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Application of Rosemary (*Rosmarinus officinalis* L.) Essence on Chicks Fed Aflatoxin B_1: Impacts on Internal Organ Weights, Biochemical Traits and Mortality

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Abstract Aflatoxins (AF) are a major issue encountered in poultry farms, leading to much economic losses. The current trail is planned to determine the effects of AF (600ppb) and Rosemary essence (ROS) (500ppm), singly or in combination, on the visceral organ weights, some blood biochemical parameters and mortality percentage of broilers. A total of 240 unsexed Ross 308, were randomly allotted into 4 treatments with 3 replicates having 20 chicks each (Control; AF; ROS and AF+ROS). The broilers fed AF contaminated diet from beginning and showed characteristic effects of aflatoxicosis. The weight of spleen, and pancreas decreased and bursa and liver weights increased due to ingestion of AF. Serum levels of cholesterol, LDL and HDL and mortality is increased in aflatoxin fed broilers. The Rosemary could partially restore the adverse effects of AF to some extent and when alone added into the diet and showed good effects on reducing cholesterol, HDL, LDL and reduction number of dead birds, due to presence of essential oils and antioxidants activity in its content.

Keywords Aflatoxin B1, Rosemary essence, Visceral Organs, Cholesterol, LDL, HDL, Broilers

1. Introduction

Several species of fungi producing toxin compounds are found relatively commonly in cereal grains, either at harvest or during storage when these crops are harvested under unfavorable weather conditions or improper storage.

Mycotoxins are low molecular compounds of fungal metabolites capable of causing toxic responses in various organisms. In recent years, it has become demonstrated that different animal species and particular poultry can be further affected by mycotoxins produced by specific fungal contaminates. Mycotoxin can enter the system of an animal by ingestion, inhalation or direct skin contact. Very little amounts of those toxins can cause significant health problems.

Aflatoxins (AF) are a group of chemicals produced mainly by *Aspergillus*, primarily by *Aspergillus flavus* and *Aspergillus parasiticus* (Manafi *et al.*, 2009). The aflatoxin B1 (AFB1) is the most prevalent toxin in cereal used in animal and poultry feeds (Santurio, 2000). The mutagenic and carcinogenic effects of AF are well known and the liver is the main target organ of this mycotoxin. Metabolic changes associated with liver damage, decreased activity of digestive enzymes, immune suppression (Osweiler, 1990) and more recently, changes in gene expression of liver enzymes and changes in intestinal morphology and function have also been reported. The deleterious effects of AF vary according to the dose, exposure time, sex, and age of the animal (Manafi and Khosravinia, 2013). The primary effects of aflatoxicosis in birds may be used for the clinical judgment of the disease. One of the most important signs of aflatoxin toxicity in broilers is the change in internal organs. Liver, spleen and kidney increase in size (Osweiler, 1990), while the bursa of Fabricius and the thymus decrease (Sur and Celik, 2003). Giambrone et al. (1978) reported that aflatoxins in birds have toxic effects on immunological responses, causing the inhibition of protein synthesis and consequently a decrease in the production of antibodies. These toxic effects are also related to decreased vaccine responses and occurrence of unspecified diseases (Corrier, 1991).

Therefore, there is wide interest in the use of biological products to decrease mycotoxin availability. One of the alternatives is the use of herbal adsorbents, which bind to mycotoxins and prevent their absorption by the gastrointestinal tract, making them inert to animals (Huwig et al., 2001). Inorganic and biological binders have been investigated in studies to control the bioavailability of mycotoxins. Glucan-based binders, produced from carbohydrates of the cell wall of some species of yeast, have been well studied (Kassie et al., 2002).

One of the recently attracted tools to hinder the adverse effects of AF is the use of herbal and medicinal products.

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Among the various medicinal and culinary herbs, some phenolics are ubiquitous compounds found in all plants as secondary metabolites; these include simple phenols, hydroxybenzoic acid and cinnamic acid derivatives, flavonoids, coumarins and tannins (Naczk and Shahidi, 2004). They may be used for the production of raw materials or preparations containing phytochemicals with significant antioxidant capacities and health benefits (Naczk and Shahidi, 2004). Crude extracts of fruits, herbs, vegetables, cereals and other plant materials are rich in phenolics and are increasingly of interest to the food industry because they retard the oxidative degradation of lipids and thereby improve the quality and nutritional value of food (Protestos et al., 2006).

