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THE EFFECT OF INCLUSION LEVELS OF VITAMIN E AND C ON PERFORMANCE OF BROILER CHICKS FED AFLATOXIN CONTAMINATED DIETS

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ABSTRACT

This experiment was conducted to evaluate the efficiency of using two levels of vitamin E (vit.E) and C (vit.C) either alone or in combination as a feed additive to ameliorate the deleterious effects of Aflatoxin (AF) on broiler performance. A total of 280 one-day old un-sexed Ross broiler chicks were housed in batteries, randomly divided into ten experimental groups as follows: The first group (C) was fed on basal diet (Control), whereas, the rest groups were received the contaminated diet with AF at 1.2 ppm/kg diet where, C^+: fed the contaminated diet, E_{100}: fed the contaminated diet with 100 mg vit.E/kg diet (E_1), E_{200}: fed the contaminated diet with 200 mg vit.E/kg diet (E_2), C_{250}: fed the contaminated diet with 250 mg vit.C/kg diet (C_1), C_{500}: fed the contaminated diet with 500 mg vit.C/kg diet (C_2), $E_{100}C_{250}$: fed the contaminated diet with E_1 + C_1, $E_{100}C_{500}$: fed the contaminated diet with E_1 + C_2, $E_{200}C_{250}$: fed the contaminated diet with E_2 + C_1 and $E_{200}C_{500}$: fed the contaminated diet with E_2 + C_2. The results indicated that, as expected, that C^+ group had significantly lower final body weight and total body weight gain; worse total feed conversion ratio and higher total mortality rate than those of the C group. On the other hand, birds which received contaminated diet with combination of vit.E and vit.C supplementation ($E_{100}C_{250}$, $E_{100}C_{500}$, $E_{200}C_{250}$ and $E_{200}C_{500}$) had significantly an improvement in productive performance as compared to those received contaminated diet (C^+). Moreover, there were no significant differences in productive performance among the birds which received control diet and those fed the contaminated diet plus combination of vit.E and C addition.

Therefore, it could be concluded that supplemental diets with the combination of vit.E and C can be used as antitoxin when the diet contaminated with aflatoxin.

Keywords: Broiler, aflatoxicosis, vitamin E, vitamin C and productive performance.

INTRODUCTION

Mycotoxins, the secondary metabolites of toxigenic fungi, are unavoidable contaminants in foods and feeds exerting harmful effects upon animal and human health (Zahoor-ul-Hassan *et al.*, 2010). The most important mycotoxins in naturally tainted foods and feeds are aflatoxins, ochratoxins, zearalenone, T-2 toxin, deoxynivalenol and fumonisins (Devegowda *et al.,* 1998; Sultana and Hanif, 2009). Aflatoxins are a group of mycotoxins produced by the strains of *Aspergillus flavus* and *Aspergillus parasiticus* (Bennett and Klich, 2003). These fungi can easily contaminate a number of grains causing both important economic losses and health problems. Among the four major groups of aflatoxin namely B_1, B_2, G_1 and G_2, aflatoxin B_1 (AFB_1) is the most toxic and a known carcinogenic. Acute or chronic aflatoxicosis in poultry birds results in decreased meat and egg production, immunosuppression and hepatotoxicosis.

Numerous strategies, such as physical separation, thermal inactivation, irradiation, microbial degradation and treatment with a variety of chemicals have been used for the detoxification or inactivation of mycotoxin-contaminated feedstuff (Goldblatt and Dollear, 1979; Anderson, 1983). Recently, producers, researchers and governments aim to develop effective, inert, cheaper, non-toxic and nutritive prevention management and decontamination technologies to minimize the toxic effects of AF. Several experimental studies have shown that vitamins C and E could ameliorate aflatoxin toxicity (Yu *et al*., 1994; Salem *et al.,* 2001).Various findings strongly suggested the enhancement of immune response due to vitamin E supplementation (Balker, 1993). At the same time, vitamin C (ascorbic acid) may scavenge peroxyl radical and inhibit cytotoxicity induced by oxidants. In addition, ascorbic acid can reduce or prevent H_2O_2-induced lipid peroxidation and the formation of OH-deoxyguanosine (Retsky and Frei, 1995; Tsou *et al.,* 1996). Therefore, the aim of the present study was to evaluate the efficiency of incorporating two levels of vitamin E and C either alone or in their combination to ameliorate the deleterious effects of Aflatoxins (AF) in broiler chick's diet.

MATERIALS AND METHODS

This experiment was carried out at Regional Center for Food and Feed. A total number of 280 one-day-old straight run broiler Ross chicks was housed in batteries and were randomly divided into ten groups (each of 28 chicks) to receive one of the experimental diets. Each group was divided into four replicates.

