Dept. of Biochemistry and Mycology,
Animal Health Research Institute, El-Mansoura Laboratory.

SOME STUDIES ON MYCOTIC ABORTION OF SHEEP FED ON CONTAMINATED FOOD

(With 4 Tables)

By

R.A. EL-RAMADY; DOAA A.H. EL-MATARY; K.A. DEEB and S.A. TAWFIK

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بعض الدراسات علي الإجهاض الفطري في الأغنام المغذاة على علائق ملوثة

رافت أحمد الرمادي ، دعاء أحمد حسين المطري ،
خالد عبد الرحمن الغريب ديب ، سيد أحمد توفيق عيد

من أهم المشاكل التي تواجه مربي الأغنام والمزارع المتخصصة الإجهاض في النعاج لما يسببه من خسائر اقتصادية كبيرة متمثلة في انخفاض الإنتاج نتيجة فقد الأجنة وأحياناً مرض أو نفوق النعاج بالإضافة إلي تكلفة العلاج. وقد استهدف هذا البحث عزل الفطريات المسببة للإجهاض في النعاج ومعرفة تاثيرها علي الصحة العامة لتلك الحيوانات بدراسة التغيرات الدموية والبيوكيميائية التي تحدث لها عند حدوث الإصابة مع تقييم إضافة مادة الكركم التي ثبت فاعليتها كعلاج مضاد للفطريات والسموم الفطرية في العلائق الملوثة لتلك الحيوانات لمنع أو تقليل حدوث الاجهاض الفطري وبخاصة في الفترة الأخيرة من الحمل. أجريت الدراسة علي عدد ٣٠ من النعاج العشار تتراوح أعمارها بين ٣ – ٥ سنوات خلال الثلاثة أشهر الأخيرة من الحمل وقسمت النعاج إلي ثلاث مجموعات كل مجموعة تشمل ١٠ حيوانات عزلت في أحواش منفصلة عن بعضها وغذيت المجموعة الأولي (الضابطة) علي علائق غير ملوثة طبيعياً بالفطريات والسموم الفطرية وغذيت المجموعة الثانية علي علائق ملوثة طبيعياً بالفطريات والسموم الفطرية وغذيت المجموعة الثالثة (المعالجة) علي نفس عليقة المجموعة الثانية الملوثة مضافاً إليها مادة الكركم بجرعة ١٠جم/رأس/يومياً طوال الثلاثة أشهر الأخيرة من الحمل. وتم أخذ عينات من العليقة المستخدمة والدريس للعزل الفطري وقياس محتواها من السموم الفطرية. هذا وقد تم متابعة جميع الحيوانات حتي الولادة وتسجيل حالات الإجهاض التي حدثت. وتم أخذ عينات دم بعد شهرين من جميع الحيوانات لعمل الفحوصات الدموية والبيوكيميائية وكذلك تم جمع مسحات من الإفرازات المهبلية والمشيمية ومعدة الأجنة المجهضة للفحص الفطري من الحيوانات التي حدث لها إجهاض مع أخذ عينات مصل لعمل الفحوص السيرولوجية المختلفة. وكانت أهم النتائج ما يلي: ١- أوضحت الدراسة حدوث ٤ حالات إجهاض في المجموعة الثانية (المغذاة علي علائق ملوثة) وحدثت حالة إجهاض واحدة في المجموعة الثالثة (المعالجة)، بينما لم تحدث أي حالات إجهاض في المجموعة الأولي (الضابطة). ٢- تم عزل فطريات الأسبراجيلس (فلافس ، فيوميجيتس، ورارازيتكس) وفطريات الميوكر والكانديدا (البيكاتر وتروبيكلس) من

محتويات معدة الأجنة المجهضة وكذلك المشيمة والإفرازات المهبلية ، وجدير بالذكر أن معظم الفطريات المعزولة من الحيوانات المريضة تم عزلها من العلائق المركزة والدريس المستخدم في تغذية تلك الحيوانات بنسب مختلفة. ٣- بالنسبة للقياسات الدموية أسفرت النتائج حدوث انخفاض معنوي في مستويات الهيموجلوبين وعدد كرات الدم الحمراء وخلايا الليمفوسيت في المجموعة المغذاة علي علائق ملوثة مقارنة بالمجموعات الأخري. ٤- بالنسبة للقياسات البيوكيميائية أظهرت نتائج التحليل حدوث ارتفاع معنوي في مستوي إنزيمات الكبد وإنزيم الفوسفاتيز القاعدي وانخفاض في قيم البروتين الكلي والجلوبيولين في المجموعة المغذاة علي علائق ملوثة مقارنة بباقي المجموعات. هذا وقد ثبت من نتائج التحاليل أن معظم القياسات الدموية والبيوكيميائية للحيوانات المعالجة كانت في الحدود الطبيعية للحيوانات السليمة أو قريبة جداً منها، وتوصي الدراسة بإضافة الكركم في علائق الأغنام العشار الملوثة أو التي يحتمل تلوثها بالفطريات بجرعة ١٠جم/رأس/يومياً في الثلاثة أشهر الأخيرة من الحمل للحد من الإجهاض الفطري في الأغنام أو تقليل نسبة الإصابة.

