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Effects of Fermented Pineapple Peel and *Indigofera zollingeriana* Leaves on Intestinal Bacterial Population, Performance, and Carcass Traits in Broiler Chickens

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ABSTRACT

The prohibition of antibiotic growth promoters has necessitated the exploration of alternative functional feed additives from agricultural waste to maintain poultry health and productivity. The current study aimed to evaluate the effects of dietary supplementation with fermented synbiotics derived from pineapple peel (*Ananas comosus*) and *Indigofera zollingeriana* leaves on growth performance, carcass traits, intestinal bacterial populations, and internal organ weights in broiler chickens. A total of 100 one-day-old broiler chickens with an average initial body weight of 42 ± 2 g were randomly allocated to five treatments with four replications in a completely randomized design. The dietary treatments initiated on day 1 continued for 28 days and consisted of a basal diet supplemented with fermented synbiotic (FS) at levels of 0% (control), 0.25%, 0.50%, 0.75%, and 1.00%. The FS powder was mixed directly into the basal diet as a top-dressing and provided daily for the entire 28-day experimental period. Feed intake and body weight were recorded weekly. Parameters measured included feed consumption, body weight gain, feed conversion ratio, live weight, carcass percentage, abdominal fat percentage, income over feed cost, relative organ weights (liver, heart, gizzard, pancreas), small intestinal pH, and populations of lactic acid bacteria and *Salmonella typhimurium*. Results demonstrated that supplementation at 1.00% significantly improved body weight gain, feed conversion ratio, live weight, carcass percentage, and income over feed cost compared to the control group. Small intestinal pH decreased from 7.03 (control) to 4.56 (1.00% FS). Lactic acid bacteria populations increased significantly from 1.58 (control) to 3.83×10^6 CFU/g (1.00% FS), while *Salmonella typhimurium* counts were reduced from 0.97 (control) to 0.17 $\times 10^6$ CFU/g (1.00% FS). No significant effects were observed on relative organ weights across all treatments compared to the control group. In conclusion, fermented synbiotics from pineapple peel and *Indigofera zollingeriana* leaves at 1.00% optimally enhanced growth performance, carcass quality, and intestinal health while improving economic returns in broiler chickens, offering a viable and sustainable alternative to antibiotic growth promoters.

Keywords: Amino acid, Broiler chicken, Fermented synbiotic, Growth performance, *Indigofera zollingeriana*, Pineapple peel

INTRODUCTION

In the past two decades, the use of functional feed additives in poultry nutrition has developed rapidly alongside increasing attention to digestive health and production efficiency. Synbiotics have gained considerable interest among feed additives. Synbiotics combine probiotics (live beneficial microorganisms) and prebiotics (substrates selectively utilized by host microorganisms) to improve gut microbiota balance, enhance nutrient absorption, and suppress pathogenic colonization in poultry (Gibson et al., 2017). Prebiotics themselves have been shown to improve growth performance (Rehman et al., 2020), modulate the immune system (Al-Baadani et al., 2025), and enhance the quality of poultry products (Dong et al., 2024). Previous studies have reported that supplementation with conventional prebiotics such as fructo-oligosaccharides (FOS), galacto-oligosaccharides (GOS), and inulin can improve the

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balance of intestinal microflora, suppress the colonization of pathogenic bacteria, and enhance feed conversion efficiency in broiler chickens (Pourabedin and Zhao, 2015; Ricke, 2018). Synbiotics offer the synergistic advantage of delivering both live beneficial bacteria and their specific substrates, thereby enhancing the survival and activity of probiotics in the gut (Śliżewska et al., 2020).

The use of synbiotics has become increasingly relevant following the ban on Antibiotic Growth Promoters (AGPs) in many countries due to the risks of antibiotic resistance and residues in animal products (Manyi-Loh et al., 2018). The AGP ban has driven the exploration of alternative prebiotic sources that are more economical and sustainable (Cheng et al., 2014). However, the high production costs of commercial synbiotics have encouraged the investigation of alternative sources, particularly from agricultural waste, to improve economic viability and sustainability.

