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INCIDENCE OF ZEARELENONE AND ZEARELENOL IN DAIRY FARMS IN ASSIUT (EGYPT)

II- COWS
(With 3 Tables)

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(Received at 5/12/1988)

معدلات بقايا الزيرالينون والزيرالينول في مزارع الأبقار لانتاج الالبان بأسبوط

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تعد الفيزوزاريموتوكسين وخاصة الزيرالينون والزيرالينول من أهم وأخطر السموم الفطرية التي تفرزها فطريات الفيزوزاريموم المتعددة وذلك لتأثيرها الخطير على الأجهزة التناسلية للحيوانات مما يهوى إلى زيادة معدلات الاجهاض وقلة الخصوبة وانخفاض انتاجية اللبن الأمر الذي ينعكس سلباً على الانتاج الحيواني . وقد اختبرت في هذه الدراسة ثلاث من مزارع الأبقار لانتاج اللبن بمحافظة أسبوط وهي مزرعة كلية الزراعة ، مزرعة أبنوب الحمام ، مزرعة بني مر وذلك لفحصها اكلينيكيًا ودراسة سجلاتها المختلفة مع تقدير نسب الزيرالينون والزيرالينول بعلائق وألبان وبول ماله الحيوانات . وقد اثبت الفحص اكلينيكي للحيوانات بالمزارع الثلاث أنها تتمتع بصحة جيدة ولا تظهر عليها أى أعراض مرضية الا أنه بفحص سجلاتها ظهر جلياً وجود انخفاض معلوي في معدلات انتاج الالبان في السنوات الأخيرة (٨٠، ٨٥، ١٩٨٦) يصاحبه انخفاض في معدل الخصوبة عامي ٨٤، ١٩٨٥ مع وجود ارتفاع في معدلات الاجهاض خلال نفس السنوات . وأظهرت نتائج تحليل العلائق الحيوانية (٦٠ عينة) باستخدام جهاز كروماتوجراف الطبقة الرقيقة وجود الزيرالينون في تسعة عشرة عينة منها ووجود الزيرالينول في تسع عينات فقط من نفس العلائق المفحوصة ، كما اثبتت النتائج عن وجود الزيرالينون في عينة واحدة من البول من بين ثلاثون عينة تم فحصها في المزارع الثلاث . أما نتائج تحليل الألبان (٦٠ عينة) فقد أظهرت الفحص وجود الزيرالينون والزيرالينول في عيتين فقط من العينات المفحوصة . وما سبق يتضح خطورة مثل هذه الفطريات لافرازها في الالبان مما يمثل تهديداً لصحة الحيوانات الرضع، والمستهلك الأدمي الأمر الذي يدفعنا لاعادة حساباتنا في طرق حصاد ونقل وتخزين وكيفية تقديم العلائق للحيوان ، كما تؤكد نتائج هذه الدراسة على ضرورة فحص هذه العلائق بين الحين والآخر للتأكد من خلوها من هذه الفطريات وسمومها وللحيلولة دون ظهور أعراض متداخلة مع أمراض أخرى يصعب تشخيصها .

SUMMARY

In the present investigation zearalenone and zearalenol mycotoxins were detected in animal ration (30 samples commercial concentrate and 30 samples rice straw), milk (60 samples) and urine (30 samples) in three dairy farms (Bani-Mur, Abnob-El-Hammam and Faculty of Agriculture Farms) in Assiut Governorate. Zearalenone was detected in 19 samples of feedstuff and a single urine sample. Zearalenol was detected in 9 samples of feedstuffs and single urine samples. All milk samples were negative for thin-layer chromatographic examination except two samples from Rani-Mur farm contained traces of zearalenone and zearalenol.

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INTRODUCTION

The study of fungal toxins and diseases caused by them dates back to the fifteenth century, where ergots invaded grain crops and ergotism affected man and animals. Mycotoxins are produced by fungal invasion on growing or stored grains during certain environmental conditions of high moisture and high or low temperature. Ingestion of sublethal quantities of mycotoxins affects reproduction, health, and growth performance in animals (SHIMODA, 1979).

Many toxic substances such as aflatoxin and fusariotoxin (zearalenone and its metabolites) may invade the human diet in cereal products or, as residues in animal tissue, animal products or other dairy products (HAGLER et al., 1980).

Dairy cattle fed on hay containing 14 ppm zearalenone showed a marked decrease fertility, and increase artificial insemination index (4) (MIROCHA et al., 1968). Loss of appetite and decreased milk production, after ingestion of feed mouldy with F. graminearum and F. culmorum, had variously been reported from Australia (FISHER et al., 1967), and in Hungary (DANKOE and TOTH, 1969). Implantation of 36 and 72 ug of zearalenone increased feed utilization, stimulated growth and improved the carcass grade of heifers (SHARP and DYER, 1971).

