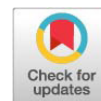


Research Article



Application of *Bacillus amyloliquefaciens* in Fermenting Water Lettuce (*Pistia stratiotes*) as a Feed Source for Mojosari Ducks

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Abstract | This study aims to evaluate the potential and effectiveness of feeding water lettuce leaves fermented with *Bacillus amyloliquefaciens* on the production performance and carcass quality of Mojosari ducks. The research was conducted using a Completely Randomized Design (CRD) with five treatments (0%, 5%, 10%, 15%, and 20% fermented water lettuce leaves) as feed for Mojosari ducks, with four replications each. The results showed that the inclusion of *Bacillus amyloliquefaciens*-fermented water lettuce leaves had a significant effect ($P < 0.05$) on feed consumption, body weight gain, protein intake, carcass percentage, and abdominal fat percentage in Mojosari ducks. Ducks fed diets containing 10% fermented water lettuce demonstrated optimal results with feed consumption ($6,451.25 \pm 53.99$ g/head), body weight gain ($1,545.21 \pm 105.49$ g/head), protein intake ($1,288.25 \pm 10.80$ g/head/period), carcass percentage ($63.78 \pm 0.75\%$), and abdominal fat percentage ($2.16 \pm 0.05\%$) compared to other treatments. The feed conversion ratio was not significantly different ($P > 0.05$) among treatments, ranging from 4.08 ± 0.22 to 4.46 ± 0.16 . The 10% inclusion level was identified as optimal, providing the best balance between production performance and feed cost efficiency. The study concludes that fermented water lettuce leaves (*Pistia stratiotes*) using *Bacillus amyloliquefaciens* can be included up to 10% in the diet without compromising the performance and carcass quality of Mojosari ducks.

Keywords | *Pistia stratiotes*, *Bacillus amyloliquefaciens*, Feed fermentation, Mojosari ducks, Production performance, Carcass quality

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INTRODUCTION

The production of local poultry, particularly Mojosari ducks, holds significant potential in supporting na-

tional food security and improving the economy of small-scale farmers. Mojosari ducks, as one of Indonesia's superior genetic resources, have a strategic value in the development of local livestock industries (Hidanah *et al.*, 2018). How-

ever, one of the main challenges in Mojosari duck farming is the high cost of feed, which can account for more than 70% of total production costs (Maulana, 2023). The heavy reliance on conventional feed ingredients such as corn and soybean meal further increase expenses, especially amidst global price fluctuations (Febrina *et al.*, 2022). Therefore, there is a need for innovations in the provision of alternative feeds that are affordable, high-quality, and accessible to local farmers (Hidayat *et al.*, 2024).

Water lettuce (*Pistia stratiotes*), an aquatic plant that grows wild and rapidly in tropical waters, is often considered an invasive weed that disrupts ecosystems (Yudhitstira *et al.*, 2015). However, its potential as a feed ingredient for Mojosari ducks lies in its nutritional content. The nutritional composition of water lettuce leaves, based on the study by Wasagu *et al.* (2013), shows that this plant contains $4.50 \pm 0.50\%$ moisture, $17.50 \pm 0.87\%$ crude fiber, $2.17 \pm 0.29\%$ crude fat, $6.96 \pm 0.13\%$ crude protein, $35.20 \pm 1.56\%$ ash, and $38.20 \pm 2.08\%$ carbohydrates, with these values calculated on a dry matter basis. In addition, water lettuce leaves contain polyphenolic compounds and exhibit antioxidant activity (Sudirman *et al.*, 2022).

Fermented feeds are increasingly being explored as functional foods in poultry nutrition, as they not only supply essential nutrients but also provide bioactive compounds that enhance gut health, immune response, and overall performance (Sugiharto, 2016). The fermentation process can reduce anti-nutritional factors, increase nutrient bioavailability, and introduce beneficial microbes that contribute to a balanced intestinal microbiota. This functional value makes fermented feed a promising innovation for sustainable poultry production.

The main limitation in using water lettuce leaves as feed for Mojosari ducks lies in their high crude fiber content, which reduces digestibility, and the presence of antinutritional compounds such as oxalates, tannins, and phytates that can interfere with digestive enzyme activity and mineral absorption (Sudirman *et al.*, 2024; Minich and Petroski, 2020). The crude fiber content of water lettuce leaves ranges from 17.50 – 20.50%. (Wasagu *et al.*, 2013). Therefore, the application of fermentation biotechnology is necessary to improve their nutritional value and reduce these limiting compounds.

