Dietary Supplementation of *Lactobacillus Sporogenes* on Performance and Serum Biochemico - Lipid Profile of Broiler Chickens

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An experiment was conducted to study the effect of dietary supplementation of probiotic *Lactobacillus sporogenes* (*L. sporogenes*) on performance and serum biochemico-lipid profile of broiler chickens. One hundred and twenty, day-old commercial broiler chicks were distributed equally into 3 groups of 40 chicks each, which was further replicated to 8 groups of 5 each. The chicks were placed on one of three dietary treatments : basal, basal with probiotic (6×10^8 spore of *Lactobacillus sporogenes* per g) at 100 or 200 mg per kg feed. Body weight gain increased and feed conversion ratio improved significantly due to dietary supplementation of 100 mg probiotic per kg diet. However, no further benefits on the above parameters could be observed by increasing the level of supplementation from 100 to 200 mg per kg. Leg abnormality score is not influenced by probiotic supplementation in the diet. However, the bone breaking strength and bone ash content increased significantly by probiotic supplementation at 100 mg per kg diet. The concentration of protein and calcium increased significantly due to dietary supplementation of *L. sporogenes*. Neither concentration of inorganic phosphorus nor activities of enzymes such as serum glutamate-oxaloacetate transminase (SGOT), serum glutamate-pyruvate transaminase (SGPT) and alkaline phosphatase (ALP) in serum were influenced due to *L. sporogenes* supplementation. Serum total cholesterol, low-density lipoprotein (LDL) cholesterol, very low-density lipoprotein (VLDL) cholesterol and triglycerides were reduced significantly by dietary supplementation of *L. sporogenes* at 100 mg per kg diet. However, high-density lipoprotein (HDL) cholesterol of birds was not influenced by *L. sporogenes* supplementation. It is concluded that dietary supplementation of *Lactobacillus sporogenes* (6×10^8 spore per g) at 100 mg per kg diet significantly enhanced body weight gain, improved feed conversion ratio, bone ash and bone breaking strength and lowered total cholesterol, VLDL cholesterol and triglycerides concentrations in the serum of broiler chickens.

Key words : Broiler Chickens, Cholesterol, *Lactobacillus sporogenes*, Performance, Serum Biochemical Profile

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Introduction

To achieve high level of economic efficiency broiler chickens are raised under intensive production system in densely populated colonies or flocks. This inflicts considerable stress due to various factors such as transport, overcrowding, vaccination, etc. Probiotics defined as live microbial feed supplement that beneficially affects the host animal by improving

its intestinal microbial balance (Fuller, 1989) have been administered to counteract such stresses which subsequently improve body weight gain, feed conversion ratio and mortality in broiler chickens (Cavazzoni *et al.*, 1998 ; Panda *et al.*, 2000 ; Haung *et al.*, 2004 ; Hooge *et al.*, 2004). Moreover, it has been shown that probiotics could protect broilers against pathogens by colonization in the gastrointestinal tract (Pascual *et al.*, 1999) and stimulation of

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systemic immune responses (Quere and Girard, 1999 ; Panda *et al.*, 2000).

The selection strategies for body weight gain in broiler chickens are resulting in lower quality poultry products due to higher body fat. The relatively larger amount of body fat in broiler carcass is not only a problem from consumer's preference point of view but also higher processing loss. Furthermore, the discarded abdominal and viscera fat increases waste management problems. Recently considerable attention has been paid to the potential of probiotics in altering lipid metabolism. This is due to the growing evidence that probiotics supplementation reduces cholesterol concentration in serum of chickens (Mohan *et al.*, 1996). Supplementation of probiotics (*Lactobacillus acidophilus*, *Bifidobacterium bifidum* and *Aspergillus oryzae*) at 100 mg per kg in the diet of broiler chickens significantly reduced the serum cholesterol concentration (Panda *et al.*, 2001). Kalavathy *et al.* (2003) reported that dietary supplementation of a mix culture of 12 strains of *Lactobacillus* at 0.1% in the basal diet of broilers resulted in higher body weight gain and lowered serum cholesterol concentration.

The most used groups in the production of probiotics are *Lactobacilli*. It is the non-spore bearing lactic acid bacteria such as *Lactobacillus acidophilus*, *Lactobacillus bulgaricus* and *Lactobacillus planatarum* widely studied as probiotics (Abdulrahim *et al.*, 1996). *Lactobacillus sporogenes*, (LS) a spore bearing lactic acid bacteria, which is stable, resistance to high temperature and tolerance to acid and bile salts (Cavazzoni *et al.*, 1998) is currently gaining momentum for use as probiotic. *Lactobacillus sporogenes* grows well, inhabits in intestine and excellent in production of biologically active lactic acid and digestive enzymes. The affinity for acidic pH and high tolerance to bile salts, the spore germinates and proliferates in the digestive tract of poultry (Han *et al.*, 1984). The information available on the use of *L. sporogenes* for enhancing performance of broiler chicken is scanty and to our knowledge the effect on altering serum lipid profile is not reported. Therefore, the present investigation is conducted to evaluate the effect of *L. sporogenes* on performance and serum biochemical and lipid profiles of commercial broiler chickens during 0 to 6 weeks of age.

