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Pathological Effects of Aflatoxin and Their Amelioration by Vitamin E in White Leghorn Layers

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ABSTRACT

White Leghorn layer breeder hens, 30 weeks of age, were divided into 12 groups (A-L). Group A was kept on basal feed and served as control, while group B was offered feed supplemented with vitamin E (100 mg/Kg). Groups C-G were offered feed containing 100, 500, 2,500, 5,000 and 10,000 µg/Kg aflatoxin B1 (AFB1), respectively, whereas groups H-L were offered same dietary levels of AFB1 along with vitamin E (100 mg/Kg). The experimental feeds were offered for three weeks and afterward all the groups were switched over to basal feed for next two weeks. Body weight, absolute and relative weights of liver and kidneys of AF fed birds were significantly higher than control group. Pathological lesions in aflatoxin (AF) fed birds included enlarged, pale and friable liver, swollen kidneys and hemorrhages on different organs. Histopathological lesions in liver included fatty change, congestion and hemorrhages, while in kidneys tubular necrosis, cellular infiltration, congestion and hemorrhages were found in groups fed AFB1 at 500 µg/Kg and higher doses. In AF fed hens, no significant ameliorative effects of vitamin E could be observed upon AF induced decrease in feed intake, gross pathology and histopathological alterations and organ weight except body weights. It was concluded that the vitamin E ameliorated the AFB1 induced toxic effects in some of parameters studied.

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INTRODUCTION

Fungi are ubiquitously present in the agricultural products and some of these produce toxic metabolites known as mycotoxins. Aflatoxins are most commonly found mycotoxins in sub-tropical regions of the world. These are secondary metabolites of *Aspergillus flavus* and *A. parasiticus* which inflict injurious effects on avian health and thus cause economic losses to the poultry industry around the world (Khan *et al.*, 1990). Pakistani climate for the greater part of the year remains hot which favours propagation of different toxigenic fungi and production and build up of injurious toxic levels of mycotoxins. Cereal crops including corn, wheat, sorghum and rice during storage are frequently contaminated by aflatoxins (Behere *et al.*, 1978; Thompson and Henke, 2000; Saleemullah *et al*., 2006). Feeding hens on a ration contaminated with injurious levels of aflatoxins (AF) particularly aflatoxin B1 (AFB1) results in drop in egg production and clinical aflatoxicosis. Being potent and hepatotoixc agent, AF suppress protein synthesis thus reduce body weight and lower egg production. The activity of several enzymes important to the digestion of starch, proteins, lipids and nucleic acids is reduced which also results in lower weight gain and poor health status (Carrillo *et al.*, 1982). Toxic effects are exhibited as anorexia, poor food conversion, retarded growth rate, and decreased weight gain and egg production. Hepatotoxicosis, haemorrhages on different organs and carcinogenesis, susceptibility to environmental and microbial stresses have also been noted (Celik *et al.*, 1996).

Many reports have documented the effects of AF upon different production and health parameters of hens. However, information is scanty about the ameliorative effects of a naturally occurring antioxidant vitamin E upon different AF induced pathological effects. This paper describes the pathological effects of AF in hens and possible amelioration of these effects by dietary administration of vitamin E.

MATERIALS AND METHODS

Production of aflatoxin

Cultures of *Aspergillus flavus* (CECT 2687, Culture Collection Center, University De Valencia, Spain) maintained in the Department of Pathology, University of Agriculture, Faisalabad were inoculated on Potato Dextrose Agar (PDA) slants and incubated at 28°C for 7 days. These cultures were inoculated on rice for the production of AF following the modified method of Shotwell *et al*. (1966). High Pressure Liquid Chromatography method 990.33 (AOAC, 2000) was used to determine the level of aflatoxins in the contents of flasks.

Experimental production of aflatoxicosis

A total of 192 laying hens, free from *Salmonella* and *Mycoplasma* infections, were procured from a layer farm. Birds were acclimatized for one week on layer feed having 16% crude protein, 2900 K.cal/Kg of energy and aflatoxin B1 levels below 1.0 µg/Kg. Birds were divided into 12 groups (A to L) having 16 birds in each, kept in the layer battery cages and offered feed contaminated with different doses of AFB1 alone and in combination with vitamin E for a period of three weeks.

