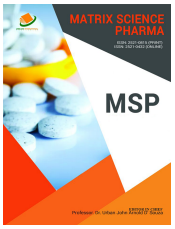
ZIBELINE INTERNATIONAL PUBLISHING

ISSN: 2521-0815 (Print)
ISSN: 2521-0432 (online)

CODEN : MSPAFY

Matrix Science Pharma (MSP)

DOI : http://doi.org/10.26480/msp.01.2018.10.13



ADVERSE EFFECT OF *OXALIS CORNICULATA*ON GROWTH PERFORMANCE OF BROILER CHICKSDURING AFLATOXICOSIS

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ARTICLE DETAILS

Article History:

Received 12 November 2017
Accepted 12 December 2017
Available online 1 January 2018

ABSTRACT

The present study was designed to investigate effect of *Oxalis corniculata* (Creeping wood sorrel) against aflatoxicosis in straight run broiler chicken (Hubbard strain). Hundred and twenty-day old broiler chicks were randomly divided into six groups with two replicates. Two different levels of *O.corniculata* (250; 500 mg/kg b.w) with and without AFB_1 (350 ppb) and a control treatment were added to the diet. Dietary treatment initiated at the end of 1st week and sustained for six weeks. *O.corniculata* supplementation in the diet during five weeks significantly reduced body weight, cumulative feed intake and feed conversion ratio of the treated groups in dose dependent manner. Dietary incorporation of *O.corniculata* 250 mg to 500mg induced negative effects on overall broiler health performance.

KEYWORDS

Oxalis corniculate, aflatoxicosis, cumulative feed intake, feed conversion ratio.

1. INTRODUCTION

Mycotoxin contaminated feed especially aflatoxins, now- a-days, is major health and production threat to poultry. Furthermore, residual mycotoxin in poultry product may also present a risk to humans and animals through their mutagenic, carcinogenic, teratogenic and immunosuppressive effects [1]. About 25% of the World's crop in the food supply contaminated with mycotoxin every year [2].

Poultry industry presents an activity of great significance Worldwide, including Pakistan, as it constitutes the major part of the animal protein available to population. Though, different crops used as poultry feed ingredients such as corn, peanut meal, sorghum, cottonseed meal, are susceptible to aflatoxin contamination, representing a greater risk for the incidence of mycotoxicosis in the poultry [3,4].

Mycotoxins are the toxic metabolites produced by molds under specific conditions. Aflatoxins are produced by various types of the fungi include *Aspergillus, Penicillium, Rhizopus, Cladosporium*and *Alterneria*. Basically, *Aspergillus flavus* and *Aspergillus paraciticus* recognized as potent toxic fungi [5]. Aflatoxin B_1, B_2, G_1 and G_2 are more accentuated, among them aflatoxin B_1 is the most toxic, classified in the class 1 of carcinogen by International Agency for Research on Cancer [6].
During metabolism it is converted into highly reactive form, aflatoxin 8, 9 epoxides, in the liver by CYP 450 enzymes that bind to proteins and DNA, form adducts and ultimately induce toxicity (aflatoxicosis) [7].

These days, research has been focused to assort these mycotoxins and protect the poultry bird from lethal effects associated with these mycotoxins. Multiple approaches have been implemented to reduce the economic losses due to these mycotoxins in poultry feed [8].

Oxalis corniculata (Family: Oxalidaceace), commonly known as creeping wood sorrel, having wide range of biological activities [9]. It is the rich source of essential fatty acids like palmitic acid, oleic acid, linoleic acid and stearic acid [10]. By phytochemical analysis, it has revealed that it contains carbohydrates, glycosides, phenolic compounds, flavonoids, phytosterols, amino acids and volatile oil. The plant is used as tonic, stimulant; beneficial in cramps, convulsion, chest pain, inflammatory tumor, piles, anemia, insomnia, tympanite's, dyspepsia and dysentery [11,12]. It also acts as a blood purifier. Reported medicinal activities of this plant are the abortifacient, wound healing and antidiarrhoeal activity [13-15]. By keeping in view the medicinal importance of *O.corniculata,* the current study designed to evaluate the efficacy of the plant against aflatoxicosis in broiler chicken.

