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Effect of supplementation of modified lignin on gut health in broilers

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Abstract
An experiment was conducted with 180 day-old Cobb broiler chicks to evaluate the effect of modified lignin on gut health. The chicks were randomly allotted into six dietary treatment groups with three replicates of ten birds each for 42 days. The treatments included a control diet as per BIS (2007) [4] (T_1), reformulated diet with 2% reduction in metabolizable energy and crude protein (T_2), and experimental diets supplemented with 0.2% (T_3, T_4) and 0.3% (T_5, T_6) modified lignin in both control and reformulated diets, respectively. The results showed that supplementation of modified lignin at 0.2 and 0.3% significantly enhanced villus height and crypt depth in duodenum, jejunum and ileocecal junction, and also reduced *E. coli* counts, and increased *Lactobacillus* counts compared to control and reformulated groups. It was concluded that dietary inclusion of modified lignin effectively improved gut health in broilers.

Keywords: *E. coli*, *Lactobacillus*, villus height, crypt depth, duodenum, jejunum, ileocecal junction

Introduction
The poultry industry in India has evolved from backyard farming to a modern organized sector, significantly contributing to the agro-livestock economy. It provides livelihood opportunities, especially in rural areas, due to its low capital requirements and high employment generation. As per BAHS (2024), India recorded a 3.17% increase in egg production, 4.95% rise in meat production, and 8.1% annual growth in poultry population, making it the second largest egg producer and fifth-largest chicken meat producer globally. However, per capita availability remains below ICMR recommendations, highlighting the need for expansion to meet nutritional demands. With FAO projecting a 70% surge in demand for animal protein, sustainable intensification is necessary, especially as feed costs remain the major limiting factor. The focus is shifting towards alternative feed resources that improve nutritional quality and sustainability. Concerns over antimicrobial resistance have driven research on prebiotics, particularly lignocellulosic derivatives, which stimulate beneficial gut bacteria and improve digestive health (Gibson *et al.*, 2017) [8]. Dietary fibers, once regarded as anti-nutritional, are now valued for promoting gut development, with lignin recognized for its natural prebiotic properties (Baurhoo *et al.*, 2009) [2].
Lignin, a complex polyphenolic polymer and key component of lignocellulose, accounts for 20-35% of dry biomass weight. It strengthens plant cell walls, resists microbial degradation, and provides antioxidant, flame-retardant, and UV-protective functions. Structurally, it is composed of syringyl (S), guaiacyl (G), and p-hydroxyphenyl (H) monomers, whose proportions vary among plant types (Norgren and Edlund, 2014) [12]. Softwood lignin is rich in guaiacyl, while hardwood lignin contains more syringyl units. Agricultural residues like sugarcane bagasse, wheat straw, and rice husk represent rich lignin sources. Extraction methods produce sulfur-containing types such as kraft lignin and lignosulfonates (Mahmood *et al.*, 2018) [10] and sulfur-free types like soda and *organosolv lignins*. Since native lignin is chemically inert, modifications such as sulfonation, oxidation, graft copolymerization, and alkylation are applied to enhance reactivity and expand applications (Mahmood *et al.*, 2018) [10].

Lignin and its derivatives have found diverse industrial and biomedical applications. They are used in dye adsorption (Silva *et al.*, 2011) [6], dispersants, plasticizers, and catalysts (Zhu *et al.*, 2019) [13]. In healthcare, lignin exhibits anticancer, antibacterial, antioxidant, and antimicrobial (Alzagameem *et al.*, 2018) [1] activities. Recent research highlights applications in surfactants, UV protection, composites (Klimek *et al.*, 2017) [9] and drug delivery systems (Cauley and Wilson, 2017) [5]. These multifunctional roles make lignin a promising candidate for sustainable livestock feed formulations and value-added industrial products.

