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Effects of substituting soybean meal with fermented rapeseed meal mixture on the growth performance, slaughter performance, meat quality, blood biochemical indices and intestinal barrier function in Langshan Chickens☆

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ABSTRACT

This study aimed to explore the effects of substituting soybean meal with a mixture of solid-state fermented rapeseed meal, apple pomace, and wheat bran on the growth performance, slaughter performance, meat quality, blood biochemical indices and intestinal barrier function of Langshan chickens. A total of 144 30-day-old Langshan chickens with similar body weights were randomly divided into three treatment groups, with six replicates per group and eight chickens per replicate: the control group (**CON**) was fed a corn-soybean meal basal diet, while the rapeseed meal mixture group (**RSM**) and the fermented rapeseed meal mixture group (**FRSM**) were fed diets substituting 5 % of soybean meal with rapeseed meal mixture and fermented rapeseed meal mixture, respectively. The trial lasted from 30 to 58 days of age. The results showed that compared to the CON group, the RSM group exhibited no significant changes in average daily feed intake (ADFI), average daily gain (ADG) and feed to gain ratio (F/G) ($P > 0.05$); the dressing percentage, half-eviscerated yield and eviscerated yield decreased ($P < 0.05$); the pH_{24h} and yellowness of breast muscle increased ($P < 0.05$); the crypt depth of the jejunum decreased, and the villus height/crypt depth ratio increased ($P < 0.05$); the serum D-lactic acid content decreased ($P < 0.05$). Compared to the CON group, the FRSM group exhibited no significant changes in ADFI, ADG and F/G ($P > 0.05$); the eviscerated yield increased ($P < 0.05$); the serum glucose and uric acid levels decreased ($P < 0.05$); the crypt depth of the jejunum decreased, and the villus height/crypt depth ratio increased ($P < 0.05$); the serum D-lactic acid content decreased ($P < 0.05$). Furthermore, compared to the RSM group, the FRSM group exhibited no significant changes in ADFI, ADG and F/G ($P > 0.05$); the dressing percentage, half-eviscerated yield and eviscerated yield increased ($P < 0.05$); the pH_{24h} of breast muscle decreased; the serum glucose and uric acid levels decreased ($P < 0.05$).In conclusion, RSM reduced the slaughter performance of Langshan chickens, while FRSM improved their slaughter performance. Both RSM and FRSM improved the jejunal morphology and intestinal permeability in Langshan chickens. In conclusion, fermentation improved the feed value of the rapeseed meal mixture; replacing part of the soybean meal diet with fermented rapeseed meal mixture helped improve the slaughter performance and intestinal barrier of Langshan chickens.

Introduction

Chicken meat, especially from high-quality local breeds, is highly favored by Chinese consumers. With the expansion of intensive farming in recent years, chicken meat production has significantly increased (Gilbert et al., 2015). Langshan Chicken is a meat and egg dual-purpose local breed unique to Jiangsu Province, China, known as one of the three high-quality local livestock breeds in the province, along with Taihu geese and Gaoyou ducks. China imports a large amount of soybeans annually to meet the livestock industry's demand for soybean meal. Developing suitable alternative ingredients is crucial to address the current shortage of protein feed materials(Supriyati et al., 2014). Rapeseed and apples are abundantly produced in China, with apple pomace, a by-product of apple juice production, consisting of apple peels, cores, and seeds. Apple pomace is not only rich in dietary fiber and minerals but also vitamins and organic acids, particularly high in

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polyphenols(Bhushan et al., 2008; Lyu et al., 2020). The by-product of rapeseed oil extraction, rapeseed meal, is a rich plant protein source (Yusuf et al., 2022). However, rapeseed meal contains various anti-nutritional factors, such as glucosinolates, oxazolidinethiones, and phytates, which can affect animal health and growth to some extent, such as glucosinolates decomposing under high temperatures to produce nitriles or isothiocyanates, leading to thyroid enlargement and other issues(Ishikawa et al., 2014; Jiang et al., 2015; Kasprowicz-Potocka et al., 2024). Phytates can reduce metal ion availability by chelating them and can also bind with proteins, lowering their digestibility and thus affecting animal growth(Ravindran et al., 2000; Zhou and Erdman, 1995).

To reduce the impact of anti-nutritional factors in rapeseed meal, appropriate processing techniques can be used to decrease their content, or specific enzyme preparations can be added to animal feed to help decompose these anti-nutritional factors(Mansour et al., 1993). For instance, phytase can degrade phytic acid present in rapeseed meal, thereby mitigating the inhibitory effect of phytic acid on the absorption of minerals(Samtiya et al., 2020).Solid-state fermentation is widely applied due to its simple equipment, low cost, and environmental friendliness. Vlassa et al. (2022) reported that solid-state fermentation could reduce the content of isothiocyanates by 55.2 % and glucosinolates by 51.6 % in rapeseed meal, and increase crude protein content by 10 %. Zhu et al. (2023) reported that fermented rapeseed meal significantly reduced glucosinolate and phytate content. Although there has been much research on solid-state fermented rapeseed meal, the effects of a mixed solid-state fermented rapeseed meal with apple pomace and bran are not yet clear.