Experimental diets:

C (control)	Basal diet (NRC recommended diet without any supplementation).
C^+	Contaminated diet with 1.2 ppm aflatoxin (AF)/kg diet.
E_{100}	Contaminated diet + 100 mg vit.E/kg diet.
E_{200}	Contaminated diet + 200 mg vit.E/kg diet.
C_{250}	Contaminated diet + 250 mg vit.C/kg diet.
C_{500}	Contaminated diet + 500 mg vit.C/kg diet.
$E_{100}C_{250}$	Contaminated diet + 100 mg vit.E+ 250 mg vit.C/kg diet.
$E_{100}C_{500}$	Contaminated diet + 100 mg vit.E+ 500 mg vit.C/kg diet.
$E_{200}C_{250}$	Contaminated diet + 200 mg vit.E+ 250 mg vit.C/kg diet.
$E_{200}C_{500}$	Contaminated diet + 200 mg vit.E + 500 mg vit.C/kg diet.

General management:

Feed and water were provided *ad_libitum*. Diets were formulated in Regional Center for Food and Feed to be isonitrogenous, isocaloric and mycotoxins-free as well as free from any medication as growth promoter or antibiotics. The diets which used were formulated to meet the nutrient requirements of the broiler chicks during starter, grower and finisher periods according to the National Research Council (NRC, 1994). All birds were fed a starter diet from one to 14 d of age containing 22.5% CP and 3063 Kcal ME/Kg diet. From 15 to 28 d of age, the birds were switched to grower diet containing 21.5% CP and 3144 Kcal ME/Kg diet. While, during 29 to 42 d of age, they were fed finisher diet containing 20.1% CP and 3195 Kcal ME/Kg diet (Table 1). The temperature was set at 32º C on the first day, gradually reduced to 24º C by the end of the third week, and until the end of experiment. The light was provided 24 hrs daily throughout the experiment. The averages of initial body weights of different groups were nearly similar. All experimented birds were vaccinated against different diseases according to the vaccination programs adopted in most Egyptian chicken broiler farmers.

Table 1. Composition and calculated analysis of the experimental diets

Ingredients	Starter	Grower	Finisher
Yellow corn (7.5% CP)	56	60	62.3
Soybean meal (45.8% CP)	32	25.5	24
Corn glutin meal (60.8% CP)	5	7.5	6.5
Vegetable oil	2.965	2.915	3.525
Dicalcium phosphate	1.73	1.96	1.76
Limestone	0.81	0.56	0.56
Vit. & Min. Mixture (1)	0.4	0.4	0.4
Salt	0.4	0.4	0.4
L-lysine-HCl	0.38	0.49	0.33
DL-Methionine	0.24	0.2	0.15
Choline chloride	0.075	0.075	0.075
Total	100	100	100
Calculated values			
CP	22.5	21.5	20.1
ME (KCal / kg)	3063	3144	3195
Lysine	1.36	1.3	1.13
Methionine	0.61	0.58	0.51
Methionine + Cystine	0.98	0.94	0.85
Calcium	0.96	0.9	0.85
Available ph	0.45	0.48	0.44

(1)Vitamins - minerals mixture supplied per kg of diet: vit. (A), 12000 I.U., vit. (D_3), 2000 I.U; vit. (E), 10 mg; vit. (K_3), 2 mg; vit. (B_1), 1 mg; vit. (B_2), 5 mg; vit. (B_6), 1.5 mg; vit. (B_{12}), 10 µg; Biotin, 50 µg; Pantothenic acid, 10mg; Niacin, 30 mg; Folic acid, 1 mg; Manganese, 60 mg; Zinc, 50 mg; Iron, 30 mg; Copper, 10 mg; Iodine, 1 mg; Selenium, 0.1 mg and Cobalt, 0.1 mg.

Aflatoxin production and assessment:

Aflatoxin production was carried out according to Davis *et al.* (1966) using liquid yeast medium and *Aspergillus flavus* NRRL (3145). The media which contain detectable amount of aflatoxin was mixed well with the basal diet to get the aflatoxin- contaminated diet. Aflatoxin in liquid medium and diet was determined according to Ross *et al.* (1997) and A.O.A.C (2005) using HPLC technique (Agillent 1100 series USA with column C 18, Lichrospher 100RP- 18, 5 µm × 25 cm). Aflatoxin was incorporated into small portion of the basal diet manually, and then this small portion was mixed with larger amount of basal diet until reaching the desired level of 1200 ppb AF/kg final feed.

Measured parameters:

Chick's performance response variables were determined according to North (1984); weekly individually body weight (wt.) and wt. gain were measured on all birds. Weekly feed consumption (g/d/bird), feed conversion ratio (FCR) (g feed/g live body wt. gain) and mortality rate were measured for each replicate. Dead birds were weighed to include their weights in the feed conversion estimation.

