Effectiveness of Rosemary (*Rosmarinus officinalis* L.) Essence on Performance and Immune Parameters of Broilers during Aflatoxicosis

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Abstract   The effect of aflatoxins B1 (AFB1) contaminated feed on performance parameters (feed intake, body weight and feed conversion ratio) and on Immune response on antibody titers of Newcastle Disease, Infectious Bronchitis and Avian Influenza content was investigated in broiler chicks using 240 unsexed Ross 308, were randomly allotted into 4 treatments with 3 replicates having 20 chicks each i.e. Control; AFB1 (600ppb); ROS (500ppm) and AF+ROS (600ppb + 500ppm). Feed contaminated with aflatoxin B1 caused a significant ($p<0.05$) increase in feed conversion ratios, a decrease in body weight and a significant ($P<0.05$) increase in feed consumption. ND, IB and Ai titer values were increased due to aflatoxin presence in the diet. The Rosemary could essence have incompletely restored the negative impacts of aflatoxin in the diet and the better values on all studied parameters were noticed in the Rosemary alone treatment group.

Keywords   Aflatoxin B$_1$, Rosemary Essence, Body Weight, Feed Consumption, FCR, Antibody titers, Broilers

1. Introduction

During the past few decades there has been a steady rise in global production of poultry meat and eggs. Although the high nutritive value of eggs and poultry meat has resulted in increasing claim, food quality and safety factors are becoming increasingly significant in determining market value of poultry products. Broilers are the major source of meat supply around the World. This massive growth and spurt in poultry production has put a great pressure on proper feeding of poultry. As mycotoxins are one of the major factors suppressing poultry productivity and also product quality, control of their impact is critical (Oguz, 2011). All mycotoxins are low-molecular-weight natural products (i.e., small molecules) produced as secondary metabolites of fungi. These metabolites compromise bird performance, increase susceptibility to infectious and parasitic diseases, and cause reproductive problems leading to huge economic harms in the poultry industry (Manafi *et al.,* 2011). According to the United Nation's Food and Agriculture Organization (FAO), approximately 25% of world's grain supply is contaminated with mycotoxins. Aflatoxin is the most commonly occurring mycotoxin across the globe. Aflatoxins are a group of secondary metabolites produced by a certain species of fungus of the genus *Aspergillus*

(especially *A. flavus* and *A. parasiticus*). These fungi are capable of growing and contaminating the grains and cereals at any time before and after the harvest, during storage, transportation and processing of feed ingredients and the formulated feeds after processing. Mycotoxins have adverse effect on both health and productivity in almost all species of domestic animals including poultry. In general, mycotoxicosis results in reduced feed intake, diminished feed conversion, decrease in production and subsequently increased susceptibility to various infections depending upon the type of toxins ingested (Xue et al., 2010). In poultry, intake of feed contaminated with aflatoxins may result in poor performance, decreased organ weight, immunosupression, irreversible liver damage, morbidity, and mortality (Ostrowski, 1984; Leeson et al., 1995). Due to these effects, poultry has commonly been considered highly susceptible to aflatoxin. However, among domestic fowl there is wide variability in specific species sensitivity to this mycotoxin. Comparative toxicological studies in avian species have shown that ducklings and turkey poults are the most sensitive species to aflatoxins and quails show intermediate sensitivity Leeson et al., 1995). In fact, some studies have reported a modest enhancement in the body weight of chickens exposed to aflatoxins in their diet. Stanley *et al.* (1993) studied the effect of aflatoxin contaminated diets with (5 ppm) from 1 to 28 days of age and found significant decreased body weight. In broilers, the effect of aflatoxins is greater in the initial growth phase, particularly during the first 21 d of age (Leeson et al., 1995). Application of some herbal extracts of plant origin like

