Research in Zoology 2014, 4(2): 43-50
DOI: 10.5923/j.zoology.20140402.02

Aflatoxicosis and Herbal Detoxification: The Effectiveness of Thyme Essence on Performance Parameters and Antibody Titers of Commercial Broilers Fed Aflatoxin B_1

Manafi M.*, M. Hedayati, M. Yari

Department of Animal Science, Faculty of Agricultural Sciences, Malayer University, Malayer, Iran

Abstract The current study is planned to investigate the effect of aflatoxin B_1 (600ppb) and the ethanolic extract of Thyme (500ppm) in broiler chickens. 240 unsexed Ross broiler chicks were randomly allotted into 4 dietary treatments with three replicates and twenty chicks per replicates and reared in cage systems for 42 days. Results showed that the body weights of broilers fed aflatoxin were reduced significantly ($P<0.05$) and feed consumption and FCR were increased significantly ($P<0.05$). The inclusion of Thyme essence have showed an improved data and addition of Thyme essence to aflatoxin fed groups could partially alleviate the adverse effects of aflatoxin in the diet. The antibody titer values against Newcastle Disease, Infectious Bronchitis and Avian Influenza of broilers fed aflatoxin were significantly ($P<0.05$) increased and incorporation of 500ppm Thyme essence could partially restore the negative impact of aflatoxin in commercial broilers.

Keywords Aflatoxin B_1, Thyme Essence, Body Weight, Feed Consumption, FCR, Antibody titers, Broilers

1. Introduction

Mycotoxins are secondary metabolites that are toxic to humans and animals and produced by many species of fungi. Most of them are in small molecular sizes [1]. Various investigations of mycotoxins have been carried out over the decade, mostly on emphasis on food intoxication and its prevention [2]. Mycotoxin food contamination causing mycotoxicosis in poultry and has been a serious problem, and remains so in some developing countries. Pathogenic *Aspergillus spp*. also produces mycotoxins which are well known for their strong and varied biological activities. For example, aflatoxin, the well-investigated mycotoxin, is known to carry the most potent carcinogenic activity as a natural product. It also carries acute toxicity to various human cells such as hepatocytes, renal cells, lung epithelioid cells, etc., as well as various immunosuppressive activities [3]. Many other mycotoxins have fairly similar activities [4]. Aflatoxins, the toxic secondary metabolites of various *Aspergillus* spp., are normally encountered in a wide range of tropical and subtropical feeds. These are furanocoumarin in compounds and mainly include aflatoxins B_1, B_2, G_1, G_2, and M_1. Till now, much work has been done to explore the hepatotoxic, carcinogenic, and immunosuppressive effects of AFB_1 [5]. When focusing on how mycotoxins play a role in food safety, attention should be given to mycotoxins that are known to be transferred from feed to food of animal origin, as this food represents a significant route of introduction for humans. Apart from their toxicological effects in affected animals, the carry-over through animal final products, such as meat, milk and eggs into the human beings food chains is an important aspect of mycotoxin impurity. FAO has estimated that up to 25% of the world's food cereals and a higher percentage of the world's animal feedstuffs are significantly contaminated by mycotoxins. Aflatoxin or ochratoxin residues in meat are infrequent [6]. However, it's more common in target organs especially liver. This organ may have its lipid content increased over three fold when 20ppm aflatoxin is incorporated in broiler diet [7]. The toxicity of AF in poultry has been widely examined by determining their teratogenic [8], carcinogenic, mutagenic and growth inhibitory effects [9]. The biochemical hematological [10], immunological [7], gross and histopathology [11] toxic effects of AF have also been well documented. Preventing of mold growth and AF contamination in feed and feedstuffs is very vital but when contamination cannot be prevented, decontamination of AF is needed before using these materials. Producers, researchers and governments aim to develop effective prevention system and decontamination techniques to minimize the toxic effects of AF. Practical and cost-effective tools of detoxifying AF-contaminated feed are in great petition. Besides of the preventive management, approaches have been employed including physical, chemical and biological treatments to cleanse AF in contaminated feeds

