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Effect of *in ovo* feeding of probiotics on serum lipid profile, carcass and conformation traits of Jabalpur colour birds

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Abstract

The present study was carried out to evaluate the effect of *in ovo* feeding of probiotic (*Lactobacillus acidophilus*) on serum lipid profile, carcass and conformation traits. A total of 240 fertile eggs were divided into 5 treatments, 48 eggs were randomly allotted to non-injected control (T₁), 48 were injected control or sham control (T₂) and remaining 144 eggs, out of which each 48 eggs were injected with 0.2 ml of 1x10⁶ CFU *Lactobacillus acidophilus* (T₃), 0.2 ml of 1x10⁹ CFU *Lactobacillus acidophilus* (T₄) and 0.2 ml of 1x10¹² CFU *Lactobacillus acidophilus* (T₅). *In ovo* injection was done on 18th day of incubation. Hatched chicks (120) were randomly divided (24 chicks in each treatment) with 8 chicks in each replicate. Serum samples were collected on 56th day of experiment for the assessment of serum lipid profile. Carcass and conformation traits were recorded at the end of the experiment. The *in ovo* fed groups showed significantly lower ($p < 0.05$) cholesterol, triglycerides, LDL and increased HDL levels compared to control and sham control groups. Conformation traits, including breast angle, keel length showed significantly better ($p < 0.05$) values in probiotic-treated groups compared to control and sham control groups and shank length, did not show any significant difference. Carcass traits such as dressing weight percentage, drawn weight percentage and eviscerated weight percentage showed significant difference ($p < 0.05$) in probiotic fed groups compared to control and sham control groups. Giblets weight and cut-up parts weight percentage did not show any significant difference among the treatments. In conclusion *in ovo* feeding of *Lactobacillus acidophilus* in the late embryonic stage alters serum lipid profile, improves body conformation traits and better carcass traits compared to control and sham control groups.

Keywords: *In ovo*, probiotic, *Lactobacillus acidophilus*, Jabalpur colour birds, lipid profile

Introduction

Probiotics are microbial feed additives that enhances the gut mucosal barrier, protecting against harmful substances while supporting the host's beneficial microbial balance. Probiotics have the ability to reduce the intensity and severity of enteric infections in broilers due to competitive inhibition, colonization, changes in pH and production of antibiotic-like substances [1]. Probiotics have the ability in reducing serum cholesterol concentrations in chicken [2, 3]. The elevated levels of cholesterol or other lipids in blood (triglycerides) as considered risk factors in developing coronary heart disease in humans [4]. Probiotic bacteria can control the absorption of lipids in the intestine and regulate serum cholesterol and triglyceride levels [5, 6]. There are some evidences proposing that *Lactobacillus* feed supplementation reduces the cholesterol and fatty acid composition of broiler chick's body [7].

The objective of the present study was to investigate the effect of *in ovo* feeding of *Lactobacillus acidophilus* on serum lipid profile, carcass and conformation traits of Jabalpur colour birds.

Materials and Methods

The proposed work was carried out at poultry farm, All India Coordinated Research Project (AICRP) on Poultry Breeding, Department of Poultry Science, College of Veterinary Science and Animal Husbandry, N.D.V.S.U., Jabalpur, Madhya Pradesh.

Preparation of probiotic culture

The probiotic strain *Lactobacillus acidophilus* was cultured in MRS broth (ATCC 314, SG Scientific, Jabalpur) for 24-48h microaerophilic environment at 37 °C to prepare the stock probiotic inoculum for *in ovo* administration. The culture was then centrifuged at 4200 g for 20 min at 4 °C to obtain the bacterial pellet. The bacterial concentration was adjusted spectrophotometrically to 600nm to obtain suspension containing 5×10^6 , 5×10^9 and 5×10^{12} CFU/ml. The number of live cells per 1 mL of suspension was determined by counting the number of colonies in each successive dilution. Plates were counted when colony growth for a 10-fold dilution was between the range of 30 to 300 colonies per plate. A 0.2 ml (corresponding to 10^6 , 10^9 and 10^{12} CFU/egg) aliquot was injected into each egg from the prepared suspension.