Materials and Methods

Venkateshwara Hatcheries Pvt. Ltd. provided 180 day-old commercial broiler chicks for this investigation, and modified lignin was procured from Phytaxis SA, Rueschlikon, Switzerland. The chicks were first evaluated based on their weight upon acquisition and then randomly divided into six experimental groups. Each group had three replicates, with 10 chicks in each replicate. Following the Bureau of Indian Standards (BIS) 2007 [4] guidelines, the control group (T_1) was fed a basal diet, while group T_2 was fed with reformulated diet 2% reduction in ME and CP in the basal diet] and the experimental groups T_3 and T_4 supplemented with 0.2% modified lignin in both basal and reformulated diets, respectively and similarly, the treatment groups T_5 and T_6 supplemented with 0.3% modified lignin in both basal and reformulated diets, respectively. The chicks were reared under standard management practices in a deep litter system until six weeks of age. The birds were vaccinated as per a standard vaccination schedule, and food and water were provided *ad libitum* throughout the trial. The study was approved by the Institutional Animal Ethics Committee, KVAFSU, Bidar, and Karnataka.

At the end of the experiment, two birds from each replicate were sacrificed, and the tissue samples from the duodenum, jejunum, and ileocecal junction were collected for histopathological studies, including measurements of villus height and crypt depth. Additionally, microbiological parameters were assessed by quantifying *Lactobacillus* and *Escherichia coli* counts from the intestinal contents and the data were statistically analyzed.

Results

Gut morphology

The results of supplementation of modified lignin on intestinal villi height and crypt depth in broilers are presented in Table 1. Statistical analysis revealed a significant difference ($p \leq 0.05$) among the treatment groups at the end of experiment. The treatment groups T_3 and T_5 showed significantly ($p \leq 0.05$) higher villi height of duodenum, jejunum and ileocecal junction than the groups T_1, T_2, T_4 and T_6 and also the treatment group T_6 showed significantly higher villi height than the groups T_1 and T_2. There was no significant difference ($p > 0.05$) in villi height among treatment groups T_3 and T_5, also among groups T_4 and T_6 and also between groups T_1, T_2 and T_4.

The treatment groups T_3 and T_5 showed significantly ($p \leq 0.05$) higher crypt depth of duodenum, jejunum and ileocecal junction than the groups T_1 and T_2. There was no significant difference ($p > 0.05$) in crypt depth among treatment groups T_3, T_4, T_5 and T_6 and also among groups T_1, T_2, T_4 and T_6.

Table 1: Effect of supplementing modified lignin on intestinal villi height and crypt depth (µm) (Mean±SE) in broilers

Experimental group	Duodenal villi height	Duodenal crypt depth	Jejunal villi height	Jejunal crypt depth	Ileocecal junction villi height	Ileocecal junction crypt depth
T_1	1169.51±15.37^{a}	171.83±14.19^{a}	909.51±15.37^{a}	164.83±14.19^{a}	662.51±15.37^{a}	157.83±14.19^{a}
T_2	1175.01±10.31^{a}	162.17±16.53^{a}	915.01±10.31^{a}	155.17±16.53^{a}	668.01±10.31^{a}	148.17±16.53^{a}
T_3	1431.83±8.11^{c}	221.51±5.31^{b}	1171.83±8.11^{c}	214.51±5.31^{b}	924.83±8.11^{c}	207.51±5.31^{b}
T_4	1209.01±24.11^{ab}	187.33±11.93^{ab}	949.01±24.11^{ab}	180.33±11.93^{ab}	702.01±24.11^{ab}	173.33±11.93^{ab}
T_5	1437.67±9.88^{c}	218.67±6.36^{b}	1177.67±9.88^{c}	211.67±6.36^{b}	930.67±9.88^{c}	204.67±6.36^{b}
T_6	1220.67±9.88^{b}	188.67±13.12^{ab}	960.67±9.88^{b}	181.67±13.12^{ab}	713.67±9.88^{b}	174.33±13.36^{ab}

