EFFECT OF ADDING *NIGELLA SATIVA* AND VITAMIN C TO RABBIT DIET CONTAMINATED WITH AFLATOXIN B_1

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SUMMARY

This work was carried to evaluate the alleviation ability of *Nigella sativa* (Ns) or vitamin C on the toxic effect of aflatoxin B_1 in rabbits' diet. Two trials were carried out, in the 1st one, thirty New-Zealand White male rabbits (average body weight 2000 ± 30 g) were used in five experimental groups (6 animal each) for six weeks. The control group was fed the basal diet, while the 2nd group fed the basal diet with 350 ppb aflatoxin B_1. The 3rd, 4th and 5th groups were fed on the basal diet with 350 ppb aflatoxin B_1 plus 0.5, 1.0 or 2.0% Ns, respectively. In the 2nd trial, forty New-Zealand White male rabbits (average body weight 1000 ± 10 g) were used in five experimental groups (8 animal each) for six weeks. The control group was fed on the basal diet, while 2nd group (T2) fed basal diet with 200 ppb aflatoxin B_1. The 3rd, 4th and 5th group fed basal diet with 200 ppb aflatoxin B_1 plus 1% Ns, 500 mg vitamin C/ kg diet and Ns plus vitamin C, respectively. Results showed that Ns (specially at 1%) or vitamin C addition alleviate significantly ($P<0.05$) the negative effect of aflatoxin B_1 on daily gain, feed intake, feed conversion, digestibility of nutrients, nutritive values and blood parameters (total protein, albumin, globulin, AST, ALT, creatinine and mortality rate (%), the best improvement was occurred by addition of Ns + vitamin C together. In conclusion, adding 1% Ns plus 500 mg vitamin C/ kg diet was better than Ns or vitamin C alone in the alleviation the toxic effect of aflatoxin B_1 in rabbits' diet.

Keywords: Nigella sativa, rabbit, vitamin C, aflatoxin B_1, daily gain, blood

INTRODUCTION

Aflatoxins are mycotoxins produced as secondary metabolites by *Aspergillus flavus* and *A. parasiticus* (Cheeke and Shull, 1985). In Egypt, the aflatoxins and other mycotoxins are frequently detected in feedstuffs (Abdelhamid, 1990, El-Gohary, 1995, Aziz *et al.*, 1997 and Hassan *et al.*, 2002). The problems with mycotoxins do not end at feed refusal or reduction of animal performance but many of these mycotoxins transfere into the meat or milk (Devegowda *et al.*, 1998).

Aflatoxins are considered to be an unavoidable naturally occurring contaminant. Therefore, to avoid the effect of this substance, increasing animal immunty must be made (Zaky *et al.*, 2000). *Nigella sativa* (Ns) has an important role in improving body health. The literature on using Ns in reducing aflatoxin effect are contraversal. Zaky *et al.*, (2000) reported that 1% Ns (crushed seed) significantly counteracted the effect of aflatoxin B_1on body weight gain and blood parameters of pekkin ducklings. On the other hand, Abdel-hamid *et al.*, (2002) reported that 1% Ns did not completely overcome the effect of aflatoxin B_1 on male rats. He concluded that more research must be carried out using graded levels of aflatoxin and Ns.

Vitamin C alleviate the aflatoxin effect on rabbits (Salem *et al.*, 2001), rats (Abd El-Mageed, 1987) and guinea pigs (Netke *et al.*, 1997). Rearly literature were carried out on using of NS plus vitamin C in the prevention of rabbit fibrosis and cirrhosis by carbon tetrachloride (produce in the body by lipid peroxidation as aflatoxin toxicity) (Turkdogan *et al.*, 2001). Also, NS plus vitamin C, E and selenium were used for monitoring and preventing the effects of N-Methyl-N-nitrosoguanidine and similar carcinogen (Gündüz *et al.*, 2002).

The present investigation was under taken to study the protective effects of different levels of Ns on aflatoxin B_1 (1st trial) and effect of Ns plus vitamin C on aflatoxin B_1 toxicity in rabbits (2nd trial).

