

Effect of feeding a *Saccharomyces cerevisiae* fermentation-derived postbiotics on growth performance and intestinal health in the crossbred broiler chickens under a low biosecurity production system

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Abstract

Open farming systems with low biosecurity conditions are very common in developing countries. In the context that many countries in the world have issued regulations prohibiting the use of antibiotics for disease prevention, finding effective alternatives becomes more important, especially for farms with low biosecurity. This study aimed to explore the effects of a *Saccharomyces cerevisiae* fermentation-derived postbiotics (SCFP) on growth performance and intestinal health of crossbred broiler chickens raised in a low bio-security production system. One hundred ninety-two 1-day-old chickens were assigned to 2 treatments. Dietary treatments were: 1) a standard basal diet and 2) a standard basal diet containing SCFP (1.25 kg/MT of Diamond V XPC™). The broilers were individually weighed every week. Feed intake was measured daily per pen. At 84 days old, the small intestine was collected for intestinal morphology analyses by hematoxylin and eosin staining. Gut integrity was evaluated by measuring the expression of tight junction genes using qRT-PCR. Results showed that supplementation of SCFP did not affect the average daily gain, feed intake, and feed conversion ratio ($P > 0.05$). Similarly, supplementation of SCFP did not change the villus height and crypt depth of intestinal tissues ($P > 0.05$). Besides, SCFP did not affect the expressions of Claudins, Occludin, ZO1, and JAM but down-regulated E-cadherin expression. Overall, supplementation of SCFP did not affect chicken performance as well as the morphology and tight junction gene expression of intestinal tissues of chickens raised in a low bio-security production system.

Keywords: growth performance, intestinal morphology, postbiotic, *Saccharomyces cerevisiae*, tight junction genes

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Introduction

Poultry products are vital protein sources for the growing global population. To meet rising demand, farmers have widely used antibiotic growth promoters (AGPs); however, their misuse has contributed to antimicrobial resistance (AMR) and antibiotic residues in meat (Roth *et al.*, 2019; Xu *et al.*, 2020; Habiba *et al.*, 2023). This threatens both animal and human health, leading to increased healthcare costs, hospitalization, and mortality. Consequently, many countries have restricted or banned AGPs – beginning with Sweden in 1986, followed by the EU in 2006, and later by nations including Mexico, New Zealand, and South Korea (Abreu *et al.*, 2023). Partial bans have also been implemented in the United States, Australia, Japan, and Canada. In Vietnam, AMR is among the highest in Asia (Torumkune *et al.*, 2022), prompting a 2020 ban on prophylactic antimicrobial use in adult terrestrial animals, with a complete ban planned by 2026 (Carrique-Mas *et al.*, 2023). Therefore, identifying effective alternatives to in-feed antibiotics is essential.

Several studies on feed additives as potential alternatives to antibiotics have been conducted, including organic acids, prebiotics, probiotics, postbiotics, and fermentation products. Among the alternatives, *Saccharomyces cerevisiae* fermentation-derived postbiotics (SCFP) is one of the potential candidates that has received research attention in recent years (Soren *et al.*, 2023). SCFPs are fermentation-derived substances containing bioactive fermentation metabolites such as organic acids, small peptides, polyamines, natural antioxidants, vitamins, and fragments of yeast cell walls (Wilson *et al.*, 2022). Supplementation with a SCFP has shown beneficial effects on growth, overall health, and the immune system in animals (Gao *et al.*, 2009; Soren *et al.*, 2024). Recently, numerous studies have focused on the effects of SCFP supplementation in broiler diets on growth performance and intestinal health. These aspects are essential because they influence the overall health of the chickens and the economic benefits of the farmers. Reports on the effects of SCFP supplementation in chicken diets have yielded inconsistent results depending on the experimental conditions. Some studies indicated that adding SCFP to chicken diets improved growth performance (Gao *et al.*, 2009). However, other studies have found that SCFP had no notable impact on growth or feed conversion ratio (FCR) (Chuang *et al.*, 2019; Lin *et al.*, 2023). This suggests that the impact of SCFP needs further investigation across various conditions to provide reliable recommendations. According to our review, most studies on the effects of SCFP supplementation in chickens were conducted under high biosecurity but not low to minimal biosecurity conditions, commonly referred to as the sector 3 production system.