SUMMARY

The aim of the present study is identification of fungi as a causative agent of abortion in sheep experimentaly fed on contaminated ration in correlates this finding with haematological and biochemical alteration before and after turmeric supplementation in ewes rations during pregnancy to evaluate these spice material in the prevention or reducing the risk of mycotic abortion especially in late of gestation period. Thirty pregnant ewes aged 3 – 5 years during the last three months of pregnancy were divided randomly into three equal groups (each of 10 animals). Animals in groups (G_1, G_2 and G_3) were housed separately in semi-roofed yards and received uncontaminated diet (control G_1), diet naturally contaminated with fungus and mycotoxins (G_2) and the same contaminated diet with curcumin spices at level of 10gm/head/day (G_3). Blood and serum samples were collected from each animal at two months post feeding trial for haematological and biochemical studies. Swabs from vaginal discharge, placenta and stomach content of the aborted foeti in addition to serum samples from aborted ewes were collected for mycological and serological examination. The obtained results showed that abortion happened at the last 2 – 3 weeks of pregnancy in 4 ewes (G_2) and only in one ewe (G_3) and was not observed in control (G_1). Mycotic examination revealed that A. flavus, *A. fumigatus, A. parasiticus, Mucor* species, *Candida albicans* and *Candida tropicalis* were isolated with different percentages from the collected samples. Most of isolated fungi were also recovered from concentrate and hay offered as ration for feeding. The results of blood

parameters should that the mean values of Hb%, RBCs count, MCH, MCHC and lymphocytes were reduced with contaminated diet G_2 when compared with control G_1 or curcumin treated group G_3. The biochemical analysis of serum revealed significant elevation in the activities of serum enzymes AST and ALP. While the levels of serum total protein and globulin were significantly reduced in contaminated diet fed group (G_2) when compared with other groups. It could be concluded that the pollution of ration with fungi and mycotoxins may be considered important cause of abortion in pregnant ewes. The dietary supplementation of curcumin for pregnant ewes fed on polluted ration with fungi and mycotoxins at dose level (10 gm/head/day) during the last 3 months of pregnancy help in reducing the risk of mycotic abortion and improve the general health condition without any toxic effects as evidenced by haematobiochemical parameters.

***Key words:** Abortion, Sheep, Aspergillus, Candida*

INTRODUCTION

Abortion in sheep and goat as in large animals is due to a wide variety of causes such as chemical, mechanical, nutritional, bacterial, viral and mycotic causes.

Aspergillus species is the main cause of mycotic abortion followed by *Mucor*, *Absidia* and *Candida* in cattle (Hoedemake and Held, 1985; Munoz and Gonazalez, 1987); and in ewes) Subhash *et al.*, 1999 and Mohamed, 2002). Abortion usually occurred during the last trimester of pregnancy (Corbel, 1988). *Aspergillus fumigatus* is a fairly common in both hay and silage (Radostits *et al.*, 2000). Mycotoxins in genital tract are spermicidal to spermatozoa (Saxena and Ishaque, 1977).

Regarding the blood chemistry and abortion in goats, Zaghloul *et al.* (1985) investigated the blood biochemical alteration of goats with chlamydial abortion and found an increase in the level of serum protein blood urea, nitrogen, creatinine and alanine aminotransferase.

Natural fungal poisons in mould grains strongly influence variation infertility and mortality rates, mycotoxins which influence fertility might reduce consumption and increase abortion as well as possible damaging in immune system (Matossian, 1996), it also retard growth and cause histopathological and biochemical changes in liver and kidneys. Various antifungal drugs have been used for treatment of aspergillosis (Helt and Riviere, 1995). Itraconazole reduced in 60 – 70%

success rates and have been widely used in human medicine and recently are used in veterinary medicine (Kelly *et al*., 1995).

One of the most important medicines which are used in prevention and treatment of various and serious diseases are medicinal herbs as turmeric which is the powdered dry rhizome of curcuma longa linn. plant of the family zingiberaceae. Curcumin (diferuloylmethane) is the major yellow pigment of turmeric, they are commonly used as spice food preservatives, yellow coloring and flavouring agents.

Extensive work has been done to establish the biological activities and pharmacological actions of turmeric and its extracts. Curcumin (diffruloylemethane) the main yellow bioactive component of turmeric has been shown to have a wide spectrum of biological actions, these include its anti-inflammatory, antioxidant, anticarcinogenic, antimutagenic, anticoagulant, antidiabetic, antibacterial, antifungal, antiviral, antiprotozoal, hypotensive, hypocholesteremic activities, curcumin has already been used to reduce post-operative inflammation. Also it has an potent immuno stimulant effects, so has been shown to have antiviral activity (Ishita *et al*., 2004).

