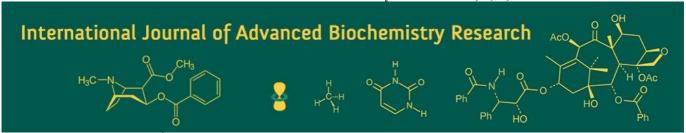
International Journal of Advanced Biochemistry Research 2025; 9(10): 470-475



ISSN Print: 2617-4693 ISSN Online: 2617-4707 NAAS Rating (2025): 5.29 IJABR 2025; 9(10): 470-475 www.biochemjournal.com Received: 08-08-2025 Accepted: 10-09-2025

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Effect of feeding fermented rapeseed meal with or without enzymes on gut health in broilers

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DOI: https://www.doi.org/10.33545/26174693.2025.v9.i10g.6064

Abstract

An experiment was conducted to evaluate the effect of dietary inclusion of fermented rapeseed meal (FRSM) with or without enzyme supplementation on the gut health of broilers. A total of 240 day-old Cobb broiler chicks were randomly assigned to eight dietary treatments with three replicates of ten birds each for six weeks period. All diets were formulated to be iso-caloric and iso-nitrogenous, as per BIS (2007) ^[3] standards. The control group (T₁) received a corn-soybean meal based basal diet without FRSM or enzymes, while T₂ was fed the basal diet with 0.02% enzyme supplementation. The treatment groups T₃, T₅ and T₇ were supplemented with FRSM at 2.5, 5.0 and 7.5%, respectively, without enzymes, whereas T₄, T₆ and T₈ received 2.5, 5.0 and 7.5% of FRSM with 0.02% enzymes, respectively. The results revealed no significant differences (*p*>0.05) among treatment groups with respect to gut morphology (villus height, crypt depth of different segments of the intestine), whereas microbial load showed significant reduction in *E. coli* counts and increased *Lactobacillus* counts compared to control group. It was concluded that dietary inclusion of FRSM up to 7.5%, with or without enzyme supplementation, has no detrimental impact on gut morphology and positively modulates the gut microbial population in broilers.

Keywords: Fermented rapeseed meal, enzymes, villus height, crypt depth, E. coli, Lactobacillus

Introduction

India's livestock sector, particularly the poultry industry, has witnessed remarkable growth in recent years, contributing substantially to agricultural output and rural employment. As per the Basic Animal Husbandry Statistics (BAHS, 2024) and the Ministry of Fisheries, Animal Husbandry and Dairying (2024), the poultry population has reached 851.81 million, with broiler meat contributing nearly half of the country's annual meat production of 10.25 million tonnes. Despite this expansion, the sector faces challenges such as escalating feed costs and sustainability concerns, largely due to the heavy reliance on soybean meal, the principal but costly protein source in poultry diets.

Rapeseed meal (RSM), a by-product of oil extraction from *Brassica napus*, offers a cost-effective and protein-rich alternative, containing 34-42% protein along with appreciable levels of choline, biotin, folic acid, and a favourable amino acid composition (Zhu *et al.*, 2019; Swati *et al.*, 2015) [14, 9]. Globally, rapeseed is the third most important oilseed crop, and RSM serves as a valuable protein source in animal feeding systems (30-45% crude protein) with high lysine and sulfur-containing amino acids (Swati *et al.*, 2015) [9]. However, its broader utilization in poultry nutrition has been limited due to the presence of antinutritional factors such as glucosinolates, erucic acid, tannins, phytates, and non-starch polysaccharides (Bellostas *et al.*, 2007; Qin *et al.*, 2017) [2, 8]. High glucosinolate levels have been linked to hypothyroidism, reduced feed digestibility, liver and kidney damage, and overall impaired bird performance (Qin *et al.*, 2017) [8]. Although breeding of low-glucosinolate cultivars has improved its nutritive value, challenges persist-particularly phosphorus utilization, as nearly 65% of phosphorus remains bound in phytate form, limiting its availability to birds (Zhu *et al.*, 2021) [13].