A sector 3 production system is a semi-commercial poultry farming system characterized by low to minimal biosecurity. This production system is very common in Vietnam and many other developing countries. Production cycles for meat birds typically last approximately 70 to 90 days, with moderate mortality rates and varying levels of efficiency. Chickens raised in this production system are more

susceptible to diseases since it is difficult to control pathogens from the environment. Therefore, the finding of alternatives to antibiotics has become even more important, especially as the use of antimicrobials for disease prevention in poultry production is being banned worldwide.

The aim of this study was to evaluate the impact of adding SCFP to the diet of F1 chickens (Ri x Luong Phuong) on growth performance, gut health, intestinal morphology, and the expression of tight junction genes in the small intestine. The study was conducted in a low biosecurity traditional production system in Vietnam to provide novel insights into the potential benefits of SCFP supplementation for chickens under this specific context.

Materials and Methods

Animals, housing, experimental design, diets: The protocol for animal study was authorized by the Ethics Committee of the Animal Research Board at Hue University (protocol code: HUVN0028, approved on August 1, 2023).

One hundred ninety-two F1 chickens, a crossbreed of local breeds (Ri and Luong Phuong) chickens, were purchased from the National Institute of Animal Sciences located in Ha Noi, Vietnam. The chickens were all one-day-old upon arrival and had a similar body weight of around 30 g per chicken ($P = 0.70$). Each chicken was leg-banded for identification purposes. The chickens received vaccinations against pox, Newcastle disease virus, H5N1, and infectious laryngotracheitis according to the manufacturer's instructions. Throughout the study period, no other medications were administered to the chickens.

The study was conducted in an open farm belonging to Hue University of Agriculture and Forestry in Hue city, Vietnam. The farm was unable to control temperature and humidity and operated under low biosecurity conditions (sector 3 production system, according to FAO classification). Temperature and humidity were measured daily using a temperature and humidity meter (Nakata NJ-2099-TH, Japan) at three intervals: 6 AM, 11 AM, and 10 PM. The average daily temperatures ($^{\circ}\text{C}$) during the experimental period were 27.2 ± 1.0 at 6 AM, 35.9 ± 3.0 at 11 AM, and 28.4 ± 1.5 at 10 PM (mean $\pm$ SD). The average daily humidity (%) was 86.2 ± 8.6 , 56.9 ± 11.7 , and 82.2 ± 8.4 , respectively. There were 32 pens, each measuring 2 m² (2.0 $\times$ 1.0 m), separated by fence nets. Each pen was equipped with a suspended plastic feeder and a water drinker. During the incubation period, a chicken brooder lamp was used in each pen to maintain the temperature between 31-35 $^{\circ}\text{C}$. Subsequently, the temperature was gradually reduced based on visual observations of the chicken's condition in the pens.

The one-day-old chickens were randomly assigned to two treatment groups. One group comprised 16 pens (96 chickens; 3 males and 3 females per pen) designated as the control, which received a standard basal broiler diet without supplementation of SCFP. The remaining 16 pens (96 chickens; 3 male and 3 female chickens/pen) were assigned to the treatment and received a standard basal broiler diet supplemented

with 1.25 kg/MT of SCFP (Diamond V XPC™, Cedar Rapids, Iowa, USA). The chickens were provided ad libitum access to feed and water. The feed ingredients were purchased and processed to formulate diets meeting the nutritional requirements of the chickens (Table 1). Feed ingredients were tested and complied

with the National Standard on Microbiology of Food and Animal Feeding Stuffs (ISO 16140). The water used for the experiment was the tap water that was adjusted to a suitable pH for chickens (5.5-6). The feed provided did not contain any antibiotic growth promoters.

Table 1 Ingredients (% as fed) and nutrient composition of the basal diet.

Ingredients	Phase 1 (0-21 days old)	Phase 2 (22-41 days old)	Phase 3 (42-84 days old)
Corn	54.79	59.35	61.78
Soybean meal	29.00	24.00	21.00
DDGS	5.00	6.80	7.00
Rice bran	5.00	3.60	4.20
Vegetable oil	2.13	2.18	2.53
CaCO ₃	1.40	1.30	1.20
Dicalcium phosphate	1.20	1.30	1.20
Vitamins and minerals ¹	0.25	0.25	0.25
NaCl	0.20	0.20	0.20
L-Lysine	0.38	0.39	0.25
L-Methionine	0.40	0.39	0.25
Threonine	0.23	0.22	0.12
Total, %	100	100	100
Nutrient composition			
DM, %	88.36	88.38	88.39
ME, Kcal/kg	2999.74	3050.60	3100.46
Crude protein, %	19.94	18.41	17.03
Crude fiber, %	4.46	4.32	4.25
Ca, %	0.89	0.87	0.81
P, %	0.56	0.57	0.54
P (easily digestible), %	0.29	0.30	0.28
SID Lys, %	1.20	1.15	0.95
SID Met+Cys, %	0.90	0.90	0.74
SID Thr, %	0.80	0.77	0.63
SID Trp, %	0.19	0.17	0.16