Fermentation using probiotic microbes such as *Bacillus amyloliquefaciens* presents a promising alternative to enhance the digestibility of plant-based feeds like water lettuce leaves. This bacterium is particularly selected for its ability to produce a wide range of digestive enzymes, including cellulase, amylase, and protease, which are specifically capable of breaking down complex cell wall components like cellulose and hemicellulose, which are abundant in water

lettuce leaves. Unlike lactic acid bacteria (LAB) and *Bacillus subtilis*, which are commonly used in fermentation processes for their general ability to improve fermentation efficiency, *Bacillus amyloliquefaciens* has demonstrated higher cellulolytic activity, making it more effective in improving the digestibility of high-fiber materials (Soeka *et al.*, 2023; Irfan *et al.*, 2017; Woldemariam *et al.*, 2020). Furthermore, its ability to survive and proliferate in harsh conditions, such as those present in the gastrointestinal tract of ducks, makes it a particularly suitable candidate for enhancing the nutritional value of feed ingredients. The urgency of this research lies in the need for innovative poultry feeds that are low-cost, efficient, and capable of improving the productivity of Mojosari ducks, especially in light of the rising costs of conventional feed ingredients and their impact on the sustainability of small-scale farming.

Based on these considerations, this study was conducted to evaluate the potential and effectiveness of feeding fermented water lettuce leaves with *Bacillus amyloliquefaciens* on the production performance of Mojosari ducks. The findings are expected to contribute to the advancement of poultry nutrition science and serve as a practical reference for farmers in formulating affordable and sustainable rations based on local feed ingredients.

MATERIALS AND METHODS

MATERIALS

This study used 200 male day-old Mojosari ducks obtained from a local hatchery, with an average age of one day at the beginning of the experiment. The ducks were reared for a total of 10 weeks. From the second week onwards, fermented water lettuce leaves were added to their feed and continued for 8 weeks.

HOUSING AND EXPERIMENTAL UNITS

The ducks were randomly divided into 20 experimental units, with each unit consisting of 10 ducks. All the ducks used in the experiment were male. The size of the cage per experimental unit was 120 x 120 x 100 cm.

ETHICAL CONSIDERATIONS AND WELFARE

This study adhered to ethical guidelines for the care and treatment of experimental animals. The research complied with institutional animal welfare standards, ensuring that the ducks were provided with adequate care, nutrition, and housing. The ducks were monitored daily for signs of stress or discomfort, and appropriate measures were taken to ensure their welfare.

ENVIRONMENTAL AND CLIMATIC CONDITIONS

The experimental location is situated in a tropical wet climate zone with an Af climate classification according to

Table 1: Feed ingredients and nutritional composition used in this study (all values are based on dry matter).

Feed Ingredients	Crude Protein (%)	Crude Fat (%)	Crude Fiber (%)	Calcium (%)	Phosphorus (%)	Lysine (%)	Methionine (%)	Metabolizable Energy (Kcal/kg)*
Ground Corn	8.58	2.66	3.50	0.38	0.19	0.30	0.19	3375.00
Fine Rice Bran	9.50	5.09	14.50	0.69	0.26	0.67	0.27	1890.00
Fish Meal	25.29	0.00	15.52	1.31	0.05	0.00	0.00	2202.94
Soybean Meal	43.35	2.49	4.50	0.63	0.36	0.60	0.50	2240.00
Meat and Bone Meal	54.00	7.50	0.90	10.00	3.00	2.50	1.80	2678.00
Coconut Oil	0.00	100.00	0.00	0.00	0.00	0.00	0.00	8600.00
Bone Meal	0.00	0.00	0.00	36.75	17.56	0.00	0.00	0.00
L-Lysin	0.00	0.00	0.00	0.00	0.00	99.00	0.00	0.00
Fermented Water Lettuce Leaves	14.26	2.46	13.21	3.50	2.75	0.81	0.73	2284.25

Description: The chemical compositions of feed ingredients and fermented water lettuce leaves were analyzed using standard methods. Moisture content was determined by oven drying at 105°C, crude protein by the Kjeldahl method, and crude fiber by the AOAC method using acid-alkali treatment. Crude fat was extracted with petroleum ether via Soxhlet, and ash content was measured by incineration at 550°C. Carbohydrates were calculated by subtracting moisture, protein, fat, and ash from 100%.

Table 2: Composition and nutritional content of the experimental diet.

Feed Ingredients	Diet (%)				
	A	B	C	D	E
Ground Corn	60.00	59.00	58.00	57.00	55.00
Fine Rice Bran	7.00	5.00	3.00	1.00	0.00
Fish Meal	8.00	6.00	4.00	2.00	0.00
Soybean Meal	8.00	8.00	8.00	8.00	8.00
Meat and Bone Meal	13.00	13.00	13.00	13.00	13.00
Coconut Oil	3.00	3.00	3.00	3.00	3.00
Bone Meal	0.50	0.50	0.50	0.50	0.50
L-Lysin	0.50	0.50	0.50	0.50	0.50
Fermented Water Lettuce Leaves	0.00	5.00	10.00	15.00	20.00
Total	100.00	100.00	100.00	100.00	100.00
Crude Protein	18.32	18.26	18.19	18.12	18.06
Crude Fat	6.13	6.12	6.12	6.11	6.13
Crude Fiber	4.83	4.86	4.88	4.91	5.04
Calcium	1.92	2.05	2.18	2.31	2.44
Phosphorus	0.64	0.77	0.90	1.03	1.16
Lysine	1.09	1.12	1.14	1.17	1.20
Methionine	0.41	0.44	0.47	0.49	0.52
Metabolizable Energy (Kcal/kg)	3118.88	3117.48	3116.08	3114.69	3114.69