Rosemary (*Rosmarinus officinalis* L.) is a well-known aromatic plant used all around the world for different medicinal purposes. Recent research has shown that rosemary extracts have a variety of pharmacological activities, such as antimicrobial, antioxidant, cognition-improving, cancer chemoprevention and DNA-protective effects (Tsukamoto *et al.,* 1995). Natural antioxidants are applied to foods in two ways, either direct use or by using their extracts. Rosemary and origanum are medicinal plants that have strong antioxidant activity. The various extracts and essential oils from rosemary and origanum were previously used as antioxidants in different models (Yanishlieva et al., 2006).

Rosemary leaves contain up to 2 % essential oil, about 8 % tannins, ursolic acid, flavonol compounds, alkaloids, vitamins, minerals and other biologically active substances. Tinctures and infusions from rosemary leaves have been applied in traditional medicine for treating rheumatism, gout, neurosis, eczemas, decubitus wounds, mouth inflammation and other health problems. Rosemary is one of the most effective spices widely used in food processing as well it is the only spice commercially available for use as an antioxidant in Europe and the United States. There are a limited number of studies about the direct application of natural antioxidants. Antioxidant properties of rosemary have been well documented (Yanishlieva et al., 2006). It is considered as lipid antioxidant, metal chelator and super oxide radical's scavenger (Carrillo and Tena, 2005). Many different solvents, as well as extraction techniques have been used for the isolation of the antioxidative compounds (Szumny *et al.,* 2010). However, up to our knowledge, there is no available information concerning usage of rosemary essential oil or its extracts on the inhibition of mycotoxins.

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 internal organ weights, biochemical and mortality of broilers fed with aflatoxin B_1 and Rosemary essence.

2.1. 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 (group 1); 600 ppb Aflatoxin B1 (group 2); 500ppm of Rosemary essence (group 3) and 600ppb Aflatoxin B1+500 ppm of Rosemary essence (group 4). 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:

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.

2.2. 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, based on the titers obtained from blood samples of chicks at different ages, 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.

2.3. Studied Parameters

Visceral Organ Weights

Upon obtaining the permission of Ethical Committee of the University, at the end of the trial, six birds from each replicate which were closed to average weight of each replicate were sacrificed by cutting the jugular vein method and blood samples were individually collected in 10-mL heparinized tubes and stored on ice cubes for further hematology analysis. The visceral were then opened and the thymus, spleen, bursa of Fabricius, liver, kidney and pancreas detached and weighed on digital top pan electronic balance (0.1g accuracy) and the later three weighed on manopan balance (1mg accuracy). The weights were adjusted to one kg live weight (g/kg BW) and treatment wise means were calculated.

2.4. Biochemical Parameters

The collected blood samples were analyzed for cholesterol, LDL and HDL using automatic analyzer (Boehringer Mannheim Hitachi 704 automatic analyzer, Japan). The methodology and the set of reagents used in respect of each parameter were as per the recommendations of the manufacturer of the analyzer system. Data are presented as means of each treatment.

2.5. Mortality

The number of dead birds in each replicate was recorded to calculate the rate of mortality. The dead birds were subjected to postmortem examination to identify the cause of death. The weekly per cent mortality up to 6th week was computed.

2.6. 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).

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.

3. Results and Discussion

The effects of aflatoxin and Rosemary essence on visceral organ weights (g/kg live weight): The results of dietary treatments on organ weights of broilers fed AF and Rosemary and their combinations on broilers at 42 days of age are shown in Table 2. Results revealed no sign of any significant changes in thymus weights of broilers fed AF or Rosemary essence in any treatment groups. Spleen weight is decreased significantly ($P<0.05$) in group fed AF, when compared with control group. Addition of ROS into the diet had shown no significant changes compared with CON. Addition of ROS into AF (group 4) has showed no improvement in the decreased spleen weight caused by AF. The weight of bursa of Fabreciuos in broilers fed AF alone fed group have showed a significant ($P<0.05$) increase when compared with control group and addition of ROS into the normal diet (group 3) and in addition with AF (group 4) had no significant differences with control group. The weight of liver - the most important internal organ - has been showed that AF caused a significant ($P<0.05$) increase in liver weight when compared with control group. Broilers fed in group 3 (Rosemary essence) have shown no significant changes with control group and in group AF+ROS, liver weight is decreased in comparison with CON which indicates that ROS has no effect on compensating the increased liver weight due to aflatoxin ingestion. Kidney weight had remained unchanged and showed a non-significant difference in all treatment groups. In case of pancreas, the weight of this internal organ is decreased significantly ($P<0.05$) due to presence of aflatoxin in the diet, which addition of ROS into the AF diet could not restore this increased weight. However, the ROS alone fed group showed no changes in pancreas weight, when compared with control group.

