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Protective effects of oregano oil on Aflatoxicosis in Japanese quail

KEYWORDS: oregano oil , aflatoxin , antioxidant , interleukin, quail.

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ABSTRACT Contamination of feeds with mycotoxins is a major problem in quail production that causes severe economic losses. This study was performed to investigate the effects of oregano oil, a volatile oil mixture, on the adverse effect of aflatoxins (AFs) on serum oxidative stress, interleukin 1 (IL-1) and interleukin 6 (IL-6) in Japanese quails. A total of 90 unsexed one week old Japanese quails chicks (Coturnix japonica) were divided into 3 groups. Group (A) was considered control quails and fed basal diet, mycotoxins free diet. Group(B) fed basal diet with 2.5 mg AFs/kg diet. Group(C) fed basal diet with 2.5 mg AFs/kg diet and 400 mg oregano oil /kg diet. The experiment was continued for 40 days. Initial and final quails' body weights were recorded. Serum reduced glutathione (GSH), catalase, superoxide dismutase (SOD) and malondialdehyde (MDA) were measured. Serum nitric oxide (NO), interleukin 1 (IL-1) and interleukin 6 (IL-6) were estimated. The level of total AFs in rice was 7.13 mg/kg , final body weight was significantly ($P<0.05$) reduced in quails treated with AFs while it was significantly ($P<0.05$) increased in group C than group B. At end of the experiment, MDA was significantly ($P<0.05$) increased in quails treated with AFs. When AFs administered with 400 mg oregano oil, MDA was decreased to a level similar to control group . Catalase, SOD and GSH were significantly ($P<0.05$) decreased in AFs treated quails and were increased in group C than group B. The levels of NO and IL-6 were increased significantly ($P<0.05$) in quails of group B, while their levels were decreased in group C. IL-1, was declined significantly ($P<0.05$) in group B than control, while its level was improved nearly similar to control one in group C. It could be concluded that oregano oil mixture has antioxidant effect and ameliorating effect to AFs immunosuppression in Japanese quails.

Introduction

Mycotoxins are secondary metabolites produced by filamentous fungi that cause a toxic response (mycotoxicosis) when ingested by animals. There are a wide variety of toxins, produced by numerous fungi, depending on the type of crop, geographical location and climatic conditions (1). Cereal plants, which are main ingredient in animals and poultry feed , may be contaminated by mycotoxins in two ways fungi growing as pathogens on plants or growing saprophytically on stored plants (1-2). Aspergillus species are the main contaminants of food and feed as they produce their famous toxin aflatoxin. Aflatoxin is mainly produced by *Aspergillus flavus* and *Aspergillus parasiticus*(3). The nomenclature of these toxins are named from the fungus producing them; that is "A" from the genus name Aspergillus, "fla" from one of the species name flavus added to toxin to give the name aflatoxin(4). There are about twenty different aflatoxins have been identified, with the major ones being B1, B2, G1, and G2, (5).Aflatoxin B1 (AFB1) is the most prevalent toxin in cereals used in feeds and presents the greatest toxigenic threat (6). Natural occurrence of aflatoxin in feed ingredients varies with growing and storage conditions, usually between 0 and 1 mg/kg or more. Higher levels of aflatoxins have been reported to cause problem and to be more serious in tropical and subtropical regions of the world where climatic conditions of temperature and relative humidity favour the growth of A. Flavus parisiticus(7).

Mycotoxins have potential effect on animals' health , especially single-stomached animals. They are incorporated into membrane structures causing various detrimental changes including lipid peroxidation and fatty acid oxidation (8). Consequently, changes in physiological functions including growth, development and reproduction occur. These changes involve reduction in efficiency of feed utilization, body weight gain, reproductive capacities and immune–suppression (7) which leads to economic losses (9-10).

