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EFFECT OF FEEDING MYCOTOXIN CONTAMINATED DIETS ON EGG PRODUCTION AND CALCIUM DEFICIENCY SYNDROME IN LOCAL BREEDS LAYERS

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ABSTRACT :

This study was designed to study the toxic effects of feeding mycotoxin-contaminated diet on the production performance and serum biochemistry in local breeds laying hens. Histopathological observations were also evaluated. A total number of 228 birds from three local breeds (Fayoumi, Mountazah and Matrouhe) of 25 weeks old were randomly divided into 6 groups, 2 groups for each breed (control and experimental) contained 33 hens + 5 cocks. The control groups were fed on basal diet for 12 weeks, while the experimental groups were fed the tested mycotoxin - contaminated diet for 8 weeks followed by 4 weeks in which the birds were fed on basal mycotoxin free diet (Clearance period).

The results showed that :

1-Through the experimental period, signs of lameness and paralysis of some hen's legs, beside thin egg shell and misshaped eggs were observed.

2-The effect of the mycotoxicosis on the blood biochemical parameters was counted, where calcium and phosphorus indicated significant decrease in their serum levels, whereas serum GPT and GOT levels were decreased in the intoxicated laying hens.

3-Hatchability % was markedly decreased in the experimental groups.

4-The histopathological study showed multiple foci of necrosis in the hepatic parenchyma while diaphysis of the long bone became more thinner and undergo necrosis in some cases.

5-The mycotoxin in contaminated diets of layers decreased egg production and feed consumption and increased mortality rate, while the eggs and shell quality were worst.

6-The control and the chicks during clearance period had an increase egg production rate, egg number, egg mass, average egg weight, yolk colour, yolk index %, albumin weight %, shell surface area, SWURA, specific gravity and egg shell volume with all breeds, while albumin index % increased with Fayoumi only at the first month (P1) and with Mountazah at the third month (P3), but yolk weight% increased with Matrouhe at P1 and with all breeds at second and third month (P2 & P3). Shell weight% increased with Fayoumi at P1 & P2 , with Mountazah at P1 & P3, while with Matrouhe the increase was recorded at P2 only. Albumin weight% raised with Matrouhe at P1 and Mountazah at P2.

7-The feed conversion improved and mortality rate reduced during the clearance period to near the level in control groups.

INTRODUCTION :

Mycotoxins in feedstuffs are recognized as a public health and economic problem of considerable importance. The production of large quantities of mycotoxins (secondary fungal metabolites) by several genera of toxogenic fungi (Mishra and Daradhiyar, 1991) and the frequent occurrence of mycotoxin contaminated food and feeds are well documented (Wyllie and Morehouse, 1978; and Abdel Fattah, 1994).

Poultry health and aflatoxicosis which is due to aflatoxins produced by certain fungi belonging to the genus Asperigillus, has been described in chickens by Gardiner and Oldroyd (1965), Hamilton (1971); and Hofacre et al (1985). They all said that it lead to lower feed efficiency, drop in egg production and increased mortality. However, Balachandran (1983) and Giambrone et al. (1985) recorded significant effect only on feed conversion but not on feed intake. Lameness and nervous signs were described by Asplin and Carnaghan (1961) in ducklings and by Okoye et al (1988) in broilers. However, alterations in biochemical parameters were clearly pronounced as marked decrease in serum concentration of protein, albumin and phosphorus were observed by Smith et al. (1992). Beura et al. (1993) experimentally recorded that 0.8mg aflatoxin per kilogram diet depressed body weight gain, retention of protein, feed intake, calcium, phosphorus levels and 1.6 mg/kg level decreased feed utilization. In intoxicated laying hens a marked decrease of calcium and phosphorus levels were obviously noticed, this beside a significant drop in egg production and together with hepatic lesions were recorded by Fernandez et al. (1994) as a result of aflatoxicosis. The hypocalcaemic effect of aflatoxin B on laying hens had been discussed also by Umesh Dimri et al. (1995). Recently, Oguz et al. (2000) reported that aflatoxin contaminated diet, clearly decreased serum total protein, albumin and inorganic phosphorus.

Fusarium species were recorded to produce powerful mycotoxin when contaminate wide variety of cereal grains, the main constituent of the poultry rations, which causing reduction of feed consumption and weight gain of the fed birds, (Hoerr et al., 1982; and Huff et al., 1988). The body weight gains were reduced and the efficiency of feed utilization was affected and serum inorganic phosphorus concentration was decreased in turkey poults fed diet contaminated with combination of mycotoxins produced by several species of Fusarium fungi (Kubena, et al., 1995).

MATERIALS AND METHODS :

This study was carried out at the experimental station of Animal Production Department AL-Azhar University, Assiut Branch, Egypt in Co-operation with Animal Health Research Institute, Assiut laboratory. Representative samples were collected from a lot of stored commercial layer rations, due to complain of the attendants from the appearance of symptoms on the fed chickens suspected to be mycotoxicosis.

The ration samples were subjected to mycological examination, then the contaminated rations utilized for feeding experiments to evaluate the alteration of the egg production, egg quality, occurrence of calcium deficiency symptoms and other performance parameters of native breed hens.

Mycological Examination:

Ten representative samples were collected separately and each sample of (50 grams) was in a labeled clean sterile plastic bag and transported to the laboratory. They were cultured for fungi using sterile technique by

inoculating 1gm of the sample in 9ml sterile saline tube, shaked, then stored for 2 hours after that 1ml was transferred into sterile petridish and mixed with 15 ml of 45^{O}C molted media (Sabauroud dextrose agar and modified Czapek Dox medium), then they were incubated at 25^{O}C. Identification of the growing fungi was based on colony characteristics (Cruickshank, et al.,1980). Proteolytic and lipolytic activities of the characterized strains of Aspergilus flavus and Fusarium were tested using skim milk and gelatin liquefaction testes according to Uliman and Blasin (1974) and Das, et al. (1979).

