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COMBATING OCHRATOXICOSIS BY SOME KNOWN ANTIOXIDANT FEED ADDITIVES

AMAL R. TOOS, A. M. HAMMAD and R. R. RHAGHEB
Animal Health Research Institute, Agricultural Research Center, Giza - Egypt

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SUMMARY

Aspergillus ochraceous was isolated from 8 (16%) of the examined 50 poultry feed samples. Two of them (25%) were high ochratoxin A producers that yielded 45 PPM of the toxin.

An experiment with a completely randomized block design was used to evaluate the use of vitamin E (100 or 1000 I.U.), vitamin C (100 or 1000 mg) alone or in combination (500 I.U. of E + 500 mg of C) / kg ration, as feed additives to broiler chickens of one-week old during exposure to ochratoxin A contaminated ration (2.5 mg / kg ration). The Production performance (daily weight gain), hematology and serum biochemistry were evaluated.

Ochratoxin (2.5 mg / kg ration) resulted in a significant reduction of body weight gain, appearance of normocytic hypochromic anemia, decreased serum proteins (total, albumin, globulins and A/G ratio), and serum lipids (total cholesterol, HDL, LDL, and phospholipids). Addition of vitamin E (1000 I.U. / kg ration), vitamin C (1000 mg / kg) alone or combination of vitamin E and vitamin C (500 I.U. + 500 mg / kg ration) corrected the adverse effects of ochratoxicosis on body weight gain and hematological parameters and bring values to about that of the control.

Ochratoxin increased serum of AP, ALT, AST activities, and uric acid, creatinine, triacyl glycerol, VLDL and free fatty acids concentrations in the exposed chickens. Addition of vitamin E (1000 I.U.) alone or in combination with vitamin C (500 I.U. + 500 mg) / kg ration improved the case except for serum AP and ALT. Addition of vitamin C alone (1000 mg / kg ration) improved the serum uric acid concentration only.

From the present investigation it could be concluded that vitamin C has a lesser pronounced antioxidant effects than those obtained with vitamin

E. Better results were obtained in groups receiving a combination of vitamin E and C (500 I.U. + 500 mg / kg ration).

INTRODUCTION

Ochratoxins comprised a group of Aspergillus ochraceous and some Penicillium species metabolites that have variable toxic properties. Ochratoxin A (OA) is widely spread as a natural contaminant of various grains, cereals, plant products and poultry rations in countries through out the world (Hamilton et al. 1982). It is generally accepted that ochratoxins are more toxic and dangerous nephrotoxic than all other mycotoxins as it has a lower LD50 value and a lower minimum dietary growth inhibitory dose than aflatoxins, T-2 toxin (Smith and Hamilton, 1970 and Huff et al. 1974).

Lipid peroxidation is one of the consequences of cellular damage in the toxicity of several mycotoxins including ochratoxin (Rizzo et al. 1994 and Shen et al. 1994). Decreased concentration of serum albumin, total proteins and cholesterol together with increased activity of creatinine kinase, increased concentration of triglycerides and uric acids were reported in chickens exposed to ochratoxicosis (Gentles et al. 1999).

Few reports have indicated that dietary addition of antioxidants, vitamins E and C can modulate the severity of toxicosis and were able to ameliorate the oxidative stress caused by ochratoxin A (Haazele et al. 1993). The dietary use of vitamin E has shown to cause significant decrease in peroxidation as it tended to counteract the OA induced increase in uric acid in the plasma of chickens (Hoehler et al. 1996).

Based on the current knowledge, the present trial was conducted to evaluate the effect of vitamin E and vitamin C, alone or in combination for protection against the toxicity of OA in broiler chickens.

MATERIAL AND METHODS

Fifty poultry feed samples from five different poultry farms were collected and mycologically examined for the presence of Aspergillus ochraceous (toxin producer strain). Ochratoxin A production from the isolated Aspergillus ochraceous was carried on synthetic liquid media according to Atalla and Nour (1990). Measurement of ochratoxin A production was performed by the fluerometric method after chloroform extraction and passage on immunaffinity column according to Hansen (1993). Aspergillus ochraceous (high toxin producer strain) was grown on sterile crushed corn for 10 days at 25(C. Ochratoxin A was determined and standardized according to Roberts and Patterson (1975). This ochratoxin contaminated corn was thoroughly mixed with broilerís ration to obtain an ochratoxin concentration of 2.5 mg / kg ration.

