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Impact of Enzymatically Hydrolyzed and Fermented Canola Meal on Broiler Performance, Intestinal Health, and Peptide Transporter Peptide Transporters Gene Expression

Research Article

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ABSTRACT

This study examined the impact of enzymatically hydrolyzed and fermented canola meal (CM) on broiler performance, carcass traits, blood serum parameters, cecal microbiota, intestinal morphology, and gene expression. A total of 540 Ross 308 broiler chicks were randomly divided into nine dietary groups: a control group (soybean meal-based), CM at 5% and 10%, CM hydrolyzed with protease at 5% and 10%, and CM fermented with *Lactobacillus reuteri* at 5% and 10%. The birds were fed these experimental diets from day 10 to day 32. Enzymatic hydrolysis and solid-state fermentation were performed using commercial protease and *L. reuteri*, respectively. The results showed that including 5% fermented CM in the diet had a minimal negative effect on broiler performance compared to other CM-supplemented diets. Additionally, this treatment resulted in the lowest abdominal fat, the highest gene expression levels (PepT1, PepT2, LEAP2), and improved small intestinal morphology. These findings indicate that enzymatic hydrolysis and fermentation can alleviate the negative impacts of CM on broiler performance and intestinal health.

KEY WORDS broiler chickens, canola meal peptides, enzymatic hydrolysis, solid-state fermentation.

INTRODUCTION

The economic success of poultry production is significantly impacted by the cost and quality of feed ingredients. While energy-dense feed materials are generally plentiful and cost-effective, protein sources, especially those with a rich amino acid profile, tend to be more scarce and costly. Soybean meal has long been the main protein source in poultry diets because of its excellent amino acid profile (Zhu *et al.* 2009). However, the growing global demand for soybean meal and its expanding role in human diets have driven the need to find alternative protein sources. Canola meal has gained attention as a potential alternative to soybean meal in poultry diets because of its high protein content. However, its broader adoption has been limited by the presence of anti-nutritional factors such as glucosinolates, phytic acid, polyphenols, and fiber (Wickramasuriya *et al.* 2015; Bastian *et al.* 2024). These compounds can adversely affect nutrient digestibility and overall bird performance. Additionally, despite the favorable amino acid profile of canola meal, its apparent metabolizable energy content is lower than that of soybean meal, and its amino acid digestibility is less efficient (Sureshkumar *et al.* 2023).

Canola meal is a more economical protein source compared to other plant and animal proteins used in livestock and poultry feed (Canola Council of Canada, 2024). Re-

search has shown that incorporating canola meal into broiler diets alongside soybean meal can improve bird performance (Elbaz *et al*. 2023). Additionally, several studies have successfully substituted a portion of soybean meal with canola meal in poultry diets (Manyeula *et al*. 2023). Peptides are short chains of amino acids produced by the hydrolysis of proteins (Jiang *et al*. 2009). Among these, bioactive peptides have specific biological functions that can enhance performance and overall health (Zaky *et al*. 2022). Plant-derived peptides exhibit a wide array of biological activities that are advantageous to human health. These activities include anti-diabetic, immune-modulatory, antimicrobial, cholesterol-lowering, antihypertensive, and antioxidant properties (Meena *et al*. 2020; Li *et al*. 2024).

In addition to their role in plant defense against invading microorganisms, antimicrobial peptides from plants have been found to exert potent antibacterial effects in humans. The positive charge and hydrophobic nature of these peptides are believed to contribute to their ability to disrupt bacterial membranes, leading to cell death (Li *et al*. 2021; Ghanbarzadeh *et al*. 2024). Because most bioactive peptides are embedded within mature proteins, enzymatic hydrolysis is the main method used to extract these compounds from various food sources (Yang *et al*. 2015). Digestive enzymes have been extensively used for this purpose. One of the key benefits of using digestive enzymes is that they allow for the oral administration of the resulting bioactive peptides (Räder *et al*. 2018). Enzymes are employed in various applications to aid in protein extraction from food. This includes breaking down cell walls, releasing starch-bound proteins, and enhancing protein solubility (Fabia and Ju, 2011). Additionally, bioactive peptides can be produced through microbial fermentation (Cian and Drago, 2022; Chourasia *et al*. 2023).

Lactobacilli, a group of beneficial bacteria commonly found in the environment and the human digestive tract, are frequently used for this purpose. The proteolytic systems of lactobacilli, including species such as *Lactococcus lactis* (Song *et al*. 2017), *Lactobacillus helveticus* (Griffiths and Tellez, 2013), and *Lactobacillus delbrueckii subsp. bulgaricus* (Zheng *et al*. 2012), are well-characterized. Solid-state fermentation (SSF) is a widely used technique for microbial fermentation (Subramaniyam and Vimala, 2012). Previous studies have demonstrated the effectiveness of SSF in reducing anti-nutritional factors in plant-based feedstuffs (Vieira *et al*. 2023). The glucosinolate content in fermented canola meal has been shown to be significantly lower than that of raw canola (Vig and Walia, 2001; Hosseinpoor *et al*. 2023). Di- and tripeptides are absorbed via H+-dependent peptide transporters PEPT1 and PEPT2 (Zwarycz and Wong, 2013). Liver-expressed antimicrobial peptide 2 (LEAP2) is recognized as a natural antagonist of the growth hormone secretagogue receptor (GHSR) and plays a crucial role in regulating feed intake and maintaining energy balance (Zheng *et al*. 2022).

This study uniquely examined the impact of fermented canola meal on broiler health, focusing on nutrient absorption and gut health. By analyzing gene expression related to nutrient absorption and changes in the gut microbiome, it provides a comprehensive understanding of how fermented canola meal affects broiler physiology. The study compares fermented canola meal with raw and hydrolyzed canola, identifying the most effective method for improving broiler health and performance. It highlights potential microbial imbalances and offers practical recommendations for including fermented canola meal in broiler diets.

MATERIALS AND METHODS

Preparation of bioactive peptides

Enzymatic hydrolysis of canola meal was carried out following the methodology described by Alashi *et al*. (2014), with minor adjustments. Similarly, solid-state fermentation was performed according to the protocol outlined by Olukomaiya *et al*. (2020), with slight modifications.

