Effect of Dietary Melatonin and L-Tryptophan on Growth Performance and Immune Responses of Broiler Chicken under Experimental Aflatoxicosis

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INTRODUCTION

Aflatoxin is the common name for a group of chemically related compounds (Moss, 1996) produced by certain strains of Aspergillus flavus and A. parasiticus in the feedstuffs as poisonous secondary metabolites. Aflatoxins are stable once formed in grain and are not degraded during normal milling and storage process (Brown, 1996) and have been demonstrated to be carcinogenic, mutagenic and teratogenic (Cole and Cox, 1981). It impairs humoral and cellular immune responses in poultry and increases susceptibility to environmental and infectious agents (Gabal and Azam, 1998) leading to severe economic loss. Among all the aflatoxins, aflatoxin B₁ (AFB₁) is the most potent and pathogenic form to poultry. Liver is considered to be the primary target organ for the aflatoxins and AFB₁ is known as a potent hepatotoxic and hepatocarcinogen. Besides, it also affects other organ systems (Coulombe et al. 1994). The most economically significant effect of aflatoxicosis in growing birds is decreased growth and poor feed efficiency. Intoxi-
The present study was undertaken to investigate the ameliorative and interactive effect of melatonin, its precursor (tryptophan) on production performance and immune responses under conditions of experimental aflatoxicosis in broilers.

**MATERIALS AND METHODS**

Aflatoxin was produced using a toxigenic strain, *Aspergillus parasiticus* NRRL 2999, this fungal strain was inoculated into potato dextrose agar and incubated at 28°C for 7-21 days before being used for toxin production. 250 mL flasks containing 50 g of rice, free from extraneous materials were autoclaved at 15 lbs pressure for 15 minutes and then inoculated with fungal spores; further processing was carried out following the procedure of Shotwell et al. (1966). Fermented rice was then steam heated to kill the fungi, the rice was then dried and grounded to a fine powder and the aflatoxin content was measured according to Pons et al. (1966) method. Aqueous acetone was used for extraction of the toxin. Analysis of individual components was done by thin layer chromatography and aflatoxin contents were finally quantified using the spectrophotometric method of Nabney and Nesbitt (1965). The aflatoxin contents were also validated by the method of AOAC (1991). The contaminated rice powder was incorporated into the uncontaminated based feed at dose rate of 0.5 mg/kg of feed. Day old broiler chickens (n=180) of Naked Neck strain were obtained from experimental broiler farm of the Central Avian Research Institute and were wing banded, weighed individually and distributed randomly into six groups. Experimental design was randomized block design with six dietary treatments having 3 replicates comprising of 10 chickens in each replicate. Different experimental groups were subjected to the following dietary treatments continuously till six weeks of age; (1) untreated control group fed on the basal feed (CTRL); (2) aflatoxin alone treated group (0.5 mg/kg feed; AF); (3) melatonin alone treated group (20 mg/kg of feed+20 mg/kg BW-i/p daily; MEL); (4) L-tryptophan alone treated group (250 mg/kg of feed; TRY); (5) combined treatment of aflatoxin and melatonin at above doses (AF+MEL); (6) combined treatment of aflatoxin and L-tryptophan at above dose (AF+TRY). Melatonin was procured from Hi Media Laboratories, Mumbai, India and L-tryptophan was sourced from Sisco Research Laboratories, Mumbai, India. All birds were reared under standard managerial conditions like water, feeder, floor space and ventilation for 0-6 weeks with natural lighting. The birds were fed with broiler starter and finisher ration for 0-3 weeks and 4-6 weeks, respectively. Ingredient and chemical composition of formulated basal diet is presented in Table 1.

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Amount (g/kg)</th>
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<tr>
<td>Rice</td>
<td>400</td>
</tr>
<tr>
<td>Corn</td>
<td>350</td>
</tr>
<tr>
<td>Wheat</td>
<td>100</td>
</tr>
<tr>
<td>Soybean</td>
<td>20</td>
</tr>
<tr>
<td>Salt</td>
<td>1</td>
</tr>
<tr>
<td>L-tryptophan</td>
<td>250</td>
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Individual body weight and group wise feed consumption in various treatments were recorded. Feed conversion ratios were calculated as the ratio between feed intake and body weight gain; and daily mortality (if any) was recorded on occurrence. Week wise livability percentages of chickens kept on different treatments were calculated. At 6 weeks of age the blood samples from each treatment group (n=6) were collected for hepatic enzymes and haemagglutination (HA) analysis. The microtitre procedure, as it was described by Siegel and Gross (1980) with slight modifications, was used to measure total HA antibody titres in chickens. The in vivo cell mediated immune (CMI) response to phytohemagglutinin (PHA-P, procured from Bangalore Genei, Bangalore, India) mitogen was evaluated...
by the method of Corrier and Deloach (1990). PHA-P (0.1 mg/bird) was injected intra-dermally in the left foot web. Right foot web of the same bird received 0.1 mL sterile phosphate buffer saline and served as control.