Group A was kept on basal feed and served as control, while group B was offered feed supplemented with vitamin E (100 mg/Kg). Groups C-G were offered feed containing 100, 500, 2,500, 5,000 and 10,000 µg/Kg aflatoxin B1 (AFB1), respectively, whereas groups H-L were offered same dietary levels of AFB1 along with vitamin E (100 mg/Kg). After three weeks, birds of all groups were kept on basal diet for another two weeks.

Parameters studied

Clinical signs, feed intake and body weight were calculated for birds of each group on weekly basis. At the end of AF feeding trial (21 days), 10 birds from each group were killed and their viscera were examined for the presence of gross lesions. Different organs were weighed to calculate their relative weights in relation to the body weight. A small portion of tissue from different organs was fixed in 10% neutral buffered formalin. Sections of 5 µm thickness were processed for staining with hematoxylin and eosin for histopathological examination (Bancroft and Gamble, 2008).

The gross lesions observed in different organs were subjectively evaluated and scored from 0 to 3 based upon severity and number of hens showing a particular lesion in a group. Similarly, a subjective evaluation of histopathological alterations in liver, kidneys, spleen and heart was made and scored from 0 to 3.

Statistical analysis

Statistical analyses were carried out using a software package "MSTATC" and different group means were subjected to comparison of analysis of variance by using Duncan's multiple range test ($P<0.05$).

RESULTS

Feed intake

Feed intake of birds fed different levels of AF alone or along vitamin E has been presented in Table 1. The mean values of feed intake of groups E, F, G, K and L were significantly lower ($P<0.05$) during the week 1 of the experiment as compared with control group A, while all other groups were non significantly different from control. In the second week, feed intake values of groups D, E, F, G, J, K and L were significantly lower, while all other groups were non significantly different from group A. At third week, values of feed intake of all the groups except B and H were significantly lower compared with group A.

After withdrawal of AF and vitamin E from the feed (week 4 of experiment), feed intake values of groups E, F, G, J, K and L were significantly lower, while all other groups were non significantly different from control group A. In week 5, feed intake values of groups F, G, K and L were significantly lower while all other groups were non significantly different from control (group A).

Body and absolute organ weights

Table 2 shows body weights and absolute organ weights of layer breeder hens after three weeks of feeding of AF with or without supplementation of vitamin E. The body weights of the breeder hens of group A were significantly higher than the groups C, D, G and H, whereas all other groups were non significantly different compared with group A.

The absolute liver weights of groups E, F, G, K and L were significantly higher than that of group A. The absolute kidneys weights of groups F, G, J, K and L were significantly higher than control. Absolute weights of spleen of groups C, D, E, F, G, J, K and L were significantly lower than that of control. The absolute weight of intestine of group A was significantly higher than the groups D, G, J and K, whereas all other groups showed non significant differences from group A. There was non significant difference among all groups in absolute weight of heart, gizzard and proventriculus.

Relative organ weights

Relative organ weights (percent of the body weight) of layer breeder hens after three weeks of feeding of AF with or without supplementation of vitamin E are given in Table 3. The mean values of relative weights of the livers of the groups D, E, F, G, K and L were significantly higher than that of group A. Relative weights of all other organs including kidneys, heart, gizzard, spleen, proventriculus and intestine were non significantly different from control (group A).

Gross lesions

Birds of group A (control) killed at the end of week 3 of the experiment did not show any gross lesion in any internal organ of the body (Table 4). Similarly, birds of groups B, C and H also did not show any gross morphological variation in any organ upon necropsy and appearance, size and consistency of internal organs were similar to those of control birds. Hemorrhages on subcutis, muscles, liver and intestines started to appear in groups D and E and lesion score increased in a dose related manner. The cumulative score of all the groups was the highest in groups G and L and decreased significantly between the groups with decrease in dietary AF level. The difference in cumulative scores between

Table 1: Feed intake (g/bird/day) of breeder hens offered AF contaminated feeds with or without supplementation of vitamin E (mean ± SD)