MATERIALS AND METHODS

This work was carried out in the Department of Animal Production, Fac. of Agric., Zagazig University, Egypt, in 2008-2009. Two trials were carried out, in the 1st one, thirty New Zeland white (NZW) male rabbits with average body weight of 2000 ± 30 g were randomly assigned to five groups (6 animal in each). The control group (T1) fed basal diet without aflatoxin B_1, 2nd group (T2) fed basal diet with 350 ppb aflatoxin B_1. The 3rd, 4th and 5th groups fed basal diet with aflatoxin B_1 plus 0.5, 1 or 2% Ns, respectively. In the 2nd trial, forty growing New Zeland white (NZW) male rabbits with average body weight of 1000 ± 10 g were randomly assigned to five groups (8 animal in each). The control group (T1) fed basal diet without aflatoxin B_1, 2nd group (T2) fed basal diet with 200 ppb aflatoxin B_1. The groups 3rd, 4th and 5th fed basal diet with aflatoxin B_1 plus 1% Ns, 500 mg vitamin C / kg diet and NS + vitamin C, respectively. Vitamin C (20%) (United Co. For Chem. & Med. Prep., Egypt) was included at 2.5 g/ kg diet to obtain 500 mg vitamin C / kg diet.

Aspergillus flavus MD 341, was obtained from the Central Lab. of Residues in Agric. Products, Agric. Pesticides Res. Centre, Dokki, Egypt, for production of aflatoxin B_1 on liquid media (2% yeast extract and 20% sucrose). The aflatoxin concentration was determined using the method A.O.A.C. (1990). The media was found to contain aflatoxin B1 alone. The media sprayed on diet to obtain aflatoxin B_1 required level. Animals in each trial were housed in individual cages under the same managerial, hygienic and environmental conditions all over the experimental period. The formula and chemical composition of basal diet are shown in Table (1). Daily fresh water was available all time. Feed intake, live body weight of animals were recorded weekly then growth rate and feed conversion values were calculated. At the last week of the trial, feed intake and feces excreted of 4 rabbits from each treatment were recorded daily for digestibility trials. At the end of the experimental feeding period, blood samples were collected at slaughter time to estimate the blood parameters. Serum total protein, albumin, aspertate amino transferase (AST), alanine amino transferase (ALT) and creatinine were analyzed using commerical kits purchased from Diamond Diognastics Company, Egypt. Also, the organs (liver, kidneys, heart and lungs) weights were determined. Proximate analysis of feed and feces were determined according to A.O.A.C. (1980). Data of the all trial alone were statistically analyzed using the General Linear Model Program of SAS (1996). Duncan's new multiple range test (Duncan, 1955) was used to determine the significant differences among treatments.

Table (1): Formulation and chemical composition (%) of basal diet.

Ingredients	%
Yellow corn	17.00
Clover hay	35.00
Wheat bran	20.00
Barley	10.00
Soybean meal	13.00
Molasses	3.00
Sodium chloride	0.20
Methionine	0.20
Vitamin and minerals	0.30
Bone meal	1.00
Limestone	0.30
Chemical composition (DM)	
OM	89.70
CP	17.00
CF	16.50
EE	2.20
NFE	54.00
Ash	10.30

RESULTS AND DISCUSSION

First trial:

Results of first trial showed that aflatoxin B_1 significantly ($P<0.05$) decreased rabbit performance (body gain and feed intake) (Table 2), digestibility of nutrients and nutritive values (Table 3). Aflatoxin B_1 had a bad effect on blood parameters (Table 4). All parameters measured were improved by Ns adding, specially level of 1%, which had the best effect.

Second trial:

1- Rabbit performance:

Data presented in Table (5) showed that aflatoxin B_1 significantly ($P<0.05$) reduced daily body gain and feed intake. Also, the worst feed conversion was occurred in rabbits fed aflatoxin B_1 contaminated diet (Table 5). These results were in agreement with those obtained by Abd El-Baki *et al.*, (2002) who reported that 125 ppb aflatoxin B_1 decreased daily gain and feed consumption of growing rabbits. Also, similar results were obtained by El-Zahar *et al.*, (1996), Salem *et al.*, (2001), Shehata, (2002), Meshreky *et al.*, (2007) and Marai and Askar (2008). The reduction in body weight by aflatoxin is not only due to depression of feed intake, but may also be due to the reduction in metabolism of protein, lipids, carbohydrate and dissolved vitamin in lipid (Cheeke and Shull, 1985 and Marai and Askar, 2008). Also, the catabolic effect of cortisol may play an important role in body weight loss, since it increased with aflatoxicosis (Hassan *et al.*, 1983).