* Corresponding author:
manafim@malayeru.ac.ir (Manafi M.)
Published online at http://journal.sapub.org/zoology

and feedstuffs. An approach to the problem has been the usage of non-nutritive and inert adsorbents in the diet to bind AF and reduce the absorption of AF from the gastrointestinal tract. Since the early 1990s, experiments with adsorbents such zeolites and aluminosilicates have proven to be successful, but high addition rates and possible potential interactions with feed nutrients are causes for concern [12, 13, 14]. Also, possible dioxin contamination may be a risk factor for using of natural clays in case of forest and trash fire near the source of them [14]. One of the approaches to overcome mycotoxicosis in poultry is using herbal products which contain essential oils. It has been well recognized that drug-metabolizing enzymes (phase-I and phase-II enzymes) and AFB_1- adduct formation can be changed by natural constituents of the diet, nutrients, phytochemicals and xenobiotics [15]. Phenolic phytochemicals are thought to promote ideal health partly via their antioxidant and free radical scavenging activities thereby protecting cellular components against free radical induced damage. But due to their diverse chemical structures, they are likely to possess different antioxidant abilities [16]. Essential oils are complex compounds, and their chemical composition and concentrations of various compounds are variable [17]. Essential oils basically consist of two classes of compounds, the terpenes and phenylpropenes, depending on the number of 5-carbon building blocks. The exact anti-microbial mechanism of essential oils is poorly understood. However, it has been suggested that their lipophilic property [18] and chemical structure [19] can play a role. It was suggested that terpenoids and phenylpropenes can penetrate the membranes of the bacteria and reach the inner part of the cell because of their lipophilicity [20]. Moreover, structural properties, such as the presence of the functional groups [19] and aromaticity [21] are also responsible for the antibacterial activity of essential oils. Thyme (*Thymus vulgaris*), a member of *Lamiaceae* family, with the main components of phenols, thymol (40%) and carvacrol (15%). This herb is also used traditionally for diseases and its beneficial value has been reported in poultry [22]. Also, yogurt, due to its probiotic potential, can be used instead of commercial antibiotics, too. The main probioticts in yogurt are lactic acid bacteria, and it has been reported, that *L. acidophilus* can absorb cholesterol from *in vitro* system. In addition, it can increase the protein digestibility and availability of minerals, viz. Cu, Mn, Ca, Fe, P for its host [23]. Thyme has been commonly used in foods mainly for the flavor, aroma and preservation and also in traditional medicine since the ancient Greeks, Egyptians and Romans. The leafy parts of thyme belonging to the *Lamiaceae* family are often added to meat, fish and food products and also used as herbal therapeutic foodstuffs. Evidence based research hypothesize that thyme possesses numerous biological activities including antispasmodic, antimicrobial, antioxidant and antifungal. Moreover, thyme extract possess antioxidant activity and hinders lipid peroxide formation [24]. The supplementation of poultry diets with essential oils led to enhanced weight gain, improved carcass quality and reduced mortality rates. The aims of the current study were to determine the activity of the ethanolic extract of thyme *in vivo* and to evaluate the protective effects of the extract on performance and antibody titers of broiler chickens fed AFs contaminated diet.

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 Thyme 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 (0-2 weeks), grower (2-4wks) and finisher (4-6 weeks) periods. The composition of diets was adopted from NRC, [25] and is presented in Table 1. The basal diet was formulated using commonly available feed ingredients which were screened for AF prior to the formulation of diets. The Aflatoxin B_1 was procured from Sigma Aldrich, USA and diluted to reach to the required level of administration. The experimental diets were prepared by adding required quantity of aflatoxin to arrive at the levels of 0 and 600ppb of AFB_1. Diets were prepared without addition of aflatoxin and Thyme essence as Control (group 1); 600 ppb Aflatoxin B_1 (group 2); 500ppm of Thyme essence (group 3) and 600ppb Aflatoxin B_1 + 500 ppm of Thyme essence (group 4). The ethanolic extraction of Thyme was prepared as per the instruction given below:

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.