Groups (AFB1 µg/Kg + Vitamin E mg/Kg feed)	Period (week) of feeding AF contaminated ration			Period (week) of feeding basal ration after withdrawal of AF and VE	
	1	2	3	4	5
A (0+0)	108.23 ± 4.79 a	110.50 ± 6.75 a	112.93 ± 3.95 a	107.38 ± 7.91 a	111.50 ± 4.36 a
B (0+100)	110.86 ± 5.51 a	114.25 ± 4.14 a	109.11 ± 6.01 a	110.45 ± 4.11 a	113.68 ± 3.71 a
C (100+0)	107.36 ± 5.23 a	109.63 ± 6.11 a	104.02 ± 7.82 b	108.77 ± 4.05 a	110.86 ± 6.30 a
D (500+0)	103.46 ± 8.53 a	96.89 ± 5.54 b	97.33 ± 4.95 bc	98.48 ± 7.24 ab	110.63 ± 4.01 a
E (2,500+0)	99.55 ± 5.80 bc	94.68 ± 6.21 b	91.43 ± 5.38c	80.00 ± 8.41 b	101.48 ± 7.83 ab
F (5,000+0)	95.52 ± 6.10 bcd	85.19 ± 5.25 c	83.84 ± 5.42 d	88.38 ± 4.87 c	93.02 ± 7.54 c
G (10,000 + 0)	83.61 ± 9.17 d	76.07 ± 5.93 d	69.43 ± 7.51ef	79.25 ± 5.23 d	88.61 ± 5.87 cd
H (100 +100)	110.68 ± 4.53 a	107.43 ± 7.37 a	109.34 ± 4.37 a	108.82 ± 4.61 a	107.30 ± 4.92 a
I (500 +100)	105.13 ± 6.44 a	107.45 ± 4.87 a	103.80 ± 5.70b	106.39 ± 4.30 a	110.73 ± 4.28 a
J (2,500 +100)	102.41 ± 4.38 ab	95.25 ± 5.79 b	91.80 ± 4.82 c	93.77 ± 6.23 b	99.23 ± 5.37 ab
K (5,000 + 100)	96.25 ± 8.61 cd	87.29 ± 6.48 c	83.94 ± 5.89d	90.55 ± 5.39 c	93.95 ± 6.90 bc
L (10,000 + 100)	88.98 ± 8.45 d	79.95 ± 7.60 d	75.27 ± 6.20e	81.95 ± 7.18 d	96.44 ± 4.31 b

Values following different alphabets in a column are significantly different (P<0.05).

Table 2: Body weight and absolute organ weights (g) of breeder hens offered AF contaminated feeds with or without supplementation of vitamin E (mean ± SD)

Group	Body weights	Organ weight						
		Liver	Heart	Kidney	Spleen	Gizzard	Proventriculus	Intestine
A	1621.76 ± 274.97a	37.32 ± 4.04c	6.53 ± 1.53	9.57 ± 1.31de	1.23 ± 0.25a	56.43 ± 13.08	8.39 ± 2.89	95.33 ± 19.40ab
B	1503.43 ± 45.09abc	39.47 ± 4.51bc	6.67 ± 1.42	9.41 ± 1.79e	1.13 ± 0.12ab	49.37 ± 5.20	7.67 ± 1.15	101.67 ± 3.21a
C	1251.69 ± 25.66c	40.18 ± 7.21bc	5.88 ± 1.05	9.92 ± 1.12de	0.90 ± 0.17bcd	47.51 ± 4.58	5.31 ± 1.53	81.18 ± 6.08bc
D	1298.37 ± 173.95bc	42.33 ± 8.62bc	7.33 ± 1.15	11.15 ± 1.25bcde	0.83 ± 0.12cd	46.23 ± 9.85	7.69 1.47	76.39 ± 14.29c
E	1470.51 ± 75.50abc	56.27 ± 3.06a	7.17 ± 0.87	11.07 ± 1.03bcde	0.97 ± 0.06bcd	49.67 ± 4.93	7.77 ± 1.15	99.61 ± 3.06a
F	1422.84 ± 51.93abc	57.49 ± 6.51a	6.85 ± 1.09	11.47 ± 1.53abcde	0.86 ± 0.13cd	46.63 ± 4.36	7.13 ± 0.87	100.53 ± 3.00a
G	1302.28 ± 45.71bc	55.67 ± 3.06a	6.23 ± 0.95	12.67 ± 1.3ab	0.73 ± 0.25d	38.56 ± 6.51	6.97 ± 0.00	73.42 ± 19.16c
H	1338.48 ± 68.98bc	37.81 ± 4.51c	7.29 ± 0.78	9.76 ± 1.24de	1.00 ± 0.00abc	45.39 ± 3.79	8.17 ± 3.61	85.23 ± 6.08abc
I	1513.62 ± 216.58ab	38.28 ± 1.53c	6.57 ± 0.69	10.46 ± 0.97cde	1.00 ± 0.00abc	46.13 ± 8.50	6.23 ± 0.69	96.19 ± 8.72abc
J	1480.92 ± 157.16abc	48.53 ± 17.35abc	7.05 ± 0.72	11.67 ± 1.44abcd	0.87 ± 0.23cd	43.28 ± 8.19	7.33 ± 0.71	76.58 ± 11.53c
K	1396.41 ± 32.15abc	51.39 ± 7.55ab	6.97 ± 0.89	12.17 ± 1.08abc	0.80 ± 0.10cd	46.45 ± 4.36	7.12 ± 0.58	70.61 ± 9.02c
L	1520.35 ± 68.12ab	58.25 ± 2.08a	6.74 ± 1.11	13.33 ± 0.58a	0.77 ± 0.15cd	50.32 ± 2.65	8.13 ± 1.30	98.56 ± 11.37ab

