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Effect of Supplementing Different Levels of Turmeric Powder on Ileal Morphology in Laying Hens

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A B S T R A C T

To investigate the effects of using different levels of turmeric powder on ileal morphology in laying hens, one hundred and forty four, 22-weeks old White Leghorn hens were divided into 6 treatments with 4 replications and each replicate had 6 birds. Control group (T_1) was fed maize-soybean meal based diet without antibiotic while birds in T_2 group were fed basal diet with antibiotic. In treatment groups T_3, T_4, T_5 and T_6 basal diet was supplemented with turmeric powder @ 0.25%, 0.50%, 0.75%, and 1% respectively. After 16 weeks of the experimental period, one bird from each replicate was slaughtered and ileal sample were collected for morphometric analysis. The mean values of villi length, villi width and crypt depth in ileum of hens were ranged 943.81 to 1148.20 microns (villi length), 192.42 to 213.29 microns (villi width), 184.65 to 213.79 microns (crypt depth) respectively. There was significant ($P<0.05$) increase in the mean values of villi length from 947.94 microns in control group T_1 to 1145.77 microns in treatment group T_6 fed highest level of turmeric powder (1.0%). Similarly there was significant ($P<0.05$) increase in villi width also from 194.78 microns in T_1 to 213.29 microns in T_6 (1.0 % turmeric powder). There was significant ($P<0.05$) increase in crypt depth from 189.32 microns in T_1 to 213.79 microns in T_6 (1.0 % turmeric powder). Thus, it can be concluded that dietary inclusion of turmeric powder reported a trend of improvement in villi length, villi width and crypt depth in gastrointestinal tract of hens.

Introduction

Poultry farming has undergone a paradigm shift in structure and operation, transforming itself from a mere backyard activity into a major commercial venture. India has emerged as the world's 2nd largest poultry market with an annual growth of more than 14%, producing 78.48 billion eggs and 3.04 million tonnes of broiler meat per year (Anonymous, 19th Livestock census all India report, 2012). Poultry farming is, however highly susceptible to infectious diseases and antibiotics are used to treat diseases and used as antimicrobial growth promoters in poultry feed worldwide for many years. However, in order to avoid the possible risk of developing resistant pathogens, as well as to meet the public pressure of antibiotic-free animal products, the use of antibiotic in poultry diet was totally

banned in European Community (Anonymous, 2003). Nevertheless, apart from preventing the potential hazard, the absence of antibiotic growth promoters in the diet has resulted in health problems in poultry (Casewell *et al.*, 2003). In order to reduce the disadvantage of sub-clinical and clinical infections, the poultry and feed industries needs to find alternatives to antibiotic growth promoters. Compared with synthetic antibiotics or inorganic chemicals, plant-derived products have proved to be less toxic, residue free and thought to be ideal feed additives in food animal production (Wang *et al.,* 1998). These natural dietary agents have drawn a great deal of attention from both the scientific community and the general public due to their various health prompting effects (Shukla and Singh, 2007). Turmeric a member Zingiberaceae family has been extensively used for the treatment of a variety of inflammatory conditions and other diseases. Curcuminoids have a wide spectrum of biological activities including antioxidant, antibacterial, antifungal, antiprotozoal, antiviral, anticoccidial and anti-inflammatory property (Masuda *et al.,* 2001).

Ethical approval

The animal experiment was conducted in accordance with guidelines approved by the Institutional Animal Ethics Committee, 12/CPCSEA Dated 6.2.2017 in the Department of Animal Nutrition, Lala Lajpat Rai University of Veterinary and Animal Sciences, Hisar.

Experimental design

Completely Randomized Design was used as experimental design at uniform and standard management practices.