The balance between antioxidants and pro-oxidants in the body. in general the cell is responsible for regulation of several metabolic pathways leading to maintenance of immune system health. Development of protection against stress conditions associated with commercial poultry production (11). This balance can be regulated by dietary antioxidants (12). Interest in naturally occurring antioxidants for food is emerging due to their positive health impacts on humans and their companion animals including poultry (13-15). Many plants have been identified as excellent poultry antioxidants; important among which is oregano (15-17).

Oregano (Origanum vulgare subsp. Hirtum) is a spices belonging to the Labiatae family,which is well known in Mediterranean countries (18).The volatile oil of oregano is obtained by steam distillation of the plant, contains more than 30 ingredients, most of which are phenolic compounds that exert various activities. The major components of oregano oil are carvacrol and thymol. These components constitute about 78–82% of the total oil and are principally responsible for its antioxidant activity (17, 19). Oregano oil was proven by several publications to have antioxidant (17, 20-22), antibacterial (23), anti-inflammatory (24) and antifungal (25) activities. Moreover, oregano oil has been shown to have positive effects on poultry performance, production and immunity (26-28).

Mycotoxins contamination of mixed feed and feed ingredients is a worldwide issue and due to ubiquitous nature of them, it is not easy to eliminate them totally from feed and feed ingredients (29). Several dietary strategies to combat the toxic effects of mycotoxins especially aflatoxins have

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been proposed among which the antioxidants. Antioxidant herbs and their extract have been also used to overcome the aflatoxins effect(30). This work aimed to investigate the effect of dietary oregano oil 400 mg/kg diet on oxidative stress and interleukin-1 (IL-1) and interleukin-6 (Il-6) levels after dietary aflatoxicosis in Japanese quail.

Material and methods:

Preparation of materials

Aflatoxins and aflatoxins level detection:

The aflatoxin used in the experiment was prepared by the Mycology Laboratory, Animal Health Research Institute, Dokki, Cairo, Egypt by fermenting parboiled rice with toxigenic fungus strain of Aspergillus parasiticus NRRL 2999. This fungal strain was inoculated into potato dextrose agar and incubated at 28˚C for 7-21 days before being used for toxin production. 250 mL flasks containing 50g of rice, free from extraneous materials were autoclaved at 15 lbs pressure for 15 minutes and then inoculated with fungal spores; further processing was carried out following the procedure of Shotwell et al. (31). Mouldy rice was autoclaved, dried and ground to fine powder. Aflatoxin levels in rice powder were measured by HPLC method in Mycotoxins Central Lab and Food Safety of the National Research Center, Dokki, Cairo. The analysis was performed according to Nabney and Nesbit (32)using Aflatoxin B1, B2, G1 and G2 standards which are purchased from Technopark1(Tulln, Austria).

Table (1):Aflatoxin B1, B2, G1, G2 levels in moulded rice obtained by HPLC analysis.

Sample	AFs (mg/kg)				
	B1	B2	G1 G1	G2	Total ppm
Aflatoxins level in rice	6.85	0.05	0.11	0.12	7.13

Oregano oil:

Oregano oil was obtained from Semieterik Oil Co., (Anatlya, Turkey).

Experimental birds and management:

A total of 90 unsexed one week old Japanese quails chicks (*Coturnix japonica*) weighing 17-21 g were purchased from Agricultural Technological Centre, Faculty of Agriculture, Cairo University, Giza, Egypt. They were kept in wire battery cages of 86 L×50 W×25 H cm which were equally partitioned into 3 pens (29x50x25cm) in Laboratory Animal House of Faculty of Veterinary Medicine, Suez Canal University. The chicks were allowed *ad libitum* access to feed and water.Ventilation and temperature (22°C-31°C) were controlled to maintain bird comfort during the grow-out. Room were electrically heated and provided with 24 hours lighting. Chicks were checked three times daily (at 7 am, 3pm and 11 pm) for food, water and mortality.

Experimental diet:

Experimental diet was formulated to meet the nutritional requirements for Japanese quail as suggested by the NRC (1994) as shown in Table 2. After preparation of the experimental diets, a 1000 g sample was collected and screened by HPLC according to method of Nabney and Nesbit (32) and it was found to be mycotoxins free.