Values following different alphabets in a column are significantly different (P<0.05).

groups fed AF alone and their counterparts fed AF with vitamin E were non significant.

Liver of the birds fed AF at 500 µg/Kg and higher doses showed swelling and increased friability compared with control birds. The color of the liver also became lighter (pale) in comparison with birds of group A. The intensity of these changes increased in a dose related manner and was more conspicuous in groups fed higher levels of AF. Birds of groups G and L showed most severe enlargement and friability of the liver, followed by groups F and K. Liver from these groups also showed hemorrhages on the surface (Fig. 1).

Kidneys of the birds fed higher doses (2,500 µg/Kg of feed and above) of AF were swollen (Fig. 2) which varied from mild to moderate enlargement in groups E, F, G, J, K and L. A severe enlargement of kidneys resulting in bulging of organ out of bony sockets was seen in groups E, F, G, J, K and L. In these groups, hemorrhages were also present on the surface of kidneys. Petechial hemorrhages were also found on surface of heart, breast and leg skeletal muscles in birds of groups G and L.

Histopathology

Liver of the AF fed hens showed fatty change characterized by round vacuoles in the cytoplasm of

Table 3: Relative organ weights (percent of the body weight) of breeder hens offered AF contaminated feeds with or without supplementation of vitamin E (mean ± SD)

Group	Liver	Heart	Kidney	Spleen	Gizzard	Proventriculus	Intestine
A	2.32 ± 0.19e	0.42 ± 0.13	0.58 ± 0.11	0.08 ± 0.03	3.43 ± 0.25	0.502 ± 0.10	5.85 ± 0.23
B	2.61 ± 0.26de	0.45 ± 0.11	0.62 ± 0.26	0.08 ± 0.01	3.26 ± 0.36	0. 51 ± 0.09	6.77 ± 0.7
C	3.20 ± 0.64bcde	0.45 ± 0.05	0.72 ± 0.07	0.07 ± 0.01	3.75 ± 0.30	0.43 ± 0.13	6.47 ± 0.47
D	3.36 ± 1.12bcd	0.58 ± 0.15	0.77 ± 0.04	0.06 ± 0.00	3.66 ± 1.34	0.60 ± 0.16	6.06 ± 2.04
E	3.84 ± 0.32ab	0.52 ± 0.02	0.86 ± 0.09	0.07 ± 0.01	3.39 ± 0.49	0.52 ± 0.05	6.80 ± 0.50
F	4.04 ± 0.52ab	0.49 ± 0.05	0.89 ± 0.11	0.06 ± 0.01	3.23 ± 0.22	0.52 ± 0.04	7.04 ± 0.47
G	4.28 ± 0.39a	0.46 ± 0.06	0.97 ± 0.07	0.06 ± 0.02	2.96 ± 0.42	0.54 ± 0.02	5.57 ± 1.30
H	2.78 ± 0.21cde	0.55 ± 0.03	0.67 ± 0.17	0.08 ± 0.00	3.40 ± 0.40	0.59 ± 0.24	6.37 ± 0.73
I	2.56 ± 0.26de	0.44 ± 0.04	0.74 ± 0.15	0.07 ± 0.01	3.08 ± 0.59	0.43 ± 0.07	6.38 ± 0.49
J	3.19 ± 0.90bcde	0.50 ± 0.07	0.78 ± 0.22	0.06 ± 0.02	2.90 ± 0.36	0 .50 ± 0.02	5.14 ± 0.63
K	3.66 ± 0.62abc	0.56 ± 0.04	0.86 ± 0.13	0.06 ± 0.01	3.30 ± 0.39	0.53 ± 0.04	5.07 ± 0.75
L	3.84 ± 0.05ab	0.53 ± 0.09	0.88 ± 0.05	0.05 ± 0.01	3.30 ± 0.31	0.55 ± 0.09	6.49 ± 0.75