Pineapple peel (*Ananas comosus*) is rich in pectin fiber, the bromelain enzyme, and oligosaccharides such as FOS, which can serve as prebiotic substrates (Hikal et al., 2021; Gómez-García et al., 2022). *Indigofera zollingeriana* leaves contain high crude protein and bioactive compounds but also contain tannins as anti-nutritional compounds that can reduce digestibility (Susanto et al., 2025). The fermentation process combining the two materials using a yogurt starter (containing *Lactobacillus delbrueckii subsp. bulgaricus* and *Streptococcus thermophilus*) produces a fermented synbiotic that contains both viable lactic acid bacteria (probiotics) and prebiotic oligosaccharides from the plant materials (Sah et al., 2016; Lu et al., 2024). Furthermore, fermentation by lactic acid bacteria produces tannase enzymes and creates an acidic environment (pH < 4.5), which hydrolyzes tannin-protein complexes and reduces anti-nutritional effects (Jeyakumar and Lawrence, 2022). The fermentable sugars and oligosaccharides from pineapple peel serve as an energy source that stimulates the growth and metabolic activity of lactic acid bacteria, thereby enhancing the bacterial production of tannase and organic acids (Parra-Matadamas et al., 2015). Consequently, the increased microbial activity facilitates more effective degradation of tannins present in *Indigofera zollingeriana* leaves compared to fermentation of *Indigofera zollingeriana* alone (García Méndez et al., 2021). The combination of pineapple peel and *Indigofera zollingeriana* leaves may yield a broader spectrum of prebiotic oligosaccharides compared to either substrate alone; pineapple peel provides FOS, while *Indigofera zollingeriana* leaves contribute other fermentable carbohydrates, potentially enhancing selective stimulation of beneficial gut microbiota.

To date, studies on pineapple peel as a feed ingredient in poultry have been reported (Sukri et al., 2023; Abba et al., 2026), while *Indigofera zollingeriana* has been investigated as a feed supplement for chickens (Mohamad Termizi et al., 2024; Tanjung et al., 2025). However, studies on the synergistic effects of the fermented synbiotic combination of both on growth performance, carcass quality, and intestinal microbial populations in broiler chickens remain limited. Therefore, the current study aimed to evaluate the effects of dietary supplementation with a fermented synbiotic from pineapple peel and Indigofera leaves on growth, carcass characteristics, intestinal pH, and the populations of beneficial and pathogenic bacteria.

MATERIALS AND METHODS

Ethical approval

An animal feeding experiment was conducted at the experimental station, Department of Animal Science, Faculty of Agriculture, Universitas Sriwijaya, South Sumatra, Indonesia. The animals were cared for according to the Indonesian Institute of Sciences Animal Welfare Guidelines. The approval of the experiment was granted by Universitas Sriwijaya with approval number KPPHP-2021-1.

Experimental design and chickens preparation

The current study employed a completely randomized design (CRD) with five treatments and four replications. The treatments consisted of a basal diet (control) supplemented with fermented synbiotic from pineapple peel and *Indigofera* leaves (FS) at the following proportions, including 100% control diet (FS0), 0.25% FS ratio (FS25), 0.50% FS ratio (FS50), 0.75% FS ratio (FS75), and 1% FS ratio (FS100). A total of 100 one-day-old broiler chickens (DOC) of the Lohman MB-202 strain with an average initial body weight of 42 ± 2 g were randomly allocated into 20 litter-floor pens (100 × 100 × 60 cm), resulting in 5 broiler chickens per pen. Initial body weight was measured for all broiler chickens before the start of the experiment, and randomization was performed without any weight-based selection to avoid bias.

Upon arrival, all chickens received drinking water mixed with 5% sugar for the first four hours as a standard practice to aid recovery from transport stress by providing an immediate energy source. The same protocol was applied uniformly to all treatment groups. After four hours, the sugar water was replaced with plain drinking water. A few hours later, the DOC was provided with the treatment diets sprinkled on paper for five days to familiarize them with the feed. Feed and drinking water were provided twice daily (morning and afternoon). The observation period lasted 28 days (4 weeks), with parameters including chicken body weight and treatment feed consumption measured weekly.