Fermented rapeseed meal has been widely applied in poultry feed. Compared to rapeseed meal, fermented rapeseed meal fed to broilers can improve average daily gain and reduced feed to gain ratio(Chiang et al., 2009). Xu et al. (2012) found that fermented rapeseed meal fed to broilers significantly increased duodenal and jejunal villus height and the jejunal villus to crypt ratio. However, studies on the impact of fermented rapeseed meal on Langshan chickens are scarce. The production application effect of fermented rapeseed meal mixture substituting soybean meal on Langshan chickens is not yet clear. Therefore, this study aimed to explore the effects of fermented rapeseed meal mixture substituting soybean meal on the growth performance, slaughter performance, meat quality, blood biochemical indices, and intestinal barrier function of the Langshan chicken.

Materials and methods

All experimental protocols were in compliance with the animal care and use guidelines approved by the Institutional Animal Care and Use Committee of Nanjing Agricultural University.

Preparation of FRSM

Rapeseed meal and bran were purchased from Nanjing Tegeili Planting Professional Cooperative (Nanjing, China), and dry apple pomace was purchased from Qianhong Medicinal Herbs Purchase Storefront (Yuncheng, China). *Lactobacillus acidophilus* powder was provided by Keweibo (Nanjing, China) Biological Technology Co., Ltd. (Nanjing, China), with a viable count $\geq 2.0\times10^{10}$ CFU/g. Rapeseed meal, bran, and dry apple pomace were mixed in a ratio of 60 %, 20 %, and 20 % (dry matter basis), respectively. The mixture was combined with water at a ratio of 1:0.8 and mixed thoroughly in a sealed plastic container. *Lactobacillus acidophilus* was used for fermentation, with an inoculation rate of 0.5 % (based on the weight of the rapeseed meal mixture) and mixed evenly. The rapeseed meal mixture was placed under anaerobic conditions in a sealed plastic container and subjected to solid-state fermentation at 30°C for 24 hours. After fermentation, the plastic container was cut open, and the contents were transferred to a tray. The mixture was then dried at low temperature (45°C) in an electric drying oven, ground, and set aside for analysis.

Chemical analysis of RSM and FRSM

All analyses were performed in duplicate, and the average of the results from three experiments was used. The crude protein content of RSM and FRSM samples was determined using an automatic protein analyzer. The content of glucosinolates in RSM and FRSM samples was measured by determining the absorbance at 540 nm using a 0.1 % sodium carboxymethyl cellulose solution and palladium chloride color reagent. Phytic acid in RSM and FRSM samples was measured using the sulfosalicylic acid-ferric chloride method, with absorbance measured at 500 nm. The amino acid composition of RSM and FRSM samples was determined using an automatic amino acid analyzer after hydrolysis with 6 mol/L HCl at 110 °C for 24 h, followed by filtration with 0.02 mol/L HCl. The changes in chemical composition before and after fermentation are shown in Table 1.

Experimental design and diets

A total of 144 male Langshan chickens at 30 days of age, with similar initial body weights, were randomly divided into three groups, each with six replicates, with eight chickens per replicate. The trial period was four weeks (30-58 days of age). The three groups were: control group (**CON**, fed the basal diet), rapeseed meal mixture substitute group (**RSM**, fed RSM diet), and fermented rapeseed meal mixture substitute group (**FRSM**, fed FRSM diet), RSM diet and FRSM diet consisted of 5 % rapeseed meal mixture substituting soybean meal and 5 % fermented rapeseed meal mixture substituting soybean meal, respectively. The diets were designed in accordance with the nutritional requirements standards for slow-growing yellow-feathered broilers. Diet formulations and nutritional components for each group are shown in Table 2. Chickens had ad libitum access to feed and water during the rearing period.

Growth trial, slaughter, and sample collection

During the feeding trial, chickens were weighed weekly by replicate, feed intake was recorded, and average daily gain (**ADG**), average daily

Table 1
Nutritional value and amino acid profile of RSM before and after solid-state fermentation.

Nutrient	RSM	FRSM	P-value
Crude protein (%)	25.15±0.35	26.15±0.33*	< 0.001
Glucosinolates (μmol/g)	23.44±1.09	18.72±0.11*	0.029
Phytates (%)	5.11±0.30	3.77±0.12*	0.002
Asp (%)	1.81±0.005	1.84±0.005*	0.001
Thr (%)	1.10±0.006	1.11±0.004*	0.025
Ser (%)	1.12±0.008	1.12±0.007	0.896
Glu (%)	4.44±0.015	4.50±0.019*	0.013
Gly (%)	1.29±0.006	1.33±0.001*	0.001
Ala (%)	1.13±0.004	1.16±0.002*	< 0.001
Val (%)	1.24±0.002	1.30±0.002*	< 0.001
Ile (%)	0.93±0.002	0.98±0.002*	< 0.001
Leu (%)	1.75±0.003	1.79±0.005*	< 0.001
Tyr (%)	0.64±0.003	0.63±0.003*	0.005
Phe (%)	1.02±0.012	1.02±0.001	0.524
Lys (%)	1.38±0.002	1.44±0.014*	< 0.001
His (%)	0.65±0.001	0.66±0.001*	< 0.001
Arg (%)	1.43±0.001	1.46±0.003*	< 0.001
Pro (%)	1.49±0.002	1.51±0.003*	0.001
Met (%)	0.41±0.010	0.46±0.012*	0.004
Cys (%)	0.61±0.012	0.67±0.017*	0.009
Trp (%)	0.30±0.006	0.33±0.006*	0.008

Note: RSM, rapeseed meal mixture; FRSM, fermented rapeseed meal mixture. *Means differ significantly ($P < 0.05$), results are represented as the mean value ± SD (n = 3).