Table (2): Effect of aflatoxin and *Nigella sativa* addition on rabbit performance

Parameters	Weeks	Control	Aflatoxin	Aflatoxin + 0.5% *Nigella sativa*	Aflatoxin + 1% *Nigella sativa*	Aflatoxin + 2% *Nigella sativa*
Body weight (g)	Initial weight	2000 ± 42	2016 ± 42	2008 ± 29	2016 ± 43	2000 ± 18
	Final w. (6 wks)	2346^{a} ± 34	1945^{b} ± 84	2402^{a} ± 45	2459^{a} ± 56	2380^{a} ±32
	Total w. gain	346^{a} ± 62	-71^{b} ± 46	394^{a} ± 19	443^{a} ± 34	380^{a} ± 26
	Daily w. change	8.24^{a} ± 1.5	-1.69^{b}±1.1	9.38^{a} ± 0.5	10.55^{a}±0.8	9.05^{a} ± 0.6
Feed intake (g/head/day)	1st week	122.20^{a} ± 3	74.60^{c} ± 6	85.00^{bc} ± 4	96.00^{b}± 5	93.00^{b} ± 3
	2nd week	136.60^{a} ± 2	81.00^{c} ± 3	104.00^{b}± 4	102.40^{b}± 6	96.00^{b} ± 3
	3rd week	139.60^{a}± 2	78.00^{c} ± 6	104.60^{b}± 3	103.00^{b}± 5	97.00^{b}± 3
	4th week	110.00^{a} ± 4	86.40^{b} ± 5	99.80^{ab}± 5	99.00^{ab}± 4	97.00^{ab}± 4
	5th week	113.00^{ab}± 4	78.00^{c} ± 6	111.00^{ab}± 3	120.00^{a}± 5	106.00^{b}± 4
	6th week	105.00^{b} ± 4	88.00^{c} ± 3	115.40^{a}± 2	118.00^{a}± 2	109.00^{ab}± 3
	Average	121.07^{a} ± 1	81.00^{c} ± 0.9	103.30^{b} ± 2	106.40^{b} ± 2	99.67^{b} ± 1
Mortality rate (%)	0	0	0	0	0	0

a,b,c.. Means in the same row bearing different letters differ significantly (P<0.05).

Table (3): Effect of aflatoxin and *Nigella sativa* addition on digestibility and nutritive values.

Items	Control	Aflatoxin	Aflatoxin + 0.5% *Nigella sativa*	Aflatoxin + 1% *Nigella sativa*	Aflatoxin + 2% *Nigella sativa*
Digestibility (%) :					
Dry matter	71.03^{a} ± 2.6	59.95^{c} ± 4.2	65.00^{b}±1.09	67.84^{b} ±0.8	64.28^{b} ± 0.4
Organic matter	73.05^{a} ± 1.7	64.04^{c} ± 1.2	67.21^{bc}±1.7	70.09^{ab} ±1.1	66.20^{bc} ± 2
Crude protein	72.34^{a} ± 1.8	66.44^{b} ± 1.8	68.42^{ab}± 1.2	73.77^{a} ± 1	70.22^{a} ± 1.1
Crude fiber	39.04^{a} ± 1.8	20.70^{d} ± 0.9	25.42^{c} ± 1.1	30.67^{b} ± 1.6	24.20^{c} ± 1.2
Ether extract	92.35 ± 1.13	89.54 ± 1.67	89.29 ± 1.70	91.60 ± 0.99	90.60 ± 1.27
Nitrogen free extract.	82.52 ± 1.82	78.37 ± 1.04	78.87 ± 1.79	79.63 ± 0.88	77.80 ± 0.97
Nutritive values (%):					
Total dige. nutrient	67.87^{a} ± 1.5	61.46^{c} ± 1.4	62.83^{bc}± 0.7	65.13^{ab}± 0.5	62.41^{bc}± 1.3
Dige. crude protein	12.30^{ab}± 0.3	11.29^{cd}± 0.3	11.63^{c} ± 0.2	12.54^{a} ± 0.2	11.94^{bc}± 0.2

a,b,c,d.. Means in the same row bearing different letters differ significantly (P<0.05).

Table (4): Effect of aflatoxin and *Nigella sativa* addition on blood parameters and internal organs weight.