Feeding Experiments :

A total number of 228 birds (198 hens plus 30 cocks) from three local breeds, Fayoumi (F), Golden Mountazah (Mon) and Matrouhe (Mat), 25-weeks-old were randomly divided into 6 groups, two for each breed (the first was considered as control group and the second as experimental one). Each group was containing 33 hens+5 cocks. The control groups of the three breeds were fed on basal mycotoxin-free diet for 12 weeks, where the other 3 experimental groups were fed on the tested mycotoxin-contaminated diet for 8 weeks followed by 4 weeks in which the birds were fed the control diet (clearance period). Both the basal and the experimental diets had the same physical formula Table (1). Birds of each group were randomly housed on floor of an individual cage in a clean healthy experimental open side pen under the same managerial conditions. The birds were maintained in the natural environment of Upper Egypt, artificial light was used beside the normal day light to provide 16 hours day photoperiod. Feed and water provided ad-libitum. Initial body weights at the beginning of the experiment were recorded to distribute the birds in groups did not significantly differ.

During the experimental period, data of egg production, egg weight, mortality and feed consumption were recorded daily while, cases of leg disorders, fertility and hatchability were reported every 4 weeks. For studying the egg quality, 6 eggs every month were taken from each group. Egg dimensions (length and width), diameter and height of albumin and yolk were measured in mm. using a digital caliber to count the egg shape, albumin and yolk index. The weight of yolk was recorded after separation and it's colour was recorded by the Roche yolk colour fan. The weight of albumin was calculated by subtracted the weight of yolk and shell from the egg weight. The shell including membrane was washed, dried then weight and thickness were recorded by a micrometer.

After 8 weeks from the beginning of the experiment, 5 blood samples from each group were withdrawn from the wing vein and centrifuged for 10 minutes at 3000 r.p.m. The obtained serum was stored at –20^{O}C for later analysis. They were analyzed for total protein, calcium, phosphorus and liver enzymes (GOT and GPT), according to Weichsalbaum (1946) and Drupt (1974) using test kits (Bio-Merieux, Bains / France).

Pathological study:

At the end of the experiment, five birds from each group were slaughtered and specimens from the spleen , liver, and long bone were taken and fixed in neutral buffer formalin. After fixation, the specimens were dehydrated, infiltrated and embedded in paraffin. The paraffin blocks were sectioned at 7μ thickness. Tissue slides were stained routinely by haematoxylin and eosin stain for routine histopathological examination according to Bancroft et al (1977).

Data for the performance response variables were statistically analyzed by Sendercor and Cochran (1980).

RESULTS :

Out of 10 ration samples of the commercial experimental ration were tested for it's fungal content, all samples were detected to contain fungi (Asperigillus flavus, Asperigillus niger and Fusarium).

Asperigillus flavus and Fusarium species showed variable degrees of proteolytic and lipolytic activities, which referred to it's ability to produce mycotoxins.

Clinical Signs:

Signs in some hens appeared at different stages of feeding were mainly lameness and paralysis of the leg. Number of eggs, showed thin shell, irregular deposition of calcium and misshaped egg. The obtained results are tabulated in tables (2-10) and Figures (1-4).

Table (1): Ingredient composition and chemical analysis of the basal diet.

Ingredient	%
Yellow corn	68.00
Soybean meal	14.50
Layer concentrate [1]	10.00
Limestone	7.25
Layer premix [2]	0.25
Total	100
Calculated analysis: [3]	
Crude protein%	17.36
Metabolizable energy (kcal/kg diet)	2831
Available P %	0.39
Calcium%	3.46
Lysine %	0.86
Methionine %	0.37
Methionine + Cystine %	0.64

1-Layer concentrate (50%) contained, crude protein 50 % fiber 2 %, fat 4.58, Ca 6 %, P 2.85 %, Methionine 1.38 %, Methionine + cystine 2.03 %, Lysine 2.75 %, NaCl 2.67 % and ME 2300 Kcal/kg.

2-Each 2.5 kg of layer premix contained, Vit. A 10m. I.U., Vit. D3. 2.25m. I.U., Vit. E. 10g, Vit k 1g. Vit B1 1g, B2 4g, B6 1.5g, B12 10mg, Pantothenic acid 10g, Niacin 20g, Folic acid 1g, Biotin 50mg, Choline Chloride 500g, Iron 30g, Manganese 40g, Zinc 45g, Cupper 3g, Cobalt 100mg, Iodine 300mg, Selenium 100mg and CaCO3 to 2500g.

3-Calculated according to NRC (1994).

Table (2): Weekly egg number, egg mass, feed consumption and mortality rate for Fayoumi layers breed.

Parameters / Weeks	Control group				Experimental group			
	Egg No.	Egg mass (kgs)	Feed cons. (kgs)	M. No.	Egg No.	Egg mass (kgs)	Feed cons. (kgs)	M. No.
1	121	4.285	26.91	-	102	4.314	26.75	2
2	129	4.695	27.00	-	99	4.197	26.85	-
P1 3	101	3.130	26.94	1	81	3.464	26.95	1
4	92	3.250	27.08	1	98	3.738	26.85	-
Total	443	15.35	107.9	2	380	15.71	107.4	3
X±SE	111±8.6	3.837±0.38	26.98±0.04		95.0±4.7	3.928±0.20	26.85±0.04	
5	103	3.665	26.96	-	77	3.456	26.97	-
6	98	3.565	26.93	1	84	3.828	26.95	-
P2 7	104	3.870	27.06	-	64	2.874	26.70	-
8	92	3.400	27.00	1	88	4.134	26.92	-
Total	397	14.50	108.0	2	313	14.29	107.6	-
X± SE	99±2.7	3.625±0.98	26.99±0.03		78.3*±5.3	3.573±0.27	26.88±0.06	
9	98	4.584	26.49	-	99	3.950	26.15	-
10	110	5.376	27.12	-	95	3.730	26.75	1
P3 11	125	6.078	26.91	-	82	3.270	26.98	-
12	110	5.424	26.98	-	73	2.915	26.97	-
Total	443	21.46	108.0	-	349	13.86	106.9	1
X± SE	111±5.5	5.365±0.31	26.99 ±0.04	-	87.3*±6.0	3.466*±0.23	26.71±0.19	

Table (3): Weekly egg number, egg mass, feed consumption and mortality for Mountazah layers breed.