Solid-state fermentation

Lactobacillus reuteri was used for solid-state fermentation (SSF). The SSF process followed the protocol described by Olukomaiya *et al*. (2020), with minor adjustments. Each SSF experiment was conducted in triplicate using 500 mL Erlenmeyer flasks. The substrates (150 g) were supplemented with 1 g of beef extract, 0.3 g of potassium chloride, 0.3 g of iron(II) sulfate, and 1 g of magnesium sulfate. Their moisture content was adjusted to 45% using reverse osmosis (RO) water before sterilization at 122 °C for 15 minutes. The cooled canola meal samples were then inoculated and incubated at 30 °C for 7 days. Non-fermented, autoclaved canola meal served as the control. All samples were subsequently dried at 65 °C for 48 hours, cooled, passed through a 0.5 mm sieve, and stored at −20 °C until further analysis.

Preparation and enzymatic hydrolysis

Enzymatic hydrolysis of canola meal was conducted using a modified version of the method described by Alashi *et al*. (2014). Unlike the original study, which focused on the hydrolysis of isolated canola meal protein, this investigation utilized whole canola meal samples. Enzymatic hydrolysis of canola meal was performed using a commercial protease enzyme (Ariana Co., Mashhad, Iran) at a substrate-to-enzyme ratio of 1:20. The hydrolysis reaction was carried out for 4 hours under optimal

conditions of pH 8.0 and 40 °C, maintained using 1 M NaOH and a thermostat. After the hydrolysis period, the enzyme was inactivated by heating at 85 °C for 20 minutes. The final hydrolysate, with a pH of approximately 4.5, was lyophilized and stored at -18 °C for subsequent analysis and experimentation.

Field experiments

This study aimed to assess the impact of various processed canola meal treatments on the growth performance of broiler chickens. A total of 540 one-day-old Ross 308 broiler chicks were randomly assigned to seven dietary treatments in a completely randomized design, with five replicates and 12 chicks per pen. The experiment lasted 42 days. Broilers received a control diet based on soybean meal from day 1 to 9. From day 10 to the end of the experiment, they were fed experimental diets containing various levels of canola meal processed by different methods.

Control: Diet based on soybean meal.
Diet 1: 5% canola meal.
Diet 2: 10% canola meal.
Diet 3: 5% canola meal hydrolyzed with commercial protease enzyme.
Diet 4: 10% canola meal hydrolyzed with commercial protease enzyme.
Diet 5: 5% canola meal fermented by *Lactobacillus reuteri*.
Diet 6: 10% canola meal fermented by *Lactobacillus reuteri*.

Experimental diets were formulated using the UFFDA diet formulation software, following commercial practices and the nutrient requirements specified in the National Research Council (NRC, 1994) tables for feed ingredients and the Ross 308 commercial strain rearing guide (Table 1). Feed and water were provided *ad libitum* throughout the experiment.

Performance and carcass evaluation

Feed intake and live weight were recorded for each cage at the end of each feeding phase. Daily feed intake, daily weight gain, and feed conversion ratio (FCR) were calculated. Mortality rates were monitored daily, and performance traits were adjusted accordingly. On day 42 of the experiment, two birds from each cage with weights close to the average were weighed and slaughtered for carcass evaluation.

Relative organ weights, microflora and tissue sampling

The relative weights of the following carcass components, digestive organs, and lymphoid organs were determined: liver, heart, gizzard, spleen, abdominal fat, bursa of Fabricius, and intestines. Additionally, intestinal length was measured. To evaluate intestinal tissue morphology, segments approximately 1.5-2 cm in length were collected from the mid-sections of the duodenum, jejunum, and ileum. These samples were washed with distilled water and preserved in a 10% formalin solution. A portion of the right liver lobe was also removed and stored in formalin for histological examination. For the analysis of cecal microflora, the distal ileum and entire cecum were immediately separated after slaughter, sealed at both ends with a special thread, and transported to the laboratory on dry ice.

Blood sampling and serum/plasma preparation

One week before slaughter (day 35 of the rearing period), the birds were fasted overnight. The next morning, blood samples were collected from the wing vein of one male and one female bird from each replicate. To isolate serum and plasma, blood samples were centrifuged at 3000 rpm for 20 minutes at 4 °C. The separated serum and plasma samples were then stored at -20 °C until laboratory analysis.

Serum antioxidant parameter analysis

The serum samples collected at the end of the rearing period (day 35) were analyzed to assess antioxidant status. The activities of the enzymes glutathione peroxidase and superoxide dismutase, as well as the concentrations of malondialdehyde and total serum antioxidants, were determined. Total antioxidant concentration was measured using Ransel kits (Randox Laboratories Ltd., Crumlin, UK) and an automated autoanalyzer (RX daytona+, Randox Laboratories Ltd., Crumlin, UK).

Malondialdehyde (MDA) assay

To assess oxidative stress, the concentration of malondialdehyde (MDA) was measured using a TBARS assay. A 250 µL aliquot of each serum sample was mixed with 25 µL of 0.2% butylhydroxytoluene (in ethanol) and 1 mL of 15% trichloroacetic acid, followed by centrifugation at 4000 rpm for 15 minutes.

The supernatant (500 µL) was then combined with 1 mL of thiobarbituric acid (TBA) (0.375% in 0.025 M hydrochloric acid) and incubated in a boiling water bath for 15 minutes. After cooling the samples in ice water, the absorbance was measured at 535 nm using a spectrophotometer (CECIL AQUARIUS) (Tsikas, 2017).

Enzyme activity assays

The activities of glutathione peroxidase and superoxide dismutase were determined using Randox kits and a spectrophotometer (Ohkawa *et al.* 1979). Measurements were performed at wavelengths of 340 nm and 505 nm, respectively, and enzyme activities were calculated according to the manufacturer's instructions.

