

قسم طب الحيوان  
كلية الطب البيطري - جامعة أسيوط  
رئيس القسم : أ.د / ابراهيم سيد أحمد

## دراسة تأثير سموم الفيوزاريوم على الجاموس المصري الحلوب

ضيفى سالم ، عادل شحاته ، ثابت عبد المنعم ، عبد العزيز شعبان

يعد التسمم الغذائى من أخطر أنواع التسمم الذي يصيب الانسان والحيوان على السواء وتعد السموم الفطرية من أهم مسببات التسمم الغذائى ، ويعد الفيوزاريوتوكسين وخاصة للزيرالينون من أهم وأخطر السموم التى تفرزها فطريات الفيوزاريوم المتعددة والتي يكمن خطرها فى تأثيرها على الاجهزة التناسلية للجنسين مما يؤدي الى انهيار اقتصاديات المزرعة . وقد تم اختيار الجاموس المصري الحلوب لهذه الدراسة لكونه المصدر الاساسى فى غذاء الانسان طيلة مراحل حياته سواء من البان أو لحوم . وقد تمت الدراسة على اثنتين من المزارع الحكومية للجاموس بمحافظة أسيوط هما :-

مزرعتا الحواتك وكلية الزراعة وذلك نتيجة للشكوي الدائمة من ارتفاع معدلات الاجهاض مع انخفاض فى معدلات الخصوبة وتناقص انتاج الالبان بهاتين المزرعتين .

وقد اثبت الفحص الاكلينيكي للحيوانات المفحوصة انها جميعا فى حالة صحية جيدة ولا تظهر عليها أي أعراض الكلينيكية للتسمم وبفحص السجلات الخاصة بهاتين المزرعتين ، اتضح وجود نقص واضح فى معدلات انتاج الالبان فى كل من فصلى الشتاء والربيع فى موسمي ١٩٨٤ ، ١٩٨٥ بالمقارنة بالعام السابق ١٩٨٣ وايضا ارتفاع فى معدلات الاجهاض مع انخفاض فى معدلات الخصوبة خلال عامي ١٩٨٤ ، ١٩٨٥ . وقد أظهرت نتائج التحليل بأستخدام جهاز كروماتوجرافيا الطبقة الرقيقة لعينات من علائق الحيوان وجود الزيرالينون فى ٣٧,٥ من العينات المفحوصة والزيرالينول فى ٢٠% كما اثبتت نتائج تحليل عينات الالبان فى كل من مزرعتي الحواتك وكلية الزراعة الى عدم وجود كل من الزيرالينون والزيرالينول فى الالبان المفحوصة وقد تم اكتشاف الزيرالينون فى عينه واحده من عينات البول المفحوصة بمزرعة الحواتك والزيرالينول فى عينتين فقط بنفس المزرعة .

من النتائج التى تم الحصول عليها من واقع المراجع العلمية مع وجود الزيرالينون والزيرالينول فى عليقة وبول الحيوانات بمزارع الحواتك وكلية الزراعة بأسيوط وعلى الرغم من تعدد الاسباب المؤدية الى ظهور حالات العقم والاجهاض ونقص ادرار اللبن الا انه يمكن القول بأن للفيوزاريوتوكسين دور بارز فى احداث هذه الاضرابات من خلال تأثيرها المباشر على معدلات الخصوبة والاجهاض والادرار .

Dept. of Animal Medicine,  
Faculty of Vet. Med., Assiut University,  
Head of Dept. Prof. Dr. I.S. Abdallah.

**INCIDENCE OF ZEARALENONE AND ZEARALENOL  
IN DAIRY FARMS IN ASSIUT (EGYPT)**

**I- BUFFALOE**

(With One Table & Two Figs.)