Description: Compiled based on Table 1. A= 0% Fermented water lettuce leaves; B= 5% Fermented water lettuce leaves; C= 10% Fermented water lettuce leaves; D= 15% Fermented water lettuce leaves; E= 20% Fermented water lettuce leaves.

Köppen–Geiger. The average daily temperature during the study ranged from 25°C to 32°C, with average relative humidity between 75–90%. The annual rainfall in this region reaches approximately 3,000 mm, and the daily sunlight intensity ranged from 8 to 10 hours.

FEEDING, CARE AND HEALTH MANAGEMENT

The ducks were fed a standard diet consisting of ground corn, fine rice bran, fish meal, soybean meal, meat and bone meal, coconut oil, bone meal, L-lysine, and fermented water lettuce leaves, with the diet adjusted to meet nutritional needs at various growth stages. Fresh drinking water was provided at all times. The feeding schedule was carried out twice daily.

For health management, the ducks were vaccinated according to standard poultry vaccination protocols, including vaccinations for common diseases such as Newcastle disease and avian influenza. Cleanliness was maintained by regularly cleaning the cages, feeders, and drinkers. Medical treatment or the use of medications was only administered when necessary and under the supervision of a licensed veterinarian. The feed ingredients and their nutritional composition used in this study are presented in Table 1. The composition and nutritional content of the experimental diet can be seen in Table 2.

METHODS

The experimental method used in this study was a Completely Randomized Design (CRD) with five treatments and four replications. The treatment variations involved the inclusion of fermented water lettuce leaves (FWLL) in the diet of Mojostari ducks as follows:

A: 0% FWLL
B: 5% FWLL
C: 10% FWLL
D: 15% FWLL
E: 20% FWLL

FERMENTATION PROCESS OF WATER LETTUCE LEAVES WITH *BACILLUS AMYLOLIQUEFACIENS*

The fermentation process of water lettuce leaves with *Bacillus amyloliquefaciens* follows a standardized protocol, which includes the following steps:

PREPARATION OF WATER LETTUCE LEAVES: 50 kg of fresh water lettuce leaves are first sterilized using an autoclave at 121°C for 15 minutes under 1 atm pressure. After sterilization, the leaves are allowed to cool to room temperature.

INOCULATION WITH *BACILLUS AMYLOLIQUEFACIENS*: A 3% (w/w) inoculum of *Bacillus amyloliquefaciens* is prepared. The bacterial strain used in this study is *Bacillus amyloliquefaciens* strain K1, which is known for its ability to produce cellulase, amylase, and protease (Irfan *et al.*, 2017). The inoculum is thoroughly mixed with the cooled water lettuce leaves to ensure even distribution.

FERMENTATION: The inoculated mixture is incubated at 40°C for 4 days to allow fermentation to occur. This temperature was selected based on optimal growth conditions for *Bacillus amyloliquefaciens*, as previously reported by Soeka *et al.* (2023).

DRYING: After the fermentation period, the fermented product is dried under direct sunlight until it reaches a moisture content of approximately 14%, ensuring the product is suitable for storage and use as feed.

The bacterial density of *Bacillus amyloliquefaciens* after fermentation is maintained at approximately 10^8 CFU/g, as determined by standard plate count methods (Woldemariam *et al.*, 2020). The specific strain of *Bacillus amyloliquefaciens* used in this process was chosen for its superior cellulolytic activity, which is crucial for breaking down the complex fibers in the water lettuce leaves.

FERMENTATION CHARACTERISTICS

During the fermentation process, several key indicators were monitored to evaluate the quality and progress of the fermentation. The final pH of the fermented water lettuce leaves was measured at approximately 5.2, indicating successful acidification which helps inhibit pathogenic microbes. The concentration of ammonium nitrogen (NH₃-N) in the final product was found to be 0.12%, reflecting moderate protein degradation and microbial activity. Volatile fatty acids (VFAs), including acetic, propionic, and butyric

acids, were detected in small concentrations, with acetic acid being the most dominant, suggesting active microbial fermentation. These characteristics support the effectiveness of *Bacillus amyloliquefaciens* in enhancing the nutritional quality and microbial safety of the fermented product.

