Research Article

Bayang Ducks Supplemented with Probiotic *Bacillus subtilis* FNCC 0059: its Effects on the Physicochemical Characteristics of Meat

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Abstract | The use of probiotics as supplements for ducks to improve meat quality has been extensively studied. This study aimed to determine the effects of probiotic administration (*Bacillus subtilis* FNCC 0059) on the proximate composition and physical characteristics (color, water-holding capacity, cooking loss, and hardness) of Bayang duck meat. This study used five treatments, with four replicates for each treatment. Probiotic supplementation at four different concentrations (76×10^6, 69×10^8, 65×10^{10}, and 53×10^{12} CFU/mL) was performed using drinking water. Probiotic supplementation resulted in a significant difference ($P<0.05$) in meat lightness (L*) from 35.21 (control) to 40.91 (treatment group) and water-holding capacity from 64.31% (control) to 72.70% (group treatment), but had no effect ($P>0.05$) on a*, b*, cooking loss, and meat hardness. The administration of probiotics (*Bacillus subtilis* FNCC 0059) also did not have a significant effect ($P>0.05$) on the moisture, ash, crude protein, and crude fat content of the meat. Administration of probiotics (*Bacillus subtilis* FNCC 0059) up to 53×10^{12} CFU/mL affected the lightness and water-holding capacity of bayang duck meat.

Keywords | *Bacillus subtilis* FNCC 0059, Bayang duck, Meat quality, Physicochemical characteristics, Probiotic

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INTRODUCTION

The use of probiotics in animal diets has become popular, especially as substitutes for antibiotics. This is because probiotics can maintain gastrointestinal health and control infections caused by pathogenic bacteria in livestock (Zhang et al., 2016). Additionally, probiotics can affect meat characteristics in various ways. Dietary inclusion of *Bacillus subtilis* could reduce cooking loss, shear force, and increase the total essential amino acids in broiler chickens (Tang et al., 2021). Stęczny and Kokoszynski, (2019) also reported that the utilisation of commercial probiotics can reduce fat content of chicken meat. The use of *Lactobacillus johnsonii* BS15 can reduce cholesterol and triglyceride levels and increase total amino acids and flavor-related amino acids in chicken meat (Liu et al., 2017). On the contrary, alternative research endeavours have yielded findings indicating that the use of probiotics does not significantly affect the nutritional value or physical quality of meat (Zhou et al., 2010; Sari et al., 2019).

Many studies have reported that feed and supplementation influence the characteristics of the resulting meat, including moisture content (Hassan and Komilus, 2020), crude fat and cholesterol content (Saturno et al., 2020), pH (Liu et al., 2017), color, drip loss, shear force (Pokoo-Aikins et al., 2022), and sensory properties (Pestana et al., 2023). Characteristics of meat are closely related to its quality, which is a concern for customers. Nutritional value, sensory attributes, processing, and hygienic-toxicological quality are the main characteristics of meat (Becker, 2000). The sensory attributes of meat are generally well regarded by consumers, who also possess knowledge of its nutritional advantages. However, they also express concerns regarding its fat content (de Araújo et al., 2022). The diet of animals is one of the most essential factors influencing meat characteristics.

Lactobacillus acidophilus is a probiotic that has been shown to have a positive effect on ducks. Zurmiati et al. (2017) studied the administration of *Bacillus amyloliquefaciens* to enhance the growth performance and feed efficiency of Pitalah ducks. However, the effects of *Bacillus sp.* probiotics on meat quality, especially in Bayang ducks, remain unknown. Bayang ducks are a local Indonesian duck breed originating from West Sumatra that has the potential to be developed as meat-producing ducks with excellent adaptability to the environment (Sarbaini et al., 2018). The Bayang duck breed is highly favored by livestock farmers because of its superior meat production in terms of both quality and quantity (Arlina and Sabrina, 2023). Duck farming using probiotics may benefit both the meat industry and consumers. This strategy may improve meat quality, encourage sustainable production, and provide safer and healthier meat products for customers. Therefore, the objective of this study was to investigate the effect of probiotic *Bacillus subtilis* FNCC 0059 supplementation on the proximate composition and physical characteristics of Bayang duck meat to optimize its use in the poultry industry.

