



## The Impact of Inclusion of Fermented Feed with Probiotic Yeast on Performance, Intestinal Histology and Intestinal Microbiota of Broilers

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Article History: 25-169

Received: 07-Jul-25

Revised: 06-Sep-25

Accepted: 07-Sep-25

Online First: 17-Sep-25

### ABSTRACT

This study aims to examine the effect of fermented feed flour (FFF) with probiotic *Saccharomyces sp.* (Sc) on performance, histology and intestinal microflora of broilers. The process of making fermented feed, namely, as much as 1% of *Saccharomyces sp.* yeast (108cfu/g) was mixed into commercial feed, then 5% molasses solution was added to the feed mixture until the water content was 35%. The mixture was then put into a basket made of bamboo and tightly closed. Then, it was incubated at room temperature (27°C) for 3 days. After 3 days, it was dried in the sun for 2 days. Before feeding, the basic feed (commercial ration) was first mixed with TPFSc and stirred until homogeneous. Broilers in Group 1 (n = 60) served as healthy controls and were fed a basic diet without the addition TPFSc. Broilers in Groups 2, 3, and 4 were fed with the addition of 5, 10, and 15% FFFSc per kg of feed. The results showed that growth, feed efficiency, villus height (VH), and the number of lactic acid bacteria in broiler Groups 2 and 3 experienced an increase (P<0.05), while the population of Coliform and *Escherichia coli* in the intestine was decreased (P<0.05). It was concluded that the addition of 10 and 15% FFFSc to commercial feed can increase growth, feed efficiency, villus height, and lactic acid bacteria population, and can reduce the pathogen population in the broiler intestine.

**Key words:** Feed fermentation, Intestinal histology, Intestinal microbiota, Broiler.

### INTRODUCTION

Fermented feed is increasingly popular in the livestock world, due to its ease of production. Fermented feed containing probiotic lactic acid bacteria can improve broiler performance, immune response and gut health by combating harmful bacteria and improving nutrient utilization (Li et al. 2020; Zhu et al. 2023). The use of FF in diets has several advantages, namely it can improve intestinal digestive function (Li et al. 2020), increase intestinal VH so that it can increase protein and dry matter digestibility (Hang et al. 2020). Probiotic microbes can increase intestinal flora and increase the quantity of *Bifidobacterium* and *Lactobacilli* in the small intestine (Rashid et al. 2023).

Fermented feed by lactic acid bacteria has been widely used in poultry production. As reported by Li et al. (2022), the inclusion of fermented feed by lactic acid bacteria at a level of 10 or 15% into the basal ration can increase the population of beneficial bacteria (*Lactobacilli* and *Bifidobacteria spp.*) and reduce *Escherichia coli* and

*Salmonella spp.* in the chicken intestine. Fermented feed has many advantages, including reducing antinutritional factors (trypsin inhibitors, phytic acid, and crude fiber), increasing nutrient digestibility, and improving the development of immune organs (Suprayogi et al. 2022; Zhang et al. 2022; Zhu et al. 2023).

Another advantage of adding fermented feed by lactic acid bacteria to the basal ration is that it has been shown to improve the histology (villi height and crypt depth) of the duodenum and jejunum (Li et al. 2020). According to Bai et al. (2020), increasing villus height and crypt depth can have an impact on the morphology of the small intestine, which reflects increased nutrient absorption. Conversely, excessive provision of fermented feed (up to 25%) can have an impact on decreasing broiler growth performance (Zhu et al. 2023).

There is still little information about commercial feed fermentation using yeast culture (*Saccharomyces sp.*) on performance, intestinal histology and microflora of the digestive tract of broilers, so it is very important to conduct this study with the aim to determine the effect of providing

**Cite This Article as:** Bidura IGNG, Wibawa AAPP, Bulkaini, Siti NW and Puspani E, 2026. The impact of inclusion of fermented feed with probiotic yeast on performance, intestinal histology and intestinal microbiota of broilers. International Journal of Veterinary Science 15(1): 92-96. <https://doi.org/10.47278/journal.ijvs/2025.102>

fermented feed with *Saccharomyces* sp. yeast on intestinal histology, the number of lactic acid bacteria and *Escherichia coli* in the digestive tract of broilers.

