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Incidence of Zearalenone and Zearalenone Producing Fungi in Cereals and Animal Feedstuffs in Jordan

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ABSTRACT. Zearalenone and zearalenone producing fungi were sought in 326 feed samples of 12 different types of cereals and animal feedstuffs collected from different localities in Jordan during March 1986 to April 1987. Some tested samples harboured zearalenone with a concentration range of 0.9-3.5 ppm. In commercially prepared animal feedstuffs and feed concentrates used for sheep, fish, layers, poultry and cows, zearalenone was detected at a level of 0.47-4.0 ppm. On the basis of microscopic and morphological identification, 77 isolates of the *Fusarium* spp. were obtained. Eleven representative isolates were identified and confirmed by the Commonwealth Mycological Institute, Kew, England. The isolates belonged to *Fusarium moniliforme Sheldon* and *Fusarium moniliforme* var. *inter*. Thirty seven of the mentioned *Fusarium* isolates were examined for their ability to produce zearalenone under laboratory conditions and only 9 (25.7%) produced zearalenone at an average level of 0.78 ppm.

Zearalenone, F-2 toxin, is a secondary metabolite produced by several *Fusarium* species including, *Fusarium roseum* and *Fusarium moniliforme* (Mirocha *et al.* 1968). Such fungi were found frequently in maize, wheat, barley and in various processed feeds (Mirocha *et al.* 1974, 1976). Feeding zearalenone contaminated feedstuffs to animals, particularly swine, cause a mycotoxicosis referred to as hyperestrogenism (Mirocha *et al.* 1968). Other signs associated with ingestion of *Fusarium* infected cereals are diarrhea, emesis, feed refusal, loss of weight and hemorrhage (Ozegovic 1970).

In Jordan, different types of imported cereals are ground and mixed with the suitable animal feed concentrates, to produce several types of animal feed rations to livestock and cattle. The wide distribution of *Fusarium* species in Jordan environment (Mamluk *et al.* 1984) together with the toxic effects of zearalenone metabolite produced by these fungi directd our interest to investigate this problem; inorder to evaluate the presence of zearalenone and zearalenone producing fungi in Jordanian cereals and animal feedstuffs.

Materials and Methods

A total of 326 animal feed and cereal samples were collected from six different localities in Jordan at bi-weekly intervals from March 1986 till April 1987. The number, type and source of collection of cereals and animal feedstuffs in presented in Table 1.

Table 1. Number of cereal and animal feed samples collected according to their source and type

Type of sample	Source						
	Provimi company	Feedina company	Al-Mashreq company	Grand floor mill	Local cooperative society	University of Jordan farm	Total
Corn	14	15	17	18	22	2	88
Soybean	10	12	9	2	9	3	45
Barley	3	5	10	9	14	–	41
Bran	1	–	6	12	12	–	31
Darnel	2	–	–	12	–	–	41
Mixed sheep Feed	2	–	–	–	–	1	3
Mixed fish Feed	1	–	1	–	–	–	2
Mixed layers Feed	10	10	7	–	–	–	27
Mixed poultry Feed	20	21	13	–	–	–	54
Mixed cow feed	12	1	1	–	–	–	14
Layers Concentrate	1	–	2	–	–	–	3
Poultry Concentrate	–	–	4	–	–	–	4
Total	76	64	70	53	57	6	326

Zearalenone detection

Zearalenone was extracted from the food samples according to either Mirocha *et al.* (1974) method or that of Blaney *et al.* (1984). However, the latter method was found to be more efficient. Thin layer chromatography of zearalenone standard and sample extracts were performed on silica gel suspension Adsorbosil-1) at a thickness of 0.25 mm, coated on glass plates (20 × 20 cm). The plates were developed first with benzene; hexane (3:1 v/v), for defatting (Betino 1985), followed by toluene, ethyl acetate: 90% formic acid (6:3:1 v/v/v) solvent system (Durackova *et al.* 1976). Zearalenone was identified on the basis of co-migration with zearalenone reference standard and by its characteristic greenish blue fluorescent colour under UV short-wave (254 nm) (Eppley 1968). The fluorescent spots of zearalenone were scraped off the TLC and eluted by absolute spectroscopic methanol for spectrophotometric measurements. The suspensions were centrifuged at 5000 rpm for 15 minutes. The supernatants were scanned for their UV absorption spectra at 200-400 nm and compared with the UV absorption spectra of the reference standard. The concentration of zearalenone was determined from the absorbance reading at 274 nm (AOAC, 26.009, 1975).