Parameters / Weeks	Control group				Experimental group			
	Egg No.	Egg mass (kgs)	Feed cons. (kgs)	M. No.	Egg No.	Egg mass (kgs)	Feed cons. (kgs)	M. No.
1	132	5.465	28.75	-	93	4.585	28.95	1
2	116	4.735	28.73	-	88	4.367	28.10	-
P1 3	76	3.300	28.77	-	47	2.322	28.85	-
4	70	2.705	28.74	1	45	2.038	29.12	1
Total	394	16.21	115.0	1	273	13.31	115.0	
X±SE	99±15	4.051±0.64	28.75 ±0.01		68.3±12	3.328 ±0.67	28.75±0.22	
5	76	4.000	28.69	-	69	2.600	28.90	-
6	88	4.563	28.72	1	68	2.755	28.02	-
P2 7	77	4.101	28.70	-	52	2.235	28.95	1
8	107	5.711	28.76	-	72	3.155	28.80	1
Total	348	18.38	115.0	1	261	10.75	114.7	2
X± SE	87±7.2	4.594±0.39	28.72 ±0.02	-	65.3 ±4.5	2.686*±0.19	28.67±0.22	
9	113	6.170	28.74	-	135	5.760	29.03	-
10	107	5.949	28.71	-	108	4.745	29.12	-
P3 11	96	5.270	28.72	-	86	3.970	29.00	1
12	98	5.305	28.75	1	60	2.710	28.95	-
Total	414	22.70	115.0	1	389	17.18	116.1	1
X±SE	104±4.0	5.674±0.23	28.73 ±0.01	-	97.3 ±16	4.296 ±0.64	29.02±0.03	

*Significant at P<0.05.
Egg No = Egg Number Egg mass = Total egg weights M. No = mortality number Feed Cons. = Feed Consumption

Table (4): Weekly egg number, egg mass, feed consumption and mortality for Matrouhe layers breed.

Parameters / Weeks	Control group				Experimental group			
	Egg No.	Egg mass (kgs)	Feed cons. (kgs)	M. No.	Egg No.	Egg mass (kgs)	Feed cons. (kgs)	M. No.
1	109	3.960	28.78	-	93	5.139	28.79	-
2	106	3.950	28.76	1	73	3.394	29.15	-
3	77	2.880	28.71	2	52	2.345	28.95	-
4	90	3.370	28.75	-	39	1.921	28.93	-
Total	382	14.16	115.0	3	257	12.80	115.8	-
X±SE	95.5±7.4	3.540±0.26	28.75 ±0.01		64.3 ±12	3.200 ±0.72	28.95±0.07	-
5	51	2.475	28.72	1	101	4.000	28.85	-
6	58	2.814	28.74	-	78	3.065	28.95	1
7	98	4.847	28.71	-	79	3.100	29.05	1
8	107	5.943	28.75	-	87	3.480	29.00	2
Total	324	16.08	115.0	1	345	13.645	115.8	-
X±SE	81±5.8	4.020±0.83	28.73±0.01		86.3±5.3	3.411±0.22	28.96*±0.04	
9	129	6.459	28.69	1	145	6.005	28.99	-
10	144	7.356	28.72	-	130	5.480	29.07	1
11	160	8.465	28.74	-	108	4.600	28.98	-
12	137	7.160	28.68	-	103	4.395	29.02	-
Total	570	29.44	115.0	1	486	20.48	116.1	1
X±SE	142±6.6	7.360±0.42	28.71 ±0.01		121±9.8	5.120± 0.38	29.01± 0.02	

*Significant at P<0.05.
Egg No = Egg Number
M. No = mortality number
Egg mass = Total egg weights
Feed Cons. = Feed Consumption

Table (5): Egg and Shell quality Measurements for the control groups.

Period	0-4 weeks P1			4-8 weeks P2			8-12 weeks P3		
Parameters	Fayoumi.	Mountazah	Matrouhe	Fayoumi.	Mountazah	Matrouhe	Fayoumi.	Mountazah	Matrouhe
Egg weight (gms.)	44.43±2.61	56.07 ±3.76	49.10 ±2.24	48.46 ±0.89	57.66 ±1.26	52.00 ±1.26	50.97±2.77	55.98±1.32	57.79 ±2.50
Yolk colour	5.80±0.20	5.2±0.2	5.40 ±0.24	5.8 ±0.2	5.6 ±0.24	6.0 ±0.45	6.0 ±0.00	7.0 ±0.32	5.60 ±0.24
Egg shape index%	71.10±0.39	71.22 ±1.4	72.93 ±1.99	72.12 ±2.04	75.54 ±1.16	72.23 ±1.4	73.15 ±0.76	74.77 ±1.34	75.03 ±1.87
Yolk index%	44.19±1.23	47.87 ±1.3	45.17 ±1.33	45.58 ±1.46	48.11 ±1.25	44.27 ±0.78	50.53 ±1.51	47.16 ±1.36	47.96 ±2.27
Albumin index%	8.53±0.67	9.56 ±0.53	8.97 ±0.68	7.61 ±0.69	7.47 ±0.40	7.83 ±0.3	9.86 ±0.6	10.19 ±0.83	8.73 ±0.83
Haugh units%	72.80±2.92	74.4 ±1.88	74.0 ±2.17	69.2 ±2.96	67.2 ±2.18	68.0 ±2.3	79.2 ±2.31	78.60 ±2.91	68.60 ±5.67
Shell weight%	11.47±0.79	10.87 ±0.07	10.02 ±0.64	12.61 ±0.36	11.10 ±0.39	12.88 ±0.63	11.99 ±0.26	11.39 ±0.46	10.89 ±0.73
Albumin weight%	53.47±0.56	58.79 ±0.88	58.01 ±3.89	52.90 ±1.39	58.27 ±1.38	54.21 ±3.27	55.15 ±0.87	56.57 ±1.01	52.54 ±0.88
Yolk weight%	35.06±0.48	30.30± 0.82	31.97 ±4.02	34.39 ±1.56	30.62 ±1.31	32.91 ±2.74	32.8 ±0.49	32.03 ±0.74	36.56 ±0.61
Shell surface area (cm^2)	57.76±2.41	68.05 ±3.15	62.50 ±1.71	61.49 ±0.80	69.40 ±3.01	64.62 ±1.11	63.66 ±2.44	68.07 ±1.13	69.58 ±2.13
SWUSA*	88.07±5.89	87.36 ±2.54	78.89 ±6.32	99.38 ±3.14	91.85 ±2.85	102.56±4.83	95.88 ±3.23	93.59 ±3.33	90.11 ±5.17
Specific gravity (gms)	1.09±0.01	1.09 ±0.01	1.08 ±0.01	1.101 ±0.01	1.092 ±0.01	1.12 ±0.01	1.08 ±0.01	1.09 ±0.01	1.09 ±0.01
Egg shell volume (cm3)	2.22±0.13	2.73 ±0.20	2.23 ±0.12	2.82±0.09	2.63±0.01	2.62±0.19	2.43 ±0.14	2.54 ±0.10	2.63 ±0.15