PREPARATION OF *BACILLUS AMYLOLIQUEFACIENS* INOCULUM

The inoculum of *Bacillus amyloliquefaciens* was prepared prior to the fermentation process. A total of 100 grams of rice bran was used as a solid substrate, sterilized at 121°C under 1 atm pressure for 15 minutes, then allowed to cool to room temperature. A pure culture of *Bacillus amyloliquefaciens* strain K1, originally isolated from the rumen of cattle by the Microbiology Laboratory, Faculty of Animal Science, Andalas University, was reactivated on nutrient agar in a Petri dish. This strain had been previously identified morphologically and biochemically and preserved in the laboratory culture collection. Ten milliliters of sterile distilled water was added to the culture and gently shaken to detach the bacterial cells. This microbial suspension was transferred into 190 mL of sterile distilled water in an Erlenmeyer flask, yielding 200 mL of suspension. This was then evenly mixed with the sterile rice bran and incubated for 24 hours at 40°C. After incubation, the mixture was oven-dried at 60°C until it formed a powder with a bacterial density of approximately 1×10^8 CFU/g, ready to be used as fermentation inoculum. Rice bran was selected as a substrate due to its affordability, nutrient content, and prior successful use in solid-state fermentation processes (Mulyawati *et al.*, 2019; Purnomo *et al.*, 2021).

PARAMETERS MEASURED

FEED CONSUMPTION (G/HEAD): Feed consumption was calculated by subtracting the remaining feed from the total feed offered during the experimental period, averaged per duck.

BODY WEIGHT GAIN (G/HEAD): Body weight gain was determined by subtracting the initial body weight from the final body weight at the end of the trial, per duck.

FEED CONVERSION RATIO (FCR): FCR was calculated as the total feed intake divided by the total weight gain for each duck during the experiment.

PROTEIN INTAKE (G/HEAD/PERIOD): Protein intake was estimated by multiplying feed consumption by the crude protein content of the diet. This study did not include dry matter or nutrient digestibility tests due to resource and time limitations, which are noted as areas for future research.

CARCASS PERCENTAGE (%): Carcass percentage was measured by calculating the ratio of carcass weight to live body weight before slaughter, multiplied by 100.

ABDOMINAL FAT PERCENTAGE (%): Abdominal fat percentage was measured by weighing the abdominal fat and dividing it by live body weight, then multiplying by 100.

INCOME OVER FEED COST (IOFC, Rp/HEAD): Income over feed cost was calculated as the difference between revenue from carcass weight and the cost of feed consumed by each duck during the trial.

DATA ANALYSIS

All collected data were analyzed using analysis of variance (ANOVA). If significant differences among treatments are detected, Duncan's multiple range test ($P < 0.05$) was applied for further comparison (Steel and Torrie, 1991).

RESULTS AND DISCUSSION

The effect of feeding water lettuce leaves fermented with *Bacillus amyloliquefaciens* on feed consumption, body weight gain, and feed conversion can be seen in Table 3.

Table 3: Feed consumption, body weight gain, and feed conversion of Mojosari ducks with fermented water lettuce leaves feed.

Treatment	Feed consumption (g/head)	Body weight gain (g/head)	Feed conversion Ratio
A(0%FWLL)	6465.88±98.42 ^a	1587.42±69.10 ^a	4.08 ± 0.22
B(5%FWLL)	6458.63±87.70 ^a	1576.15±92.18 ^a	4.11 ± 0.20
C(10%FWLL)	6451.25±53.99 ^a	1545.21±105.49 ^a	4.18 ± 0.25
D(15%FWLL)	6246.44±99.70 ^b	1412.87±83.12 ^b	4.47 ± 0.28
E(20%FWLL)	6189.56±96.16 ^b	1404.94±43.60 ^b	4.46 ± 0.16

Description: ^{a-b}Different superscripts in the same column are significantly different ($P < 0.05$). FWLL: Fermented water lettuce leaves.

The highest feed consumption in this study was observed in treatment A (0% FWLL) = 6465.88 ± 98.42, B (5% FWLL) = 6458.63 ± 87.70, and C (10% FWLL) = 6451.25 ± 53.99 g/head, while the lowest feed consumption was observed in treatment D (15% FWLL) = 6246.44 ± 99.70 and E (20% FWLL) = 6189.56 ± 96.16 g/head. The statistical analysis showed that the feeding of fermented water lettuce leaves with *Bacillus amyloliquefaciens* had a significant effect ($P < 0.05$) on feed consumption in Mojosari ducks. The Duncan Multiple Range Test (DMRT) indicated that treatment A did not differ from treatments B and C, but it differed significantly ($P < 0.05$) from treatments D and E.

The addition of water lettuce (*Pistia stratiotes*) at 5% (treatment B) and 10% (treatment C) caused a slight decrease in

feed consumption to 6458.63 ± 87.70 grams and 6451.25 ± 53.99 grams, but statistically there was no significant difference compared to the control (A). This suggests that at the addition level of up to 10%, fermented water lettuce leaves do not significantly affect the ducks' appetite or feed consumption. Reduced palatability causes the ducks to be less interested in the feed, thus reducing consumption (Nuraini *et al.*, 2022). Fermentation can also increase organic acid content and produce a distinct aroma (Maulana *et al.*, 2021), which, if too high, may decrease the ducks' appetite.