Statistical analyses:

One-way analysis of variance has been adopted using SAS software general linear model (SAS, 2004). The main factor was vitamins supplementation. Mean values were compared using the Duncan's New Multiple Range test (Duncan, 1955) when significant differences existed. Mortality rate was tested by the chi-square procedure (Snedecor and Cochran, 1993). The fixed effects model used in the analysis was as follow:

$Y_{ij} = \mu + T_i + e_{ijk}$.

Where:

Y_{ij}: the observation of the $_J{}^{th}$ chick in the $_i{}^{th}$ treatment.

μ: the overall mean.

T_i: effect of the $_i{}^{th}$ treatment (i = 1, 2….10).

e_{ijk}: the random error effect.

RESULTS AND DISCUSSION

Body weight (BW):

Results in Table (2) showed that the body weight of AF-group (C^+ group) in all experimental periods was significantly (p<0.0001) the lowest as compared to the other experimental groups. On the other hand, all vitamins treatments improved the body weight especially of those which received the mixture of vit.E and vit.C. Moreover, at 14 days of age, $E_{100}C_{250}$ and $E_{200}C_{250}$ groups had the highest (p<0.0001) body weight. Also, at 28 days of age, E_{100}, C_{500}, $E_{100}C_{250}$, E_{100} C_{500} and $E_{200}C_{500}$ groups had the significantly (p<0.0001) highest body weight. While, at 42 days of age, $E_{100}C_{250}$ and E_{100} C_{500} groups had the significantly (p<0.0001) highest body weight.

The decrease in the body weight due to aflatoxicosis in the present study was in agreement with several previous investigations using dietary AF in poultry feed (Salem *et al.*, 2001; Tedesco *et al.,* 2004; Han *et al.,* 2008; Khan *et al.,* 2010).

The decrease of body weight due to aflatoxicosis may be attributed to the biotransformation of aflatoxins which gives rise to various metabolites; the AFB_1-8,9-epoxide, in particular, is relevant because it may covalently bind to DNA and to proteins which then alters enzymatic processes such as gluconeogenesis, Krebs cycle or fatty acid synthesis (Lesson *et al.,* 1995) and inhibit the protein synthesis (Oguz *et al*., 2000a).

The improvement in body weight due to vitamins supplementation to the contaminated feed in the present study was in agreement with several previous investigations which used same vitamins in poultry feed (Salem *et al.,* 2001; El Barkouky *et al.,* 2010; Khan *et al.,* 2010). The present results also are in agreement with results of the other investigations which used vitamin E + selenium (Se) (Kumar *et al.,* 2003; Shlig, 2009).

Table 2. Effects of vit. E and C supplementation on body weight (g) of broilers fed aflatoxicosis diet

Treatment	Age (day)		
	14 days	28 days	42 days
	x¯±SE	x¯±SE	x¯±SE
C	301.83±6.58[ab]	1103.75±34.99[bc]	2372.75±10.11[abc]
C^+	251.55±6.87[d]	1016.81±33.67[d]	2159.58±33.67[e]
E_{100}	293.57±16.22[ab]	1209.76± 27.26[a]	2411.67±30.96[ab]
E_{200}	255.67±13.20[cd]	1032.00±24.71[cd]	2267.50±29.04[cde]
C_{250}	312.14±4.10[ab]	1137.86±20.21[ab]	2222.02±25.14[de]
C_{500}	284.05±13.35[bc]	1200.83±19.60[a]	2260.00±56.58[cde]
$E_{100}C_{250}$	320.60±7.15[a]	1217.38±38.72[a]	2435.48±40.91[a]
$E_{100}C_{500}$	311.43±3.09[ab]	1221.79±23.50[a]	2435.24±46.39[a]
$E_{200}C_{250}$	320.24±13.85[a]	1162.38±18.24[ab]	2315.97±19.79[bcd]
$E_{200}C_{500}$	299.17±10.49[ab]	1220.97±28.96[a]	2314.64±41.92[bcd]

[a, b, c, d, e] Means in the same column, with different superscripts, are significantly different (p<0.0001).

C=Control diet, C^+=Control diet+1.2 ppm aflatoxin (AF)/kg diet, E_{100}=Control diet+AF+100 mg vit.E/kg diet, E_{200}=Control diet+AF+200 mg vit.E/kg diet, C_{250}=Control diet+AF+250 mg vit.C/kg diet, C_{500}=Control diet+AF+500 mg vit.C/kg diet, $E_{100}C_{250}$=Control diet+AF+100 mg vit.E+250 mg vit.C/kg diet, $E_{100}C_{500}$=Control diet+AF+100 mg vit.E+500 mg vit.C/kg diet, $E_{200}C_{250}$=Control diet+AF+200 mg vit.E+250 mg vit.C/kg diet and $E_{200}C_{500}$=Control diet+AF+200 mg vit.E+500 mg vit.C/kg diet.