In ovo injection procedure

On 18th day of incubation, each egg was candled and earmarked to identify the site of the injection (Amniotic sac). After disinfection of egg shell surface with 99.90% ethyl alcohol, a pin head size hole was made using a needle fitted with a rubber stopper to prevent the needle from piercing the air cell membrane and 0.2 ml of respective treatment solution was injected into the amnion using an insulin syringe with 31 gauge needle (0.25 mm x 8 mm) to a depth of about 8 mm without disturbing the air cell, the site of injection was sealed using paraffin wax after injecting the solution. Out of 240 eggs, 48 eggs were used for non-injected control (T₁), 48 were injected control or sham control (T₂) and remaining 144 eggs, out of which each 48 eggs were injected with 0.2 ml of 1×10^6 CFU *Lactobacillus acidophilus* (T₃), 0.2 ml of 1×10^9 CFU *Lactobacillus acidophilus* (T₄) and 0.2 ml of 1×10^{12} CFU *Lactobacillus acidophilus* (T₅). After *in ovo* injection, the eggs were sealed using paraffin wax and then transferred to the hatcher machine. On day 21, chicks from each treatment (T₁ to T₅) were removed from the hatcher and divided into replicates. The required number of healthy chicks were selected at random, wing banded to preserve individual identity and weighed.

Rearing and feeding of birds

The experiment was conducted in a floor house with individual pens on either side of the central passage. The chicks were housed in individual pens as per treatment groups and reared on a deep litter system. Standard management practices including brooding, proper lighting, raking of litter, cleaning of feeders, waterers, etc. were followed throughout the experiment.

Birds were fed starter (0-3 weeks) and finisher (4-8 weeks) diets formulated as per ICAR standards [8]. The starter diet contained 21.5% crude protein and 2800 Kcal/kg ME while finisher diet contained 18% crude protein and 3050 Kcal/kg ME.

Serum lipid profile

About 2.0 ml of blood were collected aseptically from the jugular vein of two birds from each replicate at 8 weeks of age at the time of slaughter. Blood sample was transferred

into clot activator vials and serum was separated carefully after centrifugation (3000 rpm, 10 minutes).

Blood samples were collected from 2 birds per replicate and serum was harvested by centrifugation at 3000 rpm for 10 min. The data on cholesterol, triglyceride, HDL and LDL were estimated by using the commercial kits in Erba semi auto analyser.

Conformation traits

At completion of the experimental period (8th week), conformation traits were measured in two birds per replicate viz., Breast angle, keel length and shank length were measured with the help of breastometer and vernier calliper respectively.

Carcass traits

At the end of the experiment two birds from each replicate were randomly selected and sacrificed. The birds were kept 12 hrs off feed but was provided before their slaughter. Immediately after weighing, the birds were slaughtered and weight of blood, feathers, skin, giblets and cutup parts were recorded for calculating the following parameters:

$$\text{a. Dressing percentage (\%)} = \frac{\text{Dressed carcass weight (g)}}{\text{Live weight (g)}} \times 100$$

$$\text{b. Drawn weight (\%)} = \frac{\text{Drawn weight (g)}}{\text{Live weight (g)}} \times 100$$

$$\text{c. Eviscerated weight (\%)} = \frac{\text{Eviscerated weight (g)}}{\text{Live weight (g)}} \times 100$$

$$\text{d. Giblet weight (\%)} = \frac{\text{Giblet weight (g)}}{\text{Live weight (g)}} \times 100$$

$$\text{e. Cut up part weight (\%)} = \frac{\text{Weight of individual cut up parts (g)}}{\text{Eviscerated weight (g)}} \times 100$$

Statistical analysis

The experimental data were analyzed using one-way analysis of variance (ANOVA) under a Completely Randomized Design (CRD), following the procedures described by Snedecor and Cochran [9]. Differences between treatment means were tested for significance using Duncan's Multiple Range Test (DMRT) at the 5% level of probability [10]. All statistical analyses were performed using SPSS software (version 22.0). Results are presented as mean $\pm$ pooled standard error of the mean (SEM), and statistical significance was considered at $p < 0.05$.