To address these limitations, fermentation has emerged as a promising strategy for improving the nutritional efficiency of RSM. Microbial fermentation significantly reduces antinutritional compounds such as glucosinolates (up to 97%), phytates, and fiber, while

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To address these limitations, fermentation has emerged as a promising strategy for improving the nutritional efficiency of RSM. Microbial fermentation significantly reduces antinutritional compounds such as glucosinolates (up to 97%), phytates, and fiber, while enhancing protein digestibility, bioactive peptide content, and vitamin availability (Hu et al., 2016; He et al., 2014) [14, 6]. Solid-state fermentation using bacteria, fungi, and yeast has been particularly effective, as it degrades glucosinolates and non-starch polysaccharides without compromising protein quality (Zaworska *et al.*, 2023) [12]. Strains including *Bacillus* spp., Saccharomyces cerevisiae, and Wickerhamomyces anomalus have been shown to lower glucosinolate levels by up to 83% while enhancing nutrient utilization (Vlassa et 2022) [10]. In addition. exogenous supplementation (e.g., protease, xylanase, glucanase, phytase) further improves the digestibility of proteins and carbohydrates. enhancing broiler thereby growth performance and feed efficiency (Zhu et al., 2021) [13]. Fermentation also promotes hydrolysis of phytates and degradation of complex polysaccharides, improving phosphorus bioavailability and energy utilization.

Overall, these approaches establish fermented rapeseed meal as an economically viable and sustainable feed ingredient that can partially substitute soybean meal, while maintaining growth performance, gut health, immunity, and meat quality in broiler production systems (Hu *et al.*, 2016; Zhu *et al.*, 2021) [7, 13].

Materials and Methods

Venkateshwara Hatcheries Pvt. Ltd. provided 240 day-old commercial broiler chicks for this investigation, the fermented rapeseed meal was procured from Hi-gain Feeds & Farms Pvt. Ltd., Mandya feed manufacturing plant and the enzyme mixture Vetzomix® was procured from Bionnar Healthcare Pvt. Ltd., Vijayawada, Andhra Pradesh. 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 [3] guidelines, the control group (T₁) received a corn-soybean meal based basal diet without FRSM or enzymes, while T2 was fed the basal diet with 0.02% enzyme supplementation. The treatment groups T_3 , T_5 and T₇ were supplemented with FRSM at 2.5, 5.0 and 7.5%, respectively, without enzymes, whereas T_4 , T_6 and T_8 received 2.5, 5.0 and 7.5% of FRSM with 0.02% enzymes, 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, 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

1. Gut morphology

The results of the effect of feeding fermented rapeseed meal with or without enzymes on intestinal villi height and crypt depth in broilers from different treatment groups is presented in Table 1.

Statistical analysis revealed no significant difference (p>0.05) in villi height of duodenum, jejunum and ileocecal junction compared to control and other treatment groups at the end of experiment.

Statistical analysis revealed no significant difference (p>0.05) in crypt depth of duodenum, jejunum and ileocecal junction compared to control and other treatment groups at the end of experiment.

Table 1: Effect of feeding fermented rapeseed meal with or without enzymes on intestinal villi height and crypt depth (μm) (Mean $\pm SE$) in broilers

Experimental	Duodenal villi height	Duodenal crypt depth	Jejunal villi height	Jejunal crypt depth	Ileum villi height	Ileum crypt depth
group						
T_1	1155.00±9.33	158.17±3.91	890.17±5.68	141.67±8.22	646.17±15.02	145.17±9.87
T_2	1174.17±9.63	171.33±8.44	913.50±6.91	154.67±9.62	679.33±20.11	152.83±13.16
T ₃	1151.33±10.61	160.50±9.59	894.83±18.91	146.33±6.97	650.33±12.22	147.33±4.12
T_4	1186.17±16.63	175.00±4.03	922.33±18.84	165.33±6.99	686.67±8.27	156.67±9.16
T ₅	1158.67±14.95	164.17±6.12	897.67±19.87	149.83±5.36	656.00±11.62	147.83±5.86
T ₆	1192.67±7.75	178.00±7.17	932.17±5.79	170.00±4.86	692.50±6.30	158.33±10.59
T ₇	1162.17±12.06	169.00±5.86	900.67±12.70	151.83±7.62	666.67±12.97	148.67±7.14
T ₈	1200.83±15.86	178.50±2.4	940.67±5.84	173.83±7.06	700.67±9.55	158.83±9.47