Curcumin inhibits lipid peroxidation in different tissues of rat and mice (Okuda *et al*., 1993), it reduced D-galactosamine- induced glutamate oxalacetate and glutamate pyruvate transaminase level (Hikino, 1985). An either extract of C. longa have hypolipemic action in rats (Rao *et al*., 1970) and lower cholesterol, fatty acids and triglycerides in alcoholic induced toxicity (Rukkumani *et al*., 2003). Curcumin had no any adverse effect on both growth and levels of erythrocytes, leucocytes and other blood constituent as haemoglobin, total serum proteins, alkaline phosphates etc.. (Sambaiah *et al*., 1982).

Turmeric and curcumin have antifungal activity. Turmeric oil is also active against *Aspergillus flavus, A. parasiticus, Fusarium moniliforme* and *Penicillium, digitatum* (Jayaprakasha *et al*., 2001). Thus both turmeric and curcumin have the potential for the development of various diseases (Ishita *et al*., 2004).

Accordingly the present investigation was undertaken to study the identification of fungi as a causative agents of abortion in sheep experimentaly fed on contaminated retion and correlate these finding with haematological and serum biochemical alteration before and after turmeric supplementation in ewes rations during pregnancy to evaluate these spice material in prevention or reducing the risk of mycotic abortion especially in late of gestation period.

MATERIALS and METHODS

I- Animals:

The present investigation was carried out on 30 pregnant ewes aged 3-5 years during the last three months of pregnancy. These animals belonged to a special private farm located at Dakahlia Governorate. All animals were apparently healthy and free from any internal and external parasites after parasitological examination according to the methods described by Soulsby (1982).

II- Experimental design:

The chosen animals were allocated randomly into equal groups (each of 10 animals) each group were housed separately in semi-roofed yard.

The sheep groups were fed on experimental diet as following:

A- Uncontaminated diet fed to control group (G_1).

B- Diet contaminated with mycotoxin and fungus fed to the 2nd group (G_2).

C- Diet contaminated with mycotoxin and fungus treated with curcumin spices fed the third group at level of 10 gm/day according to Aggarwal *et al.*, (2003).

Feed allowances were calculated according to NRC (1985) and the amount of concentrate feed mixture (CFM) and Trifolium Alexandrium hay were estimated to cover 40 and 60% of dry matter requirement respectively. The concentrate feed mixture (CFM) consists of 20%, uncorticated cotton seed meal, 40% , yellow corn 7%, soyabean meal, 25%, wheat brains, 5% molasses, 1.5% limestone, 1% common salts and 0.5% mineral mixture.

The feeds in experimental diet (G_2 and G_3) were naturally contaminated during the storage period with toxins. The concentrated feed mixture was contaminated with 35.1 ppb mixture of aflatoxins and Trifolium Alexandrium hay contaminated with 37.2 ppb mixture of aflatoxins but the feed used in control diet (G_1) were uncontaminated with aflatoxins. Samples of feeds were analysed according to A.O.A.C. (2005).

III- Samples:

A- Swabs from vaginal discharge and parts of placenta of aborted ewes and samples from stomach contents of aborted foeti were collected under complete aseptic condition and stored at 4°C and send to laboratries of animal health research institutes as quickly as possible for mycological, microbiological and viral examination.

B- Serum samples were taken from the diseased and control parturient ewes for the serological (brucellosis and other causative bacterial agent); viral (Riftvalley fever). Blood smears were also taken for blood parasites examination.

C- Blood sampling: Two blood samples were collected from different two groups of animals under investigation after two months post treatment:

1- The first blood samples were collected in heparinized tube for the determination of total erythrocytes and total leucocytic counts, packed cells volume (PCV), haemoglobin concentration following the technique described by Coles (1986). Blood indices were also calculated.

2- The second blood samples were collected without anticoagulant for serum separation and stored at -20°C. Serum samples were used for determination of transaminases enzymes AST, ALT, alkaline phosphatase AP, total protein, albumine, total bilirubin, urea-N, creatinine. Glucose, total lipids, cholesterol triglyceride were determined by using commercial kits supplied by (Boehringer, Mannheim, Germany) for all colorimetric biochemical analysis, while serum globulin was calculated mathematically by substracting the albumin value from the total protein value.

D- Feed samples: A total of 10 samples were collected from concentrate (6) and hay (4). The examined samples were sent to the laboratory for mycological examination and they were analyzed chemically to determine the possible mycotoxins.

IV- Mycological examination of animal samples:

The collected samples were treated with 15% KOH and examined microscopically for presence of hyphae then cultivated onto sabouraud's dextrose agar with chloramphenicol then incubated at 25°C and 37°C for (2-7 days) and the isolated fungi were identified according to Refai (1979); Christensen (1981), Kozakiewicz (1989) and Moubasher (1993).

