TÜBİTAK

**Turkish Journal of Veterinary and Animal Sciences**

**http://journals.tubitak.gov.tr/veterinary/**

**Research Article**

Turk J Vet Anim Sci
(2014) 38: 207-211

doi:10.3906/vet-1303-9

# Ameliorative efficacy of citrus fruit oil in aflatoxicosis in broilers: a growth and biochemical study

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**Received:** 04.03.2013 • **Accepted:** 17.09.2013 • **Published Online:** 28.02.2014 • **Printed:** 28.03.2014

**Abstract:** An experiment was conducted to study the efficacy of citrus fruit oil on growth parameters and biochemical parameters of broilers in 4 groups with 2 replicates of 20 chicks in each. Standard broiler diet without aflatoxin was given to group A, basal diet + citrus fruit oil 2.5 g $kg^{-1}$ to group B, basal diet + 1 ppm aflatoxin to group C, and 1 ppm aflatoxin + basal diet + citrus fruit oil 2.5 g $kg^{-1}$ to group D. The results showed that supplementation in group D had a moderate effect on posttreatment growth performance, relative organ weight, and biochemical parameters. It is suggested that citrus fruit oil as a feed additive causes partial amelioration of aflatoxicosis.

**Key words:** Citrus fruit oil, aflatoxicosis, growth performance, biochemical parameters, ameliorative efficacy

## 1. Introduction

The poultry industry in India has achieved phenomenal progress in recent decades. The profit margin of the poultry industry mainly depends on the quality of feed and ingredients. Fungal contamination of agricultural products is often unavoidable and of worldwide concern. Mycotoxins are one of the major factors affecting poultry productivity and product quality. Among mycotoxins, aflatoxins are of more concern as they account for 25% of poultry feed samples and ingredients contamination (1).

Citrus fruit oil (active ingredient d-limonene) shows detoxification and antioxidant properties by increasing the level of glutathione S-transferase (2). D-Limonene, a monoterpenoid constituent of citrus fruit oil, blocks tumor induction by chemical carcinogens by preventing bioactivation of procarcinogens, has inhibitory effects on cytochrome P450 enzymes, and inhibits *p*-nitrophenol hydroxylase (pNP) and 7-ethoxyresorufin *O*-deethylase (EROD) activity in vitro in liver microsomes from acetone-, phenobarbital (PB)-, and β-naphthoflavone (BNF)-treated mice (3). Thus, the present study was designed to evaluate the ameliorative efficacy of citrus fruit oil in aflatoxicosis and its effect on growth performance and biochemical parameters in broilers.

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## 2. Materials and methods

### 2.1 Aflatoxin production

Aflatoxin was produced on rice using *Aspergillus parasiticus*, NRRL 2999 culture by the method of Shotwell et al. (4). Fermented rice was autoclaved and ground to a fine powder. The aflatoxin content in the rice powder was analyzed by a thin layer chromatography (TLC) fluorodensitometer (Camag II, Basel, Switzerland) (5). The rice powder was added to the basal diet to provide the final concentration of 1 ppm (1 mg $kg^{-1}$). Aflatoxin and citrus fruit oil (CFO) was added to feed wherever required and fed to birds from day 7 to 42 of age.

### 2.2. Chickens and feed

One hundred and sixty (n = 160) day-old Ross 308 broiler chicks of both sexes were procured from a commercial hatchery (Suguna Poultry Farm Pvt ltd) and reared in a battery cage system in experimental sheds with average temperature ranging from 27 to 31 °C and relative humidity of 59% to 62% with 16:8 ± 1 h L:D cycle of intensity of 10 to 20 lx. Individually, chicks were weighed and divided into 4 groups randomly with 2 replicates of 20 chicks in each as group A, B, C, and D after acclimatization for 7 days. The starter and grower basal diets were given to birds as recommended by the National Research Council (6). All

chicks were vaccinated on days 7 and 11 of age with the LaSota strain of the Newcastle disease virus and infectious bursal disease (intermediate strain), respectively.

**2.3. Experimental design**

A 2 × 2 factorial design was used and the experimental groups included basal diet (group A), basal diet + citrus fruit oil 2.5 g $kg^{-1}$ (group B), basal diet + 1 ppm aflatoxin (group C), and 1 ppm aflatoxin + basal diet + citrus fruit oil 2.5 g $kg^{-1}$ (group D). Citrus fruit oil containing volatile oils of citrus fruits was obtained from M/s Tetragon Chemie, Bangalore, India.

The experimental protocol used was approved by the Institutional Animal Ethics Committee, Veterinary College, Bangalore. Handling of animals was according to the guidelines of Committee for the Purpose of Control and Supervision of Experiments (CPCSEA), Ministry of Environment and Forests, Government of India.