turmeric (*Curcuma longa*), garlic (*Allium sativum*) and asafetida (*Ferula asafetida*) have shown to counteract aflatoxicosis in animals and poultry through their antioxidant activity. Several herbal products contain antioxidant substances capable of scavenging free radicals and enhancing antioxidant enzymes. Oxidative changes (increased peroxides, reduced antioxidant enzyme activity) in liver and kidney due to aflatoxin B1 were reversed in rats by feeding a root/ rhyzome extract of *Picrorhiza kurroa* (Picroliv) and a seed extract of *Silybum marianum* (Silymarin) (Weiss, 2002). Similarly, Rosamarinic acid, a phenolic component of *Boragnaceae* species of plants (sage, basil, and mint) reduced free radical oxygen formation, and inhibited protein/DNA synthesis as well as apoptosis of human hepatoma cells caused by aflatoxin B1 (Manafi, 2010). According to Gowda & Ledoux (2008) ellagic acid, a phenolic compound of strawberries and grapes showed anticarcinogenic activity and inhibited aflatoxin B1 mutagenicity. Iqbal et al. (1983) observed chemoprotective effect of piperine (1-piperoyl piperidine), an alkaloid of pepper against aflatoxin by inhibiting cytochrome P 450 bioactivation of aflatoxin B1. The protective effect of chlorophylline (a derivative of the green pigment chlorophyll) against aflatoxin B1 was also observed. The carbonyl functional groups of the curcuminoids are thought to be responsible for their antimutagenic and anticarcinogenic action. Further, strong inhibitory effect of curcumin on superoxide anion generation was noticed. Tocopherols, which are prevalent antioxidants, are potential antioxidant sources in vegetable oils. Industrially processed oils contain less tocopherol than natural oils, because the topocherol content of raw oils is diminished during processing (Foo et al., 2000). Therefore, the use of antioxidants is necessary to increase the stability of oils. Because synthetic antioxidants adversely affect health, studies about the use of natural antioxidants have been accelerated and the applications of natural antioxidants have increased. For this reason, many studies have evaluated the natural anti-oxidative properties of a wide range of plants and spices (Candan et al., 2003). Herbs have been reported to promote various functions like growth (Shalaby et al. 2006), appetite stimulation, resistance to stress (Citarasu 2010), immune responses (Ergun et al. 2011), skin coloration (Yılmaz and Ergun 2012), egg hatching rates (Yılmaz and Ergun 2012), and disease resistance (Yılmaz et al. 2011) in fish culture due to the many active components they contain. Several studies have reported that the oral administration of herbs, for example, rosemary in Nile Tilapia *O. niloticus* (Zilberg et al. 2010) and rosemary in Mozambique Tilapia (Ergun et al., 2011) improved growth performance, disease resistance, and immunity. Rosemary (*Rosmarinus officinalis* L.) is of interest for further research as an aromatic plant with medicinal properties. Rosemary is selected because it is of interest as a preservative due to its antioxidative characteristics, and its use in the pharmaceutical, food and cosmetic industries. Previous work (McEwan et al., 2002; Mitsch et al., 2004) suggested that plant extracts may be included in poultry diets as bioactive supplements. Plant extracts may have potential as natural alternatives to antimicrobial growth promoters (AGP) in animal diets, but their effects on gut health and broiler performance have not been clearly established. Garlic (*Allium sativum* L.) is noted for its bioactive properties, which include antimicrobial (Benkeblia, 2004), antifungal (Pai and Platt, 1995) and antioxidant (Yin and Cheng, 1998) effects. Various EO's distilled from culinary herbs, for example rosemary EO, have antimicrobial and bioactive properties (Viuda-Martos et al., 2008). Extracts from rosemary and some other herbs (5000 mg/kg), which were rich in rosmarinic acid, had shown no influence on feed intake and feed conversion, but cased to grew faster and improved apparent whole tract and an improved ileal digestibility of nutrients in broilers (Hernandez et al., 2004).

The effect of rosemary oils on broiler growth performances has not been well documented (Hernandez et al., 2004). Several studies indicated that the use of essential oils improved broiler feed conversion ratio (Windisch et al., 2008). However, most data available in literature are obtained from commercial products containing blends of different essential oils. Perhaps essential oils, which inhibit pathogenic and nonpathogenic bacteria, are more efficient in broiler chickens. The objective of the present study was to evaluate the antimicrobial activities of rosemary essential oils on broiler chicken performances.

2. Materials and Methods

This experiment was planned and carried out in the Department of Animal Science, Faculty of Agricultural Sciences, Malayer University, Malayer, Iran with objective of evaluating the performance and immune response of broilers fed with aflatoxin B_1 and Rosemary essence.

Experimental Design, Housing, Management and Test Diet

240 day-old unsexed Ross 308 strain of broiler chicks were wing banded, weighed and randomly spread in a completely randomized experimental design with four treatments and three replications of twenty chicks in each. Each replicate group of chicks was housed in an independent pen, conventional deep litter house. Chicks in all the replicates were kept up to six weeks of age under uniform standard conditions. Brooding was done till three weeks of age. Each pen was fitted with an automatic bell type drinker and a hanging tubular feeder. Chicks were provided *ad libitum* feed and water throughout the study. Feeding of test diets commenced at first day of age and continued till the termination of experiment at six weeks of age. The temperature was maintained at 30±1°C in the first week and reduced by 2.5°C per week to 21°C. From day one until day 4, the lighting schedule was 24 hour. At days 14-42 the dark time was gradually increased to 4 hour. Diets were prepared to meet the nutrient requirements of commercial broilers during the starter (0-2wks), grower (2-4wks) and finisher

(4-6 wks) periods. The composition of diets was adopted from NRC, (1994) and is presented in Table 1. Diets were prepared without addition of aflatoxin and Rosemary essence as Control (group1); 600 ppb Aflatoxin B1 (group2); 500ppm of Rosemary essence (group 3) and 600ppb Aflatoxin B1 + 500 ppm of Rosemary essence (group4). The Aflatoxin B1 was procured from Sigmal Aldrich, USA and diluted to reach to the required level of administration. The ethanolic extraction of Thyme was prepared as per the instruction given below:

Table 1.   Ingredients and composition of the basal diets (as-fed basis, %)

Ingredients (%)	Starting diet (0-2wk)	Growing diet (2-4wk)	Finishing diet (4-6wk)
Corm	59.00	67.36	72.01
Soybean meal	33.74	28.63	24.46
Soybean oil	1.56	0.65	0.56
Calcium carbonate	0.60	0.67	0.63
Dicalcium phosphate	1.41	1.02	0.84
Oyster shell	0.66	0.66	0.63
Common salt	0.30	0.30	0.30
Vit. And Min. Permix[1]	0.50	0.50	0.50
DL-Methionine	0.13	0.06	0.02
Lysine – HCL	0.09	0.14	0.05
Calculated analysis			
ME (Kcal/kg)	2900	2950	3000
Crude protein (%)	20.84	18.43	16.87