V- Mycological examination of feed samples:

Ten grams of each samples were weighed, diluted with 90 ml of solution prepared by adding one ml of tween 80 (Merck) to one litre sterile distilled water (0.001 tween 80) and allowed to stand for 15-30 min with intermittent shaking before being plated. Appropriate serial 10 dilution were made, 0.1 ml were surface plated for isolation of fungi, Sabouraud's dextrose agar (Difco) were used. Isolation and identification of the different fungi were done as mentioned before.

VI- Examination of certain mycotoxins from feed samples:

The production of aflatoxins, ochratoxin and zearalenone from the toxigenic strains of *Aspergillus and Fusarium* species were determined as it was described by Wander *et al.* (1965) and Edds (1973).

Statistical analysis:

The obtained data was statistically analyzed according to Senedecor and Cochran (1982) using SAS computer program.

RESULTS

Table 1: Number and percentage of fungal isolates recovered from the aborted ewes and from stomach contents of aborted foeti

Fungal spp.	Stomach contents of foeti (30)		Placenta (30)		Vaginal discharge (30)	
	No.	%	No.	%	No.	%
A. fumigatus	8	26.7	12	40	10	33.3
A. flavus	9	30	12	40	5	16.7
C. parasiticus	4	13.3	3	10	4	13. 3
C. albicans	3	10	2	6.7	2	6.7
C. tropicalis	1	3.4	-	0	-	0-
Mucor	3	10	6	20	4	13.3

Table 2: Levels of mycotoxins (PPb) produced in feed samples

Feed samples	Aflatoxin	Ochratoxin	Zearlenone
Concentrates	21	10	-
Concentrates	12	9	-
Concentrates	24	3	10
Hay	12	6	-
Hay	30	-	8

Table 3: Haematological parameters (mean ± SE) of healthy pregnant ewes as affected by different experimental diets.

Parameters	*Groups*		
	Control (G_1) n = 10	Contaminated diet (G_2) n = 10	Treated diet (G_3) n = 10
Haemoglobin "Hb" (g/dL)	10.68 ± 0.26^{a}	10.01 ± 0.21^{b}	11.23 ± 0.23^{a}
Hematocrit "HCT" (%)	33.09 ± 1.30^{a}	36.33 ± 1.76^{a}	34.6 ± 1.53^{a}
RBCs (x 10^6/uL)	12.4 ± 0.38^{a}	11.0 ± 0.26^{a}	12.03 ± 0.28^{a}
MCV (FL)	26.68 ± 0.23^{a}	33.03 ± 0.28^{a}	28.76 ± 0.24^{a}
MCH (pg)	8.61 ± 0.20^{a}	9.1 ± 0.21^{a}	9.33 ± 0.22^{a}
MCHC (%)	32.27 ± 0.74^{a}	30.30 ± 0.69^{b}	32.45 ± 0.74^{a}
WBCs (x 10^3/uL)	8.17 ± 0.33^{a}	8.96 ± 0.23^{a}	8.42 ± 0.25^{a}
Lymphocytes (%)	72.4 ± 0.89^{a}	62.8 ± 0.78^{b}	70.3 ± 0.66^{a}
Neutrophils (%)	24.0 ± 1.0^{a}	28.7 ± 0.88^{a}	24.6 ± 1.67^{a}
Monocytes (%)	3.0 ± 0.58^{a}	4.6 ± 0.88^{a}	3.2 ± 1.0^{a}
Eosinophils (%)	0.6 ± 0.12^{a}	3.2 ± 0.60^{a}	1.7 ± 0.88^{a}
Stap cells (%)	0.0 ± 0.0^{a}	0.7 ± 0.33^{a}	0.2 ± 0.09^{a}

Means in the same row with different superscribts differ significantly at ($P < 0.05$).

Means with the same letter in the same row are not significantly different

Table 4: Biochemical parameters (mean ± SE) of healthy pregnant ewes as affected by different experimental diets.

Parameters	*Groups*		
	Control (G_1) n = 10	Contaminated diet (G_2) n = 10	Treated diet (G_3) n = 10
AST (u/L)	40.03 ± 0.67^{a}	46.46 ± 3.99^{a}	41.0 ± 1.84^{a}
ALT (u/L)	28.41 ± 0.69^{b}	37.97 ± 1.27^{a}	31.51 ± 0.71^{b}
ALP (u/L)	82.89 ± 1.77^{b}	91.57 ± 1.62^{a}	79.47 ± 2.02^{c}
Total protein (g/dL)	6.90 ± 0.06^{ab}	6.53 ± 0.15^{b}	7.03 ± 0.10^{a}
Albumin (g/dL)	2.86 ± 0.12^{a}	2.72 ± 0.20^{a}	2.82 ± 0.12^{a}
Globulin (g/dL)	4.04 ± 0.07^{ab}	3.78 ± 0.06^{b}	4.17 ± 0.03^{a}
A/G	0.70 ± 0.04^{a}	0.71 ± 0.06^{a}	0.67 ± 0.03^{a}
Total bilirubin (mg/dL)	0.30 ± 0.06^{a}	0.46 ± 0.09^{a}	0.39 ± 0.10^{a}
Serum creatinine (mg/dL)	1.26 ± 0.15^{a}	1.44 ± 0.06^{a}	1.25 ± 0.09^{a}
Blood urea (mg/dL)	29.44 ± 1.29^{b}	36.05 ± 0.92^{a}	30.28 ± 0.62^{ab}
Glucose (mg/dL)	69.33 ± 2.33^{a}	64.0 ± 2.08^{a}	66.67 ± 1.70^{a}
Total lipids (mg/dL)	362 ± 10.06^{ab}	398.2 ± 13.64^{a}	341.0 ± 7.66^{b}
Cholesterol (mg/dL)	98.6 ± 3.01^{a}	93.4 ± 1.53^{a}	95.5 ± 2.85^{a}
Triglycerides (mg/dL)	88.34 ± 1.33^{a}	81.59 ± 2.33^{a}	85.4 ± 1.15^{a}