By

**D.A. SALEM; A. SHEHATA; TH.A. IBRAHIM  
and A. SHAABAN**

(Received at 13/2/1988)

**SUMMARY**

A toxicological studies were carried out to detect zearalenone and zearalenol mycotoxins in feedstuffs (20 samples commercial concentrate and 20 samples rice straw), milk (20 samples) and urine (10 samples) in two dairy farms (El-Hawatka and Faculty of Agriculture farms) in Assiut governorate, Zearalenone was detected in 15 samples of feedstuffs and a single urine sample. Zearalenol was detected in 8 samples of feedstuffs and two urine samples. Relationship between reproductive disturbance and fusariotoxicosis were discussed.

**INTRODUCTION**

The problem of mycotoxin contamination of animal feed has been widely recognized since the discovery of aflatoxins. Zearalenone (F2 toxin) and zearalenol are two mycotoxins produced by various species of fusarium. Fusariotoxicosis has been reported in different countries (WYLLIE and MOREHOUSE, 1977). The signs of zearalenone intoxication in farm animals include infertility, abortion, hyperestrogenism, feed refusal, lethargy and anemia (MIROCHA, *et al.* 1968, 1974; KORPINEN, 1972, JEMMALI, 1973). Infertility was observed in England in dairy cattle fed 14 ppm zearalenone in hay (MIROCOHA, *et al.* 1968) while abortion appeared in cattle at 12 ppm and 32 ppm in swine in United States (MIROCHA, *et al.* 1974).

In many regions throughout the world the presence of fusarium toxins in major cereal crops (corn, rice, oats, wheat, maize) are prevalent and cause losses in animal resources enough to cause heavy economic losses (CHRISTENSEN, 1979). HASSAN and SELIM (1982) reported that eleven isolates, (belonged to the genus fusarium) were isolated from 10 corn samples representing the crops harvested from different governorate in Egypt. EL-MARAGY (1984) reported that zearalenone was detected in nine wheat grains extracts. The toxin was present alone in 5 samples and together with other mycotoxins in 4 other samples. The wheat grains were collected from different localities in Egypt. MIROCHA, *et al.* (1981) reported that free and conjugated forms of zearalenone and diastereomeric zearalenole were present in cows milk receiving 25 ppm dietary zearalenone in 7 days. The cow had consumed about 1.4 gm of total zearalenone in 7 days and about 0.7% of the consumed dose was found in the milk as total metabolites.

The detection of potentially toxic substances before toxic concentrations are reached, is important for prevention and stoppage of the dangerous effect of intoxication. This is specially applicable to natural and environmental toxicity, where many toxic substances such as aflatoxin



D.A. SALEM, et al.

and fusariotoxins might enter the human diet directly in cereal products or in the form of residues in animal tissue, milk or other dairy products (HAGIER, et al. 1980).

The persistence complain in two dairy farms in Assiut governorate was a high percentage of infertility, abortion and a decrease in milk yield. This initiated the question of investigating the presence of mycotoxins, and planning for detection and estimation of zearalenone that might produce these syndroms.

### **MATERIAL and METHODS**

A total number of forty dairy buffaloes constitute the animal population in the two dairy farms (El-Hawateka and Faculty of Agriculture) at Assiut governorate were investigated in this study. Twenty samples of feedstuffs (10 from commercial concentrate mixture and 10 rice straw) were collected from each farm.

Twenty milk samples (250 ml for each) were collected randomly from each farm, in clean dry bottles and kept in the freezer (-10°C) till used for analysis.

Ten random voided urine samples were collected in McCartney bottles from each farm and preserved in the deep freeze till analysis. Determination of zearalenone and zearalenol in feed samples were determined by using thin layer chromatography according to SHOTWELL, et al. (1976). Determination of zearalenone and zearalenol in milk and urine samples were carried out according to HAGLER, et al. (1980). Statistical analysis of the data was calculated after KALTON (1967).

### **RESULTS**

The study of seasonal milk yield values (mean  $\pm$  S.E.) revealed that there was a significant decrease at faculty of agriculture and El-Hawateka farms in winter and spring of 1985. These results were statistically compared by season's of 1983 (table 1).

The clinical examination of female buffaloes showed that there was no observed clinical deviation from normal. On the other hand the percentages of infertility and aborted cases were significantly elevated in 1984 and 1985 compared with 1983 (table 1).