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.

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 B_1, BRONHOPEST B_1 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 B_1 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. Blood was centrifuged @4000 rpm for 10 min and serum separated after 8 to 10 hours as per the standard procedures [39] 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 (bio check®) 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 [26]. 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 [27].

3. Results and Discussion

The effects of aflatoxin and Thyme 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 Thyme extract have shown a non-significant changes compared with control group. In AF+THY group, the reduced body weight of chicks due to presence of AF could not be reached up to the control level. At day 14, AF fed group could significantly (P<0.05) reduce the body weight, compared with control group. In group fed Thyme essence, the maximum body weight were found and in group fed AF+THY, the minimum BW has been found. This indicates that all 3 groups of AF, THY and AF+THY have shown a significant changes in BW, when compared with control group. At day 21 and day 28, the same trend of day 14 have been found in all treatment groups. At day 28, the body weight is found to be higher in THY fed group, followed by control, AF and AF+THY fed groups. At this day, again the toxic effects of AF could not be reached by Thyme essence administration in broiler diet. At the end of trial, the control group showed a weigh of 2207.93g BW, whereas AF fed group is found 2052.60g, which indicates a significant (P<0.05) reduce in BW of broilers. Similar to other weeks, BW of group fed Thyme extract found to the higher among all treatments, compared with control group and in AF+THY fed group, the BW could not reach to the control group. This means that Thyme extract has no ability to significantly (P<0.05) reduce the adverse effects of aflatoxin in broiler diet.

The effects of aflatoxin and Thyme essence on body cumulative feed consumption (g): Table 3 is depicting the effects of feed consumption of broilers fed different dietary treatments. The negative effects 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 167.33 in control group and significantly (P<0.05) decreased. At day 14, the maximum amount of feed is consumed in AF group (535.0g) followed by THY (531.30g), AF+THY (529.40g) and CON (516.70g). At the end of week III and week IV, the same trend is continued and in AF fed group, significant (P<0.05) lower body weight was found, when compared with control group. The maximum amount of feed consumed was in THY fed group, which was significantly (P<0.05) higher than all other treatments, when compared with control group. At day35, the significant (P<0.05) lower amount of feed consumed in AF fed groups, followed by THY, CON and AF+THY groups. This shows that AF alone has reduced the feed consumption and inclusion of THY 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, AF+THY, CON and THY groups, respectively.

The effects of aflatoxin and Thyme essence on feed conversion ratio (FCR): The effect of AF on FCR is showed in Table 4 and started from day 7, where data found to be 0.005, 0.995, 0.935 and 0.835 in AF, THY, AF+THY and CON fed groups of broilers. At day 14, the FCR of AF group found to be significantly (P<0.05) higher than control group and in THY group, FCR found to be 1.183 vs. 1.207 of CON and was significantly better. When compare AF with AF+THY group, the FCR was significantly higher in AF fed groups which shows Thyme 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 THY group, the FCR data was in a similar range with control group but in AF+THY group, FCR was significantly (P<0.05) higher than control group. The FCR values for AF and AF+THY groups, when compared, revealed that Thyme essence could reluctant the adverse effect of AF significantly (P<0.05) and could be introduced as a useful tool to overcome mycotoxicosis. At the end of week IV, AF fed group have shown significantly (P<0.05) higher FCR values when compared with control group and remained at top among all treatments. In Thy group, the FCR significantly (P<0.05) lower than control group and by comparing THY with AF+THY group, the Thyme essence could not hide the adverse effect of AF. At day 35, the 1.927 FCR was found in AF vs. 1.725 of control group and was significantly (P<0.05) higher in AF fed group. Thyme essence could improve the FCR value even better that CON. In AF+THY group, the feed efficiency could be restore the harmful effects of AF significantly (P<0.05). Very interestingly, the feed conversion ratio found to be maximum in AF group followed by CON, THY and AF+THY treatment groups. The AF fed group showed a significantly (P<0.05) lower FCR compared with control group and by comparing AF with AF+THY groups, the FCR was found better in AF+THY group significantly (P<0.05).