Confirmatory tests for zearalenone

Acetate derivatives with different RF values were prepared by treating portions of the isolated toxin and the reference standard with pyridine and acetic anhydride. The test was then continued according to the method of Vorster and Purchase (1968). The identity of zearalenone was also developed by using 50% Bisdiazotized Benzidine colouring reagent sprayed on spotted TLC plates (Malaiyandi *et al.* 1976).

Mycological examination

Feedstuff samples were subjected to microbiological analysis under asceptic conditions for the isolation of fungal colonies of *Fusarium* group using potato dextrose agar or Czapek-Dox plates. The plates were incubated at 28 ± 1°C for 3-5 days. Suspected fungal colonies were transferred to Czapek slants and incubated for one week at 28 ± 1°C until sporulation. Fusaria were identified according to Booth (1971). However, representative *Fusarium* isolates were sent to the Commonwealth Mycological Institute for complete identification.

Ability of the identified isolates to produce zearalenone

Fusarium isolates were screened for zearalenone production on a chemically defined medium proposed by El-Kady and El-Maraghy (1982). The media were inoculated by ca. 10^6 *Fusarium* spores for each 100 ml of media and incubated for 10 days in the dark in a closed rotary shaker running at 150-200 rpm and 18°C.

After incubation, zearalenone was extracted from culture filtrate using El-Maraghy method (1982).

Results

Out of 326 samples of 12 different types, only 37 (11.3%) samples were found to be contaminated with zearalenone (Table 1). The highest incidence of contamination with zearalenone was found to occur in mixed fish feed, followed by mixed sheep feed (Table 2). No zearalenone was detected in layers concentrate. Zearalenone contamination levels among samples varied from 0.47 to 4.3 ppm.

As summarized in Table 3 a total of 77 (23.4%) samples were found to be contaminated with *Fusarium* spp. Eleven representative isolates were completely identified by the Commonwealth Mycological Institute, Kew, England, 7 were *identified* as *Fusarium moniliforme* Sheldon and 4 as *Fusarium moniliforme* var. *intermedium*.

A total of 37 *Fusarium* isolates were tested for their ability to produce zearalenone. Nine isolates with a mycelial dry weight range of 0.49-1.22 gm yielded zearalenone levels of 0.4-1.68 ppm (Table 4). Highest yield of zearalenone was produced by an isolate from corn samples. However, isolates

Table 2. Incidence of zearalenone in some food and animal feedstuffs

Sample Type	Total samples examined	Total samples contaminated	Average level of contaminated (ppm)	Percentage contaminated samples
Corn	88	4	4.3	2.5
Soybean	45	6	2.3	13.5
Barley	41	4	0.9	9.8
Bran	31	4	2.7	12.9
Darnel	14	2	3.5	14.3
Mixed poultry feed	54	8	1.5	14.8
Mixed layers feed	27	3	0.47	11.1
Mixed cow feed	14	1	3.0	7.1
Mixed sheep feed	3	2	4.0	66.7
Mixed fish feed	2	2	3.5	100.0
Layers concentrate	3	0	0.0	0.0
Poultry concentrate	4	1	3.9	25.0
Total	326	37		11.3

originating from corn samples yielded the highest percentage (30.8%) of toxigenic strains of *Fusarium* species (Table 4).

Discussion

The data obtained from this study indicated the presence of zearalenone in 11.3% of the food samples tested. In Australia (Blaney *et al.* 1984) found that 85% out of 293 maize samples analysed were contaminated with zearalenone at an average concentration of 0.17 mg/kg. The levels of detected zearalenone in the present study are considered biologically of significant effect on farm animals, knowing that animals fed on diet contaminated with zearalenone at levels of 1.0 ppm suffer from feed refusal and hyperestrogenism as reported by Jemmali (1973) and Mirocha *et al.* (1974). The highest contamination incidence of *Fusarium* spp. were found in samples collected from localities where storage facilities of grains were poor. However, results indicate that most of the feedstuffs used in Jordan are considered as suitable substrate for *Fusarium* development if conditions of moisture and temperature are suitable.