Items	Control	Aflatoxin	Aflatoxin + 0.5% *Nigella sativa*	Aflatoxin + 1% *Nigella sativa*	Aflatoxin + 2% *Nigella sativa*
Blood parameters:					
AST (u/l)	$35.00^{ab} \pm 2$	$40.5^{a} \pm 1.73$	$34.50^{ab} \pm 1$	$32.50^{b} \pm 0.9$	$31.37^{b} \pm 0.8$
ALT (U/l)	$21.67^{b} \pm 0.9$	$30.00^{a} \pm 1.7$	$21.77^{b} \pm 1.9$	$23.33^{b} \pm 0.9$	$24.10^{b} \pm 0.7$
Total protein (g/dl)	$6.16^{a} \pm 0.09$	$5.43^{b} \pm 0.20$	$6.03^{a} \pm 0.14$	$6.25^{a} \pm 0.14$	$6.01^{a} \pm 0.15$
Albumin (g/dl)	$4.20^{a} \pm 0.12$	$3.70^{b} \pm 0.12$	$4.04^{a} \pm 0.13$	$4.16^{a} \pm 0.1$	$4.00^{a} \pm 0.13$
Globulin (g/dl)	1.96 ± 0.08	1.73 ± 0.09	1.91 ± 0.03	2.09 ± 0.15	2.01 ± 0.04
Creatinine (mg/dl)	$0.75^{b} \pm 0.03$	$1.03^{a} \pm 0.1$	$0.92^{ab} \pm 0.1$	$0.90^{ab} \pm 0.1$	$0.94^{ab} \pm 0.1$
Organs weight (%live weight)	$2.91^{b} \pm 0.13$	$3.48^{a} \pm 0.19$	$3.40^{a} \pm 0.14$	$3.41^{a} \pm 0.09$	$3.46^{a} \pm 0.08$
Liver	0.79 ± 0.07	0.96 ± 0.10	0.88 ± 0.08	0.81 ± 0.06	0.79 ± 0.04
Kidneys	0.61 ± 0.02	0.67 ± 0.09	0.61 ± 0.06	0.60 ± 0.03	0.62 ± 0.02
Lungs	0.30 ± 0.03	0.28 ± 0.01	0.32 ± 0.01	0.29 ± 0.01	0.33 ± 0.01
Heart					

a,b,c,d.. Means in the same row bearing different letters differ significantly ($P<0.05$).

Rabbits fed diets contaminated with aflatoxin B_1 plus Ns or vitamin C showed significantly ($P<0.05$) improvement in daily gain, feed intake and feed conversion in comparison with rabbits fed contaminated diet alone. The best improvement in daily gain and feed conversion was occurred by Ns plus vitamin C where the values were significantly ($P<0.05$) higher than the values obtained by Ns or vitamin C alone. This may be in agreement with that obtained by Gündüz *et al.*, (2002) who, reported that suspension of Ns plus vitamin C, E and selenium was better than Ns alone in monitoring trace elements (zinc, copper and iron) in rabbits treated by carcinogenic compound (N-methyl-N-nitrosoguanidine). The results of Ns agree with those of Zaky *et al.*, (2000) who reported that Ns counteracted the effect of aflatoxin on body weight gain of pekkin ducklings. The improvement by Ns may be due to its content of some active compounds such as 1- nigellaone, thymoquinon and thymohydroquinon which inhibit bacteria and improve body function and performance) (Mohan *et al.*, 1996 and Abd El-Hakim *et al.*, 2002), 2- fat soluble unidentified factors and essential fatty and amino acids whic display an essential role in growth performance, 3- several macro and micro elements which are responsible for regulating all vital function in the body and improve the immunity and 4- vitamins have essential role in growth performance (thiamin, riboflavin, pyridoxine and niacin) (William, 1999 and Seleem and Rowida, 2005 and Seleem et al., 2007). Increasing daily body weight gain by vitamin C was in agreement with those obtained by Salem *et al.*, (2001). The results of vitamin C may be due to increaseing feed intake and digestibility of nutrients (Abd El-Mageed, 1987).