Fertility disturbances in dairy cows fed on grains infested with F. graminearum, F. tricinctum and F. poae were also noted in Finland (ROINE et al., 1971). Swelling of the vulva, decreased milk production with lack of appetite in cows fed on a mixture contain 5-75 ppm zearalenone in Hungary, but abortion did not occur (VANYI et al., 1974).

Administration of 25 and 100 ppm of zearalenone to Holstein cows for 42 consecutive days had no effect on the blood picture. Swollen and hyperemic external genitalia within one week after treatment was observed. Cows came into estrus normally on the day 21 of the cycle, and ovulated (MIROCHA et al., 1978).

The observations obtained by PALTÍ (1979), discussed the effects of fusarial toxins on cattle fertility and growth. Generally, cattle seem to be less susceptible to this toxin than pigs. The fact that ear implants of zearalenol are widely used as growth stimulants in beef cattle does not preclude the possibility of zearalenone or zearalenol causing deleterious effect when consumed regularly in amounts likely to be encountered naturally in feed stuffs of dairy cattle (CHRISTENSEN, 1979).

Detection and estimation of some mycotoxins (Fusariotoxins), in feed stuffs and some biological materials in the animals which might cause dairy and reproductive problems in Assiut Governorate animal farms have been likely to be investigated in this work.

MATERIAL and METHODS

A six frezian dairy cows in the three dairy farms Bani-Mur, Abnob-EI-Hammam and Faculty of Agriculture at Assiut Governorate were investigated in this study. Twenty ration samples (10 from commercial concentrate mixture and 10 rice straw) collected from each farm.

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

Ten random voided urine samples were collected in mc-cartney bottles from each farm and kept frozen till analysis.

Standard fusariotoxins were supplied by Dr. BROW, N., Department of Health, Education, and Welfare, Public health Service Washington, D.C. 20204. U.S.A.

Zearalenone and zearalenol in feed samples were detected using thin layer chromatography according to the method of 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 were calculated after KALTON (1967).

RESULTS

The clinical observations of Frezian cows at Bani-Mur farm revealed no clinical alteration than normal. Infertility percentage recorded as 16.30, 21.05 and 19.78, while the percentage of aborted cases in the pregnant cows reached to 10.19, 16.67 and 6.9 in 1983, 1984 and 1985 respectively (Table 1). Results of seasonal daily milk yield revealed a highly significant decrease in winter, 1984 and 1985, compared with the value in winter, 1983 and significantly decreased in autumn, 1984. Significant elevation ($P/0.05$) in spring, 1984 was also observed as shown in (Table 2).

At Al-Hammam newly built dairy farm, Frezian cows apparently appeared healthy. Infertility percentages, abortion percentages reached to (18.10, 10.30) and (15.0, 6.9) in 1984 and 1985 respectively (Table 1). A highly significant decrease in daily milk yield by Frezian cows in winter and spring seasons of 1985, while in autumn of the same year showed a highly significant elevation than the previous year (Table 2).

The clinical manifestations of Frezian cows of faculty of agriculture farm were examined and they clinically appeared healthy without any symptoms of toxicity. Frezian milk yield revealed a highly decrease in spring, 1985 and of a highly significant decrease in autumn, 1984 and 1985. Only significant decrease ($P/0.05$) was also observed in winter and summer seasons of 1985 (Table 2).

Detection of zearalenone and zearalenol were performed on sixty samples (30 samples of commercial concentrate mixture and 30 samples of rice straw). Zearalenone and zearalenol were determined solely in 19 and 9 samples respectively, only one sample contains the two mycotoxins (Table 3).

Analysis for zearalenone and its (metabolites mainly zearalenol) on samples of milk and urine, using thin layer chromatography revealed that only two samples from Bani-Mur farm were positive for both zearalenone and zearalenol after spotting 80 μl of the samples was also confirmed by the faint pink colour which appeared at the same standard R_f value sprayed by diazonated benzidine. At Bani-Mur farm one sample suspected to contained traces of zearalenone and another for zearalenol.