The effects of aflatoxin and Thyme essence on antibody titers against ND, IB and AI: The antibody titer against Newcastle Disease Virus, Infectious Bronchitis Virus and Avian Influenza Virus is showed in Table 5. ND titer is increased significantly (P<0.05) in AF group when compared with control group. The THY and AF+THY groups are also showed an increase in titer value compared with control group. The titer values for NDV ranged from 3.66 to 4.84. The IBV titer values was found to be significantly (P<0.05) higher in AF group (10297.16) vs. CON (8105.45). In THY and AF+THY groups, the significant (P<0.05) higher values in IB titer found and by comparing AF with AF+THY group, it could be found that the Thyme essence could rectify the adverse effects of AF into some extent. In case of AIV, titer values for AF group was found to be significantly (P<0.05) higher than control group and in THY and AF+THY groups, significant (P<0.05) higher values were found, compared with control group. Comparing AF with AF+THY groups, it is showed that AI titer values in AF+THY group could decrease from that of AF values significantly (P<0.05) and has a clear indication of positive effects of Thyme essence on AF fed broilers.

Table 2. Body weight (g) of chicks fed Aflatoxin B_1 and Thyme 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[b]	766.56±0.85[b]	1284.43±0.49[b]	1775.26±0.72[b]	2207.93±0.53[c]
AF[2]	45.02±0.76[a]	184.10±0.96[b]	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]
THY[3]	44.90±0.98[a]	197.40±0.12[a]	449.10±0.75[a]	819.50±0.23[a]	1366.30±0.74[a]	1852.70±0.93[a]	2352.10±0.27[a]
AF+THY[4]	45.00±0.16[a]	181.80±0.08[b]	350.60±0.81[d]	624.20±0.71[d]	1113.20±0.51[d]	1774.20±0.89[b]	2316.30±0.49[b]

Mean values within a row with different superscript letters (a, b and c) were significantly different ($p<0.05$).[1]CON (Control), [2]AF (Aflatoxin B_1 at 600ppb level), [3]THY (Thyme essence at 500ppm level) and [4]AF+THY (Aflatoxin B_1 and Thyme 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 Thyme 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[c]	516.70±0.33[b]	1148.93±0.48[d]	2041.06±0.92[b]	3062.32±0.38[b]	4369.49±0.49[b]
AF[2]	185.05±0.84[b]	535.00±0.19[a]	1155.50±0.29[c]	1897.00±0.17[c]	3358.95±0.23[c]	4127.77±0.31[c]
THY[3]	196.60±0.91[a]	531.30±0.79[a]	1182.10±0.72[a]	2090.50±0.65[a]	3092.150±0.29[a]	4381.96±0.62[a]
AF+THY[4]	170.00±1.04[a]	529.40±0.31[ab]	1166.40±0.32[b]	1897.00±0.69[c]	2838.72±0.19[c]	3525.40±0.43[c]

Mean values within a row with different superscript letters (a, b and c) were significantly different ($p<0.05$). [1]CON (Control), [2]AF (Aflatoxin B_1 at 600ppb level), [3]THY (Thyme essence at 500ppm level) and [4]AF+THY (Aflatoxin B_1 and Thyme 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 Thyme 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±0.28[c]	1.207±0.22[c]	1.498±0.12[c]	1.589±0.08[b]	1.725±0.01[b]	1.979±0.18[b]
AF[2]	1.005±0.27[a]	1.525±0.28[a]	1.851±0.28[a]	1.704±0.12[a]	1.927±0.19[a]	2.011±0.12[a]
THY[3]	0.995±0.19[a]	1.183±0.19[d]	1.442±0.22[c]	1.530±0.27[c]	1.669±0.26[c]	1.863±0.21[c]
AF+THY[4]	0.935±0.14[b]	1.367±0.29[b]	1.649±0.19[b]	1.557±0.23[bc]	1.600±0.11[d]	1.552±0.29[d]