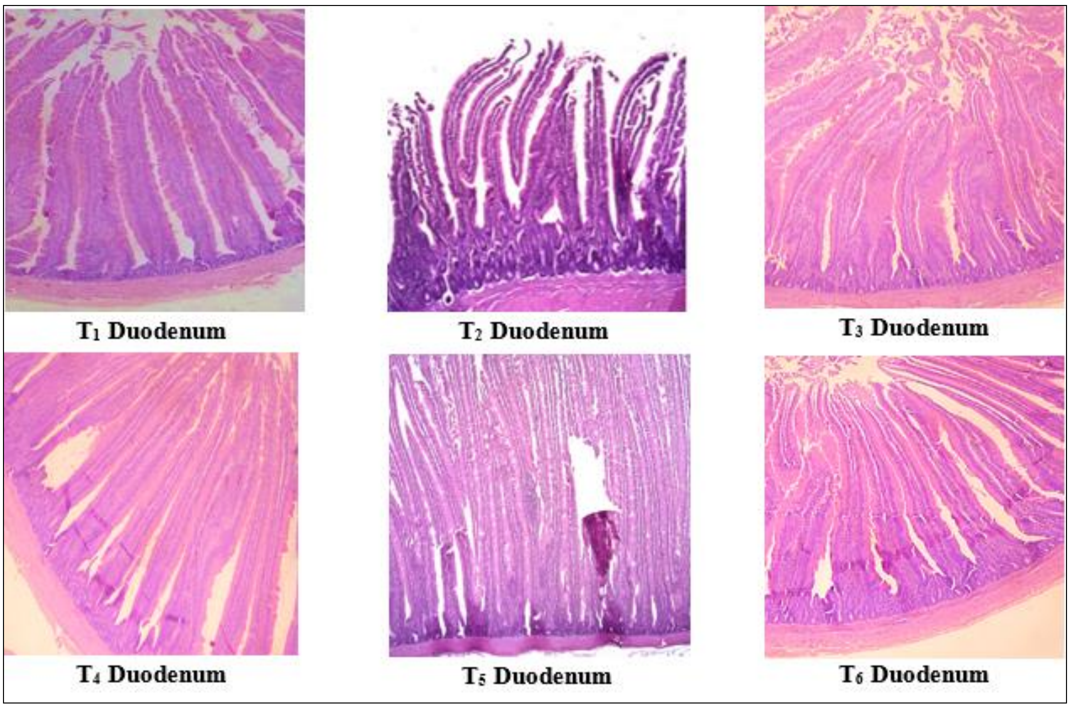

Plate 1: Section of duodenum from 42nd day old broilers fed with diets supplemented with modified lignin on duodenum villi height and crypt depth