DISCUSSION

Quantitative estimation of zearalenone and detection of zearalenol in feed-stuffs at examined farms in Assiut Governorate revealed that 31.66% of total samples (commercial concentrate mixture and rice straw) contained zearalenone, while zearalenol represented only in 15% of the total analysed feed samples. The range of zearalenone (mg/kg) in the examined samples was 0.5-1.0 (13 samples); 1.5-2.0 (6 samples); 3.0-5.0 (6 samples); and 6.0-80 (4 samples). The highest concentration reached up to 8.0 mg/kg, while the lowest concentration was not less than 0.5 mg/kg. This may be attributed to the low sensitivity of thin layer chromatography with other factors previously discussed by many authors. EPPLEY (1968) attributed this phenomena to the weak fluorescence of zearalenone in comparison to other mycotoxins and also due to interference from the oily material. The fluorescence of zearalenone is too weak to detect at higher levels (TAKEDA et al., 1978). A dietary level of 500 ug/kg of zearalenone is considered to be biologically significant and it is not usually recommended to animals used in breeding (MIROCHA et al., 1976). The levels of zearalenone found in ration at Bani-Mur farm and EL-Hammam farm (1420 and 1560 ug/kg respectively) were within the range found in feed stuffs associated with hyperestrogenism in the United States (MIROCHA et al., 1976).

The clinical examination of investigated animals revealed no obvious symptoms of intoxication. The clearly sign was the high percentage of infertility in all farms, which reached up to 21.05 % in female animals at Bani-Mur farm in 1984. At EL-Hammam farm the infertility percentage reached to 18.10% and 10.3% in 1984, 1985 respectively. The previous records of infertility percentages in the above mentioned farms through a light on 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 rations mixed with zearalenone or infested with *F.graminearum*, *F.culmorum*, *F.tricinictum*, and *F.poa*. This supports our opinion about the prevalence relationship between the detection of fusariotoxins in feed stuffs and the high percentage of infertility in the investigated farms.

The abortion incidence in pregnant animals in the investigated farms in Assiut Governorate revealed a high rate of abortion at Bani-Mur and EL-Hammam (Frezian cattle). The highly percentage (16.67%) was found at Bani-Mur farm. Abortion in cattle was observed in the United States when the animals fed a sorghum contained 12 ppm zearalenone, while abortion in swine was observed when fed a corn contained 32 ppm zearalenone (MIROCHA et al., 1974), the same author in the same year reported that infertility and abortion were observed when a pig fed on feed contained 0.01 ppm zearalenone.

Estimation of mean values of seasonal milk yield in dairy animals in the investigated farms revealed a highly significant decrease in winter and spring, 1985 in all farms, and at Bani-Mur in the same seasons of 1984. The sub-tropical climate of Assiut Governorate during spring and winter seasons usually records a high temperature at mid-day and low temperature in the night, which may enhance the production of the toxins.

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A copious amount of zearalenone induced was estimated at the same latter conditions by SHERWOOD and PEBERDY (1972), and MIROCHA et al. (1974).

Milk yield depletion is considered as one of the important record due to feed-contamination with fusario-toxins. Loss of appetite and decreased milk production, was observed after ingestion of mouldy feed with F.graminearum and F.culmorum in Australia (FISHER et al., 1967), and in Hungary (DANKOE and TOTH, 1969). VANYI et al. (1974) stated that decrease milk production and lack of appetite occurred in cows fed on a mixture contained 5-75 ppm zearalenone in Hungary. Zearalenone on concentration of 250 ug/Kg fed to dairy cattle was accompanied by off food, low milk production (MICROCHA et al., 1976).

The results of thin layer chromatographical analysis of zearalenone and zearalenol in the milk samples revealed that, two out of 60 samples were positive for both zearalenone and zearalenol. Our results obtained may be attributed to the low sensitivity of the analytical method used for detection of zearalenone or zearalenol, beside the minor secretion of these toxins via milk as parent compounds.

The two positive results of milk samples recorded at Bani-Mur farm in correlation with zearalenone range in feed stuff (1.42 mg/Kg) in the same farm, is in agreement with the results of SHREEVE et al. (1979), who reported that no residues of zearalenone ($\frac{1}{4}$ ug/Kg) were detected although its concentration was up to nearly 2 mg/Kg. in the experimental feed of lactating cows. On the other hand zearalenone, appeared not to be "carried-over" significantly into meat and milk when dietary concentrations of natural contaminants was 1-2 mg/Kg (SHREEVE et al., 1978).

The results of zearalenone and zearalenol analysis in feed stuff, milk, and urine in the investigated farms correlated with the high infertility and the abortion percentages associated with a decrease in the milk production indicated a constant relationship between the contaminated ration by fusariotoxins (zearalenone and zearalenol) and the severe economic losses of the animal production. Attention must be given to the hygienic measures of harvesting, preparation and storage of feed stuff in order to avoid fungal contamination and toxins production.

