that the AFB1 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 [53,54] elevation could potentially be attributed to hepatic degeneration and subsequent leakage of enzymes into circulation after rupture of the plasma membrane and cellular damage [55, 56]. Such hepatic toxic effect of aflatoxin attributed to its active metabolite in liver as epoxide, Netke *et al.* [57] which covalently bind to DNA and may affect structural and enzymatic protein function [58]. Moreover the elevation of serum GGT activity suggested hepatic necrosis, thickness of bile duct and intrahepatic cholestasis [59]**.**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 hydroxid. 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 alteration in tubular function [4, 60]. Also, if microbial protein synthesis in the rumen which is inhibited by mycotoxins, more free ammonia remains in the rumen, is absorbed into the blood, and is metabolized to urea, resulting in elevated blood urea concentrations and reduced hepatic fractional protein synthesis rates [61-63].

Mycotoxins in cattle and buffaloes inducing 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 [64] in pigs; Hassan and mogda [4] in quails and Madheswaran et al., [65] in chickens. Mycotoxins cause inhibition of DNA and protein synthesis as well as immunosuppressive due to the inflammation, cirrhosis of liver and kidneys [66,67]. At the same table (5) the globulin component showed drop in α1, α2, β1 and γ1 globulin in all the diseased animals, while increase γ2globulin as compared with healthy animals. The results coincided with the tune of total proteins and albumin.This may be attributed to that AFB1 causes hepatotoxic, nephrosis, hemorrhages (liver and kidneys) [68]. In addition, mycotoxins has immunosuppressive effect inhibit nearly cellular and humeral immunologic reaction [68,2,5,4]. The decrease in serum level of globulin might be due to the adverse effect of mycotoxins on synthesis of total proteins and globulin [69, 70, 11].

In this study, we have shown that the mycotoxicated animals had significant increase in serum level of malondialdehyde (MDA) MDA. Mycotoxins particularly AFB1 are well known to generate free radicals, disturbing the antioxidant status and ultimately leading to oxidative stress and carcinogenesis [71]. Lipid peroxidation plays an important role in carcinogenesis [72] and may lead to the formation of several toxic products, such as malondialdehyde (MDE) and 4-hydroxynonenal. Also, AFB1 peroxide is a very reactive and unstable metabolite of AFB1 that will bind to cellular macromolecules like DNA, RNA, lipids and proteins, leading to lipid peroxidation and cellular injury. [73]. The hepatic damage is associated with an increase in the tissue malondialdehyde (MDA) level, an indirect index of lipid peroxidation [74].

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 [75].

Our finding of low plasma retinol, and α-tocopherol, in toxicated cattle compared to apparently healthy and treated animals (table, 6) was shown in previous studies by Clarke *et al.,* [76], Vatassery et al., [77]. 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 [78,79,80].

In conclusion, previously it were recorded that the fungal diseases affect upon the growth rate and health of human being and animals including aneamia , stunted growth, carcinogenic, tremorgenic, haemorrhagic, dermatitic, pulmonary edema, immunosuppressive and hormonal effects [4,5,,6,11]. This study, reported the dangerous effects of fungal diseases and their mycotoxins and its pollution of animal feed which resulted a significant losses in animal health and cause 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 were a critical demand to safe the animal and human health.

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

Fungal isolates	Milk of mastitic animal		Nasal swabs		Vaginal swabs		Feeds	
	No. of +ve	%	No. of +ve	%	No. of +ve	%	No. of +ve	%
Aspergillus sp.	22	88	24	96	20	80	25	100
A. candiduss	6	24	1	4	0	0	15	60
A. clavatus	2	8	2	8	2	8	1	4
A .flavus	20	80	20	80	13	52	18	72
A. fumigatus	21	84	16	64	7	28	5	20
A. niger	10	40	20	8	23	92	12	48
A.. ochraceus	0	0	0	0	5	20	5	40
Fusarium sp.	10	40	16	64	4	16	6	24
F. oxysporum	10	40	1	4	0	0	6	24
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
P. citrinum	1	4	3	12	0	0	0	0
P. duclauxii	0	0	0	0	3	12	2	8
P funiculosum	0	0	10	40	2	8	0	0
P. islandicum	0	0	1	4	2	8	0	0
P. oxalicum	1	4	0	0	4	16	11	44
P.purpurogenum	0	0	0	0	0	0	2	8
Cladosporium specie	2	8	4	16	3	12	2	8
Al. alternata	1	4	1	4	2	8	7	28
C. albicans	15	60	6	24	10	40	20	80
C. tropicalis	6	24	17	68	8	32	10	40
Rhodotorula rubra	4	16	3	12	3	121	8	32