Materials and Methods

A total of one hundred and forty four single comb White Leghorn hens of commercial strain, 22 weeks of age, in the first phase of their production cycle with an average weight of 1764 g were randomly divided in to six treatment groups, having four replications with six birds in each replication. The birds were fed a maize-soybean meal based diet as per BIS standards (2007) in first treatment (T_1-negative control) and in second treatment the birds were fed with same diet added with antibiotic (T_2-positive control) and four different levels of turmeric powder were selected @ 0.25%, 0.50%, 0.75% and 1.0% in T_3, T_4, T_5 and T_6 respectively. Hens were fed the experimental diet for sixteen weeks beginning at 22 weeks of age and continued up to 38 weeks of age. All the feed ingredients were procured in one lot before the start of the experiment. The feed ingredients, feed additives and supplements used were maize, groundnut cake, soybean meal, rice polish, fish meal, mineral mixture, common salt, shell grit and vitamins. Feed additives and supplements were premixed and then mixed with weighed quantity of feed ingredients to make a homogenous mixture of rations. Chemical composition (%DM basis) and metabolizable energy (ME, Kcal/Kg) of feed ingredients used in formulation of experimental diets are presented in the Table 1. The ingredients, composition and mixing rate of feed additives/supplements used in ration formulations are presented in Table 2. The laying hens were reared in deep litter system and hens were offered feed and water *ad libitum*. The data obtained were analyzed by analysis of variance with SPSS procedure and Least Significant Difference test with significance level of 95%.

Gastrointestinal morphology

At the end, one bird from each replicate was slaughtered and ileal samples were collected for intestinal morphological examinations, cross-sections measuring 1 mm to 2 mm thick from ileum were prepared and enclosed in tissue cassettes. The tissues were fixed in 10%

neutral buffered formalin over 24 h for each intestinal segment, a 5µm section was placed onto a glass slide and stained with alcian blue and hematoxylin-eosin. Slides were viewed under Fluorescent microscope and visual measurements were taken of the villous length (µm distance from apex of villus to the junction of the villous and crypt), villous width and crypt depth (µm distance from the junction to the basement membrane of the epithelial cell at the bottom of the crypt).

Statistical analysis

Data was analysed statistically as described by Snedecor and Cochran (1994). Analysis of variance was used to study the differences among treatment means and they were compared by using Duncans Multiple Range Test 1955(DMRT) as modified by Kramer (1956).

Results and Discussion

Intestinal morphology

The mean values of villi length, villi width and crypt depth in ileum of hens were 947.94, 943.81, 948.19, 1142.44, 1145.77 and 1148.20 microns (villi length), 194.78, 192.42, 198.55, 206.85, 211.44 and 213.29 microns (villi width), 189.32, 184.65, 195.01, 198.99, 207.49 and 213.79 microns (crypt depth) are (Table 3) in treatment groups T_1, T_2, T_3, T_4, T_5 and T_6 respectively. The findings clearly indicate that, there was significant ($P<0.05$) increase in the mean values of villi length from 947.94 microns in control group T_1 to 1145.77 microns in treatment group T_6 fed highest level of turmeric powder (1.0%). Similarly there was significant ($P<0.05$) increase in villi width also from 194.78 microns in T_1 to 213.29 microns in T_6 (1.0 % turmeric powder). Results of crypt depth were also same as above measures. There was significant ($P<0.05$) increase in crypt depth from 189.32 microns in T_1 to 213.79 microns in T_6 (1.0 % turmeric powder). Thus, it can be concluded that dietary inclusion of turmeric powder reported a trend of improvement in villi length, villi width and crypt depth in gastro-intestinal tract of hens. Figure 1 and 2 represents the mean values of villi length and crypt depth under different treatments. Figure 3, 4, 5, 6, 7 and 8 depicts the histological morphometric analysis of villi length of ileal samples in T_1, T_2, T_3, T_4, T_5 and T_6 respectively.

Table.1 Chemical composition (%DM basis) and metabolizable energy (Kcal/Kg) of feed ingredients the experimental diets

Ingredients	CP	CF	EE	Ash	ME*
Maize	9.10	2.65	3.39	2.50	3309
Groundnut cake	40.90	8.90	7.94	4.52	2596
Soybean Meal	45.15	3.78	3.43	6.93	2230
Rice Polish	12.20	4.69	14.78	12.83	2737
Fish Meal	48.15	2.05	5.30	22.43	2240

Table.2 Ingredient and chemical composition of ration for layers of control group

Feed ingredients	Percentage (%)
Maize	58
Groundnut cake	10
Soybean Meal	12
Rice Polish	09
Fish Meal	06
Mineral Mixture	1.5
Salt	0.5
Shell Grit	03
Chemical composition	**% DM basis**
CP	18.04
CF	4.34
EE	3.61
NFE	66.21
Ash	7.80
Metabolizable energy(Kcal/Kg)	2697.17

*Feed additive included Intermix Regular 10 g, Meriplex d s 10 g, Toxinil 100g per 100 Kg of ration.