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

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

Treatment groups	ND	IB	AI
CON[1]	3.66±0.62[b]	8105.45±0.82[c]	3.00±0.63[c]
AF[2]	4.27±0.44[b]	10297.16±0.21[a]	4.28±0.45[a]
THY[3]	4.84±0.54[a]	5803.87±0.62[d]	3.54±0.74[b]
AF+THY[4]	4.57±0.68[a]	9150.14±0.92[b]	3.81±0.63[b]

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

Numerous reports on effects of aflatoxins on bird performance and serum biochemistry have been previously reviewed by Devegowda and Murthy [28]. There is a general covenant that dietary aflatoxin reduces weight gain, feed intake, and increase feed conversion ratio. These data indicate that AFB_1 has the capability to reduce broiler performance and increase the incidence of bruising in carcass when present at levels of more than 0.5 mg/kg diet. Dersjant-Li *et al.* [29] determined in their review that each mg of AFB_1/kg diet would decrease the growth performance of broilers by 5%. However, data published throughout last decade regarding effect of administration of low doses of

AFB_1 on weight gain is not consistent with this statement. For example, Manafi et al., [3] noted 21% decrease in final body weight at 35 days age in broilers fed on 0.3mg AFB_1/kg diet. In contrary, Tedesco *et al.* [30] noted only 10% decrease in weight gain of broilers at 28 days of exposure to 0.8 mg AFB_1/kg diet. For levels of AFB_1 of 1 mg/kg diet, 10% reduction in weight gain was noticed by Zhao *et al.* [31] at 21 days of exposure while 15% reduction at 42 days exposure was recorded by Denli *et al.* [32]. At further higher levels of 3 mg AFB_1/kg diet, only 11% reduction in weight gain at 21 days exposure was noted by Valdivia *et al.* [33]. From these reports, it is evident that both the level and length of AFB_1 exposure affect the amount of reduction in broilers weight gain. Furthermore, diverse type of and rations used in different studies make it impractical to simplify the dose-response relationship regarding weight gain. In a recent review, the effects of AFB_1 on weight gain in broilers is believed could be of biphasic nature (hormesis), *i.e.*, improvement at low doses while reduction at high doses. In the review of Denli *et al.*, [32] the maximum improvement in weight gain of broilers was stated to be 3 to 4% during exposure to low levels of AFB_1. In the above-mentioned report of Zhao *et al.* [31] these authors however noted 13% improvement in weight gain of broilers during 2nd week of exposure to 0.8mg AFB_1/kg diet. After 2nd week of exposure the weight gain of broilers started to drop under AFB_1 diet with significant effects apparent during 4th week of exposure. It therefore seems that the length of exposure to AFB_1 besides its level could also influence the type of response regarding weight gain. However these improvements in weight gain, though might be of economic importance, were never reported to be of any statistical changes. Secondary to the effects on liver, the immune-suppressive nature of AFB_1 is the best recognized part of its toxicity. Recent epidemiological reports also indicate high correlation between outbreaks of Newcastle disease (ND) and aflatoxin contamination of broiler rations [5]. 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. However, the question regarding susceptibility of modern broiler regarding immunotoxicity remains yet to be unknown. Furthermore, there is evidence regarding biphasic nature of the effects of AFB_1 on humoral immunity. Recent report indicate that humoral immune response from broilers could increase and decrease depending upon the level and duration of exposure to the toxin. During earlier studies on effects of AFB_1, Tung *et al.* [34] described the toxin-induced increase in lysosomal enzyme activity in liver and skeletal muscles of chicken. These authors assumed that this increase in lysosomal activity, besides other factors, could harmfully affect tissue integrity during aflatoxicosis. In this regard, dietary AFB_1 has been found by Çelik *et al.* [35] to result in degeneration of follicle associated epithelium (FAE) in bursa of Fabricius and destruction of thymus cortex in chicken. On the grounds of the report of Tung *et al.* [34] it was therefore urged that any impaired function of FAE might result in serious deficiencies in both cellular and antibody responsiveness of the chicken immune system. This is due to this fact that bursal follicles play a crucial role in antigen presentation to the lymphoid cell population. Besides the effects on lymphocytes, non-specific effects of the toxin on protein synthesis through inhibition of RNA polymerase, lipid peroxidation, and liver injury are also assumed to result in reduced immunoglobulin production. This indicates that AFB_1-induced modulation of humoral immunity in broilers may not be a result of the toxin's non-specific effects on protein metabolism. Higher doses of extracts from both plants showed better results than low doses. These results showed that extract oil derived from thyme and cinnamon in broiler diets improved body weight gain, feed intake and feed conversion ratio, which may be due to active materials (thymol and caracol) in these plants which are considered as digestion stimulating factors, in addition to their antimicrobial activity against bacteria found in the intestine [36]. Moreover, the improvement of body weight gain and feed conversion are due to the active materials (Cinnamaldehyde and eugenol) found in cinnamon, causing greater efficiency in the utilization of feed, resulting in enhanced growth. There is an evidence to suggest that herbs, spices and various plant extracts have appetite and digestion stimulating properties and antimicrobial effects [37]. According to the literature, the effects of plant extract on feed intake are inconsistent [23]. It has been shown that when relatively high amounts (5 g/kg) of thyme essential oil were added into diet, early feed consumption (8-14 days) was decreased in broilers, but adaptation by the birds lessened the negative effects of thyme on feed intake by birds as they got older [38]. Similarly, results in the present study confirmed the negative influence of high concentrations of ground thyme herb on feed intake at early stages in the life of the chick. It can be hypothesized that this negative effect may be due to low diet palatability when thyme is included.