Means in the same row with different superscribts differ significantly at ($P < 0.05$).

Means with the same letter in the same row are not significantly different

DISCUSSION

Careful clinical examination of pregnant ewes throughout this study revealed that abortion took place at the last 2-3 weeks of pregnancy in 4 ewes from group 2 (Non treated) and only in one case from group 3 (treated one) and was not observed in G_1 (control group). The aborted ewes showed a rise of body temperature, rapid respiration, off food and putrified vaginal discharge. The gross post mortem of aborted foeti showed the presence of characteristic skin lesion (mycotic dermatitis) on head and neck, blood tinged with fluid in both thoracic and abdominal cavities. The post mortem lesion were similar to those previously recorded by Zaghloul and Shehata, (1991); Johnson *et al.*, (1994) and El-Nagar *et al.* (1997).

Mycotic examination of stomach contents of aborted foeti and placenta and vaginal discharge of affected ewes revealed that *A. flavus, A. fumigatus, A. parasiticus and Mucor* species were isolated with a higher percentage, these results agreed with Hoedemake and Held (1985); Sheridan *et al.* (1985); Biberstin (1986); Macausland *et al.* (1987); Johnson *et al.* (1994); Subhash *et al.* (1999) and Mohamed (2002).

Candida albicans and *Candida tropicalis* were isolated from stomach content of foeti and from vaginal swab and placenta of aborted ewes (Table 1), this finding was in accordance with that reported by Sinha *et al.* (1980) and Sanford and Josephson (1988).

Results in Table 2 showed that aflatoxin was produced from feed samples more than permissible limits (15 ppb) while ochratoxin and zearlenone were in the permissible limits as recorded by FAC-Doex, (1996 & 1999) it is interesting to mention that, most of isolated fungi from the most affected dams and aborted foeti were also recovered from concentrates, hay and barssem offered as a ration for feeding, these mycotoxins may have direct effect on the liver (Mohamed, 2002).

Concerning the haematological parameters, the results in Table 3 showed that the mean values of haemoglobin (Hb%); red blood cells (RBCs); MCH; MCHC and lymphocytes cells were reduced with contaminated diet (G_2) when compared with uncontaminated (G_1) but the differences were non significant in Hb, MCHC and lymphocytes. Similar results were recorded by Allam *et al.* (2002) who recorded reduction in most of haematological parameters as a result to aflatoxicosis and most of other mycotoxins. On the other hand turmeric (curcumin) supplementation to contaminated diet in G_3 had significantly increases in Hb%, MCHC and lymphocytes cells and also the same

trend was observed in RBCs and MCH without significant. Similar results were observed by Abdelhamid *et al.* (2002) with some medicinal herbs, thyme, ginger and safflower. Aflatoxin induced immunosuppression and increased in WBCs (Harvery *et al.*, 1995) but adding medicinal herbs overcome this serve side effects and also significant increase in immune response related to herbs addition was reported by Soliman *et al.* (1999) and Dawoud *et al.* (2000). Concerning biochemical parameter Table 4 demonstrated a significant decrease in serum total protein and globulin with decrease in albumin level with G_2 (contaminated diet) compared with control group (uncontaminated diet).

These results are in accordance with those of Allam *et al.* (2002). But turmeric supplementation to the contaminated diet have been attempted with high degree of success to reduce toxicity and impairment of immune response during aflatoxicosis and ochratoxicosis in livestock, so the turmeric powder maintained both of total protein and globulin concentration and also the albumin value to the normal range. Thus the highest value of albumin/globulin (A/G) was recorded with G_3 (0.71) and the lowest value was detected in G_3 (0.67). These results are in accordance with those of Abdelhamid *et al.* (2002) due to single or compound mycotoxicosis in broiler. Huff *et al.* (1992) who reported reduction in serum total protein due synergistic action of dietary aflatoxin and ochratoxin in chicks. Reduction in serum total protein and serum albumin induced by mycotoxicosis could be due to pathological changes in liver. Kurkure *et al.* (2002) reported that treatment of chicks with curcumin and curcum longa (0.5g/kg feed) during aflatoxicosis help to maintain normal serum protein levels.

