



Optimizing Fermentation Conditions of Water Lettuce Leaves (*Pistia Stratiotes*) with *Bacillus amyloliquefaciens* for Enhanced Nutritional Quality as Poultry Feed

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Abstract | The aim of this study was to determine the optimal fermentation duration and inoculum dosage for fermenting water lettuce leaves (*Pistia stratiotes*) with *Bacillus amyloliquefaciens* to enhance its nutritional content. The study employed a completely randomized design with two factors: Factor A - fermentation duration (A1: 2 days, A2: 4 days, A3: 6 days) and Factor B - inoculum dosage (B1: 1%, B2: 3%, B3: 5%). Differences among treatments were analyzed using Duncan's Multiple Range Test (DMRT). The observed parameters included water content, dry matter, crude protein, crude fat, crude fiber, and ash content. The results indicated that fermentation duration and inoculum dosage had a significant interaction ($P < 0.05$) on crude protein, crude fiber, and ash content but showed no significant effect ($P > 0.05$) on water content and dry matter. The study concluded that the best treatment was 4 days of fermentation and 3% inoculum dosage. The nutritional composition of fermented water lettuce leaves with *Bacillus amyloliquefaciens* was as follows: water content 72.98%, dry matter 27.02%, crude protein 14.26%, crude fat 2.46%, crude fiber 13.21%, and ash content 6.57%. The fermentation of water lettuce leaves (*Pistia stratiotes*) using *Bacillus amyloliquefaciens* is an effective method to enhance the nutritional value and digestibility of feed ingredients. This process involves inoculating water lettuce leaves with microbial cultures that produce fiber- and protein-degrading enzymes, thereby reducing the content of antinutritional compounds. Optimized fermentation can increase crude protein content, improve nutrient availability, and produce bioactive compounds that support poultry digestive health. The application of this fermented product in poultry feed can enhance feed efficiency, improve production performance, and reduce farming costs, thus contributing to the sustainability of poultry farming.

Keywords | *Bacillus amyloliquefaciens*, Fermentation duration, Inoculum dosage, Nutrition, Water lettuce, Poultry feed, Nutritional enhancement, Fermentation optimization

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yloliquefaciens. The research aims to enhance the quality of water lettuce leaves (*Pistia stratiotes*) through fermentation technology for use as poultry feed.

MATERIALS AND METHODS

The materials used in this study were *Bacillus amyloliquefaciens* inoculum, water lettuce leaves, alcohol, and aquadest. Desain Eksperimental: This experiment employed a 3x3 factorial design within a completely randomized design (CRD) framework, with three replicates per treatment. The first factor (A) was the fermentation duration, consisting of three levels: 2 days, 4 days, and 6 days. The second factor (B) was the inoculum dosage, also consisting of three levels: 1%, 3%, and 5%.

PREPARATION OF *BACILLUS AMYLOLIQUEFACIENS* INOCULANT

Weigh 100 grams of rice bran, then sterilize using an autoclave for 15 minutes at 121 °C, 1 atm pressure, then cool at room temperature. Petridish dishes that have been overgrown with pure culture of *Bacillus Amyloliquefaciens* add distilled water as much as 10 mL then the petri dish is gently shaken until the microbes are separated from the media and then put into an erlemeyer tube that has contained distilled water as much as 190 mL. Sterilized rice bran was mixed with 200 mL of distilled water that already contained *Bacillus amyloliquefaciens* suspension. Incubate for 24 hours at 40°C then dry it with an oven at 60 °C, so that it takes the form of flour which is used as inoculum.

FERMENTATION OF APU-APU LEAF WITH *BACILLUS AMYLOLIQUEFACIENS*

Weigh 100 grams of apu-apu leaves sterilize in an autoclave for 15 minutes, temperature 121°C with a pressure of 1 atm then the media is cooled at room temperature. Then add *Bacillus amyloliquefaciens* inoculant with the dose according to the treatment (1, 3, and 5%) mix until homogeneous and incubated according to the treatment (2, 4, and 6 days) at 40°C. After the incubation process was complete, the media was oven dried at 60°C until dry so that the fermentation product was obtained.

PARAMETERS

The measured were water content, dry matter, crude protein, crude fiber, crude fat and ash (AOAC, 2016).

DATA ANALYSIS

All data obtained were statistically analyzed by analysis of variance. Differences between treatments were determined using Duncan Multiple Range Test (DMRT) (Steel *et al.*, 1997).