Table 1 The composition and analysis of experimental diets

Items (g/kg)	Starter (d 0 to 10)	Grower (d 10 to 28)		Finisher1 (d 28 to 35)		Finisher2 (d 35 to 49)	
		Canola meal free	Containing canola meal	Canola meal free	Containing canola meal	Canola meal free	Containing canola meal
Corn	565.50	629.50	629.50	661.50	661.50	703.00	703.00
Soybean meal (CP 42%)	385.00	325.00	175.00	290.00	140.00	250.00	100.00
Canola meal	0.00	0.00	150.00	0.00	150.00	0.00	150.00
Soybean oil	5.00	5.00	5.00	10.00	10.00	10.00	10.00
Calcium Carbonate	10.00	10.00	10.00	9.00	9.00	8.00	8.00
Mono calcium phosphate	15.85	13.21	13.21	13.21	13.21	13.21	13.21
Sodium bicarbonate	1.00	1.50	1.50	1.50	1.50	1.50	1.50
Salt	2.50	2.50	2.50	2.50	2.50	2.50	2.50
DL-methionine	2.60	2.17	2.17	2.17	2.17	2.17	2.17
L-lysine	1.39	1.16	1.16	1.16	1.16	1.16	1.16
L-threonine	0.27	0.23	0.23	0.23	0.23	0.23	0.23
Vitamin and mineral premix[1]	0.50	0.50	0.50	0.50	0.50	0.50	0.50
Acidifier	1.00	1.00	1.00	0.00	0.00	0.00	0.00
Anticoccidial	0.00	0.50	0.50	0.50	0.50	0.00	0.00
Calculated composition (g/kg)							
Metabolizable energy (kcal/kg)	2797.00	2877.00	2843.00	2951.00	2916.00	3000.00	2966.00
Crude protein	221.90	200.70	191.70	188.10	179.10	174.20	165.20
Met	6.00	5.70	4.80	4.10	3.70	4.40	3.50
Met+Cys	9.90	8.30	8.80	7.60	7.60	7.50	7.30
Lys	13.60	11.90	10.70	11.00	9.90	10.00	8.90
Thr	8.90	8.00	7.80	7.50	7.30	7.00	6.70
Ca	9.20	7.80	8.20	7.50	7.10	7.60	7.60
Available P	4.90	4.30	4.30	4.00	4.30	4.10	4.00
Na	2.10	2.10	2.10	2.10	2.10	2.10	2.10

[1] The vitamin and mineral premix supplied the following per kilogram of diet: vitamin A: 400000 IU; vitamin D3: 160000 IU; vitamin E: 2200 IU; vitamin K: 88 mg; vitamin B1: 2 mg/kg; vitamin B1: 88 mg/kg; vitamin B2: 220 mg/kg; vitamin B3: 1600 mg/kg; B5: 520 mg/kg; B6: 120 mg/kg; B9: 60 mg/kg; B12: 0.48 mg/kg; H2: 8 mg/kg; Fe: 800 mg/kg; Cu: 640 mg/kg; Mn: 4800 mg/kg; Zn: 4400 mg/kg; I: 50 mg/kg and Se: 12 mg/kg.

Relative gene expression analysis and intestinal bacteria quantification

At the end of the rearing period, two birds per pen were humanely euthanized. Immediately following euthanasia, 1 cm sections of the left liver lobe and mid-jejunum were excised and flash-frozen in liquid nitrogen at -80 °C for subsequent gene expression analysis. RNA extraction was performed using the TRIzol Reagent (Invitrogen, USA) following the manufacturer's protocol. The quality and quantity of extracted RNA were assessed using a Nanodrop spectrophotometer 2000 (Thermo Fisher Scientific, Waltham, MA, USA) by measuring the absorbance ratios at 260/280 nm (protein contamination) and 260/230 nm (phenol contamination). The cDNA synthesis was performed using an Add Bio kit (South Korea). To quantify gene expression, a reference gene with a constant copy number in the cell line and unaffected by the treatment was used for PCR product normalization. The PCR signal represents the relative transcription level of the target gene compared to the reference gene.

The $2^{(-\Delta\Delta Ct)}$ method was employed to calculate changes in gene expression in qRT-PCR, with threshold cycles as the endpoint. qRT-PCR was performed on cDNA synthesized from each sample, and Ct values were analyzed using Microsoft Excel Spreadsheet.

Real-time PCR using the 16S rRNA gene is a precise and rapid method for determining the bacterial population of the gastrointestinal tract.

The 16S rRNA gene, with its conserved and variable regions, serves as a molecular marker for identifying and classifying bacteria. In this method, bacterial DNA is first extracted from gastrointestinal samples. Then, using specific primers for the 16S rRNA gene, DNA amplification is performed.

During the amplification process, the amount of fluorescence produced in each cycle is measured, allowing for the quantitative determination of bacterial numbers (Yang *et al*. 2015). Table 2 shows the specifications of primers used for the quantification of gene expression and bacterial populations.

Table 2 The PCR conditions and the sequences of primers used to gene expression and bacteria quantification

		Tm (˚C)	Product size
Firmi-primer-F	TGAAACTYAAAGGAATTGACG	59	155 bp
Firmi-primer-R	ACCATGCACCACCTGTC		
Bact-primer-F	CRAACAGGATTAGATACCCT	59	172 bp
Bact-primer-R	GGTAAGGTTCCTCGCGTAT		
Actin-primer-F	CCGTTACTGACGCTGAGGAG	59	141 bp
Actin-primer-r	GCGGGATGCTTAACGCG		
Proteo-primer-F	CAAAKACTGACGCTSAGGTG	59	97 bp
Proteo-primer-R	GGCACAACCTBCAARTCG		
16 s f	CAGCTCGTGTCGTGAGATGT	59	150 bp
16 s r	CGTAAGGGCCATGATGACTT		
Pept1 f;	CCTGCGGTGGGATGACAACT	59	95 bp
Pept1 r;	AGTCTGCAATGAGCGCTCCC		
Pept2 f;	GAGCTGTCCTGACGCTGTATT	59	188 bp
Pept2 r;	CAGATGGCCGACCACATACAC		
Leap2 f:	CTATTCGGGGCTCTTCCAAC	59	125 bp
Leap2 r:	TGAGCAGCAGCGAGAAGAATA		
bactin f	GCCATCAGAGAGAAGATGACAC	60	141 bp
bactin r	GTAAACACCATCACCAGAGTCCA		
PCR condition		Temprature	Duration
Conditioning (initial denaturation)		95	180 s
Deanaturation		95	10 s
Anealing		59-60	30 s
Extention		72	20 s

Intestinal histomorphology

At the end of the experiment, two birds per pen were euthanized. For intestinal tissue sampling, 1-1.5 cm segments were excised from the mid-portions of the duodenum, jejunum, and ileum. The excised tissue sections were rinsed with distilled water and transferred to labeled plastic containers containing 30 mL of 10% formalin solution. Cryostat sections (15-20 µm) of frozen specimens were prepared at -45 °C. Sudan Black staining was used to evaluate the abundance of unsaturated lipids in hepatocyte cytoplasm. Hematoxylin-eosin (H&E) staining was employed to assess histomorphometric parameters. Tissue samples were fixed in 10% buffered formalin, followed by dehydration with ascending alcohols, clarification with xylene, and paraffin embedding. Paraffin blocks were sectioned at 5-7 µm using a microtome (DS4055-Iran), and sections were stained with H&E. Histomorphometric analyses were conducted using a Dino-Lite lens digital camera, Dino-capture 2 software, and ImageJ V. 1.52 software to evaluate liver tissue stainability with Sudan Black (Asadi *et al*. 2022).