MATERIALS AND METHODS

Ethical Approval

This study was conducted following the guidelines of ethical animal experimentation according to the Republic of Indonesia Law Number 18 of 2009 regulating livestock and animal health. The procedure of this study was approved by the Research Ethics Committee, Faculty of Medicine, Universitas Andalas, Indonesia (No. 520/UN.16.2/KEP-FK/2023).

Materials

The livestock used were 100 one-day-old Bayang ducks with similar body weights, obtained from a local farm in the Padang area, West Sumatra. The housing used consisted of box-type enclosures, with a total of 20 units, each housing 5 ducks. Each enclosure unit had dimensions of 80 × 60 × 60 cm. For duckling warming, a 60-watt incandescent lamp was used for each box until they reached 3 weeks of age or until their feathers grew, after which the lamp was turned on at night. The probiotic used was *Bacillus subtilis* FNCC 0059, purchased from the Food and Nutrition Culture Collection (Gadjah Mada University, Indonesia).

Feeding programme and Experimental design

The ducks were allowed a 1-week adaptation period to drinking water without probiotics. Feeding and drinking water were provided according to the ducks' needs from 1 to 8 weeks of age. The probiotic *Bacillus subtilis* FNCC 0059 was administered through drinking water once every 7 days and started in the second week and continued until the seventh week. Probiotics were administered according to the treatment groups, which were the control group without probiotics and probiotics at concentrations of 76 × 10^6 (P1), 69 × 10^8 (P2), 65 × 10^{10} (P3), and 53 × 10^{12} (P4) CFU/mL. Feed and drinking water were provided *ad libitum*. The formulation, nutrient content and metabolic energy of the feed are shown in Table 1. After the eighth week, four ducks from each group were slaughtered for breast meat sample collection. Samples were stored in polyethylene bags at 4°C until further analysis.

Table 1: Feed ingredients, nutrient content, and metabolic energy of Bayang duck feed

Feed ingredients	Composition, nutrient content and metabolic energy
Corn (%)	59.00
Rice bran (%)	13.00
Soybean meal (%)	18.5
Fish meal (%)	7.00
Premix (%)	0.70
Coconut oil (%)	1.80
Total (%)	100
Crude protein (%)	20.25
Crude fibre (%)	5.53
Crude fat (%)	4.67
Calcium (%)	0.83
Phosphorus (%)	0.38
Metabolic energy (Kcal/kg)	2905.90

Proximate analysis

The determination of the proximate composition of the samples was conducted in accordance with the guidelines provided by the Association of Official Analytical Chemists (AOAC) in 2016. The moisture content was assessed via the oven method, the protein content was determined utilising the Kjeldahl method, the fat content was determined utilising the Soxhlet extraction method, and the ash content was calculated employing the dry-ashing method.

Water Holding Capacity

Water-holding capacity was determined according to the method described by Carvalho et al. (2017), with slight modifications. The samples were cut into cubes of 1.0 g. The samples were positioned between two sheets of filter paper and subjected to a 1-kilogram force for 5 min. The samples were measured in terms of weight, and the water holding capacity (WHC) was calculated using the equation: 100 - [(a - b/a) x 100], where a represents the beginning weight of the sample and b represents the final weight.

Cooking Loss

Cooking Loss was determined according to the method described by Liu et al. (2022). The weighing of each sample was followed by their placement in polyethylene bags, after which they were subjected to heating in water at a temperature of 80°C for 60 min. The samples were subjected to a cooling process, followed by blot drying, and then weighed. The cooking loss was obtained by determining the percentage difference in weight before and after the cooking process.