Diet and the synbiotic production process

The synbiotic powder used in the present study was produced through a liquid fermentation process combining pineapple peel (*Ananas comosus*) and *Indigofera zollingeriana* leaves, following the method described by Palupi et al. (2020) with modifications to ensure standardization.

Pineapple peel waste was collected from local farmers in Indralaya, South Sumatra, Indonesia, and *Indigofera* leaves were harvested from the experimental farm of the Department of Animal Science, University of Sriwijaya, Indralaya, Indonesia.

The yogurt starter used in the present study was a commercial product (Cimory, Indonesia). According to the product label, the yogurt contains a consortium of lactic acid bacteria, including *Lactobacillus delbrueckii* subsp. *Bulgaricus*, *Streptococcus thermophilus*, *Lactobacillus acidophilus*, and *Bifidobacterium* spp. The starter contained a high density of viable cells, typically expected in commercial yogurt products, which is sufficient to drive fermentation. To ensure standardization, all fermentation batches used the same product batch number, and the starter was added at a consistent volume of 300 mL per 12 L of fermentation medium.

The fermentation ingredients consisted of 5,640 g of ground pineapple peel, 360 g of ground *Indigofera* leaves, 300 mL of yogurt starter, 90 g of granulated sugar, and 12 L of distilled water. All materials were mixed in a 20-L fermentation jar, sealed, and incubated at room temperature (±28°C) for 72 hours, with daily stirring to maintain homogenization. After fermentation, the biomass was separated using a sterile strainer, oven-dried at 50°C for 48 hours, ground to pass a 120-mesh sieve, and stored in airtight containers at 4°C until diet preparation.

For dietary supplementation, the synbiotic powder was mixed into the basal diet as a top-dressing (added on top, not substituted for any basal ingredient such as corn or soybean meal) at levels of 0% (control), 0.25%, 0.50%, 0.75%, and 1.00% (w/w) and provided daily for the 28-day experimental period. The same basal diet was used for all treatments; only the FS supplementation level differed. The basal diets were commercial broiler starter (days 1-21) and finisher (days 22-28) feeds obtained from PT Japfa Comfeed Indonesia Tbk. According to the manufacturer, the diets are formulated to meet or exceed the nutrient requirements for broiler chickens as recommended by the NRC (1994) and are suitable for the Lohman MB-202 strain. The nutrient composition of the basal diets is presented in Table 1.

Table 1. Nutrient composition of the commercial basal diets (as a feed basis) for starter and finisher

Nutrient	Starter feed (1-14 days of age, BR I)*	Finisher feed (15-28 days of age, BR II)*
Water content (%)	12	12
Crude protein (%)	22.00	20.00
Crude fiber (%)	5.00	5.00
Fat (%)	5.00	5.00
Ash (%)	7.00	7.00
Calcium (%)	1.00	0.90
Phosphorus (%)	0.50	0.45
Metabolizable energy (kcal/kg)	3,000	3,100
Aflatoxin (µg/kg)	50.00	50.00

*PT Japfa Comfeed Indonesia Tbk. Diets are formulated to meet NRC (1994) recommendations and are suitable for Lohman MB-202 broilers. BR I: Broiler starter feed (1-21 days), BR II: Broiler finisher feed (22-28 days).

Data collection and laboratory measurements

Feed consumption, body weight gain, and feed conversion ratio were measured following the methods described by Quintana-Ospina et al. (2023). Carcass percentage and abdominal fat were determined using the approach outlined by Maynard et al. (2023). The calculation of Income Over Feed Cost (IOFC) referred to the procedures reported by Anggitasari et al. (2016). Internal organ weights (liver, heart, gizzard, and pancreas) were measured according to Henry et al. (2023). The pH of small intestine digesta was determined following the protocols described by Yardimci et al. (2006) and Nkukwana et al. (2015). Intestinal bacterial populations (lactic acid bacteria and *Salmonella typhimurium*) were enumerated using the standard serial dilution and colony-forming unit (CFU) method on selective agar media (Makras et al., 2006; Kowalska et al., 2020).