Body weight gain (BWG):

Results in Table (3) showed that body weight gain of C^+ group was significantly (p<0.0001) decreased as compared to most experimental groups throughout the studied experimental periods. On the other hand, supplementing diets with vitamins improved body weight gain and could ameliorate the toxicity of AF. At 14 days of age, either of $E_{100}C_{250}$ and $E_{200}C_{250}$ groups recorded the highest (p<0.0001) weight gain; while, at 28 days of age, E_{100}, C_{500}, $E_{100}C_{500}$ and $E_{200}C_{500}$ groups had the highest (p<0.0001) values; but, at 42 days of age, control group (C) occupied first (p<0.01). Finally, results showed that both $E_{100}C_{250}$ and $E_{100}C_{500}$ groups harvested the significantly (p<0.0001) highest weight gains through the entire experimental period.

The decrease in body weight gain due to aflatoxicosis in the present study was in agreement with several previous investigations in poultry (Parlat *et al.,* 2001; Han *et al.,* 2008) while, disagree with results which obtained by Zaghini *et al*. (2005) who found no apparent effect of feeding 44-wk-old laying hens at contaminated diet by aflatoxin B_1 (2.5 ppm/kg of feed) for four weeks on the body weight gain. This disagreement may be due to variation of species (laying hens have more resistance to aflatoxicosis than broiler) and the age (the younger birds have more susceptibility to aflatoxicosis than mature ones).The decrease of body weight gain due to aflatoxicosis may be attributed to the same reasons of the decrease in body weight. The improvement in body weight gain due to vitamins supplementation to the contaminated feed in the present study was in agreement with results recorded by Shlig (2009).

Table 3. Effects of vit. E and C supplementation on weight gain (g) of broilers fed aflatoxicosis diet

Treatment	Age (day)			
	0-14 days	15-28 days	29-42 days	0-42 days
	$\bar{x}$±SE	$\bar{x}$±SE	$\bar{x}$±SE	$\bar{x}$±SE
C	246.23±16.58^{bc}	801.92±35.53^{c}	1269.00±37.84^{a}	2317.15±10.11^{abc}
C^{+}	211.55±16.87^{d}	765.26±38.31^{c}	1142.78±43.10^{abcd}	2119.59±33.67^{e}
E_{100}	253.57±16.22^{ab}	916.19±19.21^{a}	1201.91±35.58^{abc}	2371.67± 30.96^{ab}
E_{200}	215.67±13.20^{cd}	776.33±20.85^{c}	1235.50±29.68^{ab}	2227.50±29.04^{cde}
C_{250}	272.14±14.10^{ab}	825.72±19.48^{bc}	1084.17±27.87^{cd}	2182.02±25.14^{de}
C_{500}	244.05±13.35^{bc}	916.79±19.57^{a}	1059.17±54.64^{d}	2220.00±56.58^{cde}
$E_{100}C_{250}$	280.60±17.15^{a}	896.79±34.91^{ab}	1218.10±79.47^{abc}	2395.48±40.91^{a}
$E_{100}C_{500}$	271.43±13.09^{ab}	910.36±21.96^{a}	1213.45±40.47^{abc}	2395.24±46.39^{a}
$E_{200}C_{250}$	280.24±13.85^{a}	842.14±30.40^{abc}	1153.58±35.04^{abcd}	2275.97±19.79^{bcd}
$E_{200}C_{500}$	259.17±10.49^{ab}	921.80±19.73^{a}	1093.68±15.92^{bcd}	2274.64±41.92^{bcd}

a, b, c, d, e Means in the same column, with different superscripts, are significantly different ($p<0.01$).
C=Control diet, C^{+}=Control diet+1.2 ppm aflatoxin (AF)/kg diet, E_{100}=Control diet+AF+100 mg vit.E/kg diet, E_{200}=Control diet+AF+200 mg vit.E/kg diet, C_{250}=Control diet+AF+250 mg vit.C/kg diet, C_{500}=Control diet+AF+500 mg vit.C/kg diet, $E_{100}C_{250}$=Control diet+AF+100 mg vit.E+250 mg vit.C/kg diet, $E_{100}C_{500}$=Control diet+AF+100 mg vit.E+500 mg vit.C/kg diet, $E_{200}C_{250}$=Control diet+AF+200 mg vit.E+250 mg vit.C/kg diet and $E_{200}C_{500}$=Control diet+AF+200 mg vit.E+500 mg vit.C/kg diet.

Feed consumption (FC):

Results in Table (4) showed that feed consumption through the first 14 days of age was significantly ($p<0.001$) higher of all experimental groups compared with control group. But, from 15 to 28 days of age, there were no significant differences among groups. While, from 29 to 42 days of age, feed consumption of all groups of vitamin E and vitamin C (either alone or in combination) were significantly ($p<0.001$) decreased compared with C^{+} group. Whereas, from 0 to 42 days of age, $E_{100}C_{250}$, $E_{200}C_{250}$ and $E_{200}C_{500}$ groups addition to control group had the lowest ($p<0.05$) feed consumption.

