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Dietary supplementation of probiotic on growth, serum cholesterol and gut microflora of broilers

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Probiotic supplementation in the diet of broilers is gaining attention as growth promoter after the ban on antibiotics because of presence of antibiotic residues in animal products and the development of drug resistant microorganisms in humans (Jin *et al.* 1997). Probiotics act as growth promoters, feed savers, nutritional bio-regulators and help in improving performance and health. Continuous use of probiotics reinforces the nonspecific immune system of animals, and consequently, anti-infectious treatments are reduced by probiotics (Khajarern and Ratanasethakul 1998). Mulder (1991) observed that lactobacilli have a bactericidal or bacteristatic effect towards *Salmonella*, *E. coli* and *Campylobacter*, etc. Detrimental effect of cholesterol in human health is a well-established phenomenon. Poultry industry is already defensive due to the high cholesterol content of meat. There is a need to reduce the cholesterol content and probiotics can help in it too (Mohan *et al.* 1995). The present experiment is, therefore, undertaken to evaluate the effect of dietary supplementation of probiotic on growth, serum cholesterol and gut microflora of broilers.

Day-old commercial broiler chicks (120) were wing banded, weighed and randomly distributed into 2 groups of 60 chicks in each. Subsequently each group was distributed to 12 replicates of 5 chicks each. The chicks were vaccinated against Marek's disease and were reared in raised wire floor battery brooders till the end of the experiments. Chicks in each group were placed on a basal diet containing 2900 kcal/kg ME and 22% CP. Probiotic was supplemented @ 100mg/kg diet in 1 group whereas the other group fed only basal diet, served as control. Water and feed were offered *ad lib.* throughout the experiment. All chicks were vaccinated against RD, IBD and fowl- pox as per the routine vaccination schedule.

The probiotic used in the study was a commercial probiotic preparation containing *Lactobacillus acidophilus, L.casei, Bifidobacterium bifidum, Aspergillus oryzae, Streptococcus faceium* and *Torulopsis* sps with 27 billion colony forming units per 100 g of the product.

Data on biweekly growth of chicks were measured up to 6 weeks of age. On day 42, blood was collected from 2 replicates (10 birds) per group for serum cholesterol estimation. The serum cholesterol (Zlatkis *et al.* 1953), HDL cholesterol (Demacker and Hifmans 1980), serum triglyceride (Fossati and Lorenzo 1982) were estimated by using the respective diagnostic kits. VLDL cholesterol was calculated from triglyceride by dividing with the factor 5. The LDL cholesterol was calculated by using the following formula:

LDL cholesterol = total cholesterol - HDL cholesterol - triglycerides/5

On the same day another 2 replicates (10 birds) per group were sacrificed by stunning the head and their crop and caecum removed quickly and stored at 4°C for *Escherichia coli* counts. The crop was incised to expose inner lining, which was thoroughly scrubbed with normal physiological saline to make up to 10 ml. The *E.coli* counts were made in aliquots drawn from the saline extract. Caecal content (1g) from each bird was collected and suspended in 9 ml of nutrient broth. Serial dilutions of each sample were made in nutrient broth and *E. coli* counts were made on MacConkey's agar and EMB agar (American Public Health Association 1984) by surface spread method. Statistical analysis of data was made using the general linear model of SAS (6.1) and comparison of means were made by Student t test. Significance of data was considered at $P < 0.05$ level.

The effect of probiotic supplementation on body weight gain, crop and caecal *E. coli* counts and serum cholesterol is presented in Table 1. There was no significant effect of probiotic supplementation on body weight gain of broilers at 6 weeks of age. Similarly Mudalgi *et al.* (1993) reported no significant effect of probiotic culture *(Lactobacillus acidophilus* and *L. bulgaricus)* on growth of broilers fed diet containing 20 or 21.8% protein. Panda *et al.* (1999) also indicated insignificant effect of probiotic on body weight gain in broilers at six weeks of age.

Dietary supplementation of probiolac has a significant effect on reducing the *E. coli* numbers in the crop but not in

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Table 1. Dietary supplementation of probiotic on body weight gain, *E. coli* counts and serum cholesterol of broilers

Parameters	Probiotic (mg/kg of diet)	
	0	100
Body weight gain (g)	1235±20	1272±24
E. coli numbers		
Crop*	11.3×10^{4b}	1.9×10^{4a}
Caecum**	4.1×10^{6}	3.2×10^{6}
Total serum cholesterol (mg/100ml)	146.06^{b}±8.87	122.06^{a}±6.45
HDL cholesterol (mg/100ml)	46.36±4.09	45.34±5.09
VLDL cholesterol (mg/100ml)	12.37±1.22	9.15±0.94
LDL cholesterol (mg/100ml)	87.31±9.53	67.56±8.78
Triglycerides (mg/100ml)	61.88±4.14	45.79±4.68

*Count expressed/ml of saline extract, **count expressed/g of caecal content means with different superscript in a row differ significantly ($P<0.05$).

the caecum. Concomitant to the finding of the experiments, Miles *et al.* (1981) reported a reduction in coliforms in probiotic fed chicks with no appreciable effect on coliforms in the caecum. Fuller (1973) reported that lactobacilli when present in sufficient numbers were directly involved in preventing the unrestricted growth of *E. coli* in crop by lowering the pH of the crop and bactericidal effect. The same worker also observed a significant reduction in number of *E.coli* of the caecum by feeding probiotics. However, in this experiment, the difference was nonsignificant in the caecum and it might be due to the more complex microbial population in the caecum than that in crop (Singh *et al.* 1994).

Supplementation of probiotic @ 100mg/kg in the diet of broiler significantly ($P<0.05$) reduced the total serum cholesterol. Similar cholesterol depressing effect was observed in rat by Rao *et al.* (1981). Nelson and Gilliand (1984) reported that some of the organisms present in the probiotic preparation could assimilate the cholesterol present in the gastrointestinal tract for their own metabolism, thus reducing the amount absorbed. This reduction in serum cholesterol could be attributable to reduced absorption and/or synthesis of cholesterol in the gastrointestinal tract by probiotic supplementation (Mohan *et al.* 1995). Further, it could be explained as *Lactobacillus acidophilus* reduces cholesterol in the blood by deconjugating bile salt in the intestine thereby preventing them from acting as precursor in cholesterol synthesis and reducing the serum cholesterol. Probiotic supplementation had no effect on various fraction of cholesterol like HDL cholesterol, VLDL cholesterol and LDL cholesterol and serum triglycerides.

Our findings suggest that supplementation of probiotic significantly reduced *E.coli* counts in the crop. There was also a significant reduction in the total serum cholesterol by supplementation of probiotic. This cholesterol depressing effect needs further investigation to confirm the observation obtained from the present study and examine the possible mode of action of probiotics.

SUMMARY

The effect of dietary supplementation of probiotic on growth, serum cholesterol and gut microflora of broilers was estimated. Water and feed were offered *ad lib.* throughout the experimental period of 6 weeks. There was no effect of probiotic supplementation on body weight gain at 6 weeks of age. Dietary supplementation of probiotic had a significant effect on reducing the total serum cholesterol concentration, but no effect was found on HDL cholesterol, VLDL cholesterol, LDL cholesterol and serum triglycerides. There was a significant reduction in *E. coli* count in the crop by supplementation of probiotics, however, the reduction was not significant in the caecum.

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