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Table (1): Reproductive status of female dairy animals in milk production farms at Assiut Governorate of Beni-Kur and El-Hannam).

Year	Farms name	total Number of femals	No. of pregnant femals	No. of infertile femals	Early birth animals	Early inseminated animal	Infertility %	abortion %
1985 [‡]	Beni-Kur	379	171	75	53	80	19.78	5.85
	El-Hannam	233	116	24	44	49	10.80	6.9
1984	Beni-Kur	380	156	80	56	88	21.05	16.67
	El-Hannam	232	80	42	24	86	18.103	15
1983	Beni-Kur	350	157	57	50	86	16.30	10.19
	El-Hannam ^{‡‡}	-	-	-	-	-	-	-

[‡] Data available only for the first nine months.

^{‡‡} Newly builded and began in milk production from January, 1984.

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Table (2): Mean values of seasonal daily milk yield at Beni-Mur, El-Hammam, and faculty of agriculture farms.

Year	Farm	Season	No. of lactating animals	Beni-Mur		El-Hammam		Faculty of agriculture	
				Milk yield/k.g./day	Mean \pm S.E	Milk yield/k.g./day	Mean \pm S.E	Milk yield/k.g./day	Mean \pm S.E
1985		Winter	249	11.194 \pm 0.415 ^{***}	10.079 \pm 0.291 ^{**}	7.349 \pm 0.538 [*]			
		Spring	288	12.365 \pm 0.239	12.605 \pm 0.474 ^{***}	8.963 \pm 0.394 ^{***}			
		Summer	249	10.797 \pm 0.369	16.247 \pm 0.599	6.588 \pm 0.349 [*]			
		Autumn	221	10.858 \pm 0.446	15.062 \pm 0.751	6.377 \pm 0.452 ^{**}			
1984		Winter	262	11.647 \pm 0.319 ^{***}	12.971 \pm 1.126	8.876 \pm 0.418			
		Spring	307	11.662 \pm 0.295 ^{**}	16.344 \pm 0.417	10.547 \pm 0.415			
		Summer	265	10.646 \pm 0.462	16.25 \pm 0.217	6.818 \pm 0.296			
		Autumn	237	9.394 \pm 0.32 ^{***}	12.027 \pm 0.504	6.38 \pm 0.378 ^{***}			
1983		Winter	254	13.207 \pm 0.318		8.467 \pm 0.334			
		Spring	273	12.556 \pm 0.406		11.215 \pm 0.441			
		Summer	268	10.761 \pm 0.358		7.364 \pm 0.283			
		Autumn	236	10.388 \pm 0.319		9.269 \pm 0.467			

S.E : Standard error
^{***} Significant at P \leq 0.01
^{**} Significant at P \leq 0.05

Table (3): Estimation of zearalenone and detection of zearalenol in feed-stuffs at Bani-Mur, Abnab El-Hammam, and Faculty of agriculture.

No. of sample	Farm	Type of feed sample	Bani-Mur		Abnab El-Hammam		Faculty of agriculture	
			Zearal- enone mg/kg	Zearg- lenol	Zearal- enone mg/kg	Zearg- lenol	Zearal- enone mg/kg	Zearal- enol
1		Commercial concentrate mixture	2 mg	-	4 mg	-	-	-
2	"	"	-	-	-	-	-	-
3	"	"	3 mg	-	-	-	-	-
4	"	"	-	-	2 mg	-	-	-
5	"	"	-	-	1.5 mg	-	-	-
6	"	"	-	-	0.7 mg	-	-	-
7	"	"	1 mg	-	-	-	-	-
8	"	"	-	-	-	-	-	-
9	"	"	-	-	-	-	-	-
10	"	"	8 mg	-	3 mg	-	-	-
11	"	"	0.5 mg	+ve	6 mg	+ve	-	+ve
12	"	"	8 mg	+ve	4 mg	-	2 mg	+ve
13	"	"	-	-	-	-	-	-
14	"	"	-	-	-	-	-	-
15	"	"	-	-	-	-	-	-
16	"	"	2 mg	+ve	6 mg	+ve	2 mg	+ve
17	"	"	4 mg	+ve	-	-	-	-
18	"	"	-	-	4 mg	-	-	-
19	"	"	-	-	-	-	-	-
20	"	"	-	-	-	-	-	-
Incidence			40 %	20 %	45 %	10 %	10 %	10 %
Mean + S.E			1.425±0.319	1.56±0.369	1.56±0.369	0.2±0.045	0.2±0.045	0.2±0.045