Results and Discussion

Serum lipid profile

The serum lipid profile of Jabalpur colour birds supplemented with *in ovo* probiotic (*Lactobacillus acidophilus*) are shown in Table 1.

Table 1: Effect of *in ovo* feeding of probiotic (*Lactobacillus acidophilus*) on serum lipid profile (mg/dl) of Jabalpur colour birds (Mean±SE)

Treatment	Cholesterol (mg/dl)	Triglyceride (mg/dl)	HDL (mg/dl)	LDL (mg/dl)
T ₁	234.83 ^a ±2.20	354.22 ^a ±25.99	55.75 ^c ±3.99	108.24 ^a ±5.09
T ₂	228.63 ^a ±2.92	349.50 ^a ±26.57	57.86 ^c ±2.11	100.87 ^a ±5.08
T ₃	195.22 ^b ±2.47	177.53 ^c ±23.23	93.60 ^a ±2.03	66.12 ^b ±5.30
T ₄	175.73 ^c ±3.16	233.38 ^b ±22.83	75.52 ^b ±3.85	53.53 ^c ±4.95
T ₅	171.03 ^c ±3.27	172.77 ^c ±28.31	74.00 ^b ±4.04	62.48 ^{bc} ±3.25

^{a,b,c} Means bearing different superscripts in a column differ significantly ($p < 0.05$)

Total serum cholesterol was significantly reduced ($p < 0.05$) in all probiotic-treated groups compared to the control and sham control. Triglyceride values significantly decreased ($p < 0.05$) in probiotic treated groups compared to control groups. HDL values increased significantly ($p < 0.05$) in probiotic fed groups. LDL values significantly reduced ($p < 0.05$) in probiotic fed groups, while significantly increased ($p < 0.05$) in control and sham control groups than the *in ovo* fed groups.

Consistent with our results, Panda ^[11] also reported a significant reduction in total serum cholesterol following probiotic supplementation, although they observed no changes in HDL, LDL and triglycerides. Likewise, Mayahi ^[12] reported decreased cholesterol and triglycerides in broilers supplemented with *Enterococcus faecium* and *Bifidobacterium*, further reinforcing the lipid-lowering role of probiotics.

The findings of our study also closely match those of Kanagaraju ^[13] who observed that *in ovo* injection of *Lactobacillus acidophilus* significantly reduced triglycerides, total cholesterol and LDL, while increasing HDL trend seen in all probiotic fed groups.

Contrary to the consistent lipid-lowering patterns in most studies, Guo ^[14] observed that *Lactobacillus* treatment transiently increased total cholesterol at 1 day of age and

increased triglycerides at later ages without significant changes in HDL or LDL.

The present study confirms that *in ovo* supplementation with *Lactobacillus acidophilus* produces a beneficial modification of serum lipids by reducing harmful components (Total Cholesterol, Triglyceride and LDL) and elevating protective HDL. The mechanisms underlying probiotic-mediated cholesterol reduction are not fully understood. Some studies suggested a decrease in endogenous cholesterol synthesis, whereas others propose enhanced degradation and excretion of cholesterol by probiotics. The possible mechanisms include bile salt deconjugation by certain probiotic strains, assimilation of cholesterol onto the surface of *Lactobacillus* cells with subsequent removal from the surrounding environment, production of intra and extracellular cholesterol dehydrogenase or isomerase enzymes and inhibition of the 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase enzyme (Shokryazdan *et al.*, 2017) ^[15].

Conformation traits

The conformation traits of Jabalpur colour birds fed with *in ovo* probiotic (*Lactobacillus acidophilus*) at different concentrations are presented in Table 2.

