Comparison of natural lactic acid fermentation of cassava leaves, *Manihot esculenta*, and duckweed, *Lemna* sp., as potential dietary substitutes for pond loach, *Misgurnus anguillicaudatus*

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Abstract. Natural lactic acid fermentation is a unique method of food preservation and processing that results in distinct flavors, textures, and an extended shelf life for various foods. The current study aims at evaluating the natural lactic acid fermentation of cassava leaves (*Manihot esculenta*) and duckweed (*Lemna* sp.) and its utilization in the palatability test for juvenile loach, *Misgurnus anguillicaudatus*. An assessment of microbial, chemical and physical analyses of fermented *M. esculenta* and fermented *Lemna* sp. as a potential dietary substitute for *M. anguillicaudatus* was performed to examine the effect of natural lactic acid fermentation. Fermented cassava leaves had a higher ash, protein and nitrogen content than in fermented duckweeds. The hydrocyanic acid level in fermented cassava leaves (0.086±0.002%) was slightly higher than in the fermented duckweeds (0.085±0.001%). The pH value in fermented cassava leaves and duckweeds were acidic (3.50 to 3.53 pH), while the temperature increased slightly (23.9 to 24.0°C). Fermented duckweeds had a higher total titratable acidity (2.31±0.27%) than fermented cassava leaves (1.86±0.11%). The lactic acid bacteria count was higher in fermented duckweeds (1.0 x 10^8 to 9.1 x 10^8 cfu g^{-1}) than in fermented cassava leaves (2.0 x 10^8 to 5.8 x 10^8 cfu g^{-1}). *M. anguillicaudatus* consumed higher diet in fermented duckweeds (unconsumed feed, 2.40 to 3.17 g) than fermented cassava leaves (unconsumed feed, 5.71 to 5.86 g) with a given amount of 6 g in each aquarium. Fermented cassava leaves and duckweeds had comparable effects on the fermentation process in terms of ash, crude protein, nitrogen, and cyanide content. The pH values between total titratable acidity were positively correlated while pH values between temperatures were inversely correlated. The use of natural lactic acid fermentation was adequate for the reduction of cyanide content.

Key Words: microbial, chemical, physical analyses, fish diet, hydrocyanic acid.

Introduction. Cassava (*Manihot esculenta*) production on the African continent which is 54% of the global production as a whole has been growing faster than in other regions for the past 5 years (Lukuyu et al 2014). Globally, its production reached 315 million tonnes in 2021 which marks a 9% increase from 2017 (Otekunrin 2024). Further, Nigeria was the leading producer accounted for approximately 63 million tonnes, representing 31% of African production and 20% of global production. According to Afuye & Mogaji (2015), cassava is a very important staple food in most developing countries, originated in South America. It is an available energy and protein source, existing in the South Pacific region, used in commercial feed production, but which has not received much attention (Diarra et al 2017). There are also opportunities to intensify the utilization of cassava, particularly unused or underused fractions and residues, within applied animal feeding programs (Lukuyu et al 2014). A by-product of cassava leaf may make up to 30% of the root yield at harvest which is a moderate source of protein (Diarra et al 2017). According to Lukuyu et al (2014), leaves comprise only 6% of the cassava plant and they can be harvested above ground without damage during root development from 3 to 4 months of age in 60-75 days cycles or can be trimmed with the stems to 40 cm before tuber harvest then chopped by hand or in a stationary forage chopper.

Intrinsically, leaves and stems, that constitute 40% of plant wet weight, have a substantial amount of potential as green feed, fresh or dried, in cassava regions. Nevertheless, cassava leaf is abundant and significantly less expensive compared to maize (Odo et al 2016). The widespread utilization of cassava as a primary feed ingredient in livestock feeding programs has been limited due to the presence of toxic cyanogenic compounds in various fractions and cultivars, high fiber and ash levels in peels, deficiencies of specific nutrients other than energy, fatty acids, minerals, and vitamins (Lukuyu et al 2014). According to Priyadarshani et al (2004), many toxic effects arising mainly from thiocyanate resulting from metabolism of cyanide have been reported. Hence, several technologies have been used to improve the utilization of cassava by-products by monogastric animals (Zendrato et al 2020). The cyanide content of cassava is often measured as hydrocyanic acid (HCN). According to Jorgensen et al (2005), the whole unbruised plant with the cyanogenic glucoside remains intact as linamarin and lotaustralin in a ratio of 93:7. When the cellular structure is disrupted, the intracellular glucoside becomes exposed to the extracellular enzyme, linamarase, then cyanide (HCN) was produced. According to Chiwona-Karltun et al (2022), fermentation can be used to make cassava products that are free of cyanogenic toxins. It is used as a traditional method for detoxification (Tachia et al 2016).

