Significance of Postbiotics with Low molecular weight whey peptides on growth efficiency, haematological parameters, serum biochemistry, faecal microbiota, and metagenomic profiling of Broilers chicken

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Introduction

❖Antibiotic-resistant strains of pathogenic bacteria are increasingly prevalent in nature and environment.

❖Uses of antibiotics as growth promoters in the poultry industry is of main concern to researchers and consumers.

❖From last decades the demand for meat and poultry-based products has considerably increased, but antibiotic free egg and meat production is the demand from the consumer side.

❖Liquid whey from cheese industry are full of nutrients. But Fermented whey are more effective due to the production of metabolites like amino acids, vitamins, enzymes, and also peptides (Dimidi *et al*., 2019).

❖Use of whey peptides with postbiotics 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 (Ashour *et al*., 2019).

Objectives

➢ To evaluate the effect of *Lactobacillus helveticus* MTCC 5463 as **postbiotics with low molecular whey peptides** (PLMWP) feeding on growth parameters of Broilers up to 42 days.

➢ To analyze the **hematological parameters**, **lipid profiles** and **histopathological** status of intestine, heart and liver tissues of broilers after 42 days study.

➢ To determine the viable **fecal lactobacilli, Enterococcus** and **coliform** counts as well as **Short chain fatty acids (SCFA)** in broilers after 42 days study.

➢ To analyze the **metagenomic analysis of cecal sacs** of the broilers after 42 days.

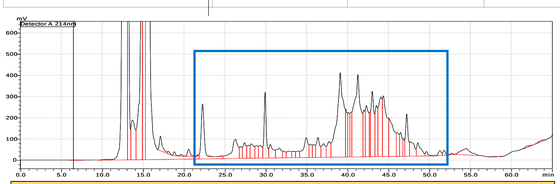
Methods

- **96 Broilers** weighing 45-50g (Venky's India Ltd.) were grouped into **four different treatments** (each having 24 broilers):
  - ❖T1: Control: basal diet with **immunomodulator and commercial probiotics**
  - ❖T2: Basal diet with **postbiotics with low molecular weight whey peptides <1 kDa**
  - ❖T3: Basal diet with **postbiotics with low molecular weight whey peptides <2 kDa**
  - ❖T4: Basal diet with **postbiotics with low molecular whey weight peptides <3 kDa**
- Broiler performance including **body weight, daily feed consumption ratio**, and **mortality rate** were determined up to 42 days during the study (Ashour *et al*., 2019).
- **Hematological analysis, blood glucose and lipid profiling of blood** samples of broilers after 42 days were estimated (Fenita *et al*., 2021).
- **Histopathological examination** of **intestine, liver and heart** tissues of broilers after 42 days were also evaluated (Malik *et al*., 2015).
- **Enumeration of fecal samples** (*Lactobacillus*, *Enterococcus* and Coliforms) **and SCFA contents** of broilers after 42 days was done (Hati *et al*., 2023).
- **Metagenomic study of cecal contents** of broiler groups was also studied

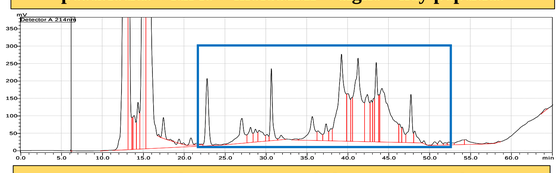
Results

Physico-chemical and bio functional properties of postbiotics with low molecular weight whey peptides

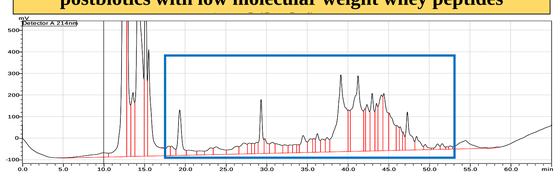
	1KDaPermeate	2KDa Permeate	3KDa Permeate
pH	4.7	4.66	4.58
Acidity (% LA)	0.63	0.65	0.69
Antioxidant activity(%)	50.41± 0.66	56.32 ± 0.39	65.81 ± 0.55
Proteolytic activity (mg/ml)	7.11 ± 0.12	7.27 ± 0.17	7.84 ± 0.13
Acetic acid	44.18^{b} ± 0.32	45.99^{a} ± 1.03	46.68^{a} ± 0.40
Propionic acid	20.43^{d} ± 0.15	21.27^{c} ± 0.47	21.59^{c} ± 0.19
Butyric acid	4.95^{e} ± 0.05	5.22^{e} ± 0.07	5.27^{e} ± 0.04