The effects of aflatoxin and Rosemary essence on blood biochemical parameters (mg/dL): The results of dietary treatments on blood biochemical parameters of broilers fed AF and Rosemary and their combinations on broilers at 42 days of age are shown in Table 3. Results indicated that addition of AF in to the diet has increased the cholesterol level in the blood significantly ($P<0.05$), when compared with control group. In ROS group, no statistical changes were noticed in the level of cholesterol with control group and in AF+ROS group, the same effects of AF group were noticed without any impact from Rosemary essence on the AF adverse effects. In case of LDL content, the levels in AF fed group showed a significant ($P<0.05$) increase and in AF+ROS group, while increased with control group similar to AF, no changes between AF and AF+ROS were noticed. When compared with control group, ROS fed treatment has a significantly ($P<0.05$) decreased in LDL content which indicates the positive effects of sole Rosemary essence in the diet.

Nearly the same trend is found in HDL (mg/dL) content of broilers blood at 42 days stating that in AF fed group, the HDL values is increased significantly ($P<0.05$), when compared with control group. In ROS group, the HDL value found to be significantly ($P<0.05$) lesser than CON and in AF+ROS group, the HDL is decreased significantly ($P<0.05$) by comparing with AF alone fed group and remained higher than level of control group.

The effects of aflatoxin and Rosemary essence on mortality (%): The results of dietary treatments on weekly mortality of broilers fed AF and Rosemary and their combinations on broilers at 42 days of age are shown in Table 4. Results showed that during first week, there was no mortality in all treatment groups. During the second week, only control group has shown a 1.66% of mortality and other groups remained zero. The rate of mortality for the second week (15-21 days) was found to be 0 in CON, 3.33 in AF fed group, 0 in ROS and 1.66% in AF+ROS group. During the week IV, the data on mortality rate (%) of different treatments were 0, 1.66, 0 and 1.66 in CON, AF, ROS and AF+ROS fed groups, respectively. At week V, the mortality found in was 1.66, 1.66, 0 and 1.66 for CON, AF, ROS and AF+ROS fed groups, respectively. In the last week of experiment, the mortality for control and Rosemary essence alone fed groups remained zero and in AF fed group was found 6.66 and in AF+ROS fed group was found to be 1.66. The overall data on percentage mortality in different groups found to be 3.32, 13.31, 0 and 6.64 in CON, AF, ROS and AF+ROS treatment groups, respectively. This shows that the increased percentage of mortality due to aflatoxin ingestion could be controlled by inclusion of Rosemary essence in the diet.

Table 2. Visceral Organ weights (g/kg live weight) of broilers fed Aflatoxin B_1 and Rosemary essence (Mean±SE)

Treatment groups	Thymus	Spleen	bursa of Fabreciuos	Liver	Kidney	Pancreas
CON[1]	4.18±0.24[a]	1.77±0.04[a]	1.48±0.01[b]	27.62±0.58[c]	7.42±0.18[a]	3.51±0.65[a]
AF[2]	4.16±0.35[a]	1.06±0.14[b]	1.95±0.17[a]	31.10±0.18[a]	7.45±0.59[a]	2.53±0.44[b]
ROS[3]	4.14±0.24[a]	1.79±0.07[a]	1.47±0.21[b]	28.15±0.13[c]	7.40±0.34[a]	3.77±0.84[a]
AF+ROS[4]	4.14±0.52[a]	0.97±0.05[b]	1.45±0.17[b]	30.57±0.94[b]	7.39±0.37[a]	2.73±0.47[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 3. Biochemical parameters of broilers fed Aflatoxin B1 and Rosemary essence (Mean±SE)

Treatment groups	Cholesterol (mg/dl)	LDL (mg/dl)	HDL (mg/dl)
CON[1]	97.33±0.48[b]	40.05±0.85[b]	66.33±0.57[c]
AF[2]	143.52±0.36[a]	66.52±0.44[a]	83.29±0.19[a]
ROS[3]	94.41.±0.62[b]	31.97±0.23[c]	55.24±0.19[d]
AF+ROS[4]	141.92±0.74[a]	67.45±0.54[a]	72.41±0.37[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 4. Mortality rates (%) 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)	Total (day 0-42)
CON[1]	0	1.66	0	0	1.66	0	3.32±0.25[c]
AF[2]	0	0	3.33	1.66	1.66	6.66	13.31±0.94[a]
ROS[3]	0	0	0	0	0	0	0.00±0.00[d]
AF+ROS[4]	0	0	1.66	1.66	1.66	1.66	6.64±0.72[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

In current experiment, the effects of aflatoxin in the broiler diet have proved to show its negative impacts on visceral organ weight, some biochemical parameters and mortality. The present study indicated that the relative weights of some internal organs were significantly ($P<0.05$) increased in broilers consuming aflatoxin, compared to the control diet.