Slaughter procedure

At the end of the 28-day experimental period, all broiler chickens were fasted for 6 hours before slaughter to ensure empty digestive tracts, with water provided *ad libitum*. Each broiler was manually restrained, and slaughter was performed by severing the jugular veins and carotid arteries using a sharp knife (exsanguination). Broiler chickens were allowed to bleed for 2-3 minutes until death. The carcasses were then scalded in hot water at 55-60°C for 1-2 minutes, and feathers were removed manually. After defeathering, the carcasses were eviscerated, and the feet, chicken, and neck were removed.

Feed consumption

The investigated parameters included consumption of ration (g/chicken/day), which was measured based on the difference between the ration given (g) and the rest of the ration remaining (g) during a specific period (days; Quintana-Ospina et al., 2023).

Body weight gain

Body weight gain (g/chicken/day) was measured by weighing the difference between body weight at the end of the study (g) and the initial body weight (g), then dividing by the length of growing time (days; Quintana-Ospina et al., 2023).

Feed conversion ratio

Feed conversion ratio was measured based on the ratio between weight gain and feed consumption (Quintana-Ospina et al., 2023).

Live weight

The quality of the broiler carcass included the measurement of live weight, which was measured based on the results of weighing at the end of the study (Quintana-Ospina et al., 2023).

Caracas percentage

The percentage of the carcass was calculated based on the percentage of weight comparison of broilers without blood, feathers, chicken, legs, and digestive organs (g) divided by live weight (g). All 5 broilers per pen were used for carcass measurement (no broilers remained; Maynard et al., 2023).

Abdominal fat

Percentage of abdominal fat (%), which is calculated based on the percentage comparison between the weight of abdominal fat contained in the abdominal cavity and fat attached to the digestive organs (g) and the live weight of broilers (g; Maynard et al., 2023).

Income over feed cost

Income over Feed Cost (IOFC) was calculated based on the difference between the revenue obtained from the sale of live broilers (IDR/kg live weight) and the feed cost incurred during the rearing period (IDR/kg live weight). The IOFC value represents the economic efficiency of feed utilization in generating profit. A higher IOFC indicates more optimal conversion of feed expenditure into income from broiler production (Anggitasari et al., 2016).

Internal organ weight

The percentage of internal organ weight was measured at the end of the rearing period. Organs assessed included the liver, heart, gizzard, and pancreas. The percentage was calculated by comparing the weight of each organ (g) to the live body weight of the broiler (g), and then multiplying the ratio by 100% (Henry et al., 2023). Relative organ development in relation to overall body growth is indicated by these percentages.

pH of small intestine digesta

The pH of small intestine digesta was measured in broilers at 28 days of age. Broilers were fasted for 6 hours before slaughter (Yardimci et al., 2006). After dissection, the small intestine was removed, and the digesta was collected into a container for pH determination using a pH meter. For measurement, 1 g of digesta was homogenized with 10 mL of distilled water. The electrode was rinsed, dried, and then immersed in the sample solution. Readings were taken once the values stabilized, and the pH was recorded.

Measurement of small intestinal bacteria

Samples for bacterial enumeration were collected from the same broilers used for pH measurement. Approximately 1 g of ileal digesta was serially diluted (10^{-1} to 10^{-6}) in sterile 0.85% NaCl (Reuben et al., 2019). Lactic acid bacteria (LAB) were enumerated by pouring 1 mL from dilutions 10^{-4}-10^{-6} onto DeMan-Rogosa-Sharpe (MRS) agar (selective medium), followed by aerobic incubation at 37°C for 48 hours (Li et al., 2024). *Salmonella typhimurium* was quantified by spreading 100 μL from dilutions 10^{-1}-10^{-3} onto Xylose Lysine Deoxycholate (XLD) agar (selective and differential), then incubating aerobically at 37°C for 24 hours (Velasquez et al., 2018). All bacterial counts were transformed to $\log_{10}$ CFU/g before statistical analysis.

Statistical analysis

All data were analyzed using SPSS software (version 20), based on the design used. Data were subjected to one-way analysis of variance (ANOVA). When a significant difference was detected among treatment means, further comparison was performed using Duncan's multiple range test (Steel and Torrie, 1980). The significance level was set at less than 0.05.