The incidence of zearalenone producing fungal isolates reported in the present study are similar to that reported in other countries such as Egypt (El-Kady and El-Maraghy 1982).

Table 3. Incidence of contaminated samples by Fusarium spp.

Sample Type	Total samples tested	Total samples contaminated with Fusarium spp.	Percentage contamination
Corn	88	24	27.3
Soybean	45	8	17.8
Barley	41	6	14.6
Bran	31	6	19.6
Darnel	14	3	21.4
Mixed poultry feed	54	15	27.8
Mixed layers feed	27	7	25.9
Mixed cow feed	14	6	42.9
Mixed sheep feed	3	1	33.3
Mixed fish feed	2	0	0
Layers concentrate	3	1	33.3
Poultry concentrate	4	0	00
Total	326	77	23.4

Table 4. Incidence of isolated toxigenic fungi and its ability to produce zearalenone

Sample	Total suspected isolates	Mycelia dry weight (gm)	Total toxigenic isolates	Toxigenic isolates (%)	Level of zearalenone production (ppm)
Corn	13	0.87	4	30.8	1.68
Barley	4	0.56	1	25	0.4
Darnel	1	0.49	0	0	0
Bran	3	0.8	0	0	0
Soybean	2	1.1	1	50	.69
Mixed cow feed	2	1.22	0	0	0
Mixed layers feed	4	0.57	1	25	0.69
Mixed poultry feed	6	0.67	1	33.3	0.44
Soil	1	0.75	0	0	0
Tomato	1	0.97	0	0	0
Control	2	0.5	2	100	0.93
Total	37		9	24.3	–

In conclusion, the present study revealed the existence of zearalenone and fungi producing it in commercially marketed animal feedstuffs and cereals imported to Jordan during 1986. The incidence and the extent of contamination is variable among the various foods analysed and is associated with the storage practices. However, more surveys are needed in order to set up standards and specification for such imported commodities.

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وجود السم الفطري زيرالينون والفطريات المفرزة له في الحبوب وأغذية الحيوانات المستخدمة في الادرن

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شملت هذه الدراسة ثلاثمائة وست وعشرين عينة من أثنى عشر نوعاً مختلفاً من الحبوب وأغذية الحيوانات المستعملة في الاردن تم جمعها من مصادر مختلفة في الاردن، في الفترة مابين آذار ١٩٨٦ ونيسان ١٩٨٧.

تم تحليل هذه العينات للكشف عن وجود السم الفطري زيرالينون، وكذلك أجريت الفحوص المناسبة للكشف عن الفطريات المفرزة للسم المذكور، وقد تبين من خلال الدراسة وجود الزيرالينون بكميات ملموسة في الحبوب المختلفة بتراكيز تتراوح مابين ٠,٩ ـ ٣,٥ جزء في المليون وفي خلطات ومركزات أعلاف الأغنام والأسماك والأبقار بكميات تتراوح مابين ٠,٤٧ ـ ٤,٠٠ جزء في المليون، مع العلم انه لم يلاحظ وجود كميات من السم المذكور في كل من عينات القمح ومركزات الدجاج البياض.

وعند التحري عن وجود الفطريات التي يشتبه أن لها قدرة على أفراز السم «زيرالينون» وباستخدام فحوص الشكل الظاهري تم عزل ٧٧ عزلة من جنس فيوزاريوم منها أحدى عشر عينة من نوع فيوزاريوم مونيليفورم *Fuasarium moniliforme* المعروفة بافرازها لهذا السم، ولدى اختبار قدرة سبع وثلاثين عزلة

من فطر الفيوزاريوم على انتاج الزيرالينون تحت ظروف مخبرية وباستخدام الأوساط الغذائية المناسبة تبين مقدرة تسع عينات على افراز مقادير بحدود ٠,٧٨ جزء في المليون.