[1]The vitamin and mineral premix provide the following quantities per kilogram of diet: vitamin A, 10,000 IU (all-trans-retinal); Vit. D3 (cholecalciferol), 2,000 IU; vitamin E, 20 IU (α-tocopherol); vitamin K3, 3.0 mg; riboflavin, 18.0 mg; niacin, 50 mg; D-calcium pantothenic acid, 24 mg; choline chloride, 450 mg; vitamin B12, 0.02 mg; folic acid, 3.0 mg; manganese, 110 mg; zinc, 100 mg; iron, 60 mg; copper, 10 mg; iodine, 100 mg; selenium, 0.2 mg and antioxidant, 250 mg.

Plant Material

Collective samples of the aerial parts from *Thymus capitatus* growing wild in Khoramabad region within Lorestan province in Iran were collected during the Sept. 2013. Collected plant materials were dried in the shade, and the plant leaves were separated from the stem, and grounded in a grinder to small particles.

Extraction

Maceration Extraction

The powder of *T. capitatus* (leaves and stems) young flowers was macerated with 70% ethanol (1:20, w/v) at room temperature for 2 days and filtered through a Whatman no.1 filter paper. Other portions of the solvent were added to the marc and the extraction was repeated until the last extract was colorless. The extracts were combined and concentrated under reduced pressure at 65ºC, 15 rpm and 90 minutes, using a rotary vacuum evaporator. The crude extract was then evaporated on a boiling water bath (HANSHIN Scientific Co, South Korea) until a constant weight was obtained to afford the maceration extract.

Steam Distillation Extraction

Air-dried of *T. capitatus* leaves were submitted for 3 h to steam distillation using a Clevenger apparatus to produce the essential oil in a yield of 5.6% (w/w). Oil was dried over anhydrous sodium sulphate and after filtration, stored at 4°C until used.

Vaccination Schedule

The local office of Iranian Veterinary Organization has proposed the required vaccination which is modulated by the veterinarian of Department of Animal Science, Malayer University, as below:

Vaccination for Newcastle Disease (ND) virus happened three times: first spray at one days old of chicken in breeder farm, second on the 13th day as B1, BRONHOPEST B1 SPF (VETERINA, GENERA®), Zagreb, Croatia) and (CEVA SANTE ANIMALE, Libourne, France) in drinking water and their booster on 20th day as clone-30 (HIPRAVIAR® CLON, Amer, Spain) through drinking water. Vaccination against Bronchitis virus happened in two times as the following: first spray at commencement of the experiment and it's booster in drinking water on the 10th day, both as H-120 (CEVA SANTE ANIMALE, Libourne, France). Vaccination against Infectious Bronchitis (IB) virus happened in two times: first on day 16 and the second on the 23th day, both as Gambo-l (CEVA SANTE ANIMALE, Libourne, France) in drinking water. The sera were applied to HI test in 28 days, to determine Ab to NDV. In titers lower than 5, the booster B1, BRONHOPEST B1 SPF (VETERINA, GENERA®), Zagreb, Croatia) was administrated in drinking water for broilers.

Studied Parameters

Performance parameters

Body weight and cumulative feed consumption were recorded and feed conversion ratio (FCR) were calculated week wise. All chickens were weighed individually at the end of each week till week VI, by digital electronic top pan balance with 0.01g accuracy to record body weight. Feed consumption was recorded replicate-wise each week in all pens till 6 weeks of age and feed consumption per bird was calculated. Weekly FCR was calculated up to 6 weeks, as feed consumed per unit body weight gain.

Immunity Parameters

At the end of the trials, upon obtaining the permission of Ethical Committee of the University, six birds from each replicate were sacrificed by cutting the jugular vein and blood samples were individually collected in 10-mL heparinized tubes and stored on ice for hematology analysis. Serum was separated after 8 to 10 hours as per the standard procedures (Calnek et al. 1992) and was stored at –20ºC for subsequent analysis. The individual serum samples were analyzed for antibody titers against Newcastle disease (ND), Infectious Bronchitis (IB) and Avian Influenza (AI) by ELISA technique and using an automatic analyzer

(Boehringer Mannhein Hitachi 704 automatic analyzer, Japan). Treatment-wise means of titers were computed.

Statistical Analysis

The total experimental data were statistically analyzed using the General Linear Model procedure of the Statistical Analysis System (SAS®) software (SAS Institute, USA, 2000). Overall data were analyzed using one way ANOVA test. Duncan multiple range test at 0.05 probability level was employed for comparison of the means (Duncan, 1955).

