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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 ($P\leq0.05$) 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 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 B_1 (AFB_1) is the most potent and pathogenic form to poultry. Liver is considered to be the primary target organ for the aflatoxins and AFB_1 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 AFB_1-8, 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 (Pieri *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 Siopes, 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, *Aspergilius 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 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 haemmagglutination (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.

Table 1 Ingredient (%) and chemical composition of basal diet

Ingredient	Starter	Finisher
Maize	56.55	65
DORB	2.5	0
Soya bean	28	21.19
Sunflower	4	5
Fish meal	6	6
Mineral mixture (ISI)	1.5	1.5
Marble chips	0	0.3
Limestone	0.3	0.3
Di-calcium phosphate	0.55	0.15
DL-methionine	0.06	0
Lysine	0.05	0.1
Salt	0.1	0.1
Trace mineral premix[1]	0.1	0.1
Vitamin premix[2]	0.15	0.15
Choline chloride	0.015	0.015
B Complex[3]	0.06	0.4
Coccidiostat	0.02	0.02
Analyzed chemical composition of basal diet		
Crude protein (%)	22.94	19.91
ME (kcal/kg)*	2807.13	2895.41
Calcium (%)	1.17	1.14
Available phosphorus (%)	0.50	0.42
Lysine (%)	1.31	1.18
Methionine (%)	0.50	0.41

[1] Trace mineral (10g/100g): $FeSO_4.7H_2O$: 8 g; $ZnSO_4.7H2O$: 10 g; $MnSO_4.H_2O$: 10 g; $CuSO_4.5H_2O$: 1 g; KI: 30 mg and Maize: 70.97 g.
[2] Vitamin A B_2D_3K each g contains: vitamin A: 82500 IU; vitamin B2: 50 mg; vitamin D3: 12000 IU and vitamin K: 10 mg.
[3] B-complex: each g contains: vitamin B_1: 8 mg; vitamin B_6: 16 mg; vitamin B_1: 2 mg; Niacin: 120 mg; Cal. Pantothenate: 80 mg; vitamin E 50%: 160 mg; L-lysine: 10 mg and Dl-methionine: 10 mg.
* Calculated value.

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 (Spinreact, Spain). Activities of marker hepatic enzymes in serum like alanine aminotransferase (ALT) and aspartate aminotransferase (AST) indicating the degree of hepatolysis were assayed using commercial kits (Labkit, Spain). Nine birds from each group were sacrificed after 6th week of experimental trial and liver, kidney, spleen, bursa of Fabricius were carefully excised from the carcass, weighed and expressed as percentage of body weight. The data pertaining to various parameters was compiled and statistically analyzed using Randomized Block Design and various means were compared following the methods of Snedecor and Cochran (1989) and differences among treatment means were determined by Duncan's multiple range test and significance was accepted at $P \leq 0.05$.

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).

Table 2 Effect of melatonin and its precursor (L-tryptophan) on body weight gain (g) of broiler chickens under experimental aflatoxicosis (Mean±SE)

Groups	1st week	2nd week	3rd week	4th week	5th week	6th week
CTRL	75.03±0.89^{ab}	201.88±2.90^{ab}	388.07±5.78^{ab}	528.12±8.12^{a}	784.25±11.62^{ab}	1061.40±15.55^{a}
AF	65.92±2.53^{c}	184.72±2.64^{d}	338.43±4.23^{e}	468.23±6.95^{c}	728.23±10.92^{d}	946.40±14.09^{b}
MEL	76.50±3.68^{a}	205.27±5.04^{a}	392.19±3.90^{a}	532.44±11.00^{a}	799.18±14.44^{a}	1060.36±17.38^{a}
TRY	76.09±1.51^{a}	203.35±2.99^{a}	388.16±5.28^{ab}	526.30±5.82^{a}	782.11±10.96^{abc}	1060.75±13.78^{a}
AF+MEL	70.45±1.27^{abc}	192.52±3.08^{bcd}	359.69±5.80^{cd}	490.34±8.57^{bc}	741.21±13.30^{cd}	962.43±18.8^{b}
AF+TRY	69.38±1.59^{bc}	190.77±2.97^{cd}	350.36±5.94^{de}	472.30±8.42^{c}	734.55±11.30^{d}	950.66±13.28^{b}