The skin thickness of foot webs (right and left) from injected birds of each group was measured by a micrometer at 0 and 24 hours after injection of mitogen. Sera samples separated and stored at -20 °C were also analyzed for total serum protein by Biuret method using commercial kits (Labkit, Spain). Activities of marker hepatic enzymes in serum by the method of Corrier and Deloach (1990). PHA-P (0.1 mg/kg) or L-tryptophan (250 mg/kg) in basal diet resulted in significantly (P<0.05) higher weight gain as compared to toxin fed group and were comparable to controls (Table 2). Supplementation of melatonin to aflatoxin incorporated diet (AF+MEL) resulted in significantly higher weight gain compared to toxin alone treated group (AF) at the end of 3rd week of trial indicating its beneficial role. Supplementing L-tryptophan to aflatoxin incorporated diet (AF+TRY) resulted in numerically higher weight gain (non-significant) compared to toxin alone treated group (AF) during the entire trial period. Melatonin or its precursor supplementation to basal diet made no significant changes in feed consumption pattern of the birds. The inclusion of aflatoxin in the basal diet markedly (P<0.05) reduced the feed intake (Table 3) at all stages of the study. Supplementation of melatonin or its precursor (L-tryptophan) to basal diet caused slight non-significant improvement in feed conversion ratio (FCR) of birds. But inclusion of melatonin to toxin incorporated diets resulted in significantly better FCR after completion of 3 weeks of study. Melatonin supplementation inhibits spontaneous and serotonin induced smooth muscle contraction in gut (Bubenik, 2002), which might have contributed to relatively slower feed transit. Melatonin supplementation was also accompanied by increased activity of digestive enzymes (Thakur, 2004). Hence, both these factors might have contributed for better FCR obtained in melatonin-supplemented groups. Besides melatonin is reported to have hypnotic effect and reduces physical activity leading to decreased heat production (Zeman, 2001) which might have also contributed to improvement in FCR. Aflatoxin incorporation to the basal diet significantly (P<0.05) affected FCR adversely at successive weeks of trial. Results of studies conducted by several researchers indicated that dietary aflatoxin at levels of 0.5 mg/kg and beyond in commercial broilers adversely affected growth in a dose related fashion (Beura et al., 1993; Verma, 1994; Rosa et al. 2001). Poor FCR is a common feature in broilers suffering from aflatoxicosis. Raju and Devecgowda (2000) reported poor FCR in broilers at 0.3 mg/kg level of dietary aflatoxin. Other researchers have also reported a dose dependent reduction in feed efficiency at different levels of dietary aflatoxin (Reddy et al. 1982).