¹Vitamin A (minimum): 3,200,000 IU/kg; vitamin E (minimum): 12,000 mg/kg; vitamin B1 (minimum): 400 mg/kg; vitamin D3 (minimum): 1,000,000 IU/kg; vitamin K3 (minimum): 660 mg/kg; vitamin B3 (minimum): 12,000 mg/kg; Fe (minimum - maximum): 10,200-13,800 mg/kg; Zn (minimum - maximum): 25,500-34,500 mg/kg; Cu (minimum - maximum): 3,200-4,800 mg/kg; Mn (minimum - maximum): 25,500-34,500 mg/kg.

Average daily gain, feed intake, and feed conversion ratio measurement: The chickens were individually weighed once every week before the morning feeding (7:00 AM). The amount fed and left over was measured daily per pen. Feed intake = Feed provided - Feed leftovers in the feeder. Provided and leftover feed were weighed at 7 AM daily. The average daily weight gain and feed conversion ratio (feed/gain) were determined based on feed intake and weight gain (Lin *et al.*, 2023).

Intestinal morphology observation: At the end of the experiment, one chicken per pen with an average pen body weight (8 females and 8 males per treatment) was euthanized by intravenous injection of pentobarbital sodium (30 mg/kg body weight). The small intestine, including the duodenum, jejunum, and ileum (5-cm segments from each region), was collected and fixed in 10% neutral buffered formalin for 24 h before Hematoxylin and Eosin (H&E) staining. The duodenum sample was collected from the middle portion of the duodenal loop, which typically encircles the pancreas. The jejunum was taken from the midpoint between the duodenal loop and Meckel's diverticulum. The ileum sample was obtained from the midpoint between Meckel's diverticulum and the junction with the ceca. In brief, intestines fixed in formaldehyde were cut into 1 cm segments and embedded in paraffin. The samples were then cut into sections with a thickness of 5 µm using a microtome

(Leica, KD-2260). The sections were stained with H&E. For analysis, three H&E-stained specimens from each intestinal part (with 200 µm intervals) were examined under a light microscope (Optika) equipped with an OptikamB1 digital camera (Italy) to assess villus height and crypt depth. Villus height and crypt depth were measured in 16 well-oriented villi and their corresponding crypts per section. Villus height was measured as the distance from the tip to the base, while crypt depth was defined as the distance from the top of the villus crypt to the muscularis mucosa (Adjji *et al.*, 2019).

Measurement of gene expression levels of tight junction genes: When the chickens were euthanized for sample collection, a 5 cm segment from the middle part of the small intestine was taken from 16 chickens per treatment (one chicken per cage) and placed in a 1.5 mL tube. The samples were then promptly frozen at -80°C for gene expression analysis. Total RNA was extracted from the tissues using Freezol Reagent (Nanjing Vazyme Biotech Co., Ltd) according to the manufacturer's guidelines. The RNA concentrations were subsequently measured using a Nanodrop Lite Spectrophotometer (Thermo Scientific). The RNA concentrations of the samples were around 600-1000 ng/µL with A260/280 from 1.8-2.2. A total of 1 µg of RNA was reverse transcribed using the FIREScript RT cDNA Synthesis MIX with oligo (dT) and random

primers (Solid Biodyne, Estonia), in accordance with the manufacturer's instructions. All cDNA samples were stored at -20°C until further analysis. The QuantStudio™ 5 Real-Time PCR System (Thermo Fisher Scientific) was used to amplify the cDNA through real-time reverse-transcriptase quantitative polymerase chain reaction (RT-qPCR) using a PCR Supermix Kit (Solid Biodyne, Estonia). Expression levels were normalized to RPS17 in the same sample. The primer sequences for the genes analyzed (E-cadherin, Occludin, Zonula occludens (ZO)-1, Junctional adhesion molecule (JAM), Claudin-1, Claudin-2, Claudin-3) are provided in Table 2. The samples were initially heated at 95°C for 1 minute, followed by 40 cycles of denaturation at 95°C for 15 seconds and annealing/elongation at 60°C for 1 minute (Pham *et al.*, 2021). Amplifications were performed on 16 independent samples per group, with each sample analyzed in triplicate on the same plate. The relative

mRNA levels were determined using the 2- $\Delta\Delta C_t$ method (Livak and Schmittgen, 2001).