Table 2
Experimental diet formula and nutrient level.

Items	CON diet	RSM diet	FRSM diet
Ingredients (%)			
Corn	70.04	68.06	68.20
Soybean meal	25.20	20.20	20.20
Corn gluten meal	0.10	2.10	1.94
Soybean oil	0.73	0.80	0.80
RSM	0.00	5.00	0.00
FRSM	0.00	0.00	5.00
Dicalcium phosphate	2.01	1.85	1.90
Limestone meal	1.00	1.05	1.00
L-Lysine hydrochloride	0.19	0.24	0.24
DL-Methionine	0.10	0.07	0.08
L-Threonine	0.00	0.00	0.01
Salt	0.30	0.30	0.30
50 % Choline chloride	0.10	0.10	0.10
Premix[1]	0.23	0.23	0.23
Calculated Nutrient levels (%)			
Metabolizable energy (MJ/kg)	12.29	12.22	12.22
Crude protein	17.52	17.53	17.51
Lysine	0.94	0.94	0.94
Methionine	0.39	0.38	0.39
Threonine	0.64	0.64	0.65
Tryptophan	0.19	0.19	0.19
Calcium	0.91	0.91	0.91
Non-phytate phosphorus	0.41	0.40	0.41

Note:[1]Vitamin premix provided per kilogram of diet: vitamin A, 12000 IU; vitamin D3, 2500 IU; vitamin E, 30 IU; menadione, 1.3 mg; thiamine, 2.2 mg; riboflavin, 6 mg; nicotinamide, 50 mg; D-pantothenic acid, 12 mg; pyidoxine·HCl, 4 mg; biotin, 0.04 mg; folic acid, 1.25 mg; vitamin B12, 0.02 mg.; Mineral premix provided per kilogram of diet: iron, 80 mg; copper, 10 mg; manganese, 80 mg; zinc, 75 mg; iodine, 0.35 mg; selenium, 0.15 mg. RSM, rapeseed meal mixture; FRSM, fermented rapeseed meal mixture.

feed intake (**ADFI**), and feed to gain ratio (**F/G**) were calculated. During the first week of the Langshan chicken rearing period, the temperature was maintained at 33-35°C with a humidity of 65-75 %. The temperature was reduced by 3°C per week, and the humidity was reduced by 5 % per week. After day 28 of the rearing period, the temperature was lowered to room temperature (no lower than 18°C in winter), and the humidity was reduced to 45-55 %.Two chickens from each replicate weighing close to the average weight of the replicate were selected at 58 days of age. An insertion of a blood collection needle into a vacuum tube was used to collect blood from the wing vein of the chickens, which was centrifuged at 3000 rpm for 10 minutes and the supernatant was used for further analysis. The selected chickens from each replicate were stunned with carbon dioxide and bled to death by cutting the jugular vein. The abdominal cavity was quickly opened, and organs were separated and feathers, abdominal fat, keratin, sharp beaks, and blood were removed. All carcasses were weighed to calculate the dressing percentage, half-eviscerated yield and eviscerated yield, breast muscle yield, and thigh muscle yield. The left breast muscle was completely skinned and kept stored in temperature at 4°C for meat quality determination. Jejunum was completely separated and weighed, its length was measured, and a 2 cm section of the mid-section of the jejunum was collected, flushed with 0.75 % of saline, and then immersed in 4 % paraformaldehyde for fixation and used for morphometric analyses. Subsequently, the mucosa of the jejunum was scraped using a glass slice that was clean, placed in a nitrogen solution, and kept at -80°C for assaying.

Meat quality

The pH value of breast muscle was measured using a pH meter (FiveGo F2 Mettler Toledo Instruments, China) at 45 min and 24 h after slaughter, three times for each sample. Brightness (L*), redness (a*) and yellowness (b*) of breast muscles at 24 h post-slaughter were measured using a CR410 colorimeter (Konica Minolta Sensing, Japan) according to Zhang et al. (2017). After 24 h post-slaughter, drip loss and cooking loss were measured according to the method of Zhang et al. (2014).

Blood biochemical analysis

A 7020 automatic biochemistry analyzer (Hitachi High-Tech Crop, Japan) was used to determine the serum levels of total protein (**TP**), albumin (**ALB**), glucose (**GLU**), uric acid (**UA**),as well as the activities of alanine transaminase (**ALT**) and aspartate transaminase (**AST**).

Jejunal tissue morphology examination

The prepared jejunal tissue was fixed in 4 % polyformaldehyde, dehydrated and dried, embedded in paraffin blocks, and sectioned to prepare approximately 6 μM jejunal cross-sections. Hematoxylin and eosin staining was performed, and the slides were sealed. A virtual microscope was used at 20x magnification to select complete and representative areas on the slide for photography. Six villus heights (**VH**) and crypt depths (**CD**) were measured, and statistical data were used to calculate villus height/crypt depth ratio (**VH/CD**). Jejunal Index Calculation Formula: Relative jejunal length (cm/kg) = jejunal length (cm) / corresponding Langshan chicken body weight (kg); Relative jejunal weight (g/kg) = jejunal weight (g) / corresponding Langshan chicken body weight (kg); Unit length weight of the jejunum (g/cm) = jejunal weight (g) / jejunal length (cm).