3. Results and Discussion

The effects of aflatoxin and Rosemary essence on body weight (g): The results of dietary treatments on weekly body weight of broiler chicks are shown in Table 2. Results showed that the weight of day-old chicks were uniform and in a similar range. At day7, the AF fed group have shown a significantly (P<0.05) lower body weight, compared with control group. The group fed Rosemary extract have shown a non-significant changes compared with control group. In AF+ROS group, the reduced body weight of chicks due to presence of AF could not be reached up to the control level, however, it has been significantly (P<0.05) improved in this group, when compared with both AF and CON groups. At the end of second week, in AF alone fed group, significant (P<0.05) reduction in body weight was noticed, compared with control group. In group fed Rosemary essence alone, no changes in body weight were found with control group and in AF+ROS group, the body weight was increased significantly, when compared with AF group, but could not reach to that of control group. At day 21, day 28 and day 35, the same trend of day 14 have been continued in all treatment groups. At the

termination of trial (day 42), the body weight found to be maximum in ROS fed group, followed by CON, AF+ROS and AF. In this week, Rosemary essence has showed a significantly (P<0.05) higher body weight, compared with control group which is clearly indicates the possessiveness of Rosemary essence alone in the diet. When AF added into the normal diet, the significant reduction in BW was noticed and when AF+ROS is applied, the body weight could be restored significantly (P<0.05) comparing with AF alone fed group.

The effects of aflatoxin and Rosemary essence on cumulative feed consumption (g): Table 3 is depicting the effects of feed consumption of broilers fed different dietary treatments. The negative impacts of presence of AF in feed of broilers have been started from the first week itself. At day 7, 185.05g of feed consumed in AF fed group, whereas it was 167.53 in control group and significantly (P<0.05) decreased. In ROS group, feed consumption was statistically equal to CON and in case of AF+ROS, the effects of AF could partially restored. At day 14, the maximum amount of feed is consumed in AF group (535.0g) followed by ROS (484.10g), AF+ROS (539.95g) and CON (516.70g). At the end of week III and week IV, the same trend is continued and in AF fed group and significant (P<0.05) lower body weight was found, when compared with control group. The maximum amount of feed consumed was in CON fed group, which was significantly (P<0.05) higher than all other treatments. At day 35, the significant (P<0.05) lower amount of feed consumed in AF+ROS fed group, followed by ROS, CON and AF groups. This shows that AF alone has increased the feed consumption and inclusion of ROS along with AF could not reach to that of control group. The same trend continued till termination of experiment at 42 days and again lower amount of feed consumed in AF+ROS, AF, CON and ROS groups, respectively.

Table 2. Body weight (g) of chicks fed Aflatoxin B_1 and Rosemary essence (Mean±SE)

Treatment groups	Week 0 (day 1)	Week I (day 7)	Week II (day 14)	Week III (day 21)	Week IV (day 28)	Week V (day 35)	Week VI (day 42)
CON[1]	45.10±0.25[a]	202.93±0.77[a]	428.06±0.35[a]	766.56±0.85[a]	1284.43±0.49[a]	1775.26±0.72[b]	2207.93±0.53[b]
AF[2]	45.02±0.76[a]	184.10±0.96[c]	387.20±0.39[c]	707.10±0.46[c]	1217.80±0.37[c]	1743.10±0.23[c]	2052.60±0.18[d]
ROS[3]	44.85±0.14[a]	200.62±0.84[a]	425.60±0.82[a]	760.35±0.71[a]	1281.10±0.19[a]	1842.75±0.62[a]	2386.80±0.17[a]
AF+ROS[4]	44.90±0.29[a]	191.30±0.25[b]	398.15±0.29[b]	732.45±0.28[b]	1253.15±0.18[b]	1745.57±0.08[c]	2175.10±0.27[c]

Mean values within a row with different superscript letters (a to d) were significantly different (p<0.05). [1]CON (Control), [2]AF (Aflatoxin B_1 at 600ppb level), [3]ROS (Rosemary essence at 500ppm level) and [4]AF+ROS (Aflatoxin B_1 and Rosemary essence at 600ppb and 500ppm levels, respectively). SEM: Standard Error of the Means

Table 3. Cumulative Feed Consumption (g/bird) of chicks fed Aflatoxin B_1 and Rosemary essence (Mean±SE)

Treatment groups	Week I (day 7)	Week II (day 14)	Week III (day 21)	Week IV (day 28)	Week V (day 35)	Week VI (day 42)
CON[1]	167.53±0.96[bc]	516.70±0.33[b]	1148.93±0.48[b]	2041.06±0.92[a]	3062.32±0.38[b]	4369.49±0.49[b]
AF[2]	185.05±0.84[a]	535.00±0.19[a]	1155.50±0.29[a]	1897.00±0.17[c]	3358.95±0.23[a]	4127.77±0.31[c]
ROS[3]	158.70±0.72[c]	484.10±0.24[c]	1109.95±0.18[c]	2009.65±0.42[b]	3058.75±0.38[b]	4442.45±0.85[a]
AF+ROS[4]	170.50±0.28[b]	539.95±0.31[a]	1159.20±0.62[a]	1897.00±0.33[c]	2662.00±0.85[c]	3556.90±0.24[d]

Mean values within a row with different superscript letters (a to d) were significantly different (p<0.05). [1]CON (Control), [2]AF (Aflatoxin B_1 at 600ppb level), [3]ROS (Rosemary essence at 500ppm level) and [4]AF+ROS (Aflatoxin B_1 and Rosemary essence at 600ppb and 500ppm levels, respectively). SEM: Standard Error of the Means