Dose of aflatoxin was selected based on previous reports (7,8). The citrus fruit oil dose selected was based on pilot experiments conducted and it was found that 2.5 g $kg^{-1}$ of broiler feed for 35 days was suitable.

Six birds selected randomly from each group were weighed individually and sacrificed on days 7, 14, 21, 28, and 35 posttreatment (Table 1) and feed conversion ratio (FCR) was calculated (Table 2). Blood was collected from each bird at each interval from wing veins using a syringe and needle for serum biochemical estimation and stored at −20 °C. A detailed necropsy was conducted; the liver was removed and weighed. Relative organ weight (liver, kidneys, spleen, bursa of Fabricius, thymus) was calculated as grams of organ per 100 grams of body weight.

$$\text{R Wt of organ} = [\text{Wt of the organ (g)}/\text{body Wt (g)}] \times 100$$

Individual serum samples were analyzed for aspartate transferase (AST), alkaline phosphatase (ALP), alanine amino transferase (ALT), total protein (TP), and total albumin (TA) using standard kits (Spam Diagnostics Pvt ltd, Surat, India) by automatic analyzer (Tables 3–7).

**2.4. Statistical analysis**

The experimental data (i.e. relative organ weights and serum biochemistry (ALT, AST, total protein and total

**Table 1.** Effect of feeding aflatoxin-contaminated diet supplemented with or without CFO on body weights (g) of broiler chicks[a].

| Day posttreatment | Group A | Group B | Group C | Group D |
|---|---|---|---|---|
| Body weight (g) | | | | |
| 7 | 116 ± 1.36[a] | 116 ± 1.38[a] | 114 ± 1.40[a] | 115 ± 1.34[a] |
| 14 | 325 ± 5.72[a] | 322 ± 4.15[a] | 320 ± 4.99[a] | 327 ± 4.11[a] |
| 21 | 683 ± 17.44[a] | 663 ± 13.98[a] | 551 ± 14.30[b] | 657 ± 11.28[a] |
| 28 | 1105 ± 23.45[a] | 1117 ± 25.78[a] | 927 ± 31.22[b] | 1060 ± 21.19[a] |
| 35 | 1620 ± 44.40[a] | 1574 ± 39.92[a] | 1315 ± 23.19[b] | 1584 ± 28.96[a] |

[a]Mean ± SEM values with different superscripts within a row differ significantly at $P < 0.05$.

**Table 2.** Effect of feeding aflatoxin-contaminated diet supplemented with or without CFO on feed conversion ratio (FCR) of broiler chicks[a].

| FCR (%) | | | | |
|---|---|---|---|---|
| 7 | 1.713 | 1.812 | 1.881 | 1.736 |
| 14 | 1.620 | 1.621 | 2.407 | 1.692 |
| 21 | 2.168 | 2.104 | 2.466 | 2.213 |
| 28 | 2.013 | 1.992 | 2.943 | 2.352 |
| 35 | 1.992 | 2.412 | 3.173 | 2.666 |

[a]Mean ± SEM values with different superscripts within a row differ significantly at $P < 0.05$.

**Table 3.** Effect of feeding aflatoxin-contaminated diet supplemented with or without CFO on aspartate amino transferase (AST) of broiler chicks[a].

| Day posttreatment | Group A | Group B | Group C | Group D |
|---|---|---|---|---|
| AST (I.U.) | | | | |
| 7 | 229.0 ± 11.28[a] | 212.9 ± 10.73[a] | 267.1 ± 14.45[a] | 231.9 ± 2.03[a] |
| 14 | 204.0 ± 1.33[a] | 207.2 ± 5.13[a] | 270.1 ± 11.91[b] | 220.4 ± 14.29[ab] |
| 21 | 245.0 ± 20.32[a] | 211.8 ± 16.54[a] | 319.5 ± 6.00[b] | 241.7 ± 4.44[a] |
| 28 | 206.4 ± 6.68[a] | 221.5 ± 7.74[a] | 273.0 ± 12.33[b] | 244.6 ± 3.99[ab] |
| 35 | 269.0 ± 5.83[a] | 227.5 ± 10.27[a] | 271.9 ± 11.17[b] | 251.7 ± 5.79[ab] |

[a]Mean ± SEM values with different superscripts within a row differ significantly at $P < 0.05$.