Diamine oxidase and lactate concentration measurement

A 7020 automatic biochemistry analyzer (Hitachi High-Tech Crop, Japan) was used to measure the serum diamine oxidase (**DAO**) activity. A commercial ELISA kit was used to measure serum D-lactic acid (**D-Lac**) concentration, purchased from Beijing Solai Bao Science & Technology Co., Ltd. (Beijing, China).

Total RNA extraction and real-time PCR

The total RNA of jejunal mucosa was extracted using Trizol reagent (Takara Bio Technology Co., Ltd., China), and the A206/A280 ratio of the RNA samples was measured using a Nanodrop ND-1000 Spectrophotometer (Thermo Scientific DE), with a ratio between 1.8 and 2.0 being considered appropriate. Total RNA was reverse transcribed to generate cDNA using a Prime-Script RT Master Mix kit (Takara Biotechnology, Inc.) RT-qPCR was performed on a Quan Studio® PCR instrument using a SYBR EX Taq RR420A kit (Applied Biosystems, CA). The system comprised 20 μL of the following components: upstream primer (10 μM) 0.4 μL, downstream primer (10 μM) 0.4 μL, cDNA 1 μL, sterile double-distilled water 8.2 μL, and SYBR Premix Ex Taq II (2×) 10 μL. Reaction conditions were as follows: one cycle of 30 s at 95°C, followed by 40 cycles of 10 s at 95°C, 30 s at 60°C, and 15 s at 95°C. *β-actin* was used as an internal reference gene and mRNA expression levels were calculated using the $2^{-\Delta\Delta Ct}$ method (Livak and Schmittgen, 2001). The target genes and primer sequences are listed in Table 3.

Table 3
Primer sequences for RT-qPCR.

Genes[1]	Accession number	Primer sequence	Product size, bp
β-actin	NM_205518.1	F:ATCCGGACCCTCCATTGTC R:AGCCATGCCAATCTCGTCTT	120
ZO-1	XM_015278981.2	F:CTTCAGGTGTTTCTCTTCCTCCTC R:CTGTGGTTTCATGGCTGGATC	131
occludin	XM_025144248.1	F:TCATCGCCTCCATCGTCTAC R:TCTTACTGCGCGTCTTCTGG	240
claudin 1	NM_001013611.2	F: GACCAGGTGAAGAAGATGCGGATG R:CGAGCCACTCTGTTGCCATACC	107

Note: [1]ZO-1, zonula occludens-1.

Statistical analysis

Experimental data were analyzed using SPSS software (Version 20.0 for Windows, SPSS Inc., IL) using one-way analysis of variance (ANOVA), with multiple comparisons conducted using Duncan's method. Results are presented as mean values and standard deviation (SD), with $P < 0.05$ as the criterion for significance.

Results

Growth performance

As shown in Table 4, Compared to the CON group, both the RSM and FRSM groups showed a decrease in ADFI and ADG, along with an increase in the F/G, but none of these changes were statistically significant ($P > 0.05$). Compared to the RSM group, the FRSM group exhibited a reduction in ADFI and F/G, and an increase in ADG, but these differences also did not reach statistical significance ($P > 0.05$).

Carcass traits

As shown in Table 5, compared to the CON group, the RSM group significantly decreased dressing percentage, half-eviscerated yield, and eviscerated yield ($P < 0.05$), while the FRSM group had a significantly increased eviscerated yield ($P < 0.05$); compared to the RSM group, the FRSM group significantly increased dressing percentage, half-eviscerated yield, and eviscerated yield ($P < 0.05$).

Meat quality

As shown in Table 6, compared to the CON group, the RSM group significantly increased breast muscle pH_{24h} and yellowness ($P < 0.05$), while the FRSM group showed no significant changes in indices ($P > 0.05$); compared to the RSM group, the FRSM group had significantly decreased breast muscle pH_{24h} ($P < 0.05$).

Blood biochemical indices

As shown in Table 7, there were no significant differences between treatment groups in TP, ALB, ALT, and AST levels ($P > 0.05$); the FRSM group had significantly lower serum UA and GLU levels compared to the CON and RSM groups ($P < 0.05$).

Jejunal index

As shown in Table 8, there were no significant differences in the relative length, relative weight, and Relative length/relative weight of the jejunum among the treatment groups ($P > 0.05$).

Jejunal morphology

As shown in Table 9, compared to the CON group, the RSM and FRSM groups significantly increased the VH/CD and significantly decreased

Table 4
Effect of fermented rapeseed meal mixture on the growth performance of Langshan chicken.