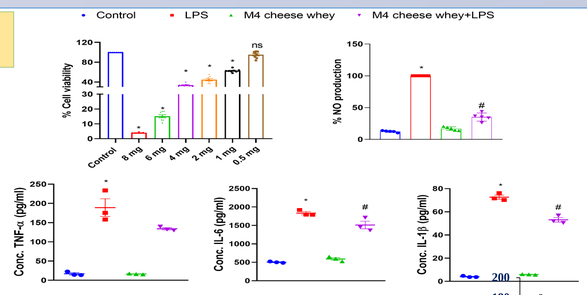
RP – HPLC chromatogram of 1kDa permeate from postbiotics with low molecular weight whey peptides



RP – HPLC chromatogram of 2kDa permeate from postbiotics with low molecular weight whey peptides



RP – HPLC chromatogram of 3kDa permeate from postbiotics with low molecular weight whey peptides



Anti-inflammatory activity of postbiotics with low molecular whey peptides

42 days old

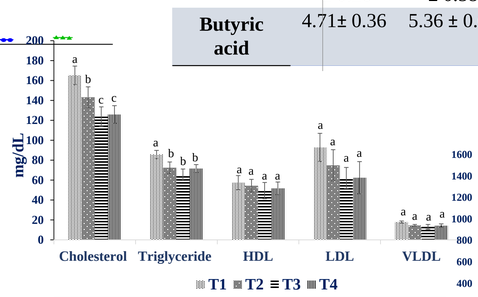
1 day old

21 days old

Growth phase of broilers

Short chain fatty acids (SCFA) of fecal samples broiler chickens

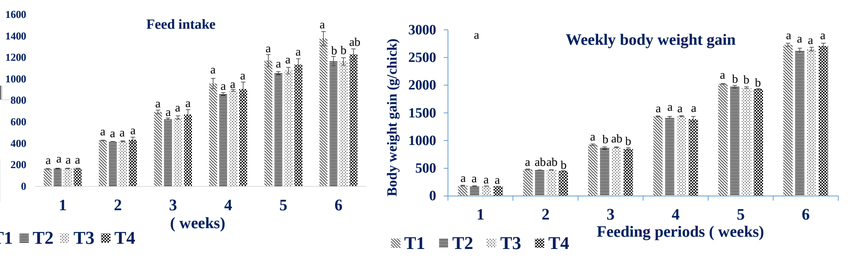
SCFA (µl/ml)	T1	T2	T3	T4
Acetic acid	10.65^{b} ± 0.78	12.49^{b} ± 0.58	12.05^{b} ± 0.75	16.72^{a} ± 1.08
Propionic acid	9.89^{b} ± 0.26	12.64^{a} ± 0.58	12.37^{a} ± 0.61	12.97^{a} ± 0.90
Butyric acid	4.71± 0.36	5.36 ± 0.67	5.72 ± 0.45	7.26 ± 0.66



Lipid profiling of broilers (after 42 days)

Peptide Sequences from Whey fermented with MTCC 5463

Whey fractions	Sequences
Alpha-lactalbumin	LDQWLCEK
	VGINYWLAHK
	ALCSEKLDQWLCEKL
Beta-lactoglobulin	ALPMHIR
	LSFNPTQLEEQCHI
	IDALNENK
	LSFNPTQLEEQCHV
	VYVEELKPTPEGDLEILLQK
Albumin	TVMENFVAFVDK