RESULTS AND DISCUSSION

Production performance

Feed consumption showed a gradual increase with the rising level of FS (Table 2). Although the average consumption (76.62-77.11 g/broiler/day) was lower compared to studies using pure prebiotics (Rehman et al., 2020; Xiao et al., 2024), the increasing pattern at levels of 0.75% and 1.00% indicated an improvement in palatability. The primary mechanism involved is the degradation of tannins during the fermentation process. Tannins are known to have astringent properties that reduce palatability by binding to salivary proteins and taste receptors (Wina et al., 2005). Fermentation by lactic acid bacteria (LAB) has been shown to degrade tannins into phenolic compounds with lower molecular weights that are no longer astringent (Mullen et al., 2015). Furthermore, organic acids, particularly lactic acid produced during fermentation, impart an acidic taste associated with a drop in pH, thereby helping to inhibit the growth of pathogenic bacteria in the digestive tract (Gao et al., 2021). Acidic conditions are expected to increase feed intake due to the accelerated rate of degradation.

The significant increase in daily body weight gain (BWG) and decrease in feed conversion ratio (FCR) at the 1.00% FS level indicate an enhanced efficiency of nutrient utilization. The mechanism is explained through two synergistic actions of the components from pineapple peel and *Indigofera* leaves. Pineapple peel contains FOS, which functions as a selective prebiotic, while the viable LAB from the yogurt starter acts as a probiotic, together forming a fermented synbiotic (Gibson et al., 2017; Yang et al., 2025). Fructo-oligosaccharides are not digested in the small intestine but are fermented by the colonic microbiota, primarily *Bifidobacterium* and *Lactobacillus* (Ricke, 2018). Fermentation of FOS produces short-chain fatty acids (SCFAs) that lower the intestinal pH, thereby inhibiting the proliferation of pathogenic bacteria such as *Salmonella* and *Escherichia coli*, while simultaneously stimulating the proliferation and differentiation of intestinal villous epithelial cells (Wu et al., 2022). An increased ratio of villus height to crypt depth significantly expands the surface area for nutrient absorption (Awad et al., 2008).

Additionally, *Indigofera* leaves are rich in protein, but their tannin content constitutes a major anti-nutritional factor (Choi and Kim, 2020). Tannins can form insoluble complexes with feed proteins and digestive enzymes, such as trypsin and pepsin, thereby reducing the digestibility of protein and amino acids (Wina et al., 2005). The fermentation process with a consortium of LAB breaks down tannin-protein bonds through the activity of tannase enzymes and the acidic conditions (Jiménez et al., 2014; Jeyakumar and Lawrence, 2022). This degradation liberates the bound protein and amino acids, increasing their availability for absorption in the small intestine (Mullen et al., 2015). Thus, fermentation transforms *Indigofera* leaves from a protein source with limited availability into a more readily digestible protein source. The synergy between the prebiotic action of FOS from pineapple peel and the enhanced nutrient availability from *Indigofera* leaves explains the improvement in growth performance and feed efficiency at higher FS levels. This synergistic action is supported by studies demonstrating that synbiotic supplementation (combining a prebiotic such as FOS with a probiotic, such as *Lactobacillus plantarum*) results in superior outcomes. For instance, Song et al. (2022) reported that a synbiotic composed of *Lactobacillus plantarum* and FOS significantly improved average daily gain and the digestibility of calcium and phosphorus in broilers, while Soumeh et al. (2019) found that adding MOS to fermented soybean meal had synergistic effects, leading to greater body weight gain and enhanced intestinal morphology. The increased lactic acid bacteria populations, decreased intestinal pH, and improved nutrient digestibility observed in the present study collectively support the proposed dual mechanism of action.

The increase in final live weight and carcass percentage at the high supplementation levels (0.75-1.00%) reflected the efficiency of nutrient utilization. The synergy between the enhanced intestinal absorption capacity through FOS and the increased availability of protein substrate from *Indigofera* leaves, liberated from tannin complexes, directs energy and amino acids more efficiently towards the anabolic pathways of muscle protein synthesis, rather than storage as adipose tissue or utilization for basal metabolic requirements. The improved digestibility and nutrient availability, particularly the balance of essential amino acids, optimize the effective energy-to-protein ratio in the diet, thereby reducing excess energy that could potentially be deposited as fat (Leeson and Summers, 2005; Musigwa et al., 2024).