Seventy one-week old broiler chickens were used

and equally grouped into 7 groups: Group I, negative control. Group II, received ration containing ochratoxin (2.5 mg / Kg). Group III, fed on ration containing mixture of ochratoxin (2.5 mg) and vitamin E (100 I.U.) / kg. Group IV received a ration containing mixture of ochratoxin (2.5 mg) and vitamin E (1000 I.U.) / kg. Group V fed on ration containing mixture of ochratoxin (2.5 mg) and vitamin C (100 mg) / kg. Group VI received a ration containing mixture of ochratoxin (2.5 mg) and vitamin C (1000 mg) / kg. Group VII fed on ration containing mixture of ochratoxin (2.5 mg) and vitamin E and C (500 I.U. + 500 mg) / kg.

All chickens were weighed on the 7th and 15th days in order to calculate the daily weight gain.

Blood samples were collected on the 15th days from the chickens of all groups. Blood hemoglobin, PCV, and RBcs counts were determined and anemia indices were calculated according to the methods described by Natt and Herrick (1952) and Ampbell (1988).

The obtained serum samples were used for the determination of alkaline phosphatase (AP) activity according to Kilichling and Freiburg (1951), aminotransferases (Reitman and Frankel, 1957), uric acid (Archibold, 1957), creatinine (Husdan and Rapoport, 1968), total proteins (Hoffamann and Richterrich, 1970), albumin and globulins (Doumas et al. 1971), total lipids (Knight et al. 1972), total cholesterol (Watson, 1960), triacyl glycerol (Fossati and Principe, 1982), free fatty acids (Schuster, 1979), phospholipids (Zilvermit and Davies, 1950), and the high density lipoproteins (HDL), low density lipoproteins (LDL), very low density lipoproteins (VLDL) concentrations were measured according to Lopez-Virella et al. (1977).

Statistical analysis of the obtained data for mean, standard error and studentís ìtî test for significant differences between test values and those of control group were done according to Snedecor and Cochran (1976).

RESULTS

Table (1) presents the incidence of A.ochraceous in the examined poultry feed samples in relation to the amount of ochratoxin A production.

Table (2) shows the effect of individual and combined supplementation of vitamins E and C on body weight gain.

Table (3) illustrates the effect of individual and combined supplementation of vitamins E and C on hematological parameters and anemia indices.

Tables (4, 5 and 6) present the data obtained as serum concentrations of the studied biochemical parameters among broiler chickens received the ration containing ochratoxins alone or in combination with the antioxidant vitamins E, C or both together.

Table 1: Incidence of Aspergillus ochraceous in the examined poultry feed samples (n = 50) in relation to ochratoxin A production.

Amount of ochratoxin A produced	No. of isolated strains
Less than 10 PPM.	1
11-20 PPM.	2
21-30 PPM	1
31-40 PPM.	2
Over 41 PPM.	2
Total	8
% of total	16

Table 2: Effect of individual and combined supplementation of vitamins E and C on body weight gain of broiler chickens fed ochratoxin contaminated rations (2.5 mg / kg ration).

Time	Body weight in grams						
	GI n=10	GII n=10	GIII n=10	GIV n=10	GV n=10	GVI n=10	GVII n=10
After One Week	484.4 ± 13.84	391.0*** ± 12.28	454.4 ± 12.31	478.0 ± 8.90	399.0** ± 26.01	420.7* ± 17.78	455.0 ± 12.88
After Two Weeks	625.0 ± 15.90	473.7*** ± 17.24	557.8** ± 18.80	621.0 ± 22.43	496.0*** ± 20.81	580.0 ± 21.98	610.0 ± 22.43
Daily weigh gain	20.09 ± 1.19	11.82*** ± 0.57	14.77** ± 0.79	20.43 ± 0.87	13.81*** ± 0.59	22.75 ± 1.22	22.74 ± 0.68