Table (6): Egg and Shell quality Measurements for the experimental groups.

Period	0-4 weeks P1			4-8 weeks P2			8-12 weeks P3		
Parameters	Fayoumi.	Mountazah	Matrouhe	Fayoumi.	Mountazah	Matrouhe	Fayoumi.	Mountazah	Matrouhe
Egg weight (gms.)	35.29±0.08	38.71±1.31	35.59±0.69	36.67±0.79	40.63±1.01	39.32±1.06	43.23±1.16	43.27±3.14	46.48±2.34
Yolk colour	4.30±0.10	4.00±0.37	4.33±0.21	5.00±0.26	5.50±0.22	5.67±0.21	5.33±0.21	5.67±0.33	5.33±0.33
Egg shape index%	76.24±1.12	74.85±1.92	74.84±1.74	74.83±1.49	74.21±1.36	73.99±0.88	75.85±1.31	74.94±1.72	71.160.2.45
Yolk Index%	40.32 ±2.53	43.57±2.24	38.49±1.65	43.19±1.24	43.09±0.88	41.82 ±0.87	44.34 ±0.69	45.60 ±0.88	44.63 ±0.64
Albumin index%	7.78 ±0.80	8.98 ±1.17	8.87 ±0.79	9.41±0.85	8.01±0.62	9.33 ±0.81	10.68 ±0.47	8.25 ±0.73	9.64 ±0.73
Haugh units%	74.83 ±2.70	77.33±4.25	76.16 ±2.24	79.6±2.12	71.00±1.98	77.30±1.73	80.83 ±1.45	74.80 ±2.98	76.16±1.76
Shell weight%	9.39±0.27	9.61±0.18	10.8 ±0.30	11.84±0.27	12.28 ±0.58	10.73 ±0.62	12.00±0. 51	11.06 ±0.35	11.24±0.73
Albumin weight%	61.22 ±0.88	59.60±1.22	57.75 ±0.96	60.52 ±2.06	58.09 ±1.23	59.841.39±	58.01 ±0.59	57.11±1.3	59.21±0.79
Yolk weight%	29.39 ±0.78	30.78±1.34	31.45±0.99	27.65±1.94	29.63 ±0.85	29.42 ±0.96	29.990. ±58	31.84 ±1.19	29.63±0.26
Shell surface area (cm^2)	49.17 ±0.67	52.45±1.25	49.46±0.68	50.51±0.76	54.29 ±0.94	53.05±1.02	56.72±1.09	56.62 ±2.95	59.63±2.17
SWUSA*	67.41 ±1.93	70.83±1.09	77.69±2.13	85.89±1.92	91.76 ±4.11	79.45±4.41	91.47±4.34	84.00 ±3.18	86.92±4.15
Specific gravity (gms)	1.082 ±0.00	1.08±0.01	1.09±0.00	1.09±0.00	1.10 ±3.3	1.09±0.00	1.09±0.00	1.09 ±0.00	1.09±0.00
Egg shell volume (cm3)	1.78±0.02	1.65±0.04	1.66±0.05	1.99±0.03	2.17 ±0.08	2.09±0.05	2.39 ±0.03	2.42 ±0.02	2.23±0.09

*SWUSA: Shell weight per unite surface area.

Table (7):Statistical mean values of egg production performance, feed conversion, hatchability %, mortality % and leg disorder cases parameters of Fayoumi breed.

Parameters	Control Group				Experimental Group			
	1st. month	2nd. month	3rd month	X±SE	1st. month	2nd. month	3rd month	X±SE
Egg No./hen	13.52	12.75	14.80	13.69±0.60	12.11	10.40	11.81	11.44 ±0.53
Egg mass/hen (gms)	481	466	715	554±80.6	512	476	469	485±13.3
Egg production rate %	48.29	45.54	52.86	48.90±2.13	43.25	37.14	42.18	40.86±1.88
Average egg weight(gms)	35.61	36.52	48.43	40.19±4.13	42.31	45.81	39.74	42.62±1.76
Feed conversion	7.03	7.45	5.03	6.5±0.75	6.83	7.53	7.71	7.36±0.27
Hatchability %	86.67	76.67	70.00	77.78±4.84	35.00	40.00	50.00	41.67±4.41*
Mortality %	5.26	5.26	-	10.52@	7.98	-	2.63	10.61@
Leg disorder cases%	-	-	-	-	5.26	5.26	5.26	15.78

Table (8):Statistical mean values of egg production performance, feed conversion, hatchability %, mortality % and leg disorder cases parameters of Golden Mountazah breed.