Items	Diets treatment[1]			P-value
	CON	RSM	FRSM	
BW at d 30 (g/bird)	309.75 ± 8.02	313.21 ± 9.89	312.00 ± 6.18	0.763
BW at d 58 (g/bird)	898.02 ± 32.99	878.86 ± 22.14	883.63 ± 55.40	0.681
ADFI (g/bird/day)	61.82 ± 1.20	61.73 ± 1.19	60.28 ± 1.31	0.585
ADG (g/bird/day)	21.01 ± 0.97	20.20 ± 0.80	20.42 ± 2.02	0.084
F/G (g/g)	2.95 ± 0.10	3.06 ± 0.01	2.96 ± 0.25	0.419

Note: [1]CON, control group; RSM, fed a diet with 5 % rapeseed meal replacing soybean meal mixture; FRSM, fed a diet with 5 % fermented rapeseed meal replacing soybean meal mixture; BW, body weight; ADFI, average daily feed intake; ADG, average daily gain; F/G, feed to gain ratio. (n=6). The results are represented as the mean value ± SD (n = 6).

Table 5
Effect of fermented rapeseed meal mixture on the slaughter performance of Langshan chicken.

Items	Diets treatment[1]			P-value
	CON	RSM	FRSM	
Dressing percentage (%)	88.47 ± 3.04[a]	85.6 ± 1.83[b]	89.05 ± 1.33[a]	0.034
Half-eviscerated yield (%)	82.32 ± 2.90[a]	79.39 ± 1.82[b]	82.88 ± 1.20[a]	0.024
Eviscerated yield (%)	77.38 ± 0.53[b]	75.27 ± 1.01[c]	78.95 ± 0.88[a]	< 0.001
Breast muscle yield (%)	12.03 ± 1.15	11.87 ± 1.01	11.62 ± 0.65	0.767
Thigh muscle yield (%)	15.36 ± 1.25	15.79 ± 0.51	15.10 ± 1.37	0.569

Note: [a, b, c] Means without common superscript letters differ significantly($P < 0.05$); [1] CON, control group; RSM, fed a diet with 5 % rapeseed meal mixture replacing soybean meal; FRSM, fed a diet with 5 % fermented rapeseed meal mixture replacing soybean meal.The results are represented as the mean value ± SD (n = 6).

Table 6
Effect of fermented rapeseed meal mixture on the breast muscle meat quality of Langshan chicken.

Items	Diets treatment[1]			P-value
	CON	RSM	FRSM	
pH_{45min}	6.50 ± 0.19	6.51 ± 0.18	6.43 ± 0.24	0.757
pH_{24h}	5.59 ± 0.07[b]	5.74 ± 0.04[a]	5.58 ± 0.07[b]	< 0.001
L* (Lightness)	51.42 ± 4.45	50.36 ± 1.60	51.99 ± 1.10	0.603
a* (redness)	2.37 ± 0.53	3.13 ± 1.23	2.32 ± 0.67	0.226
b* (yellowness)	5.66 ± 1.34[b]	7.40 ± 1.35[a]	6.36 ± 0.52[ab]	0.054
Drip loss (%)	2.59 ± 0.15	2.49 ± 0.71	2.13 ± 0.29	0.205
Cooking loss (%)	11.26 ± 2.78	12.58 ± 1.79	13.65 ± 1.25	0.162

Note: [a, b,] Means without common superscript letters differ significantly($P < 0.05$); [1] CON, control group; RSM, fed a diet with 5 % rapeseed meal mixture replacing soybean meal; FRSM, fed a diet with 5 % fermented rapeseed meal mixture replacing soybean meal.The results are represented as the mean value ± SD (n = 6).

Table 7
Effect of fermented rapeseed mixture meal on the blood biochemical indexes of Langshan chicken.

Items	Diets treatment[1]			P-value
	CON	RSM	FRSM	
TP (g/L)	43.95 ± 3.53	47.64 ± 2.39	46.77 ± 3.58	0.149
ALB (g/L)	14.56 ± 0.70	14.95 ± 0.90	14.10 ± 0.76	0.213
UA (μmol/L)	240.00 ± 49.76[a]	203.33 ± 50.17[a]	119.67 ± 40.36[b]	0.002
GLU (mmol/L)	13.21 ± 0.43[a]	13.01 ± 0.32[a]	12.45 ± 0.40[b]	0.010
AST (U/L)	259.55 ± 8.52	269.82 ± 8.71	262.64 ± 18.50	0.385
ALT (U/L)	11.82 ± 1.91	10.91 ± 1.94	11.91 ± 3.35	0.749

Note: [a, b,] Means without common superscript letters differ significantly($P < 0.05$); [1] CON, control group; RSM, fed a diet with 5 % rapeseed meal mixture replacing soybean meal; FRSM, fed a diet with 5 % fermented rapeseed meal mixture replacing soybean meal; TP, total protein; ALB, albumin; UA, uric acid; GLU, glucose; AST, aspartate transaminase; ALT, alanine transaminase.The results are represented as the mean value ± SD (n = 6).

Table 8
Effect of fermented rapeseed meal mixture on the jejunal index of Langshan chicken.

Items	Diets treatment[1]			P-value
	CON	RSM	FRSM	
Relative length (cm/kg)	59.94 ± 5.07	61.93 ± 2.47	63.95 ± 6.34	0389
Relative weight (g/kg)	15.48 ± 1.89	15.51 ± 1.31	15.54 ± 2.53	0.999
Relative length/relative weight (g/cm)	0.26 ± 0.02	0.26 ± 0.03	0.25 ± 0.06	0.880

Note: [1] CON, control group; RSM, fed a diet with 5 % rapeseed meal mixture replacing soybean meal; FRSM, fed a diet with 5 % fermented rapeseed meal mixture replacing soybean meal.The results are represented as the mean value ± SD (n = 6).