The increase or no alteration in feed consumption due to aflatoxicosis in the present study was in agreement with several previous investigations in broiler chicks (Oguz *et al*., 2000b; Celik *et al*., 2005; LI Juan-juan *et al*., 2010). At the same time, these results were in agreement with results reported by Tedesco *et al*. (2004), who found no alteration in feed consumption of birds fed on diet contaminated by 0.8 ppm AFB_1/kg diet as compared to control birds at the first three weeks of experiment. Whereas, the results disagreed with the results which recorded by Salem *et al*. (2001); Safameher and Shivazad (2007), they found decrease of feed consumption rate in aflatoxicosis poultry. The differences of results may be attributed to variation of species, age and type of aflatoxin.

Table 4. Effects of vit. E and C supplementation on feed consumption (g) of broilers fed aflatoxicosis diet

Treatment	Age (day)			
	0-14 days	15-28 days	29-42 days	0-42 days
	$\bar{x}\pm SE$	$\bar{x}\pm SE$	$\bar{x}\pm SE$	$\bar{x}\pm SE$
C	290.09±27.02^{b}	1252.09±52.90^{Ns}	2216.33±51.39^{bc}	3758.51±65.35^{b}
C^{+}	328.32±16.45^{a}	1259.23±87.34^{Ns}	2485.63±85.50^{a}	4073.18±78.28^{a}
E_{100}	351.00±12.15^{a}	1319.62±27.35^{Ns}	2251.67±72.04^{b}	3922.29±91.45^{ab}
E_{200}	331.67±22.87^{a}	1277.00±36.60^{Ns}	2275.08±45.42^{b}	3883.75±81.66^{ab}
C_{250}	376.43±15.52^{a}	1270.00±37.33^{Ns}	2132.62±36.44^{bc}	3779.05±41.07^{ab}
C_{500}	346.55±14.66^{a}	1349.86±64.04^{Ns}	2242.50±70.81^{b}	3938.90±39.08^{ab}
$E_{100}C_{250}$	368.74±19.61^{a}	1208.04±59.17^{Ns}	2150.76±52.60^{bc}	3727.54±69.78^{b}
$E_{100}C_{500}$	365.72±25.95^{a}	1301.43±44.65^{Ns}	2177.44±78.47^{bc}	3844.59±80.74^{ab}
$E_{200}C_{250}$	364.92±19.25^{a}	1262.86±55.53^{Ns}	2094.47±46.22^{bc}	3722.24±69.74^{b}
$E_{200}C_{500}$	358.81±25.23^{a}	1348.69±52.82^{Ns}	2007.00±58.55^{c}	3714.50±60.44^{b}

[a, b, c,] Means in the same column, with different superscripts, are significantly different (Ns= no significant) and ($p<0.05$).

C=Control diet, C^{+}=Control diet+1.2 ppm aflatoxin (AF)/kg diet, E_{100}=Control diet+AF+100 mg vit.E/kg diet, E_{200}=Control diet+AF+200 mg vit.E/kg diet, C_{250}=Control diet+AF+250 mg vit.C/kg diet, C_{500}=Control diet+AF+500 mg vit.C/kg diet, $E_{100}C_{250}$=Control diet+AF+100 mg vit.E+250 mg vit.C/kg diet, $E_{100}C_{500}$=Control diet+AF+100 mg vit.E+500 mg vit.C/kg diet, $E_{200}C_{250}$=Control diet+AF+200 mg vit.E+250 mg vit.C/kg diet and $E_{200}C_{500}$=Control diet+AF+200 mg vit.E+500 mg vit.C/kg diet.

The increase in feed consumption due to aflatoxicosis may be attributed to the activity of some digestive enzymes can be altered toward rising by aflatoxin exposure (Lesson *et al*., 1995). It was found that the specific activities of pancreatic chymotrypsin, amylase and lipase, but not trypsin increased significantly by aflatoxin (Richardson and Hamilton, 1987).

The improvement (restored to as recorded in control) of feed consumption in some of vitamin groups, especially, in combination groups was in agreement with Salem *et al*. (2001).