Parameters	Control Group				Experimental Group			
	1st. month	2nd. month	3rd month	X±SE	1st. month	2nd. month	3rd month	X±SE
Egg No./hen	12.05	11.20	13.49	12.25±0.67	8.24	8.49	13.49	10.07±1.71
Egg mass/hen (gms)	496	589	738	608±70.48	406	347	596	450±75.12
Egg production rate %	43.04	40.00	48.18	43.74±2.39	29.43	30.32	48.18	35.98±6.11
Average egg weight(gms)	41.13	52.56	54.69	49.46±4.21	49.29	40.86	44.19	4478±2.45
Feed conversion	7.09	6.26	5.07	6.14±0.59	8.64	10.67	6.76	8.69±1.13
Hatchability %	80.00	73.33	76.00	76.44±1.94	70.00	50.00	46.76	55.59±7.27
Mortality %	2.63	2.63	2.63	7.98 @	2.63	2.63	2.63	7.98 @
Leg disorder cases%	-	-	-	-	7.89	5.26	2.63	15.78

Table (9): Statistical mean value of egg production performance, feed conversion, hatchability %, mortality % and leg disorder cases parameters of Matrouhe breed.

Parameters	Control Group				Experimental Group			
	1st. month	2nd. month	3rd month	X±SE	1st. month	2nd. month	3rd month	X±SE
Egg No./hen	11.85	10.14	18.40	13.46±2.52	7.79	11.85	17.51	12.38±2.82
Egg mass/hen (gms)	439	502	951	631±161	388	467	738	531±106
Egg production rate %	42.32	36.21	65.71	48.08±8.99	27.82	42.32	62.45	44.20±10.05
Average egg weight(gms)	37.05	49.54	51.69	46.09±4.56	49.79	39.43	42.14	43.79±3.10
Feed conversion	8.12	7.15	3.91	6.39±1.27	9.05	8.49	5.67	7.73±1.04
Hatchability %	73.33	66.67	70.00	70.00±1.92	55.00	65.00	63.33	61.11±3.09
Mortality %	7.9	2.63	2.63	13.16@	-	5.26	2.63	7.89@
Leg disorder cases%	-	-	-	-	5.26	5.26	2.63	13.15

@= Total mortality % *Significant at P<0.05.

Table (10): Statistical estimated means of total protein, calcium, phosphorus, GOT and GPT in blood serum of the layer groups.

Parameters	Fayoumi		Mountazah		Matrouhe	
	Control	Exper.	Control	Exper.	Control	Exper.
Total protein g/100 ml	5.20±0.16	5.40±0.0.3	5.00 ±0.90	5.10±0.80	5.30 ±0.36	5.60 ±0.20
Calcium mg/dl	9.89±1.90	6.87±0.90	10.20±0.82	6.06**±0.75	10.00±0.56	6.90**±0.80
Phosphorus	5.18±0.79	2.35*±0.23	4.20±0.95	2.13 ±0.43	4.50±0.60	2.74 ±0.42
GOT U/l	30.30±6.30	27.00±1.67	63.3±4.20	60.50 ±2.00	71.60±5.10	67.40 ±1.55
GPT U/l	20.50±6.50	7.33 ±0.67	12.00 ±6.20	8.20 ±2.44	18.20 ±3.10	10.55 ±1.63

* Significant at < 0.05 ** Significant at < 0.01

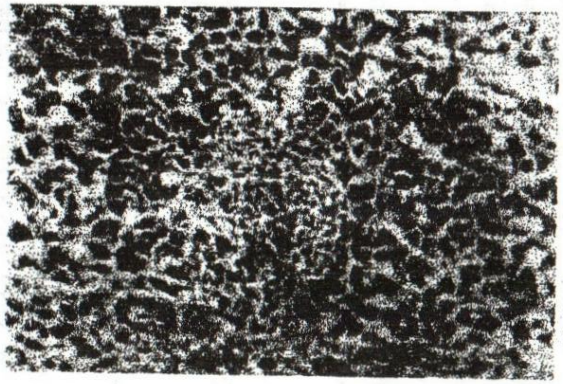

Fig.(1) : Liver from infected group showing focal necrotic foci infiltrated with mono- nuclear cells H&E 10 X40.

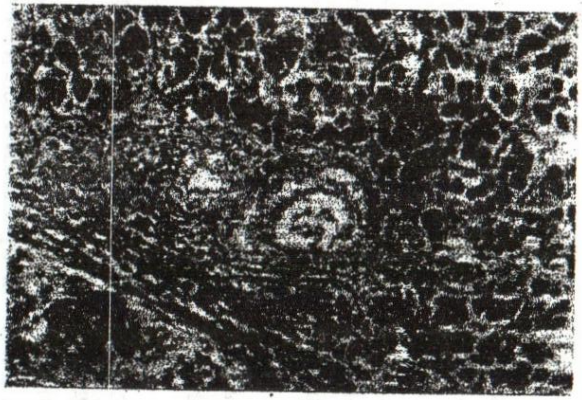

Fig.(2) : Liver from infected group showing hyperplasia of the bile duct epithelium in the portal area with leukocytic cellular infiltration.

Fig.(3) : Long Section of long bone showing normal diaphysis. (Control group).H&E 10X40

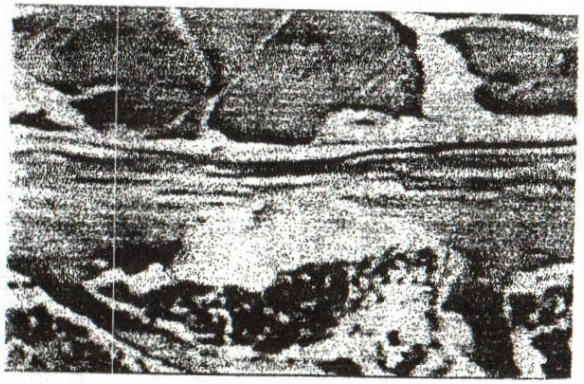

Fig.(4) : Longitudinal section of long bone showing necrosis and thinning on the diaphysis, (infected group).H & E. 10X40.

DISCUSSION :

The effect of mycotoxin contaminated diet on laying hens is summarized in Tables (2, 3 & 4) showed obvious decrease in weekly egg number, egg mass, feed consumption and increased mortality rate compared to the control group while the birds in clearance period (last month) which fed control diet had marked increase in egg number and egg mass with the all breeds while feed consumption and mortality rate were reduced with Mountazah and Matrouhe hens. However, the Fayoumi hens recorded increased feed consumption and mortality rate was constant.