Duckweed is a new type of animal feed ingredient, and the proportion of duckweed replacing traditional feed may affect the digestibility and absorption of nutrients by animals (Takacs et al 2025). It is also rich in macro- and micro-minerals such as calcium and chlorine. Duckweed is an aquatic macrophyte distributed worldwide and it has also been used for ducks, fish, and other animals. Additionally, it can be used as a source of proteinaceous food with a favorable profile of important amino acids (Goopy & Murray 2003). There is an increasing demand of these freshwater macrophytes that it can replace fishmeal and fish oil without affecting the survival, growth performance, and quality of the farmed products (Shrivastav et al 2022). According to a study by Flores-Miranda et al (2014b), the fermentation of duckweed (*Lemna polyrhiza*) flour by *Bacillus* sp. decreases fiber and anti-nutritional factors. It is important to consider that the presence of anti-nutritional factors such as tannins and phytic acid, among others in plants negatively affects their nutritional value and, consequently, the growth of cultured animals. According to Ayuni et al (2019), fermented duckweed improved the quality of quail diet up to 10% by increasing protein and reducing fiber contents with mixed culture probiotics. Moreover, Herawati et al (2020) demonstrated that 2.5% fermented *Lemna minor* meal, as a substitution in fish feed, resulted in the best growth performance of tilapia with a weight gain of 33.03 g and highest relative growth rate of 2.01%.

Nowadays, there is an increasing demand for quality ingredients that can replace fishmeal and fish oil without affecting the survival, growth performance, and quality of farmed products. As a substitute for fish meals, plant protein is widely used in aquaculture as well as the poultry and swine feed industries. Based on the study of Han et al (2022), when the replacement rate of duckweed powder was 20% the weight gain, specific growth rate, feed conversion rate, protein efficiency ratio and other parameters had no difference with the control. Fermented and unfermented dried powder of duckweed was used to feed rohu (*Labeo rohita*) fingerlings. The feed combination was best at 30% fermented fraction, indicating that the fermentation allows duckweed inclusion at a higher rate.

According to the study of Balogun et al (2021), fermentation of cassava involves the steeping of cassava roots in water for 3 to 4 days, which softens the root to disintegrate the tissue structures in contact with the linamarin located in the cell walls, under the action of linamarase, that breaks down the glucosides into hydrogen cyanide (HCN). Traditional technologies have been developed in Central Africa to eliminate cyanohydric acid in cassava leaves, in order to make these suitable for consumption (Kobawila et al 2005). This technology aimed at improving the quality of fish feed by increasing protein and reducing fiber content (Al-Souti et al 2019).

The pond loach is typically found in muddy, still or slow-moving habitats, both in native and introduced communities (Ashton & Ciccotto 2010). Their total length is $\leq$20 cm for the native specimens from East Asia, North Russia, Korea, Japan, China, Vietnam and Burma (Keller & Lake 2007). The pond loach (*Misgurnus anguillicaudatus*) is popular among the Chinese populations, due to its rich nutrients and pleasant taste. Furthermore, it is widely consumed and favored in Asia due to its high nutritional value and excellent flavor (Ke et al 2022). According to Lausan et al (2023), these species are very active right before a thunderstorm when the barometric pressure drops, that causes a pressure change in their swim bladder. The characteristics of pond loach are: an omnivorous food habit, rapid growth, tolerance to high stocking density and utilization of atmosphere oxygen for respiration in oxygen-depleted water, that make them a desirable cultivar of high market value (Wang et al 2009). Additionally, they feed primarily on aquatic insects, as well as on crustacea, Mollusca, and zooplankton while in unfavorable environmental conditions with restricted food resources, detritus can contribute as a major component to the diet (Pyrzanowski et al 2021). This study was conducted to provide a potential diet for *M. anguillicaudatus*. Moreover, this study aimed to evaluate the lactic acid fermentation of cassava leaves and duckweeds.

Material and Method

Study period and location. The study was conducted from June to July 2022 at the Bureau of Fisheries and Aquatic Resources-Cordillera Administrative Region La Trinidad Regional Fish Farm, Benguet, Philippines.