Statistical analysis

The collected data were statistically analyzed using SAS 9.4 software (SAS, 2013). Appropriate analysis of variance (ANOVA) procedures were employed, including completely randomized and factorial designs, as well as general linear models. The following statistical model was used:

$$Y_{ij} = \mu + T_i + \varepsilon_{ij}$$

Where:

Y_{ij}: value for the j^{th} observation in the i^{th} treatment.

µ: overall mean.

Ti: effect of the i^{th} treatment.

εij: random error associated with the j^{th} observation in the i^{th} treatment.

If the F-test was significant, means were separated using Duncan's multiple range test.

RESULTS AND DISCUSSION

The effects of the experimental treatments on the production traits of broiler chickens are shown in Table 3. The results of this study demonstrated that the inclusion of canola meal in broiler diets led to increased feed intake across all experimental groups compared to the control group. During the growth, finishing, and overall rearing periods, diets containing 10% hydrolyzed canola meal and 5% or 10% fermented canola meal exhibited comparable weight gain to the control group. However, other treatments resulted in lower weight gain. Notably, the group fed 10% raw canola meal displayed the lowest weight gain. Overall, the inclusion of canola meal in diets resulted in a higher feed conversion ratio, with the exception of the group fed 5% fermented canola meal, which exhibited a feed

conversion ratio comparable to the control group during the growth and finishing periods.

The group fed 10% raw canola meal demonstrated the highest feed conversion ratio. The control group exhibited superior performance, characterized by the lowest feed consumption and the highest weight gain. The group supplemented with 5% fermented canola meal displayed comparable results to the control group, indicating significant improvements in weight gain and feed conversion efficiency. Conversely, groups receiving other canola meal treatments experienced a reduction in weight gain despite increased feed intake, leading to a higher feed conversion ratio. These findings suggest that while canola meal inclusion generally increased feed intake, the processing method significantly influenced the overall performance of the animals.

Previous studies have shown that high inclusion levels of canola meal in poultry diets can lead to reduced weight gain due to the presence of anti-nutritional factors (Hameed *et al*. 2002; Ajao *et al*. 2022). In another report, replacing 10% of soybean meal with canola meal did not affect bird body weight. Research indicates that partial replacement of soybean meal with canola meal in poultry diets does not significantly affect bird body weight, making it a viable alternative protein source (Elbaz *et al*. 2023). Including 10% canola meal in diets had no effect on the growth performance of broilers during the early growth phase (Woyengo *et al*. 2011; Gopinger *et al*. 2014). Additionally, Rabie *et al*. (2015) found that replacing 10% of the diet with canola meal did not affect broiler growth performance.

However, Elbaz *et al*. (2023) observed that including 10% canola meal in the diet from 1 to 21 days of age significantly affected daily feed intake and weight gain in broilers. In contrast, including 20% canola meal had negative effects on broiler growth performance at 21 and 42 days of age, characterized by reduced daily feed intake, lower body weight gain, and higher feed conversion ratio. Elbaz *et al*. (2023) also suggested that including 10% canola meal in the diet could significantly reduce feed intake and increase weight gain in broilers. In this study, the dietary inclusion of fermented canola meal at a 5% level resulted in performance similar to the control group. Metabolites derived from glucosinolates have adverse effects on bird intestinal function, leading to reduced nutrient digestion and absorption (Manyeula *et al*. 2023), consequently reducing bird performance.

The performance results underscore the significant impact of including canola meal and its processing methods on the production traits of broiler chickens. The findings indicate that while canola meal generally increases feed intake, the method of processing is crucial. Canola meal contains anti-nutritional factors such as glucosinolates and sinapine, which can negatively affect bird performance by impairing nutrient digestion and absorption. The microbial fermentation process effectively reduces these anti-nutritional factors, thereby enhancing the nutritional value of the canola meal.

This process not only reduces anti-nutritional factors but also increases the bioavailability of nutrients. Studies have shown that microbial fermentation can improve the digestibility of amino acids and small peptides, which are essential for the growth and development of broilers (Elbaz *et al*. 2023).

Fermented canola meal exhibits improved digestibility of amino acids and small peptides, which are crucial for protein synthesis and growth in broilers. This enhanced nutritional quality contributes to better weight gain and feed conversion efficiency. Fermentation enriches the canola meal with probiotics, amino acids, peptides, and feed growth promoters, which aid in the digestion and absorption of nutrients (Wu *et al*. 2020). The inclusion of fermented canola meal in the diet improves gut health by promoting a favorable microbial balance. The presence of lactic acid bacteria enhances the gut environment, leading to better nutrient absorption and overall health of the birds. Improved gut health is associated with better growth performance, as it ensures efficient nutrient utilization and reduces the risk of gastrointestinal issues (Cherian *et al*. 2024).

The results presented in Table 4 indicate that the various experimental treatments did not significantly affect the relative weights of internal organs, such as carcass weight, heart, liver, spleen, bursa of Fabricius, and intestinal length. However, abdominal fat content was significantly influenced. The highest abdominal fat content was observed in the group fed 10% raw canola meal, while the lowest was found in the group fed 5% fermented canola meal.

A notable finding in carcass composition was the reduced abdominal fat in birds fed fermented canola meal, which did not differ significantly from the control group, unlike the group fed raw canola meal. Fermented feeds often contain lactic acid bacteria, which have beneficial effects on animal health. These bacteria reduce fat synthesis by decreasing the activity of acetyl-CoA carboxylase, the rate-limiting enzyme in hepatic fatty acid synthesis. The reduced activity of this enzyme leads to decreased fatty acid synthesis, which in turn limits the availability of fatty acids for esterification into triglycerides for storage in adipose tissue. Consequently, adipose tissue, particularly in the abdominal region, is reduced (Kalavathy *et al*. 2010; Vieco-Saiz *et al*. 2019).