GI : Negative control.
GII : Fed ration containing ochratoxin alone.
GIII : Fed ration containing ochratoxin + vitamin E (100 I.U) kg.
GIV : Fed ration containing ochratoxin + vitamin E (1000 I.U) kg.
GV : Fed ration containing ochratoxin + vitamin C (100 I.U) kg.
GVI : Fed ration containing ochratoxin + vitamin C (1000 I.U) kg.
GVII: Fed ration containing ochratoxin + vitamins E and C (500 I.U. + 500 mg)/kg.
* : Signifcant at $P < 0.05$
** : Signifcant at $P < 0.01$
*** : Signifcant at $P < 0.001$

Table 3: Effect of individual and combined supplementation of vitamins E and C on hematological parameters and anemia indices of broiler chickens fed ochratoxin contaminated rations (2.5 mg / kg ration)

Time	GI n=10	GII n=10	GIII n=10	GIV n=10	GV n=10	GVI n=10	GVII n=10
Hemoglobin gm/dl	14.80 ± 2.12	7.80** ± 1.27	9.81* ± 0.82	14.00 ± 2.56	9.62* ± 1.12	13.23 ± 2.4	14.07 ± 2.22
PCV%	32.30 ± 1.62	24.00** ± 1.42	27.10* ± 1.53	28.2 ± 1.50	24.50** ± 1.42	26.00** ± 1.54	28.3 ± 1.73
RBCs $10^6/\mu l$	4.50 ± 0.28	3.00*** ± 0.20	3.54** ± 0.17	4.05 ± 0.26	3.25** ± 0.16	3.69* ± 0.18	4.18 ± 0.18
MCH Pg.	32.17 ± 1.67	26.53** ± 0.91	27.71* ± 0.78	34.57 ± 1.16	29.6 ± 0.82	35.85 ± 1.90	33.66 ± 0.98
MCV FI	71.72 ± 2.85	80.00 ± 3.28	76.55 ± 2.05	69.63 ± 1.98	74.77 ± 2.03	70.46 ± 2.37	67.70 ± 1.17
MCHC gm/dl	45.82 ± 1.86	32.50*** ± 1.82	36.20*** ± 1.56	49.65 ± 1.70	39.37* ± 1.49	50.88 ± 1.68	49.72 ± 1.64

Table 4: Effect of individual and combined supplementation of vitamins E and C on Liver and Kidney function test parameters of broiler chickens fed ochratoxin contaminated rations (2.5 mg / kg ration).

Time	GI n=10	GII n=10	GIII n=10	GIV n=10	GV n=10	GVI n=10	GVII n=10
AP mmol/l	1.05 ± 0.10	1.90*** ± 0.18	1.65** ± 0.14	1.45* ± 0.15	1.84*** ± 0.16	1.50** ± 0.13	1.46* ± 0.15
ALT U/l	23.07 ± 0.52	32.30*** ± 1.18	29.00*** ± 0.83	25.12* ± 0.74	31.10l*** ± 0.84	27.60* 1.07	26.80* ± 1.01
AST U/l	45.30 ± 2.41	65.32*** ± 2.32	55.80** ± 2.74	48.80 ± 2.21	60.30*** ± 3.31	55.40** ± 2.54	50.10 ± 2.40
Uric acid mg/dl	6.14 ± 0.30	9.39*** ± 0.75	8.48** ± 0.58	6.25 ± 0.19	8.25** 0.16	6.66 ± 0.27	6.31 ± 0.24
Creatinine mg/l	15.95 ± 0.19	17.50*** ± 031	17.15* ± 0.41	16.13 ± 0.43	17.21* ± 0.42	16.87* ± 0.33	16.00 ± 0.24

GI : Negative control.
GII : Fed ration containing ochratoxin alone.
GIII : Fed ration containing ochratoxin + vitamin E (100 I.U) kg.
GIV : Fed ration containing ochratoxin + vitamin E (1000 I.U) kg.
GV : Fed ration containing ochratoxin + vitamin C (100 I.U) kg.
GVI : Fed ration containing ochratoxin + vitamin C (1000 I.U) kg.
GVII: Fed ration containing ochratoxin + vitamins E and C (500 I.U. + 500 mg)/kg.
* : Signifcant at $P < 0.05$
** : Signifcant at $P < 0.01$
*** : Signifcant at $P < 0.001$

Table 5: Effect of individual and combined supplementation of vitamins E and C on the proteinogram parameters of broiler chickens fed ochratoxin contaminated rations (2.5 mg / kg ration).