Table (5): Effect of aflatoxin and *Nigella sativa* & vitamin C addition on rabbit performance

Item	Weeks	Control	Aflatoxin	Aflatoxin + *Nigella sativa*	Aflatoxin + Vitamin C	Aflatoxin + *Nigella sativa* + Vitamin C
Body weight (g)	Initial	1010.00 ± 18	1005.00 ± 8	1000.00 ± 14	1005.00 ± 8	1000.00 ± 11
	1 week	1155.11^{a} ± 22	1083.05^{b} ± 12	1106.75^{ab}±18	1118.75^{ab}±10	1129.50^{a} ± 7
	2 week	1311.77^{a} ± 27	1157.46^{c} ± 18	1225.75^{b}± 25	1232.50^{b} ± 8	1248.50^{ab}±16
	3 week	1468.15^{a} ± 34	1255.71^{c} ± 19	1364.00^{b} ± 30	1358.50^{b} ± 15	1383.25^{b} ± 14
	4 week	1608.15^{a} ± 46	1315.21^{c} ± 20	1469.00^{b} ± 35	1465.25^{b} ± 22	1497.00^{b} ± 19
	5 week	1746.40^{a} ± 46	1364.21^{c} ± 18	1572.25^{b} ± 41	1580.75^{b} ± 15	1619.50^{b} ± 16
	6 week	1882.90^{a} ± 46	1420.21^{d} ± 19	1659.75^{c} ± 45	1678.75^{bc}±13	1736.75^{b}± 15
Daily body gain (g)	1 week	20.73^{a} ± 1.50	11.15^{d} ± 0.84	15.25^{c} ± 0.63	16.25^{bc}± 0.48	18.50^{ab}± 0.65
	2 week	22.38^{a} ± 1.22	10.63^{c} ± 0.75	17.00^{b} ± 1.08	16.25^{b} ± 1.03	17.00^{b} ± 1.29
	3 week	22.34^{a} ± 1.38	9.75^{c} ± 0.86	16.75^{b} ± 0.75	18.00^{b} ± 1.58	19.25^{ab}± 0.48
	4 week	20.00^{a} ± 2.08	8.50^{c} ± 0.65	15.00^{b} ± 0.92	15.25^{b} ± 1.03	16.25^{b} ± 0.75
	5 week	19.75^{a} ± 1.98	7.00^{c} ± 0.90	14.75^{b} ± 0.85	16.50^{ab}± 1.90	17.50^{ab}± 1.66
	6 week	19.50^{a} ± 0.65	8.00^{d} ± 0.41	12.50^{c} ± 0.65	14.00^{c} ± 0.92	16.75^{b} ± 1.18
	Average	20.78^{a} ± 0.65	9.17^{d} ± 0.30	15.21^{c} ± 0.30	16.04^{bc}± 0.45	17.54^{b} ± 0.37
Feed intake (g/head /day)	1 week	99.75^{a} ± 3	63.75^{d} ± 2	95.00^{ab} ± 2	91.25^{bc} ± 2	87.50^{c} ± 1
	2 week	103.75^{a}± 4	63.25^{b} ± 2	95.00^{a} ± 2	102.25^{a} ± 4	95.50^{a} ± 2
	3 week	118.25^{a}± 1	67.25^{d} ± 2	103.00^{c} ± 1	110.50^{b} ± 2	104.50^{c} ± 2
	4 week	119.50^{a}± 2	67.00^{c} ± 3	99.00^{b} ± 1	115.00^{a} ± 2	101.00^{b} ± 4
	5 week	117.50^{a}± 2	65.00^{c} ± 2	112.50^{a} ± 2	115.75^{a} ± 2	104.25^{b} ± 3
	6 week	120.00^{a} ± 2	66.50^{d} ± 3	108.25^{c} ± 1	117.50^{ab}± 1	113.25^{bc}± 2
	Average	113.13^{a} ± 0.8	66.13^{c} ± 0.7	102.13^{b} ± 0.4	108.71^{ab} ± 0.7	101.00^{b} ± 0.8
Feed conversion (feed/ gain)	1 week	4.81^{b} ± 0.20	5.72^{ab} ± 0.7	6.23^{a} ± 0.30	5.62^{ab} ± 0.1	4.73^{b} ± 0.17
	2 week	4.64^{b} ± 0.22	5.95^{a} ± 0.53	5.59^{ab} ± 0.2	6.29^{a} ± 0.43	5.62^{ab} ± 0.5
	3 week	5.29^{b} ± 0.36	6.90^{a} ± 0.68	6.15^{ab} ± 0.3	6.14^{ab} ± 0.6	5.43^{b} ± 0.10
	4 week	5.98^{b} ± 0.60	7.88^{a} ± 0.35	6.60^{ab} ± 0.5	7.54^{ab} ± 0.5	6.22^{b} ± 0.30
	5 week	5.95^{b} ± 0.68	9.29^{a} ± 1.57	7.63^{ab} ± 0.5	7.02^{ab} ± 0.9	5.96^{b} ± 0.62
	6 week	6.15^{c} ± 0.30	8.31^{a} ± 1.00	8.66^{b} ± 0.52	8.39^{b} ± 1.0	6.76^{bc} ± 0.36
	Average	5.47^{b} ± 0.15	7.34^{a} ± 0.4	6.81^{ab} ± 0.2	6.83^{ab} ± 0.2	5.79^{b} ± 0.06