2. MATERIALS AND METHODS

This experiment was planned and carried out in the Department of Poultry Science, Faculty of Animal husbandry, University of Agriculture, Faisalabad, Pakistan, with objective of evaluating the growth performance of broilers fed with aflatoxin B_1 and *O.corniculata.*

2.1 *Oxalis corniculata* extract preparation

Oxalis Corniculata at maturity was collected from District Faisalabad (Pakistan), identified and a specimen vide voucher no. 312-3-16 was submitted at herbarium, Department of Botany, University of Agriculture, Pakistan. All parts of the plant (leaves, stem, flowers and roots) were shade dried for two weeks, chopped, and grinded mechanically of mesh size 1 mm to make 2 kg powder which was extracted with 4.0 litres of ethanol with occasional shaking and filtered. The filtrate was evaporated through rotary vacuum evaporator at 40°C to obtain *Oxalis corniculate* ethanolic extract (OCEE). The extract was stored at 4 ºC till further use.

2.2 Aflatoxin Production

Pure culture of *Aspergillus flavus* (CECT 2687) was used to produce aflatoxin B_1 through basmati rice fermentation by following the method in a study [16]. After 6 days of incubation at 28ºC, the aflatoxin was extracted and quantified through HPLC.

2.3 Experimental Animal

A total of hundred and twenty day-old unsexed (Hubbard) broiler chicks were purchased from the local hatchery and acclimatized in an open shed environment for the period of one week. On day seven, chicks were wing banded, weighed and randomly spread in a completely randomized

experimental design with six treatments and two replications of ten 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. A corn soy meal-based feed having 22% crude protein and 3000 Kcal/kg metabolizable energy was prepared without addition of any toxin binder, vitamins, mineral supplements and antibiotics. This diet was used as basal diet for all treatments, aflatoxin contaminated rice powder was used for inclusion of aflatoxicosis mixed according to required level. The dose of OCEE was adjusted with the help of literature.

2.4 Experimental Design

Feeding of test diets commenced at seven days of age and continued till the termination of experiment at six weeks of age. The chicks were assigned to the following treatment groups: 1). CON (control), 2); AF (aflatoxin B_1 at 350 ppb); 3). OC_{250mg} (*O. corniculate* at 250 mg);4). AF_{350ppb}+ OC_{250mg} (Aflatoxin B_1 and *O. corniculate* at 350 ppb and 250 mg, respectively); 5). OC_{500mg} (*O. corniculata*at500mg);6).AF_{350ppb}+ OC_{500mg} (Aflatoxin B_1 and *O. corniculate* at 350 ppb and500mg, respectively). Broiler Chicks were reared under standard management conditions and provided feed and water *ad libitum* throughout the study.

2.5 Parameters studied

2.5.1 Body weight and feed conversion ratio

The observations on body weight, feed intake, feed conversion ratio and mortalities were recorded at the end of each week, for the whole experimental period of seven weeks. Four birds from each replicate randomly selected and weighed with the help of an electric balance. Birds were offered feed daily and refusal was recorded on the next day. Record of weekly feed intake was kept separately and recorded to compute feed conversion ratio per bird per week as described in a study.

2.6 Statistical Analysis

The data obtained were subjected for analysis of variance (ANOVA) by using General Linear Model procedure and Duncan's new multiple range test was applied to compare means by using SPSS version. 22. Significant differences in all analysis were based on $P < 0.05$ [17].