Table 4. Feed conversion ratio (FCR) of chicks fed Aflatoxin B_1 and Rosemary essence (Mean±SE)

Treatment groups	Week I (day 7)	Week II (day 14)	Week III (day 21)	Week IV (day 28)	Week V (day 35)	Week VI (day 42)
CON[1]	0.825±0.08[b]	1.207±0.12[c]	1.498±0.32[c]	1.589±0.28[b]	1.724±0.01[c]	1.978±0.18[bc]
AF[2]	1.005±0.07[a]	1.381±0.28[a]	1.634±0.28[a]	1.657±0.32[a]	1.926±0.29[a]	2.010±0.42[a]
ROS[3]	0.791±0.01[b]	1.137±0.17[d]	1.459±0.16[d]	1.568±0.15[c]	1.659±0.23[d]	1.861±0.29[c]
AF+ROS[4]	0.891±0.02[b]	1.356±0.14[b]	1.582±0.37[b]	1.597±0.22[b]	1.825±0.23[b]	1.935±0.28[b]

Mean values within a row with different superscript letters (a to d) were significantly different (p<0.05). [1]CON (Control), [2]AF (Aflatoxin B_1 at 600ppb level), [3]ROS (Rosemary essence at 500ppm level) and [4]AF+ROS (Aflatoxin B_1 and Rosemary essence at 600ppb and 500ppm levels, respectively). SEM: Standard Error of the Means

Table 5. Antibody titers of broilers fed Aflatoxin B_1 and Rosemary essence at 42 days (Mean±SE)

Treatment groups	ND	IB	AI
CON[1]	3.66±0.62[b]	8105.45±0.82[b]	3.00±0.63[b]
AF[2]	4.27±0.44[a]	10297.16±0.21[a]	4.28±1.05[a]
ROS[3]	3.35±0.62[b]	6466.23±0.18[c]	3.12±0.41[b]
AF+ROS[4]	4.22±0.96[a]	10652.23±0.81[a]	4.22±0.28[a]

Mean values within a row with different superscript letters (a to d) were significantly different (p<0.05). [1]CON (Control), [2]AF (Aflatoxin B_1 at 600ppb level), [3]ROS (Rosemary essence at 500ppm level) and [4]AF+ROS (Aflatoxin B_1 and Rosemary essence at 600ppb and 500ppm levels, respectively). SEM: Standard Error of the Means. ND: Newcastle disease; IB: Infectious Bronchitis and AI: Avian Influenza. SEM: Standard Error of the Means

The effects of aflatoxin and Rosemary essence on feed conversion ratio (FCR): The effect of AF on weekly FCR is showed in Table 4. At day 7, where data found to be 0.825, 1.005, 0.791 and 0.891 in CON, AF, ROS and AF+ROS fed groups of chicks. In this week, only FCR of AF fed group is increased significantly (P<0.05). At day 14, the FCR of AF group found to be significantly (P<0.05) higher than control group and in ROS group, FCR found to be 1.137 vs. 1.207 of CON and was significantly better. By comparing AF with AF+ROS group, the FCR was significantly higher in AF fed groups which shows Rosemary essence can have useful effect to improve FCR of AF fed groups. At day 21, the higher FCR was found in AF group, where, found to be significantly (P<0.05) higher than control group. In ROS group, the FCR data was significantly (P<.05) decreased when compared with control group. In AF+ROS group, the FCR was significantly (P<0.05) better than AF alone fed group. At day 28, FCR was found to be maximum in AF group and found to be significantly (P<0.05) higher when compared with control group. In ROS group, FCR was significantly (P<0.05) altered with control group and in AF+ROS group, the FCR was numerically improved. At the end of week V, FCR in CON was 1.724 and in AF group, 1.926 and was significantly (P<0.05) increased. In ROS group, the FCR was significantly (P<0.05) better compared with control group, and in AF+ROS group, the FCR was found to be better than AF alone fed group and could restore partially the adverse effects of AF in the diet. At the termination of trial, the maximum FCR is found to be in AF group and significantly (P<0.05) higher than CON group. FCR value in ROS group, was significantly (P<0.05) better than all fed groups.

The effects of aflatoxin and Rosemary essence on antibody titers against ND, IB and AI: The antibody titer against Newcastle Disease, Infectious Bronchitis Virus and Avian Influenza Viruses are shown in Table 5. ND titer is increased significantly (P<0.05) in AF group when compared with control group. The ROS group was as equal as CON and AF+ROS was almost similar to that of AF group. The titer values for NDV ranged from 3.35 to 4.27. The IB titer values was found to be significantly (P<0.05) higher in AF group (10297.16) vs. CON (8105.45). In ROS group, the value is decreased significantly (P<0.05) when compared with control group. In AF+ROS group, the significant (P<0.05) higher values in IB titer was found. It could be found that the Rosemary essence could rectify the adverse effects of AF in IB titer value to some extent. In case of AI, titer values for AF group was found to be significantly (P<0.05) higher than control group and in AF group. This increased titer value could not be restored in AF+ROS group and in case of ROS alone group, the AI titer values remained non-significant when compared with CON group.