Color

The color analyses were conducted following the method described by Stęczny and Kokoszynski, (2019), utilising the Hunter colour system approach and using a colorimeter (HunterLab ColorFlex EZ, USA). The digital colorimeter was calibrated using white standards, yielding the following calibration values: L= 94.76, a= -0.795, and b= 2.200. The colour parameter that was measured in this study included the L* value, which represents lightness, the a* value, which represents redness, and the b* value, which represents yellowness.

Hardness

Hardness was determined following the method described by Stęczny and Kokoszynski (2019). Samples of breast meat were cut into pieces measuring 1 x 1 x cm and were measured using a texture analyzer (CT-3 Brookfield, USA) with some modifications, utilizing a single compression cycle and a 10 mm probe.

Data analysis

A randomized block design was used in the study, and results were provided in mean and standard error of the mean (SEM). Statistical analysis was conducted using SPSS software version 16.0 (SPSS Inc., USA), and assessment of significant differences was performed at a 5% level of probability using Duncan's multiple range test (DMRT).

RESULTS AND DISCUSSION

Proximate composition

The proximate composition of Bayang duck breast meat supplemented with Bacillus subtilis FNCC 0059 is shown in Table 2. The findings indicated that probiotic supplementation did not have a significant impact ($P>0.05$) on moisture, ash, protein, and fat content among the treatment groups. In terms of ash content, the groups supplemented with probiotics tended to have lower ash content than the control group (p-value 0.25). There was a slight increase in crude protein content with higher levels of probiotic supplementation, although the difference was not statistically significant.

Interestingly, in this study, the crude fat content of Bayang duck breast meat treated with various concentrations of probiotics tended to be higher than that of the control group (p-value 0.30). Pietrzak et al. (2009) reported similar results where feeding with Enterococcus faecium NCIMB 10415 resulted in high fat content in chicken breast meat. Hascik et al. (2011) reported that the supplementation with Lactobacillus fermentum in hybrid chicks (Ross 308, Hubbard JV, Cobb 500) thigh meat tended to increase protein and fat content in the probiotic group compared to the control, although it was not statistically significant, which aligns with the pattern observed in this study.

Several studies have reported that the administration of probiotics may reduce fat digestibility by inhibiting pancreatic lipase activity, which plays a pivotal role in the absorption of fat in the intestines. This inhibition occurs as a result of deconjugation of bile salts or bile acids, induced by the bile salt hydrolase activity of lactobacilli within the intestinal tract (Sharifi et al., 2012; Knarreborg et al., 2003). Therefore, the indirect reduction in fat absorption is contingent on the bile salt hydrolase activity of lactobacilli in the intestines. This mechanism can explain the phenomena observed in this study, where it is possible that the administration of Bacillus subtilis FNCC 0059 at a concentration of 53×10^{12} CFU/mL may not yet provide the necessary bile salt hydrolase activity to inhibit pancreatic lipase activity in the intestines.

Other studies have reported that probiotic supplementation can reduce fat content in chicken meat, especially in the thigh meat (Stęczny and Kokoszynski, 2019; Tang et al., 2021). However, the impact of probiotics on the proximate composition of meat remains to yield diverse outcomes among reported studies. The observed disparities in results can be attributed to variances in cultural durations, the age of experimental animals, and the various approaches employed for probiotic delivery (Zhou et al., 2010; Stęczny and Kokoszynski, 2019).

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Table 2: The effect of probiotic supplementation on the proximate composition of Bayang duck meat

Parameters	Control	P1	P2	P3	P4	SEM	*P*-value
Moisture (%)	74.83[a]	74.12[a]	75.31[a]	72.74[a]	74.11[a]	0.38	0.26
Ash (%)	1.63[a]	1.48[a]	1.08[a]	1.44[a]	1.32[a]	0.08	0.25
Crude Protein (%)	20.02[a]	20.58[a]	20.75[a]	21.60[a]	21.56[a]	0.27	0.30
Crude Fat (%)	2.24[a]	2.74[a]	2.80[a]	2.88[a]	2.71[a]	0.10	0.30

* Significant differences (P<0.05) are indicated by means with distinct superscripts in the same row. Probiotics concentrations: 76 × 10^6 (P1), 69 × 10^8 (P2), 65 × 10^{10} (P3), and 53 × 10^{12} (P4) CFU/mL.