Values following different alphabets in a column are significantly different (P<0.05).

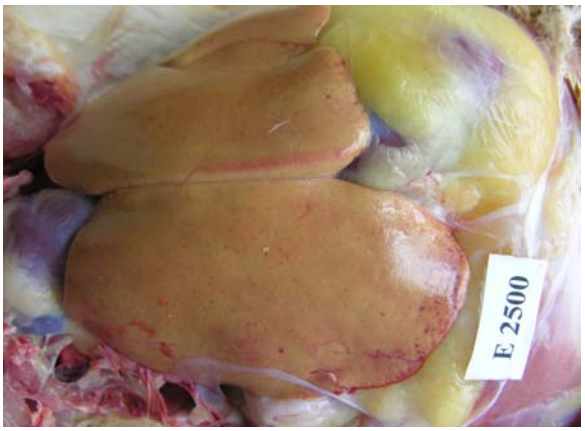

Fig. 1: Photograph of liver of a breeder hen fed 2500 µg/Kg AFB1 for 3 weeks. Liver is enlarged, hemorrhagic and pale in color.

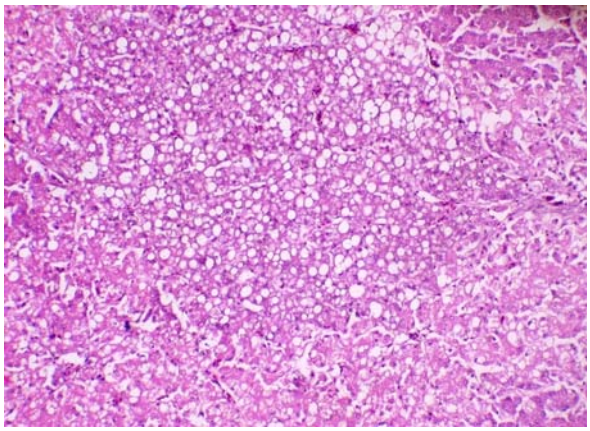

Fig. 3: Photomicrograph of liver of a breeder hen fed 5000 µg/Kg AFB1 for 3 weeks. Fatty change of hepatocytes is present. (H & E stain, 200X).

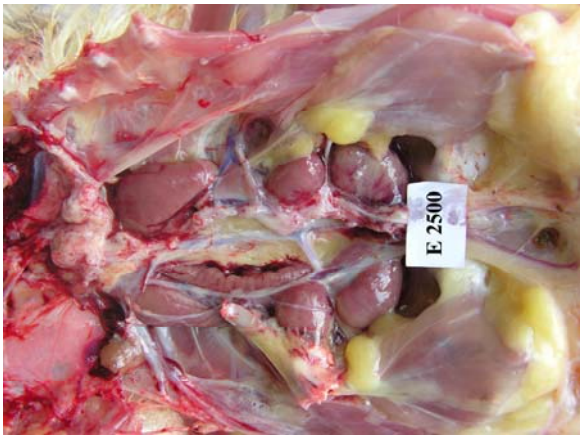

Fig. 2: Photograph of a breeder hen fed 2500 µg/Kg AFB1 (group E) for 3 weeks. Kidneys are swollen.

