Influence of supplementation of *Lactobacillus* cultures on growth performance, fecal microbiota, blood profile and cholesterol contents in broilers

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Introduction

- Considering the worldwide pressure from consumers, the scientific community and international regulatory agencies, to remove or decrease the use of antibiotics as performance enhancers and the rational use of the therapeutic form in poultry production, maintenance and taking food safety into consideration has been a challenge (Bonato and Borges, 2019).
- The feed supplementing lactic acid bacteria with antimicrobial activity, non-toxic to the host and survival to the intestinal barrier and promoting the host could be an alternative to replace conventional antibiotics as growth promoting substances (Park and Kim, 2015).
- *Lactobacillus* strains have a high ability to attach to the intestinal epithelium and are able to establish in the chicken intestine within a day after hatching, so they are considered to be normal bacterial flora of the gastrointestinal tract (GIT) of chickens (Shokryazdan et al., 2016).
- Use of LAB as feed additives to replace antibiotic-associated growth stimulator and their effect on the quality of the meat and eggs is the major area of research (Kizerwetter-Swida et al., 2005).
- Here, efficacy of two indigenous LAB strains: *L. plantarum* KGL3A and *L. fermentum* KGL4 as a growth stimulant and their cholesterol-lowering potential on broilers is studied.

Objectives

- To check the effect of *Lactobacillus* feeding on growth parameters of Broilers up to 42 days.
- To analyze the hematological parameters and lipid profiles of broilers after 42 days study.
- To study the histopathological status of intestine and liver tissues of broilers after 42 days study.
- To determine the viable fecal lactobacilli, enterococcal and coliform counts in broilers after 42 days study.

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Methods

- **96 Broilers** weighing 45-50g (Cobb 430Y, Venky's India Ltd.) were grouped into **four different treatments** (each having 24 broilers):
  - **T1** (control): basal diet + antibiotic as growth promoter (BMD-100) and immunomodulatory factor (Immunowall/Zist(S))
  - **T2:** basal diet without having antibiotic as growth promoter and immunomodulatory factor + *L. plantarum* KGL3A (10^8 CFU/ml)
  - **T3:** basal diet without having antibiotic as growth promoter and immunomodulatory factor + *L. fermentum* KGL4 (10^8 CFU/ml)
  - **T4**: basal diet without having antibiotic as growth promoter and immunomodulatory factor +combination of T3 and T4 bacterial strains.
- Broiler performance including **body weight, daily feed consumption ratio**, and **mortality rate** were determined up to 42 days during the study (Timmerman et al., 2006).
- **Hematological analysis and lipid profiling of blood** samples of broilers after 42 days were conducted (Timmerman et al., 2006).
- **Histopathological examination** of **intestine and liver** tissues of broilers after 42 days were also evaluated (Wang et al., 2019).
- **Enumeration of fecal samples** (*Lactobacilllus*, *Enterococcus* and coliforms count) of broilers after 42 days was done (Loh et al., 2010).

Discussion

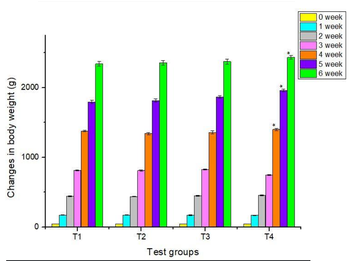
- During the entire study, higher bodyweight was observed among the *Lactobacillus* fed broilers groups (T4: 2433 g, T3: 2371 g, T2: 2355 g ($P<0.05$)) as compared to control group (T1: 2339 g).
- Lipid profile analysis further confirmed the significant decrease in low-density lipoprotein (LDL) content of T4 (19%) and T3 (16%) groups than the control group (T1) while more than 10% increase in high-density lipoprotein HDL content was observed in T4 and T3 groups than the control group (T1).
- The histopathological examinations of the fine macroscopically examined intestinal and liver tissues suggested well-organized epithelial lining and villi structure in *Lactobacillus* fed broiler groups (T2, T3, T4) and control group (T1).
- Further, the decrease in fecal coliforms and enterococcus counts and an increase in *Lactobacillus* counts in treatment groups compared to the control group were found after 42 days of study.