Materials and Methods

Stock, Husbandry and Diet

One hundred and twenty, day-old commercial broiler chicks (Krishibro) were wing banded, vaccinated against Marek' disease and distributed in a completely randomized design into 3 groups of 40 chicks each, which was further replicated to 8. The chicks of each replicate were housed at random in the pens of wire floored battery brooders having provision of feeders, waterers and faecal trays from 1 to 42 days of age. The chicks were placed on one of three dietary treatments : basal, basal with probiotic supplementation at a rate of 100 or 200 mg per kg feed. The group not supplemented with probiotic served as control. The probiotic used in the study was a commercial preparation containing *Lactobacillus sporogenes* with 6, 000 million spores per g of the product. The experimental diets were formulated to meet the Bureau of Indian Standard nutrient recommendations (BIS, 1992) and the composition is presented in (Table 1). All birds were reared under uniform managemental conditions throughout the experimental period.

Experimental Procedures

Body Weight and Feed Conversion Ratio

Individual body weight of chicks and replicate-wise feed intakes were recorded at weekly intervals through out the experimental period. Feed conversion ratio was calculated as the weight gain per feed intake. Mortality was monitored on daily basis.

Serum Bio-chemical Parameters

On 35th day of experimental feeding 3 ml of blood was collected from brachial vein from 12 birds in each treatment. Subsequently serum was separated and the levels of Ca (AOAC, 1990), P (Fiske and Subba Row, 1925), protein (Doumas *et al.*, 1971), SGOT, SGPT (Reitman and Frankel, 1957) and alkaline phosphatase (ALP) activity (Bergmeyer, 1974) in the serum were estimated. On 42nd day, another 3 ml of blood from 12 birds in each dietary treatment was collected for estimation of serum cholesterol. The serum cholesterol (Zlaktis *et al.*, 1953), HDL cholesterol (Demacker and Hifmans, 1980), serum triglycerides (Fossati and Lorenzo, 1982) were estimated by using respective diagnostic kits (Qualigens India. Pvt. Ltd., Catalogue no. 72201-04). VLDL cholesterol was calculated from triglycerides by dividing the factor 5. The LDL

Table 1. Composition of experimental diets (per 100 kg)

Ingredient	Starter (0–3 wk)	Finisher (4–6 wk)
Maize	57.08	65.80
Soybean meal	37.00	29.50
Deoiled rice bran	2.30	1.51
Oyster shell grit	0.90	1.00
Dicalcium phosphate	1.90	1.38
Common salt	0.40	0.40
DL-Methionine	0.17	0.08
Choline chloride	0.10	0.10
Vitamin premix ¹	0.05	0.05
Mineral premix ¹	0.10	0.10
Nutrient composition		
Analyzed (%)		
Crude protein	23.08	20.10
Calcium	1.05	0.90
Available phosphorus	0.45	0.35
Calculated		
Metabolizable energy (kcal/kg)	2802	2910
Lysine (%)	1.20	1.01
Methionine (%)	0.50	0.38

¹Supplies per kg diet : Vitamin A, 16,500 IU ; vitamin D₃, 3200 ICU ; vitamin E, 12 mg ; vitamin K, 2 mg ; vitamin B₁, 1.2 mg ; vitamin B₂, 10 mg ; vitamin B₆, 2.4 mg ; vitamin B₁₂, 12 µg ; niacin, 18 mg ; pantothenic acid, 12 mg ; Mn, 90 mg ; Zn, 72 mg ; Fe, 60 mg ; Cu, 10 mg ; I, 1.2 mg.

cholesterol was calculated by using the formula :
 $\text{LDL cholesterol} = \text{Total cholesterol} - \text{HDL cholesterol} - \text{VLDL cholesterol}$.

Leg Abnormality Score

On 43rd day of the experimental feeding, leg scoring was done (Watson *et al.*, 1970) to find out leg abnormalities, if any, by giving the numerical score as : 0—Completely normal leg ; 1—Slightly swelling of the tibio-metatarsal joint ; 2—Marked swelling of the tibio-metatarsal joint ; 3—Swelling and slight slipping of the achilles tendon ; 4—Swelling combined with marked degree of slipping of the achilles tendon.