At higher levels of water lettuce addition, 15% (D) and 20% (E), the feed consumption dropped more sharply to 6246.44 ± 99.70 grams and 6189.56 ± 96.16 grams, respectively. The factors affecting the decrease in Mojosari duck feed consumption include the increased crude fiber content in water lettuce, even after fermentation. High crude fiber content can slow down digestion and reduce nutrient absorption efficiency, ultimately decreasing the ducks' desire to consume more feed (Tholib Zaqi *et al.*, 2019). Other factors that can reduce feed consumption include anti-nutritional factors (Supriatman *et al.*, 2017).

Fermentation with *Bacillus amyloliquefaciens* aims to reduce anti-nutritional compounds such as oxalates, tannins, and lignin in water lettuce. Specific measurements of these compounds before and after fermentation are required to confirm the degree of reduction. Preliminary results showed a decrease in oxalates by 35% (from 5.0% to 3.25%) and tannins by 40% (from 2.5% to 1.5%) after fermentation. However, at higher inclusion levels (15% and 20%), residues of these compounds may still be present in sufficient amounts to potentially affect feed palatability. From this study, it can be concluded that the addition of up to 10% fermented water lettuce in the diet is still acceptable to Mojosari ducks without significantly reducing feed consumption, but any addition beyond 10% tends to decrease consumption. Therefore, the use of fermented water lettuce in feed should be limited to a maximum of 10% to maintain optimal feed consumption.

The highest body weight gain in this study was observed in treatment A (0% FWLL) = 1587.42 ± 69.10, B (5% FWLL) = 1576.15 ± 92.18, and C (10% FWLL) = 1545.21 ± 105.49 g/head, while the lowest body weight gain was observed in treatment D (15% FWLL) = 1412.87 ± 83.12 and E (20% FWLL) = 1404.94 ± 43.60 g/head. The statistical analysis showed that feeding fermented water lettuce leaves with *Bacillus amyloliquefaciens* had a significant effect ($P < 0.05$) on body weight gain in Mojosari ducks. The DMRT stated that treatment A did not differ ($P > 0.05$) from treatments B and C, but differed significantly ($P < 0.05$) from treatments D and E.

This study showed that the use of 5% and 10% fermented water lettuce with *Bacillus amyloliquefaciens* resulted in body weight gains of 1576.15 ± 92.18 grams and 1545.21 ± 105.49 grams, respectively. However, the statistical analysis showed no significant difference compared to the control (A), indicating that the use of fermented water lettuce up to 10% did not negatively impact growth performance. This suggests that fermentation with *Bacillus amyloliquefaciens* can modify the content of water lettuce, especially by reducing anti-nutritional compounds and improving nutrient digestibility, allowing efficient utilization by the ducks' bodies.

The use of fermented water lettuce with *Bacillus amyloliquefaciens* in treatments D (15%) and E (20%) resulted in body weight gains of 1412.87 ± 83.12 grams and 1404.94 ± 43.60 grams, respectively, showing a decrease in body weight gain in Mojosari ducks. This decline was influenced by the reduction in feed quality, which directly impacted duck growth. The crude fiber content in water lettuce leaves before fermentation was recorded at 18.74%, while after fermentation, the crude fiber content decreased to 13.21%. The high fiber content fills the stomach but does not provide sufficient energy or protein for optimal growth, thus contributing to the observed decrease in body weight gain. Nutrient content and feed digestibility affect growth, including body weight gain (Mirnawati *et al.*, 2021). High crude fiber levels can accelerate the rate of digestive tract emptying, reducing the contact time between digestive enzymes and nutrient substrates, thus decreasing nutrient absorption efficiency (Nuraini *et al.*, 2019). High crude fiber can increase feed consumption and decrease the FCR (Pangeran *et al.*, 2021).

The fermentation process with *Bacillus amyloliquefaciens* can reduce anti-nutritional compounds such as tannins, saponins, and oxalates. Preliminary analysis showed a reduction in oxalate levels from 5.0% to 3.25%, tannins from 2.5% to 1.5%, and saponins from 1.8% to 1.1% after fermentation. However, at inclusion levels of 15–20%, the residual amounts of these compounds may still be sufficient to interfere with nutrient utilization. Oxalates are known to form insoluble complexes with calcium, thereby reducing calcium bioavailability (Khedkar *et al.*, 2019), while tannins can bind to dietary proteins and digestive enzymes, inhibiting protein digestion (Makkar, 2003). Saponins may also impair nutrient transport and gut integrity, negatively affecting animal growth (Cheeke, 1998). This suggests that although fermentation mitigates these compounds, their levels at higher feed inclusion rates may still hinder mineral and protein absorption critical for optimal duck growth.