Similar results in terms of reduced abdominal fat have been reported in studies using yeast species such as *Candida tropicalis* and *Saccharomyces cerevisiae* (Nie *et al*. 2015).

Table 3 Effects of supplementation of different treatments in diet on growth performance of broiler chickens

Item	Treatments							SEM	P-value
	T1	T2	T3	T4	T5	T6	T7		
Starter (1-10 d)									
Feed intake (g)	295.57	295.64	297.50	297.10	297.56	295.17	298.09	0.81	0.06
Body weight gain (g)	262.46	254.14	258.25	260.78	258.30	254.14	254.96	3.66	0.56
FCR (g/g)	1.15	1.16	1.15	1.14	1.15	1.16	1.17	0.016	0.06
Grower (11-28 d)									
Feed intake (g)	1071.68^{b}	1112.10^{a}	1119.06^{a}	1117.57^{a}	1119.31^{a}	1114.09^{a}	1121.30^{a}	3.26	0.001
Body weight gain (g)	677.82^{a}	640.00^{bc}	663.80^{ab}	677.52^{a}	658.30^{ab}	633.32^{bc}	619.18^{c}	11.57	0.006
FCR (g/g)	1.58^{d}	1.74^{abc}	1.9^{bc}	1.65^{cd}	1.70^{bc}	1.76^{ab}	1.81^{a}	0.029	0.001
Finisher (29–49 d)									
Feed intake (g)	2885.14^{b}	2986.87^{a}	3005.7^{a}	3001.56^{a}	3006.24^{a}	2992.21^{a}	3011.58^{a}	8.18	0.001
Body weight gain (g)	1405.4^{a}	120.46^{b}	1342.72^{ab}	1381.10^{a}	1343.80^{ab}	1299.54^{b}	1275.46^{b}	25.31	0.008
FCR (g/g)	2.05^{c}	2.33^{a}	2.24^{ab}	2.17^{bc}	2.24^{ab}	2.30^{ab}	2.35^{a}	0.043	0.001
Total (1-49 d)									
Feed intake (g)	4242.40^{b}	4394.61^{a}	4422.13^{a}	4416.23^{a}	4423.11a	4402.47^{a}	4430.97^{a}	12.14	0.001
Body weight gain (g)	2350.40^{a}	2169.60^{bc}	2264.80^{ab}	2319.40a	2260.40^{abc}	2187.00^{bc}	2164.60^{c}	31.18	0.001
FCR (g/g)	1.81^{d}	2.03^{ab}	1.95^{bc}	1.90c	1.96^{bc}	2.01^{ab}	2.05^{a}	0.025	0.001

T1: the control diet based on soybean meal; T2: canola meal previously hydrolyzed using commercial protease enzyme 5%; T3: canola meal previously hydrolyzed using commercial protease enzyme 10%; T4: canola meal fermented by *Lactobacillus reuteri* 5%; T5: canola meal fermented by *Lactobacillus reuteri* 10%; T6: canola meal 5% and T7: canola meal 10%.
FCR: feed conversion ratio.
The means within the same row with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of the means.

Table 4 Effects of supplementation of different treatments in diet on relative weight of internal organs of broiler chickens

Item	Treatments							SEM	P-value
	T1	T2	T3	T4	T5	T6	T7		
Heart	0.48	0.49	0.52	0.52	0.46	0.48	0.49	0.01	0.64
Liver	1.95	1.89	2.01	1.94	2.04	1.88	2.00	0.05	0.78
Abdominal fat	1.01^{bc}	1.14^{ab}	1.04^{bc}	0.8^{c}	1.26^{ab}	1.10^{abc}	1.35^{a}	0.05	0.004
Spleen	0.09	0.09	0.09	0.10	0.10	0.10	0.10	0.004	0.80
Bursa of fabricius	0.16	0.16	0.16	0.18	0.18	0.18	0.16	0.01	0.20
Carcass	68.05	65.40	68.18	66.55	66.35	6.43	65.74	0.40	0.10
Intestine length	9.41	9.27	8.99	9.50	9.01	9.47	9.87	0.23	0.75

T1: the control diet based on soybean meal; T2: canola meal previously hydrolyzed using commercial protease enzyme 5%; T3: canola meal previously hydrolyzed using commercial protease enzyme 10%; T4: canola meal fermented by *Lactobacillus reuteri* 5%; T5: canola meal fermented by *Lactobacillus reuteri* 10%; T6: canola meal 5% and T7: canola meal 10%.
The means within the same row with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of the means.

These findings suggest that the improvement in carcass characteristics observed with the use of hydrolyzed proteins may be attributed to the formation of peptides generated during the hydrolysis of protein sources. A significant sex effect was observed on carcass weight, bursa of Fabricius, and heart weight, with females exhibiting higher weights compared to males. Overall, the results indicate that the inclusion of canola meal, whether hydrolyzed or fermented, did not negatively impact the relative weights of most internal organs. The significant differences observed in abdominal fat weights highlight the importance of processing methods in influencing fat metabolism and overall performance in broilers (Shoaib *et al*. 2023).

The results in Table 5 highlight the effects of different dietary treatments on the serum antioxidant capacity of broiler chickens. Here is a comprehensive discussion of the findings: The overall antioxidant capacity did not show significant differences across treatments (P>0.05). This suggests that the inclusion of canola meal, whether hydrolyzed or fermented, did not significantly alter the antioxidant capacity of the serum. The stability in antioxidant capacity indicates that the treatments were well-tolerated by the broilers. The levels of SOD, an important antioxidant enzyme, were not significantly different across treatments (P>0.05). This indicates that the dietary treatments did not have a notable impact on the activity of SOD in the serum.

Table 5 Effects of supplementation of different treatments in diet on serum antioxidant capacity

Item	Treatments							SEM	P-value
	T1	T2	T3	T4	T5	T6	T7		
Antioxidant	1.89	1.78	1.76	1.79	1.84	1.69	1.76	0.08	0.36
SOD	10.68	11.79	10.89	11.28	10.65	10.82	11.4	0.49	0.46
Protein	65.56	64.70	70.20	65.50	66.70	62.10	63.90	2.60	0.47
Gpa	66.06	73.70	66.50	61.90	63.30	6.60	67.60	3.25	0.25
MDA	3.91^{abc}	3.69^{bc}	4.74^{a}	3.35^{bc}	3.06^{c}	3.26^{bc}	4.09^{ab}	0.33	0.01

T1: the control diet based on soybean meal; T2: canola meal previously hydrolyzed using commercial protease enzyme 5%; T3: canola meal previously hydrolyzed using commercial protease enzyme 10%; T4: canola meal fermented by *Lactobacillus reuteri* 5%; T5: canola meal fermented by *Lactobacillus reuteri* 10%; T6: canola meal 5% and T7: canola meal 10%.
SOD: superoxide dismutase; Gpa: glutathione peroxidase and MDA: malondialdehyde.
The means within the same row with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of the means.