Data obtained from the present study showed that, the activities of ALT, ALP and blood urea levels were increased significantly while AST, serum total bilirubin and creatnine showed a non significant increase in G_2 (contaminated diet) in comparison with control one, this finding indicated that aflatoxin ochratoxin combination had hepatotoxic and effects on ewes.

The observed results are in agreement with Mohamed (2002) who mentioned that biochemical analysis of the serum of the affected dams with mycotic abortion revealed a significant increase in the levels of albumin compared with those the apparently healthy ones. In the present study there was a significant increase in total lipids value and non significant reduction in serum chlolestrol, triglyceride level and glucose value due to dietary aflatoxin ochratoxin and feeding of these mycotoxins in combination to G_2 (contaminated diet). Increase of serum cholesterol during aflatoxicosis, a similar tend was reported earlier by

Mani *et al.* (1993) and Stoev *et al.* (2000) during ochratoxicosis due to combined mycotoxicosis, similar results were recorded by Huff *et al.* (1992). Reduction in serum glucose, total cholesterol and triglyceride levels on the other side total lipid elevation during induced mycotoxicosis reflects impaired liver metabolism leading to reduced synthesis of cholesterol and triglyceride and may be due to loss of appetite or depraved metabolic processes which consequently reflected upon glucose metabolism and its levels. The significant improvement of these parameters of mycotoxicated ewes (G_3) supplemented with turmeric powder in indicative of range its protective role and maintained their values to the normal ranges with the finding reported by Abdelhamid *et al.* (2002) who reported that blood protein and iron concentrations improved while the concentration of total lipids and the activity of ALP and ALT were reduced as a result of adding some medicinal herbs to the contaminated diet. Curcumin reduces low density lipoprotein significantly in plasma and total cholesterol level in liver along with an increase of α-tocopherol level in rat plasma, suggesting in vivo interaction between curcumin and α-tocopherol that may increase the bioavailability of vitamin E and decrease cholesterol level (Kamal Eldin *et al.*, 2000). Turmeric supplementation to G_3 (treated group) was seen to resist the change induced by mycotoxins on the studied biochemical parameters and was also made to as certain. The protective role of herbal preparation curcumin longa during induced mycotoxicosis, similar results was reported by Ashry (2003) who indicated that turmeric powder and its products produced their reductive effects on lipid peroxidation in the different tissues of rats as protective agent against cancer. Serum urea, creatinine, AST and ALT activities were discussed and revealed that turmeric powder either 10 or 20% had no any significant side effect on these parameters. Similar observation were reported by Deshpande *et al.* (1998) and Park *et al.* (2000) who recorded that the cureumin and turmeric returned the elevated levels of AST and ALT activities to normal levels in rats treated with either formadhyde or Ccl_4.

It can be concluded that the dietary supplementation of turmeric powder 10g /head/day for the last three months in pregnant ewes feeding on the contaminated diet with mycotoxins and polluted with fungi proved to be effective as fungicidal agent and also in detoxification of the mycotoxins and improving the general health condition as seen by haematobiochemical parameters and had no toxic effects as evidenced by their effects on liver and kidney functions.

REFERENCES

A.O.A.C. (2005): Official Methods of Analysis. 16th Ed., Association of Official analytical Chemists, Washington, D.C. USA.

Abdelhamid, A.M.; Sallam, A.E.; AbdAllah, G.A. and El-Samra, S.H. (2002): Effect of feeding male rats on aflatoxic diets without or with medicinal herbs (thyme, safflower, ginger, black cumin and/or garlic). Proc. 2nd Conf. Food-borne Contamination and Egyptian's Health. April, El-Mansoura, Egypt.

Aggarwal, B.B.; Kumar A. and Bharti, A.C. (2003): Anticancer potential of curcumin. Preclinical and clinical studies. Anticancer Res., 23: 363-398.

Allam, S.M.; Soliman, A.A.; Ahmed, M.E. and Shebl, M.A. (2002): Aflatoxicosis in Egyptian sheep. 2- clinical chemistry and chemistry and quality. Proc. 2nd Conf., Foodborne Contamination and Egyptians' Health, 23 – 24 April, 2002, El-Mansoura, Egypt.

Ashry, K.M. (2003): Biochemical studies on turmeric powder with special reference to antioxidant effect and possible roles in protection against chemical carcinogenesis. Assiut Vet. Med. J. 49: 99.

Biberstin, E.L. (1986): Systemic mycosis in current veterinary therapy. P. 611 – 619. 1st Ed. Edited by B.L. Howard Saunders Comp. Philadelphia..

Christensen, M. (1981): A synoptic key and evaluation of species in the Aspergillus flavus group. Mycoses. 73: 1056 – 1084.

Coles, E.H. (1986): Veterinary Clinical. 4th Ed., W.B. Saunders Comp.

Corbel, M.J. (1988): Production of antibodies to placental antigens by pregnant ewes experimentally infected with Aspergillus fumigatus. Br. Vet. J., 128, X1iv = X1vi.