The percentages of infertility in El-Hawateka farm were 7-19% in 1983, 15.3% in 1984, and 16-18% in 1985.

Analysis for zearalenone and zearalenol was performed in forty samples of feed stuffs. Overall, 37.5% (15 samples) were found to contain zearalenone and 20% (8 samples were found to contain zearalenol (Table 2).

Analysis for zearalenone and detection of zearalenol in milk and urine samples by using thin layer chromatography revealed that only one sample of urine at El-Hawateka farm contained zearalenone, and two samples contained zearalenol.



## ZEARALENONE &amp; ZEARALENOL RESIDUES

## DISCUSSION

Zearalenone and zearalenol are amongst the most common mycotoxins found in feed stuffs, which has been suspected to cause mycotoxicosis. In order to obtain a true picture of the real danger in this respect, a study was carried out to investigate the occurrence of the toxins in feedstuffs, milk, and urine at El-Hawateka and Faculty of Agriculture dairy buffaloes farms at Assiut governorate.

The estimation of zearalenone and detection of zearalenol in feedstuffs of both two farms revealed that 37.5% of total samples (commercial concentrate mixture and rice straw) contained zearalenone, while zearalenol was detected in 20% of the total examined feed samples.

Non detectable lower concentrations less than 0.5 mg/kg may attributed to the low sensitivity of thin-layer chromatography, as previously discussed by many authors (EPPLEY, 1968) attributed this phenomena to the weak fluorescence of zearalenone in comparison to other mycotoxins and to the interference of the oily material.

The clinical examination of animals revealed no obvious symptoms of intoxication. The clear sign the high percentage of infertility in the two farms. The previous records of high infertility percentages through a light to the relationship between infertility and contamination of feed by fusariotoxins. MIROCHA, *et al.* (1968); ROINE, *et al.* (1971); KORPINEN (1972); JEMMALI (1973) and MIROCHA, *et al.* (1974), recorded a marked decrease in both cattle and swine fertility, when fed on a ration mixed with zearalenone or infested with fusarium species. This support our opinion about the relevance relationship between the detection of fusariotoxins in feed stuffs and the high percentage of infertility in the investigated farms.

The evidence of abortion in the two farms is in agreement with MIROCHA, *et al.* (1974) who reported that abortion in cattle was observed in the United States when animals were fed sorghum contained 12 ppm zearalenone.

Our data recorded a significant decrease in milk yield in winter and spring which is considered as one of the important records due to feed contamination with fusariotoxins. Loss of appetite and decreased milk production were observed after ingestion of mouldy feed with fusarium species in Australia (FISHER, *et al.* 1967) and in Hungary (DANKO and TOTH, 1969). The subtropical climate of Assiut governorate during spring and winter seasons usually record a high temperature in the morning and low at night in the same day which enhance the production of the toxin by fusarium molds.

The sensitivity of the analytical method for detection of trace amounts of zearalenone or zearalenol beside the minor excretions of these toxins via milk and the fastly excretion of them from the body (SHREEVE, *et al.* 1978), clarified the negative result of zearalenone and zearalenol in milk obtained in our results. Also due to the previous causes we can attribute the low positively results obtained in urine analysis.

## REFERENCES

- Christensen, C.M. (1979): "Zearalenone" in conference on Mycotoxins in Animal feeds and Grains Related to Animal health, Shimoda, W. (Ed.), pp 300, Food and Drug Administration, Rockville MD. June 8, report FDA/BVM 79: 139, pp 1-76.
- Dank'o, G.; and Toth, O. (1969): Toxicity caused by fusarium graminearum (Schwabe) in hog, cattle and sheep. *tudományos közlemények* 2, 1-18 (Hungarian).



