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Effect of Supplementing Different Levels of Turmeric Powder on Gut Microbiota in Laying Hens

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ABSTRACT

To investigate the effects of using different levels of turmeric powder on ileal micro-biota 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 contents were collected aseptically, weighed (1g each), transferred to sterile tubes and homogenized with sterile 0.9% normal saline solution (1:1) and mixed on vortex. 10 fold serial dilutions of samples were made up to six dilutions and 0.1 ml of each dilution was poured and spread uniformly on Mc Conkey Lactose Agar (MLA) for *E. coli* and MRS for *Lactobacillus*. Plates were incubated at 37 ºC for 24 hours. Bacterial colonies were counted by pour plate method. The average number of colonies was multiplied by reciprocal of the dilution factor and expressed as log cfu/g of contents. The mean values of *E. coli* ranged 4.85 to 6.89 (log cfu/g) in treatment groups, there was significant ($P<0.05$) decrease in the mean values of *E. coli* count from 6.89 log cfu/g in negative control group T_1 to 4.85 log cfu/g in treatment group T_6 fed highest level of turmeric powder (1.0%). The mean values of *E. coli* (log cfu/g) decreased significantly ($P<0.05$) as the level of turmeric powder inclusion in the diet increased from 0.25 % to 1.0 %. The mean values of *Lactobacillus* ranged 5.48 to 7.17 (log cfu/g) in treatment groups, there was significant ($P<0.05$) increase in *Lactobacillus* sp. from 5.88 log cfu/g in T_1 to 7.17 log cfu/g in treatment T_6 fed highest level of turmeric powder (1.0%). The mean values of *Lactobacillus* sp. (log cfu/g) decreased significantly ($P<0.05$) as the level of turmeric powder inclusion in dietary feed increased from 0.25 % to 1.0 %. Thus, it can be concluded that dietary inclusion of turmeric powder shows a trend of improvement in gut micro flora environment.

Introduction

Poultry farming is, highly susceptible to infectious diseases and antibiotics are used to treat these diseases. These antibiotics have been used as antimicrobial growth promoters in poultry feed worldwide for many years to improve food safety by improving animal health and reducing or removing exogenous pathogens. 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). Compared with synthetic antibiotics or inorganic chemicals, these plant-derived products have proved to be less toxic, residue free and are 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 rhizome as such contains 6.3% proteins, 5.1% fat, 3.5% minerals and 69.4% Carbohydrates (Chattopadhyay *et al.*, 2004). It has both oil parts as well as coloring pigment part. The coloring material is a rich source of the *curcumin*, bis demethoxy *curcumin* and demethoxy curcumin, collectively referred to as curcuminoids which act as powerful antioxidants, (Haung *et al.,* 1995). *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%.

Bacteriological assay

One bird from each replicate was slaughtered at the end of experiment and ileal contents were collected aseptically. Samples were weighed (1g each), transferred to sterile tubes and homogenized with sterile 0.9% normal saline solution (1:1). Then the solutions were mixed on vortex. 10 fold serial dilutions of samples were made up to six dilutions and 0.1 ml of each dilution was poured and spread uniformly on MLA for estimation of *Coliform* count and MRS for *Lactobacillus*. Plates were incubated at 37 ºC for 24 hours. Bacterial colonies were counted by pour plate method (Quinn *et al.,* 1992). The average number of colonies was multiplied by reciprocal of dilution factor and expressed as log cfu/g of contents.