Statistical analyses: The cage served as the experimental unit for measuring average daily weight gain, feed intake, and feed conversion ratio. Individual chickens were the experimental unit for intestinal morphological observations and gene expression analysis. Results are presented with the arithmetic mean and standard deviation. The data were checked for normality and homogeneity of variance. If the data showed a normal distribution and homogeneous variances, a t-test was performed to evaluate the differences between the control and treatment groups. If the assumptions were not met, the Kruskal-Wallis test was used. Differences were considered statistically significant at $P < 0.05$. Data were analyzed using Minitab Statistical Software, version 16.0 (Minitab Inc., State College, PA, USA).

Table 2 The sequence of primer pairs is used for amplification of target genes.

Gene Name		Primer sequences (5' to 3')	Genbank Accession No.
Housekeeping genes			
RPS17	Forward	AAGCTGCAGGAGGAGGAGAGG	NM_204217.1
	Reverse	GGTTGGACAGGCTGCCGAAGT	
Junctional molecular genes			
E-Cadherin	Forward	TCACGGGCAGATTCTAT	NM_001039258.2
	Reverse	CACGGAGTTCGGAGTTTA	
Occludin	Forward	ACGGCAAAGCCAACATCTAC	NM_205128.1
	Reverse	ATCCGCCACGTCTTCAC	
ZO-1	Forward	AAGTGGGAAGAATGCCAAAA	XM_015278981.2
	Reverse	GGTCCTTGGATCCCGTATCT	
JAM	Forward	AGACAGGAACAGGCAGTGCT	XM_025149444.1
	Reverse	TCCAATCCCATTGAGGCTA	
Claudin-1	Forward	AAGGTGTACGACTCGTGCT	NM_001013611.2
	Reverse	CAGCAACAAACACACCAACC	
Claudin-2	Forward	CCTGCTCACCTCATTGGAG	NM_001277622.1
	Reverse	GCTGAACTCACTCTTGGGCT	
Claudin-3	Forward	GCCAAGATCACCATCGTCTC	NM_204202.1
	Reverse	CACCAGCGGGTTGTAGAAAT	

Results

Effects of feeding SCFP on performance of F1 (Ri x Luong Phuong) crossbred broiler chickens: Effects of adding a SCFP on body weight of F1 (Ri x Luong Phuong) crossbred broiler chickens from one-day old to 12 weeks old are presented in Table 3. The body weight of chickens at 12 weeks old in the treatment group was about 50 g/chicken higher than that in the control, 1940 g compared to 1892 g/chicken; however, it was not statistically different ($P = 0.18$). The body weight of chickens at different growing phases was also not different between the control and treatment ($P > 0.05$).

The daily feed intake (g/chicken/day) during the experimental period between one day old and 12 weeks old was similar between the control and SCFP ($P = 0.21$), around 62 g/chicken/day. The average daily gain for the entire experimental period was not statistically different between the control and SCFP ($P = 0.19$), around 22 - 23 g/day/chicken (Table 3). Similarly, there was no difference ($P = 0.16$) in the feed conversion ratio between the control and SCFP in the experimental period from one-day-old until 12 weeks old, with values ranging from approximately 2.7 - 2.8 kg feed/kg weight gain (Table 3).