Experimental procedures. Two to three months old cassava leaves were harvested to the local farmers of Virac, Itogon, Benguet, Philippines. After harvesting, the cassava leaves were exposed under the sun for 2 to 3 hours. Then stalks and petioles were removed, and the leaves were cut into fragments. Cut leaves of cassava were then cleaned in water, then drained, and finally fermented naturally with a starter culture of lactic acid bacteria, *Lactobacillus* sp., for 7 days at 37°C, according to the studies of Kobawila et al (2005), Barati et al (2019), Miller et al (2013), and Ikeda et al (2013).

Microbial analysis. The microbial analysis was carried out at the First Analytical Services and Technical Cooperative (FAST) Laboratories, Angeles City, Pampanga, Philippines, according to the standard method, namely the pour plate method.

Determination of the pH values and temperature. 25 g each of cassava leaves and duckweed sampled during fermentation were pounded in a blender, then suspended in 50 mL of distilled water. The pH value and temperature were measured according to the procedure described by Kobawila et al (2005). Furthermore, fermentation of cassava leaves and duckweeds was monitored on daily basis for 7 days, as described by Miller et al (2013).

Chemical analyses. The ash, crude protein, and nitrogen contents of fermented cassava leaves and duckweeds were determined using ignition-gravimetric method and Kjeldahl method. The cyanide content and percentage total titratable acidity were analyzed using distillation / titrimetric method. All chemical analyses were done at FAST Laboratories.

Assessment of palatability. Six aquaria of 2 L capacity were used to assess the palatability of the fermented cassava leaves and duckweeds to *M. anguillicaudatus*. 60 pieces of adult *M. anguillicaudatus* (mean weight=4.349 g) were randomly distributed in 6 aquaria. *M. anguillicaudatus* were allowed to acclimatize for 15-20 minutes. Subsequently, 6 g of test feed diet were introduced into each aquarium and the loach was given 5 minutes to consume the feed. At the end of 5 min, the unconsumed feed diet was siphoned out, dried and weighed. Each feed was tested four times on two days at predefined times at 9:00 h and 14:00 h (Al-Souti et al 2019). The palatability test was carried out at La Trinidad Regional Fish Farm and Loach Hatchery of the Department of Agriculture-Bureau of Fisheries and Aquatic Resources Cordillera Administrative Region.

Dry matter of unconsumed feed diets was analyzed at the Department of Agriculture Feed Chemical Analysis Laboratory-Cordillera Administrative Region.

***Data analysis**.* Mann-Whitney U test was utilized to identify differences between treatments of the fermentation process at a 95% confidence interval ($P<0.05$), while Shapiro-Wilk test was used for normality test of the treatments' data. Data were analyzed using Jamovi 2.3.21 for Windows. The treatments' data were presented as means ±SD.

Results. The chemical contents of fermented cassava leaves and duckweed in terms of ash, crude protein, nitrogen and cyanide content were analyzed. The mean ash content of fermented duckweeds (0.71±0.03%) was comparable to that of fermented cassava leaves (0.94±0.06%) (Table 1). The mean protein content of fermented duckweeds (0.93±0.07%) was also comparable to that of fermented cassava leaves (2.71±0.04%). Regarding the mean nitrogen, fermented duckweeds (0.15±0.01%) and fermented cassava leaves (0.45±0.07%) had comparable contents. The mean cyanide content/hydrocyanic acid of fermented duckweeds (0.085±0.001%) and fermented cassava leaves (0.086±0.002%) were similar. Analysis using t-Test revealed that the mean difference on ash content, crude protein and nitrogen was significantly different ($P<0.05$) while its cyanide content was not significantly different between the two treatments ($P>0.05$).

Table 1

Mean (±SD) percentage of ash, crude protein, nitrogen, and cyanide contents of fermented duckweeds and fermented cassava leaves

Chemical parameters	*Fermented duckweed*	*Fermented cassava leaves*
Ash content (%)	0.71 ± 0.03^{a}	0.94 ± 0.06^{b}
Crude protein (%)	0.93 ± 0.07^{a}	2.71 ± 0.04^{b}
Nitrogen (%)	0.15 ± 0.01^{a}	0.45 ± 0.07^{b}
Cyanide (%)	0.085 ± 0.001^{a}	0.086 ± 0.002^{a}

Means in a row superscripted with the same letter are not significantly different ($P>0.05$)

The physical parameters of fermented cassava leaves and duckweed in terms of pH, total titratable acidity and temperature were analyzed. The mean pH of fermented duckweeds (3.53±0.03) was comparable to that of fermented cassava leaves (3.50±0.08) (Table 2). The mean total titratable acidity of fermented duckweeds (2.31±0.27) was also comparable to that of fermented cassava leaves (1.80±0.11). The mean temperatures of fermented duckweeds (23.9±0.06) and fermented cassava leaves (24.0±0.06) were similar. The Mann-Whitney U test revealed that the mean differences of pH, total titratable acidity and temperature values were not significantly different ($P>0.05$).