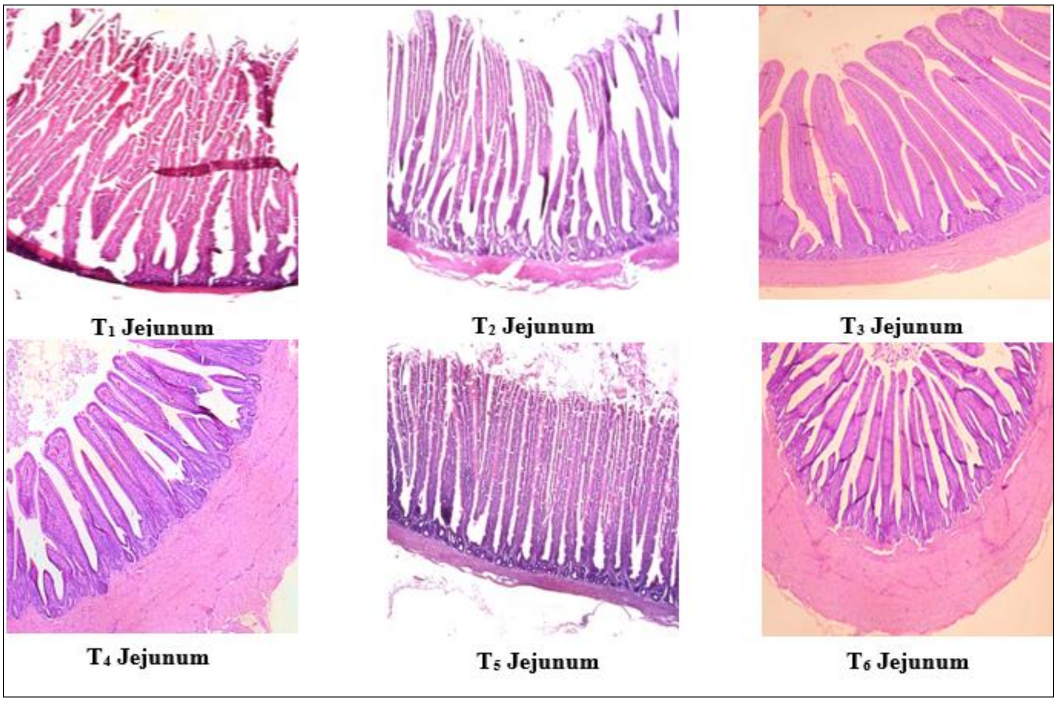

Plate 2: Section of jejunum from 42nd day old broilers fed with diets supplemented with modified lignin on jejunum villi height and crypt depth

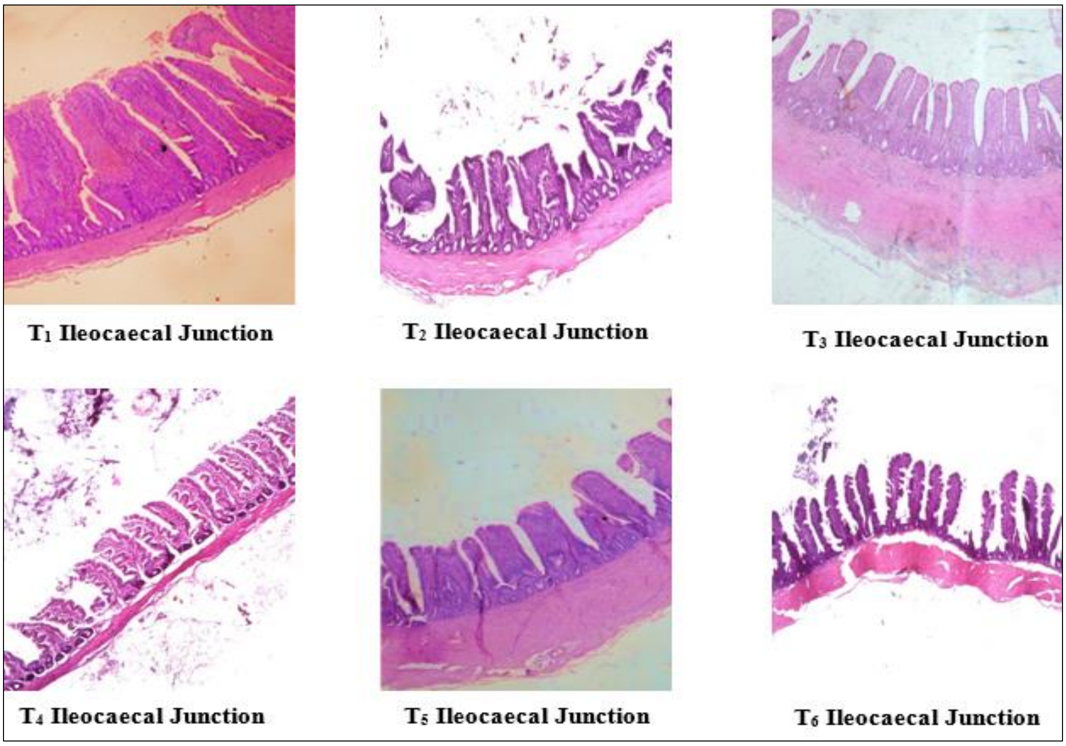

Plate 3: Section of Illeocaecocolic junction from 42nd day old broilers fed with diets supplemented with modified lignin on Illeocaecocal junction villi height and crypt