Table 3: The effect of probiotic supplementation on the color, water-holding capacity, cooking loss, and hardness of Bayang duck meat

Parameters	Control	P1	P2	P3	P4	SEM	*P*-value
Color							
L*	35.21[a]	40.52[b]	40.91[b]	39.83[b]	39.67[b]	0.68	0.03
a*	10.23	10.40	10.08	10.44	9.63	0.30	0.94
b*	9.10	9.89	10.34	9.32	9.52	0.20	0.35
Water Holding Capacity (%)	64.31[a]	67.53[ab]	72.70[b]	69.09[ab]	70.26[b]	0.90	0.02
Cooking loss (%)	41.70	38.56	39.56	37.48	37.60	1.09	0.78
Hardness (N)	15.78	13.22	14.46	16.36	16.91	0.56	0.22

* Significant differences (P<0.05) are indicated by means with distinct superscripts in the same row. Probiotics concentrations: 76 × 10^6 (P1), 69 × 10^8 (P2), 65 × 10^{10} (P3), and 53 × 10^{12} (P4) CFU/mL.

Color

Color is a factor that affects consumer preference for meat (Adeyemi and Sazili, 2014). Our research found that supplementation with Bacillus subtilis FNCC 0059 had a significant effect (P<0.05) on the L* value (lightness) but did not yield a significant impact (P>0.05) on the a* (redness) and b* value (yellowness) (Table 3). The lightness of the meat in all groups that received probiotic supplementation was significantly higher than that of the control group, but there was no significant difference among the probiotic groups.

The lightness of meat color is related to the type of muscle fiber it possesses (Liu et al., 2023). Duck meat is characterized by the presence of muscle fiber type IIA, which imparts a bright red color (Kim et al., 2008; Lu et al., 2023). This suggests that probiotic supplementation in this study may help maintain the bright red color characteristic of meat. The higher lightness values in this study tended to correspond to a brighter red color, although regrettably, this was not accompanied by an increase in the a* value. Similar with our findings, Abdulla et al. (2017) documented that probiotic supplementation increased the L* value in chicken meat, but was also accompanied by a significant decrease in the a* value compared to the control, which was not observed in our study.

On the other hand, an increase in meat lightness is associated with the ability of probiotics to enhance myosin content, leading to increased lightness and a corresponding increase in protein mass in meat (Abdurrahman et al., 2016). In contrast, Liu et al. (2018) reported different findings, wherein the administration of Clostridium butyricum probiotics significantly increased the a* value in Peking duck meat. This increase was associated with the capacity of probiotics to counteract pH decline, which can lead to protein denaturation in the muscle, potentially affecting meat color (Carvalho, 2017).

Water Holding Capacity

The water-holding capacity (WHC) of meat is a significant characteristic pertaining to the quality of meat. It signifies the meat's potential to retain water during several stages, including storage, preparation, and cooking. Supplementation with Bacillus subtilis FNCC 0059 had a significant effect (P<0.05) on water-holding capacity (Table 3). The groups that received probiotic supplementation had higher water-holding capacity values than the control group (64.31%), especially in the groups receiving probiotics with concentrations of 69 × 10^8 and 53 × 10^{12} (72.70% and 70.26%, respectively). The findings of this study are in line with those previously reported by Mohammed et al. (2021), where the WHC value of broiler chicken meat increased with higher concentrations of Bacillus subtilis supplementation. The addition of 0.35% and 0.45% probiotics to the feed resulted in a significant increase in the WHC of Cherry Valley duck meat (Hassan and Komilus, 2020).