Direct use of water lettuce leaves in chicken rations cannot be in large quantities, a maximum of only 15% because it contains high fiber, namely 21.86%. (Sitompul *et al.*, 2020). Water lettuce leaves have potential as poultry feed due to their high nutritional content (Jeksi *et al.*, 2022). The nutritional composition of water lettuce leaves includes 88.66% dry matter, 13.06% crude protein, 2.15% crude fat, 2778.55 kcal/kg metabolizable energy, and 21.86% crude fiber (Sitompul *et al.*, 2020). The direct use of water lettuce leaves as poultry feed has some major limitations, one of which is the high crude fiber content. The high crude fiber content in water spinach leaves can reduce feed digestibility, thus lowering the efficiency of nutrient utilization by poultry. In addition, excessive crude fiber can affect poultry growth, leading to decreased body weight and egg production.

Manu *et al.* (2023), the reduction of crude fiber in poultry feed can be achieved through fermentation, a bioconversion process utilizing microorganisms that produce fiber-degrading enzymes. The bacteria commonly used in the fermentation process of poultry feed include *Bacillus amyloliquefaciens* (Supriyati *et al.*, 2015). During fermentation, *Bacillus amyloliquefaciens* produces enzymes such as amylase (Zurmiati *et al.*, 2017), Protease and lipase (Ramadhan *et al.*, 2021), cellulase (Chistyakov *et al.*, 2015). Factors influencing fermentation include inoculum dosage, fermentation duration, substrate composition, and substrate thickness (Maulana *et al.*, 2021). The advantage of fermentation is its ability to improve the digestibility of feed, particularly waste-based feed materials (Hidayat *et al.*, 2024). Inoculum dosage refers to the amount of inoculum introduced into the fermentation substrate, while fermentation duration refers to the time period over which fermentation occurs.

Fermentation using microorganisms such as *Bacillus amyloliquefaciens* has become a promising approach to improve poultry feed quality, especially for feed ingredients with high crude fiber content like water lettuce leaves. *Bacillus amyloliquefaciens* is known for its ability to produce fiber-degrading enzymes, such as amylase and cellulase, which can effectively break down fiber in feed ingredients. By using *Bacillus amyloliquefaciens* for the fermentation of water lettuce leaves, the crude fiber can be effectively degraded, thus improving the digestibility and overall quality of the feed. Therefore, the use of *Bacillus amyloliquefaciens* for fermenting water lettuce leaves offers a new solution to address the limitations of directly using water lettuce leaves as poultry feed, while simultaneously enhancing feed efficiency and sustainability. Thus, this study was conducted to determine the optimal fermentation duration and inoculum dosage for water lettuce leaves using *Bacillus am-*

WATER CONTENT (%)

Table 1 the water content of *Bacillus amyloliquefaciens*-fermented water lettuce leaves ranges from 67.76% to 73.01%. This study indicates no interaction ($P>0.05$) between factor A (fermentation duration) and factor B (inoculum dose) on water content. However, factor B (inoculum dose) showed a very significant effect ($P<0.01$) on water content. This study also demonstrates that as the fermentation duration increases, microbial activity within the fermentation substrate enhances, leading to an increase in water content. Microbial activity during fermentation not only increases the water content in the material but also generates metabolic water. This metabolic water serves as an important indicator of the sustainability of the fermentation process. Steinkrauss (1995), noted that during soybean meal fermentation, microbes break down carbohydrates, producing water. Additionally, Rachman (1989) emphasized that water is a byproduct of aerobic fermentation.

Table 1: Effect of fermentation duration and inoculum dosage on water content of fermented water lettuce leaves.

Fermentation Duration (Factor A)	Inoculum Dose (Factor B)			Average
	B1 (1%)	B2 (3%)	B3 (5%)	
A1 (2 Day)	67.76	71.02	72.81	70.53
A2 (4 Day)	68.28	72.98	72.80	71.35
A3 (6 Day)	70.21	72.99	73.01	72.07
Average	68.75 ^a	72.33 ^a	72.87 ^b	
SEM				0.53

^{a-b} Superscripts that are different in the same column are not significantly different ($P>0.05$), SEM: Standard error of the mean.

Table 2: Effect of fermentation duration and inoculum dosage on dry matter of fermented water lettuce leaves.