The adverse effects of Aflatoxin in broilers have been previously well described by different scientists. Phillips *et al.* (1988) reported that addition of 7.5ppm of AFB1 in boilers from 0 to 3 weeks revealed friable, enlarged and pale livers. Kubena *et al.* (1990) reported that administration of 3.5 ppm of AFB1 in broilers for first 4 weeks of age showed increase in relative weights of all internal organs. Espada *et al.* (1992) on a 0-4 weeks of study on broiled fed 0.5ppm of AFB1 reported pale yellow livers, edema of gall bladder, multi focal areas of congestion in kidneys. In another study by Manafi and Khosravinia (2013), low levels of 0.1, 0.2 and 0.4 ppm of AFB1 in the broilers diet up to six week showed enlarged, friable, pale and congested livers. Raju and Devegowda (2000) on broiler fed 0.3ppm of aflatoxin B1 for

5 weeks reported that AF significantly increased both liver and kidney weights. In a study by Arvind *et al.* (2003) on broilers for 0-5 weeks reported that addition of 0.168ppm of AFB1 could increase relative weights of liver, kidney, gizzard and spleen significantly. In another study using higher dosage of AFB1 by Manafi, (2010), it has been reported that 2ppm of AF could increase relative weight of liver and gizzard. Manafi, (2012) shown that each mg of AFB1/kg diet would decrease the growth performance of broilers by 5%. It has been postulated that AF toxicity is expressed by disruption of protein synthesis through conversion to a 2-3 epoxide binding to DNA and inhibiting RNA synthesis, while T-2 toxin can affect protein synthesis through the inactivation of initiation and termination, possibly through binding to ribosome (Ueno, 1991). Thus AF primarily inhibit protein synthesis during transcription and T-2 toxin primarily effects protein synthesis during translation which may account for their synergistic toxicity (Manafi and Khosravinia, 2013). The reduced cumulative feed consumption in the toxin fed groups over the mycotoxin free groups was due to the impaired hepatic metabolism and interference with phosphenolpyruvate carboxylase (Ueno, 1991). Decreased feed intake during mycotoxicosis has been reported earlier by Hoehler and Marquardt (1996) for T-2 and Kubena *et al.* (1997), Raju and Devegowda (2002) and Manafi et al., (2011), for combined mycotoxicosis. Addition of Rosemary essence into the AF fed groups has shown fractional positive effects of these studied parameters, which may be due to active materials (carnosol and rosmanol) in this plant which are considered as digestion stimulating factors, in addition to their antimicrobial activity against bacteria found in the intestine (Mansoori and Acamovic, 2009). Moreover, the improvement of body weight gain and feed conversion are due to the active materials found in rosemary, causing greater efficiency in the utilization of feed, resulting in enhanced growth. Oil of rosemary herb supplement normally contains 165 g/kg camphor, which is the principal component in Artemisia annua oil noted for its strong antifungal and antimicrobial activity (Foo et al., 2000). The existence of high borneol and camphene in rosemary oil also suggest its antibacterial value. It is believed that terpenes or other compounds are responsible for the positive effects on the studied parameters. The extracts contain a large proportion of A-type condensed tannins, while mimosa is composed of 5-deoxy tannins and this oil contains variable numbers of gallic acid groups linked to the condensed tannins (Bento et al., 2005). Cranberries, normallycontais flavonoid compounds, including proanthocyanidins, which may be mainly epicatechin units (Foo et al., 2000). Cranberries were also reported to contain mainly quercetin and myrectin flavanols, and anthocyanidins. The rosemary is known to have varying abilities to precipitate proteins and affect nutrient digestibility. It is clear from these results that CT structure can have a profound and differential effect in the poultry gut. However these improvements in weight gain, though might be of economic importance, were never reported to be of any statistical changes. Generally, the immune-toxic dose of AFB_1 is considered as less than the dose required eliciting a reduction on performance. Though several inconsistent reports are available, the threshold dose of AFB_1 may be generalized to be 0.4 and 1 mg/kg for the negative effects on cell mediated and humoral immunity, respectively. There is an evidence to suggest that herbs, spices and various plant extracts have appetite and digestion stimulating properties and antimicrobial effects (Pai and Platt, 1995). According to the literature, the effects of plant extract on feed intake are inconsistent (Carrillo *et al.,* 2005). It has been shown that when relatively high amounts (5g/kg) of essential oil were added into diet, early feed consumption (8-14 days) was decreased in broilers. It is worthy to mention that since the previous studies on aflatoxin with rosemary essence are scanty, we are failed to compare our results with the findings of other scientists. However, reducing the adverse effects of aflatoxin with a blend of EO's are studied before, but as commercial products, might not be suitable to compare, since the sole rosemary essence is not reported. It can be hypothesized that this negative effect may be due to low diet palatability when Rosemary is included.

4. Conclusions

Tit could be concluded that aflatoxin B1 in the broiler diet can influence the performance and immune response and addition of Rosemary essence as a herbal feed additive could partially reestablish these harmful effects on broilers. Nevertheless, there is lack in the proof of its beneficial impacts in nutrient digestibility and gut function of broilers..

ACKNOWLEDGEMENTS

REFERENCES

[1]   Arvind, K.L., V.S. Patil, G. Devegowda, B. Umakantha and S.P. Ganpule. 2003. Efficacy of Esterified glucomannan to counteract Mycotoxicosis in naturally contaminated feed on performance, serum biochemical and hematological parameters in broilers. Poult. Sci., 82: 571-576.