Time	Body weight in grams						
	GI n=10	GII n=10	GIII n=10	GIV n=10	GV n=10	GVI n=10	GVII n=10
T.proteins g/dl	4.55 ± 0.20	3.09*** ± 0.18	3.81* ± 0.18	4.21 ± 0.12	3.75** ± 0.16	4.2 ± 0.18	4.40 ± 0.17
Albumin g/dl	2.11 v0.08	1.36*** ± 0.06	1.69*** ± 0.05	1.89* ± 0.06	1.36*** ± 0.04	1.72** ± 0.06	2.00 ± 0.05
Globulins g/dl	2.44 ± 0.10	1.73*** ± 0.06	2.12 ± 0.12	2.23 ± 0.07	2.39 ± 0.08	2.30 ± 0.05	2.40 ± 0.09
A/G Ratop	0.86 ± 0.02	0.79** ± 0.01	0.80* ± 0.02	0.85 ± 0.04	0.57*** ± 0.02	0.75** ± 0.03	0.83 ± 0.01

Table 6: Effect of individual and combined supplementation of vitamins E and C on the lipid profile of broiler chickens fed ochratoxin contaminated rations (2.5 mg / kg ration).

Time	GI n=10	GII n=10	GIII n=10	GIV n=10	GV n=10	GVI n=10	GVII n=10
T. lipids mg/dl	490.1 ± 7.4	415.8*** ± 6.9	450.8** ± 7.2	470.1* ± 6.6	430.3*** ± 8.6	565.5* ± 6.1	470.0* ± 5.5
Phosphlipids mg/dl	180.6 ± 2.5	138.1*** ± 3.5	160.1** ± 2.3	170.6* ± 2.3	150.1*** ± 2.5	165.3** ± 3.1	168.8* ± 3.5
Triglycerides mg/dl	70.8 ± 4.6	145.9*** ± 5.9	90.7** ± 2.7	74.9* ± 3.2	110.1*** ± 5.77	86.5* ± 4.8	78.0 ± 5.1
T.cholesterol mg/dl	164.2 ± 2.5	142.5*** ± 2.8	152.2** ± 2.6	166.2 ± 2.3	145.5*** ± 2.9	155.2* ± 2.6	157.6 ± 2.9
H.D.L. mg/dl	32.84 ± 1.67	28.46* ± 1.17	30.44 ± 1.80	32.12 ± 1.93	28.70 ± 1.20	31.04 ± 1.12	31.52 ± 2.90
L.D.L. mg/dl	117.8 ± 1.9	84.7*** ± 1.6	103.0** ± 2.8	113.5 ± 2.2	90.8** ± 2.0	109.6** ± 1.7	116.5 ± 1.9
V.L.D.L. mg/dl	13.55 ± 1.79	29.18*** ± 2.16	18.74* ± 0.97	16.98 ± 1.58	22.03* ± 1.78	17.30 ± 1.98	15.61 ± 1.80
Free Farry A. m.mol	1.39 ± 0.11	2.47*** ± 0.12	1.94** ± 0.10	1.67 ± 0.13	2.26*** ± 0.14	1.79* ± 0.13	1.53 ± 0.19

GI : Negative control.
GII : Fed ration containing ochratoxin alone.
GIII : Fed ration containing ochratoxin + vitamin E (100 I.U) kg.
GIV : Fed ration containing ochratoxin + vitamin E (1000 I.U) kg.
GV : Fed ration containing ochratoxin + vitamin C (100 I.U) kg.
GVI : Fed ration containing ochratoxin + vitamin C (1000 I.U) kg.
GVII: Fed ration containing ochratoxin + vitamins E and C (500 I.U. + 500 mg)/kg.
* : Signifcant at $P < 0.05$
** : Signifcant at $P < 0.01$
*** : Signifcant at $P < 0.001$

DISCUSSION

Table (1) showed that eight (16%) of the examined poultry feed samples were contaminated with Aspergillus ochraceous (toxin producer strains). Ochratoxin A was isolated from all isolated A.ochraceous strains. The highest toxin producer strain yielded 45 PPM. This result comes in agreement with the results obtained by Atalla and Nour El-Din (1990) and Abou El-Magd (1991).