Table 9
Effect of fermented rapeseed meal mixture on the jejunal morphology of Langshan chicken.

Items	Diets treatment[1]			P-value
	CON	RSM	FRSM	
VH (μm)	1196.72 ± 70.11	1259.84 ± 149.68	1323.95 ± 60.51	0.129
CD (μm)	296.73 ± 58.60[a]	210.30 ± 57.23[b]	203.92 ± 42.60[b]	0.015
VH/CD	4.18 ± 0.93[b]	6.30 ± 1.43[a]	6.69 ± 1.22[a]	0.006

Note: [a, b,] Means without common superscript letters differ significantly($P < 0.05$); [1] CON, control group; RSM, fed a diet with 5 % rapeseed meal mixture replacing soybean meal; FRSM, fed a diet with 5 % fermented rapeseed meal mixture replacing soybean meal; VH, villus heights; CD, crypt depths; VH/CD, villus height/crypt depth ratio. The results are represented as the mean value ± SD (n = 6).

CD ($P < 0.05$). There were no significant differences in these indicators between the RSM and FRSM groups ($P > 0.05$).

Serum D-lactate concentration and diamine oxidase activity

As shown in Fig. 1(A), there were no significant differences in serum DAO activity among the treatment groups ($P > 0.05$). As shown in Fig. 1 (B), compared to the CON group, the RSM and FRSM groups significantly reduced serum D-Lac content ($P < 0.05$). There were no significant differences in D-Lac content between the RSM and FRSM groups ($P > 0.05$).

Jejunal tight junction-related protein mRNA expression levels

As shown in Fig. 2, there were no significant changes in the expression levels of *ZO-1, Occludin*, and *Claudin 1* in the jejunum among the treatment groups ($P > 0.05$).

Discussion

The presence of glucosinolates and oxazolidinethiones as anti-nutritional factors in rapeseed meal is one of the main reasons affecting its application in poultry production. These substances with anti-nutritional properties can reduce animal feed intake and disrupt the effective absorption and utilization of nutrients by animals, interfering through mechanisms such as damaging the thyroid (Bell, 1984; Tripathi and Mishra, 2007). The levels of glucosinolates and phytates in rapeseed meal mixtures decrease after fermentation, and the crude protein and amino acid content increase, proving that the feeding value of fermented rapeseed meal mixtures is higher than that of rapeseed meal mixtures. Chiang et al. (2009) demonstrated that feeding broilers with fermented canola meal had no significant impact on growth performance. The results of the study showed that fermented rapeseed meal mixture had no significant effect on growth performance of Langshan chickens, in line with the findings of Chiang et al. (2009).

Slaughter performance is an important reference indicator for the level of broiler production performance, and higher slaughter performance is often associated with higher economic benefits (Li et al., 2024). The results of this experiment showed that at a 5 % substitution ratio, the fermented rapeseed meal mixture improved Langshan chicken slaughter performance, significantly increasing the eviscerated yield, while the rapeseed meal mixture reduced Langshan chicken slaughter performance. Fermentation significantly reduces the content of anti-nutritional substances in rapeseed meal, such as phytates and glucosinolates, and also breaks down proteins into smaller, more digestible molecular forms (Hu et al., 2016). Smaller molecular forms may promote the absorption and utilization of nutrients by Langshan chickens, thereby improving their slaughter performance.

Meat color, muscle texture, tenderness, and juiciness are factors that directly influence consumer purchasing decisions and eating experience. Improving meat quality is key to the sustainable development of the broiler industry and to meeting market demands. Ashayerizadeh et al. (2018) demonstrated that, compared to the control group, broilers fed with 50 % fermented rapeseed meal showed an increase in breast muscle lightness and yellowness values, and a decrease in redness value, though