From an economic perspective, the significant increase in Income Over Feed Cost (IOFC) in the FS75 and FS100 treatments indicated that the additional cost incurred by FS supplementation was offset by the increased revenue from chickens with higher live weights and carcass yields. The significantly higher final body weight and carcass yield in the FS75 and FS100 groups support Bollido (2020), who demonstrated that direct profitability is largely determined by final body weight gain. Thus, despite the increase in feed consumption, the proportional increase in body weight and carcass quality resulted in a positive net financial return. Overall, FS supplementation at the 1.00% level was not only effective in leveraging the prebiotic FOS and the probiotic LAB to enhance biological performance but also proved to be economically viable within a commercial broiler production system.

Table 2. Production performance of broiler chickens as affected by dietary fermented synbiotic supplementation

Variables	Treatments				
	FS_0	**FS_{25}**	**FS_{50}**	**FS_{75}**	**FS_{100}**
Feed consumption (g/chicken/day)	76.62 ± 1.48^{a}	76.70 ± 1.63^{ab}	76.94 ± 0.47^{b}	77.05 ± 1.45^{bc}	77.11 ± 1.66^{c}
Body weight gain (g/chicken/day)	56.12 ± 1.78^{a}	56.57 ± 0.58^{a}	57.04 ± 1.50^{a}	57.40 ± 0.77^{a}	59.69 ± 0.41^{b}
Feed conversion ratio (FCR)	1.58 ± 0.10^{b}	1.55 ± 0.05^{b}	1.52 ± 0.03^{ab}	1.50 ± 0.08^{ab}	1.42 ± 0.04^{a}
Live weight (g)	$1{,}625.95 \pm 42.50^{a}$	$1{,}673.00 \pm 51.73^{ab}$	$1{,}687.10 \pm 7.93^{b}$	$1{,}699.25 \pm 20.90^{b}$	$1{,}724.00 \pm 39.08^{b}$
Carcass percentage (%)	71.99 ± 0.90^{a}	72.72 ± 2.85^{a}	72.21 ± 2.21^{a}	74.35 ± 0.91^{a}	77.76 ± 1.60^{b}
Abdominal fat (%)	1.23 ± 0.22^{a}	1.30 ± 0.25^{a}	1.26 ± 0.27^{a}	1.09 ± 0.17^{a}	1.01 ± 0.18^{a}
Income over feed cost (USD)	0.52 ± 0.05^{a}	0.58 ± 0.06^{ab}	0.59 ± 0.01^{ab}	0.60 ± 0.02^{b}	0.62 ± 0.05^{b}

FS_0: Control ratio, FS_{25}: 0.25% FS ratio, FS_{50}: 0.50% FS ratio, FS_{75}: 0.75% FS ratio, FS_{100}: 1% FS ratio. Different superscripts in the same row indicate significant differences ($p < 0.05$)

Organs and the digestive tract

The results showed that supplementation with fermented prebiotics from pineapple peel and *Indigofera zollingeriana* leaves (FS) did not have a significant effect on the relative weights of internal organs, including the liver, heart, gizzard, and pancreas (Table 3; $p > 0.05$). The finding indicated that the supplementation strategy did not impose a physiological burden or toxic effects on these vital organs. Specifically, the relative liver weight ranged from 2.13-2.34%, which falls within the normal range (2.5-3.5%) for healthy broiler chickens (Iqbal et al., 2014). The relative weights of the heart (0.37-0.47%), gizzard (1.92-2.09%), and pancreas (0.19-0.25%) were also consistent with standards reported in the previous studies (Emiola et al., 2007; Hafsan et al., 2021).

