

# 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 ( $P\leq 0.05$ ) 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 cellu-

lar 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<sub>1</sub> (AFB<sub>1</sub>) is the most potent and pathogenic form to poultry. Liver is considered to be the primary target organ for the aflatoxins and AFB<sub>1</sub> 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.

AFB<sub>1</sub> is oxidized by microsomal mixed function oxidase (cytochrome P450) to several water-soluble metabolites. The formation of AFB<sub>1</sub>-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 peroxy 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 Siope, 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-*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.

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.

**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 <sup>1</sup>	0.1	0.1
Vitamin premix <sup>2</sup>	0.15	0.15
Choline chloride	0.015	0.015
B Complex <sup>3</sup>	0.06	0.4
Cocciostat	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

<sup>1</sup> Trace mineral (10g/100g): FeSO<sub>4</sub>.7H<sub>2</sub>O: 8 g; ZnSO<sub>4</sub>.7H<sub>2</sub>O: 10 g;

MnSO<sub>4</sub>.H<sub>2</sub>O: 10 g; CuSO<sub>4</sub>.5H<sub>2</sub>O: 1 g; KI: 30 mg and Maize: 70.97 g.

<sup>2</sup> Vitamin A B<sub>2</sub>D<sub>3</sub>K each g contains: vitamin A: 82500 IU; vitamin B<sub>2</sub>: 50 mg; vitamin D<sub>3</sub>: 12000 IU and vitamin K: 10 mg.

<sup>3</sup> B-complex: each g contains: vitamin B<sub>1</sub>: 8 mg; vitamin B<sub>6</sub>: 16 mg; vitamin B<sub>12</sub>: 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 6<sup>th</sup> 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≤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 3<sup>rd</sup> 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	1 <sup>st</sup> week	2 <sup>nd</sup> week	3 <sup>rd</sup> week	4 <sup>th</sup> week	5 <sup>th</sup> week	6 <sup>th</sup> week
CTRL	75.03±0.89 <sup>ab</sup>	201.88±2.90 <sup>ab</sup>	388.07±5.78 <sup>ab</sup>	528.12±8.12 <sup>a</sup>	784.25±11.62 <sup>ab</sup>	1061.40±15.55 <sup>a</sup>
AF	65.92±2.53 <sup>c</sup>	184.72±2.64 <sup>d</sup>	338.43±4.23 <sup>c</sup>	468.23±6.95 <sup>c</sup>	728.23±10.92 <sup>d</sup>	946.40±14.09 <sup>b</sup>
MEL	76.50±3.68 <sup>a</sup>	205.27±5.04 <sup>a</sup>	392.19±3.90 <sup>a</sup>	532.44±11.00 <sup>a</sup>	799.18±14.44 <sup>a</sup>	1060.36±17.38 <sup>a</sup>
TRY	76.09±1.51 <sup>a</sup>	203.35±2.99 <sup>a</sup>	388.16±5.28 <sup>ab</sup>	526.30±5.82 <sup>a</sup>	782.11±10.96 <sup>abc</sup>	1060.75±13.78 <sup>a</sup>
AF+MEL	70.45±1.27 <sup>abc</sup>	192.52±3.08 <sup>bcd</sup>	359.69±5.80 <sup>cd</sup>	490.34±8.57 <sup>bc</sup>	741.21±13.30 <sup>cd</sup>	962.43±18.8 <sup>b</sup>
AF+TRY	69.38±1.59 <sup>bc</sup>	190.77±2.97 <sup>cd</sup>	350.36±5.94 <sup>de</sup>	472.30±8.42 <sup>c</sup>	734.55±11.30 <sup>d</sup>	950.66±13.28 <sup>b</sup>

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

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

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

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

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 findings 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 aflatoxin 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 a potent 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 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

**Table 4** Effect of melatonin and its precursor (L-tryptophan) on haemagglutination 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 <sub>2</sub> )	Liver (% b.w.)	Kidney (% b.w.)	Bursa (% b.w.)	Spleen (% b.w.)
CTRL	0.411±0.018 <sup>a</sup>	9.11±0.42 <sup>ab</sup>	2.53±0.08 <sup>c</sup>	0.64±0.05	0.248±0.006 <sup>ab</sup>	0.24±0.02
AF	0.316±0.011 <sup>c</sup>	5.78±0.40 <sup>c</sup>	3.05±0.15 <sup>a</sup>	0.72±0.04	0.203±0.004 <sup>c</sup>	0.26±0.02
MEL	0.436±0.014 <sup>a</sup>	9.22±0.43 <sup>a</sup>	2.55±0.09 <sup>c</sup>	0.64±0.04	0.259±0.005 <sup>a</sup>	0.27±0.01
TRY	0.414±0.008 <sup>a</sup>	8.67±0.33 <sup>abc</sup>	2.52±0.15 <sup>c</sup>	0.69±0.05	0.234±0.005 <sup>bc</sup>	0.26±0.02
AF+MEL	0.362±0.011 <sup>b</sup>	8.22±0.32 <sup>abcd</sup>	2.82±0.14 <sup>bc</sup>	0.62±0.04	0.218±0.006 <sup>cde</sup>	0.29±0.03
AF+TRY	0.376±0.006 <sup>b</sup>	7.89±0.35 <sup>bcd</sup>	2.88±0.08 <sup>ab</sup>	0.65±0.05	0.212±0.004 <sup>de</sup>	0.28±0.02

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

**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 <sup>b</sup>	13.8±0.67 <sup>b</sup>	3.5±0.25 <sup>ab</sup>
AF	142.8±3.07 <sup>a</sup>	19.8±2.41 <sup>a</sup>	2.6±0.19 <sup>c</sup>
MEL	127.5±4.95 <sup>b</sup>	14.5±0.82 <sup>b</sup>	3.5±0.20 <sup>ab</sup>
TRY	125.5±6.50 <sup>b</sup>	14.5±0.80 <sup>b</sup>	3.7±0.19 <sup>a</sup>
AF+MEL	126.9±4.16 <sup>b</sup>	14.5±0.78 <sup>b</sup>	3.1±0.17 <sup>bc</sup>
AF+TRY	133.3±5.30 <sup>ab</sup>	18.2±1.63 <sup>ab</sup>	3.0±0.13 <sup>bc</sup>

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

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.

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