Fermentation Duration (Factor A)	Inoculum Dose (Factor B)			Average
	B1 (1%)	B2 (3%)	B3 (5%)	
A1 (2 Day)	32.24	28.98	27.19	29.47 ^a
A2 (4 Day)	31.72	27.02	27.20	28.65 ^{ab}
A3 (6 Day)	29.79	27.01	26.99	27.93 ^b
Average	31.25 ^a	27.67 ^{ab}	27.13 ^b	
SEM				0.53

^{a-b} Different superscripts in the same column are not significantly different ($P>0.05$), SEM: Standard error of the mean.

DRY MATTER (%)

Table 2 the dry matter content of *Bacillus amyloliquefaciens*-fermented water lettuce leaves ranges from 26.99 - 32.24%. This study showed no interaction ($P>0.05$) between factor A (fermentation duration) and factor B (inoculum dose) on dry matter. However, factors A (length of

fermentation) and B (dose of inoculum) showed a significantly different effect ($P<0.01$) on dry matter.

The low content of dry matter in treatment A3 (fermentation duration of 6 days) which is 27.93% compared to other treatments is due to the long fermentation duration that increases microbial activity in remodeling the substrate as a result of high H₂O production during fermentation, dry matter goes down when the water content is high. The results of research by Mirzah and Muis (2015) state that the length of fermentation time causes a lot of material to be overhauled by enzymes that are sterilized by microbes. Added by Nuraini *et al.* (2019), a long fermentation time makes microbes in the fermentation substrate to multiply optimally so that the enzymes produced are higher as a result of the overhaul of the substrate from complex components to simple ones to be optimal. The fermentation process increases water activity because the microorganisms growing in the substrate produce H₂O as a waste product of feed degradation (Firdaus, 2020).

CRUDE PROTEIN (%)

Table 3, the crude protein content of *Bacillus amyloliquefaciens*-fermented water lettuce leaves ranges from 11.01% to 14.88%. This study shows a significant interaction ($P<0.05$) between factor A (fermentation duration) and factor B (inoculum dose) on crude protein content. Both factor A (fermentation duration) and factor B (inoculum dose) also significantly affect crude protein content ($P<0.01$).

Table 3: Effect of fermentation duration and inoculum dosage on crude protein of fermented water lettuce leaves.

Fermentation Duration (Factor A)	Inoculum Dose (Factor B)			Average
	B1 (1%)	B2 (3%)	B3 (5%)	
A1 (2 Day)	11.01 ^e	12.44 ^d	14.11 ^{abc}	12.52
A2 (4 Day)	11.06 ^e	14.26 ^{ab}	14.49 ^{ab}	13.27
A3 (6 Day)	13.12 ^{cd}	14.87 ^{ab}	14.88 ^a	14.29
Average	11.73	13.86	14.49	
SEM				0.32

^{a-b} Different superscripts in the same column are significantly different ($P<0.05$), SEM: Standard error of the mean.

The highest crude protein content was observed in the treatments A2B2 = 14.26%, A3B2 = 14.87%, A1B3 = 14.11%, A2B3 = 14.49%, and A3B3 = 14.88%. The high crude protein content in these treatments was due to the interaction between fermentation duration and inoculum dose, which optimized the growth of *Bacillus amyloliquefaciens*. The growth of microorganisms contributes to a higher protein yield (Djulardi *et al.*, 2023). The increase in protein content is due to microbial bodies, which are single-cell proteins, and the enzymes produced, which are also proteins. The cell structures of microorganisms are composed of single-cell proteins (Rahmatullah *et al.*, 2020).

This study focuses on optimizing fermentation conditions to improve the nutritional quality of water lettuce leaves (*Pistia stratiotes*) as poultry feed by using *Bacillus amyloliquefaciens* as the main microorganism. The optimization involves varying inoculum doses and fermentation time to achieve optimal nutritional enhancement. The novelty of this research lies in combining fermentation condition optimization with the utilization of *Bacillus amyloliquefaciens*, which has not been widely explored in poultry feed based on water lettuce leaves.

Previous studies mostly highlighted the benefits of fermenting other feed materials, such as rice bran, soybean meal, or agricultural waste, but have rarely focused specifically on water lettuce leaves as poultry feed. Additionally, the microorganisms used for improving feed nutrition in earlier research were predominantly *Lactobacillus spp.* or *Saccharomyces spp.*, with limited exploration of fermentation parameter optimization.