3. RESULTS AND DISCUSSION

3.1 Effect of *Oxalis corniculata* on body weight and FCR

The effects of aflatoxin and *Oxalis corniculate* on broilers have been shown in Table 1. At the time of initiation, it was found that all chicks were almost in similar range and uniform. They were provided the same feed to acclimatize them according to open shed environment. After first week, the chicks were divided into groups of similar body weight. It was observed that at the end of second week, there was a significant reduction in body weight of broilers fed AFB_1as compared to control group. In group *Oxalis corniculate* supplemented with 500 mg per kg body weight, significant difference was observed as compared to control group. In AFB_1+OC, at both levels adverse effects of aflatoxin which was seen in AF group significantly (P<0.05) increased in dose dependent manner. At day 21, the AFB_1fed group had significantly (P<0.05) lower body weight, compared with control group. The OC (500 mg) along aflatoxin fed group has shown the least body weight among all other treatments. At the end of 28 days, The AFB_1fed group brought down the body weight significantly (P<0.05) up to 63 grams and addition of OC (500 mg) also influenced the negative impact on over all broiler health and could decline the body weight equivalent to 52 grams. However, the body weight of OC (250 mg) and AFB_1+OC (250 mg) were not significantly different which indicates the fact that OC (250mg) is not able to ameliorate the toxic effects of $AFB_1$and decreased the body weight. The maximum BW seen in the control group that ensured that it had adverse effect on over all broiler health. At 35th day of the animal trial, the gap between the $AFB_1$and control group has been increased and addition of OC also could significantly (P<0.05) deleteriously effect the BW of broilers. At the end day of 6th week, the final BW of broilers fed $AFB_1$was found to be (1899 g) and compared with that of control group (2159 g), there was a significant decrease in AFB_1fed groups. So, it has been confounding that addition of OC (500 mg) to AFB_1contaminated diet had significantly (P<0.05) reduced the BW of broilers at 42 days but not closer to the aflatoxin fed group. *O. corniculata* not able to ameliorate the toxic effects of aflatoxin. It was used as medicinal plant, but not able to remove the toxic effects of aflatoxin in this research study. Though, *O. corniculata* (500 mg) along with aflatoxin has shown the lowest BW (1922 g) which stands for the least body weight among all other treatments used in this experiment.

The effects of cumulative feed consumption of broilers fed different dietary treatments are shown in Table 2. At the end of first week the chicks fed almost similar diet. Not very prominent difference in feeding habit observed among the chicks during the acclimatized period. So, when they grouped according to different dietary conditions significant (P<0.05) difference in their feeding habits have been observed. At the end of second week, the feed intake was significantly (P<0.05) increased in AFB_1fed group and OC at both levels (250 mg, 500 mg) could enhanced the adverse effects ofAFB_1. At day 21, the OC (250 mg) alone fed group showed a higher feed consumption and addition of OC could significantly (P<0.05) increased this parameter. The feed intake of broilers fed in control group was minimum followed by OC (250 mg) in this week. At the end of 4th and 5th week, comparable trend was followed, and maximum feed consumption was found OC (500 mg) in group. The addition of OC into AFB_1group could significantly (P<0.05), increased the feed consumption in dose dependent manner, although, feed intake OC groups were found to be significantly (P<0.05) greater than control group. At the end of the 5th week, the trend was changed. It has been observed that feed consumption in AFB_1fed group significantly (P<0.05) decreased as compared to the control group, however, the feed consumption in *O. corniculata*fed groups at both levels were also significantly (P<0.05) lower than control group followed by *O. corniculate* (500 mg) and (250 mg) at both levels.

The effects of cumulative feed consumption of broilers fed different dietary treatments are shown in Table 3. At the end of first week the chicks fed almost similar diet. Not very prominent difference in feeding habit observed among the chicks during the acclimatized period. So, when they grouped according to different dietary conditions significant (P<0.05) difference in their feeding habits have been observed. At the end of second week, the feed intake was significantly (P<0.05) increased in AFB_1fed group and OC at both levels (250 mg, 500 mg) could enhanced the adverse effects ofAFB_1. At day 21, the OC (250 mg) alone fed group showed a higher feed consumption and addition of OC could significantly (P<0.05) increased this parameter. The feed intake of broilers fed in control group was minimum followed by OC (250 mg) in this week. At the end of 4th and 5th week, comparable trend was followed, and maximum feed consumption was found OC (500 mg) in group. The addition of OC into AFB_1group could significantly (P<0.05), increased the feed consumption in dose dependent manner, although, feed intake OC groups were found to be significantly (P<0.05) greater than control group. At the end of the 5th week, the trend was changed. It has been observed that feed consumption in AFB_1fed group significantly (P<0.05) decreased as compared to the control group, however, the feed consumption in *O. corniculata*fed groups at both levels were also significantly (P<0.05) lower than control group followed by *O. corniculate* (500 mg) and (250 mg) at both levels.

Aflatoxins are notorious food and feed contaminants in the poultry feed across the globe [18-21]. Aflatoxin B_1 is directly associated with mal absorption of the nutrients especially macronutrients because of decreased activity of digestive enzymes during aflatoxicosis [22]. But a conspicuous difference in the vulnerability among various poultry strains has been noticed, it might be due to variation in metabolic rates of these birds, type and sensitivity of analytical methods available at the time of study.