The stability of these internal organ weights can be explained by the indirect mechanism of synbiotic action. FS supplementation, particularly at higher levels, increased the population of LAB and decreased the pH of the small intestine (Table 3). A more optimal intestinal environment supports the efficiency of digestion and nutrient absorption, thereby preventing accessory digestive organs such as the liver and pancreas from undergoing hypertrophy due to nutrient deficiencies or the toxic burden from pathogens (Lan et al., 2022; Ringseis and Eder, 2022). Furthermore, the fermentation process that degrades tannins in Indigofera leaves plays a crucial role in maintaining organ function, as dietary tannins have been shown to induce hepatic inflammation (Yuan et al., 2023) and reduce the relative weight of vital organs, including the liver, bursa of Fabricius, and spleen (Choi and Kim, 2020; Hidayat et al., 2021). Undegraded tannins can form complexes with proteins and minerals, potentially inducing oxidative stress and burdening the detoxification function of the liver (Wina et al., 2005). By reducing tannins through fermentation, the risk of anti-nutritional effects can be minimized, thereby maintaining stable organ function and morphology, as reported in other studies using natural ingredients (Choi and Kim, 2020; Mahawan et al., 2025).

Compared to the control group, FS supplementation at 0.75% and 1.00% significantly decreased small intestinal pH, and supplementation at 0.50%, 0.75%, and 1.00% significantly increased LAB populations ($p < 0.05$). Small intestinal pH decreased significantly from a neutral condition (7.03 in the control) to a more acidic one (4.56 in FS100), indicating increased fermentative activity by LAB ($p > 0.05$). The pH reduction is driven by two synergistic factors; the FOS substrate from pineapple peel fermented by LAB produces lactic acid as the main product (Cao et al., 2019; Yang et al., 2025), and the inoculation of active LAB from the fermented product, which used yogurt as a starter, means that viable LAB continue to contribute to organic acid production in the digestive tract (Ayivi and Ibrahim, 2022; Li et al., 2024). The decrease in pH creates a selective environment that inhibits the growth of pathogens sensitive to acidic conditions, such as *Salmonella*, while simultaneously supporting the proliferation of LAB (Neal-McKinney et al., 2012; El Baaboua et al., 2018). A lower pH condition enhances the activity of endogenous digestive enzymes and improves intestinal mucosal health (Ma et al., 2021; Han et al., 2023).

The LAB population was significantly higher in the FS50, FS75, and FS100 groups compared to FS0 and FS25 ($p < 0.5$), with no significant differences among FS50, FS75, and FS100 ($p > 0.05$). The highest LAB population (3.83×10^6 CFU/g) was observed in FS100. This increase confirmed the effectiveness of FS as a prebiotic substrate, where FOS from pineapple peel serves as a selective "food" for indigenous LAB (Al-Khalaifah, 2018; Kaewarsar et al., 2023). Furthermore, the reduction in tannin content due to fermentation also supports LAB colonization, as tannins are known to possess antimicrobial activity that can inhibit the growth of both beneficial and pathogenic bacteria (Reverón et al., 2017; Jeyakumar and Lawrence, 2022). Reducing tannin content through fermentation removes an inhibitory barrier, thereby allowing LAB to colonize more effectively and compete for nutrients and adhesion sites (Zhang et al., 2023). The increase in the LAB population creates a biological barrier effect through competitive exclusion of pathogens (Adeyemi and Nahashon, 2026). Conversely, the *Salmonella typhimurium* population decreased drastically with increasing FS levels, from 0.97×10^6 CFU/g (control) to 0.17×10^6 CFU/g (FS100). The suppression of *Salmonella typhimurium* results from a multifaceted assault by LAB, supported by the modified intestinal environment. The decreased pH is bactericidal or bacteriostatic to enteropathogens (Ringseis and Eder, 2022), while LAB produce antimicrobial metabolites such as bacteriocins, hydrogen peroxide, diacetyl, and reuterin that specifically inhibit pathogens (Chen et al., 2025). Additionally, LAB compete with pathogens for nutrients and adhesion sites on the intestinal epithelium (Hammami et al., 2019) and produce SCFAs from FOS fermentation, which serve as an energy source for colon epithelial cells, strengthening tight junction integrity and reducing pathogen translocation (Lan et al., 2022). Thus, FS supplementation not only increases the LAB population and decreases small intestinal pH but also can effectively suppress *Salmonella typhimurium* colonization through comprehensive biological mechanisms.