- 25 samples of each were examined

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

Animal	Total No.	Aflatoxin B1			Zearalenone		
		Positive cases	%	Mean levels ppb	Positive cases	%	Mean levels ppb
Apparently healthy cattle	20	2	10	2.5±0.01	1	5	5.0±0.0
Diseased cattle	20	12	60	15.20±0.01	16	80	62.0±0.1

Ppb : Part per billion

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

Mycotoxin in feeds	Aflatoxin B1			Ochratoxin A			Zearalenon			T-2			Fumonisin BI		
	Positive		Mean of levels ppm	Positive		Mean of levels ppm	Positive		Mean of levels ppm	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): Biochemical changes due to mycotoxicosis in cattle

Chemical tests	Apparently healthy cattle	Diseased cattle groups	Treated cattle groups
AST	49.11±4.26	90.45 ± 7.21***	63.33±3.2*
ALT	30.67± 3.84	51.34± 3.29***	43.33±3.8*
ggt u/l	22.64 ± 1.15	64.18±4.49***	49.9±3.54*
Urea	43.48±1.67	58.86±3.79**	48.65±1.6
Creatinine	0.94±0.05	1.14±0.06*	0.96±0.96

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

Table (5): Paterns of protein electrophoresis in diseased cattle.

Paterns of protein	Apparently healthy cattle	Diseased cattle groups	Treated cattle groups
T.protein	6.16 ± 0.09	5.42 ±0.16***	6.04±0.21
Albumin	2.38 ±0.04	1.98 ±0.08***	2.26±0.07
Alpha1	0.82 ±0.02	0.63 ±0.01***	0.72±0.03*
Alpha2	0.35 ±0.03	0.29 ±0.01	0.34±0.05
T-alph	1.17 ±0.04	0.92 ±0.01***	1.06±0.03*
Beta1	0.66 ±0.02	0.56 ±0.01***	0.66±0.03
Beta2	0.40 ±.02	0.438 ±0.02	0.48±0.06
T.beta	1.06 ±0.01	0.998 ±0.02*	1.14±0.05
Gamma1	1.26 ±0.03	1.19 ±0.02**	1.29±0.13
Gamma2	0.29 ±0.01	0.33 ±0.02	0.29±0.02
T.Gamma	1.55 ±0.04	1.52 ±0.05	1.58±0.13
T.globulin	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 Buffaloes and 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)	12.4±6.8	18.2±5.7**	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

1. HASSAN A.A. (1994). Detection and control of ochratoxin in food and food-stuffs. Thesis, Ph.D. *Fac. Vet. Med.,* Cairo University.
2. 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.

3. 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.
4. Hassan, H.A. ; Ramadan M. Khoudair and El sayed E. Youniss (2009): The Effect of Some Mycotoxins on Immunity of Cattle Vaccinated against Brucellosis and Guinea Pigs Experimentally Vaccinated With S19 Vaccine. *Egypt. J. Appl. Sciences* , Vol. 24 No. (**2**) (1-13).
5. 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.
6. 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 B1 in rates . *Int. J. Life Sciences,* Vol. 7 No. (**3**) (148-153).
7. Hassan , . A. A. (1998): Mycosis in turkeys.**5** th Scientific Congress proceeding, Fac. of Vet. Med., Cairo University, Vet. Med. J. Giza, 46.(**4B**): 857-865.
8. 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.
9. 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.
10. 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.
11. 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 rats .*Egypt. J. Appl. Sciences,* Vol. 24 No. (**9**),86-100.
12. 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.
13. 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.
14. 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.
15. 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, 217-221.
16. 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.
17. CHU, F. S. AND LI, G. Y. (1994): Simultaneous occurrence of fumonisin B9 and other mycotoxins in moldy corn collected from the peoples of republic of China in regions with high incidence of oesophageal cancer. *Appl. Environ.Microbiol.,* 60, 847-852.
18. 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
19. 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.
20. Hansen TJ. (1993): Quantitative testing for mycotoxins. Am, Assoc, *Cereal Chemist.* Inc., 38 (5): 5.
21. Reitman, S. and Frankel, S. (1957): "Acolorimetric determination of serum glutamic oxaloacetic acid and glutamic pyruvic transaminase." *Am. J. Clin. Path*., 28: 56- 58.
22. Wybenga, D.R.; Digigorgio, J. and Piliggi, V.J. (1971): "Automated method for urea measurement in serum." *Clin. Chem*., 97: 891- 895.
23. Sonnenwirth, A. and Jareet, L. (1980): " Garduals Clinical Laboratory Methods and Diagnosis." Vol.9,8th Ed., Mosby.
24. Davis, B. (1964): Disk electrophoresis. II Method and application to human serum protein. *Ann. N.Y. Acad. Sci*., 929: 404-427.
25. Yoshioka, T.; Kawada, K.; Shimada, T. and Mori, M. (1979): Lipid peroxidation in maternal and cord blood and protective mechanism aganist actived oxygen toxicity in the blood. *Am J Obstet Gynecol*, 135, 372-376.
26. Beutler, E.; Dubon, O .and Kelly, BM (1963): Improved method for the determination of blood glutathione. *J Lab Clin Med* 61: 882-888.
27. Henry, R.J. (1974): "Clinical chemistry, principles and techniques." 2ndEd., Harport and Rowhogerstown, M.D. 862.
28. Burgner, D.; Rockett, K and Kwiatkowski, D (1999): Nitric oxide and infectious diseases. Arch Dis Child, 81, 185–188.
29. 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.
30. 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
31. 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.
32. 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.