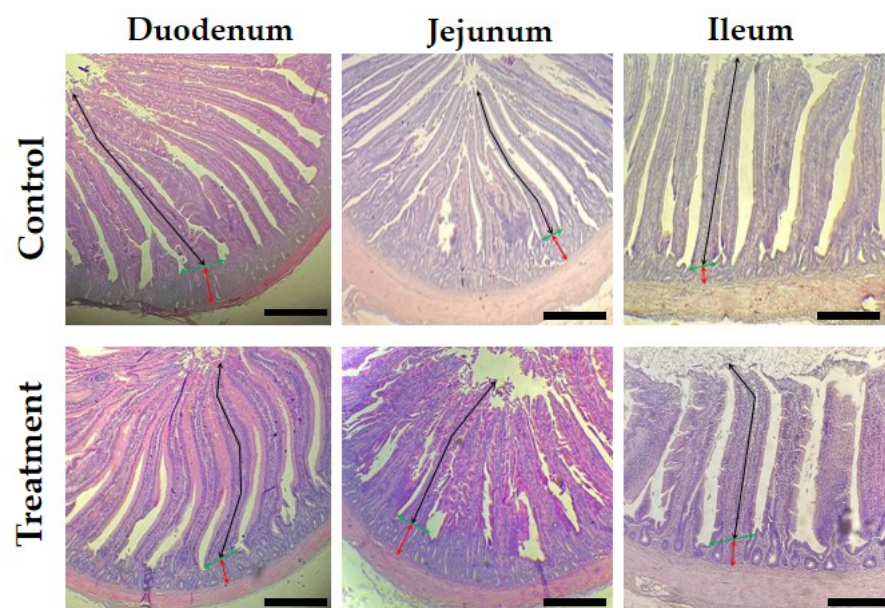
Effect of feeding a SCFP on gut morphology: The effects of SCFP on the gut morphology (villus length and crypt depth) of the F1 (Ri x Luong Phuong) chickens on day 84 are presented in Table 4 and Fig. 1. The average lengths of villi in the duodenum, jejunum, and ileum of control chickens were higher than that of the treatment, 1616.28±269.41 μ m, 1313.36±122.32 μ m, and 953.64±227.17 μ m in control chickens, respectively compared to 1591.54±233.38 μ m, 1270.99±223.32 μ m, and 910.72±149.89 μ m in treatment chickens. In contrast, the average depths of crypts in the duodenum, jejunum, and ileum of control chickens were lower than that of the treatment, 252.95 ± 38.31 μ m, 303.83 ± 60.35 μ m, and 181.45 ± 55.92 μ m in control chickens compared to 258.26 ± 46.41 μ m, 273.57 ± 65.21 μ m, and 172.37 ± 49.56 μ m in treatment chickens. The VH/CD indexes in the duodenum, jejunum, and ileum were similar between control and treatment chickens. No statistically significant differences were observed in villus length, crypt depth, or VH/CD ratio in the small intestine between the control and treatment groups (Table 4 and Fig. 1).

Table 3 Effects of feeding a SCPF on growth performance (mean $\pm$ standard deviation) of F1 (Ri x Luong Phuong) crossbred broiler chickens (n = 16).

Item	Control ¹	Treatment ²	P-value
Starter (1-3 weeks)			
BW ³ at 3 weeks (g)	213.74 $\pm$ 26.71	206.10 $\pm$ 30.24	0.46
ADG ⁴ (g/day)	8.75 $\pm$ 1.28	8.37 $\pm$ 1.45	0.45
ADFI ⁵ (g/day)	18.45 $\pm$ 3.04	17.84 $\pm$ 3.14	0.59
FCR ⁶	2.11 $\pm$ 0.16	2.14 $\pm$ 0.19	0.68
Grower (4-6 weeks)			
BW at 6 weeks (g)	737.63 $\pm$ 45.18	740.73 $\pm$ 66.45	0.88
ADG (g/day)	24.95 $\pm$ 1.46	25.46 $\pm$ 2.06	0.42
ADFI (g/day)	52.61 $\pm$ 1.36	53.11 $\pm$ 3.17	0.57
FCR	2.12 $\pm$ 0.12	2.10 $\pm$ 0.20	0.78
Finisher (7-12 weeks)			
BW at 12 weeks (g)	1891.72 $\pm$ 93.66	1940.43 $\pm$ 107.70	0.18
ADG (g/day)	27.48 $\pm$ 1.78	28.56 $\pm$ 1.42	0.07
ADFI (g/day)	87.69 $\pm$ 4.37	89.99 $\pm$ 5.62	0.20
FCR	3.20 $\pm$ 0.16	3.16 $\pm$ 0.17	0.53
Total (1-12 weeks)			
ADG (g/day)	22.16 $\pm$ 1.12	22.74 $\pm$ 1.29	0.19
ADFI (g/day)	61.61 $\pm$ 2.24	62.86 $\pm$ 3.15	0.21
FCR	2.78 $\pm$ 0.10	2.77 $\pm$ 0.11	0.16

¹Control = Basal control diet; ²Treatment = Basal diet containing 1.25 kg/MT (2.5 lb/t) of Diamond V XPCTM; ³BW = Body weight;⁴ADG = Average Daily Gain; ⁵ADFI = Average Daily Feed Intake; ⁶FCR = Feed Conversion Ratio.**Table 4** Effect of feeding a *Saccharomyces cerevisiae* fermentation product on gut morphology of chickens at 84 days old (mean $\pm$ standard deviation).