Bone Ash and Bone Breaking Strength

On day 43rd, one bird from each replicate in each dietary treatment was selected at random and sacrificed by cervical dislocation. Both the tibiae were freed from soft tissue and diaphysis, defatted by soaking in petroleum ether for 48 h and dried at 100°C for 12 h. The right and left tibiae were used for determination of bone ash and bone strength, respectively. Dried bone samples were ashed at 600 ± 30°C for 12 h for estimation of bone ash (AOAC, 1990). Breaking strength on the left tibia was determined by universal testing machine (EZ test,

120891-04, Shimadzu-Japan).

Statistical Analysis

Data were subjected to statistical analysis under completely randomized design employing one-way analysis of variance (Snedecor and Cochran, 1989). The means of different treatments were compared with Duncan multiple range tests (Duncan, 1955). Significance was considered at $P < 0.05$ level.

Results

The performance of broiler chickens fed diet supplemented with *Lactobacillus sporogenes* (probiotic) is depicted in Table 2. Body weight gain (BWG) increased significantly by dietary supplementation of 100 mg probiotics per kg diet. However, no further benefits on BWG could be observed by increasing the level of supplementation from 100 to 200 mg per kg. The feed conversion ratio (FCR) improved significantly in the 100 mg probiotic supplemented dietary group as compared to control. The FCR was intermediate in the dietary group supplemented with 200 mg probiotic per kg diet. Mortality was not influenced due to supplementation of probiotic in the diet. Leg abnormality score (Table 2) was not influenced due to *L. sporogenes* supplementation in the diet. However, the bone breaking strength and bone ash content increased significantly due to supplementation of *L. sporogenes* at 100 mg per kg diet. No further benefits in these two parameters could be observed by increasing the supplementation from 100 to 200 mg per kg diet.

The serum concentration of protein and calcium (Ca) (Table 3) increased significantly due to dietary supplementation of *L. sporogenes*. However, no difference could be noticed between the levels of supplementation. Neither concentration of inorganic phosphorus (iP) nor activities of enzymes such as SGOT, SGPT and ALP in serum were influenced due to *L. sporogenes* supplementation.

The effect of dietary supplementation of *L. sporogenes* on serum lipid in broiler chicken is shown in Table 4. Serum total cholesterol was significantly lowered in broilers supplemented with *L. sporogenes* at 100 mg per kg diet. There was no significant difference in HDL cholesterol of birds fed with or without *L. sporogenes*. However, the serum LDL cholesterol was reduced significantly by dietary supplementation of *L. sporogenes*. Serum VLDL cholesterol and triglycerides followed the same trend as

Table 2. Dietary supplementation of *Lactobacillus sporogenes* on performance of broiler chicken (0-6 wks)

Attributes	Probiotic (mg/ kg diet)			SEM
	0	100	200	
Body weight gain (g)	1398 ^b	1508 ^a	1497 ^a	15.31
Feed conversion ratio	2.27 ^a	1.97 ^b	2.07 ^{ab}	0.05
Mortality (%)	5.00	5.00	7.50	—
Leg abnormality score	1.00	1.00	0.58	0.74
Tibia breaking strength (Newton)	59.42 ^b	61.89 ^a	61.98 ^a	0.29
Bone ash (%)	37.49 ^b	39.69 ^a	39.64 ^a	0.24

^{a,b} Means with different superscripts in a row differ significantly ($P < 0.05$)

Table 3. Dietary supplementation of *Lactobacillus sporogenes* on serum biochemical parameters of broiler chicken

Attributes	Probiotic (mg/ kg diet)			SEM
	0	100	20	
Protein (mg/dl)	4.25 ^b	4.84 ^a	4.90 ^a	0.06
Calcium (mg/dl)	8.26 ^b	8.68 ^a	8.72 ^a	0.04
Phosphorus (mg/dl)	4.68	4.62	4.59	0.12
Enzymes (IU/L)				
SGOT	50.13	48.24	48.03	0.61
SGPT	25.65	25.28	24.91	0.25
ALP	38.97	38.51	38.61	0.22

^{a,b} Means with different superscripts in a row differ significantly ($P < 0.05$).

SGOT-Serum glutamic oxalo acetate transminase ; SGPT-Serum glutamic pyruvate transaminase ; ALP-Alkaline phosphatase

Table 4. Dietary supplementation of *Lactobacillus sporogenes* on serum lipid concentrations (mg/dl) of broiler chicken

Attributes	Probiotic (mg/ kg diet)			SEM
	0	100	200	
Total cholesterol	126.70 ^a	119.08 ^b	119.49 ^b	0.72
HDL cholesterol	74.27	72.24	72.20	0.94
LDL cholesterol	39.46 ^a	35.02 ^b	35.49 ^b	0.23
VLDL cholesterol	12.97 ^a	11.82 ^b	11.80 ^b	0.06
Triglycerides	64.89 ^a	59.14 ^b	59.10 ^b	0.30

^{a,b} Means with different superscripts in a row differ significantly ($P < 0.05$).