Earlier, it has been supposed that AFB_1dose more than 1.25 ppm in the diet is able to cause the negative impact on growth performance; but the current literature suggests that the administration of lower dosage (0.02 ppm), can damage the cells and reduce growth performance in animals [23,24]. Poultry and fish are extremely sensitive to AFB_1 and even respond to low dose as 15-30 ppb [18]. Thus, the reasoning for these differences in earlier and current reports could be the difference in the performance of broilers at the time of the study. Now-a- days newly established generations of broiler are known to gain more weight by utilizing less feed in shorter period of time. The dose of AFB_1 (350 ppb) used in this study, had shown a direct negative impact on broiler overall performance including body weight, feed intake and FCR directly.

Phytochemicals are the secondary metabolites produced by medicinal plants. These phytochemicals include phenolics, flavonoids, tannins and alkaloids. Antioxidants like vitamin C and E used to decrease the toxicity of chemical toxins. Recently, plant derived polyphenols gained more intention to use them as antioxidants [25]. Phytochemical studies of *O. corniculata* have indicated the presence of tannins, alkaloids, saponins, flavonoids, linoleic acid, stearic acid and palmitic acid. It is also the rich source of vitamin C, niacin and oxalates [9]. In the current study, despite of its medicinal significance, results revealed that *O. corniculta* produced negative effects on the health of broiler chicks and these effects become more synergetic, when administered along with aflatoxin B_1. So, it might be due to alkaloids, saponins and oxalates, constitutes of the plant. Synergetic actions basically based on nature of the chemical compound

that interact with single or multiple targeted sites, responsible for a physiological response.

4. CONCLUSION

The results indicate the adverse effect of the medicinal plant (*O. corniculata*). It appears that broiler chicks may be more sensitive to the toxicity of oxalic acid content of plant relative to other animals, directly affect the health performance of the chicks. Particularly, fast growing broiler chicken may be more sensitive to toxic challenge of oxalic acid content because of the high metabolic rate associated with rapid growth.

ACKNOWLEDGEMENTS

The authors are grateful to Higher Education Commission of Pakistan for funding this research under HEC indigenous Ph. D fellowship program.

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Table 1: Body weight (g) of chicks fed aflatoxin and different levels of *Oxalis corniculata* (Mean±SE)

Treatment	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	43.25±0.97 ^{a}	150.05±1.45 ^{a}	370.35±2.42^{a}	740.65±2.90^{a}	1211.20±2.10^{a}	1746.25±1.95^{a}	2159.90±2.75^{a}
AF_{350ppb}	43.20±0.55 ^{a}	150.20±0.27 ^{a}	295.25±2.35^{d}	691.30±1.71^{d}	1148.55±1.33^{cd}	1588.05±0.99^{e}	1899.20±1.58^{f}
OC_{250mg}	43.80±0.97 ^{a}	149.80±2.06 ^{a}	375.40±2.97^{a}	728.65±3.73^{b}	1191.50±12.9^{ab}	1708.75±4.56^{ab}	2078.95±9.50^{b}
OC_{500mg}	43.60±1.15 ^{a}	148.75±1.40 ^{a}	348.75±2.41^{b}	705.95±4.93^{cd}	1159.30±13.5^{bc}	1727.70±9.63^{b}	2021.30±6.73^{c}
OC_{250mg} +AF_{350ppb}	43.75±0.83 ^{a}	149.35±1.88 ^{a}	339.05±3.48^{b}	718.95±2.94^{bc}	1181.75±10.4^{b}	1671.85±13.0^{c}	1952.85±14.5^{d}
OC_{500mg} +AF_{350ppb}	43.85±0.89 ^{a}	149.45±2.19 ^{a}	313.55±3.09^{c}	675.85±7.87^{e}	1122.45±12.4^{d}	1640.05±3.92^{d}	1922.70±6.28^{e}

Mean values within a row with different superscript letters (a to f) were significantly different (P<0.05). CON (control), AF (aflatoxin B_1 at 350ppb), OC_{250mg} (*Oxalis corniculata*at 250 mg), OC_{500mg} (*Oxalis corniculata*at 500 mg), AF_{350ppb}+ OC_{250mg} (Aflatoxin B_1 and *Oxalis corniculata*at 350ppb and 250 mg, respectively), AF_{350ppb}+ OC_{500mg} (Aflatoxin B_1 and *Oxalis corniculata*at 350ppb and 500 mg, respectively). SE: (Standard Error)