[2]   Benkeblia, N. (2004) Antimicrobial activity of essential oil extracts of various onions (Allium cepa) and garlic (*Allium sativum*). Lebensmittel-Wissenschaft und-Technologie: Food Science & Technology, 37: 263–268.

[3]   Bento, M.H.L., Makkar, H.P.S. and Acamovic, T. 2005. Effect of mimosa tannin and pectin on microbial protein synthesis and gas production during *in vitro* fermentation of N-15 labelled maize shoots. Animal Feed Science Technology, 123: 365–377.

[4]   Calnek, B.W. H.J. Barnes, C.W. Beard, W.M. Reid and H.W. Yolder. 1992. Journal of Diseases of Poultry. 9th Edition.. Wolfe Publication Ltd., USA.

[5] Candan, F., Unlu, M., Tepe, B., Daferera, D., Polissiou, M., Sokmen, A. and Akpulat, A. 2003. Antioxidant and antimicrobial activity of the essential oil and methanol extracts of Achillea milllefolium subsp. millefolium Afan. (Asteraceae). Journal of Ethnopharmacology, 87: 215–220.

[6] Carrillo, J.D. and Tena, M.T. (2005). Determination of volatile compounds in antioxidant rosemary extracts by solid phase micro extraction and gas chromatography. Anal. Lett., 38: 1193-1212.

[7] Citarasu, T. 2010. Herbal biomedicines: a new opportunity for aquaculture industry. Aquaculture International 18:403–414.

[8] Duncan, D.B., 1955. Multiple range and multiple F tests. Biometrics. 11:1-42.

[9] Ergun, S., S. Yılmaz, Y. Sevdan, andM. Yigit. 2011. Effects of thyme, rosemary and fenugreek on some hematological and immunological parameters of tilapia, Oreochromis mossambicus. Pages 311–312 in Aquaculture Europe 2011: "Mediterranean Aquaculture 2020." European Aquaculture Society, Oostende, Belgium.

[10] Eralsan, G., D. Essz, M. Akdogan, F. Sahindokuyucu and L. Altrintas. 2005. the effects of aflatoxin and sodium bentonite and alone on some blood electrolyte levels in broiler chickens. Turk Veterinerlik ve Hayvanclk Dergisi. 29: 601-605.

[11] Espada, Y., M. Domingo, J. Gomez and M.A. Calvo. 1992. Pathological lesions in following an experimental intoxication with aflatoxin B1 in broiler chickens. Res. Vet. Sci., 53: 2275-2279.

[12] Foo, L.Y., Lu, Y., Howell, A.B. and Vorsa, N. 2000. The structure of cranberry proanthocyanidins which inhibit adherence of uropathogenic P-fimbriated Escherichia coli in vitro. Phytochemistry, 54: 173–181.

[13] Gowda, N.K.S. and Ledoux, D.R. 2008. Use of antioxidants in amelioration of mycotoxin toxicity: a review. Anim. Nutr. Food Tech., 8: 1-11.

[14] Hernandez, F., Madrid, J., Garcia, V., Orengo, J. and Megias, M.D. 2004. Influence of two plant extracts on broilers performance, digestibility, and digestive organ size. Poultry Science, 83: 169–174.

[15] Hoehler, D. and R.R. Marquardt. 1996. Influence of vitamins E and C on the toxic effect of ochratoxin A and T-2 toxin in chicks. Poult. Sci., 65: 1508-1515.

[16] Iqbal, Q.K., P.V. Rao, and S.J. Reddy, 1983. Dose response relationship of experimentally induced aflatoxicosis in commercial layers. Indian J. Anim. Sci., 53: 1277-1280.

[17] Kubena, L.F., R.B. Harvey, W.E. Huff, D.E. Carier, T.D. Phillips and G.E. Rottinghaus. 1990. Efficacy of hydrated sodium calcium aluminosilicate to reduce the toxicity of aflatoxin and T-2 toxin. Poult. Sci., 69: 1078-1086.

[18] Kubena, L.F., T.S. Edrington, R.B. Harvey, S.A. Buckley, T.D. Phillips, G.E. Rottinghaus, and H.H. Caspers. 1997. Individual and combined effects of Fumonisin B1 present in Fusarium moniliforme culture material and deoxynivalenol in broiler chicks. Poult. Sci., 76: 1239-1247.

[19] Leeson, S., G. J. Diaz, and J. D. Summers. 1995. Poultry Metabolic Disorders and Mycotoxins. Univ. Books, Guelph, Ontario, Canada.

[20] Manafi, M. 2010. Effect of AFlatoxicosis in Hatching Eggs Quality and Immune Status in Broiler Breeders. In: World Poult. Sci. J. (Spp.), 66: 704-705.

[21] Manafi, M., K. Mohan, and M. Noor Ali. 2011. Effect of Ochratoxin A on Coccidiosis-Challenged Broiler Chicks. World Mycotoxin Journal. 4(2): 177-181.

[22] Manafi, M. 2012. Counteracting Effect of High Grade Sodium Bentonite during Aflatoxicosis in Broilers. Journal of Agricultural Science and Technology. 14: 539-547.