### RESULTS AND DISCUSSION

In the first week, inclusion of aflatoxin at 0.5 mg/kg in feed significantly (P<0.05) reduced the body weight gain in comparison to control. Supplementation of melatonin (40 mg/kg) or L-tryptophan (250 mg/kg) in basal diet resulted in significantly (P<0.05) higher weight gain as compared to toxin fed and were comparable to controls (Table 2). Supplementation of melatonin to aflatoxin incorporated diet (AF+MEL) resulted in significantly higher weight gain compared to toxin alone treated group (AF) at the end of 3rd week of trial indicating its beneficial role. Supplementing L-tryptophan to aflatoxin incorporated diet (AF+TRY) resulted in numerically higher weight gain (non-significant) compared to toxin alone treated group (AF) during the entire trial period. Melatonin or its precursor supplementation to basal diet made no significant changes in feed consumption pattern of the birds. The inclusion of aflatoxin in the basal diet markedly (P<0.05) reduced the feed intake (Table 3) at all stages of the study. Supplementation of melatonin or its precursor (L-tryptophan) to basal diet resulted in significantly better FCR after completion of 3 weeks of study. Melatonin supplementation inhibits spontaneous and serotonin induced smooth muscle contraction in gut (Bubenik, 2002), which might have contributed to relatively slower feed transit. Melatonin supplementation was also accompanied by increased activity of digestive enzymes (Thakur, 2004). Hence, both these factors might have contributed for better FCR obtained in melatonin-supplemented groups. Besides melatonin is reported to have hypnotic effect and reduces physical activity leading to decreased heat production (Zeman et al. 2001) which might have also contributed to improvement in FCR. Aflatoxin incorporation to the basal diet significantly (P<0.05) affected FCR adversely at successive weeks of trial. Results of studies conducted by several researchers indicated that dietary aflatoxin at levels of 0.5 mg/kg and beyond in commercial broilers adversely affected growth in a dose related fashion (Beura et al. 1993; Verma, 1994; Rosa et al. 2001). Poor FCR is a common feature in broilers suffering from aflatoxicosis. Raju and Devecgowda (2000) reported poor FCR in broilers at 0.3 mg/kg level of dietary aflatoxin. Other researchers have also reported a dose dependent reduction in feed efficiency at different levels of dietary aflatoxin (Reddy et al. 1982).
Dietary aflatoxin at 0.5 mg/kg levels significantly (P<0.05) decreased the haemagglutination titre against sheep RBCs in comparison to melatonin and tryptophan alone treated birds (Table 4). Under experimental aflatoxicosis, reduced humoral immune response has also been observed in previous studies (Virdi et al., 1989; Bakshi, 1991). Aflatoxin inclusion in the basal diet significantly (P<0.05) decreased the haemagglutination titre against sheep RBC and CMI response to PHA-P. Whereas melatonin supplementation showed significant increases in HA titre against sheep RBC and CMI response to PHA-P (Table 4). Such a reduction in bursal size was also observed at 0.5 mg/kg level of dietary aflatoxin (P<0.05) decrease in the relative weight of bursa of Fabricius (Calvo et al., 2001). Melatonin has a role in the development and maturation of immune system and in the progression of immune response (Krystyna, 2002). The influence of melatonin on immune response may also be due to stimulation of cytokine (Th2 cell cytokines, such as IL-4 and IL-10) production (Maestroni, 1995). Aflatoxin inclusion significantly (P<0.05) alleviated the CMI response. Whereas melatonin supplementation showed significant increases in HA titre against sheep RBC in comparison to melatonin and tryptophan alone treated birds (Table 4). Under experimental aflatoxicosis, reduced humoral immune response has also been observed in previous studies (Virdi et al., 1989; Bakshi, 1991). Aflatoxin inclusion in the basal diet significantly (P<0.05) decreased the liver weight whereas supplementation of melatonin or its precursor in toxin added diet significantly (P<0.05) reduced the liver weight in comparison to negative control. Aflatoxin being potently hepatotoxic resulted in enlarged liver with increased content of lipids as noted previously by Chen et al. (1985) and Verma (1994).

The inclusion of aflatoxin in basal diet induced a significant (P<0.05) hypoproteinemnic state (Table 5) in birds as previously observed by Reddy et al. (1982), Bakshi (1991) and Verma (1994). This decline in serum proteins may be due to decline in protein biosynthesis as aflatoxin forms adducts with DNA, RNA and protein and also inhibits RNA synthesis and DNA-dependent RNA polymerase activity as well as causing degranulation of endoplasmic reticulum (Groopman et al., 1996). Supplementation of melatonin or L-tryptophan numerically increased the serum protein levels as compared to toxin fed birds.

This may be due to ability of melatonin to protect liver tissues from toxic effects of aflatoxin and its resultant inhibition of protein synthesis. Dietary aflatoxin also significantly increased activities of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) in serum (Table 5), indicating liver damage, such changes were also observed by Balachandran and Ramakrishnan (1987), Verma (1994) and Nath (2008).

Melatonin inclusion significantly (P<0.05) alleviated this aflatoxin induced increase in AST and ALT activities.
whereas L-tryptophan supplementation brought about slight reduction in AST and ALT activities when compared to toxin treated birds.

CONCLUSION

In conclusion our findings suggest that dietary L-tryptophan was partially as effective as dietary melatonin in alleviating aflatoxin induced growth retardation and immunosuppression in broiler chickens.

REFERENCES


The means within the same column with at least one common letter, do not have significant difference (P≤0.05).

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the feed and immunization against selected infectious diseases in poultry. II. Effect on one-day-old chick simultaneously vaccinated against Newcastle disease, infectious bronchitis and infectious bursal disease. *Avian Pathol.* 27, 290-295.