Table (2): Components of quails' basal experimental diet

Ingredient	Concentration (kg/100kg diet)
Ground yellow corn	55.780
Soya bean meal	31.960
Fish meal	1.000
Corn gluten	7.450
Bran	1.000
Dicalcium Phosphate(22%Ca&19%P)	0.710
Limeston(38% Ca)	1.300
Lysine (purity 98%)	0.170
DL – Methionine (purity 98%)	0.070
Iodized sodium chloride	0.300
Mineral & Vitamin premix	0.300

Experimental design:

The experimental birds were randomly divided into 3 groups. Group A, was considered control quails and fed basal diet only.Group B, fed basal diet with 2.5 mg aflatoxins /Kg diet (2.5 ppm) according to Eraslan et al ., (33). Group C fed basal diet with 2.5 mg aflatoxin /Kg diet (2.5 ppm) and oregano oil(400 mg/kg diet).

Body weight gain of each bird was determined at the beginning and at the end of experiment.

Blood sampling:

After 20 days and 40 days treatments, 15 birds/ group were slaughtered and blood samples were collected in sterilized plain tubes to separate serum. Sera were stored at -20°C for determination of MDA , GSH , catalase , SOD, NO , IL-1and IL-6.

Estimation of serum malondialdehyde (MDA):

The MDA in serum which is measure of the intensity of lipid peroxidation, was assayed calorimetrically at wave length (λ = 532 nm) using commercial kit (Biodiagnostic, Egypt) according to the method described by Ohkawa et al., (34). All procedures were carried out according to manufacturer protocol.

Serum superoxide dismutase (SOD), reduced glutathion (GSH)and catalase assays:

Serum SOD, GSH and catalase activity were determined in sera from all groups using commercial kits (Biodiagnostic, Egypt) according to Nishikimi et al.(35),Tietez(36)and Aebi(37), respectively.

Nitric oxide assay:

Serum nitric oxide (NO) level in each tested sample was measured using the method described by Rajaraman et al.(38). Briefly, a total volume of 100 µl of each serum sample including the negative control was incubated with an equal volume of Griess reagent (1% sulphanilamide, 0.1% N-Naphthyl-ethylenediamine, 2.5% phosphoric acid) in a 96 microtiter plate well with flat bottom and incubated for 10 minutes at 27 °C. After incubation, the optical density was measured spectrophotometrically at 570 nm using an ELISA reader. Molar concentrations of NO2 were calculated from a standard curve generated from a graded series of NaNO2 concentrations.

Interleukin assay:

Serum interleukin 1 and 6 (IL1& IL6) levels were determined using commercial ELISA kits (Biosource, USA) according to manufacturer illustrated protocol.

Statistical analysis:

All values were expressed as mean ± standard error. Differences were evaluated by One-way analysis of variance was

Volume : 6 | Issue : 3 | March 2016 | ISSN - 2249-555X | IF : 3.919 | IC Value : 74.50

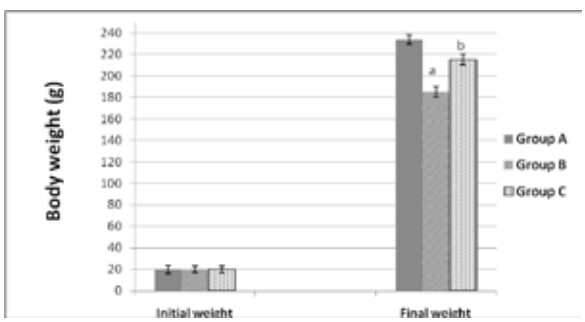
used for examining differences among groups. Inter-group comparisons were made with followed by Tukey Kramer multiple comparison tests.*P* value of < 0.05 was considered to indicate significance. All the analyses were done using GraphPad Prism (Version 5.01, GraphPad Software, San Diego, USA).