Table 4: Effect of fermentation duration and inoculum dosage on crude fat of fermented water lettuce leaves.

Fermentation Duration (Factor A)	Inoculum Dose (Factor B)			Average
	B1 (1%)	B2 (3%)	B3 (5%)	
A1 (2 Day)	3.03	3.16	4.74	3.65
A2 (4 Day)	2.57	2.46	6.83	3.95
A3 (6 Day)	3.50	2.73	6.06	4.09
Average	3.03 ^b	2.78 ^a	5.88 ^a	
SEM				0.47

^{a-b}Different superscripts in the same column are significantly different (P<0.05), SEM: Standard error of the mean.

CRUDE FAT (%)

Table 4 the crude fat of *Bacillus amyloliquefaciens*-fermented water lettuce leaves ranges from 2.46 - 6.83%. This study showed no interaction (P>0.05) between factor A (fermentation duration) and factor B (inoculum dose) on crude fat. However, factor B (inoculum dose) showed a significantly different effect (P<0.01) on crude fat. The high fat content in treatment B3 (5% inoculum dose) with an average of 5.88% is due to the large dose of inoculum so that the microbial biomass that grows more and more so that the development in the fermentation substrate is faster. The development of high microbial biomass causes high fat content because the microbial body itself contains fat. According to Saputro *et al.* (2015), fat content increases along with the increasing length of fermentation time, where the longer the fermentation that takes place *Trametes sp.* mushrooms will continue to grow and develop and be able to convert high-carbohydrate substrates into fat. According to Iba and Padang (2021), microbial cultures and microalgae can produce biomass containing fatty acids, making them a potential source for the production of

single-cell oils and a new potential for poultry feed. These microorganisms and microalgae are known for their ability to synthesize high-value oils, such as omega-3 fatty acids, which are essential for animal health. The use of such biomass in poultry feed can enhance the nutritional quality of the feed, improve poultry performance, and contribute to more sustainable feed production systems.

CRUDE FIBER (%)

Table 5, the crude fiber content of *Bacillus amyloliquefaciens*-fermented water lettuce leaves ranges from 13.07% to 17.68%. This study shows a significant interaction (P<0.05) between factor A (fermentation duration) and factor B (inoculum dose) on crude fiber content. Both factor A (fermentation duration) and factor B (inoculum dose) also significantly affect crude fiber content (P<0.01).

Table 5: Effect of fermentation duration and inoculum dosage on crude fiber of fermented water lettuce leaves.

Fermentation Duration (Factor A)	Inoculum Dose (Factor B)			Average
	B1 (1%)	B2 (3%)	B3 (5%)	
A1 (2 Day)	17.23 ^a	15.04 ^b	13.84 ^{bc}	15.37
A2 (4 Day)	17.68 ^a	13.21 ^c	13.10 ^c	14.67
A3 (6 Day)	14.82 ^b	13.55 ^{bc}	13.07 ^c	13.81
Average	16.58	13.93	13.34	
SEM				0.50

^{a-b}Different superscripts in the same column are significantly different (P<0.05), SEM: Standard error of the mean.

The lowest crude fiber content was observed in the treatments A2B2, A3B2, A3B1, A2B3, and A3B3. The low crude fiber content in these treatments is due to the high cellulase enzyme activity produced by microorganisms, which is maximized due to optimal growth. The growth is influenced by the appropriate fermentation duration and inoculum dose. The reduction in crude fiber content during fermentation is attributed to the high cellulase enzyme activity produced by bacteria during the fermentation process. Cellulase enzymes play a role.

in breaking down crude fiber (Maulana *et al.*, 2021). The high cellulase enzyme activity is the result of optimal fermentation duration and inoculum dose for *Bacillus amyloliquefaciens* growth on the water lettuce leaves leaf substrate, leading to maximal enzyme production. The factors determining enzyme activity during fermentation include the microorganism biomass, inoculum dose, and fermentation duration, which align with the microorganisms' growth needs (Wahyuni *et al.*, 2023).

This study has significant potential to support the development of more sustainable and economical poultry feed by utilizing water lettuce leaves, often considered a weed.

The findings offer a solution to reduce poultry feed costs, as processing through fermentation can decrease crude fiber content, which otherwise hinders the absorption of other nutrients in poultry feed. The use of *Bacillus amyloliquefaciens* as a fermentation agent represents a significant innovation. This microorganism is known to enhance crude protein content, improve feed digestibility, and reduce crude fiber. Another potential of this study lies in mitigating the negative impacts of aquatic plant waste, which often poses environmental problems in water bodies.