a,b,c.. Means in the same row bearing different letters differ significantly (P<0.05).

Table (6): Effect of aflatoxin and *Nigella sativa* & vitamin C addition on digestibility and nutritive values

Items	Control	Aflatoxin	Aflatoxin + *Nigella sativa*	Aflatoxin + Vitamin C	Aflatoxin + *Nigella sativa* + Vitamin C
Digestibility (%):					
DM	$69.77^{a} \pm 0.7$	$58.2^{b} \pm 1.15$	$69.4^{a} \pm 0.68$	$68.52^{a} \pm 0.5$	$68.9^{a} \pm 0.85$
OM	$71.5^{a} \pm 0.75$	$62.20^{b} \pm 0.7$	$70.9^{a} \pm 0.59$	$70.11^{a} \pm 0.7$	$70.60^{a} \pm 0.9$
CP	$71.50^{a} \pm 0.8$	$64.00^{b} \pm 0.8$	$72.1^{a} \pm 0.58$	$71.75^{a} \pm 0.7$	$72.15^{a} \pm 0.6$
CF	$38.06^{a} \pm 0.9$	$25.0^{c} \pm 0.77$	$30.61^{bc} \pm 0.9$	$31.00^{bc} \pm 0.55$	$34.10^{ab} \pm 0.7$
EE	90.65 ± 0.72	88.0 ± 0.59	90.69 ± 0.73	89.90 ± 0.55	91.15 ± 0.56
NFE	83.00 ± 0.90	80.69 ± 1.1	81.25 ± 0.81	80.20 ± 0.76	82.20 ± 0.73
Nutritive values (%):					
TDN	$67.75^{a} \pm 1.0$	$62.94^{b} \pm 1.2$	$65.68^{a} \pm 0.9$	$65.08^{a} \pm 0.7$	$66.80^{a} \pm 1.4$
DCP	$12.16^{a} \pm 0.3$	$10.88^{b} \pm 0.2$	$12.26^{a} \pm 0.3$	$12.20^{a} \pm 0.3$	$12.27^{a} \pm 0.3$

a,b,c.. Means in the same row bearing different letters differ significantly ($P<0.05$).

2- Digestibility and nutritive values:

Aflatoxin B_1 decreased ($P<0.05$) the digestibility of dry matter (DM), organic matter (OM), crude protein (CP), crude fiber and caused insignificantly decrease in ether extract and nitrogen free extract (NFE) digestibility (Table 6). The nutritive values as TDN (%) and DCP (%) showed similar trend of digestibility (Table 6). These results were in agree with those reported by Abd El-Mageed, (1987), Ibrahim (2000), Abd El-Baki *et al.*, (2002) and Shehata, (2002). This bad effect of aflatoxin may be due to interfering with utilization of dietary nutrients (Smith, 1982, Cheeke and Shull, 1985 and Diekman and Green, 1992).

Addition of Ns or vitamin c alone or Ns plus vitamin C improved significantly ($P<0.05$) the digestibility of nutrients and nutritive values which negatively affected by aflatoxin B_1. The results of Ns may be due to its contents, which regulate digestion and absorbtion and fight the internal parasites (Nasr *et al.*, 1996; Medenica *et al.*, 1997; Abdel-Azzem *et al.*, 1999 and Abd El-Hakim *et al.*, 2002). The improvement in digestibility by vitamin C was in agreement with those obtained by Abd El-Mageed (1987), these results may be due to biological role of vitamin C in digestive enzyme biosynthesis and activation (Earp *et al.*, 1970).