Result:;

Initial and final body weights:

Figure(1), showed that initial body weight was non-significantly affected in all groups at the beginning of the experiment. However, final body weights revealed a significant (P<0.05) reduction in AFs treated group (group B) than control (group A). Treatment with diet containing AFs and 400 mg/kg oregano oil (group C) significantly (P<0.05) improved the final body weight than AFs treated group (group B).

Figure(1): Effect of AFs and oregano oil co-administration with AFs on initial and final body weight of Japanese quail.



Different superscripts between columns indicate significance at P<0.05.

Lipid peroxidation and oxidative stress enzyme:

At 20th day of treatment there was no significant difference observed between experimental group in MDA, catalase and SOD. However GSH was significantly reduced in AFs treated group than control and oregano oil with AFs treated group.

At 40th day of the experimental period, MDA was significantly (P<0.05) increased in quails treated with AFs (group B) than control. When oregano oil co-administered with 400 mg/kg oregano oil, MDA was significantly (P<0.05) decreased than group B. The catalase, SOD and GSH were significantly (P<0.05) decreased in group B and were improved significantly (P<0.05) with oregano oil when co-administered with AFs (Table 3).

NO and interleukins assays:

At 20th day of experimental period, levels of NO and IL-6 didn't show any significant difference between groups. IL-1 was significantly reduced in AFs treated group (group B) than control (group A) and oregano oil with AFs treated group (group C).

At 40th day of experimental period, Levels of NO and IL-6 were significantly (P<0.05) increased in quails of group B than those of control, while their levels were decreased in group C than group B . IL-1, was declined significantly (P<0.05) in AFs treated quails than those of control, while its level was improved toward control value when oregano oil co-administrated with AFs(group C).

***Table (3):* Effects of AFs and oregano oil co-administration with AFs on NO, MDA, Catalase, GSH, SOD, IL-1 and IL-6 in Japanese quails.**

		Group A	Group B	Group C
NO(μM)	20th day	10.45±2.79	12.98±3.39	10.08±0.78
	40th-day	15.08±0.95[a]	22.83±3.48[b]	16.65±2.13[a]
MDA (μmol/L)	20th day	2.19±0.4	3.01±0.84	2.9±0.13
	40th day	2.81±0.38[a]	3.89±0.24[b]	2.97±0.31[a]
Catalase (U/ml)	20th day	18.7±2.5	15.46±1.7	17.54±1.27
	40th day	19.87±1.55[a]	13.25±2.4[b]	16.59±1.21[a]
GSH (U/ml)	20th day	14.49±0.33[a]	10.01±0.11[b]	12.53±0.92[a]
	40th day	16.34±1.22[a]	9.15±0.89[b]	15.74±0.77[c]
SOD (U/ml)	20th day	200.45±9.12	191.91±6.81	190.75±10.01
	40th day	179.83± 11.98[a]	149.38 ± 11.28[b]	171.04 ±6.06[a]
IL-1 (Pg/ml)	20th day	41.54 ±5.90[a]	32.53 ±0.95[b]	43.29 ±1.35[a]
	40th day	38.22±1.40[a]	30.66±1.66[b]	35.28±3.18[a]
IL-6 (Pg/ml)	20th day	135.99±6.24	119.08±10.11	133.70±15.14
	40th day	130.07±1.24[a]	139.83±2.12[b]	125.78±4.11[a]

Different superscripts between columns indicate significance at P<0.05.

Discussion:

There is an increased interest about the natural products which may alleviate the bad effects of environmental toxic compounds and prevent multiple diseases. In this concern, there are a lot of natural products have been recognized as antioxidants that exert immune-protection against toxic compounds in the feed of animals and poultry. In the current study, we evaluated the ability of oregano oil in a dose of 400 mg/kg in diet to protect Japanese quails from the toxic effects of AFs.