**Intermix Regular - Each g contained vitamin A- 82,500 IU, vitamin D_3-16,500 IU, vitamin B2- 50 mg and vitamin K- 10 mg. (10 g/quintal)*

*Meriplex- DS - Each g contained vitamin B1- 8 mg, vitamin B_6 -16 mg, Niacin- 120 mg, vitamin B_{12}- 80 mcg, Calcium D Pantothenate- 80 mg, vitamin E_{50} -80 mg and Calcium- 88 mg. (10 g/quintal)

*Toxinil - Organic acid, mannon oligosaccharide (MOS), activated charcoal, sodium bentonite, HSCAS (hydrated sodium calcium allumino silicate), copper oxine, *Bacillus subtilis* (100 g/quintal)

*Mineral mixture - Mineral mixture for poultry: composition (w/w): moisture- 3% (maximum), Calcium- 32% (minimum), Phosphorus- 6% (minimum), Manganese- 0.27% (minimum), Iodine- 0.01% (minimum), Zinc- 0.26% (minimum), Fluorine- 0.03% (maximum), Copper- 0.001% (minimum) and Iron- 0.001% (minimum). (1.5 kg/quintal).

Table.3 Mean values of villi length, villi width and crypt depth in gastro-intestinal tract in layers under different treatments

Treatments	Villi length (microns)	Villi width (microns)	Crypt depth (microns)
T_1	$947.94^{ab} \pm 2.44$	$194.78^{ab} \pm 0.50$	$189.32^{ab} \pm 2.95$
T_2	$943.81^{a} \pm 0.27$	$192.42^{a} \pm 0.26$	$184.65^{a} \pm 1.12$
T_3	$948.19^{b} \pm 0.32$	$198.55^{b} \pm 0.97$	$195.01^{b} \pm 1.42$
T_4	$1142.44^{c} \pm 0.63$	$206.85^{c} \pm 1.14$	$198.99^{bc} \pm 1.87$
T_5	$1145.77^{cd} \pm 2.12$	$211.44^{cd} \pm 3.52$	$207.49^{cd} \pm 1.86$
T_6	$1148.20^{d} \pm 0.85$	$213.29^{d} \pm 0.28$	$213.79^{d} \pm 6.54$

The mean values in same column with different superscripts differ significantly ($P < 0.05$)

Fig.1 Mean values of villi length (microns) in layers under different dietary treatments