ASH (%)

Table 6, the ash content of *Bacillus amyloliquefaciens*-fermented water lettuce leaves ranges from 5.70% to 6.83%. The result of analysis of variance showed that there was no interaction between fermentation duration and inoculum dose. From this study, it can be seen that the ash content is not affected by the fermentation process, but it can increase the availability of minerals, especially phosphorus because some microorganisms, namely *Bacillus amyloliquefaciens*, produce the enzyme phytase, which binds phosphorus. As phytic acid breaks down, certain minerals (magnesium, iron, calcium and zinc) become more available. The higher the yeast concentration, the higher the ash content. The observed fermentation results, presented in graphical form, can be seen in Figure 1 (crude protein) and Figure 2 (crude fiber).

Table 6: Effect of fermentation duration and inoculum dosage on ash of fermented water lettuce leaves.

Fermentation Duration (Factor A)	Inoculum Dose (Factor B)			Average
	B1 (1%)	B2 (3%)	B3 (5%)	
A1 (2 Day)	5.83	5.70	5.74	5.76
A2 (4 Day)	6.46	6.57	6.83	6.62
A3 (6 Day)	6.06	5.50	6.06	5.87
Average	6.12	5.92	6.21	
SEM				0.54

^{a-b}Different superscripts in the same column are significantly different ($P < 0.05$), SEM: Standard error of the mean.

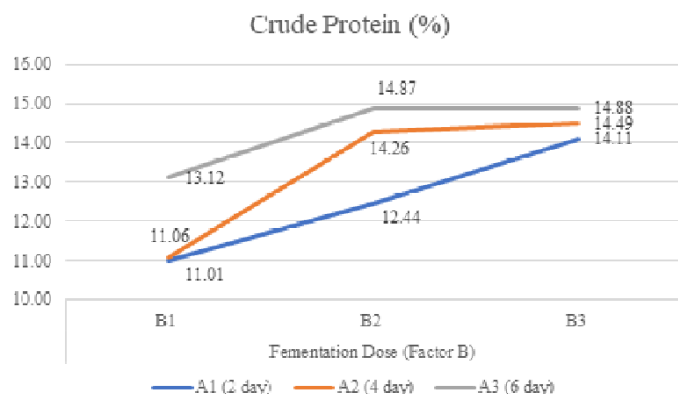


Figure 1: Graph effect of fermentation duration and inoculum dosage on crude protein of fermented water lettuce leaves.

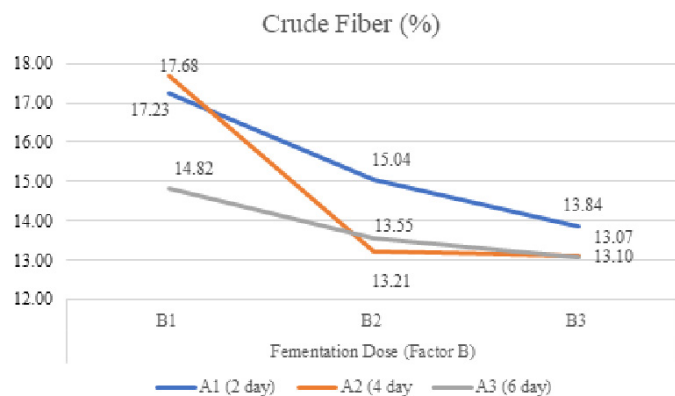


Figure 2: Graph effect of fermentation duration and inoculum dosage on crude fiber of fermented water lettuce leaves.

CONCLUSIONS AND RECOMMENDATIONS

From this study, it can be concluded that the best treatment for fermenting water lettuce leaves with *Bacillus amyloliquefaciens* is a fermentation duration of 4 days and an inoculum dose of 3%. The nutrient content of water lettuce leaves fermented with *Bacillus amyloliquefaciens* is as follows: water content 72.98%, dry matter 27.02%, crude protein 14.26%, crude fat 2.46%, crude fiber 13.21% and ash 6.57%. The fermentation of water lettuce leaves (*Pistia stratiotes*) using *Bacillus amyloliquefaciens* is an effective method to enhance the nutritional value and digestibility of feed ingredients. This process involves inoculating water lettuce leaves with microbial cultures that produce fiber and proteindegrading enzymes, thereby reducing the content of antinutritional compounds. Optimized fermentation can increase crude protein content, improve nutrient availability, and produce bioactive compounds that support poultry digestive health. The application of this fermented product in poultry feed can enhance feed efficiency, improve production performance and reduce farming costs, thus contributing to the sustainability of poultry farming.