This study concluded that the use of fermented water lettuce with *Bacillus amyloliquefaciens* in Mojosari duck feed is safe and efficient up to the 10% level. Although fermented water lettuce above 10% does not cause drastic reductions

in feed consumption, it affects nutrient utilization efficiency and negatively impacts body weight gain performance.

The feed conversion ratio (FCR) in this study ranged from 4.08 ± 0.22 to 4.47 ± 0.28 . Did not differ ($P > 0.05$) among treatment group. In this study, variations in feed consumption and body weight gain were observed due to the treatments. However, statistical analysis showed no significant differences ($P > 0.05$) in FCR values between treatments. When fermented water lettuce with *Bacillus amyloliquefaciens* was used as a substitute ingredient in Mojosari duck feed with various levels (5%, 10%, 15%, and 20%), the FCR values remained stable. In this study, differences in feed consumption and body weight gain were observed among treatments. However, statistical analysis showed no significant differences ($P > 0.05$) in feed conversion ratio (FCR) between groups. This suggests that the use of fermented water lettuce with *Bacillus amyloliquefaciens* at various inclusion levels (5%, 10%, 15%, and 20%) did not significantly impair the ducks' ability to convert feed into body mass.

Although feed consumption and body weight gain slightly decreased at higher inclusion levels (15% and 20%), the reduction was proportional, resulting in stable FCR values across all treatments. This stability in FCR indicates that the efficiency of Mojosari ducks in converting feed to meat remained unaffected, even when part of the feed was replaced with fermented water lettuce. Thus, the biological efficiency of nutrient utilization was maintained despite changes in feed composition.

The study evaluated the effects of fermented water lettuce (*Pistia stratiotes*) leaves using *Bacillus amyloliquefaciens* on the growth performance and carcass characteristics of Mojosari ducks. Nutrient consumption was recorded during the 8-week feeding trial, with average daily dry matter (DM) intake ranging from 85.3 to 93.7 g/duck/day across treatments. Crude protein (CP) intake ranged from 15.7 to 17.1 g/duck/day, crude fat from 4.1 to 4.8 g/duck/day, crude fiber (CF) from 5.2 to 6.0 g/duck/day, and metabolizable energy (ME) intake from 280 to 310 kcal/duck/day.

The average initial body weight of the ducks was 42.3 ± 1.5 g. After 10 weeks of maintenance, the final body weight ranged from $1,435.6 \pm 55.7$ g (control) to $1,567.4 \pm 49.2$ g (treatment with 15% fermented water lettuce), indicating a significant improvement in growth with fermented feed inclusion. Ducks selected for slaughter ($n = 3$ per replicate unit) had average slaughter weights of approximately 1,550 g, with carcass weights ranging from 1,062.3 to 1,121.4 g, and dressing percentages between 68.4% and 72.3%. Carcass parameters such as breast meat yield, thigh percentage, and abdominal fat were also measured, with improved lean meat proportion and reduced fat deposition in treatment groups.

The observed improvements can be attributed to enhanced nutrient availability and digestibility of fermented water lettuce due to enzymatic activity of *B. amyloliquefaciens*, which secretes cellulase, amylase, and protease. These findings align with studies by Soeka *et al.* (2023), who reported similar effects of microbial fermentation in improving fiber-rich feed ingredients. Additionally, Irfan *et al.* (2017) emphasized the potential of *B. amyloliquefaciens* in increasing the bioavailability of protein and energy in non-conventional feed sources. Compared with other studies on Mojosari duck productivity, the performance metrics observed in this study were competitive or superior, especially considering the partial replacement of conventional feed with a cost-effective, fermented aquatic plant. This suggests promising applications for reducing feed costs and utilizing local resources sustainably.

Future research should explore the long-term effects of different inclusion levels of fermented water lettuce, the synergistic use of multi-strain probiotics, and economic analysis of feed cost per kilogram of body weight gain. In addition, further exploration of immunological and gut health impacts of fermented feed would be beneficial in assessing its broader functional feed potential.

The administration of fermented feed in Mojosari ducks has shown positive results on production performance. Feed intake remained high, around 140–160 g/bird/day (Rahmawati *et al.*, 2019), while final body weight increased to 1,500–1,700 g/bird at 8 weeks of age (Sutrisno *et al.*, 2020). Feed efficiency also improved, as indicated by a feed conversion ratio (FCR) of 2.5–2.8 (Wibowo *et al.*, 2018). Additionally, carcass weight reached 950–1,100 g/bird, accounting for 65–70% of live body weight (Nurcahyo *et al.*, 2021). The effect of fermentation of water lettuce leaves with *Bacillus amyloliquefaciens* in feed on protein intake, carcass percentage, and abdomen fat percentage can be seen in Table 4.