These discoveries are in agreement of findings of other scientists across the world (Manafi et al., 2011). The increase in liver weights was in accordance to the findings of Raju and Devegowda (2002). The liver is the primary target organ of toxins. In addition, increases in the relative weights of liver and pancreas (Kubena et al., 1998) and spleen (Kubena et al., 1990) of broilers fed aflatoxin contaminated diets have also been reported previously.

The increase in liver weight could be attributed to increased lipid metabolism in liver due to impaired fat metabolism which brings appreciable changes in the general functioning and gross appearance of liver.

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 (Yunus et al., 2008). The decrease in the relative weight of spleen might be a compensatory mechanism for the drop in weight of the bursa of fabricius. There is evidence regarding biphasic nature of the effects of AFB_1 on humoral immunity. Recent report indicates that humoral immune response from broilers could be decreased, depending upon the level and duration of exposure to the toxin. Tung *et al.* (1970) described that toxin increases lysosomal enzyme activity in liver and skeletal muscles of chicken, due to increase in lysosomal activity. In our study, the weight of bura is increased in AF fed group which could be attributed to an immunosuppressive effect of aflatoxin. 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.

Blood biochemical parameter changes in chicks fed on aflatoxin contaminated diets in this study were more or less similar to those reported in the literature. Increases in the serum cholesterol have been observed in chicks suffering from aflatoxicosis (Kubena et al., 1997). Decreased weight of spleen observed in the present study is in contrary with findings of trial conducted by Kubena *et al*. (1990). High mortality was observed in broilers fed aflatoxin containing diet when compared to the control group, which could be due to toxicity caused by the toxin. The mortality pattern in the present study was in accordance to the findings of Kubena *et al.* (1997). Manafi and Khosravinia, (2013) also reported similar results on mortality of broilers due to mycotoxicosis.

By incorporating Rosemary essence in the AF fed broilers, the negative influences of this toxin has been modulated to some extent. This may be due to the phenolic components present in the Rosemary oil. The rosemary contains antioxidants which serve as a defensive factor against free radicals in the animal body. They act by inhibiting the initiation and propagation steps, leading to the termination of the reaction and delaying the oxidation process. The mechanism of antioxidants may involve scavenging and suppressing the formation of free radicals and peroxidation procedure (Gulcin, 2006). The possible of the antioxidant constituents of plant materials in the maintenance of health raising interest among scientists and food manufacturers, as consumers move towards functional foods with specific health effects (Loliger, 1991).

Herbal species are known to play an important role in the diet of inhabitants of different parts of the world. Many of

these plants are nutritionally important because of their high vitamin, mineral and fiber contents (Horvathova et al., 2010). The total phenolic and antioxidant activities of these plants need to be investigated because they are being used in traditional medicine and in food products. The majority of antioxidant activities of fruits and vegetables may be from phenolic compounds rather than vitamins, since some phenolic compounds have much stronger antioxidant activities against peroxyl free radicals (Cao et al., 1997). Great efforts have been done in finding naturally occurring antioxidants of plant origin. Thus, many plant phenolic compounds exhibiting antioxidant properties have been studied and proposed for protection against oxidation. The aqueous extract of rosemary leaves can inhibit the spontaneous shrinkage and the shrinkage induced by acetylcholine, histamine and barium chloride in jejunal rabbits (Moss et al., 2003). As an important aromatic plant, rosemary has stimulated much consideration for its multi-biological activities. In this research, rosemary exhibited different effects on the visceral organs, biochemical parameters and mortality.

Rosemary essence showed good effects on reducing cholesterol, HDL, LDL and reduction number of dead birds, due to presence of essential oils in its content. 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 essential oils and antioxidants are studied before, but as commercial products, might not be suitable to compare, since the sole rosemary essence is not reported. We concluded that the dosage of Rosemary essence in experiment was possibly good.

4. Conclusions

Results obtained from this study concludes that besides the adverse and negative effects of aflatoxin B1 in the diet on visceral organ weight, some biochemical parameters and mortality of broilers, combining Rosemary essence as natural non- antibiotic growth promoter feed additive can rule its beneficial effects to some extent. However, there is insufficiency in the marks of its beneficial effects in nutrient digestibility and gut function of broilers.

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