- El-Maraghy, S.S. (1984): Natural occurrence of zearalenol and zearalenone producing fungi isolated from wheat grains, flour and bread in Egypt. Ph.D. thesis botany Dept. Assiut Univ.
- Eppley, R.M. (1968): Screening method for zearalenone, Aflatoxins, and ochratoxin JAOAC 51: 74-78.
- F.A.O. (1979): Perspective on mycotoxins FAO. Food and nutrition paper, number 13 food and agriculture organization of the United nations. pp. 44-120.
- Fisher, E.E.; Kollock, A.W. and Wellington, N.M. (1967): Toxic strains of *Fusarium culmorum* (W.G.Sm) Sacc. from zeamays associated with sickness in dairy cattle. *Nature* 215:322.
- Hagler, W.M.; Dank'o, G.; Horrath, L.; Palyusik, M. and Mirocha, C.J. (1980): Transmission of zearalenone and its metabolite into ruminant milk. *Acta Vet. Hung.* 28 (2): 209-216.
- Hassan, M.N. and Selim, S.A. (1982): Some toxinogenic fungi associated with stored corn in Egypt. *J. Egypt. Vet. Med. Assoc.* 42 (4): 5-12.
- Jemmali, M. (1973): Présence d'un facteur oestrogenique. D'origine fongique dans la zearalenone ou F-2 comme contaminant naturel dans du maïs. *Microbiol. (Inst. Pasteur)* 124 (B): 109-114.
- Kalton, G. (1967): In "introduction to statistical ideas from social scientists" 2nd Ed. Acad. press (London).
- Korpinen, E.L. (1972): Natural occurrence of F.2 and F-2 producing *Fusarium* strains associated with field cases of bovine and swine infertility. I.U.P.A.C. Symposium "control of mycotoxins" Kungälv, Sweden, p. 21.
- Mirocha, C.J.; Harrison, J.; Nichols, A.A. and Mc Clintock, M. (1968): Detection of a fungal estrogen (F-2) in hay associated with infertility in dairy cattle. *Appl. Microbiol.* 16: 797-798.
- Mirocha, C.J.; Christensen, C.M. and Nelson, G.H. (1972): Analysis of zearalenone (F-2) and its naturally occurring derivatives. I.U.P.A.C. symposium, "control of Mycotoxins" Kungälv, Sweden, p. 30.
- Mirocha, C.J.; Beth Schauerhamer and Pathre, S.V. (1974): Isolation, and quantitation of zearalenone in maize and barley. *JAOAC* 57, 1104-1110.
- Mirocha, C.J.; Pathee, S.V. and Robison, T.S. (1981): Comparative metabolism of zearalenone and transmission into bovine milk. *Food, Cosmet., Toxicol.* 19: 25-30.
- Roine, K.; Korpinen, E.L. and Kallela, K. (1971): Mycotoxicosis as a probable cause of infertility in dairy cows. *Nordisk Veterinär Medicin* 23: 628-633.
- Shimoda, W. (1979): "Introduction in conference on mycotoxins in Animal feeds and Grains Related to Animal Health. Shimoda, W. (Ed)., pp. 300-300, Food and Drug Administration, Rockville, MD. June 8, report, FDA/BVM 79/139 pp (iii).
- Shotwell, O.L.; Goulden, M.L. and Bennett, G.A. (1976): Determination of zearalenone in corn collaborative study *JAOAC* 59, 666-670.
- Shreeve, B.J.; Patterson, D.S.P.; Roberts, B.A. and Wrathall, A.E. (1978): Effect of "Mouldy" feed containing zearalenone on pregnant sows. *Br. Vet. J.* 134 (5): 421-427.
- Wyllie, T.D. and Mourehouse, L.G. (1977) (Ed): In mycotoxic fungi, Mycotoxins, Mycotoxicoses an Encyclopedic hand book Vol. 2 Mycotoxicoses of domestic and laboratory Animals, poultry and aquatic invertebrates and vertebrates. Marcel Dekker, Inc., New York and Basel, pp. 86.