3- Blood parameters:

Aflatoxin B_1 decreased significantly ($P<0.05$) serum total protein, albumin and globulin values (Table 7). These findings agreed with those reported by Pier, (1992), El-Zahar *et al.*, (1996), Nowar *et al.*, (1996) and Abd El-Baki *et al.*, (2002). Decreasing of serum protein may be attributed to degeneration of endoplasmic reticulum and inhibition of protein synthesis (Srivastava, 1984). The activities of aspertate amino transferase (AST)

and alanine amino transferase (ALT) enzymes were increased by aflatoxin B_1 (the increase was significantly only with ALT). These results agreed with those reported by Nowar *et al.*, (1996), El-Zahar *et al.*, (1996) and Zaky *et al.*, (2000). Increasing ALT activities may be due to hepatocellular necrosis or increasing the permeability of cell membrane (Zaky *et al.* 2000). The creatinine value was higher ($P<0.05$) in serum of rabbits fed aflatoxin B_1 diet in comparison with that of control. These results agreed with those obtained by Nowar *et al.* (1996) and Abd El-Baki *et al.* (2002).

Table (7): Effect of aflatoxin and *Nigella sativa* & vitamin C addition on blood constituents

Items	Control	Aflatoxin	Aflatoxin + *Nigella sativa*	Aflatoxin + Vitamin C	Aflatoxin + *Nigella sativa* + Vitamin C
AST (u/l)	$30.00^{ab} \pm 1.0$	$34.5^{a} \pm 0.95$	$27.00^{b} \pm 1.00$	$28.50^{b} \pm 0.55$	$28.5^{b} \pm 0.55$
ALT (U/l)	$20.00^{b} \pm 0.95$	$28.00^{a} \pm 1.73$	$20.33^{b} \pm 1.25$	$21.00^{b} \pm 0.87$	$21.00^{b} \pm 0.50$
Total protein (g/dl)	$5.95^{a} \pm 0.50$	$4.95^{b} \pm 0.42$	$5.72^{a} \pm 0.50$	$5.55^{a} \pm 0.40$	$5.63^{a} \pm 0.13$
Albumin (g/dl)	$4.10^{a} \pm 0.25$	$3.50^{b} \pm 0.15$	$3.92^{a} \pm 0.14$	$4.00^{a} \pm 0.13$	$3.98^{a} \pm 0.07$
Globulin (g/dl)	$1.85^{a} \pm 0.25$	$1.45^{b} \pm 0.15$	$1.80^{a} \pm 0.14$	$1.55^{ab} \pm 0.13$	$1.65^{ab} \pm 0.09$
Creatinine (mg/dl)	$1.13^{b} \pm 0.06$	$1.33^{a} \pm 0.03$	$1.12^{b} \pm 0.05$	$1.10^{b} \pm 0.05$	$1.10^{b} \pm 0.06$

a,b,c.. Means in the same row bearing different letters differ significantly ($P<0.05$).

Table (8): Effect of aflatoxin and *Nigella sativa* & vitamin C addition on internal organs weight (% live weight)

Items	Control	Aflatoxin	Aflatoxin + *Nigella sativa*	Aflatoxin + Vitamin C	Aflatoxin + *Nigella sativa* + Vitamin C
Liver	$2.36^{b} \pm 0.05$	$2.90^{a} \pm 0.2$	$2.6^{ab} \pm 0.2$	$2.7^{ab} \pm 0.03$	$2.65^{ab} \pm 0.14$
Kidneys	0.72 ± 0.04	0.73 ± 0.04	0.75 ± 0.03	0.65 ± 0.09	0.70 ± 0.04
Lungs	0.49 ± 0.02	0.50 ± 0.03	0.53 ± 0.04	0.56 ± 0.03	0.55 ± 0.03
Heart	0.26 ± 0.04	0.24 ± 0.03	0.26 ± 0.02	0.26 ± 0.01	0.26 ± 0.02
Mortality rate (%)	12.50	37.50	12.50	12.50	12.50

a,b,c.. Means in the same row bearing different letters differ significantly ($P<0.05$).

Addition of Ns, vitamin C, or the two additivies together improved significantly ($P<0.05$) all blood parameters studied, whereas, the values of blood parameters for additivies groups become nearly similar to those of control group. These results agreed with those reported by Zaky *et al.* (2000) who reported that 1% crushed seed in diet of pekkin ducklings improved blood parameters (globulin, AST, LDH, ALT) that negatively affected by aflatoxin B_1. Also, Abdelhamid *et al.* (2002) found that 1% Ns improved the immunity of rats and its health condition by improving blood parameters. The

improvement of liver function by Ns may be due to the increase of glutathion and glutathion-S- transferase by NS (Zaky *et al.* 2000).