[23] Manafi, M. and H. Khosravinia. 2013. Effects of Aflatoxin on the Performance of Broiler Breeders and Its Alleviation by Herbal Mycotoxin Binder. Journal of Agricultural Science and Technology. 15: 55-63.

[24] Mansoori, B. and Acamovic, T. 2009. Influence of tannic acid and polyethylene glycol on the excretion and digestibility of amino acids in gelatin-fed broilers. British Poultry Science, 50: 199–206.

[25] McEwan, N.R., Graham, R.C., Wallace, R.J., Losa, R., Williams, P. and Newbold, C.J. 2002. Effect of essential oils on ammonia production by rumen microbes. Reproduction Nutrition Development, 42: S65.

[26] Mitsch, P., Zitterl-Eglseer, K., Kohler, B., Gabler, C., Losa, R. and Zimpernik, I. 2004. The effect of two different blends of essential oil components on proliferation of Clostridium perfringens in intestines of broiler chickens. Poultry Science, 83: 669–675.

[27] NRC, National Research Council. 1994. Nutrient Requirements of Poultry. 9th ed. Natl. Acad. Sci., Washington.

[28] Oguz, H. 2011. A review from experimental trials on detoxification of aflatoxin in poultry feed. Eurasian J. Vet. Sci., 27 (1): 1-12.

[29] Ostrowski, H. 1984. Biochemical and physiological responses of growing chickens and ducklings to dietary aflatoxins. Comp. Biochem. Physiol. C 79:193–204.

[30] Pai, S.T. and Platt, M.W. 1995. Antifungal effects of *Allium sativum* (garlic) extract against Aspergillus species involved in otomycosis. Letters in Applied Microbiology, 20: 14–18.

[31] Phillips, T.D., L.F. Kubena, R.B. Harvey, Taylor, and N.D. Heidelbaugh. 1988. Hydrated sodium calcium aluminosilicate: a high affinity sorbent for aflatoxin. Poult. Sci., 67: 243-247.

[32] Raju, M.V.L.N. and G. Devegowda. 2000. Influence of esterified glucomannan on performance and organic morphology, serum biochemistry and hematology in broilers exposed to individual and combined mycotoxicosis (aflatoxin, ochratoxin and T-2 toxin). Br. Poult. Sci., 41: 640-650.

[33] Raju, M. V. L. N. and G. Devegowda. 2002. Esterifed glucomannan in broiler chicken diets contaminated with aflatoxin, ochratoxin and T-2 toxin: Evaluation of its binding ability (in vitro) and Efficacy as immunomodulator. Asian-Aust.J.Anim.Sci.15: 1051-1056.

[34] SAS. Institute. 2000. SAS® User's Guide: Statistics. Version & Edition. SAS Institute Inc., Cary, NC, USA.

[35] Shalaby, A. M., Y. A. Khattab, and A. M. Abdel Rahman. 2006. Effects of garlic (Alliumsativum) and chloramphenicol

on growth performance, physiological parameters and survival of Nile Tilapia (Oreochromis niloticus). Journal of Venomous Animals and Toxins 12:172–201.

[36] Stanley, V.G., Roje. S. Woldesenbet and D.H. Hutchinson, 1993. The use of scharo myces cerevisiae to suppress the effect of aflatoxicosis in broiler chicks. Poult. Sci., 72: 1867-1872.

[37] Ueno, Y. 1991. Biochemical mode of action of mycotoxins. In: Mycotoxins and Animal Foods. Eds: J.E. Smith and R.S. Henderson, CRC Press, Boca Raton, pp. 437-453.

[38] Viuda-Martos, M., Riuz-Navajas, Y., FERNANDEZ-LOPEZ, J. and Perez-Alvarez, J.A. 2008. Antibacterial activity of different essential oils obtained from spices widely used in the Mediterranean diet. International Journal Food Science & Technology, 43: 526–531.

[39] Weiss, W.P. 2002. Amntioxidants nutrients and milk quality. In: Roche symposium, pacific Northwest nutrition conference, Vancouver, British Columbia, Canada.

[40] Windisch, W., K. Schedle, C. Plitzner, and A. Kroismayr. 2008. Use of phytogenic products as feed additives for swine and poultry. J. Anim. Sci. 86(E. Suppl.):E140–E148.

[41] Xue, C.Y., G.H., Wang, F. Chen, X.B. Zhang and Y.C. Cao. 2010. Immunopathological Effects of Ochratoxin A and T-2 Toxin Combination on Broilers. Poult. Sci., 89: 1162-1166.

[42] Yılmaz, S., and S. Ergun. 2012. Effects of medicinal herb extracts on egg hatching of the angel fish (Pterophyllum scalare). Kafkas Universitesi Veterinerlik Fak¨ultesi Dergisi 18:185–189. (In Turkish with English abstract.).

[43] Yin, M.C. and Cheng, W.S. 1998. Antioxidant activity of several Allium members. Journal Agriculture & Food Chemistry, 46: 4097–4101.

[44] Zilberg, D., A. Tal, N. Froyman, S. Abutbul, N. Dudai, and A. Golan-Goldhirsh. 2010. Dried leaves of Rosmarinus officinalis as a treatment for streptococcosis in tilapia. Journal of Fish Diseases 33:361–369.