4. Conclusions

Based on the founded data in this trial and available reports, it could be concluded that the addition of aflatoxin in broiler diet can have a negative impact on the performance and immune response of broilers and Thyme essence can be used as natural non-antibiotic feed additive on broilers. Nevertheless, there is scarcity in the evidence of its beneficial effects in nutrient digestibility and gut function of broilers fed with aflatoxin and Thyme essence.

ACKNOWLEDGEMENTS

This study was funded by Directorate of Research, Malayer University, Malayer, Iran.

REFERENCES

[1] Tabata, S, 2002. General aspects of mycotoxin. In: Hosogai Y, Matsumoto M (eds). Mycotoxin. Tokyo: Chuo Hoki, 71.

[2] Fung, F. and R. Clark, 2004. Health effects of mycotoxins: a toxicological overview. Journal of Toxicology and Clinical Toxicology, 42: 217_/234.

[3] 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.

[4] 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.

[5] Yunus, A. W., M. K. Nasir, U. Farooq, and J. Bohm, 2008. Prevalence of poultry diseases and their interaction with mycotoxicosis in district Chakwal: 1. Effects of age and flock size. J. Anim. Plant Sci. 18:107–113.

[6] Biswas, A.K., N, Kondaiah, A.S.R, Anjaneyulu, and P.K., Mandal, 2010. Food Safety Concerns of Pesticides, Veterinary Drug Residues and Mycotoxins in Meat and Meat Products. Asian Journal of Animal Sciences, 4, 46-55.

[7] Zaghini, A., G, Martelli, P, Roncada, M, Simioli, and L, Rizzi 2005. Mannanoligosaccharides and aflatoxin B_1 in feed for laying hens: effects on egg quality, aflatoxins B_1 and M_1 residues in eggs, and aflatoxin B_1 levels in liver. Poultry Science, 84(6): 825-32.