Table 2: Cumulative Feed Consumption (g/bird) of chicks fed aflatoxin and different levels of *Oxalis corniculata* (Mean±SE)

Treatment	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	167.23±0.91^{a}	501.70±0.21^{f}	1125.01±0.19^{e}	2039.15±0.30^{f}	3045.43±0.18^{f}	4340.75±0.21^{a}
AF_{350ppb}	167.05±0.82^{a}	548.80±0.71^{d}	1210.50±0.81^{d}	2148.60±0.29^{e}	3164.50±0.13^{e}	4120.67±0.91^{d}
OC_{250mg}	166.02±0.67^{a}	525.10±0.53^{e}	1101.34±0.31^{f}	2199.44±0.15^{d}	3198.10±0.43^{d}	4261.24±0.32^{b}
OC_{500mg}	168.01±0.74^{a}	578.68±0.19^{b}	1242.10±0.56^{c}	2289.06±0.56^{b}	4076.13±0.87^{b}	4222.12±0.51^{c}
OC_{250mg} +AF_{350ppb}	166.08±0.83^{a}	556.23±0.43^{c}	1282.12±0.71^{b}	2235.23±0.34^{c}	4023.11±0.33^{c}	4080.53±0.76^{e}
OC_{500mg} +AF_{350ppb}	167.01±0.61^{a}	623.78±0.86^{a}	1302.18±0.93^{a}	2356.90±0.81^{a}	4089.14±0.43^{a}	3989.13±0.32^{f}

Mean values within a row with different superscript letters (a to f) were significantly different (P<0.05). CON (control), AF (aflatoxin B_1 at 350ppb), OC_{250mg} (*Oxalis corniculata*at 250 mg),OC_{500mg} (*Oxalis corniculata*at 500 mg)AF_{350ppb}+ OC_{250mg} (Aflatoxin B_1 and *Oxalis corniculata*at 350ppb and 250 mg, respectively), ,AF_{350ppb}+ OC_{500mg} (Aflatoxin B_1 and *Oxalis corniculata*at 350ppb and 500 mg, respectively). SE: (Standard Error)

Table 3: Feed Conversion Ratio (FCR) of chicks fed aflatoxin and different levels of *Oxalis corniculata* (Mean±SE)

Treatment	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	1.11±0.90^{a}	1.34±0.13^{d}	1.51±0.16^{c}	1.68±0.15^{d}	1.74±0.89^{d}	2.00±0.98^{c}
AF_{350ppb}	1.11±0.11^{a}	1.87±0.81^{b}	1.75±0.51^{b}	1.87±0.66^{c}	1.99±0.73^{b}	2.16±0.18^{a}
OC_{250mg}	1.10±0.31^{a}	1.39±0.76^{d}	1.51±0.13^{c}	1.84±0.76^{c}	1.87±0.19^{c}	2.04±0.91^{c}
OC_{500mg}	1.12±0.16^{a}	1.65±0.56^{c}	1.75±0.19^{b}	1.97±0.77^{b}	2.35±0.67^{a}	2.08±0.72^{b}
OC_{250mg} +AF_{350ppb}	1.11±0.51^{a}	1.64±0.67^{c}	1.78±0.15^{b}	1.89±0.81^{c}	2.40±0.87^{a}	2.08±0.89^{b}
OC_{500mg} +AF_{350ppb}	1.11±0.18^{a}	1.98±0.41^{a}	1.92±0.34^{a}	2.09±0.15^{a}	2.49±0.52^{a}	2.07±0.56^{b}

Mean values within a row with different superscript letters (a to d) were significantly different (P<0.05). CON (control), AF (aflatoxin B_1 at 350ppb), OC_{250mg} (*Oxalis corniculata*at 250 mg),OC_{500mg} (*Oxalis corniculata*at 500 mg),AF_{350ppb}+ OC_{250mg} (Aflatoxin B_1 and *Oxalis corniculata*at 350ppb and 250 mg, respectively), AF_{350ppb}+ OC_{500mg} (Aflatoxin B_1 and *Oxalis corniculata*at 350ppb and 500 mg, respectively). SE: (Standard Error)