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NOVELTY STATEMENTS

This study focuses on optimizing fermentation conditions

to improve the nutritional quality of water lettuce leaves (*Pistia stratiotes*) as poultry feed by using *Bacillus amyloliquefaciens* as the main microorganism. The optimization involves varying inoculum doses and fermentation time to achieve optimal nutritional enhancement. The novelty of this research lies in combining fermentation condition optimization with the utilization of *Bacillus amyloliquefaciens*, which has not been widely explored in poultry feed based on water lettuce leaves. Previous studies mostly highlighted the benefits of fermenting other feed materials, such as rice bran, soybean meal, or agricultural waste, but have rarely focused specifically on water lettuce leaves as poultry feed. Additionally, the microorganisms used for improving feed nutrition in earlier research were predominantly *Lactobacillus* spp. or *Saccharomyces* spp., with limited exploration of fermentation parameter optimization.

AUTHOR'S CONTRIBUTIONS

The authors contributed to the study as follows: Wizna conceptualized the research, prepared the initial manuscript draft, and carried out statistical analyses. Yan Heryandi was responsible for conducting experiments and gathering field data. Rusfidra processed and interpreted the experimental data while contributing to the methodology section. Meri Wulandari refined the manuscript to ensure technical precision and uniformity in writing style. Rika Safitri oversaw the research process and handled correspondence with the journal.

CONFLICT OF INTERESTS

The authors declare no conflict of interest regarding the publication of this article. All authors affirm that there are no personal, professional, or financial relationships that could influence the work reported in this manuscript.

REFERENCES

- AOAC (2016). Official methods of analysis of the association of the official analytical chemists. 20th ed. Washington, D.C, USA.
- Chistyakov V, Melnikov V, Chikindas ML, Khutsishvili M, Chagelishvili A, Bren A, Kostina N, Cavera V, Elisashvili V (2015). Poultry-beneficial solid-state *Bacillus amyloliquefaciens* B-1895 fermented soybean formulation. *Biosci. Microbiota, Food Health*, 34(1): 25–28. <https://doi.org/10.12938/bmfh.2014-012>
- Djulardi A, Mirnawati, Ciptaan G, Kurnia R, Srifani A, Adriani L, Makmur M (2023). Improving the Quality and Nutritional Value of a Mixture of Sago Pith and Indigofera Leaves Fermented with *Rhizopus oligosporus*. *World's Vet. J.*, 13(4): 580–586. <https://doi.org/10.54203/scil.2023.wvj62>
- Firdaus M (2020). The effect of the fermentation process on feed quality and water content in animal feed.. *Jurnal Ilmu Peternakan*, 15(2): 110-115. <https://ejournal.uin-suska.ac.id/>