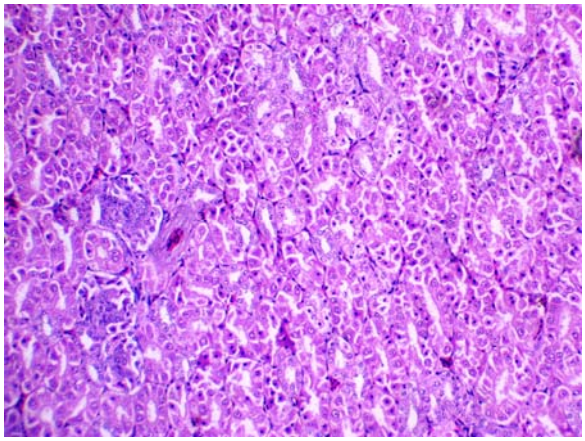

Fig. 4: Photomicrograph of kidney of a breeder hen fed 500 µg/Kg AFB1 for 3 weeks. Pyknotic nuclei of tubular epithelial cells are present (H & E stain, 200X).

Table 4: Gross lesion scores in the layer breeder after 3 week of AF intoxication

Lesions		Maximum possible score	Groups											
			A	B	C	D	E	F	G	H	I	J	K	L
Pallor of liver		96	0	0	21d	33c	58b	69ab	77a	18d	27c	52b	60b	71a
Friable liver		96	0	0	0	28d	52bc	63b	71a	0	22d	49c	58b	66a
Haemorrhages	Subcutis	96	0	0	0	18c	48ab	57a	61a	0	26c	42b	51ab	58a
	Muscle	96	0	0	0	33c	63b	71ab	76a	0	22cd	59b	67b	71ab
	Liver	96	0	0	0	27c	79b	81b	93a	0	21c	72b	77b	89ab
	Kidneys	96	0	0	0	21c	57b	68b	84a	0	17c	51b	62b	79ab
	Intestine	96	0	0	0	25c	68ab	75ab	79a	0	22c	63ab	69ab	72a
	Heart	96	0	0	0	0	0	12	14	0	0	0	12	14
Enlargement of organs	Liver	96	0	0	0	35d	64c	79b	93a	0	28d	58c	72b	87ab
	Kidneys	96	0	0	0	25d	58c	75b	84a	0	19d	54c	71b	79ab
Cumulative score		960	0	0	21d	245c	547b	650b	779a	18d	204c	500b	599b	729a

Values following different alphabets in a column are significantly different (P<0.05).

Table 5: Histopathological scoring of layer breeders fed and AF and vitamin E for three weeks

Organs	Histopathological lesions	Maximum score possible	Groups											
			A	B	C	D	E	F	G	H	I	J	K	L
Livers	Fatty change	96	0	0	11e	22d	51c	67b	86a	9e	19d	50c	64b	83a
	Congestion	96	0	0	4d	18c	38a	33a	36a	3d	15c	34a	31b	33a
	Dilated sinusoidal spaces	96	0	0	6d	12c	45b	55ab	61a	5d	10c	40b	51ab	57a
	Necrosis	96	0	0	3d	7c	19b	28ab	37a	1d	5c	17b	26ab	35a
	Mononuclear cells infiltration	96	0	0	7c	12c	19b	35a	42a	5c	10c	18b	34a	40a
	Hetrophilic cells infiltration	96	0	0	3d	7c	13b	16ab	19a	2d	6c	11b	15ab	18a
	Total score	576	0	0	34e	78d	185c	234b	281a	25e	65d	170c	221b	266a
Kidneys	Congestion/haemorrhage	96	0	0	8c	17c	38b	57ab	69a	6c	15c	35b	55ab	67a
	Cellular infiltration	96	0	0	4d	11c	23b	21b	32a	3d	8c	22b	19b	28a
	Tubular necrosis	96	0	0	13d	28c	67b	77a	91a	8d	21c	65b	74a	86a
	Total score	288	0	0	25d	56c	128b	155b	192a	17d	44c	122b	148b	181a
Cumulative score (liver+kidney)		864	0	0	59e	144d	313c	389b	473a	42e	109d	292c	369b	447a

Values of groups in each row followed by different alphabets are significantly different ($p<0.05$).

hepatocytes as a prominent feature. Liver parenchyma also showed individual cell necrosis, congestion, dilated sinusoidal spaces and cellular infiltration around triads and blood vessels (Fig. 3). Kidneys of the AF fed hens had tubular necrosis as a prominent feature along with cellular infiltration, congestion and hemorrhages in the parenchyma (Fig. 4).