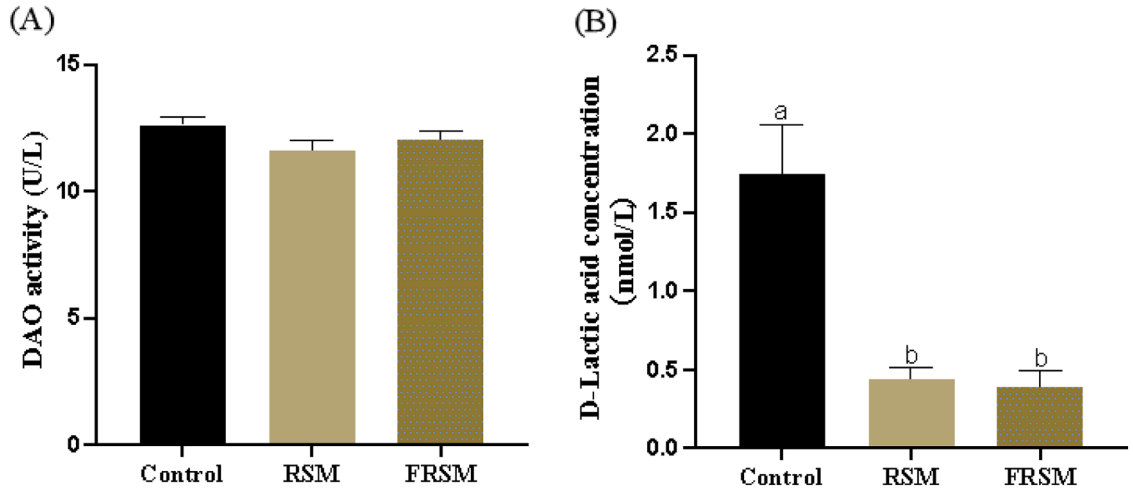

Fig. 1. Effects of fermented rapeseed meal mixture on the serum activity of (A) DAO and levels of (B) D-Lac in Langshan chicken. [a, b,] Means without common superscript letters significantly differ ($P < 0.05$); RSM, fed a diet with 5 % rapeseed meal mixture replacing soybean meal; FRSM, fed a diet with 5 % fermented rapeseed meal mixture replacing soybean meal.The results are represented as the mean value ± SD (n = 6).

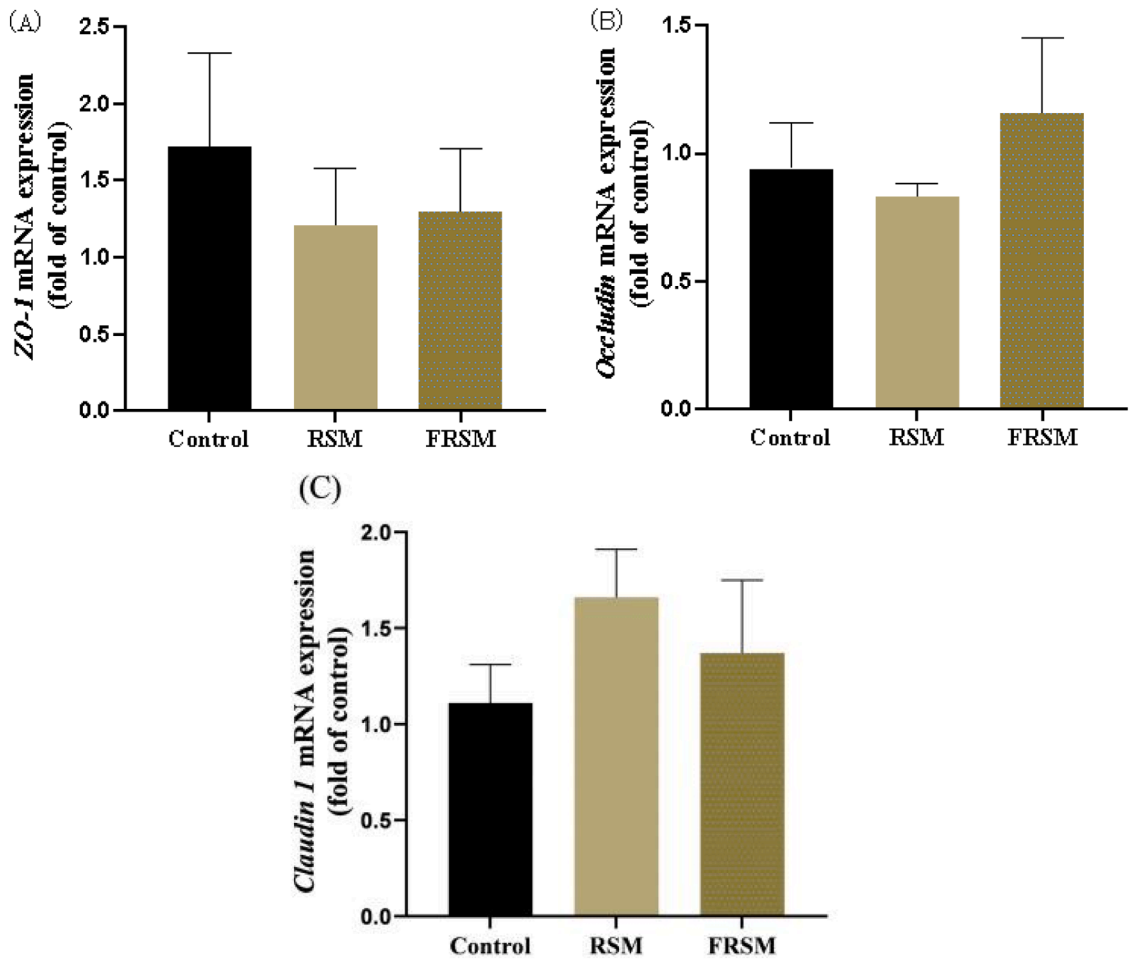

Fig. 2. Effects of fermented rapeseed meal mixture on the mRNA expression of (A) *ZO-1*, (B) *Occludin*, and (C) *Claudin-1* in the jejunum of Langshan chicken. RSM, fed a diet with 5 % rapeseed meal mixture replacing soybean meal; FRSM, fed a diet with 5 % fermented rapeseed meal mixture replacing soybean meal.The results are represented as the mean value ± SD (n = 6).

these differences were not statistically significant, indicating that feeding fermented rapeseed meal does not significantly affect the breast muscle quality of broilers. In the present study, compared to the CON group, the FRSM group exhibited an increase in breast muscle lightness and yellowness values, and a decrease in redness value, though none of these differences reached statistical significance. This is consistent with the findings of Ashayerizadeh et al., (2018).