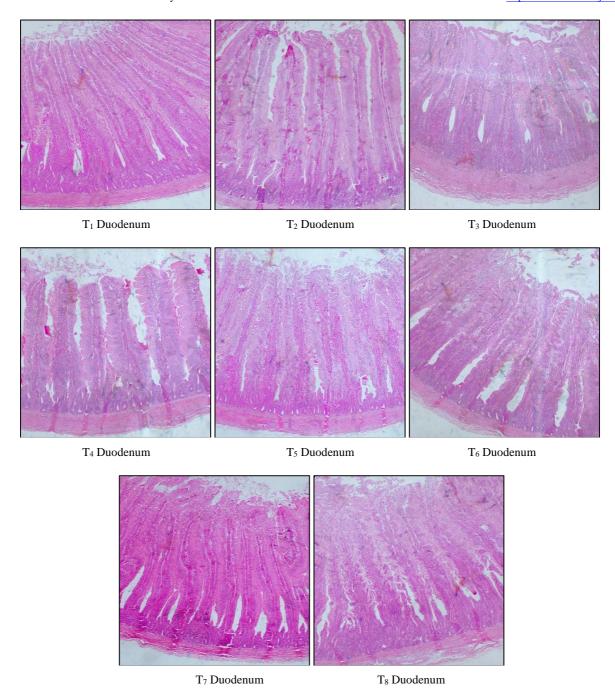
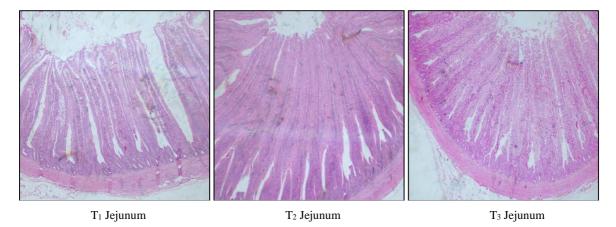


Plate 1: Section of duodenum from 42nd day old broilers fed with diets containing fermented rapeseed meal with or without enzymes on duodenum villi height and crypt depth.



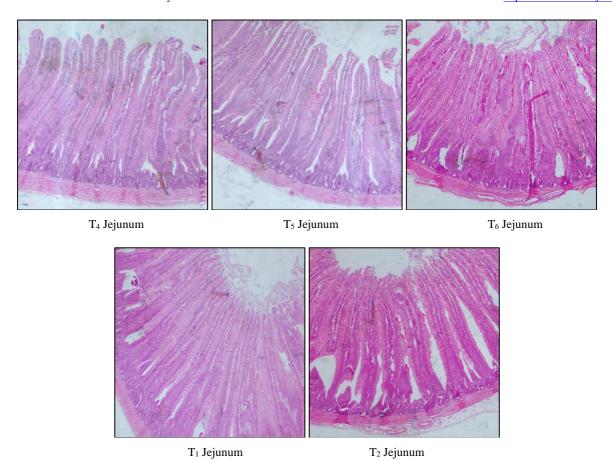
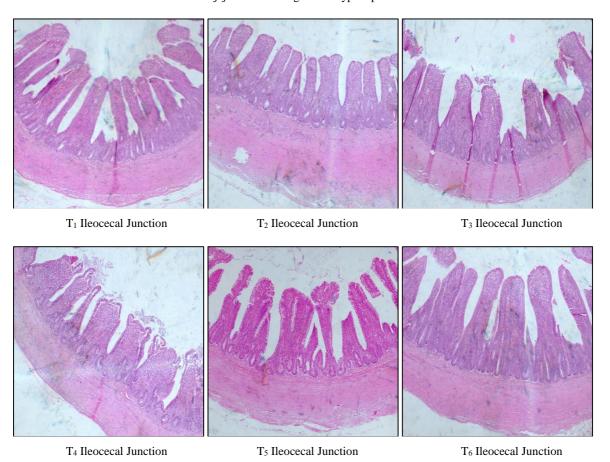
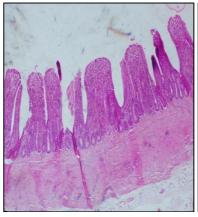
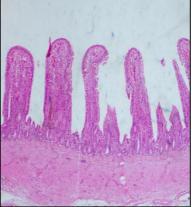


Plate 2: Section of jejunum from 42nd day old broilers fed with diets containing fermented rapeseed meal with or without enzymes on jejunum villi height and crypt depth.