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

Bacteriological assay

Data pertaining to *E. coli* and *Lactobacillus* sp. count of ileal content of the experimental birds under different dietary treatments are presented in Table 3. The mean values of *E. coli.* in ileum of hens were 6.89, 5.01, 5.94, 5.24, 5.15 and 4.85 log cfu/g 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 ($P<0.05$) decrease in the mean values of *E. coli* count from 6.89 log cfu/g in negative control group T_1 to 4.85 log cfu/g in treatment group T_6 fed highest level of turmeric powder (1.0%). The mean values of *E. coli* (log cfu/g) decreased significantly ($P<0.05$) as the inclusion level of turmeric powder increased from 0.25 % to 1.0 %. Significant reduction in the *E. coli* count was observed in T_2 and T_6 group in comparison to the control group. Results of present study are in agreement with the findings of Miquel *et al.,* (2002) observed that turmeric powder has been reported to exhibit antimicrobial properties and ethanol turmeric extract demonstrated high potential to inhibit some pathogenic bacteria of chickens. Thus like antibiotics, turmeric powder could control and limit the growth and colonization of numerous pathogenic and non-pathogenic species of bacteria in the bird's gut resulting in balanced gut microbial ecosystems that lead to better feed utilization reflected by improved feed conversion ratio. Similarly, Samarasinghe *et al.,* (2003) reported that colony forming units of *Coliform* bacteria, yeast and mould as well as total viable microbes in broiler gut contents were markedly reduced ($P<0.05$) when the diet was supplemented with turmeric powder, mannonoligosaccharide or virginiamycin.

The mean values of *Lactobacillus* sp. in ileum of hens were 5.58, 5.48, 6.08, 6.64, 6.81 and 7.17 (log cfu/g) in treatment groups T_1, T_2, T_3, T_4, T_5 and T_6 respectively. The values of bacteriological count (log cfu/g) clearly indicate that there was significant ($P<0.05$) increase in *Lactobacillus* sp. from 5.88 log cfu/g in T_1 to 7.17 log cfu/g in treatment T_6 fed highest level of turmeric powder (1.0%). The mean values of *Lactobacillus* sp. (log cfu/g) increased significantly ($P<0.05$) as the level of turmeric powder inclusion in dietary feed increased from 0.25 % to 1.0 %. Thus, it can be concluded that dietary inclusion of turmeric powder shows a trend of improvement in gut micro flora environment.

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

*Calculated value (Singh and Panda, 1992)

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 *E. coli* and *Lactobacillus* sp. count in gastro-intestinal tract in layers under different treatments

Treatments	Ileum	
	***E. coli* (log cfu/g)**	***Lactobacillus sp.* (log cfu/g)**
T_1	6.89 [c] ±0.08	5.58 [a] ±0.34
T_2	5.01 [ab] ±0.18	5.48 [a] ±.028
T_3	5.94 [b] ±0.48	6.08 [bc] ±0.26
T_4	5.24 [ab] ±0.33	6.64 [bcd] ±0.26
T_5	5.15 [ab] ±0.28	6.81 [cd] ±0.10
T_6	4.85 [a] ±0.25	7.17 [d] ±0.01

Fig.1 Mean values of *E. coli* (log cfu/g) in layers under different dietary treatments