GLU is one of the main energy sources for broilers, and blood GLU levels reflect the availability of energy and metabolic levels in animals (JøRgensen et al., 1996). Appropriate blood GLU levels are necessary to maintain growth, production, and normal physiological functions(Braun and Sweazea, 2008). This experiment found that compared to the control and rapeseed meal mixture groups, the fermented rapeseed meal mixture group had a significant decrease in serum GLU levels in broilers. Wu et al. (2022) showed that serum GLU levels were lower in the fermented rapeseed meal group than in the rapeseed meal group at the same level of substitution, which is in agreement with the findings of the experiment. However, Hu et al. (2016) showed that fermented rapeseed meal significantly increased serum GLU levels in broilers, possibly because different fermentation conditions affect the conversion efficiency and final composition of nutrients in rapeseed meal, including the content and type of digestible carbohydrates, which may directly affect the concentration of GLU in broiler serum. The serum UA content in poultry is an important indicator for assessing protein metabolism status. UA is the final product of nitrogen metabolism, and the metabolism of amino acids produces UA as waste excreted from the body, reflecting the utilization rate and efficiency of amino acid metabolism (Donsbough et al., 2010; Simoyi et al., 2002). This experiment found that the fermented rapeseed meal mixture significantly reduced serum UA levels in Langshan chickens. Fermentation can improve the quality and digestibility of protein in rapeseed meal by degrading anti-nutritional factors, increasing amino acid availability, and higher amino acid utilization rates mean fewer amino acids are converted into UA through metabolic pathways, leading to a decrease in serum UA levels.

The intestine plays a crucial role in the animal body, not only is responsible for the digestion and absorption of nutrients but also is essential for maintaining overall physiological function (Montagne et al., 2003). The impact of fermented rapeseed meal on broiler intestinal morphology has been reported in many studies. Studies have shown that feeding diets with fermented rapeseed meal and bran mixture improved the jejunal and ileal VH, CD, and VH/CD in broilers aged 24-42 days (Chiang et al., 2009). Fazhi et al. (2011) fed broilers with 5 % fermented rapeseed meal diet, and broiler jejunal VH and VH/CD increased, while CD decreased. The experimental results showed that rapeseed meal mixture and fermented rapeseed meal mixture had no significant effect on jejunal index of Langshan chickens. Compared to the control group, the fermented rapeseed meal mixture significantly improved the jejunal mucosal morphology of Langshan chickens, although the rapeseed meal mixture also improved jejunal mucosal morphology, the effect was not as good as that of the fermented rapeseed meal mixture, which is consistent with previous research. The activity of microorganisms during the fermentation process produces a series of beneficial substances, such as short-chain fatty acids, which can promote intestinal health, increase the growth rate of villi, and may optimize the

renewal and repair processes of intestinal cells directly or indirectly, leading to a decrease in CD (Cook and Sellin, 1998; Venegas et al., 2019).

Serum DAO activity and D-Lac levels are used to assess intestinal permeability, and changes in their levels in the serum can reflect intestinal health status, especially the degree of damage to the intestinal mucosa and the integrity of intestinal barrier function (Cai et al., 2024). Serum DAO activity and D-Lac levels increase, indicating that the intestinal mucosa may be damaged and intestinal barrier function affected (Li et al., 2002; Song et al., 2009). The results of this experiment showed that both rapeseed meal mixture and fermented rapeseed meal mixture had no significant impact on serum DAO activity in Langshan chickens, but significantly reduced serum D-Lac levels. Beneficial bacteria in fermented rapeseed meal can inhibit the number of intestinal pathogens, reducing intestinal inflammation and damage caused by pathogens, thereby reducing serum D-Lac levels, as D-Lac mainly enters the blood under conditions of impaired intestinal barrier and excessive bacterial fermentation (Qiu et al., 2024). Beneficial bacteria in fermented rapeseed meal may indirectly promote intestinal health and microbial balance, indirectly promoting the health of the intestinal mucosa, but this improvement may not be sufficient to cause significant changes in serum DAO activity. This indicates that feeding fermented rapeseed meal mixture did not cause damage to the intestinal mucosa of Langshan chickens, could maintain the integrity of the intestinal mucosa. ZO, occludin, and claudin are some of the main proteins that constitute tight junctions in the intestine, a special structure located between intestinal epithelial cells that prevents harmful substances such as pathogens and toxins from crossing the intestinal wall into the bloodstream (Fanning et al., 1999; González-Mariscal et al., 2003). ZO is a cytoplasmic protein located beneath the cell membrane and connected to the cytoskeleton (Furuse et al. 1993), occludin is a transmembrane protein in tight junctions directly involved in the barrier function of tight junctions (Balda et al. 1996), and claudin is the basic structure of tight junctions (Günzel and Yu, 2013). The results of this study found that both rapeseed meal mixture and fermented rapeseed meal mixture had no significant impact on the expression levels of jejunal tight junction proteins *ZO, occludin*, and *claudin* in Langshan chickens.

Conclusion

RSM reduced the slaughter performance of Langshan chicken, and improved the morphology of the jejunum mucosa and intestinal permeability; FRSM enhanced the slaughter performance of Langshan chicken, reduced the serum levels of uric acid and glucose, and improved the morphology of the jejunum mucosa and intestinal permeability.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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