[8] Sur E, and I, Celik 2003. Effects of AFB_1 on the development of the bursa of Fabricius and blood lymphocyte acid phosphatase of the chicken. British Poultry Science, 44: 558–566.

[9] Oguz H, and V, Kurtoglu, 2000. Effect of CLI on fattening performance of broiler chickens during experimental aflatoxicosis. British Poultry Science, 41: 512–517.

[10] Basmacioglu, H, H, Oguz M, Ergul R, Col and Y.O, Birdane, 2005. Effect of dietary EGM on performance, serum biochemistry and hematology in broilers exposed to AF. Czech Journal of Animal Science, 50: 31-39.

[11] Ortatatli M, and H, Oguz, 2001. Ameliorative effects of dietary CLI on pathological changes in broiler chickens during aflatoxicosis. Research in Veterinary Science, 71: 59–66.

[12] Dwyer, M.R., L.F., Kubena, R.B., Harvey, K, Mayura, A.B., Sarr, S, Buckley, R.H., Bailey, and T.D., Phillips, 1997. Effects of inorganic adsorbents and cyclopiazonic acid in broiler chickens. Poultry Science, 76: 1141-1149.

[13] Phillips, TD, 1999. Dietary clay in the chemoprevention of AF-induced diseases. Toxicology Letters, 52(Suppl): 118-126.

[14] Rosa, C.A.R., R, Miazzo, C, Magnoli, M, Salvano, S.M., Chiacchiera, S, Ferrero, M, Saenz, E.C., Carvalho, and A, Dalcero, 2001. Evaluation of the efficacy of bentonite from the south of Argentina to ameliorate the toxic effects of AF in broilers. Poultry Science, 80: 139–144.

[15] Dapkevicius, A., T.A. van Beek, G.P. Lelyveld, A van Veldhuizen, A. de Groot, J.P.H Linssen, and R. Venskutonis, 2002. Isolation and Structure Elucidation of Radical Scavengers from Thymus vulgaris Leaves. Journal of Natural; Production, 65: 892-896.

[16] Abdel-Wahhab, M.A., N.S. Hassan, A.A. El-Kady, Y.A. Khadrawy, A.A. El-Nekeety, S.R Mohamed, HA Sharaf and F.A. Mannaa, 2010. Red ginseng extract protects against aflatoxin B_1 and fumonisins-induced hepatic pre-cancerous lesions in rats. Food Chemestry and Toxicology, 48: 733-742.

[17] Lee, K.W., H. Everts, H.J. Kappert, H. Wouterse, M. Frehner and A.C. Beynen, 2004. Cinnamanaldehyde, but not thymol, counteracts the carboxymethyl cellulose-induced growth depression in female broiler chickens. International Journal of Poultry Science, 3: 608- 612.

[18] Ghasemi H. A., K, Taherpour, I, Hajkhodadadi, and H, Akhavan-Salamat, 2014. Comparative Effects of Nettle (*Urtica Dioica*) and Commercial Feed Additives on Productive Performance and Blood Lipid Profile of Broiler Chickens, Journal of Animal Science Advances, 4(1): 633-640.

[19] Farag, R.S., Z.Y. Daw and S. H. Abo-Raya, 1989. Influence of some spice essential oils on Aspergillus parasiticus growth and production of aflatoxins in a synthetic medium. Journal of Food Science, 54: 74-76.

[20] Helander, I.M., H.L. Alakomi K. Latva-Kala, T. Mattila-Sandholm, I. Pol, E.J. Smid, L.G. M. Gorris and A. Von-Wright, 1998. Characterization of the action of selected essential oil components on Gram-negative bacteria. Journal of Agricultural Food Chemistry, 46: 3590-3595.

[21] Bowles, B.L. and A.J. Miller, 1993. Antibouulinal properties of selected aromatic and aliphatic aldehydes. Journal of Food Production, 56: 788-794.