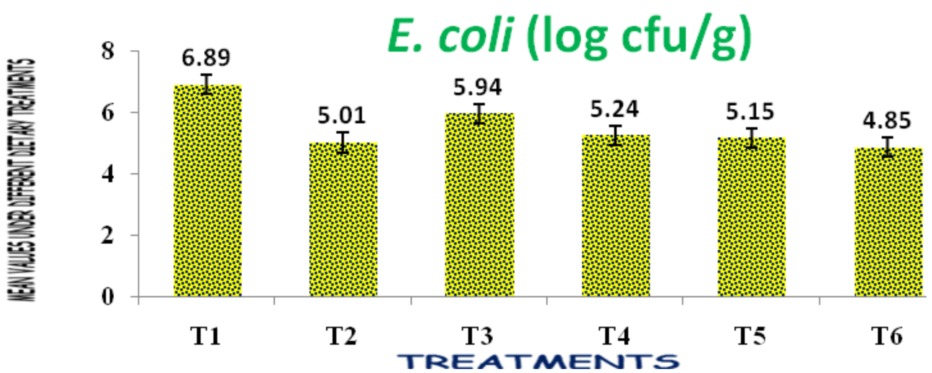
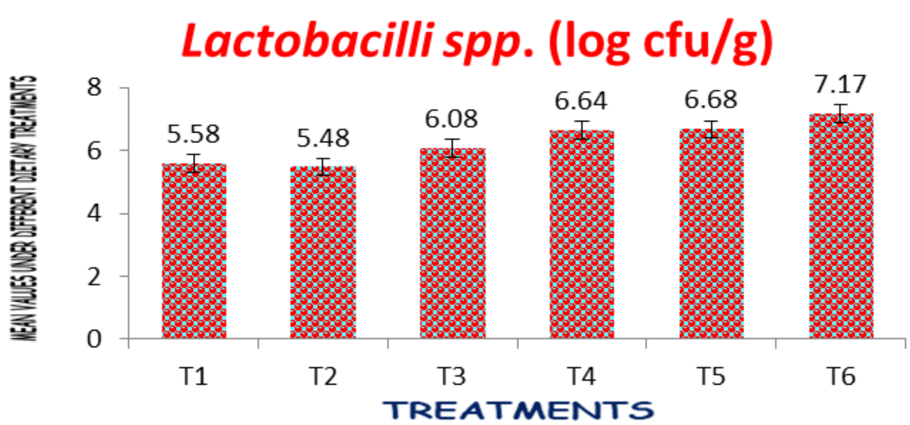


Fig.2 Mean values of *Lactobacillus* sp. (log cfu/g) in layers under different dietary treatments



Significant increase in *Lactobacillus* sp. count was observed in T_2 and T_6 group in comparison to the control group. Figure 1 and 2 represents the *E. coli* and *Lactobacillus* sp. count (log cfu/g) under different dietary treatments similarly Namagirilakshmi *et al.*, (2010) stated that intestinal microbial count was significantly reduced (P<0.01) by 45, 54, 56 and 48 % in 0.25%, 0.50 % 0.75% and 1.0% turmeric fed groups respectively compared to control. This reduction in microbial load of broiler chickens could be due to the antibacterial effect of turmeric on intestinal micro biota. Similarly, *Lactobacillus* sp. count was significantly higher (P<0.01) in 1.0% turmeric fed group compared to other groups. These observations differed from the reports of Araujo and Leon (2001) found that turmeric alcoholic extract (10-200mg/ml) inhibited the growth of *Lactobacillus* sp. in vitro. The higher number of *Lactobacillus* sp. count in 1% turmeric fed chicks might be due to stimulatory effect at higher level of turmeric in feed.

Jamroz *et al.,* (2005) reported that plant extract supplement also significantly increases the *Lactobacillus* sp. numbers following an application of natural plant extract. Similarly Siddiqui *et al., (*2015) studied the effect of different dietary levels of *Nigella sativa* seed powder on *E. coli* and total viable bacterial count in excreta of broilers. Both *E. coli* and total bacterial counts were significantly decreased by *Nigella sativa* seed powder supplemented diets irrespective of inclusion levels. Contrary to these findings Kumar *et al.,* (2017) stated that the counts of total bacteria, *E. coli*, *Lactobacillus* sp., and *Clostridium* sp. were not affected by BCS (black cumin seed) and AB (antibiotic growth promoters) compared with the control group. But, *Salmonella* sp. decreased linearly (P = 0.05) with increasing doses of BCS.

In conclusion, antibacterial effects of turmeric powder on gram negative bacteria and beneficial effect on gram positive bacteria at 1% inclusion level results in production of more lactic acid by *Lactobacilli* in gut which further leads to decreased pH in intestine thereby at low pH *Coliforms* are not able to colonise themselves within intestinal mucosa, which interns cause less sloughing of tissue due to decreased toxin produced by them. Thus like antibiotics, turmeric powder could control and limit the growth and colonization of numerous pathogenic and non-pathogenic species of bacteria in the bird's gut resulting in balanced gut microbial ecosystems that lead to better feed utilization reflected by improved feed conversion ratio.

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