Gut microbial count

The results of supplementation of modified lignin on gut microbial load in broilers are presented in Table 2. Statistical analysis revealed a significant difference ($p \leq 0.05$) among the treatment groups at the end of experiment. The treatment groups T_3 and T_5 showed significantly ($p \leq 0.05$) lower *E. coli* count and higher *Lactobacillus* count than the groups T_1, T_2, T_4 and T_6 and also the treatment groups T_4 and T_6 showed significantly lower *E. coli* count and higher *Lactobacillus* count than the groups T_1 and T_2. There was no significant difference ($p \leq 0.05$) in *E. coli* count and *Lactobacillus* count among groups T_3 and T_5, also among groups T_4 and T_6 and also among groups T_1 and T_2.

Table 2: Effect of supplementation of modified lignin on gut microbial load (*log10* CFU/g) (Mean ± SE) in broilers

Experimental group	*E. coli*	*Lactobacillus*
T_1	7.09 ± 0.043^{c}	6.68 ± 0.051^{a}
T_2	7.07 ± 0.011^{c}	6.71 ± 0.049^{a}
T_3	6.47 ± 0.055^{a}	7.21 ± 0.027^{c}
T_4	6.88 ± 0.031^{b}	7.06 ± 0.022^{b}
T_5	6.51 ± 0.059^{a}	7.21 ± 0.022^{c}
T_6	6.89 ± 0.031^{b}	6.96 ± 0.064^{b}

$^{a, b, c}$ Means in the same column with no common superscript differ significantly ($p \leq 0.05$)

Discussion

There was a significant difference ($p \leq 0.05$) in gut morphology of the birds supplemented with modified lignin compared to the control group at the end of the experiment.

The results of the present study were in agreement with Baurhoo *et al.* (2007) [3] who conducted a trial by supplementing Alcell lignin at different levels in broilers and reported that there was a significant increase in jejunum villi height and a higher number of goblet cells per villus in birds supplemented with 1.25% of Alcell lignin compared to the control group. They concluded that this can be explained by the action of indigenous microbes, which stimulate vascularization and the development of intestinal villi, thereby enhancing the efficiency of digestion and nutrient absorption. Additionally, goblet cells produce mucins that bind to pathogenic microorganisms, reducing their ability to colonize the gut mucosa. The results of the present study were in disagreement with Carvalho *et al.* (2024) [7] who conducted an experiment in broilers, fed with diet containing different levels of sugarcane bagasse lignin and reported that there were no significant effect on gut histomorphometry in comparison to control group.

There was a significant difference ($p \leq 0.05$) in gut microbial count of the birds supplemented with modified lignin compared to the control group at the end of the experiment.

The results of the present study were in agreement with Makivic *et al.* (2019) [11] who observed significant increase in counts of *Lactic acid bacteria* and *Bifidobacterium* spp., while significant decrease in the population of *Escherichia coli* in the ileum and cecum in broilers fed diet with 0.6% lignocellulose compared to those on a control diet. They summarized that this result may be due to the abrasive effect of insoluble fibers, which prevent pathogenic bacteria from adhering to the intestinal mucosal surfaces. Moreover, lignin contains various phenolic monomers that exert biological activities, including antibacterial effects.

The results of the present study were in disagreement with Carvalho *et al.* (2024) [7] who conducted an experiment in broilers, fed with diet containing different levels of sugarcane bagasse lignin and reported that there was no significant difference in counts of *Lactic acid bacteria* and *Bifidobacterium* spp., between the treatments groups and in comparison to control group.

Conclusion

Based on the results, it was concluded that the addition of 0.2 and 0.3% modified lignin to the basal diet improved gut health in broilers. However, the supplementation of 0.2 and 0.3% modified lignin in reformulated diet showed similar results like that of control diet. Hence, it was concluded that supplementing 0.2% modified lignin in both control and reformulated diets (2% reduction in ME and CP) was beneficial in improving gut health

Conflict of Interest: Not available

Financial Support: Not available

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