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

**Research Article**

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**ABSTRACT**

The aim of the present work was to determine whether the administration of melatonin or L-tryptophan (a precursor of melatonin) affects the immune responses and performance of broilers during induced exposure to aflatoxins in feed. The study was conducted from 0-6 weeks comprising six dietary treatments in triplicate with 10 chickens in each replicate. The diets were formulated to supply 23% crude protein (CP) and 2800 kcal ME/kg in starter ration and 20% CP and 2900 kcal ME/kg in finisher ration. The experimental diets were offered ad libitum with free access to water throughout the entire experiment. Inclusion of aflatoxin in the feed at 0.5 mg/kg feed caused a significant reduction in the growth performance of broilers. Supplementation of melatonin (20 mg/kg in feed and 20 mg/kg body weight through i.p. route) or its precursor (L-tryptophan at 250 mg/kg feed) in aflatoxin fed broilers resulted in numerically improved performance. Aflatoxin inclusion in the feed also caused a significant reduction in haemagglutination titer against sheep RBC and cell mediated immune responses to phytohemagglutinin (PHA-P) in broilers. Melatonin or L-tryptophan inclusion in toxin incorporated feed significantly improved both humoral and cell mediated immunity. No significant (P>0.05) differences were observed among various groups with respect to kidney and spleen weight but liver weight increased significantly (P≤0.05) and weight of bursa significantly decreased upon aflatoxin inclusion. Our study suggests that L-tryptophan was partially as effective as melatonin in alleviating aflatoxin induced growth retardation and immunosuppression in broiler chicken.

**KEY WORDS** aflatoxin, broilers, immunity, L-tryptophan, melatonin, performance.

**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 feed-stuffs 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 hepatotoxin 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-
cated adult hens have decreased egg production and the hatchability, whereas insemination of hens with affected male has shown decreased fertility (Brown, 1996). Besides, aflatoxins also pose a significant public health hazard because of the possible transmission of residual toxin through poultry egg and meat to humans.

AFB1 is oxidized by microsomal mixed function oxidase (cytochrome P450) to several water-soluble metabolites. The formation of AFB1-8, 9-epoxide, an active metabolite, and its subsequent covalent binding to DNA, RNA and proteins play a critical role in both acute and chronic toxicity (Choy, 1993). It has been implicated that oxidative stress following aflatoxin metabolism together with hepatotoxicity and carcinogenicity can be inhibited by the use of dietary antioxidants (Firozi and Bhattacharya, 1995). Melatonin (N-acetyl-5-methoxy tryptamine) is the main secretory product of the pineal gland. Biosynthesis of melatonin takes place in the pinealocytes present in the pineal gland and begins with the uptake of the amino acid tryptophan. Melatonin is a potent antioxidant and scavenger of various free radicals especially hydroxyl and peroxyl radicals with enhancement of antioxidative enzyme activities in many tissues (Pier et al. 1994). Melatonin added to the drinking water of quail resulted in an increase in total white blood cells (WBC), an increase in the percentage of lymphocytes, a decrease in the percentage of heterophils, and a decrease in the heterophil/lymphocyte (H/L) ratio (Moore and Siospes, 2000). Gopi (2006) observed that under experimental aflatoxicosis, melatonin supplementation at 40 mg/kg feed resulted in a significant (P<0.05) improvement in humoral antibody titre against sheep RBCs (specify the meaning of this acronym) and cell mediated immune (CMI) response to phytohemagglutinin (PHA-P) in broiler chickens.

Herichova et al. (1998) tested effects of oral administration of tryptophan (150 mg/kg) and they observed an increased availability of melatonin. Esteban et al. (2004) revealed that the synthesis of serotonin and melatonin, as well as the innate immune response, can be modulated by oral ingestion of tryptophan in rats. With this background, 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−ip 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 managemental 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.

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 vitro 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.

### 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 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 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 Devegowda (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. 1985; 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. Tryptophan supplementation showed significant increases in HA titre against sheep RBC and CMI response to PHA-P. Melatonin inclusion significantly (P<0.05) alleviated aflatoxicosis (Mean±SE) (Table 5) in birds as compared 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).

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<th>Table 4 Effect of melatonin and its precursor (L-tryptophan) on body weight gain (g) of broiler chickens under experimental aflatoxicosis (Mean±SE)</th>
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The means within the same column with at least one common letter, do not have significant difference (P≥0.05).

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<th>Table 5 Effect of melatonin and its precursor (L-tryptophan) on cumulative feed intake (g) of broiler chicken under experimental aflatoxicosis (Mean±SE)</th>
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<td>Groups</td>
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The means within the same column with at least one common letter, do not have significant difference (P≥0.05).

An in vivo study (Brennan et al. 2002) revealed that melatonin not only enhanced circulating white blood cells (WBC) counts but also activities of B and T-lymphocytes in immature male chickens. Aflatoxin inclusion in the basal diet significantly (P<0.05) increased 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).
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.

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