## MATERIALS AND METHODS

### Experimental design and feeding trial

This study used 240 one-day-old male broiler strain CP 707 produced by a Limited Liability Company (PT.) Caroen Phokphan Tbk, Mojokerto, Jawa Timur, Indonesia, divided into four treatment groups and 6 replications. Before feeding, the basic feed (commercial ration) was first mixed with FFFSc and stirred until homogeneous. Broilers in Group 1 (n=60) served as healthy controls and were fed a basic diet without the addition of FFFSc. Broilers in Groups 2, 3 and 4 were fed with addition of 5, 10 and 15% FFFSc per kg of feed.

During the experimental period, all birds were fed and watered *ad libitum*. All birds were housed in controlled cages. Lighting was provided 24 hours a day.

### Wet and dry fermentation feed process.

The basic feed used is commercial feed brand CP 511 for broiler chickens aged 0-5 weeks. Commercial feed CP 511 is produced by PT. Charoen Phokphan Tbk, Indonesia. The nutrient composition is presented in Table 1. The process of making fermented feed, namely: 1% ( $10^8$ cfu/g) yeast starter culture was inoculated into the commercial feed substrate (CP 511) for fermentation, which was then mixed and added with 5% molasses solution in sterile water until the water content was 35%. The mixture was then put into a bamboo basket and incubated at 27°C for approximately 60 hours. After approximately 60 hours of incubation, the FF was then opened and dried in the sun or oven at a temperature <35°C until dry (Zhu et al. 2023).

Before feeding, the basic feed (commercial ration) was mixed with dry fermented feed by *Saccharomyces* sp. (FFFSc) with different proportions (5, 10 and 15%). This mixed feed was used as experimental feed, while the control was a commercial ration without FFFSc. All treatments were repeated 6 times and each repetition used 40 DOC broiler strain CP 707 with homogeneous body weight.

### Observed variables

Measurements of BW, LWG, feed intake (FI), and feed efficiency (FE= FI/LWG) were carried out on d 56. Histological observation of the jejunum organs was carried

out by slaughtering 6 birds from each treatment and observation according to the procedure (Ermayanti et al. 2021). Duodenal tissue was processed for the preparation of histological preparations with Hematoxylin and Eosin staining; 200x magnification. Histological examination of the duodenum was carried out by measuring the height of the villi (VH), crypt depth (CD) and the ratio of VH to CD. The total number of microbes was determined using Plate Count Agar (PCA), a non-selective medium for growing mesophilic aerobic bacteria. Coliforms and *E. coli* were isolated using the spread method on EMBA (Endo-Metallic Bile Agar), a selective differential medium designed to detect these bacteria based on the procedure of Sudatri (2021).

Meanwhile, the method used to obtain the total Coliform and *E. coli* bacteria was the spread method, using EMBA media according to the procedure (Sudatri 2021).

### Statistical Analysis

Data were analyzed by one-way ANOVA. Differences between treatments were compared using Duncan's method. Significance was done at  $P \leq 0.05$  (Steel and Torrie 2015).

## RESULTS

As shown in Table 2, the addition of FFFSc in commercial rations showed a significant effect ( $P < 0.05$ ) on BW, LWG, and feed efficiency (FI/LWG). Broiler groups 3 and 4 had BW, namely: 11.62 and 10.24% higher ( $P < 0.05$ ) than broiler group 1. Likewise, LWG in broiler groups 3 and 4, namely: 011.89 and 10.49% was significantly higher than broiler groups 1 and 2. Feed efficiency in broiler groups 3 and 4, namely: 6.59 and 5.99% significantly higher than broiler group 1.

The addition of FFFSc at levels of 10 and 15% in broiler rations from 0-5 weeks of age can increase ( $P < 0.05$ ) the population of lactic acid bacteria and reduce pathogens in the intestine (Table 3).

As presented in Table 4, the addition of FFFSc to commercial feed has an effect ( $P < 0.05$ ) on the height of duodenal villi. The addition of FFFSc in commercial feed at levels 5; 10 and 15% significantly ( $P < 0.05$ ) increased the height of duodenal villi in broilers, namely: 25.62, 47.04 and 39.92% higher than the control. However, at levels 5; 10; and 15% the addition of TPFSc in commercial feed had no effect ( $P > 0.05$ ) on villus width, crypt depth, and the ratio of villus height to crypt depth. More details are presented in Table 4.

**Table 1:** Nutritional content of CP 511 broiler feed

Chemical composition#	FFFSc levels in commercial rations (g/100g)				Standard
Gross energy, kcal/kg	4275	4272	4269	4265	-
Crude protein, %	22.42	22.51	22.61	22.70	Min 18%
Ash, %	6.86	6.87	6.88	6.88	
Crude fibre, %	5.53	5.54	5.55	5.55	Max 6.0%

# Results of Animal Feed Nutrition Laboratory analysis, Udayana University, Denpasar, Indonesia; Indonesian National Standard (SNI), 2006.