Table 6 Effects of supplementation of different treatments in diet on gene expression of LEAP2، Pep T1, Pep T2

Item	Treatments							SEM	P-value
	T1	T2	T3	T4	T5	T6	T7		
LEAP2	1.00^{b}	0.67^{b}	2.04^{ab}	3.12^{a}	1.04^{b}	2.02^{ab}	0.99^{b}	0.42	0.001
Pept1	1.00^{bc}	0.37^{c}	1.58^{bc}	5.44^{a}	0.94^{bc}	2.49^{b}	0.73^{c}	0.47	0.001
Pept2	1.00^{c}	0.61^{c}	1.94^{c}	6.53^{a}	5.11^{ab}	4.43^{b}	1.23^{c}	0.61	0.001

T1: the control diet based on soybean meal; T2: canola meal previously hydrolyzed using commercial protease enzyme 5%; T3: canola meal previously hydrolyzed using commercial protease enzyme 10%; T4: canola meal fermented by *Lactobacillus reuteri* 5%; T5: canola meal fermented by *Lactobacillus reuteri* 10%; T6: canola meal 5% and T7: canola meal 10%.
The means within the same row with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of the means.

Table 7 Effects of supplementation of different treatments in diet on intestinal Morphology in broiler chickens

Item	Treatments							SEM	P-value
	T1	T2	T3	T4	T5	T6	T7		
Duodenum									
Villus height (µm)	1046.57^{a}	923.02^{bc}	956.72^{ab}	871.43^{bcd}	838.01^{cd}	814.04^{d}	1019.86^{a}	34.19	0.001
Villus thickness (µm)	108.03^{b}	106.60^{b}	77.21^{c}	141.23^{a}	94.066^{bc}	164.29^{a}	99.39^{bc}	9.28	0.001
Crypt depth (µm)	165.89^{bc}	230.90^{ab}	232.82^{ab}	216.48^{ab}	165.89^{c}	253.17^{a}	242.83^{a}	15.51	0.001
Crypt diamete	9.05	8.87	6.87	8.51	8.78	11.80	9.43	1.22	0.21
Villus height:crypt depth	5.65^{a}	4.14^{bc}	4.36^{b}	4.15^{bc}	5.13^{ab}	3.26^{c}	4.39^{b}	0.35	0.001
Jejunum									
Villus height (µm)	913.30^{b}	762.82^{c}	759.91^{c}	88.71^{c}	807.91^{bc}	1117.05^{a}	926.06^{b}	48.88	0.001
Villus thickness (µm)	115.21^{b}	124.07^{ab}	107.2^{b}	102.73^{b}	98.91^{b}	149.40^{a}	103.57^{b}	9.54	0.001
Crypt depth (µm)	183.76^{b}	185.75^{b}	187.34^{b}	122.21^{c}	164.76^{b}	256.88^{a}	187.35^{b}	11.75	0.001
Crypt diamete	8.25	7.76	8.15	7.57	8.05	7.84	8.72	1.49	0.10
Villus height:crypt depth	5.18^{ab}	4.14^{b}	4.13^{b}	5.80^{a}	5.01^{ab}	4.41^{b}	5.83^{a}	0.47	0.03
Ileum									
Villus height (µm)	616.00^{b}	474.55^{c}	537.73^{bc}	570.33^{bc}	1045.38^{a}	553.21^{bc}	473.42^{c}	36.00	0.001
Villus thickness (µm)	121.28^{ab}	110.81^{bc}	142.99^{a}	88.67^{c}	103.47^{bc}	119.79^{ab}	85.33^{c}	8.99	0.001
Crypt depth (µm)	196.07^{b}	214.99^{ab}	254.68^{a}	216.60^{ab}	213.54^{ab}	221.94^{ab}	116.64^{c}	14.37	0.001
Crypt diamete	8.53^{ab}	5.89^{c}	5.15^{c}	7.34^{abc}	9.23^{a}	5.72^{c}	6.24^{bc}	0.83	0.001
Villus height:crypt depth	3.19^{c}	2.22^{d}	2.34^{d}	2.69^{cd}	5.03^{a}	2.52^{d}	4.11^{b}	0.22	0.001

T1: the control diet based on soybean meal; T2: canola meal previously hydrolyzed using commercial protease enzyme 5%; T3: canola meal previously hydrolyzed using commercial protease enzyme 10%; T4: canola meal fermented by *Lactobacillus reuteri* 5%; T5: canola meal fermented by *Lactobacillus reuteri* 10%; T6: canola meal 5% and T7: canola meal 10%.
The means within the same row with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of the means.

SOD plays a crucial role in protecting cells from oxidative damage by catalyzing the dismutation of superoxide radicals into oxygen and hydrogen peroxide. The serum protein levels did not show significant differences across treatments (P>0.05). This suggests that the inclusion of canola meal, whether hydrolyzed or fermented, did not adversely affect the protein metabolism of the broilers. Protein levels in the serum are indicative of the overall nutritional status and health of the birds.

The levels of Gpa, another important antioxidant enzyme, were not significantly different across treatments (P>0.05). This indicates that the dietary treatments did not have a significant impact on the activity of Gpa in the serum. Gpa plays a vital role in protecting cells from oxidative damage by reducing hydrogen peroxide and organic hydroperoxides. The levels of MDA, a marker of lipid peroxidation, showed significant differences across treatments (P=0.01). The group fed 10% hydrolyzed canola meal (T3) exhibited the highest MDA levels, indicating increased lipid peroxidation and oxidative stress. Conversely, the group fed 10% fermented canola meal (T5) showed the lowest MDA levels, suggesting reduced oxidative stress and better antioxidant protection.

Oxidative stress, which results from severe damage to cells and metabolic processes, harms proteins, nucleic acids, and other biomacromolecules. This leads to the production of large amounts of malondialdehyde, which damages tissues and increases susceptibility to diseases (Elbaz *et al*. 2023). Previous studies have shown that hydrolyzed products, such as soybean meal (Xu *et al*. 2015) and camel milk (Soleymanzadeh *et al*. 2016), can reduce serum free radical levels.