Data in Tables (5&6) showed that the control group had increased egg weight, yolk colour, yolk index %, shell surface area, SWURA, egg shell volume and specific gravity, while albumin index % raised with all breeds at P_1 (first experimental monthes) and with Mountazah at P_3, also yolk weight % raised with all breeds at P_2 & P_3 (second and third experimental month) and with Matrouhe at P_1 but shell weight % was higher in Fayoumi at P_1 & P_2 and in Mountazah at P_1 & P_3 , also in Matrouhe at P_2. Moreover albumin weight % was surpassed the Matrouhe at P_1 and Mountazah at P_2 while Haugh units was lower with all breeds during the three periods when compared to the experimental group. There is no available literature regarding the effect of mycotoxin contaminated diet on egg and shell quality.

On the other hand, the data in Table (7) illustrated that the Fayoumi control group had increased egg number and egg production rate at all periods and increased egg mass and average egg weight at P_3 together with improved feed conversion and reduced mortality rate compared to the other two groups. Furthermore, egg number, egg mass, egg production rate and average egg weight were increased and improved feed conversion and decreased mortality rate in the clearance period.

Increased egg mass, egg number, egg production rate and average egg weight and improved feed conversion were recorded in the Mountazah control group (Table, 8). While clearance period for that breed had increased egg mass and average egg weight and improved feed conversion and mortality rate, but egg number and egg mass were constant.

Data in Table (9) indicated that the Matrouhe control group had increased egg mass during all periods, while egg production rate and egg number increased at P_1 & P_3 but the egg weight on average increased at P_2 & P_3. Also feed conversion was improved and the mortality rate decreased at P_2. Moreover the clearance period had great effect in increasing egg number, egg mass, egg production rate, egg weight beside, feed conversion was improved and mortality rate was constant.

These findings are in agreement with those reported by Hamilton (1971) and Hofacre et al, (1985) who found that the aflatoxicosis cause in chickens resulted in lowered feed efficiency, increased mortality, pale comb, diarrhea, and drop in egg production. Prabaharan et al. (1999) found that total feed consumption of broiler was decreased in treated group with aflatoxin when compared to the control group, while Fernandez et al, (1994) demonstrated significant decrease ($P<0.001$) in egg production in laying hens fed 5 parts/10^6 of aflatoxin which started to recover during the clearance periods.

There was a marked decrease in the hatchability % in the experimental groups due to feeding the contaminated diet as shown in Tables (7,8 & 9). This effect may be due to impairment of reproductive tracts which lead to reduce fertility, whereas decreased hatchability

of fertile eggs and this was found in local chickens strains by Abu Sree et al. (1999), Tiwari et al. (1989), Rizk et al. (1993), and Abdel Hamid et al. (1995), whereas Kim and Lee (1994) reported that new toxins from Fusarium species was toxic to chicken embryos.

Results in Table (10) show the effect of the mycotoxixosis on the blood biochemical parameters. In intoxicated laying hens, the total protein levels in the experimental groups of the three breeds were not significantly differed than the control. These results disagreed with that of Smith, et al. (1992), Genedy et al. (1999) and Oguz, et al. (2000), however Kubena, et al. (1997) reported an increase in the serum concentration of total protein. Data of serum calcium and phosphorus levels in present study indicated significant changes in their levels in the experimental groups, where marked decrease in their levels were obtained than that of the control groups. These findings were clearly in accordance to those of Kubena et al. (1988), Smith, et al. (1992); Beura et al. (1993) and Fernandez et al. (1994). A decrease in serum calcium and phosphorus concentrations, observed in this study has several practical implication, because both elements are important in egg shell formation. The low calcium level is a sensitive indicator of intoxication and may discussed as the main cause of the signs of lameness, leg disorders and egg quality alterations which have observed.

Enzyme activities in laying hens that had been fed the contaminated ration in the three breeds of the experiment were variable. So the mycotoxin had no significant effect on serum GOT, where slight decease in the levels was noticed, while GPT levels were lowered in the intoxicated groups than in the control ones. The obtained data were similar to the observations of Fernandez et al. (1994), but conflicting with the data which detected by Kubena et al. (1997) and Genedy et al. (1999).

The depressive effect was much more pronounced in the case of mycotoxin contaminated diet on productive, reproductive performance and some blood biochemical parameters. This may be attributed to the important changes in hepatic metabolism that effect protein, lipid and enzyme synthesis (Tung et al., 1972). Aflatoxin may be a cause of the fatty liver syndrome (Hamilton & Garlich, 1971) , which is characterized by an enlarged, yellowish and friable liver. Moreover aflatoxin cause immunosuppression (Pier et al., 1979), it is likely that this was the cause of the lymphocytic depletion observed in the bursa and spleen.

The histopathological examination of the affected groups revealed pathological changes in the liver and bone. Liver showed multiple foci of necrosis in the hepatic parenchyma infiltrated with mononuclear cells (Fig. 2). Hyperplasia of the bile duct epithelium was also noticed in most of the infected cases (Fig. 2). These lesions were agreed with those reported by Okoye and Okeke (1986), Abdel-Hamid and Hassieb (1988); Okoye et al (1988) and Fernandez et al (1994). Examination of the long bones showed that the diaphysis became more thinner and undergo necrosis in some cases (Fig. 4). The histopathological changes in long bone was documented by our results indicating calcium deficiency in birds fed myco-contaminated diet as well as the appearance of leg disorders.

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REFERENCES :

1-Abdel-Fattah A.M. (1994). Public health hazards of myco-contamination of some cereals to human and animal heath. Assiut Vet. Med. Jr - Vol. 31, No.61, 1994.

2-Abdel-Hamid, H.S and Hassieb, M.M. (1988). Pathogenicity of Asperigillus Flavus and Asperigillus niger isolated from poultry feedstuffs in chickens. Paper presented to Alex. Univ. 1st scientific Symposium, 23-24 March, 1988.