Feed conversion ratio (FCR):

Results in Table (5) showed that feed conversion ratio in C^{+} group was significantly ($p<0.0001$) the worst when compared to all experimental groups during all periods studied. On the other hand, all vitamins treatments significantly ($p<0.0001$) improved feed conversion ratio as compared to C^{+} group in the total period. The $E_{100}C_{250}$ group recorded the significantly lowest value at 28 days of age ($p<0.05$) and in the entire period ($p<0.0001$) compared with other groups. At 14 days of age, control group had the significantly ($p<0.0001$) best FCR, while, $E_{200}C_{250}$ group recorded the significantly ($p<0.0001$) lowest FCR of all vitamins groups. The FCR was restored to or lower than recorded in C group in E_{100}, C_{250}, C_{500}, $E_{100}C_{500}$, $E_{200}C_{250}$ and $E_{200}C_{500}$ groups at 28 days of age and in E_{100}, E_{200}, $E_{100}C_{250}$, $E_{100}C_{500}$, $E_{200}C_{250}$ and $E_{200}C_{500}$ groups at 42 days of age.

Table 5. Effects of vit. E and C supplementation on feed conversion ratio (g feed/ g weight gain) of broilers fed aflatoxicosis diet

Treatment	Age (day)			
	0-14 day	15-28 day	29-42 day	0-42 day
	x̄±SE	x̄±SE	x̄±SE	x̄±SE
C	$1.18±0.08^{c}$	$1.56±0.06^{ab}$	$1.75±0.08^{b}$	$1.62±0.02^{d}$
C^{+}	$1.55±0.02^{a}$	$1.67±0.18^{a}$	$2.17±0.04^{a}$	$1.92±0.07^{a}$
E_{100}	$1.39±0.05^{ab}$	$1.44±0.04^{ab}$	$1.87±0.04^{b}$	$1.65±0.01^{bcd}$
E_{200}	$1.54±0.04^{a}$	$1.64±0.03^{a}$	$1.84±0.05^{b}$	$1.74±0.04^{bc}$
C_{250}	$1.38±0.03^{ab}$	$1.53±0.03^{ab}$	$1.97±0.04^{ab}$	$1.73±0.01^{bc}$
C_{500}	$1.43±0.01^{ab}$	$1.47±0.06^{ab}$	$2.12±0.09^{a}$	$1.77±0.02^{b}$
$E_{100}C_{250}$	$1.31±0.01^{b}$	$1.34±0.02^{ab}$	$1.79±0.12^{b}$	$1.56±0.05^{d}$
$E_{100}C_{500}$	$1.35±0.03^{b}$	$1.43±0.05^{ab}$	$1.79±0.06^{b}$	$1.60±0.04^{d}$
$E_{200}C_{250}$	$1.30±0.03^{b}$	$1.49±0.04^{ab}$	$1.82±0.04^{b}$	$1.63±0.03^{cd}$
$E_{200}C_{500}$	$1.38±0.11^{ab}$	$1.46±0.05^{ab}$	$1.83±0.04^{b}$	$1.63±0.05^{cd}$

[a, b, c, d] Means in the same column, with different superscripts, are significantly different ($p<0.05$).

C=Control diet, C^{+}=Control diet+1.2 ppm aflatoxin (AF)/kg diet, E_{100}=Control diet+AF+100 mg vit.E/kg diet, E_{200}=Control diet+AF+200 mg vit.E/kg diet, C_{250}=Control diet+AF+250 mg vit.C/kg diet, C_{500}=Control diet+AF+500 mg vit.C/kg diet, $E_{100}C_{250}$=Control diet+AF+100 mg vit.E+250 mg vit.C/kg diet, $E_{100}C_{500}$=Control diet+AF+100 mg vit.E+500 mg vit.C/kg diet, $E_{200}C_{250}$=Control diet+AF+200 mg vit.E+250 mg vit.C/kg diet and $E_{200}C_{500}$=Control diet+AF+200 mg vit.E+500 mg vit.C/kg diet.

The inferior feed conversion ratio due to aflatoxicosis in the present study was in agreement with several previous investigations in poultry (Safameher and Shivazad, 2007; Han *et al.,* 2008; Shi *et al*., 2009). In broiler chicks, the results were in agreement with Shi *et al.* (2006); Pasha *et al.* (2007); LI Juan-juan *et al.* (2010). Also, Our results were in agreement with results of Oguz *et al.* (2000b) when they fed broiler chicks on contaminated diet by 100 ppb AF/kg diet, and found significantly inferior FCR compared to control group whereas, the results were in disagreement with them when they fed broiler chicks on contaminated diet by 50 ppb AF/kg diet, they found no notable differences in FCR compared to control group. Also, the results were in disagreement with Tedesco *et al.* (2004), who reported that feed conversion ratio of commercial broilers (14-d-old and were adapted for five days) was not influenced by fed on 0.8 mg AFB_1/kg diet for 35 days might be because of increase of age and decrease the duration of exposure to aflatoxin.

The increase of feed conversion ratio in birds which fed on AF contaminated diet may be attributed to anorexia, listlessness, inhibition of protein synthesis and lipogenesis (Kubena *et al.,* 1998; Oguz and Kurtoglu, 2000; Oguz *et al.,* 2000a). Impairing liver functions and protein/lipid utilization mechanisms due to aflatoxicosis may have also affected the growth performance and general health (Espada *et al.,* 1992; Fernandez *et al.,* 1994).