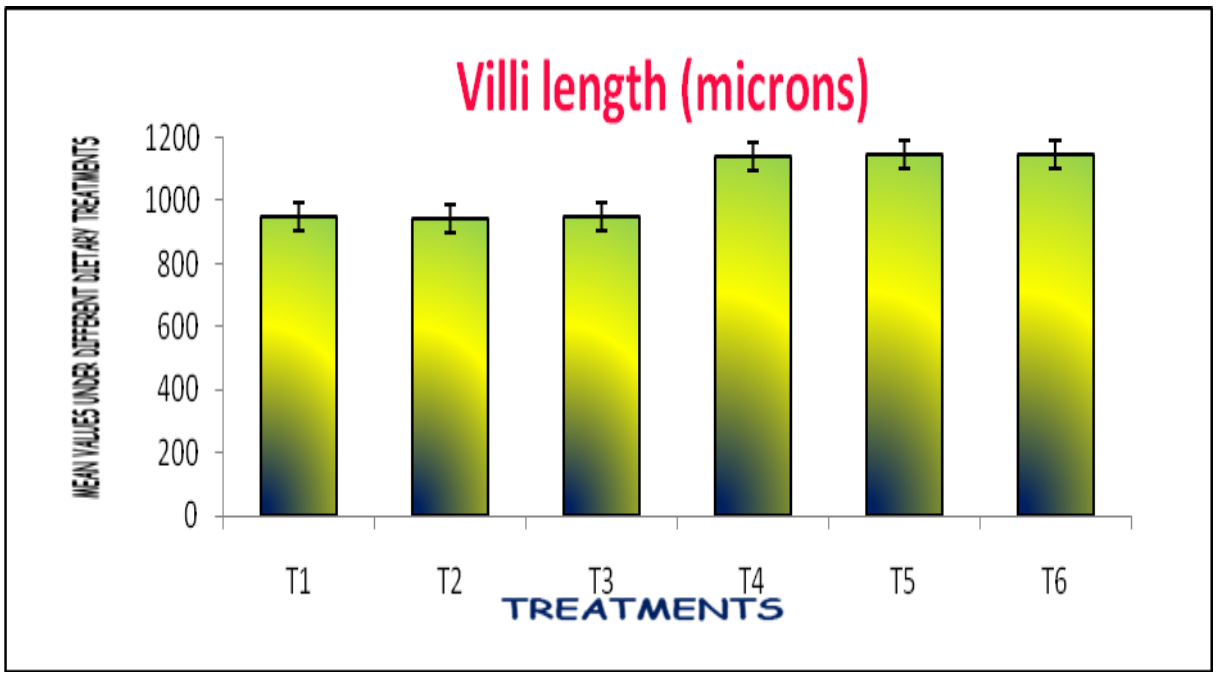


Fig.2 Mean values of crypt depth (microns) in layers under different dietary treatments