Results

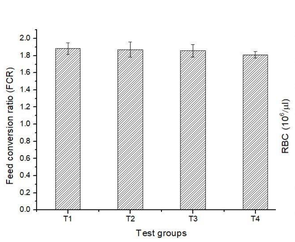
Techno-functional characterization of the *Lactobacillus* isolates

Probiotic Attributes	*L. plantarum* KGL3A	*L. fermentum* KGL4
Acidic pH tolerance (log CFU/ml)	5.4 after 3h incubation	5.5 after 3h incubation
Bile salt hydrolase activity	white hallo of precipitation found due to bile salt hydrolase activity	white hallo of precipitation found due to bile salt hydrolase activity
Cholesterol assimilation (%)	56%	58%
Antimicrobial activity against	*S. dysenteriae* (NCDC 107): 25mm, *E. coli* (ATCC 25922): 28mm, *S. typhi* (NCTC 5017): 32mm, *B. cereus* (ATCC 14459)- 24mm, *S. aureus* (MTCC 114)-18mm	*S. dysenteriae* (NCDC 107): 24mm, *E. coli* (ATCC 25922): 22mm, *S. typhi* (NCTC 5017): 20mm, *B. cereus* (ATCC 14459): 28mm, *S. aureus* (MTCC 114): 30mm
Antibiotic susceptibility	Susceptible: A10µg, E15µg, TE30µg, R15µg, MET15µg, OX1µg, K30µg, G30µg, S10µg. Resistant: VA30µg, NX10µg	Susceptible: A10µg, E15µg, TE30µg,R15µg, MET15µg , OX1µg, K30µg, G30µg, S10µg. Resistant: VA30µg, NX10µg
Cell surface hydrophobicity (%)	64.90	67.70
Cellular auto-aggregation (%)	72.70	69.90
EPS production (mg/ml)	03.72	03.64
Antioxidant activity (%)	41.00	41.50
Survival in stimulated gastric juice conditions(%)	74.45	74.00
SCFAs production (µg/ml)	Acetate: 05.16, Lactate: 16.00, Butyrate: 0.096	Acetate: 02.27, Lactate: 13.95, Butyrate: 0.075
B-vitamins production (µg/ml)	B2: 0.41, B9: 0.74, B12: 0.081	B2: 0.72, B9: 0.80, B12: 0.046

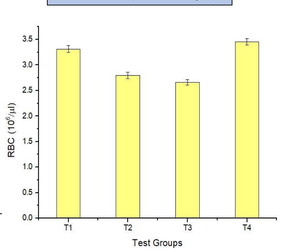
Changes in body weight(g) of broilers (after 42 days)



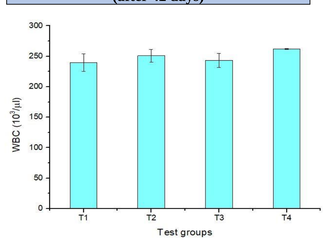
Feed conversion ratio of broilers (after 42 days)



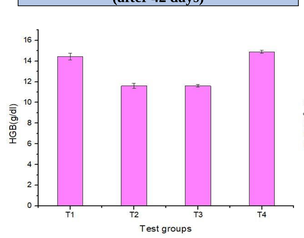
RBC content in blood samples of broilers (after 42 days)



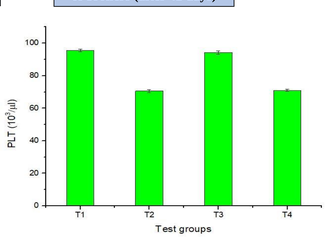
WBC content in blood samples of broilers (after 42 days)