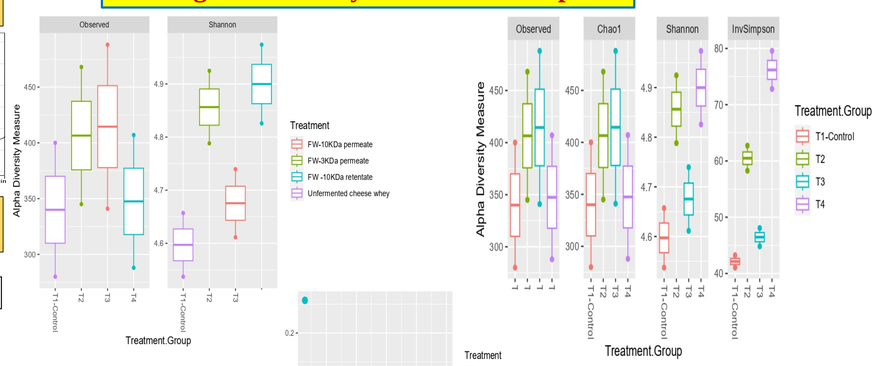


Body weight gain and feed intake of broiler groups at 42 day

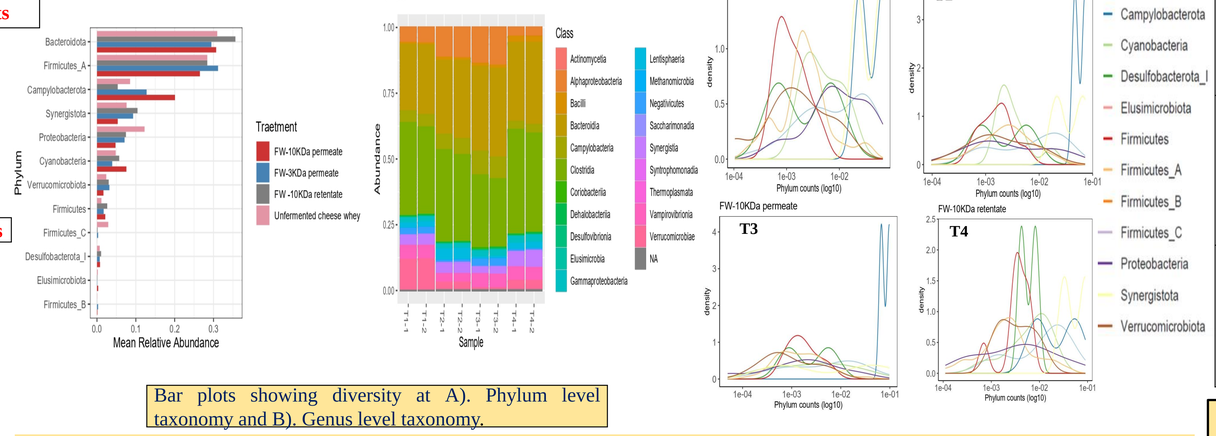
	T1 (Control)	T2	T3	T4
Body weight(g)	2730.13 ± 28.277	2626.38 ± 40.740	2651.63 ± 32.980	2706.88 ± 51.349
FCR	1.95 ± 0.13	1.78 ± 0.09	1.67 ± 0.04	1.68 ± 0.09
WBC (10^3/ uL)	21.42^{b} ± 0.426	22.65^{a} ± 0.240	22.26^{a} ± 0.336	22.34^{a} ± 0.155
RBC (10^6 /uL)	2.20 ± 0.107	2.32 ± 0.032	2.37 ± 0.100	2.39 ± 0.083
HCT (%)	25.83 ± 0.622	26.58 ± 0.387	26.84 ± 0.653	26.45 ± 0.782
MCV (fL)	132.98 ± 1.410	133.24 ± 0.819	134.08 ± 0.798	136.18 ± 0.522
MCH (pg)	39.05 ± 0.290	39.17 ± 0.445	39.20 ± 0.495	40.25 ± 0.332
MCHC (g/dL)	30.19 ± 0.400	30.37 ± 0.460	30.95 ± 0.362	30.85 ± 0.231
PLT (uL)	120.01^{b} ± 1.291	125.01^{a} ± 1.080	124.75^{a} ± 1.931	126.25^{a} ± 0.854
Hb (g/dL)	12.17 ± 0.167	12.15 ± 0.185	12.49 ± 0.487	12.43 ± 0.523
Glucose (mg/dl)	204.27 ± 1.71	192.54 ± 0.89	196.29 ± 1.94	197.66 ± 2.60

Growth performance and haematological traits of broiler chickens fed with whey peptides

Metagenomic analysis of Caeca samples

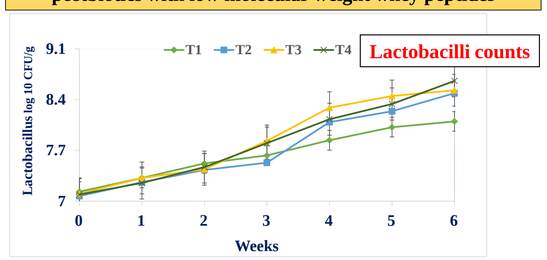


NMDS plots plotted from Bray–Curtis distances calculated from the relative abundances of all samples

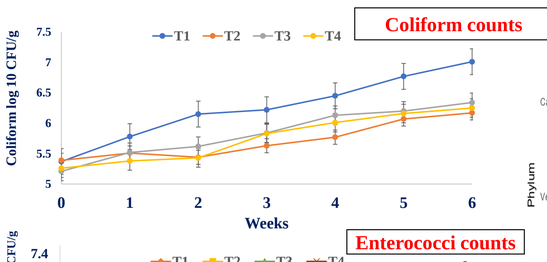


Bar plots showing diversity at A). Phylum level taxonomy and B). Genus level taxonomy.