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T₆ Ileocecal Junction

T₆ Ileocecal Junction

Plate 3: Section of Ileocaecocolic junction from 42nd day old broilers fed with diets containing fermented rapeseed meal with or without enzymes on Ileocaecocolic junction villi height and crypt depth.

2. Gut microbial count

The results of the effect of feeding fermented rapeseed meal with or without enzymes on gut (intestinal) microbial load (log10 CFU/g) in broilers is represented in Table 2. Statistical analysis revealed a significant difference ($p \le 0.05$) among the treatment groups at the end of experiment. The treatment groups T_5 , T_6 , T_7 and T_8 showed significantly ($p \le 0.05$) lower $E.\ coli$ count and higher Lactobacillus count than the groups T_1 , T_2 , T_3 and T_4 . There was no significant difference (p > 0.05) in $E.\ coli$ count and Lactobacillus counts among groups T_5 , T_6 , T_7 and T_8 and also among groups T_1 , T_2 , T_3 and T_4 .

Table 2: Effect of feeding fermented rapeseed meal with or without enzymes on gut microbial load (log_{10} CFU/g) (Mean±SE) in broilers

Experimental group	E. coli	Lactobacillus spp.
T_1	6.636±0.059 ^b	6.665±0.052a
T_2	6.648±0.087 ^b	6.672±0.100a
T_3	6.656±0.070 ^b	6.678±0.057a
T_4	6.658±0.042 ^b	6.688±0.023a
T_5	6.416±0.047 ^a	6.972±0.053 ^b
T_6	6.405±0.050a	6.979±0.042 ^b
T_7	6.409±0.039a	6.982±0.048 ^b
T_8	6.408±0.026 ^a	6.994±0.040 ^b

a.bMeans in the same column with no common superscript differ significantly ($p \le 0.05$)

Discussion

There was no significant difference $(p \le 0.05)$ in gut morphology of the birds fed with fermented rapeseed meal with or without enzymes compared to the control group at the end of the experiment.

The results of the present study were in agreement with Fahimi *et al.* (2023) ^[5] who observed supplementation of fermented rapeseed meal at 50% in broiler diet showed no significant effect on intestinal villi characteristics compared to the control group.

The results of the present study were in disagreement with Wu *et al.* (2022) [11] who observed supplementation of fermented rapeseed meal at 10% in broiler diet showed significantly increased duodenal villus height and villi height/crypt depth ratio compared to control group There was a significant difference ($p \le 0.05$) in gut microbial count of the birds supplemented with modified lignin compared to the control group at the end of the experiment.

There was a significant difference ($p \le 0.05$) in gut microbial count of the birds fed with fermented rapeseed meal with or without enzymes compared to the control group at the end of the experiment.

The results of the present study were in agreement with Ashayerizadeh *et al.* (2018) [1] who reported that supplementation of fermented rapeseed cake at all levels in broiler diets showed significantly higher population of lactic acid bacteria in the crop and a lower population of coliform bacteria in the ileum compared to the control group. They attributed this effect to the unique characteristics of fermented feeds, particularly their high lactic acid content, which helps to acidify the upper gastrointestinal tract. This creates favourable conditions for the growth and establishment of beneficial bacteria, such as lactic acid bacteria. These beneficial microbes contribute to a competitive exclusion effect, forming a natural defence barrier against infections and pathogenic bacteria, including *Salmonella* and coliforms.

The results of the present study were in disagreement with Drazbo *et al.* (2020) [4] who observed supplementation of fermented rapeseed cake at 150 g/kg in turkey diet showed significantly decreased *Lactobacillus* counts in the ceca compared to the control group.

Conclusion

Based on the findings of the present study, it was concluded that inclusion of fermented rapeseed meal up to 7.5%, with or without enzyme supplementation at 0.02% in the diet, has no detrimental impact on gut morphology and positively modulates the gut microbial population in broilers. Hence, the incorporation of fermented rapeseed meal up to 7.5% in broiler diets, with or without enzyme supplementation, can be recommended without any adverse effects.

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