Histopathological lesion scoring

Histopathological lesion scoring of breeder hens fed AF and vitamin E is shown in Table 5. Fatty change score in hepatocytes were significantly higher in groups G and L than all other groups. Groups F and K showed moderate fatty change. Groups C and H showed significantly lower lesion scores than all other groups. Groups E, F, G, J and L showed significantly higher values of congestion than all other groups. Dilated sinusoidal spaces and necrosis of hepatocytes of groups G and L were significantly higher than all other groups, except groups F and K. Infiltration of mononuclear and heterophilic cells was significantly higher in groups G and L than all other groups, except F and K. Total liver scores of groups G and L were significantly higher and for groups C and H was significantly lower than all other groups. In kidneys, congestion and hemorrhages score were significantly higher in groups G and L than all other groups, while groups C, D, H and I showed significantly lower scores than all other groups. Cellular infiltration scores of group G and L differed non significantly among each other while it was significantly higher than all other groups. Tubular necrosis scores of groups F, G, K and L were non significantly different from each other, but were significantly higher than all other groups. Groups E and K showed moderate changes. Total kidneys scores of groups G and L were significantly higher than all other groups. Cumulative scores of liver and kidney of groups G and L were significantly higher, while those of C and H were significantly lower than all other groups.

DISCUSSION

A decrease in the feed intake of AF fed hens as observed in the present study has been reported by many authors (Azzam and Gabal, 1998; Verma *et al.,* 2003; Pandey and Chauhan, 2007). Administration of vitamin E had no ameliorating effect on feed intake.

A significant decrease in the body weight of group fed 10,000 µg/Kg AF was rendered as non significant from control by concurrent feeding of vitamin E, suggesting its ameliorating effect. An increase in the liver relative weight was observed in birds fed 2,500 µg/Kg or higher AF levels. Similar observation has been reported by Stanley *et al.* (2004). The increase in the relative weight of liver might have occurred due to swelling of liver, which is a characteristic feature in aflatoxicosis (Huff and Doerr, 1981; Ortatatli *et al.,* 2005). A concurrent feeding of vitamin E did not ameliorate the toxic effects of AF in hens as determined by relative weight of liver.

Different lesions present in AF fed hens included swollen, pale and friable liver, swollen kidneys and hemorrhages on different organs. Similar gross lesions have been reported during aflatoxicosis in chicken (Endrington *et al*., 1997; Shivachandra *et al.,* 2003; Karaman *et al.,* 2005; Ortatatli *et al.,* 2005). Enlargement in liver due to aflatoxicosis has also been reported in other avian species including Japanese quails (Parlat *et al.,* 1999), ducks (Ostrowski-Meissner, 1984) and water fowls (Robinson *et al.,* 1982). A significant increase in cumulative score of gross lesions and scores of liver and kidneys occurred with increase in dose and duration of AFB1 administration. No ameliorating effect of vitamin E was observed upon severity of gross lesions in the present study.

Microscopically, fatty change of hepatocytes was the most conspicuous alteration accompanied by individual cell necrosis in liver parenchymal haemorrhages and mononuclear and heterothallic cellular infiltration around blood vessels in liver. Many workers also reported similar findings in the chicken (Carnaghan *et al.,* 1966; Asim *et al.,* 1990; Ortatatli and Oguz, 2001; Ortatatli *et al.,* 2005), turkeys and Japanese quail (Jakhar and Sadana, 2004), ducklings (Newberne and Butler, 1969), snow geese and mallards (Robinson *et al.,* 1982).

Kidneys of AF intoxicated birds revealed degeneration and necrosis of tubular epithelial cells, congestion and hemorrhages of the parenchyma. Such changes have also been reported by many researchers to occur along with enlargement of livers in birds intoxicated with aflatoxins (Chen *et al.,* 1985; Arshad *et al.,* 1992; Abo-Norag *et al.,* 1995; Edrington *et al.,* 1997; Raju and Devegowda 2000; Ortatatli and Oguz, 2001).

In conclusion, the present study described the AFB1 induced pathological effects in layer breeder hens. In AF fed hens, an ameliorative effect of vitamin E was observed upon AF induced decrease in body weight. However, no significant ameliorative effects of vitamin E could be observed upon AF induced decrease in terms of feed intake and organ weights.

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