Dawoud, A.S.; Salem, F.S.; Edress, N.M. and Nawar, A. (2000): Clinicopathological and Immunological studies on the effect of Nigella astiva extracts in IBDV vaccinated chicken. Proc. 5th Vet. Med. Zag. Conf., 132 – 145, Sept., Sharm El-Sheikh, pp. 75.

Deshpande, U.R.; Gdre, S.G.; Raste, A.S.; Pillai, D.; Bhide, S.V. and Longa, L. (1998): Extraction carbon tetrachloride-induced liver damage in rats. Indian J. Exp. Biol. 36 (6): 573 – 577.

Edds, G.T. (1973): Acute aflatoxicosis A. Review J. Am. Vet. Asocc. 162 – 304.

El-Nagar, A.L.; Ahmed, Y.F.; Ibrahim, F.A. and Refai, M.K.M. (1997): Mycotic abortion in small ruminants induced by Aspergillus

fumigatus in Egypt. Egyptian J. of Comparative Pathology and Clinical Pathology. 10 (2): 59 – 76.

FAC "Codex Committee on Blood Additive and Contaminants" (1996): Codex alimentary Joint. FAO/WHO food standers program 31 session CX-FAC 66/15 poisoning program 31 session CX-FAC 99/15 poisoning paper on Zeazrlenone. Netherlands.

FAC-Codex "Committee on Food Additives and Contaminants" (1999): Codex alimenterius Joint FAO/WHO food standers program 31 session CX FAC 99/14. Poisoning paper on ochratoxin A. Netherlands.

Harvey, R.B.; Edrington, T.S.; Kubena, L.F.; Corrier, D.E. and Elissada, M.H. (1995): Influence of the antibiotic lincomycin and tylosin on aflatoxicosis when added to aflatoxin-contaminated diets of growing swine. J. Vet. Diagn. Invest. 7 (3): 374.

Helt, M.C. and Riviere, J.M.E. (1995): Antifungal and antiviral drugs. In veterinary pharmacology and therapeutics Ed. Adams R. 7th ed Lowa State University Press. Ames. PP. 855 – 883.

Hikino, H. (1985): Antihepatotoxic activity of crude drug. Yakugaku Zasshi, 105: 109 – 118.

Hoedemake, M. and Held, T. (1985): Mycotic abortion in German block pied cow (short clinical communication). Praktische-Tierarzt. 66 (6): 488 – 489.

Huff, W.E.; Kubena, L.F.; Harvey, R.B. and Phillip, T.D. (1992): Efficacy of hydrated sodium calcium alumino silicate to reduce the individual and combined toxicity of aflatoxin and ochratoxin A. Poult., 71: 64 – 69.

Ishita, C.; Kaushik, B.; Uday, B. and Ranajit, K. (2004): Turmeric and curcumin: Biological actions and medicinal applications. Current Science, 87 (1): 44 – 53.

Jayaprakasha, G.K.; Negi, P.S.; Anadharmakrishnan C. and Sakariahk, K. (2001): Chemical composition of turmeric oil a by products from turmeric oleoresin industry and its inhibitory activity against different fungi. Z. Nature Forch 65: 40 – 44.

Johnson, C.T.; Lupsons, G.R. and Lawrence, K.E. (1994): The bovine placentome in bacterial and mycotic abortion. Veterinary Record. 134 (11): 263 – 266.

Kamal-Eldin, A.; Frank, J.; Razdan, A.; Tengblad, S.; Basu, S. and Vessby, B. (2000): Effect of dietary phenolic compounds on tocopherol, cholesterol and fatty acids in rats. Lipids, 35: 427 – 435.

Kelly, S.E.; Shaw, S.E. and Clark, W.T. (1995): Long term survival of four dogs with disseminated Aspergillus terrus infection treated with itraconazole. Aust. Vet. J. 72: 313.

Kozakiewicz, Z. (1989): Aspergillus species on stored products. Mycological papers; 16: 1 – 188, C.A.B. International Mycological Institute Ferry Lane, Kew, Surrey, UK.

Kurkure, N.V.; Pawar, S.; Kognole, S.M.; Ganorkar, A.G. and Kalory, D.R. (2002): Ameliorative effect of turmeric curcuma Longa induced aflatoxicosis in cockerels. Ind. J. Vet. Pathol., 24: 26 – 28.

Mani, K.; Narhari, D.; Kumara, R. and Ramamoorthy, J.N. (1993): influence of dietary aflatoxin Bion certain haematological and biochemical characters of broiler chicken. Ind. Vet. J., 70: 801 – 804.

Matossian, M. (1996): Effect of natural fungal toxins on fertility and mortality in Connetient 1660 – 1900. J. Nutritional Med., 6: 285 – 300.

Macausland, I.P.; Slee, K.J. and Hirst, F.S. (1987): Mycotic abortion in cattle. Australian Veterinary Journal 64 (5): 129 – 132.