Table 3. Effect of fermented synbiotic supplementation on digestive organ weights, intestinal pH, and microbial populations in broiler chickens

Variables	Treatments				
	FS_0	**FS_{25}**	**FS_{50}**	**FS_{75}**	**FS_{100}**
Liver weight percentage (%)	2.34 ± 0.34^a	2.20 ± 0.16^a	2.54 ± 0.22^a	2.13 ± 0.19^a	2.28 ± 0.42^a
Heart weight percentage (%)	0.42 ± 0.02^a	0.37 ± 0.07^a	0.44 ± 0.01^a	0.40 ± 0.12^a	0.47 ± 0.04^a
Gizzard weight percentage (%)	2.04 ± 0.34^a	2.09 ± 0.15^a	2.08 ± 0.50^a	1.92 ± 0.16^a	1.99 ± 0.24^a
Pancreas weight percentage (%)	0.25 ± 0.05^a	0.19 ± 0.06^a	0.23 ± 0.02^a	0.24 ± 0.02^a	0.25 ± 0.03^a
Small intestinal pH	7.03 ± 0.38^b	6.64 ± 0.88^b	6.46 ± 0.18^b	5.10 ± 0.33^a	4.56 ± 0.36^a
Lactic acid bacteria ($\times 10^6$ CFU/g)	1.58 ± 0.49^a	2.37 ± 0.23^a	3.49 ± 0.34^b	3.53 ± 0.20^b	3.83 ± 0.56^b
Salmonella typhimurium ($\times 10^6$ CFU/g)	0.97 ± 0.24^b	0.85 ± 0.43^b	0.67 ± 0.20^b	0.20 ± 0.10^a	0.17 ± 0.06^a

FS0: Control ratio, FS25: 0.25% FS ratio, FS50: 0.50% FS ratio, FS75: 0.75% FS ratio, FS100: 1% FS ratio. Different superscripts in the same row indicate significant differences ($p < 0.05$).

CONCLUSION

Supplementation with fermented synbiotics from pineapple peel and *Indigofera zollingeriana* leaves at high levels (1.00%) was shown to improve growth performance, carcass quality, and income over feed cost (IOFC), while also decreasing small intestinal pH, increasing the lactic acid bacteria (LAB) population, and suppressing pathogenic bacterial growth without affecting relative organ weights. Therefore, this supplementation value is recommended to achieve maximum biological and economic benefits with minimal negative impacts. However, the short duration (28 days) and controlled experimental conditions limit the generalizability of these findings to long-term commercial production systems. Future studies should investigate the underlying molecular mechanisms of the synbiotic's action, including its effects on intestinal gene expression and immune modulation. Long-term feeding trials under commercial conditions are also recommended to confirm the economic benefits and assess the stability and shelf life of the synbiotics.

DECLARATIONS

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Authors' contributions

Rizki Palupi provided the original text, contributed to the experimental design, carried out the experiments, and conducted the statistical analysis. Sofia Sandi and Fitra Yosi contributed to both the statistical analysis and the design of the experiments. Anggriawan Naidilah Tetra Pratama and M. Akbar Wijaya Fikri assisted in carrying out the experiments and collecting the data. All authors analyzed and reviewed the results and then composed the final manuscript. All authors have reviewed and approved the final edition of the manuscript for publication.

Availability of data and materials

The data obtained in the present study can be obtained from the corresponding author upon reasonable request.

Competing interests

The authors have declared no competing interests.

Ethical considerations

Plagiarism, consent to publish, misconduct, data fabrication and/or falsification, double publication and/or submission, and redundancy have been checked by all the authors. During the preparation of this work, the authors used Grammarly solely for language and grammar improvement. No generative artificial intelligence tools were used to generate data, results, or conclusions. The authors take full and sole responsibility for all use of artificial intelligence in the preparation of this manuscript. The authors further affirm that they are solely responsible for the accuracy, originality, and integrity of the final content both before initial submission, during the revision process, and upon final publication. The authors have checked and verified all scientific data, interpretations, and conclusions without reliance on AI for intellectual content.

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