Hydrolyzed protein sources have been shown to reduce lipid peroxidation in tissues and increase serum antioxidant capacity. This is attributed to the bioactive peptides generated during the hydrolysis process, which possess antioxidant properties (Akbarian *et al*. 2022; Nemati *et al*. 2024).

The improvement in antioxidant capacity when using hydrolyzed protein sources can vary depending on the specific bioactive peptides derived during hydrolysis. Different peptides have distinct antioxidant activities, which can influence the overall effectiveness (Aloo and Oh, 2022).

Therefore, it can be concluded that using hydrolyzed protein sources is likely to reduce lipid peroxidation in tissues, increase serum antioxidant capacity, and overall decrease oxidative damage (Hu *et al*. 2016). The improvement in antioxidant capacity in the body when using hydrolyzed protein sources may depend on the type of bioactive peptides derived during hydrolysis (Damgaard *et al*. 2015). In a study by Elbaz *et al*. (2023), it was reported that feeding broilers diets containing fermented canola meal supplemented with exogenous enzymes improved antioxidant status by significantly increasing superoxide dismutase levels and decreasing malondialdehyde. Hosseinpoor *et al*. (2023) reported that canola-derived peptides obtained from protease (alkalase or proteinase) hydrolysis increased activity.

During canola protein hydrolysis, the protein structure may undergo changes, exposing more reactive amino acid side chains. The findings of the present study demonstrated that adding 200 mg of canola peptides per kilogram of diet increased antioxidant activities. Bischoff (2019) showed that canola fermentation can enhance oxidative resistance and antioxidant capacity. These results confirm that cells were not under stress, as evidenced by the decreased levels of malondialdehyde. Studies have shown that amino acids such as tyrosine, methionine, and lysine can act as antioxidants.

These amino acids help reduce oxidative stress by neutralizing free radicals (Jové *et al*. 2020; Monteiro *et al*. 2022). The fermentation process can increase the levels of free amino acids, which in turn can contribute to the reduction of malondialdehyde levels due to their antioxidant properties (Torkova *et al*. 2015; Wunderle *et al*. 2024).

The low level of malondialdehyde could be attributed to the increased levels of free amino acids, such as tyrosine, methionine, and lysine, resulting from the fermentation process. These amino acids can act as antioxidants (Wang and Gonzalez De Mejia, 2005). The action of microbial glucosidases during fermentation can produce lipophilic aglycones, which are more effective free radical scavengers than their corresponding glycosides. This leads to increased oxidative activity (Michlmayr and Kneifel, 2014; Elbaz *et al*. 2023).

Malondialdehyde (MDA) is widely recognized as a significant marker of lipid peroxidation in biological systems. It is produced as a result of oxidative stress and the peroxidation of polyunsaturated fatty acids (Morales and Munné-Bosch, 2019). High levels of MDA are known to cause tissue damage and are associated with various diseases. This is due to the oxidative stress induced by free radicals (Abdel-Wareth *et al*. 2024; Urban *et al*. 2024). In broiler production, the use of high-energy diets leads to increased fat storage in the body. This elevated fat content results in higher production of free radicals, which in turn increases the levels of MDA (Desbruslais and Wealleans, 2022; Cordiano *et al*. 2023).

The results of this experiment demonstrated that, despite no changes in serum antioxidant activity in the chicks, malondialdehyde levels decreased, indicating a reduction in lipid peroxidation. In this regard, Liu *et al*. (2022) reported that using neutrase-hydrolyzed cottonseed meal, the produced peptides could effectively prevent the autoxidation of linoleic acid.

In this study, the inclusion of 5% fermented canola in the diet significantly upregulated the expression of the LEAP2, PepT1, and PepT2 genes. Additionally, raw canola at a 5% level also increased the expression of the LEAP2 gene (Table 6). The growth and development of organisms are directly linked to the absorption of various nutrients, including protein transporters involved in nutrient uptake. The peptide transporter PepT1 is responsible for the absorption of dipeptides and tripeptides in enterocytes and is regulated by dipeptides in mammals (Tokutake *et al*. 2021).

Peptide transporters, such as PepT1 and PepT2, are integral membrane proteins that mediate the cellular uptake of dipeptides, tripeptides, and peptide-like drugs. They belong to the proton-coupled oligopeptide transporters (POTs) family and play a crucial role in the absorption and conservation of nitrogen from dietary protein (Talevi and Bellera, 2021). Studies have shown that the mRNA abundance of PepT1 and PepT2 genes varies across different developmental stages and dietary compositions. This variation is influenced by factors such as amino acids, peptides, and growth factors (Abele and Tampé, 2018).

Dietary supplementation with peptides has been shown to increase the gene expression of peptide transporters in the intestine (Sheng *et al*. 2022). In broilers, intestinal PepT1 gene expression increases with diet protein quality and levels, as well as feed restriction (Gilbert *et al*. 2008; Osmanyan *et al*. 2018; Abdelli *et al*. 2021). Gilbert *et al*. (2008) stated that the composition and concentration of dietary peptides influence the activity and gene expression of PepT1, contributing to intestinal absorption dynamics. In the present study, the use of fermented and raw canola significantly increased the gene expression of peptide transporters compared to the control group.

In the present study, no positive effects of raw, fermented, or hydrolyzed canola on intestinal morphology were observed (Table 7). The intestinal villi are the primary site of nutrient absorption, and the epithelial cells of the villi are the functional cells of digestion and absorption, which are highly desirable for increased enzyme activity and nutrient uptake. Villus height, crypt depth, villus height-to-crypt depth ratio, and intestinal wall thickness are significant indicators for evaluating the absorptive capacity of the small intestine (Montagne *et al*. 2007). However, smaller crypts indicate a slower tissue turnover rate, resulting in a lower demand for new tissue regeneration (Leeson *et al*. 1987). Zhang *et al*. (2022) reported a decrease in villus height and the villus height-to-crypt depth ratio, along with an increase in crypt depth in the intestines of broilers fed diets containing 10% and 20% canola. Elbaz *et al*. (2023) demonstrated a significant increase in ileal villus height in broilers fed a fermented canola diet. However, broilers fed the fermented canola diet had a lower crypt depth in the ileum.