## ZEARALENONE &amp; ZEARALENOL RESIDUES

Table (1)  
 Mean values (+ S.E.) of seasonal daily milk yield and infertility %  
 at El-Hawateka and Faculty of Agriculture farms

Year	Season	El-Hawateka farm		Faculty of agriculture farm	
		Milk yield (kg/day)	Infertility %	Milk yield (kg/day)	Infertility %
1985	Winter	3.740 $\pm$ 0.188**	18.61	6.126 $\pm$ 0.256*	-
	Spring	4.412 $\pm$ 0.133**		4.882 $\pm$ 0.168**	
	Summer	4.098 $\pm$ 0.880		5.043 $\pm$ 0.530	
	Autumn	4.141 $\pm$ 0.259		6.335 $\pm$ 0.397	
1984	Winter	4.847 $\pm$ 0.133*	15.30	6.628 $\pm$ 0.284*	-
	Spring	4.915 $\pm$ 0.118*		5.270 $\pm$ 0.370*	
	Summer	4.024 $\pm$ 0.201		5.218 $\pm$ 0.374	
	Autumn	4.307 $\pm$ 0.205		5.430 $\pm$ 0.631	
1983	Winter	5.197 $\pm$ 0.177	7.10	8.243 $\pm$ 0.600	-
	Spring	5.128 $\pm$ 0.120		7.065 $\pm$ 0.940	
	Summer	4.274 $\pm$ 0.111		6.115 $\pm$ 0.335	
	Autumn	4.549 $\pm$ 0.217		5.213 $\pm$ 0.229	

\*\* Significant at P/ 0.01

\* Significant at P/ 0.05



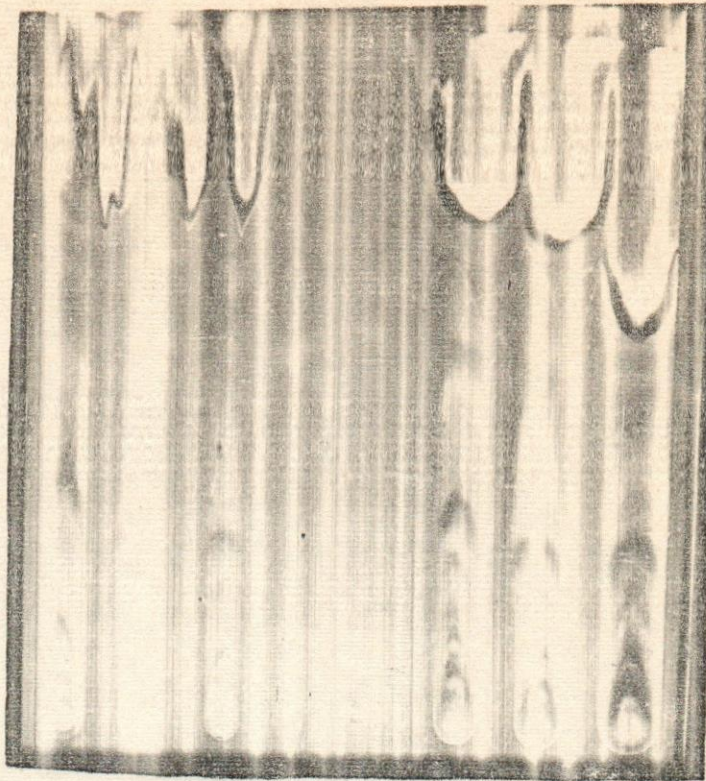


Fig. (1)

TLC plate showing zearalenone + ve at the three concentrations of the sample after comparison with zearalenone standard