The significant improvement of feed conversion ratio in vitamins groups was in agreement with results obtained by He *et al.* (2013).

Mortality Rate:

The results in Table (6) showed no significant differences among all experimental groups in mortality rate at 14 and 28 days of age. Whereas, the mortality rate at 42 days of age and total period of C^{+} group was significantly the highest as compared to all experimental groups. Moreover, at 42 days of age, E_{100} and $E_{100}C_{250}$ groups had the significantly lowest rate of mortality (did not record any mortality) while, in a cumulative period, $E_{100}C_{250}$ group had the significantly lowest rate of mortality.

Table 6. Effects of vit. E and C supplementation on Mortality Rate of broilers fed aflatoxicosis diet

Treatment	Age (day)			
	0-14 days	15-28 days	29-42 days	0-42 days
	(%)	(%)	(%)	(%)
C	3.57	3.70	3.85	10.71
C^+	10.71	4.00	25.00	35.71
E_{100}	7.14	3.85	0.00	10.71
E_{200}	7.14	3.85	4.00	14.29
C_{250}	0.00	0.00	7.14	7.14
C_{500}	10.71	4.00	8.33	21.43
$E_{100}C_{250}$	3.57	0.00	0.00	3.57
$E_{100}C_{500}$	0.00	3.57	3.70	7.14
$E_{200}C_{250}$	10.71	0.00	8.00	17.86
$E_{200}C_{500}$	7.14	3.85	4.00	14.29

No significant difference between treatments in starter period (χ^2 =7.57, P = 0.57)
No significant difference between treatments in growing period (χ^2 =3.15, P = 0.95)
Difference between treatments in finishing period was significant (χ^2=19.20, P = 0.02)
Difference between treatments in the entire period was significant (χ^2=17.50, P = 0.04)
C=Control diet, C^+=Control diet+1.2 ppm aflatoxin (AF)/kg diet, E_{100}=Control diet+AF+100 mg vit.E/kg diet, E_{200}=Control diet+AF+200 mg vit.E/kg diet, C_{250}=Control diet+AF+250 mg vit.C/kg diet, C_{500}=Control diet+AF+500 mg vit.C/kg diet, $E_{100}C_{250}$=Control diet+AF+100 mg vit.E+250 mg vit.C/kg diet, $E_{100}C_{500}$=Control diet+AF+100 mg vit.E+500 mg vit.C/kg diet, $E_{200}C_{250}$=Control diet+AF+200 mg vit.E+250 mg vit.C/kg diet and $E_{200}C_{500}$=Control diet+AF+200 mg vit.E+500 mg vit.C/kg diet.

The increase in mortality rate due to aflatoxicosis at 42 days of age and in the entire period of experiment of this study was in agreement with that reported by Pasha *et al*. (2007). The increase of mortality rate due to aflatoxicosis at 42 days of age and in the entire period of experiment of this study may be attributed to reducing disease resistance, the gradually increase of chronic toxic effects (Oguz *et al*., 2000b), or, severely inhibiting the immune system of the birds (Pasha *et al*., 2007). While, the results obtained were in disagreement with the results of Oguz *et al*. (2000b) and Tedesco *et al*. (2004). This disagreement may be due to the variation of age, dose, sex, duration of exposure and immune response.

The significant decrease in mortality rate due to vitamins supplementation, in the present study, was in agreement with previous investigations in broiler chicks fed mycotoxins contaminated diets with vitamin E or C (Mubarak *et al*., 2009; El Barkouky *et al*., 2010).

In the present study the improvement in productive performance due to vitamins supplementation to the contaminated feed may be attributed to that vitamin E (α-tocopherol) has long been recognized as being the major lipid-soluble and chain breaking antioxidant that prevents free radicals from initiating peroxidative tissue damage (Verma *et al*., 2007). Several studies have also shown that α-tocopherol inhibits free radical formation (Kalender *et al*., 2004; Kalender *et al*., 2005) and may effectively minimize lipid peroxidation in biological systems (Kalender *et al*., 2002).Vitamin E is known for its antioxidant properties and has been shown to modulate immune functions in various species (Konjufca *et al*., 2004). Vitamin C (ascorbic acid) is a well-known low molecular weight antioxidant that protects the cellular compartment from water-soluble oxygen and nitrogen radicals (Jurczuk *et al*., 2007). Vitamin C efficiently inhibits *in vitro* lipid peroxidation due to a combination of direct radical interception and interaction with α-tocopherol as a co-antioxidant (Verma *et al*., 2007). Kultu, (2001) reported that ascorbic acid supplementation increased body weight gain, carcass weight and concluded that ascorbic acid supplementation improved the productive performance of broiler chicks with experimentally induced hypothyroidism. The beneficial influences of ascorbic acid (AA) noted in the present study can be attributed to the fact that AA is a very efficient antioxidant, and a scavenger of oxygen free radicals, which are toxic by-products of many metabolic processes (Dawson *et al*., 1990). Also, Ascorbic acid was reported to inhibit the binding of AFB_1 to DNA (Yu *et al*., 1994). In addition, AA is required for the hydroxylation of essential amino acids and of several oxidase enzymes (Tucker and Halver, 1984; Dawson *et al*., 1990).