**Table 4.** Effect of aflatoxin and supplementation of CFO in the diet on alkaline phosphatase (ALP) of broiler chicks[a].

| ALP (I.U.) | | | | |
|---|---|---|---|---|
| 7 | 2057 ± 362.5[a] | 2289 ± 194.3[a] | 3684 ± 260.8[b] | 3391 ± 614.6[ab] |
| 14 | 1896 ± 103.2[a] | 2239 ± 245.8[a] | 3877 ± 416.7[b] | 2898 ± 262.8[ab] |
| 21 | 2737 ± 362.5[a] | 1906 ± 194.3[a] | 4096 ± 260.8[b] | 3061 ± 614.6[a] |
| 28 | 1713 ± 103.2[a] | 1528 ± 245.8[a] | 3823 ± 416.7[b] | 2698 ± 262.6[ab] |
| 35 | 1672 ± 327.7[a] | 1656 ± 82.5[a] | 3828 ± 527.9[b] | 2546 ± 442.5[ab] |

[a]Mean ± SEM values with different superscripts within a row differ significantly at $P < 0.05$.

**Table 5.** Effect of aflatoxin and supplementation of CFO in the diet on alanine transferase (ALT) of broiler chicks [a].

| ALT (I.U.) | | | | |
|---|---|---|---|---|
| 7 | 16.59 ± 3.27[a] | 13.95 ± 1.43[a] | 23.78 ± 2.19[a] | 21.13 ± 3.41[a] |
| 14 | 6.86 ± 0.59[a] | 7.57 ± 1.47[a] | 24.49 ± 2.07[b] | 16.17 ± 1.28[a] |
| 21 | 8.52 ± 1.23[a] | 18.06 ± 1.08[a] | 43.29 ± 7.00[b] | 22.20 ± 0.82[a] |
| 28 | 14.32 ± 1.74[a] | 14.19 ± 2.12[a] | 38.41 ± 5.98[b] | 21.22 ± 0.99[a] |
| 35 | 13.96 ± 3.87[a] | 14.84 ± 3.57[a] | 19.16 ± 2.99[a] | 11.885 ± 2.21[a] |

[a] Mean ± SEM values with different superscripts within a row differ significantly at $P < 0.05$.

**Table 6.** Effect of aflatoxin and supplementation of CFO in the diet on total protein (g $dL^{-1}$) of broiler chicks[a].

| Day posttreatment | Group A | Group B | Group C | Group D |
|---|---|---|---|---|
| | Total protein (g %) | | | |
| 14 | 2.68 ± 0.18[a] | 2.37 ± 0.09[a] | 1.82 ± 0.09[b] | 2.37 ± 0.14[ab] |
| 21 | 2.48 ± 0.12[a] | 2.53 ± 0.14[a] | 1.69 ± 0.04[b] | 2.35 ± 0.09[ab] |
| 28 | 2.56 ± 0.13[a] | 2.75 ± 0.11[a] | 1.81 ± 0.24[b] | 2.48 ± 0.06[a] |
| 35 | 2.55 ± 0.15[a] | 2.57 ± 0.07[a] | 1.85 ± 0.18[b] | 2.73 ± 0.07[a] |

[a]Mean ± SEM values with different superscripts within a row differ significantly at $P < 0.05$.

**Table 7.** Effect of aflatoxin and supplementation of CFO in the diet on total albumin (g $dL^{-1}$) of broiler chicks[a].

| Total albumin (g %) | | | | |
|---|---|---|---|---|
| 14 | 1.52 ± 0.02[ac] | 1.44 ± 0.04[a] | 0.77 ± 0.04[b] | 1.7 ± 0.08[c] |
| 21 | 1.65 ± 0.04[a] | 1.48 ± 0.03[a] | 0.91 ± 0.02[b] | 1.41 ± 0.02[a] |
| 28 | 1.50 ± 0.02[ac] | 1.41 ± 0.02[ac] | 0.95 ± 0.02[b] | 1.33 ± 0.04[ac] |
| 35 | 1.80 ± 0.05[a] | 1.71 ± 0.03[ac] | 1.06 ± 0.12[b] | 1.41 ± 0.02[c] |

[a]Mean ± SEM values with different superscripts within a row differ significantly at $P < 0.05$.

albumin)) were analyzed statistically as per Snedecor and Cochran (9) using one-way ANOVA. Statements of statistical significance are based on $P \leq 0.05$ significance level.

## 3. Results

Dietary treatments had no significant ($P \geq 0.05$) effect on body weight, FCR, relative organ weights, or biochemistry in groups A and B throughout the experimental study. Reduction in body weight was observed in the broiler chicks fed with aflatoxin (group C) as well as in the birds fed with the toxin and supplemented with citrus fruit oil (group D) as compared to the control groups from day 14 posttreatment. The FCR of the aflatoxin-fed birds (group C) revealed a numerical increase from day 7 posttreatment and was highest on day 35 posttreatment, whereas in group D (aflatoxin + citrus oil) it was appreciable only from day 21 posttreatment.