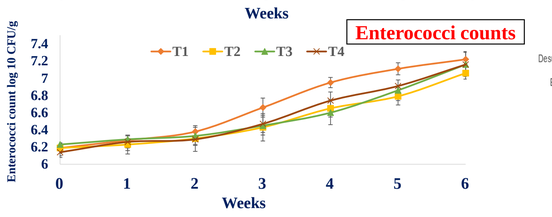
There was no significant difference observed at any taxonomic level from genus to phylum among various treatment group. Moreover, there was no significant difference observed between any of the groups at Phylum and Genus level



Lactobacilli counts

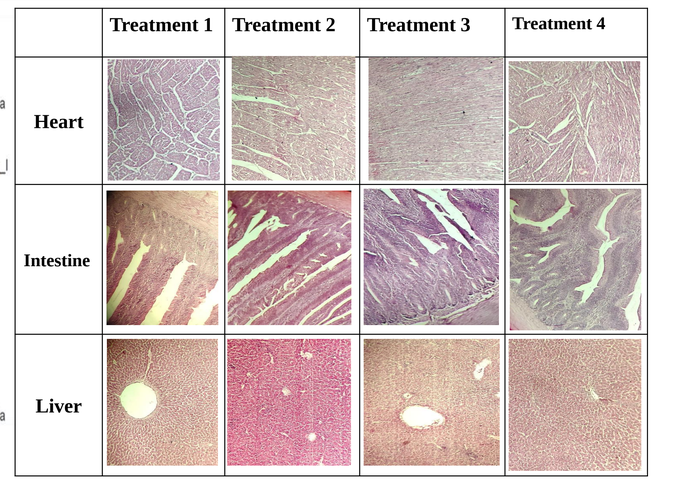


Coliform counts



Enterococci counts

Fecal sample analysis of broilers up to 42 days



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

Discussions

- During the entire study, control group (T1) birds had higher feed intake, compared to whey peptide treated groups. There was no significance difference was observed in T4 diet group body weight (2707.88 ± 51.349g) and control diet group body weight (2730.13 ± 28.277g) of chicken birds. FCR was also highest in control diet group (1.95) compared to T4 diet group (1.68) respectively.
- Lower Cholesterol content was observed in T3 and T4 (25.04 %) groups than the control group (T1). Similarly, 23.78 % reduction in triglyceride content was observed in T3 group and 5.74 % reduction in blood glucose observed in T2 group than the control group.
- The histopathological examinations of the fine macroscopically examined intestinal, liver and heart tissues suggested well-organized epithelial lining and villi structure, normal central vein and hepatic cords of liver tissue in all the groups (T2, T3, T4) and control group (T1).
- ***Lactobacillus helveticus* MTCC 5463 as postbiotics with low molecular weight whey peptides** supplementation exhibit no significant influence on red blood corpuscles (RBC), haemoglobin, hematocrit, mean corpuscular volume, mean corpuscular haemoglobin, mean corpuscular haemoglobin concentration while exhibit significant impact on leukocytes (WBC) and platelets.
- The metagenomic analysis of ceca samples revealed no significant differences was observed in the relative proportions of genus or phylum across different groups. The effect of adding whey peptides in basal diet decreased the number of *E. coli* and enterococci counts and increased Lactic acid bacteria counts in stools of broiler chickens.
- T4(36.64 + 1.34) group exhibited hights overall SCFA production comparison to control group (24.45 ± 1.68) respectively.
- **Peptides: LDQWLCEK, VGINYWLAHK, ALCSEKLDQWLCEKL** were present in the postbiotics of fermented whey with ***Lactobacillus helveticus*** MTCC 5463.

Conclusions

- The supplementation of ***Lactobacillus helveticus* MTCC 5463 as postbiotics with low molecular weight whey peptides** as feed supplements to the broilers had overall positive effects on broilers growth performance in this study without providing commercial probiotic and immunomodulator.
- Further, more studies are required to validate the claim for ***Lactobacillus helveticus* MTCC 5463 as postbiotics with low molecular weight whey peptides**.

Key Message

***Lactobacillus helveticus* MTCC 5463 as postbiotics with low molecular weight whey peptides** could be considered as an alternative for **antibiotic free meat and egg production** in broilers in future.