33. 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.
34. 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.
35. 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.
36. Moubasher, A.H. (1995): Soil fungi are an active patner of our ecosystem.
37. 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
38. Panangala; V.S.; Fish, N.A. and Barnum, D.A. (1978): Microflora of the cervico-vaginal mucus of repeat breeder cows. Canadian Veterinary Journal, 19 (**4**): 83-89.
39. 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.
40. 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
41. 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.
42. 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.
43. 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.
44. Morsy, A. A. A. (2007): Mycotic causes of infertility in farm animals. Ph.D. Thesis, Fac. Vet. Med. Assiut University, Egypt.
45. 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
46. 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
47. 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.
48. 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.
49. 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.
50. 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.
51. Nesheim, S. (1971): Ochratoxin : occurrence, production, analysis and toxicity. 85 th. ANNM. Meeting AOAC. Washington, D.C.A. pp,23.
52. Epply, R.M. and Baily, WJ.(1993): 12,13, Epoxy Trichocethine, as the propaple mycotoxins responsible for stachybotryotoxicosis. *Science*, 181:758-760.
53. Orsi RB, Oliveira CA, Dilkin P, Xavier JG, Direito GM, 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**):201-8.
54. Nayak, S., and Sashidhar, RB. (2010): Metabolic intervention of aflatoxin B1 toxicity by curcumin. J Ethnopharmacol. 17; 127(3):641-4.
55. 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.
56. Afzali, N., and Devegowda. G. (1999): Ability of modified mannanoligosaccharide to counteract aflatoxicosis in broiler breeder hens. Poult. Sci. 78:228.
57. 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.
58. Cullen, J.M. and Newbern, P.M. (1994): The toxicity of aflatoxin human health, veterinary and agricultural significance. 2nd Ed., Academic Press, Philadelphia.
59. Kaneko, J. J; Harvey, J. W. and Bruss, M. (1997):Clinical biochemistry of domestic animals. academic press. California, USA.
60. 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):
61. 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.
62. Danicke, S., K. Matthaus, P. Lebzien, H. Valenta, K. Stemme, K.-H. Ueberschar, E. Razzazi-fazeli, J. Bohm, and G. 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.
63. Danicke, S., T. Goyarts, S. Doll, N. Grove, M. Spolders, and G. Flachowsky(2006): Effects of the Fusarium toxin deoxynivalenol on tissue protein synthesis in pigs. Toxicol. Lett. 165:297–311
64. Osuna O, and Edds GT(1982):Toxicology of aflatoxin B1, warfarin, and cadmium in young pigs: clinical chemistry and blood coagulation. *Am J Vet Res.* 43(**8**):1387-94.

65. 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
66. 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.
67. 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.
68. Tietz, N. W. (1996): Fundamentals of clinical chemistry 4th Ed., Vol 9, (Moss, D.W and Hendersson, A. R.) W. B Saunders company.
69. 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.
70. Agag M(2004):mycotoxins in foods and feeds 1-aflatoxins Ass. Univ. Bull. Environ. Res. Vol. 7 No. 1, March 2004
71. 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.
72. 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.
73. Stresser, DM.; Bailey, GS.AND Williams DE.(1994): Indol-3-carbinol and beta-naphthoflavone induction of aflatoxin B1 metabolism and cytochromes P-450 associated with bioactivation and detoxication of aflatoxin B1 in the rat. *Drug Metab Dispos* 1994; 22:383-91.
74. 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 B1-intoxicated rat liver. *Singapore Med J* 2010; 51(4): 326-331.
75. 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.
76. Clarke ED, Wardman P, and Wilson I. (1984): Cis-trans isomerization of the (5-nitro-2-furyl)acrylamide, AF-2, initiated by ascorbate, glutathione, Fe(II) and OH-. *Biochem Pharmacol. Jan* 1; 33(**1**):83-7.
77. 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*,11,1419-142.
78. Lee DH, and Jacobs DR Jr. (2005): Association between serum gamma-glutamyltransferase and C-reactive protein. *Atherosclerosis* 178:327-330.
79. Lee DH, Blomhoff R, and Jacobs DR Jr. (2004): Is serum gamma glutamyltransferase a marker of oxidative stress*? Free Radic Res* 38:535-539
80. 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.