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

Table 3 Effect of melatonin and its precursor (L-tryptophan) on cumulative feed intake (g) of broiler chicken under experimental aflatoxicosis (Mean±SE)

Groups	1st week	2nd week	3rd week	4th week	5th week	6th week
CTRL	111.2±0.64^{ab}	322.4±0.76^{a}	650.2±8.61^{a}	955.0±17.19^{a}	1510.5±22.18^{ab}	2194.4±2.88^{a}
AF	107.0±0.86^{d}	309.7±1.18^{b}	616.2±7.11^{c}	902.4±6.92^{d}	1459.2±7.42^{d}	2088.4±12.83^{d}
MEL	110.1±0.54^{abc}	320.8±8.24^{a}	646.8±0.60^{a}	945.6±8.04^{ab}	1501.1±4.43^{abc}	2160.3±22.97^{ab}
TRY	110.1±0.64^{abc}	319.2±2.90^{ab}	650.7±4.05^{a}	954.4±5.60^{a}	1514.1±7.56^{a}	2188.7±1.68^{a}
AF+MEL	108.9±1.15^{bcd}	316.5±1.69^{ab}	628.4±2.89^{bc}	916.4±3.40^{cd}	1470.2±6.90^{cd}	2090.4±5.68^{d}
AF+TRY	108.6±1.12^{cd}	312.5±2.28^{ab}	622.7±3.93^{c}	908.7±2.17^{cd}	1477.5±9.93^{cd}	2103.6±18.01^{cd}

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

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) down regulated the CMI response. Whereas melatonin supplementation showed significant increases in HA titre against sheep RBC and CMI response to PHA-P. These finding are in agreement with Moore and Siopes (2000) where in them it was reported that both humoral and cellular responses significantly increased by 22 and 34%, respectively upon melatonin exposure compared to quails receiving diluents only. The immunomodulatory role of melatonin could be due to the presence of its binding sites in lymphocytes and 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), which in turn enhances lymphocyte activities. Melatonin also augments T-helper cell and natural killer cell activity, interleukin and interferon (IFN-gamma) production in monocytes (Maestroni, 1995). A significant (P<0.05) decrease in the relative weight of bursa of Fabricius was observed at 0.5 mg/kg level of dietary aflatoxin (Table 4). Such a reduction in bursal size was also observed by Chattopadhyay *et al*. (1985) at doses of 0.5 and 0.625 mg/kg of aflotoxin in feed. Relative weight of bursa in Fabricius increased slightly with melatonin or L-tryptophan supplementation in birds fed aflatoxin incorporated ration.

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).

The inclusion of aflatoxin in basal diet induced a significant (P<0.05) hypoproteinemic 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 or 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

Table 4 Effect of melatonin and its precursor (L-tryptophan) on haemogglutination titre against sheep RBCs, cell mediated immune response to PHA-P (foot web index) and relative weights of visceral organs in broilers under experimental aflatoxicosis

Groups	Foot web index	HA titre (log_2)	Liver (% *b.w.*)	Kidney (% *b.w.*)	Bursa (*% b.w.*)	Spleen (% *b.w.*)
CTRL	0.411±0.018^{a}	9.11±0.42^{ab}	2.53±0.08^{c}	0.64±0.05	0.248±0.006^{ab}	0.24±0.02
AF	0.316±0.011^{c}	5.78±0.40^{c}	3.05±0.15^{a}	0.72±0.04	0.203±0.004^{c}	0.26±0.02
MEL	0.436±0.014^{a}	9.22±0.43^{a}	2.55±0.09^{c}	0.64±0.04	0.259±0.005^{a}	0.27±0.01
TRY	0.414±0.008^{a}	8.67±0.33^{abc}	2.52±0.15^{c}	0.69±0.05	0.234±0.005^{bc}	0.26±0.02
AF+MEL	0.362±0.011^{b}	8.22±0.32^{abcd}	2.82±0.14^{bc}	0.62±0.04	0.218 ±0.006^{cde}	0.29±0.03
AF+TRY	0.376±0.006^{b}	7.89±0.35^{bcd}	2.88±0.08^{ab}	0.65±0.05	0.212±0.004^{de}	0.28±0.02

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

Table 5 Effect of melatonin and its precursor (L-tryptophan) on serum aspartate aminotransferase (AST), alanine aminotransferase (ALT) and serum protein levels of broiler chickens under experimental aflatoxicosis