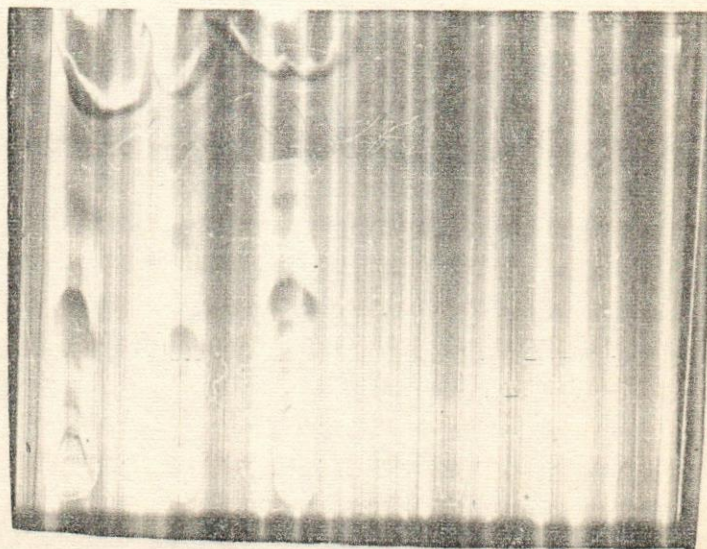
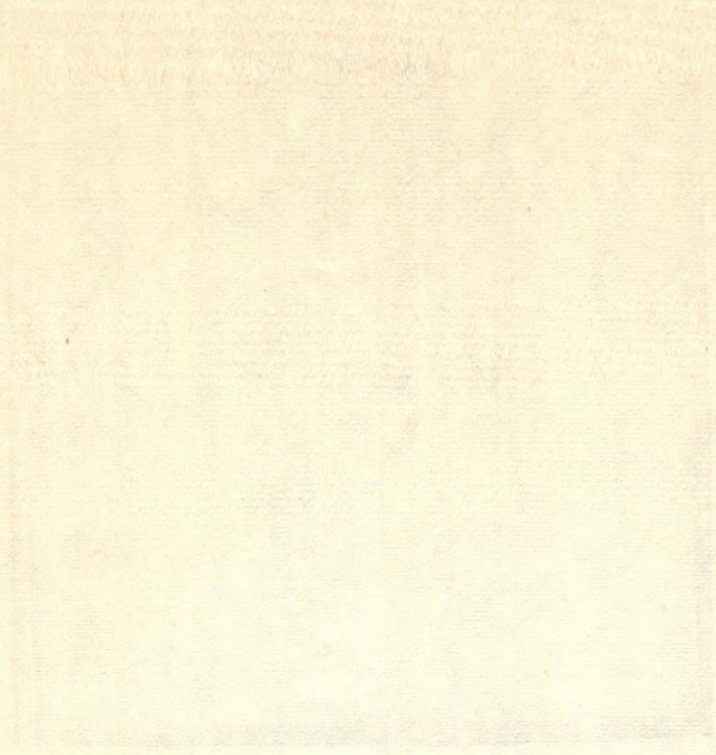


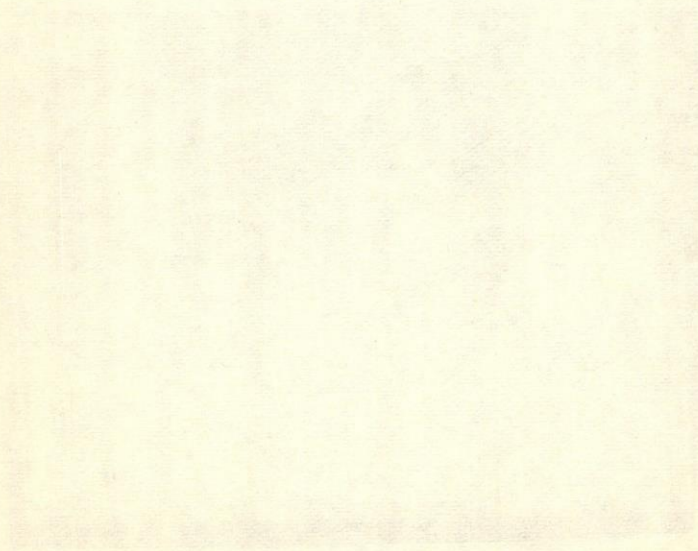
Fig. (2)

TLC plate showing zearalenone + ve at two concentrations of the samples, one on each side of zearalenone standard





Faint, illegible text centered below the top stamp.



Faint, illegible text centered below the middle stamp.