**Table 2:** Effect of adding FFFSc in diet on broiler performance

Variable	FFFSc levels in commercial rations (g/100 g)				SE
	0	5	10	15	
Initial body weight, g/head	47.06 <sup>a</sup>	46.95 <sup>a</sup>	47.19 <sup>a</sup>	46.98 <sup>a</sup>	0.513
FI, g/head/35 days	3284.37 <sup>a</sup>	3276.99 <sup>a</sup>	3432.86 <sup>a</sup>	3411.53 <sup>a</sup>	59.194
BW, g/head	2013.75 <sup>a</sup>	2021.04 <sup>a</sup>	2247.74 <sup>b</sup>	2219.93 <sup>b</sup>	29.641
LWG, g/head/35 days	1966.69 <sup>a</sup>	1974.09 <sup>a</sup>	2200.55 <sup>b</sup>	2172.95 <sup>b</sup>	27.078
FCR (FI/LWG)	1.67 <sup>a</sup>	1.66 <sup>a</sup>	1.56 <sup>b</sup>	1.57 <sup>b</sup>	0.025

Note: Values with different letters in the same row indicate significant differences ( $P < 0.05$ ).

**Table 3:** Effect of giving FFFSc in broiler rations aged 0-5 weeks on the number of Coliform and *E. coli* bacteria in the intestine

Variable	FFFSc levels in commercial rations (g/100 g)				Normal
	0	5	10	15	
Total Coliform, cfu/g	4.72 x 10 <sup>5</sup> ± 0.29 x 10 <sup>5</sup> a	3.98 x 10 <sup>5</sup> ± 0.21 x 10 <sup>5</sup> a	5.63 x 10 <sup>4</sup> ± 0.13x10 <sup>4</sup> b	5.29 x 10 <sup>4</sup> ± 0.17 x 10 <sup>4</sup> b	4.0 x 10 <sup>6</sup> – 9.4 x 10 <sup>6</sup>
Total <i>E. Coli</i> , cfu/g	7.91 x 10 <sup>3</sup> ± 0.13 x 10 <sup>3</sup> a	6.98 x 10 <sup>3</sup> ± 0.21 x 10 <sup>3</sup> a	4.92 x 10 <sup>3</sup> ± 0.19 x 10 <sup>3</sup> b	3.85 x 10 <sup>3</sup> ± 0.17 x 10 <sup>3</sup> b	10 <sup>4</sup> - 10 <sup>5</sup>
Total lactic acid bacteria, cfu/g	1.35x10 <sup>5</sup> 0.09x10 <sup>5</sup>	2.07x10 <sup>5</sup> ± 0.12x10 <sup>5</sup>	3.98x10 <sup>6</sup> ± 0.08x10 <sup>6</sup> b	2.87x10 <sup>6</sup> ± 0.11x10 <sup>6</sup> b	-

Note: Values with different letters in the same row indicate significant differences (P<0.05); Cfu = colony forming units.

**Table 4:** Effect of inclusion of fermented feed in broiler rations from 0-5 weeks of age on jejunum histology (200x magnification)

Variable	FFFSc levels in commercial rations (g/100 g)				SE
	0	5	10	15	
Villi height, µm	1001.70a	1258.37b	1472.86b	1401.54b	59.651
Villi width, µm	174.68a	187.17a	197.14a	141.5a	31.037
Crypt depth, µm	66.39a	104.74a	139.68a	121.54a	35.526
Ratio VG/CD	15.09a	12.01a	10.54a	11.53a	2.732

Note: Values with different letters in the same row indicate significant differences (P<0.05).

## DISCUSSION

The inclusion of FFFSc feed in commercial rations at a level of 10-15% significantly improves broiler performance (LWG and feed efficiency). This increase is due to the fermentation product containing probiotics that help maintain the health of the broiler digestive tract (Nurrady et al. 2018) and more palatable feed (Nurhayati et al. 2025). In addition, fermented feed products cause more bacteria to grow, so that more enzymes are produced, especially cellulase enzymes that can help digest crude fiber (Sudarmono et al. 2016).