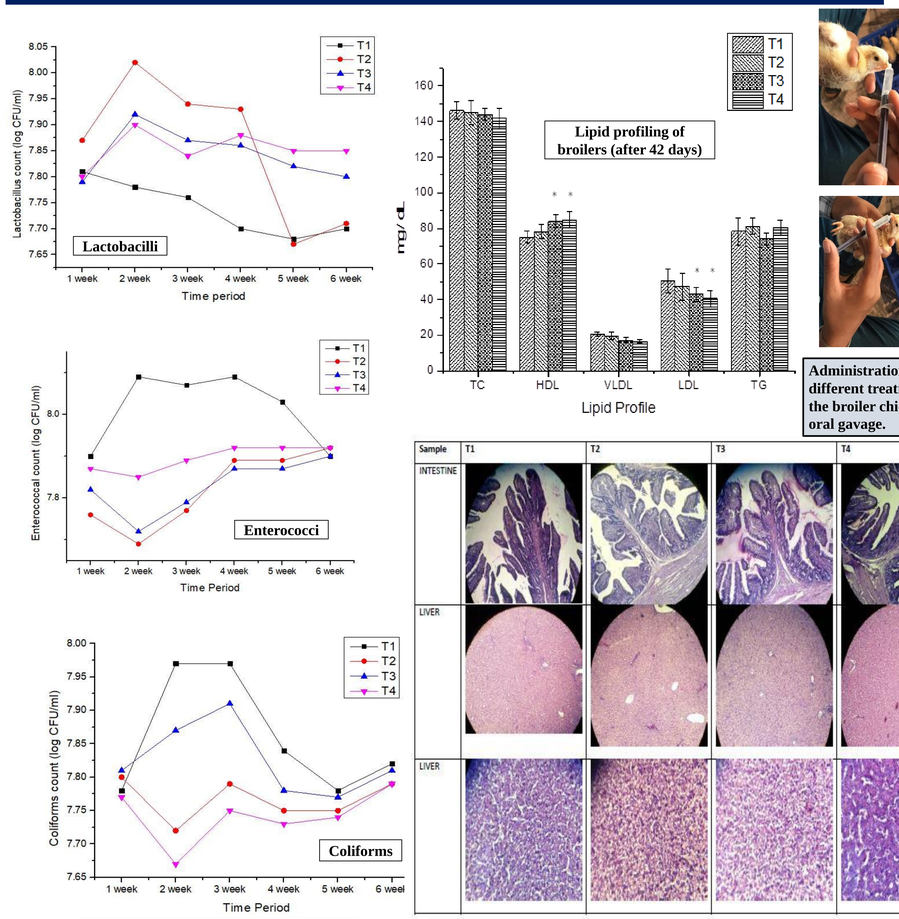
HGB content in blood samples of broilers (after 42 days)



PLT content in blood samples of broilers (after 42 days)



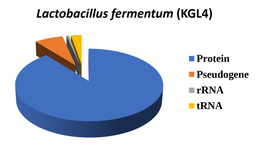
Results

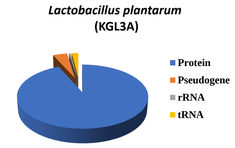


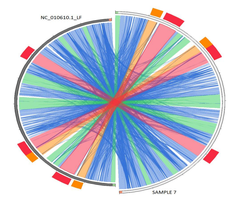
Administration with different treatments to the broiler chickens by oral gavage.

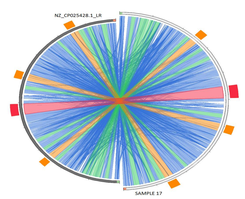
Fecal sample analysis of broilers up to 42 days

Histopathological examination of intestine and liver tissues of broilers after 42 days





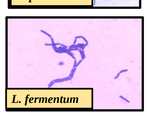




Genetic features of isolate KGL4 and KGL3A from reference genome comparison. Whole genome sequencing of KGL4 & KGL3A was performed through NextSeq500 with 2X150bp chemistry. High quality data between 838 mb-1.25 Gb was generated.

Phenotypic & Genotypic characterization of the *Lactobacillus* isolates

Culture code	Species	Genbank Accession No.	Growth against 6.5% NaCl	Catalase Test	Hemolytic Test
KGL4	*L. fermentum*	MF951099	-ve	-ve	-ve
KGL3A	*L. plantarum*	MG722814	-ve	-ve	-ve



Conclusion

- The supplementation of *Lactobacillus* isolates viz. KGL4 & KGL3A as feed supplements to the broilers had overall positive effects on broilers growth performance in this study without providing growth promoter as antibiotic.
- Further, more studies are required to validate the claim for the two specific *Lactobacillus* cultures (KGL4 & KGL3A).

Key Message

- ✓ Lactic acid bacteria could be considered as an alternative for antibiotic free meat and egg production in broilers in future.