HDL cholesterol-High density lipoprotein cholesterol ; LDL cholesterol-Low density lipoprotein cholesterol
VLDL cholesterol-Very low density lipoprotein cholesterol.

that of LDL cholesterol. No further decrease in total cholesterol, LDL cholesterol or triglycerides concentration in serum was observed by increasing the supplementation from 100 to 200 mg.

Discussion

Dietary supplementation of *Lactobacillus sporogenes* at 100 mg per kg diet significantly improved the body weight gain and feed conversion ratio in this experiment. Similarly, Han *et al.* (1984) found

significant improvement in body weight gain and feed conversion ratio by supplementing chicken diets with *L. sporogenes* and *Clostridium butyricum*. Consistent improvements in body weight gain of chickens by feeding a culture of *L. sporogenes* have also been reported by Kalbande *et al.* (1992). In two separate trials with day old broiler chicks on *L. sporogenes* supplementation, Cavazzoni *et al.* (1998) reported significant improvement in growth and feed conversion ratio during 1 to 49 days of age. *L.*

sporogenes are functional in prophylactic and curative treatments without causing any pathological and pharmacological side effects and are good source of certain digestive enzymes such as amylase, protease and lipase for optimum digestion and absorption of nutrients. Thus, the significant improvement in body weight gain and feed conversion ratio in this experiment could be attributed to a better microbial environment in the gut, which in turn might have enhanced digestion, absorption and utilization of nutrients (Panda *et al.*, 2000).

No information in literature is available with regard to the influence of *L. sporogenes* on bone breaking strength and bone ash content. However, our finding suggests that *L. sporogenes* had a positive influence in these two parameters. This could be attributed to higher assimilation of calcium (Mohan *et al.*, 1995) in the bone, which might have created a greater resistance resulting in higher strength. This hypothesis is further supported by the increased concentration of Ca in serum (Table 3) in probiotic-supplemented group.

Dietary supplementation of probiotic in the present study resulted in higher serum concentration of protein and Ca. This could be attributed to the favourable environment in intestinal tract due to feeding of *L. sporogenes*, which might have helped to digest and absorb more nitrogen and calcium (Mohan *et al.*, 1995) resulting in higher concentration in serum. Similar trend was observed in our earlier studies (Panda *et al.*, 2003). The non difference in concentration of inorganic phosphorus (iP) and activities of enzymes (SGOT, SGPT and ALP) due to *L. sporogenes* supplementation was in agreement with the findings of Baidya *et al.* (1994).

Similar cholesterol depressing effect due to *L. sporogenes* supplementation in broiler chicken was observed by Joy and Samuel (1997). Probiotic supplementation significantly reduced the serum LDL cholesterol (Kalavathy *et al.*, 2003) and triglycerides (Santoso *et al.*, 1995) in broiler chicken. The findings of our study and also from previous studies indicated that probiotic feeding has a cholesterol depressing effect in broiler chickens. However the mechanism(s) of action for producing hypocholesterolemic effect is not yet fully understood. Nelson and Gilliland (1984) reported that some of the microorganisms present in the probiotic preparation could utilize the cholesterol present in the gas-

trointestinal tract for their own metabolism, thus reducing the amount absorbed. Mohan *et al.* (1995) reported that the reduction in serum cholesterol could be attributable to reduced absorption and/or synthesis of cholesterol in the gastro-intestinal tract by probiotic supplementation (Mohan *et al.*, 1995). *Lactobacillus acidophilus* reduces the cholesterol in the blood by deconjugating bile salts in the intestine, thereby preventing them from acting as precursors in cholesterol synthesis (Abdulrahim *et al.*, 1996). *Lactobacillus* has a high bile salt hydrolytic activity, which is responsible for deconjugation of bile salts (Surono, 2003). Deconjugated bile acids are less soluble at low pH and less absorbed in the intestine and is more likely to excreted in faeces (Klaver and van der Meer, 1993). Probiotic microorganism inhibits hydroxymethyl-glutaryl-coenzyme A, an enzyme involved in the cholesterol synthesis pathway there by decrease cholesterol synthesis (Fukushima and Nakano, 1995).

In conclusion, it is concluded that dietary supplementation of *Lactobacillus sporogenes* (6×10^8 spore per g) at 100 mg per kg diet significantly enhanced body weight gain, improved feed conversion ratio, bone ash and bone breaking strength and lowered total cholesterol, VLDL cholesterol and triglycerides concentrations in the serum of broiler chickens.

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