Table 2: Effect of *in ovo* feeding of probiotic (*Lactobacillus acidophilus*) on conformation traits of Jabalpur colour birds (Mean±SE)

Treatment	Breast angle (o)	Keel length (cm)	Shank length (cm)
T ₁	55.63 ^b ±0.50	8.77 ^c ±0.11	5.50±0.17
T ₂	55.28 ^b ±1.12	8.77 ^c ±0.19	5.47±0.15
T ₃	58.83 ^a ±0.86	9.57 ^{ab} ±0.16	5.85±0.17
T ₄	58.28 ^a ±1.02	9.32 ^b ±0.15	5.67±0.18
T ₅	58.33 ^a ±0.70	9.75 ^a ±0.21	5.65±0.16

^{a,b,c} Means bearing different superscripts in a column differ significantly ($p < 0.05$)

The breast angle was significantly ($p < 0.05$) higher in all probiotic-treated groups respectively, compared to the control groups. All the probiotics supplemented group reported significantly higher ($p < 0.05$) values for the keel length than control and sham control groups. In case of shank length, the differences were not statistically significant ($p > 0.05$) among the treatments, though birds receiving probiotic supplementation showed numerically higher shank lengths than the control groups. These findings agree with Softić, ^[16] who reported significant positive effects of probiotics on breast angle, keel length and shank length, demonstrating that probiotics support better musculoskeletal growth through improved nutrient absorption and gut health.

Carcass traits

The carcass characteristics of Jabalpur colour birds supplemented *in ovo* with *Lactobacillus acidophilus* at different concentrations are presented in Table 3.

Carcass traits such as dressing weight percentage, drawn weight percentage and eviscerated weight percentage

showed significant difference in probiotic fed groups compared to control and sham control groups. Giblets weight and cut-up parts weight percentage did not show any significant difference among the treatments.

These findings contrast with those of El-Moneim ^[17] who reported that *in ovo* administration of probiotics did not significantly affect carcass weight or dressing percentage. This outcome of cutup parts aligned with the results of Berrecoso ^[18] who reported that *in ovo* administration of probiotics, prebiotics or synbiotics did not produce significant changes in the relative weights of carcass components such as the breast muscle, drumstick, proventriculus, gizzard and giblets. The present study suggests that *in ovo* feeding of *Lactobacillus acidophilus* may exert a stronger influence on gut health, nutrient absorption and muscle deposition, leading to improved carcass yield, but does not affect the proportional distribution of cut-up parts. This difference may be due to variations in probiotic strain, dosage, injection timing, or breed-specific responses and experimental conditions.

Table 3: Effect of *in ovo* feeding of probiotic (*Lactobacillus acidophilus*) on carcass traits of Jabalpur colour birds (Mean±SE)

Treatment	Dressing%	Drawn%	Eviscerated%	Giblet%	Cut up parts					
					Neck%	Breast%	Wings%	Legs%	Thigh%	Back%
T ₁	73.02 ^c ±0.13	67.27 ^b ±0.20	62.22 ^b ±0.21	5.03±0.12	10.54±0.04	28.24±0.12	15.12±0.17	14.82±0.04	13.86±0.15	17.41±0.12
T ₂	73.63 ^{bc} ±0.16	66.80 ^b ±0.33	61.63 ^b ±0.33	5.20±0.13	10.17±0.05	28.78±0.16	15.36±0.14	15.17±0.02	13.12±0.11	17.21±0.10
T ₃	74.97 ^a ±0.13	70.13 ^a ±0.32	64.90 ^a ±0.32	5.21±0.06	10.32±0.08	28.92±0.11	15.25±0.19	14.94±0.09	13.32±0.12	17.25±0.11
T ₄	74.30 ^{ab} ±0.11	69.63 ^a ±0.35	64.50 ^a ±0.30	5.14±0.08	10.34±0.06	29.14±0.17	14.89±0.17	14.89±0.04	13.31±0.13	17.43±0.14
T ₅	74.13 ^{ab} ±0.14	69.90 ^a ±0.23	64.73 ^a ±0.23	5.19±0.10	10.39±0.05	29.16±0.10	14.83±0.13	14.90±0.05	12.91±0.12	17.80±0.12

^{a,b,c} Means bearing different superscripts in a column differ significantly ($p < 0.05$)

Conclusion

The results of the present study suggest that *in ovo* feeding of probiotic (*Lactobacillus acidophilus*) with 1×10^6 , 1×10^9 and 1×10^{12} CFU *Lactobacillus acidophilus* helps in improving the serum lipid profile, conformation tarits and carcass traits compared to control groups.

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