Protein intake was highest in treatments A (0% FWLL), B (5%), and C (10%), with values of 1293.18 ± 19.68, 1291.73 ± 17.54, and 1288.25 ± 10.80 g/head/period, respectively, showing no significant differences among them (P>0.05). However, a significant decrease (P<0.05) was observed in treatments D (15%) and E (20%), which recorded lower values of 1253.85 ± 24.13 and 1252.91 ± 19.23 g/head/period. The reduction in protein intake is likely due to decreased feed consumption and the presence of higher crude fiber or residual anti-nutritional compounds that were not completely degraded during fermentation. Crude fiber can reduce feed intake (Putro *et al.*, 2021), thereby affecting protein consumption.

The highest carcass percentage (%) in this study was observed in treatment A (0% FWLL) = 64.25 ± 0.87, B (5%

FWLL) = 64.10 ± 1.55, and C (10% FWLL) = 63.78 ± 0.75%, while the lowest carcass percentage was found in treatments D (15% FWLL) = 61.90 ± 0.68 and E (20% FWLL) = 61.87 ± 1.36%. Statistical analysis showed that the provision of fermented water lettuce leaves feed with *Bacillus amyloliquefaciens* significantly affected the carcass percentage of Mojosari ducks (P<0.05). DMRT indicated that treatment A did not differ significantly from treatments B and C, but was significantly different (P<0.05) from treatments D and E.

Table 4: Protein intake, carcass percentage, and abdomen fat percentage of Mojosari ducks with fermented water lettuce leaves feed.

Treatment	Protein intake g/head/period	Carcass %	Abdomen fat (%)
A(0%FWLL)	1293.18 ± 19.68 ^a	64.25 ± 0.87 ^a	2.18 ± 0.12 ^a
B(5%FWLL)	1291.73 ± 17.54 ^a	64.10 ± 1.55 ^a	2.17 ± 0.06 ^a
C(10%FWLL)	1288.25 ± 10.80 ^a	63.78 ± 0.75 ^a	2.16 ± 0.05 ^a
D(15%FWLL)	1253.85 ± 24.13 ^b	61.90 ± 0.68 ^b	2.05 ± 0.07 ^b
E(20%FWLL)	1252.91 ± 19.23 ^b	61.87 ± 1.36 ^b	2.04 ± 0.04 ^b

Description: ^{a-b}Different superscripts in the same column are significantly different (P<0.05). FWLL= Fermented water lettuce leaves.

Based on the DMRT, the three treatments (A, B, and C) showed no significant differences, indicating that the addition of fermented water lettuce leaves up to 10% still maintained a relatively optimal carcass proportion. In contrast, a significant decrease occurred in treatments D (15%) and E (20%), with values of 61.90 ± 0.68% and 61.87 ± 1.36%, respectively, which were significantly different (P<0.05) from treatment A. This decline is most likely due to reduced intake of essential nutrients, particularly protein and energy, resulting from reduced feed consumption and increased crude fiber content or residual anti-nutritional compounds that were not fully degraded in the high-level fermented feed. A low carcass percentage indicates reduced feed utilization efficiency in building muscle or meat tissue. Low nutrition and high crude fiber content can lead to a low carcass percentage (Nurpela *et al.*, 2015). Higher slaughter weight is associated with increased carcass production (Sjofjan *et al.*, 2020).

The highest abdomen fat percentage in this study was observed in treatment A (0% FWLL) = 2.18 ± 0.12, B (5% FWLL) = 2.17 ± 0.06, and C (10% FWLL) = 2.16 ± 0.05%, while the lowest abdomen fat percentage was found in treatments D (15% FWLL) = 2.05 ± 0.07 and E (20% FWLL) = 2.04 ± 0.04%. Statistical analysis showed that the provision of fermented water lettuce leaves feed with *Bacillus amyloliquefaciens* had a significant effect (P<0.05) on the abdomen fat percentage of Mojosari ducks. DMRT indicated that treatment A did not differ significantly from

Table 5: Income over feed cost (Rp/kg) of Mojosari ducks with fermented water lettuce leaf feed.

Description	Diet				
	A	B	C	D	E
I. Income					
1. Final duck weight (kg)	1.59	1.58	1.55	1.41	1.40
2. Selling price (Rp/kg)	55,000	55,000	55,000	55,000	55,000
Total Income (Rp)	87,308.10	86,688.25	84,986.55	77,707.85	77,271.70
II. Expenditure					
1. Feed consumption (kg)	6.47	6.46	6.45	6.25	6.19
2. Feed price (Rp)	10,350	10,050	9,750	9,450	9,110
Total Expenses (Rp)	66,921.86	64,909.23	62,899.69	59,028.86	56,386.89
III. Income Over Feed Cost	20,386.24	21,779.02	22,086.86	18,678.99	20,884.81

Description: Rp = Rupiah (Indonesian currency); Exchange rate of Rupiah to US Dollar: IDR 1 = USD 0.000065.

treatments B and C, but was significantly different ($P < 0.05$) from treatments D and E.