Groups	**AST** (IU/L)	**ALT** (IU/L)	**Total protein** (g/dL)
CTRL	126.3±4.79^{b}	13.8±0.67^{b}	3.5±0.25^{ab}
AF	142.8 ±3.07^{a}	19.8 ±2.41^{a}	2.6±0.19^{c}
MEL	127.5±4.95^{b}	14.5±0.82^{b}	3.5±0.20^{ab}
TRY	125.5±6.50^{b}	14.5±0.80^{b}	3.7±0.19^{a}
AF+MEL	126.9±4.16^{b}	14.5±0.78^{b}	3.1±0.17^{bc}
AF+TRY	133.3±5.30^{ab}	18.2±1.63^{ab}	3.0±0.13^{bc}

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

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

AOAC. (1991). Official Methods of Analysis. Vol. I. 15th Ed. Association of Official Analytical Chemists, Arlington, VA.

Bakshi C.S. (1991). Studies on the effect of graded dietary levels of aflatoxin on immunity in commercial broilers. MS Thesis. Indian Veterinary Research Institute. lzatnagar, U.P., India.

Balachandran C. and Ramakrishnan R. (1987). An experimental study on the pathology of aflatoxicosis in broiler chickens. *Indian Vet. J.* **64,** 911-914.

Beura C.K., Johri I.S., Sadagopan V.R. and Panda B.K. (1993). Interaction of dietary protein level on dose-response relationship during aflatoxicosis in commercial broilers. *Indian J. Poult. Sci.* **28,** 170-177.

Brennan C.P., Hendricks G.L., EI-Sheikh T.M. and Mashaly M.M. (2002). Melatonin and the enhancement of immune response in chickens. *Poult. Sci.* **81,** 371-375.

Brown T. (1996). Fungal diseases. Pp. 305-315 in Poultry Diseases, F.T.W. Jordan and M. Puttison Eds. Jordan, Saunders W.B. Ltd. Site of publication.

Bubenik G.A. (2002). Gastrointestinal melatonin. Localization, function and clinical relevance. *Dig. Dis. Sci.* **47,** 2336-2348.

Calvo J.R., Reiter R.J., García J.J., Ortiz G., Tan D.X. and Karbownik M. (2001). Characterization of the protective effects of melatonin and related indoles against alpha naphthyl isothiocyanate-induced liver injury in rats. *J. Cell. Biochem.* **80,** 461-470.

Chattopadhyay S.K., Taskar P.K., Schwabe O., Das Y.T. and Brown H.D. (1985). Clinical and biochemical effects or aflatoxin in feed ration of chicks. *Cancer. Biochem. Biophys.* **8**, 67-75.

Chen C., Pearson A.M., Coleman T.H., Gray J.I. and Wolzak A.M. (1985). Broiler aflatoxicosis with recovery after replacement of the contaminated diet. *Brit. Poult. Sci.* **26,** 65-71.

Choy W.N. (1993). A review of the dose-response induction of DNA adducts by aflatoxin B_1 and its implications in quantitative cancer risk. *Mut. Res*. **296,** 181-198.

Cole R.J. and Cox R.H. (1981). The aflatoxins. Handbook of Toxic Fungal Metabolites. Academic Press, New York, NY. Pp. 1-66.

Corrier D. and DeLoach J.R. (1990). Evaluation of cell mediated cutaneous basophil hypersensitivity in young chickens by an interdigital skin test. *Poult. Sci*. **69,** 403-408.

Coulombe R.A. (1994). Non hepatic disposition and effects of aflatoxin B_1. Pp. 89-101 in The Toxicology of Aflatoxins: Human Health, D.L. Eaton and J.D. Groopman Eds. Veterinary and Agricultural Significance, Academic Press, San Diego, California, USA.

Esteban S., Nicolaus C., Garmundi A., Rial R.V., Rodríguez A.B., Ortega E. and Ibars C. (2004). Effect of orally administered L-tryptophan on serotonin, melatonin, and the innate immune response in the rat. *Mol. Cell. Biochem.* **267,** 39-46.

Firozi P.F. and Bhattacharya R.K. (1995). Effects of natural polyphenols on aflatoxin B_1 activation in a reconstituted microsomal monooxygenase system. *J. Biochem. Toxicol.* **10,** 25-31.