Attribute	Control ¹	Treatment ²	P-value
Duodenum (μm)			
Villus height (VH)	1616.28 $\pm$ 269.41	1591.54 $\pm$ 233.38	0.79
Crypt depth (CD)	252.95 $\pm$ 38.31	258.26 $\pm$ 46.41	0.62
VH/CD	6.60 $\pm$ 1.17	6.55 $\pm$ 1.34	0.84
Jejunum (μm)			
Villus height (VH)	1313.36 $\pm$ 122.32	1270.99 $\pm$ 223.32	0.90
Crypt depth (CD)	303.83 $\pm$ 60.35	273.57 $\pm$ 65.21	0.16
VH/CD	4.79 $\pm$ 1.14	5.05 $\pm$ 1.04	0.46
Ileum (μm)			
Villus height (VH)	953.64 $\pm$ 227.17	910.72 $\pm$ 149.89	0.35
Crypt depth (CD)	181.45 $\pm$ 55.92	172.37 $\pm$ 49.56	0.70
VH/CD	5.64 $\pm$ 1.26	5.98 $\pm$ 1.72	0.78

¹Control = Basal control diet; ²Treatment = Basal diet containing 1.25 kg/MT (2.5 lb/t) of Diamond V XPCTM. VH/CD ratios were calculated individually, and the mean value was used.**Figure 1** The effects of SCPF on the gut morphology of chickens. Control = Basal control diet; Treatment = Basal diet containing 1.25 kg/MT (2.5 lb/t) of Diamond V XPCTM. Black double-headed arrows indicate the length of villi. Red double-headed arrows indicate the crypt depth. Green double-headed arrows indicate the boundary of villi and crypts. Scale bar = 500 μ m.

Effect of feeding SCFP on the mRNA expression levels of tight junction genes: The relative mRNA expression levels of junctional molecules were measured to assess the impact of SCFP supplementation on chicken gut health (Fig. 2). The results indicate that the mRNA expression levels of Claudin-3 and ZO-1 were similar between the control and the treatment group. The gene expression of Claudin-1, Claudin-2, Occludin, and JAM was modestly increased with SCFP supplementation in the diet. The mRNA levels of

Claudin-1, Claudin-2, Occludin, and JAM in the SCFP group were 1.06, 1.12, 1.11, and 1.14 times higher, respectively, compared to the control group. Despite their moderate increase, statistical analysis revealed that the differences are not significant ($P > 0.05$). Among the 7 genes in this study, only E-cadherin expression was altered by adding SCFP to the chicken diet. Its expression level was significantly decreased, about 2.5 times, in chickens supplemented with SCFP, compared to that in the control group ($P < 0.05$).

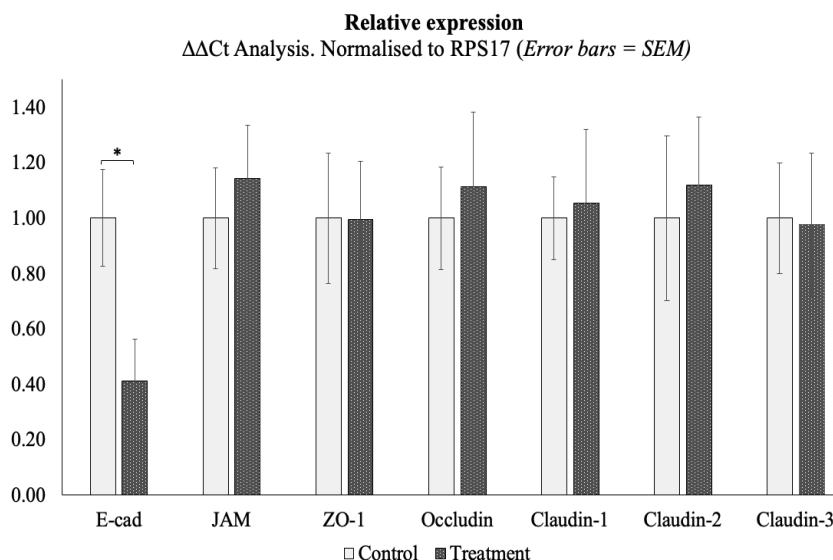


Figure 2 mRNA expression levels of tight junction genes in the small intestine of chicken. Control = Basal control diet; Treatment = Basal diet containing 1.25 kg/MT (2.5 lb/t) of Diamond V XPC™.