Results indicated that initial body weights were non-significantly altered which indicated the homogeneity of the initial weights and similarity between quails at the beginning of the experiment. Ingestion of AFs for 40 days produced a significant (P<0.05) reduction in quails' final body weight than control. These results agreed with Nazar et al.,(39). The decrease in body weight in the quail fed AFs-contaminated diet alone may be due to the effects of AFs regulate the homeostatic loop of body weight regulation, leading to cachexia through altering the balance between orexigenic and anorexigenic circuits (40). The treatment with oregano oil with AFs improved significantly (P<0.05) the final body weight than AFs treated one. These results coincide with Çabuk,et al.(28) and Eraslan et al., (33)This effect may be related to digestion stimulating effects as well as palatability of oregano volatile oils (41-42). On other hand, Botsoglou et al., (16) reported that oregano oil have no effect on quails body weight.

In the present study, animals fed AFs contaminated diet had much more lipid peroxidation and oxidative stress manifested by the significant (P<0.05) increase of lipid

Volume : 6 | Issue : 3 | March 2016 | ISSN - 2249-555X | IF : 3.919 | IC Value : 74.50

peroxidation (MDA) and the significant (P<0.05) decrease of enzymatic antioxidant such as catalase, SOD and GSH. These results agreed with Citil et al. (43) and Ma et al., (44). AFs produced oxidative stress through production of reactive oxygen species (ROS) such as superoxide anion, hydrogen peroxide and hydroxyl radicals, that exceeds the tolerance capacity of the cellular antioxidant defence system. MDA contents in blood, which is the end product of lipid peroxidation, elevated following the occurrence of oxidative stress. The free radicals peroxidated the lipid membranes which are highly susceptible to peroxidation, and MDA was generated. In addition, MDA can also cause peroxidation itself, and increase further peroxidation by synergism with free radicals (45).Thus it causes immunopathologies in host immune cells(46).The effect of AFs was improved with their combination with oregano oil (400mg/kg diet) that represented by significant (P<0.05) decrease in MDA and increase in catalase, SOD and GSH.These results are in harmony with BOU et al. (47)who demonstrated the antioxidant effect of oregano oil. The serum NO level was significantly (P<0.05) increased in AFs group than control augmented the results of MDA and oxidative stress enzymes. NO was considered as an intracellular messenger (48) that mediates a number of physiological processes but because it contains unoccupied coupled electrons in its molecular orbits, so it is known as a free radical. Therefore, it can yield dangerous effects (49). The administration of oregano oil here as natural antioxidant had reversed the adverse effect of AFs.

Serum IL-1 was significantly (P<0.05) reduced in AFs group than control. These results are coincide with Moon et al. (50)that revealed the immuno-suppressive effect of AFs. IL-1 is produced by poly morph nuclear leukocytes (PMN) and considered an vital messenger in array signalling toward infection (51) through the activation of range of cells including macrophages and T lymphocytes.These array signals lead to production of other cytokines and chemokines(52). Moreover, PMN were adversely affected by the oxidative stress and free radicals induced by AFs that was reversed by the antioxidant effect of oregano oil.

On other side, AFs significantly (P<0.05) increased IL-6 at 40th day of treatment than those of control. These contradictory results may be explained by the ability of AFs to produce stress (39) that could increase some inflammatory cytokines including IL-6 (53).Where the time of exposure could probably affect the AFs effect on body (39) as IL-6 tended to decrease with AFs administration until 20th day of the experiment then by the 40th day of the experimental period; it was significantly (P<0.05) increased.

According to current results, the administration of AFs in the diet had a depressing effect on immunity and induction of oxidative stress that could ameliorate by oregano oil administration with AFs . These effects were reflected on the final body weight.

Conclusion:

It could be concluded that oregano oil 400-mg/kg diet can protect against aflatoxicosis in Japanese quails. The mechanism of action of these extracts might be through the scavenging of ROS and should be considered as an accessible source of natural antioxidants.

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Volume : 6 | Issue : 3 | March 2016 | ISSN - 2249-555X | IF : 3.919 | IC Value : 74.50

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