[22] Mikaili, P., M.A Mohammad Nezhady., J Shayegh and M.H Asghari, 2010. Study of antinociceptive effect of nepeta meyeri, raphanus sativas and origanum vulgare extracts in mouse by acute pain assessment method, International Journal of Academic Research, 2(6): 126-128.

[23] Kirkpinar, F., H.B., Unlu, and G, Ozdemir, 2011. Effects of oregano and garlic essential oils on performance, carcass, organ and blood characteristics and intestinal microflora of broilers. Livestock Science, 137:219-225.

[24] Hutchins, J.E. and W.M Jr. Hagler, 1983. Rapid liquid chromatographic determination of aflatoxins in heavily contaminated com. J. Assoc. Off. Anal. Chem., 66: 1458-1465.

[25] N.R.C., National Research Council. 1994. Nutrient Requirements of Poultry. 9th ed. Natl. Acad. Sci., Washington.

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

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

[28] Devegowda, G. and T.N.K., Murthy, 2005. Mycotoxins: Their effects in poultry and some practical solutions. In The Mycotoxin Blue Book; Diaz, D.E., Ed.; Nottingham University Press: Nottingham, UK, pp. 25–56.

[29] Dersjant-Li, Y., M.W.A., Verstegen, and W.J.J., Gerrits, 2003. The impact of low concentrations of aflatoxin, deoxynivalenol or fumonisin in diets. Nutrition Research Reviews, 16(2): 223-39.

[30] Tedesco, D, S, Steidler, S, Galletti, M, Tameni, O, Sonzogni, and L, Ravarotto, 2004. Efficacy of silymarin-phospholipid complex in reducing the toxicity of aflatoxin B_1 in broiler chicks. Poultry Science, 83: 1839–1843.

[31] Zhao, J., R.B., Shirley, J.D., Dibner, F, Uraizee, M, Officer, M, Kitchell, M, Vazquez-Anon, and C.D., Knight, 2010. Comparison of hydrated sodium calcium aluminosilicate and yeast cell wall on counteracting aflatoxicosis in broiler chicks. Poultry Science, 89: 2147–2156.

[32] Denli, M., J.C., Blandon, M.E., Guynot, S, Salado, and J.F., Perez, 2009. Effects of dietary AflaDetox on performance, serum biochemistry, histopathological changes, and aflatoxin residues in broilers exposed to aflatoxin B_1. Poultry Science, 88: 1444–1451.

[33] Valdivia, A., A, Martinez, F, Damian, T, Quezada, R, Ortiz, C, Martinez, J, Llamas, M, Rodriguez, L, Yamamoto, and F, Jaramillo, 2001. Efficacy of N-acetylcysteine to reduce the effects of aflatoxin B_1 intoxication in broiler chickens. Poultry Science, 80: 727–734.

[34] Tung, H.T., W.E., Donaldson, and P.B., Hamilton, 1970. Effects of aflatoxin on some marker enzymes of lysosomes. Biochim. Biophys. Acta Gen. Subj. 222, 665–667.

[35] Çelik, I., H, Oğuz, Ö, Demet, H.H., Dönmez, M, Boydak, and E, Sur, 2000. Efficacy of polyvinylpolypyrrolidone in reducing the immunotoxicity of aflatoxin in growing broilers. British Poultry Science, 41: 430–439.

[36] Cabuk, M., A. Alcicek, Bozkurt and N. Imre, 2003. Antimicrobial properties of essential oils isolated from aromatic plants and using possibility as alternative feed additives. 11. National Animal Nutrition Congress, pp: 184-187.

[37] Kamel, C., 2001. Tracing methods of action and roles of plant extracts in non-ruminants. In: Recent Advances in Animal Nutrition (eds.). Garns Worthy, P. C. and J. Wiseman, Nottingham University Press, Nothingham, UK.

[38] Cross, D.E., R.M., McDevitt, K, Hillman, and T, Acamovic, 2007. The effects of herbs and their associated essential oils on performance, dietary digestibility and gut microflora in chickens from 7 to 28 days of age. British Poultry Science, 48: 496-506.

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