CONCLUSION

The present results indicated that, aflatoxin is one of the most potent important mycotoxins in broilers due to its toxicity. Aflatoxin produces large deleterious and negative effects on broiler's productive performance. The addition of vit.E and vit.C in combination form may be efficient in reducing the negative effects of aflatoxin and enhance broiler's tolerance to toxicity. However, results showed that addition of combination of vit.E and vit.C at 100 mg/kg and 250 mg/kg aflatoxin contaminated diet, respectively, was more efficient in reducing the negative effects of aflatoxin on broiler's productive performance.

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تـأثير إضـافة فيتـامين هـ وفيتـامين ج علـى الأداء الإنتـاجي لـدجاج التسـمين المغذاه علـى علائق ملوثة بالأفلاتوكسين

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أجريت هذه التجربة لتقييم فاعلية إضافة مستويين من فيتامين (هـ) وفيتامين (ج) سواء في صورة منفردة أو خليطة كإضافات غذائية في تخفيف الآثار الضارة للأفلاتوكسين على الأداء الإنتاجي لدجاج التسمين. حيث تم استخدام عدد 280 كتكوت تسمين سلالة روص (Ross) عمر يوم واحد غير مجنسة وتم تقسيمها عشوائياً في بطاريات إلى 10 مجموعات كالتالي: C: غذيت على عليقة أساسية كاملة الإحتياجات الغذائية وبدون أي إضافات (المقارنة). أما باقي المجموعات فقد تم تغذيتها جميعاً على عليقة ملوثة بالأفلاتوكسين بتركيز 1,2 جزء في المليون لكل كجم عليقة أساسية حيث أن، C^+: غذيت على العليقة الملوثة، E_{100}: غذيت على العليقة الملوثة و مضافاً إليها 100 ملجم فيتامين (هـ) / كجم عليقة (E_1)، E_{200}: غذيت على العليقة الملوثة و مضافاً إليها 200 ملجم فيتامين (هـ)/ كجم عليقة (E_2)، C_{250}: غذيت على العليقة الملوثة و مضافاً إليها 250 ملجم فيتامين (ج)/ كجم عليقة (C_1)، C_{500}: غذيت على العليقة الملوثة و مضافاً إليها 500 ملجم فيتامين (ج)/ كجم عليقة (C_2)، $E_{100}C_{250}$: غذيت على العليقة الملوثة و مضافاً إليها[E_1+C_1]، $E_{100}C_{500}$: غذيت على العليقة الملوثة و مضافاً إليها [E_1+C_2]، $E_{200}C_{250}$: غذيت على العليقة الملوثة و مضافاً إليها [E_2+C_1]، و $E_{200}C_{500}$: غذيت على العليقة الملوثة و مضافاً إليها [E_2+C_2]. وأظهرت نتائج التجربة وكما كان متوقعاً، فإن مجموعة الأفلاتوكسين (C^+) سجلت إنخفاضاً معنوياً في وزن الجسم النهائي والزيادة الوزنية الإجمالية، وأسوأ معامل تحويل غذائي وأعلى معدل نفوق وذلك بالمقارنة بمجموعة المقارنة (C). على الجانب الآخر، الطيور التي غذيت على العليقة الملوثة ومضافاً إليها فيتامين (هـ) وفيتامين (ج) في الصورة الخليطة ($E_{100}C_{250}$, $E_{100}C_{500}$, $E_{200}C_{250}$ and $E_{200}C_{500}$) سجلت تحسناً معنوياً في الأداء الإنتاجي وذلك مقارنة بالطيور التي غذيت على العليقة الملوثة بالأفلاتوكسين (C^+). الأكثر من ذلك، لم يلاحظ أي إختلافات معنوية في قياسات الأداء الإنتاجي بين الطيور التي غذيت على عليقة المقارنة والطيور التي غذيت على العليقة الملوثة ومدعمة بالصور الخليطة من فيتامين (هـ) و فيتامين (ج).

يمكن إستنتاج أن إضافة الصور الخليطة من فيتامين (هـ) وفيتامين (ج) وبالمستويات التي تم استخدامها في التجربة إلى العليقة من الممكن أن تستخدم كمضاد للتسمم الفطري في حالة ما إذا كانت العليقة ملوثة بالأفلاتوكسين.

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