Chickens fed ochratoxin A (OA) contaminated ration (2.5 mg / kg) showed significantly decreased body weight than that of the control group (table 2). The toxic effects of (OA) produced by Aspergillus ochraceous are well established (Huff et al. 1992 and Kubena et al. 1994).

The OA-induced growth depression was consistent with the findings of Devegowda et al. (1998), El-Nabrawy et al. (1999) and Prakash et al. (2000) and might be attributed to the inhibition of protein dependant physiologic processes (Gentles et al. 1999). Addition of vitamin E or C alone or in combination to the contaminated diet resulted in a significantly increased body weight compared to those given OA contaminated diet alone. Addition of vitamin E (1000 I.U. / kg ration) alone or with vitamin C (500 I.U. + 500 mg / kg ration) increased body weight of the OA exposed chickens in such a manner that there were no significant body weight variation between them and the control group. On the other hand, higher doses of vitamin C alone (1000 mg / kg ration) were needed to increase body weight of the exposed chickens to about that of the control group at the second week of the experiment. These results agreed with those reported by Hoehler et al. (1996), who stated that addition of vitamin E tended to counteract the oxidative effects of OA and Haazle et al. (1993) and Prakash et al. (2000) who showed that vitamin C moderately ameliorate the negative effects of ochratoxin.

Table (3) showed that broilers exposed to 2.5 mg OA / kg ration had decreased hemoglobin concentration, PCV% and RBCs counts. The calculated anemia indices exhibited a significantly decreased MCH and MCHC value indicative to normocytic hypochromic anemia. The obtained results are in agreement with those reported by Baily et al. (1990), Rama-Devi et al. (1998), Stoev et al. (1999) and Rama-Devi et al. (2000). On the other hand, Huff et al. (1979) reported that OA induced hypochromic microcytic anemia typical of nutritional iron deficiency. However, the decreased RBCs counts and hemoglobin concentration during ochratoxicosis might be attributed to the adverse effects of OA on the hemopietic system. Addition of vitamin E (1000 I.U. / kg ration) or vitamin C (1000 mg / kg ration) or in combination (500 I.U. + 500 mg / kg ration) counteracted the bad effects of OA on the hemopoietic system and brought hemoglobin concen-

tration and RBCs counts of groups IV, VI and VII to about that of the control group.

Few of the reports have indicated that dietary addition of vitam E or C can modulate the severity of ochratoxicosis on RBCs counts and hemoglobin concentration. However, Harvey et al. (1994) found that vitamin E partially barrows against aflatoxin-induced changes in RBCs count, hematocrit and hemoglobin concentration. Vitamin E and C can modulate the severity of toxicosis and was able to ameliorate the oxidative stress caused by ochratoxin A (Haazele et al. 1993).

Table (4) showed that broiler chickens exposed to OA (2.5 mg / kg ration) had significantly increased serum alkaline phosphatase (AP), alanine aminotransferase (ALT), aspartate aminotransferase (AST) activities, uric acid and creatinine concentrations. Similar results were also reported by Hoehler et al. (1996), El-Bagoury and Abdel-Khalek (1997), Gentles et al. (1999), and Rama-Devi et al. (2000).

The adverse effects of ochratoxin on the liver and kidney functions might be attributed to their effect on the enzymes involved in phenylalanine metabolism, mitochondrial function and their effects on lipid peroxidation (Marquardt and Frolich, 1992). Addition of vitamin E (1000 I.U. / kg ration) or in combination with vitamin C (500 I.U. + 500 mg / kg ration) reduced the adverse effects of ochratoxin on the liver and kidneys and brought serum AST, uric acid and creatinine values of groups IV and VII to about that of the control (G 1). On the other hand, addition of vitamin C alone (1000 mg / kg ration) only improved serum uric acid concentration.