The higher WHC in the group receiving probiotics may be attributed to their ability to inhibit the post-slaughter

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pH decline in meat, thereby slowing down protein denaturation. Protein denaturation results in loss of the ability of the protein to bind water (Huff-Lonergan and Lonergan, 2005), leading to reduced WHC. Barbut et al. (2008) also reported that WHC is closely associated with denaturation of sarcoplasmic and myosin proteins, which can lead to a decrease in their water-binding capacity. Higher WHC values provide an advantage for retaining water in meat, which in turn affects meat tenderness.

Cooking Loos

Supplementation with Bacillus subtilis FNCC 0059 had no significant effect (P>0.05) on cooking loss in Bayang duck breast meat (Table 3). This finding is in line with several other studies that have reported that probiotic supplementation does not significantly affect the cooking loss value, although it tends to decrease the value in probiotic treatment groups compared to the control (Liu et al., 2022; Sari et al., 2019; Tang et al., 2021). Abdulla et al. (2017) reported that probiotic supplementation significantly reduced cooking loss in broiler chicken breast meat.

According to Mohammed et al. (2021), cooking loss is related to meat WHC, and probiotic diet supplementation can increase WHC and reduce cooking loss. However, this was not observed in this study. The increase in cooking loss due to prebiotic supplementation may be attributed to increased absorption of crude fiber in the small intestine, leading to increased intramuscular fat binding, which can suppress the excretion of liquid during the thermal treatment of meat. This increase in fiber content is due to the high lactic acid produced by lactic acid bacteria (Natsir et al., 2023). Similar to WHC, cooking loss also affects meat characteristics, particularly tenderness.

Hardness

Supplementation with *Bacillus subtilis* FNCC 0059 did not have a significant effect (P>0.05) on the hardness of Bayang duck breast meat in any of the treatment groups (Table 3). A comparable result was documented by Abdulla et al. (2017), who found that the addition of *Bacillus subtilis* probiotic supplements did not significantly affect the hardness of chicken meat. Other studies have also reported that probiotic supplementation in Pegagan ducks did not affect WHC, cooking loss, or meat hardness (Sari et al., 2019). In contrast, Liu et al. (2018) reported that the administration of *Clostridium butyricum* to Peking ducks resulted in a significant decrease in meat hardness compared with the control.

Meat hardness was influenced by WHC and cooking loss. In this study, although probiotic supplementation led to a significant increase in WHC percentage, it was not accompanied by a decrease in cooking loss and meat hardness. Changes in meat hardness are related to changes in collagen concentration (Maiorano et al., 2012). This suggests that supplementation with *Bacillus subtilis* FNCC 0059 probiotics may not affect the concentration of collagen in the meat. Hardness is a meat quality attribute that affects consumer preference, as consumers tend to prefer meat with low hardness values, indicating tender meat (de Araujo et al., 2022). However, the hardness of duck meat has been reported to vary in other studies, ranging from 4.21 to 37.36 N, and is influenced by the genetic characteristics of duck types and the age at slaughter (Kokoszynski et al., 2020; Muhlisin et al., 2013).

CONCLUSION

Supplementation of Bayang ducks with *B. subtilis* FNCC 0059 in drinking water resulted in brighter breast meat color in all treatments compared to the control. The probiotic treatment groups also had higher water holding capacity values than the control, although this did not have a significant impact on cooking loss and meat hardness values, as well as a* (redness) and b* (yellowness) values. In terms of proximate composition, no parameters (moisture, ash, protein, and fat content) differed significantly between the treatment and control groups. However, this study only examined meat quality and its impact on meat products and consumer preferences remains unknown. It is essential to conduct additional studies on the incorporation of meat into processed meat products to evaluate the sensory attributes of the resulting meat, with a special emphasis on comprehending its impact on customer preferences.

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NOVELTY STATEMENT

No studies have been published on the impact of probiotic Bacillus subtilis FNCC 0059 on the physicochemical properties of bayang duck meat.

AUTHORS CONTRIBUTIONS

The authors collectively participated in the implementation of experimental trials, performed statistical analysis, and contributed to the composition and revision of the text.

CONFLICT OF INTEREST

All authors declare no conflicts of interest and consent to the submission of this work to the journal.

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