3-Abdel-Hamid, A.M.; Arief, H.S. M.; El-Keraby, F. and Dorra, T. M. (1995). Effect of some dietary supplements to aflatoxin - diets of chickens. II. On the tissues analysis. J. Agric. Sci. Mansoura Univ. 20 (7):3227-3231.

4-Abdel-Hamid Y.A.E. and Mabrouk, Y.M.A. (1999): Aflatoxin contaminated hen diets and its effect on: 2-DNA content and micronucleic. Egypt Poult. Sci. Vol. 19(1) March. 1999 (85-96).

5-Abu Sree, Y.H., Salwa G.K.Gendi, El-Nagger, N.M. and Qota., E.M.A. (1999). Effect of feeding aflatoxin diet with and without decontaminating agents on its residues in tissues, reproductivity and semen quality of local chicken strains.

6-Asplin, F.D. and Carnaghan, B.A. (1961) The toxicity of certain groundnut meals for poultry with special reference to their effect on ducklings and chickens. Veterinary Record, 73:1215-1218.

7-Balachandran, C. (1983). Aflatoxicosis in Broiler chicken, Thesis submitted to the TNAU.

8-Bancroft, J.D.; Stevens, A.S. and Douson, J.M.P. (1977). Theory and practice of histologic techniques. Churchill Livingstone. Edinburg, Newyork.pp22 Beura, C. K.; T. S. Jouhri, V. R.; Sadagopan, and B.

9-Beura, C. K.; Johri, T. S.; Sadagopan, V. R. and Panda, B. K. (1993) : Interaction of dietary protein level on dose response relationship during aflatoxicosis in commercial broilers. 1. Physical responses, livability and nutrient retention. Indian Journal of Poultry Sci. (1993) 28 (3) 170-177.

10-Cruickshank, R,; Duguid, J.P.; Marmion, B.P. and Swain, R.H. (1980). Medical microbiology, 12th. Ed., Vol reprinted Churichill Livingestone & Robert Stevenson Edinburgh, EH I, 3AF.

11-Das, A.; Charrerjee, M. and Ray, A. (1979). Enzymes of some higher fungi. Mycologia 71:530-536.

12-Drupt, F.F. (1974): Calorimetric determination of serum albumin and globulin. Pharm. Biol., Vol. 9 : 77-79.

13-Fernandez, A.; Verde, M.T.; Gascon, M.; Ramos, J.; Gomez, J.; Luco, D.F. and Charez, G. (1994): Variation of clinical biochemical parameters of laying hens and broiler chickens fed aflatoxin - containing feed. Avian Pathology (1994) 23, 37-47.

14-Gardiner, M.r. and Oldroyd, B. (1965). Avian aflatoxicosis. Australian Veterinar J., 41:272-276.

15-Genedy, Salwa G.K.; El-Naggar, N.M.; Isshak, N.S. and Gota, E.M.A. (1999): Effect of aflatoxins decontaminatry agents on performance blood constituents and some tissues of local poultry strains. Egypt Poult. Sci. 4 : 96-101.

16-Giambrone, J. J.; Diener, U. L.; Davis, N. D.; Panangala V. S. and Hoerr, F.J. (1985). Effects of aflatoxin on young turkeys and broiler chickens. Poultry sci. 64: 1678-1684.

17-Hamilton, P.B. (1971). A natural and extremely severe occurrence of aflatoxicosis in laying hens, Poultry Sci 50:1880-1882.

18-Hamilton, P B. and Garlich, J. D. (1971). Aflatoxin as a possible cause of fatty liver syndrome in laying hens. Poult. Sci, 50;800-804.

19-Hoerr, F.G.; Carlton, W.W.; Yagen,B. and Joffe, A.Z. (1982). Mycotoxicosis caused by either T-2 toxin or diacetoxyscirpenol in the diet of broiler chickens. Fundam. Appl. Toxicol. 2:121-124.

20-Hofacre, C.L.; Page,R.K. and Fletcher,O.J. (1985). Suspected mycotoxicosis in laying hens. Avian Diseases, 29:846849.

21-Huff, W.E.; Harvey,R.B.; Kubena,L.F. and Rottinghaus, G.E. (1988).Toxic synergism between aflatoxin and T-2 toxin in broiler chickens. Poultry Sci. 67:1418-1423

22-Kim, J.C. and Lee, Y.W. (1994). Sambutoxin, a new mycotoxin produced by toxic Fusarium isolates obtained from rotted potato tubers. App. Environ. Microbiol., Vol. 60, No.12 4380-4386.

23-Kubena, L. F., Huff, W.E.; Harvey, R.B.; Corrier, D.E.; Philips, T.D. and Creger, C.R. (1988). Influence of Ochratoxin A and deoxynivalenol on growing broiler chicks. Poultry Sci. 67:253-260.

24-Kubena, L F.; Edrington,T.S.; Kamps - Holtzapple,C.; Harvey,R.B.; Elissalde, M.H. and Rottinghaus,G.E. (1995). Influence of fumonisin B_1 present in Fusarium maniliforme culture material and T-2 toxin in Turkey poults. Poultry Sci. 74 : 306-313.

25-Kubena, L. F.;Edrington,T.S.; Harvey,R.B.; Buckley,S.A.; Phillips,T.D.; Rottinghaus, G.E. and Casper, H.H. (1997). Individual and combined effects of Fumonisin B_1 present in Fusarium moniliforme culture material and T-2 toxin or Deoxyinvaleral in broiler chicks. Poultry Science 76 : 1239-1247.

26-Mishra, N.K., and Daradhiyar,S.K. (1991). Mold flora and aflatoxin contamination of stored and cooked samples of pearl millet in the paharia tribal belt of santhal paragana, Bihar, India. Appl. Environ. Microbiol. 57 : 1223- 1226.

27-National Research Council (1994). Nutrient requirements of poultry. Ninth revised edition, National Academy Press, Washington, D.C.

28-Oguz, H.; Kececi, T.; Birdane, Y.O; Onder , F. and Kurtoglu, V. (2000). Effect of clinoptilolite on serum biochemical and haematological characters of broiler chickens during aflatoxicosis. Research Veterinary SCI. 69 (1) 89-93.