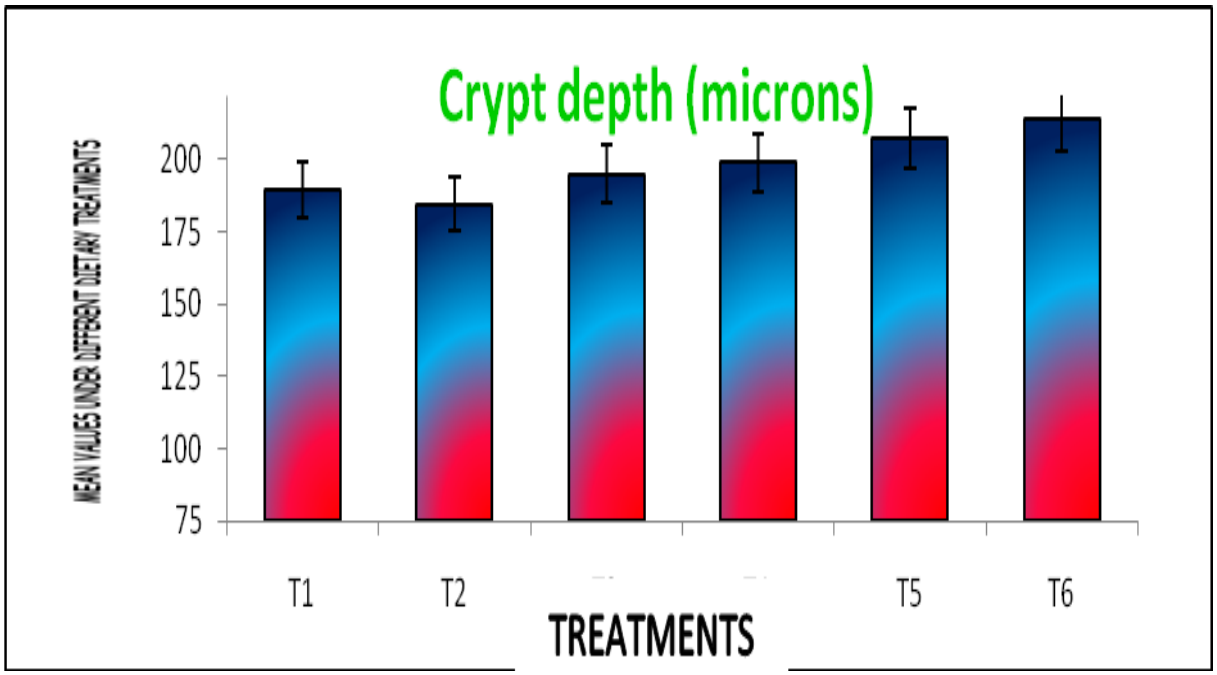


Fig.3 Villi length of ileum in group T_1

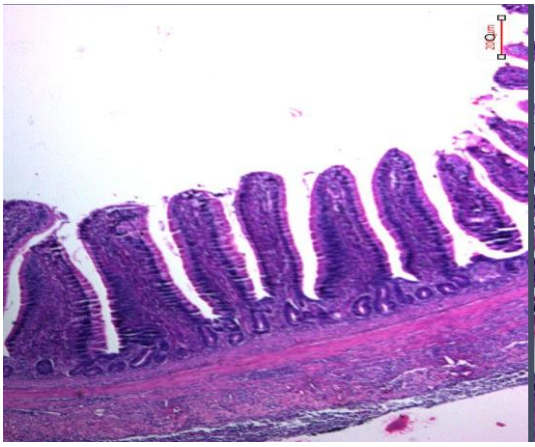


Fig.4 Villi length of ileum in group T_2

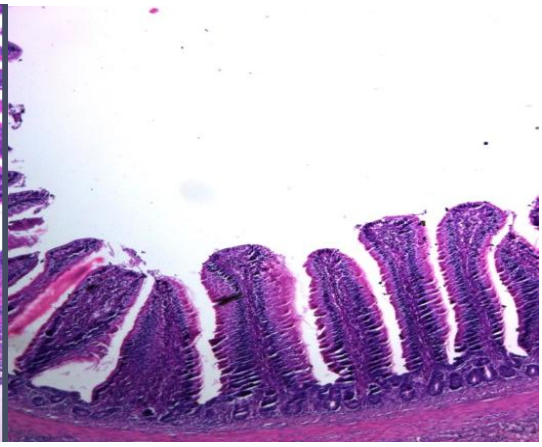


Fig.5 Villi length of ileum in group T_3

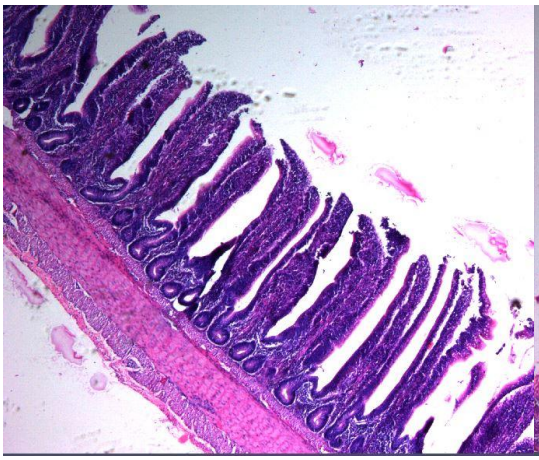


Fig.6 Villi length of ileum in group T_4

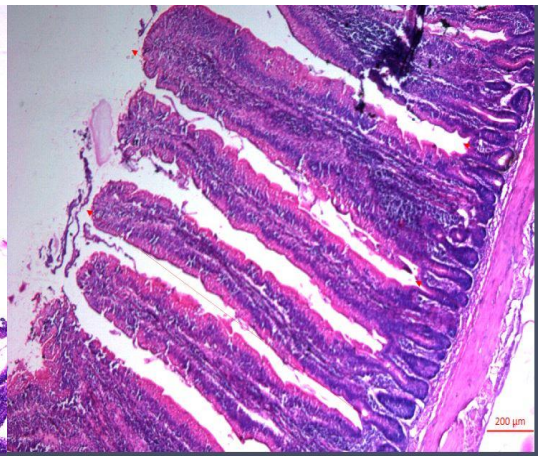


Fig.7 Villi length of ileum in group T_5

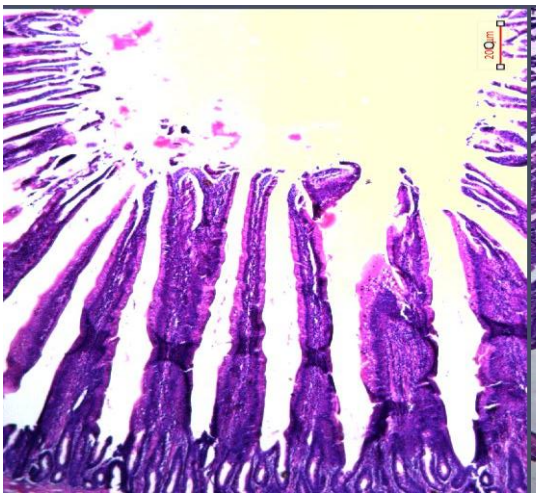
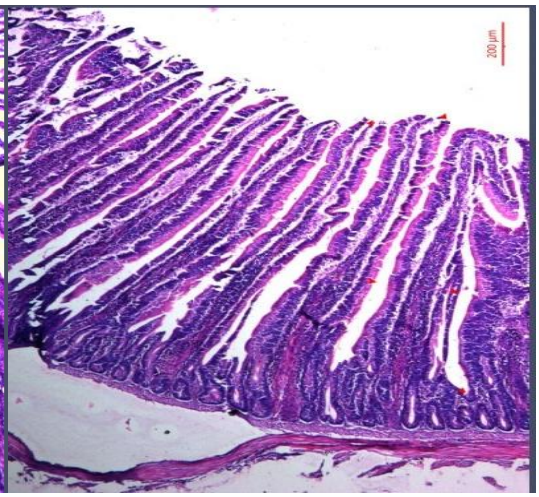