Among the aflatoxin-fed groups, the aflatoxin control (group C) had significantly ($P \leq 0.05$) reduced body weight than supplemented group D. There was a significant increase ($P \leq 0.05$) in relative liver weight in aflatoxin-fed birds (group C) from day 14 to 28 posttreatment, whereas birds fed with aflatoxin in combination with citrus oil (group D) showed only a numerical increase when compared to the untreated control. Relative weights of the kidneys, heart, and lymphoid organs (bursa of Fabricius, spleen, and thymus) of all the treatments did not show any significant ($P \geq 0.05$) difference among the groups throughout the experimental study.

Serum AST and ALP levels in the aflatoxin alone treated birds (group C) were increased significantly ($P \leq 0.05$) as compared to the control birds of group A throughout the study, whereas birds from group D (aflatoxin + citrus oil) showed a significant ($P \leq 0.05$) decrease compared to the control group from day 14 of treatment onwards. Aflatoxin-fed birds showed a significant ($P \leq 0.05$) increase in serum ALT activity as compared to the controls on days 14, 21, and 28 and it was maximum on day 21 of treatment. A significant ($P \leq 0.05$) decrease in ALT levels towards normal levels was seen in supplemented group D.

## 4. Discussion

Aflatoxin-intoxicated birds (group C) as well as citrus oil supplementation along with aflatoxin intoxication (Group D) showed reduced body weight gain and increased FCR. These findings are in agreement with previous studies (7,10). The reduced body weight gain and increased FCR can be attributed to anorexia, inhibitory effect of aflatoxin on protein synthesis, and lipogenesis (11).

Among the aflatoxin-fed groups, the aflatoxin control (group C) had significantly ($P \leq 0.05$) reduced body weight than supplemented group D. This could be attributed to the possible partial amelioration of aflatoxin by the citrus oil in the study. There was a significant increase ($P \leq 0.05$) in relative liver weight in aflatoxin-fed birds (group C). The increase in the relative weight of the liver in aflatoxin-fed birds could be attributed to the AFB-1-induced impaired fat metabolism in the liver with an increase in the fat content of the hepatocytes (12,13), and the reduction in relative weight in toxin- and citrus-oil–supplemented birds (group D) could be related to the incorporation of citrus oil in the diet and its possible role in ameliorating aflatoxin. Significant ($P \leq 0.05$) reductions in the total proteins and albumin levels were observed in aflatoxin-fed birds (14). This could be due to degeneration of endoplasmic reticulum in hepatocytes and covalent binding of aflatoxin metabolites to template RNA, which causes inhibition of transcription in protein synthesis (15), whereas supplementation of citrus oil along with toxin caused a marginal increase in total protein and albumin levels in comparison with aflatoxin-alone–fed birds, which could be due to partial alleviation of toxic effects. The improved body weight and FCR further supported this assumption.

Serum ALT and AST levels in aflatoxin-treated birds were higher throughout the study in comparison with control birds. Supplementation of citrus oil reduced these enzyme levels significantly from 14 days of treatment. This could be attributed to seepage of enzymes due to membrane damage from cytosol (16). ALT levels were maximum at younger age, which was attributed to microsomal AFB1 activation to the reactive AFB1-8-9 epoxide (AFBO)

being most efficient in younger birds and deficiency in cytosolic glutathione S-transferase, which prevents free radical injury (17). Reduced ALT levels in supplemented birds group D (aflatoxin + citrus oil) clearly indicates the protective effects of citrus oil in aflatoxicosis.

From these results, it is suggested that supplementation of citrus fruit oil reduced toxicity by decreasing relative organ weight, and improved enzyme levels, FCR, and body weight. The beneficial effect of citrus fruit oil used in this study could be attributed to the presence of d-limonene in the citrus fruit oil, which shows detoxification and antioxidant properties by increasing the level of glutathione S-transferase (3). D-Limonene, a monoterpenoid constituent of citrus fruit oil, has inhibitory effects on cytochrome P450 enzymes (4). Microsomal cytochrome P450 (CYP) enzymes CYP2A6 and to a lesser extent CYP1A1 are responsible for bioactivation of AFB1 into epoxide form, which forms AF-DNA adduct in the liver of chicken and quail (18,19). Bioactivation of aflatoxin could be reduced by d-limonene present in citrus fruit oil due to its inhibitory effects on cytochrome P450 enzymes. Citrus fruit oil also had no adverse effects on body weight, FCR, relative organ weights, or biochemistry as compared to the healthy controls. Keeping the importance of public health in mind, there is an urgent need to look for an alternate approach to counteract aflatoxicosis and herbal feed supplements appeared to be a suitable component for better management of aflatoxicosis.

**Acknowledgments**

We are thankful to M/s Tetragon Chemie, Bangalore, Karnataka, India, for providing the necessary facilities and the Indian Council of Agricultural Research (ICAR) for providing a junior research fellowship.

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