29-Okoye, J. O. A.; Asuzu, I.U and Gugnani, H.C. (1988). Paralysis and lameness associated with aflatoxicosis in broilers. Aviant Pathology, 17 : 731-734.

30-Okoye, J.O.A. and Okeke, C.N. (1986) . Pathogenicity of an isolated Aspergillus flavus in chickens. Avian pathology, 15: 259-270.

31-Pier, A. C.; Richard, J. L., and Thurston, J R. (1979). The influence of mycotoxins on resistance and immunity. In. Interaction of Mycotoxins in Animal production, pp. 56-66. Washington. D. C.National Academy of Science.

32-Prabaharan, V.; Titus George and Balasubramaniam, G.A. (1999). Influence of dietary aflatoxin and coccidiosis on growth rate in broiler chicken. Indian. Vet. J. Sept., 76: 827-828.

33-Rizk, R. E.; El-Sayed, N. A.; Abd Allah, G. A. and El-Deeb, S.A. (1993). The residue of low dietary aflatoxin B_1 and its effect on productivity and reproductivity of local chicken strains. Egypt. Poult. Sci. Vol. 13:301-306.

34-Sendercor, G.W. and Cochran, W.G. (1980) Statistical methods, Lowa State Univ. Press. Ames, Lowa, U.S.A.

35-Smith, E.E.; Kubena, L.F.; Braithwaite C. E. and Barvey, R.B. (1992). Toxicological Evaluation of Aflatoxin and Cyclopiazonic Acid in Broiler Chickens. Poultry Sci. 71: 1136-1144.

36-Tiwari, R.P.; Virdi, J.S.; Gupta, L. K.; Saini, S.S. and Vadehra, D V. (1989). Development of chicks exposed to aflatoxin B_1 during embryogenesis. Ind. J. Anim. Sci., 59; 1473-1479.

37-Tung, H. T.; Donaldson, W. E.and Hamilton, P. B. (1972). Altered lipid transport during aflatoxicosis. Toxicology and Applied Pharmacology, 22 : 97-104.

38-Uliman, U. and Blasin, C. (1974) : A simple medium for the detection of different lipolytic activity of microorganisms. Zentralb. Bakteriol. Hyg. A. ABT. 229 : 264-267.

39-Umesh Dimri; RAO, V. N. and Joshi, H. C. (1995). Effect of acute aflatoxicosis on serum calcium, magnesium and phosphorus levels in chickens. Indian Journal of Vet. Med. (1995).

40-Weichselbaum, P. E. (1946): An Accurate and rapid method for detection of protein in small amount of blood serum and plasma. A. J. Clinic Path. 16 : 40-42.

41-Wyllie, T.D., and Morehouse L.G. (1978). Mycotoxic Fungi, Mycotoxins and Mycotoicosis, Vol. 2. Marcel Dekker, Inc., New York, Ny.

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أثر التغذية علي علائق ملوثة بسموم الفطريات علي إنتاج البيض وحدوث أعراض نقص الكالسيوم للسلالات البياض المحلية

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أجريت هذه التجربة ، لدراسة التأثير السام للتغذية على عليقة ملوثة بسموم الفطريات على وظائف الإنتاج وبعض العناصر الكيميائية لسيرم الدم ، لدجاجات من سلالات محلية مستنبطة ، كذلك تم ملاحظة الأعراض الظاهرية ، والفحص الهستوباثولوجى .

إجمالى عدد ٢٢٨ طائر من ثلاث سلالات محلية مستنبطة (الفيومى — المنتزة — المطروح) لعمر ٢٥ إسبوع ، تم تقسيمها عشوائيا لستة مجموعات، مجموعتين لكل سلالة احداهما ضابطة والأخرى تجريبية، وكل مجموعة تحتوي علي ٣٣ فرخة + ٥ ديوك، وغذيت المجموعات الضابطة من السلالات الثلاثة على عليقة خالية من سموم الفطريات لمدة ١٢ أسبوع ، بينما المجموعات التجريبية ، تم تغذيتها لمدة ٨ أسابيع على علف ملوث بسموم الفطريات والذى تم إختباره قبل التجربة ، تبعتها ٤ أسابيع أخرى تم التغذية خلالها على علف المجموعات الضابطة والخالى من السموم .

خلال فترة التجربة، ظهرت أعراض عرج وتأثر بالأرجل لبعض دجاجات المجموعات التجريبية ، وكذلك وجود بعض البيض ضعيف القشرة والبيض غير منتظم الشكل والمنتج من هذه المجموعات، كما أن نسبة الفقس انخفضت .

أظهرت النتائج أيضا تأثير التسمم بالفطريات على قياسات سيرم الدم ، فقد لوحظ نقص معنوي في مستوى الكالسيوم والفوسفور وانخفاض مستوي قياسات إختبار وظائف الكبد .

وبالدراسة الهستوباثولوجية ، وجد تأثيرا واضحا فى المجموعات المغذاة على العلف الملوث حيث وجد بؤر متنكرزة فى خلايا الكبد، وكذا تضخم فى النسيج المبطن للقناة المرارية ، أما العينات المأخوذة من العظم ، فأظهرت تغيرات مورفولوجيكية ، كذا ترقق فى بعض أجزاء العظم يصل إلى التنكرز فى بعض الأحيان .

أظهر أيضا تأثير التغذية على العلف الملوث إنخفاض إنتاج البيض وإستهلاك العليقة وزيادة النافق وكذلك أدت التغذية بالعلف الملوث الي عدم إنتظام صفات جودة البيض والقشرة مع قلة وزن البيضة ولون الصفار والنسبة المئوية لمعامل الصفار ووزنه، وكذلك قلة النسبة المئوية لمعامل البياض ووزنه والنسبة المئوية لوزن القشرة ومساحة مسطح القشرة والكثافة النوعية ووزن القشرة لكل وحدة مساحة، كما أظهرت النتائج أن المجموعات المغذاة على علف خالى من سموم الفطريات لمدة شهر زاد فيها أنتاج البيض وتحسن معامل التحويل الغذائى وصفات جودة البيض والقشرة وقلت نسبة النافق فيها .