Gabal M.A. and Azzam A.H. (1998). Interaction of aflatoxin in

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

Gopi K. (2006). Influence of melatonin on aflatoxicosis in broiler chicken. MS. Thesis. Deemed University, IVRI, Izatnagar.

Groopman J.D., Wang J.S. and Scholl P. (1996). Molecular biomarkers for aflatoxins: from adducts to gene mutations to human liver cancer. *Can. J. Physiol. Pharmacol*. **74,** 203-209.

Herichova I., Zeman M. and Veselovsk J. (1998). Effect of tryptophan administration on melatonin concentrations in the pineal gland, plasma and gastrointestinal tract of chickens. *Acta. Vet. Brno*. **67,** 89-95.

Krystyna S.S. (2002). Melatonin in immunity: comparative aspects. *Neuroendocrinol. Lett*. **23,** 61-66.

Maestroni G.J.N. (1995). T-Helper-2 lymphocytes as a peripheral target of melatonin. *J. Pineal. Res*. **18,** 84-89.

Moore C.B. and Siopes T.D. (2000). Effects of light conditions and melatonin supplementation on the cellular and humoral immune responses in Japanese quail. *Gen. Comp. Endocrinol*. **119,** 95-104.

Moss M.O. (1996). Centenary Review: Mycotoxins. *Mycol. Res*. **100,** 513-523.

Nabney J. and Nesbitt B.F. (1965). A spectrophotometric method for determining the aflatoxins. *Analyst*. **90,** 155-160.

Nath R. (2008). Changes in the serum enzymes during experimental aflatoxicosis in broiler chicken. *Indian Vet. J.* **85,** 10-12.

Pieri C., Marra M., Moroni F., Recchioni R. and Marcheselli F. (1994). Melatonin: a peroxyl radical scavenger more effective than vitamin E. *Life. Sci*. **55,** 271-276.

Pons W.A., Cucullua F.A., Lee L.S., Robertson J.A., Franz S.O. and Goldblatt L.A. (1966). Determination of aflatoxins in agricultural products: use of aqueous acetone for extraction. *J. Assoc. Off. Anal. Chem*. **49,** 554-562.

Raju M.V.L.N. and Devegowda G. (2000). Influence of modified glucomannan on performance, organ morphology, serum biochemistry and hematology in broilers exposed to individual and combined mycotoxicosis. *Br. Poult. Sci*. **41,** 640-650.

Reddy A.R., Reddy V.R., Rao P.V. and Yadagiri B. (1982). Effect of experimentally induced aflatoxicosis on the performance of commercial broiler chicks. *Indian J. Anim. Sci*. **52,** 405-410.

Rosa C.A., Miazzo R., Magnoli C., Salvano M., Chiacchiera S.M., Ferrero S., Sáenz M., Carvalho E.C. and Dalcero A. (2001). Evaluation of the efficacy of bentonite from the south of Argentina to ameliorate the toxic effects of aflatoxin in broilers. *Poult. Sci*. **80,** 139-144.

Shotwell O.L., Hesseltine C.W., Stubblefield R.D. and Sorenson W.G. **(**1966). Production of aflatoxin on rice. *Appl. Environ. Microbiol*. **14,** 425-428.

Siegel P.B. and Gross W.B. (1980). Production and persistence of antibodies in chicken to sheep erythrocytes. 1. Directional selection. *Poult. Sci*. **59,** 1-5.

Snedecor G.W. and CochranW.G. (1989). Statistical Methods. 8th Ed. Oxford and ISH Publishing Co., Calcutta.

Thakur R. (2004). Effect of pinealectomy and exogenous melatonin on jejunal melatonin and digestive enzyme levels in broiler. MS Thesis. Deemed University, IVRI, Izatnagar.

Verma J. (1994). Studies on the effect of dietary aflatoxin, ochratoxin and their combinations on performance energy and protein utilization in poultry. Ph D. Thesis. Deemed University, IVRI, Izatnagar.

Virdi J.S., Tiwari R.P., Saxena M., Khanna V., Singh G., Saini S.S. and Vadehra D.V. (1989). Effects of aflatoxin on the immune system of the chick. *J. Appl. Toxicol*. **9,** 271-275.

Zeman M., Buyse J., Herichova I. and Decuypere E. (2001). Melatonin decreases heat production in female broiler chickens. *Acta Vet. Brno*. **70,** 15-18.