Mohamed, A. Balata (2002): Investigation of suspected cases of mycotic abortion in does and ewes under semi aird condition. 6th Vet. Med. Zag. Conference (7 – 9 Sep., 2000) Hurghada. 153 – 160.

Moubasher, A.H. (1993): Soil fungi in Qatar other Arab countries. The Scientific and Applied Researcher Center, University of Qatar, Doha, Qatar, 566 pp.

Munoz, M.C. and Gonzalez, M. (1987): Association of mycotic agents with bovine abortion. Rerista-de-sauld- Animal. 9 (4): 366.

NRC (1985): Nutrient requirement of domestic animals. Nutrient requirement of sheep. National Research Council Washington, D.C. USA.

Okuda, Y.; Yoshida, T. and Hatano, T. (1993): Antioxidant Phenolics in oriental medicine. Cited after Yagi K. (1991): editor of active oxygen lipid peroxides and antioxidant. PP. 333-346. Japan. Sci. Press. Tokyo CRC Press, Boca Raton.

Park, E.J.; Jeon, C.H.; Ko, G.; Kim, J. and Sohn, D.H. (2000): Protective effect of curcumin in rat liver injury induced by carbon tetrachloride. J. Pharm. Pharmacol., 52 (4): 437 – 440.

Patnaik, N.; Mohanty, B.N.; Ray, S.K.H.; Mohanty, D.N. and Mishra, P.R. (1992): Clinical report on lugol's iodine treatment of mycotic abortion on bovine. Indian Vet. J. 69: 1029 – 1031.

Radostits, O.M.; Gay, C.C.; Blood, D.C. and Hinchcliff, K.W. (2000): Veterinary Medicine 9th Ed. W. B. Saunders Company Ltd, London, New York, Philadelphia, San Francisco, St Louis, Sydney.

Rao, S.D.; Chandrashekhara, N.; Satyanarayana, M.N. and Srinivasan, M. (1970): Effect of curcumin on serum and liver cholesterol levels in the rate. J. Nutr., 100: 1307-1315.

Refai, M.K. (1979): Isolation and Identification of fungi. Published by Faculty of Vet. Med., Cairo University.

Rukkumani, R.; Sribalasubashini, M. and Menon, V.P. (2003): Protective effect of curcumin and photo-irradiated curcumin on circulatory lipids and lipid peroxidation products in alcohol and polyunsaturated fatty acid induced toxicity. Phytother Res., 17: 925 – 929.

Sambaiah, K.; Ratankumar, S.; Kamanna, V.S.; Satyamarayana, M.N. and Rao, M.V.L. (1982): Influence of turmeric and curcumin on growth, blood constituent and serum enzymes in rats. J. Food Sc. Technol., 19: 187 - 190.

Sanford, S.E. and Josephson, G.K.A. (1988): Candida parapsilosis abortion in cow. Candidian Veterinary Journal. 29 (5): 458.

Saxena, S.C. and Ishaque, M. (1977): Therapeutic evaluation of antimycotic drugs in repeat breeding bovines due to mycotic infection. Current Science, 46: 780 – 782.

Sendecor, G.W. and Cochran, W.G. (1982): Statistical Methods. 7th Ed. Iowa State Univ. Press, Ames. Iowa, USA.

Sheridan, J.J.; White, D.S.C.; McGrvie, Q.D. (1985): The occurrence of organisms concerned with bovine abortion in Israel. Israel Journal of Medical Science. 23 (7): 864.

Soliman, A.Z.M.; Ghazalah, A.A.; El-Samra, S.H.; Atta, A.M. and Abdo, Z.M.A. (1999): The synergistic effect of either black seed or garlic with fat and broiler performance and immunity. Egypt. J. Nutr. Feeds (Special Issue), 603.

Soulsby, E.J. (1982): Helminths, arthropods and Protoza ofdomesticated animal. 7th Baillier Tindall and Casselltd.

Stoev, S.D.; Anguleov, G., Ivano, I. and Pavlov, D. (2000): Influence of OA and an extract of artichoke on the vaccinal immunity and health in broiler chicks. Exp. Toxicol. Pathol., 52: 43 – 55.

Subhash Vetma; Ramesh C. Katoch; Satish K. Jand. and Parag Nigam (1999): Fungi associated with abortions and infertility in does and ewes. Veterinarski Arhiv 69 (1): 1 – 5.

Wander, K.J.; Steyn, P.S.; Fouris, L.; Scott, De. B. and Theron, J.J. (1965): Ochratoxin A, A toxin metabolites produced by A. ochraceus. Wilth Nature 205: 1112.

Zoghloul, A.H; Abdel Aal, Th.S. and Nafie, Th.S (1985): Some biochemical alteration in blood serum of goats with chlamydial abortion In Abstracts of the Second National Conference of Biochemistry, Cairo.Pp.123 – 124.

Zoghloul, A.M. and Shehata, S.H. (1991): The clinical picture of mycotic abortion in buffalo cows. Assiut Vet. Med. J., 25 (49): 203 – 210.