Discussion

The body weight of F1 (Ri x Luong Phuong) crossbred broiler chickens at one day old and at 12 weeks old, as well as their ADG, ADFI, and FCR over the entire experimental period from one day old to 12 weeks of age, were similar to other studies on this type of crossbred broiler chickens (Tran *et al.*, 2017; Tran *et al.*, 2020; Tran *et al.*, 2022). The SCFP supplementation did not affect the ADG, ADFI, and FCR over the entire experimental period. This is consistent with the findings of Chuang *et al.* (2019), who reported that feeding Ross 308 broiler chickens with SCFP (1 kg/MT feed) from 0 to 5 weeks of age under controlled environmental conditions did not significantly affect ADG, ADFI, or FCR. Similar results were observed by Lin *et al.* (2023), where Ross 308 broilers supplemented with *Saccharomyces cerevisiae* hydrolysate at 0.5 kg/MT feed during the starter and grower phases and 0.25 kg/MT feed during the finisher phase (0–6 weeks of age) showed no improvement in growth performance. Taken together, these studies and the present findings suggest that relatively low supplementation levels (≤ 1.25 kg/MT feed) of SCFP or related yeast-derived products may not be sufficient to enhance growth performance in broiler chickens. However, our findings are not in agreement with Soren *et al.* (2024), who supplemented Vencobb commercial broilers with SCFP at the same dosage (1.25 kg/MT feed) used in this study and reported positive effects on ADG and ADFI during the first two weeks of age as well as an overall improvement in FCR throughout the trial.

Similarly, Gao *et al.* (2009) demonstrated that SCFP supplementation at higher inclusion levels (2.5 and 5 kg/MT feed) improved ADG and FI in Arbor Acres broilers from 0 to 6 weeks of age. These discrepancies indicate that the efficacy of SCFP may depend not only on the dietary dose but also on the breed of chickens. Compared with fast-growing commercial strains such as Vencobb or Arbor Acres, the crossbred chickens used in the present study have lower feed intake capacity, which likely reduced their effective intake of SCFP and may partly explain the lack of improvement in growth performance observed.

Healthy intestines can be identified by villus length and crypt depth. Studies suggested that increased villus length and shallower crypt depth play a role in augmenting nutrient absorption within the gastrointestinal tract. A reliable measure for evaluating intestinal integrity is the villus height to crypt depth ratio. A rise in this ratio is linked to enhanced processes of digestion and absorption (Caspary, 1992). Morphometric analyses revealed no differences in villus height, crypt depth, and villus-height-to-crypt-depth ratio in the small intestine between the control and treatment. The results of the present study are similar to those of Chuang *et al.* (2019), who reported that supplementing Ross 308 broilers with a relatively low dose of SCFP (1 kg/MT feed) did not affect villus height or the villus-to-crypt ratio. In contrast, Soren *et al.* (2024) observed that SCFP supplementation at the same dosage used in our study (1.25 kg/MT feed) significantly increased villus height and the villus height-to-crypt depth ratio in Vencobb broilers.

Similarly, Gao *et al.* (2008) demonstrated that higher inclusion levels of *Saccharomyces cerevisiae* culture (2.5–7.5 g/kg feed) improved villus height in broilers. Taken together, these findings suggest that, as with growth performance, the effects of SCFP on intestinal morphology are not consistent across studies. A possible explanation is the relatively low effective dosage received by the birds in our study, combined with the use of crossbred chickens that have lower feed intake than fast-growing commercial strains, which likely limited the amount of SCFP reaching the intestine and contributed to the lack of observable effects.