Several researchers (Hasan et al. 2016; Su et al. 2022; Liu et al. 2023) stated that in fermented feed products by probiotics, there will be an increase in probiotic microbes, enzymes, decompose antinutrients into more easily digestible compounds and can increase the crude protein content of feed (Nurhayati et al. 2020). The increase in broiler performance due to the administration of FFFSc is due to the increase in protein, phosphorus, and acid content in the feed (Arsene et al. 2021) and the increase in dry matter and dietary protein digestibility (Hang et al. 2020), increasing the activity of digestive enzymes (Rashid et al. 2023) and duodenal protease enzyme activity (Li et al. 2020). This research is supported by several researchers (Hasan et al. 2016; Moniño et al. 2023; Khonyoung et al. 2025) who reported that the inclusion of fermented feed products by probiotics (*Saccharomyces* sp.) significantly increases LWG and feed efficiency.

There was an increase in the number of lactic acid bacteria in broiler groups 3 and 4, and conversely there was a decrease in the pathogen population in the digestive tract of broilers. The decrease in the number of pathogens in the intestines and the increase in the number of intestinal lactic acid bacteria can contribute to increased feed efficiency (Momtazan et al. 2011). Li et al. (2020) also reported that the addition of probiotic fermented feed to commercial feed at levels of 10% or 15% significantly increased the population of beneficial *Bifidobacteria* spp. while decreasing the population of pathogenic *E. coli* and *Salmonella* spp. in a dose-dependent manner, indicating a positive linear relationship between the amount of fermented feed and the reduction of these pathogens. Research, including work by Rashid et al. (2023), supports the idea that probiotics can help regulate and

enrich the gut microbiota by increasing beneficial bacteria, such as *Lactobacillus* and *Bifidobacterium*, in the poultry gut.

Understanding the complex ecology of the gut is critical for breakthroughs in this form of therapy (Rashid et al. 2023), as immune regulation can be enhanced by changes in the microbial population induced by probiotics (Anwar and Rahman 2016). According to Zhu et al. (2023), the inclusion of fermented feed in the diet can accelerate the flow of digestion into the cecum so that the production of lactic acid is greater which causes a decrease in intestinal acidity. Probiotics have good effects on poultry, such as increasing the concentration of beneficial bacteria and decreasing the concentration of *E. coli* bacteria (Zurmiati et al. 2014).

The height of broiler jejunal villi increased with the presence of FFFSc in the feed. Short crypt depth, villus height, greater VH/CD ratio and other intestinal morphological factors indicate increased intestinal health in the absorption of nutrients (Shroyer and Kocoshis 2011). The addition of FFFSc to the diet can increase protein digestibility and duodenal VH (Hang et al. 2020), improve digestive function, so that nutrient utilization can be more optimal (Li et al. 2020). According to Rashid et al. (2023), probiotic supplementation in feed can increase digestive enzyme activity, and improve FI. In line with Ismael et al. (2022), Sc supplementation in feed can improve intestinal health as measured by increased VH. According to Bai et al. (2020), a higher VH: CD ratio is associated with better nutrient absorption in the poultry digestive tract. In contrast, Anand Laxmi et al. (2017) reported that supplementation of fermented yeast culture in feed had no effect on jejunal morphology of laying hens.

## Conclusion

The addition of 10 and 15% FFFSc in commercial feed can increase growth, feed efficiency, villus height, lactic acid bacteria, and reduce the pathogen population in the broiler intestine. So, the inclusion of FFFSc in feed is very potential as an alternative to antibiotics. The types of probiotic microbes used as fermentation inoculants, and their combinations, and the level of addition in the ration need to be studied for future research.

## DECLARATIONS

**Funding:** This research article was funded by the Rector of Udayana University, Denpasar, under Senior Academic Research Grant Number: B/229.230/UN14.4.A/PT.01.03/2025.

**Acknowledgement:** The researcher would like to thank the Rector, Udayana University, Denpasar, Indonesia who has provided research funds, through the Udayana Excellent Research Grant, so that this research can be completed properly.

**Conflict of Interest:** None.

**Data Availability:** Data supporting this study are available from the corresponding author upon reasonable request.

**Ethics Statement:** This research has received permission from the Animal Ethics Commission number: B/99/UN14.2.9/PT.01.04/2025, from the Faculty of Veterinary Medicine, Denpasar, Indonesia.

**Author's Contribution:** IGNGB, NLPS, B, NW and EP, all jointly participated in the research and the preparation of this manuscript.

**Generative AI Statement:** The authors declare that no Gen AI/DeepSeek was used in the writing/creation of this manuscript.

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