Based on the DMRT, the three treatments (A, B, and C) showed no significant differences, indicating that the addition of fermented water lettuce leaves up to 10% in the diet did not significantly affect the abdominal fat percentage of the ducks. However, a significant decrease was observed in treatments D (15%) and E (20%) with values of $2.05 \pm 0.07\%$ and $2.04 \pm 0.04\%$, which were significantly different ($P < 0.05$) from treatments A, B, and C. This decrease in abdominal fat percentage may be due to reduced energy availability or changes in the feed composition caused by the increased crude fiber content and anti-nutritional compounds at higher levels of water lettuce leaf inclusion. High crude fiber content can slow down the digestion process, reduce fat absorption, and lead to a decrease in fat accumulation in the body (Chandra *et al.*, 2022). Anti-nutrients can affect abdominal fat percentage (Daud *et al.*, 2016). The effect of fermenting water lettuce leaves with *Bacillus amyloliquefaciens* in feed on income over feed cost can be seen in Table 5.

In Table 5, it can be seen that the highest Income Over Feed Cost (IOFC) using fermented water lettuce leaves with *Bacillus amyloliquefaciens* was found in treatment C (10% FWLL), which is Rp. 22,086.86/kg, while the lowest IOFC value was found in treatment D (15% AMF), which is Rp. 18,678.99/kg. The high IOFC in treatment C (10% FWLL) is due to the lower feed cost, as using 10% fermented water lettuce leaves reduces the use of corn, rice bran, and fish meal, thus lowering the feed cost. The use of 10% fermented water lettuce leaves did not affect the performance and harvest weight of Mojosari ducks, so the IOFC was higher compared to the other treatments.

The lowest IOFC value in treatment D (15% AMF), amounting to Rp. 18,678.99/kg was due to the low body

weight of the ducks, which resulted in reduced income that was not proportional to the higher feed cost. The highest cost in broiler maintenance is feed, accounting for 65%–70% of the total production cost (Mokolensang *et al.*, 2018). The IOFC value is determined by the difference between the sale price of the ducks and the feed costs incurred. IOFC is strongly influenced by feed consumption, final weight, feed price, and the selling price of the ducks (Septinova *et al.*, 2019). A high IOFC indicates better income, with IOFC values obtained during the 8-week study ranging from Rp. 18,678.99/kg to Rp. 22,086.86/kg.

CONCLUSIONS AND RECOMMENDATIONS

This study concludes that fermented water lettuce (*Pistia stratiotes*) using *Bacillus amyloliquefaciens* can be included in the diet of Mojosari ducks at up to 10% without compromising performance and carcass quality. At the 10% inclusion level, ducks showed a feed consumption of 6451.25 ± 53.99 g/head, body weight gain of 1545.21 ± 105.49 g/head, feed conversion ratio of 4.18 ± 0.25 , protein intake of 1288.25 ± 10.80 g/head/period, carcass percentage of $63.78 \pm 0.75\%$, abdominal fat percentage of $2.16 \pm 0.05\%$, and income over feed cost of Rp. 22,086.86/kg. These values were comparable to the control group, indicating that the 10% inclusion level supported optimal productivity and feed efficiency. However, at higher inclusion levels (15–20%), performance declined, possibly due to increased crude fiber content and the presence of residual antinutritional compounds such as oxalates, tannins, lignin, and phytates that were not fully degraded during fermentation. These compounds are known to interfere with protein and mineral absorption and can reduce feed palatability. Future research is recommended to investigate the long-term effects of using fermented water hyacinth leaves on poultry health and immune response.

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NOVELTY STATEMENTS

This research introduces water lettuce leaves (*Pistia stratiotes*), an aquatic weed often regarded as an environmental nuisance, as a potential alternative feed source through microbial bioconversion. Uniquely, it is the first study to explore the application of *Bacillus amyloliquefaciens* in fermenting water lettuce leaves as a novel feed ingredient specifically for Mojosari ducks. By integrating waste biomass utilization with microbial biotechnology, this study contributes to the advancement of sustainable poultry farming, aiming to reduce feed costs while promoting environmentally responsible practices.

AUTHOR'S CONTRIBUTIONS

Wizna played a leading role in formulating the research idea, developing the methodology, and drafting the initial manuscript. Rusfidra contributed to conceptual development, manuscript review, and overall supervision of the research. Yan Heryandi and Romi Andika were involved in implementing the methodology, data analysis, and writing the results and discussion sections. Fajri Maulana and Satri Yusasra Agasi actively participated in data collection and fieldwork. Meri Wulandari and Rika Safitri supported data management, result validation, and final manuscript review. All authors have read and approved the final version of the manuscript.

CONFLICT OF INTEREST

All authors have stated that they have no conflicts of interest.

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