Ochratoxin A catalytically enhances lipid peroxidation (Rahimtula et al. 1988 and Omar et al. 1990). The observation that vitamin E and C protects the cell membrane lipids by preventing the formation of lipid hydroperoxides (Hoekstra 1975 and Hoehler et al. 1996) explains the normal serum AST, uric acid and creatinine levels among groups exposed to diets containing OA (2.5 mg / kg ration) and supplemented with vitamin E (1000 I.U. / kg ration) alone or in combination with vitamin C (500 I.U. + 500 mg / kg ration).

Table (5) presents the average values of total proteins, albumin, globulins and albumin / globulin ratio. Presence of ochratoxin in the diet significantly reduced serum proteins, albumin and globulins concentrations.

The significantly decreased serum total proteins and albumin levels during ochratoxicosis were also reported by Gentles et al. (1999) and Rama-Devi et al. (2000). Disruption of serum protein levels appears to be a result of inhibition of protein synthesis through the competitive inhibition of phenyalanine-t-RNA synthetase (Uena, 1991). Addition of vitamin E (1000 I.U. / kg), alone or in combination (500 I.U. + 500 mg / kg ration),

greatly reduced the adverse effect of ochratoxin on protein synthesis and led to increased serum protein concentration in groups IV, and VII to about that of the control. Similar results were obtained by Haazele et al. (1993) who reported that, dietary addition of vitamin E and C can modulate the severity of toxicosis and were able to ameliorate the oxidative stress caused by ochratoxin A. The improved serum globulins concentration in groups III, IV, and VII could be attributed to the enhancement of humeral immune response due to vitamin E supplementation reported by Tengerdy et al. (1973). Vitamin E stimulates the helper activity of T lymphocytes increasing cooperation between the T cells and B cells in immunoglobulin production (Tanaka et al. 1979). On the other hand vitamin C is the most important water-soluble antioxidant that inhibits the destructive oxidative reactions (Halliwell, 1990) caused by OA.

Table (6) showed that ochratoxin A caused an increased serum concentration of triglycerides, VLDL and free fatty acids together with decreased serum concentration of total lipids, total cholesterol, phospholipids, HDL and LDL among broiler chickens fed ration containing OA (2.5 mg / kg). Serum triglycerides concentration was only improved by diet supplementation with a mixture of vitamins E and C (500 I.U. + 500 mg / kg ration). Addition of vitamin E (1000 I.U.) alone or in combination with vitamin C (500 I.U. + 500 mg / kg ration) improved serum cholesterol and free fatty acids concentration. The VLDL concentration was improved in the groups given vitamin E (1000 I.U.), vitamin C (1000 mg) alone or in combination (500 I.U. + 500 mg / kg ration). Feeding ration supplemented with vitamin E (100 or 1000 I.U), vitamin C (100 or 1000 mg) alone or in combination (500 I.U. + 500 mg / kg ration) significantly increased serum concentration of HDL to about that of the control.

The significantly decreased serum lipids and cholesterol in broiler chickens fed ration contaminated with ochratoxin A were consistent with the results obtained by Rama-Devi et al. (2000) and might be attributed to the general reduction of lipogenesis or transport mechanism (Manning and Wyatt, 1984). The increased serum triglycerides within the same group agreed with Gentles et al. (1999). Increased blood level of the VLDL, which are mainly triglycerides, have been found in human beings and rats with chronic renal failure. The elevations are apparently due to increased liver production and decreased peripheral uptake of the VLDL (Kaneko et al. 1997). Supplementation of the ration with the antioxidant vitamins E or C (alone or in combination) reduced the lipid peroxidation mechanism of ochratoxin. The metabolic interactions between OA and these antioxidant vitamins is possible through the cytochrome P-450 and 4-hydroxy-OA formation suggesting that they protected

poultry against the toxic effect of OA by reducing the degree of lipid peroxidation (Haazele et al. 1993).

On the basis of the current information we can conclude that, ochratoxin toxicity can be controlled by the antioxidant vitamins E or C. Vitamin C has a lesser pronounced protective effects than those obtained with vitamin E and a mixture of lower doses from both gives better results than using each one alone.

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