Elbaz *et al*. (2023) demonstrated that feeding broilers a fermented canola meal diet resulted in increased villus height. The increased intestinal weight could be explained by the increased villus height in the ileum of broilers fed fermented canola. Beneficial changes in intestinal morphology led to an increased surface area for absorption, resulting in improved nutrient absorption, which could explain the improved production performance. Chiang *et al*. (2010) reported that adding 10% canola had no adverse effects on intestinal morphology, except for a decrease in villus height of the small intestine in broilers at 21 days of age and an increase in the villus height-to-crypt depth ratio in broilers at 42 days of age. In another study, Chiang *et al*. (2010) reported that the use of fermented canola improved the intestinal morphology of broilers. The improvement in intestinal morphology may be largely due to the removal of toxins and the breakdown of large proteins into smaller peptides after fermentation.

The present study demonstrated that the inclusion of 5% fermented canola meal in broiler diets resulted in an increased population of Firmicutes, Bacteroidetes, and Actinobacteria (Table 8). A higher abundance of Firmicutes and Bacteroidetes is typically associated with improved gastrointestinal function and health, while Proteobacteria and Actinobacteria may indicate unhealthy or disease-associated conditions. Therefore, the increased population of Firmicutes could suggest improved nutrient absorption and gut health.

Conversely, Bacteroidetes contribute to the breakdown and digestion of fiber and the production of short-chain fatty acids, which are beneficial for gut health. However, a high abundance of Proteobacteria and Actinobacteria typically indicates microbial imbalance and unhealthy conditions in the gut tissue.

In this study, the population of gut bacteria, except for Actinobacteria, showed a significant increase, which positively impacts gut health. However, the population of Proteobacteria also increased in the same treatment, indicating signs of microbial imbalance, which is not beneficial for gut health (Table 8).

Elbaz *et al*. (2023), investigating the effects of fermented canola on the microbial population of the gastrointestinal tract of broilers, showed that the population of lactic acid bacteria in the crop and coliforms in the ileum of broilers fed diets containing fermented canola meal was significantly higher and lower, respectively. Additionally, the total population of anaerobic bacteria in the ceca of broilers fed fermented canola meal was lower compared to the control group.

Table 8 Effects of supplementation of different treatments in diet on caecal bacterial count (log10 CFU) in broiler chickens

Item	Treatments							SEM	P-value
	T1	T2	T3	T4	T5	T6	T7		
Firmicutes	1.00^{b}	0.75^{b}	0.88^{b}	1.87^{ab}	1.19^{b}	2.8^{a}	1.54^{b}	0.35	0.001
Bacteroidetes	1.00c	1.15^{c}	1.71^{c}	3.25^{ab}	1.03^{c}	4.21a	1.69^{ab}	0.55	0.004
Proteobacteria	1.00	0.56	0.93	1.15	1.02	1.05	0.88	0.58	0.11
Actinobacteria	1.00^{c}	0.55^{c}	0.99^{c}	2.35^{b}	1.07^{c}	3.99^{a}	1.95^{b}	0.25	0.001

T1: the control diet based on soybean meal; T2: canola meal previously hydrolyzed using commercial protease enzyme 5%; T3: canola meal previously hydrolyzed using commercial protease enzyme 10%; T4: canola meal fermented by *Lactobacillus reuteri* 5%; T5: canola meal fermented by *Lactobacillus reuteri* 10%; T6: canola meal 5% and T7: canola meal 10%.
The means within the same row with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of the means.

Chiang *et al*. (2010) reported that the population of Lactobacillus in the cecum of broilers fed a diet containing 10% fermented canola meal was significantly higher compared to broilers fed a control diet or a diet containing 10% raw canola meal. In Sun *et al*. (2013) experiment, replacing soybean meal with fermented cottonseed meal in the diet increased Lactobacillus and decreased coliforms in the cecum of broilers at 21 days of age. Engberg *et al*. (2009) investigated the effect of feeding fermented feeds on the gastrointestinal microbiota of laying hens. They reported that feeding fermented feed to laying hens increased lactic acid bacteria in the crop and decreased coliform populations in the ileum.

Microbial activities in the gastrointestinal tract significantly impact the performance and overall health of broilers (Niba *et al*. 2009). Fermented feed, due to its high concentration of lactic acid and the presence of lactic acid bacteria, can help balance the microbial flora of the host's gastrointestinal tract. These feeds, by acidifying the upper gastrointestinal tract, not only maintain its hygiene and sanitation but also provide the necessary environmental conditions for the establishment and growth of beneficial bacteria such as lactic acid bacteria. The beneficial microbial population formed by reducing the gastrointestinal pH (through the production of short-chain fatty acids, especially lactic and acetic acids) and the phenomenon of competitive exclusion forms a natural barrier against infections and pathogenic bacteria such as Salmonella and coliforms (Engberg *et al*. 2009).

CONCLUSION

The results of this study demonstrate that the inclusion of 5% fermented canola meal in broiler diets significantly improves several growth performance and health-related parameters. Specifically, feed intake, body weight, and feed conversion ratio in groups receiving 5% fermented canola meal were comparable to the control group, indicating that this dietary inclusion does not compromise growth performance. Additionally, fermented canola meal reduced abdominal fat in broilers, suggesting an improved carcass composition. This reduction in abdominal fat is likely due to the enhanced nutrient digestibility and metabolism resulting from the fermentation process. Furthermore, the increased expression of genes related to nutrient absorption and the higher populations of beneficial gut bacteria, such as Firmicutes and Bacteroidetes, suggest a positive impact of this processing method on gut health and nutrient absorption. However, the increased populations of Proteobacteria and Actinobacteria in some treatments may indicate a potential microbial imbalance, which could be detrimental to health. Therefore, while the use of fermented canola meal, especially at a level of 5%, can significantly enhance broiler growth performance, it is essential to monitor the gut microbiome balance to avoid potential negative effects.

Future studies could investigate the effects of different levels of fermented canola meal on gut microbiome balance and its long-term effects on overall broiler health. Additionally, exploring the molecular mechanisms associated with increased expression of nutrient absorption genes and their relationship with growth performance could improve feed formulation. Moreover, studies examining the effects of fermented canola meal on immune parameters and disease resistance could help optimize the utilization of this feed resource. These investigations would provide a more comprehensive understanding of the benefits and potential risks associated with the inclusion of fermented canola meal in broiler diets.

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