Fig.8 Villi length of ileum in group T_6



It may be due to the decreased bacteriological count of harmful microorganisms in the GI tract of birds; these microorganisms produce toxins which causes the sloughing off the intestinal epithelium. Turmeric powder neutralizes these toxins, so the intestinal epithelium renews itself. This ultimately results in increased villi length, width. The morphometric analysis results in the present study showed that the supplementation of antioxidants increased villi height and villi width. Also phenolic compounds administration like curcumin may reduce gut inflammation, improve digestibility of nutrients and metabolism (Giannenas *et al.*, 2010). The villi:crypt depth ratio is an indicator of the likely digestive capacity of the small intestine. An increase in this ratio corresponds to an increase in digestion and absorption (Montagne *et al.*, 2003). Increased intestinal villi height and the ratio of villi height to crypt depth is an indication of vast area for nutrient absorption and higher absorption function (Sieo *et al.*, 2005). Thinning of the gastrointestinal walls tract may be due to the inhibition of the microbial production of polyamines and volatile fatty acids, known to increase enterocyte turnover rate and activity. This increased net energy committed to maintaining the luminal tissue comes at the expense of more productive purposes such as muscle accretion (Bedford, 2000).

These findings are in consistent agreement with Rajput *et al.,* (2013) found that the duodenal villi heights were significantly higher in birds fed turmeric powder at level of 200 mg/kg at 21 and 42 day. The jejunum villi heights were significantly higher in birds fed turmeric powder at level of 200 mg/kg at 21 day and in birds fed turmeric powder at level of 100mg/kg feed at 42 day. While, ileum villi height were significantly higher in birds fed turmeric at 150 and 200 mg/kg levels, in comparison with control and 100 mg/kg level groups at 21 and 42 day. He also found that the duodenal villi width was significantly higher in birds fed turmeric at 200 mg/kg level at 21 day; while, at 42 day, control and group fed turmeric at 150mg/kg exhibited more width. Furthermore, jejunum villi width at 42 day was significantly more in birds fed turmeric at 150 mg/kg and 200 mg/kg level, as compared to control group. Namagirilakshmi *et al.,* (2010) reported that the turmeric feeding to birds significantly increased ($P<0.01$) the intestinal villi length than control. This increase in the intestinal villi length could be attributed to the turmeric effect on gut health by reducing intestinal pH, bacterial load and selectively increasing *Lactobacillus* count (Sieo *et al.,* 2005). Contrary to these findings Rajput *et al.,* (2013) stated that depth of intestinal crypts in curcumin supplemented groups was significantly less for all segments except for jejunum at 42 day of small intestine, as compared to those of control group. While, Kumar *et al.,* (2017) stated that intestinal morphology in the duodenum, jejunum and ilium were not generally affected by BCS (black cumin seed).

In conclusion, the morphometric analysis results in the present study showed that the supplementation of antioxidants increased villi height and villi width, antioxidants present in turmeric powder may effectively scavenge the generated free radicals caused by stress, consequently resulting in improved ileal morphology. Also phenolic compounds like curcumin present in turmeric may reduce gut inflammation, improve digestibility of nutrients and metabolism. The villi: crypt depth ratio is an indicator of the likely digestive capacity of the small intestine. Increased intestinal villi height and the ratio of villi height to crypt depth is an indication of vast area for nutrient absorption and higher absorption function results in efficient production.

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