The mucosal layer that covers intestinal epithelial cells serves as a protective barrier, effectively separating intestinal microbes from the epithelial cells (Broom and Kogut, 2018). The width of the gaps between intestinal epithelial cells is mainly regulated by tight junction (TJ) proteins, such as those in the Occludin, Claudins, and Zonula occludens (ZO) families. Claudins and Occludin are transmembrane proteins that play a crucial role in regulating the size of the intercellular space (Suzuki, 2012). These proteins "connect" adjacent epithelial cells, ensuring the integrity of the tight junctions. ZO-1, found in intestinal epithelial cells, binds to Claudins and Occludin, further strengthening the stability of the tight junctions (Tang *et al.*, 2015). Claudins, Occludin, and ZO-1 are essential for maintaining the integrity and tightness of the intestinal barrier, preventing pathogens from penetrating the epithelial cells (Suzuki, 2012; Tang *et al.*, 2015; Broom and Kogut, 2018). The results presented in Figure 2 indicated that the gene expressions of Claudin-1, Claudin-2, Claudin-3, Occludin, JAM, and ZO-1 were slightly induced by adding SCFP to the diet, but it was not significantly different in SCFP chickens compared with those of the control. This result contrasts with previously published studies, which support the idea that yeast products can enhance gut barrier integrity through an upregulation of Claudin-1, Occludin, and ZO-1 (Kim *et al.*, 2022; Lin *et al.*, 2023; Gong *et al.*, 2025).

E-cadherin is a crucial tight-junction protein that promotes the cell-cell adhesion of intestinal epithelial cells. Several studies have demonstrated that probiotics can exert protective effects on this molecule; for instance, *Saccharomyces boulardii* supernatant has been shown to maintain or restore E-cadherin localization in intestinal epithelial cells (Terciolo *et al.*, 2017). In contrast, our results (Fig. 2) show that E-cadherin expression was significantly lower in chickens supplemented with SCFP. One possible explanation is that SCFP may modulate intestinal epithelial cell turnover. Some studies suggest that probiotic or postbiotic supplementation can accelerate epithelial renewal, which may temporarily reduce adhesion proteins like E-cadherin during tissue remodeling (Aggarwal *et al.*, 2022; Di Luccia *et al.*, 2022). Another potential factor is natural pathogen exposure. Chickens in both control and SCFP groups were unexpectedly infected with *Eimeria* spp. from 5 to 12 weeks of age (data not shown). Fecal examination indicated that oocyst shedding in the SCFP group peaked on day 84 and was markedly higher than in controls (135.5×10^3 vs. 54.0×10^3 oocysts per gram of

feces, respectively). The timing of tissue sampling may therefore coincide with peak oocyst excretion, contributing to the pronounced downregulation of E-cadherin. Supporting this, Pham *et al.* (2021) reported decreased E-cadherin expression in the cecum of chickens experimentally infected with *Eimeria tenella*. To our knowledge, this is the first study reporting a reduction in E-cadherin gene expression following SCFP supplementation. However, given that the trial was conducted under low-biosecurity farming conditions, where environmental factors, including uncontrolled coccidial infection, may have influenced the results, further controlled studies are required to clarify whether this reduction is directly attributable to SCFP or secondary to pathogen-induced intestinal damage.

Overall, supplementing the diet with SCFP did not affect the performance of F1 chickens (Ri x Luong Phuong) in terms of average daily gain, feed intake, or feed conversion ratio. Likewise, SCFP supplementation did not alter intestinal morphology, including villus height and crypt depth ($P > 0.05$). Furthermore, *Saccharomyces cerevisiae* fermentation product supplementation did not affect the expressions of Claudins, Occludin, ZO1, and JAM but down-regulated the E-cadherin expression in the small intestine. To the best of our knowledge, this is the first study to evaluate the effects of SCFP supplementation on growth performance and intestinal health of broiler chickens raised under low-biosecurity conditions (sector 3). In contrast to previous reports conducted under more controlled environments, our study differed in two important aspects. First, the trial was carried out under low-biosecurity conditions, where natural pathogen exposure occurred and contributed to health challenges during the experimental period. Second, the use of local chicken breeds with relatively low feed intake meant that the actual amount of SCFP consumed per bird was lower compared to fast-growing commercial broilers commonly used in controlled studies. These differences may explain why our results did not show the expected positive outcomes. Therefore, these findings do not indicate a failure of SCFP; rather, they suggest that its efficacy in low-biosecurity systems may be influenced by several factors, particularly the dosage used and the overall health management practices. Further studies evaluating higher supplementation levels are needed to better assess the effectiveness of SCFP in chickens raised under sector 3 production systems. In addition, additional research should test the hypothesis that natural pathogen exposure under open-farming conditions could modify the effects of SCFP on growth performance, gut morphology, and tight-junction gene expression.

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