<index.php/peternakan/article/download/30721/11312v>

- Hidayat R, Khusairi A, Zakhiya M, Maulana F, Febrina P, Sandri D, Susalam MK (2024). Nutritional Improvement of Food Scraps and Organic Waste from Pesantren Kitchens in the Regency Tanah Laut. *J. Peternakan Indones.*, 26(3): 128–137. <https://doi.org/10.25077/jpi.26.3.128-137.2024>
- Iba W, Padang CR (2021). The production of single-cell protein from the microalga *Chlorella vulgaris* using broiler chicken waste and its application in feed. *Jurnal Ilmu dan Teknologi Pangan*, 26(11): 1-10.
- Jeksi S, Daud M, Yaman MA (2022). Effect of Kiapu (*Pistia Stratiotel L*) in Fermented Diet on Feed Consumption, Final Body Weight, Feed Conversion, Feed Efficiency and Cholesterol Content of Breast and Leg Muscle on Selected Local Chicken . *Proc. 6th Int. Semin. Anim. Nutr. Feed Sci.*, (ISANFS 2021): 21(Isanfs 2021): 46–50. <https://doi.org/10.2991/absr.k.220401.011>
- Manu KR, Mulyantini NGA, Kallau NHG, Telupere FMS, Detha AIR (2023). The fermentation feed based on local materials in the form of pellet and mash on performance, carcass and intestinal organs of broiler. *J. Kajian Vet.*, 11(2): 198-217. <https://doi.org/10.35508/jkv.v11i2.12596>
- Maulana F, Nuraini N, Mirzah M (2021). Nutrient Content and Quality of Fermented Palm Waste with *Lentinus edodes*. *Jurnal Peternakan Indonesia. Indones. J. Anim. Sci.*, 23(2): 174. <https://doi.org/10.25077/jpi.23.2.174-182.2021>
- Mirzah M, Muis H (2015). Improving the Nutritional Quality of Cassava Bark Waste through Fermentation Using *Bacillus amyloliquefaciens*. *Jurnal Peternakan Indonesia, Indones. J. Anim. Sci.*, 17(2): 131. Available at: <https://doi.org/10.25077/jpi.17.2.131-142.2015>
- Nuraini NA, Djulardi, Trisna A (2019). Palm Kernel Cake Fermented with *Lentinus edodes* in the Diet of Quail. *Int. J. Poult. Sci.*, 18(8):387–392. <https://doi.org/10.3923/ijps.2019.387.392>
- Prayogi G, Tafsini M, Wahyuni TH (2021). Protein digestibility and metabolizable energy of palm kernel cake fermented by Starbio on chicken. *IOP Conf. Ser. Earth Environ. Sci.*, 782(2): 6–11. <https://doi.org/10.1088/1755-1315/782/2/022096>
- Rachman A (1989). Fermentation technology. Ministry of Education and Culture Directorate General of Higher Education Inter-Centre Ipb University of Food and Nutrition. Bogor.
- Rahmatullah R, Hasnudi, Mirwandhono E, Patriani P, Ginting N, Siregar GAW (2020). The effects of fermentation time and em4 dose on nutrient content of kepok's peel as animal feed. *J. Phys. Conf. Ser.*, 1542(1). <https://doi.org/10.1088/1742-6596/1542/1/012030>
- Ramadhan RF, Wizna Y, Marlida, Mirzah, Supratman H (2021). Content and Nutritional Quality of Mixed Bovine Blood and Agricultural Waste Fermented by *Bacillus Amylolyquefaciens* as Broiler Feed. *Jurnal Peternakan. Vol 18 (1): 77-86.* <https://doi.org/10.24014/jupet.v18i1.12060>
- Saputro R, Fathul F, Widodo Y (2015). Effect of fermentation duration with *Trametes* Sp. media on organoleptic, moisture and fat content of pineapple leaf waste in Lampung Tengah. *Jurnal Ilmiah Peternakan Terpadu*. 3(1): 68-74.
- Sitompul R, Erwan E, Saleh E (2020). Pemanfaatan Tepung Daun Apu-apu (*Pistia stratiotes*) dalam Ransum Basal

- terhadap Organ Pencernaan Ayam Ras Pedaging. Jurnal Peternakan. 17 (1): 17-24. <https://doi.org/10.24014/jupet.v17i1.7439>
- Steel RGD, Torrie JH, Dickey D (1997). Principles and procedures of statistics: a biometric approach.
- Steinkraus KH. (1995). Handbook of Indigenous Fermented Foods. Marcel Dekker, New York.
- Supriyati, Haryati T, Susanti T, Susana IWR (2015). Nutritional Value of Rice Bran Fermented by *Bacillus amyloliquefaciens* and Humic Substances and Its Utilization as a Feed Ingredient for Broiler Chickens. Asian Australas. J. Anim. Sci., 28(2): 231-238. <https://doi.org/10.5713/ajas.14.0039>
- Wahyuni T, Prestiani N, Rusmana D, Hasbuna A (2023). Effect of Inoculum Dosage and Fermentation Duration by *Bacillus subtilis* on Nutrient Content of Chicken Feather Flour. Jurnal Nutrisi Ternak Tropis Dan Ilmu Pakan, 5(3): 135. <https://doi.org/10.24198/jnttip.v5i3.49792>
- Zurmiati, Wizna MH, Abbas ME, Mahata, Fauzano R (2017). Effect of *Bacillus amyloliquefaciens* as a Probiotic on Growth Performance Parameters of Pitalah Ducks. Int. J. Poult. Sci., 16 (4): 147-153. <https://doi.org/10.3923/ijps.2017.147.153>