4- Mortality rate and internal organs weight :

Aflatoxin B_1 increased the mortality rate (%) which was 37.50% in comparison with 12.5% for other groups (Table 8). The incidence of death may be due to the disturbance of organs function and decreased the immune responsiveness (Lovell, 1991). The weight of liver, kidneys and heart as % of body weight increased by aflatoxin B_1 (Table 8). These results agreed with those reported by Nowar *et al.*, (1996); Santurio *et al.*, (1999) and Shehata, (2002). No significant ($P<0.05$) differences were found between organs weight of rabbits fed aflatoxin B_1 with or without NS or vitamin C, the significant was only in liver of rabbits fed aflatoxin B1 comparing with those of control group. Literature showed that Ns caused an increase in the percent of organs weight of rabbits fed uncontamined diets (Seleem *et al.* 2007).

It could be concluded that adding Ns (at 1%) or 500 mg vitamin C/ kg diet alleviate the toxic effect of aflatoxin B_1. The improvement of Ns plus vitamin C was better than that of Ns or vitamin C alone in alleviation the hazard effect of aflatoxin B_1 in rabbits.

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تأثير إضافة حبة البركة وفيتامين ج إلى عليقة الأرانب الملوثة بالأفلاتوكسين B_1

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تم إجراء هذا العمل لمعرفة مقدرة حبة البركة ، فيتامين ج على تقليل التأثير السام لعليقة الأرانب الملوثة بالأفلاتوكسين B_1. تم إجراء تجربتين، فى التجربة الأولى استخدم ٣٠ ذكر أرنب نيوزيلندى أبيض (متوسط الوزن ٢٠٠٠± ٣٠ جم) وزعت على خمس مجموعات (٦ أرنب/ مجموعة) لمدة ٦ أسابيع. تغذت المجموعة الأولى (الكنترول) على العليقة الأساسية، بينما تغذت المجموعة الثانية على العليقة الأساسية مضاف لها ٣٥٠ جزء فى البليون أفلاتوكسين B_1 . وتغذت المجموعات الثالثة، الرابعة والخامسة على العليقة الأساسية المضاف لها ٣٥٠ جزء فى البليون أفلاتوكسين B_1 بالإضافة إلى ٠,٥ ، ١ ، ٢% حبة بركة على التوالى. فى التجربة الثانية تم استخدام ٤٠ ذكر أرنب نيوزيلندى أبيض (متوسط الوزن ١٠٠٠± ١٠ جم) وزعت على خمس مجموعات (٨ أرنب / مجموعة) لمدة ٦ أسابيع. تغذت المجموعة الأولى (الكنترول) على العليقة الأساسية، بينما تغذت المجموعة الثانية على العليقة الأساسية مضاف لها ٢٠٠ جزء فى البليون أفلاتوكسين B_1 . وتغذت المجموعات الثالثة، الرابعة والخامسة على العليقة الأساسية المضاف لها ٢٠٠ جزء فى البليون أفلاتوكسين B_1 بالإضافة الى ١ % حبة بركة، ٢,٥ جم فيتامين ج / كجم عليقة، ١ % حبة بركة + ٥٠٠ ملجم فيتامين ج / كجم عليقة على التوالي.

أوضحت النتائج أن إضافة حبة البركة (بمعدل ١%) أو فيتامين ج قللت معنويا (مستوى ٥%) التأثير السيئ للأفلاتوكسين B_1 على معدل النمو، الغذاء المأكول، كفاءة التحويل الغذائى، هضم المركبات الغذائية، القيم الغذائية وقياسات الدم (البروتين الكلى، الألبيومين، الجلوبيولين، إنزيم الأسبرتيت أمينوترانزفيريز، إنزيم الألانين أمينو ترانزفيريز، الكرياتينين) والنسبة المئوية للنفوق، أفضل تحسن تم الحصول عليه بإضافة حبة البركة مع فيتامين ج.

التوصية: إضافة ١% حبة بركة + ٥٠٠ ملجم فيتامين ج أفضل من إضافة حبة البركة أو فيتامين ج بمفردهم فى تخفيف الأثر السام للأفلاتوكسين فى علائق الأرانب.