Table 2

Mean (±SD) percentage of pH, total titratable acidity, and temperature of fermented duckweeds and fermented cassava leaves

Physical parameters	*Fermented duckweed*	*Fermented cassava leaves*
pH (%)	3.53 ± 0.03^{a}	3.50 ± 0.06^{a}
Total titratable acidity (%)	2.31 ± 0.27^{a}	1.80 ± 0.11^{a}
Temperature (%)	23.9 ± 0.06^{a}	24.0 ± 0.06^{a}

Means in a row superscripted with the same letter are not significantly different ($P>0.05$)

The microbial analysis of fermented duckweeds and fermented cassava leaves in terms of lactic acid bacterial count was analyzed. The mean lactic acid bacterial count of fermented duckweeds (5.6333333 x 10^8 cfu g^{-1}) was comparable to that of fermented cassava leaves (3.5 x 10^8 cfu g^{-1}) (Table 3), which was not significantly different ($P>0.05$) between the two treatments.

Table 3
Mean (±SD) lactic acid bacteria count of fermented duckweeds and fermented cassava leaves

Microbial analysis	*Fermented duckweed*	*Fermented cassava leaves*
Lactic acid bacterial count (%)	5.63333333 x 10^8 [a]	3.5 x 10^8 [b]

Means in a row superscripted with the same letter are not significantly different (P>0.05)

Palatability. The palatability test of fermented duckweeds and fermented cassava leaves in terms of dry matter on the unconsumed feed was analyzed. It was tested four times on two days at predefined times at 9:00 a.m. and 2:00 p.m. (Al-Souti et al 2019). The mean dry matter of fermented duckweeds (2.90±0.44) was comparable to that of fermented cassava leaves (4.79±1.80) (Table 4) which was significantly different (P<0.05) between the two treatments. The test diet consumption by adult loach is higher for the fermented duckweeds than for the fermented cassava leaves.

Table 4
Mean (±SD) percentage of dry matter of fermented duckweeds and fermented cassava leaves

Parameter	*Fermented duckweed*	*Fermented cassava leaves*
Dry matter (%)	2.90±0.04[a]	4.79±1.80[a]

Means in a row superscripted with the same letter are not significantly different (P>0.05)

Discussion. According to the study of Setiyatwan et al (2019), the highest ash content in fermented duckweed (using *Trichoderma harzianum* and *Saccharomyces cerevisiae*) was of 22.76%. In the present study, the result of ash content of treatment 1 (fermented duckweeds) is 0.71%, determined by using lactic acid bacteria. Lower ash content of duckweeds indicated the lower amount of non-biodegradable fraction of carbon and higher digestibility (Negassa & Fikadu 2021). Based on the reported study of Febrini et al (2023), the *Lemna* sp. before fermentation has 15.92% ash content while after fermentation it has 20.76%. According to Iskandar et al (2019), the increase in ash content using *Saccharomyces* sp. for fermentation indicates the high mineral content of the substrate. While in fermented cassava leaves, it ranged from 2.86 to 7.44% using natural *S. cerevisiae* and *Lactobacillus plantarum* inoculum from the study of Terefe, Omwamba & Nduko (2022). In the present study, the result of ash content in treatment 2 (fermented cassava leaves) was 0.94%, by using lactic acid bacteria.

Based on the study of Leng et al (1995), the crude protien content of duckweed ranges from 35 to 43% compared to the fermented duckweed, which ranges from 84.8 to 97.9%. Nevertheless, the protein content of *Lemna* sp. before fermentation process is 19.17% while after fermentation is 23.47% (Febriani et al 2023). Moreover, a fermentation process for seven days will increase the crude protein by 5.60%. Based on the study of Andriani et al (2019), the fermented *Lemna* sp. has increased protein content and a decreased crude fiber, which causes an increase in the daily growth rate of catfish and tilapia, in line with Handajani (2007), who stated that the fermentation process increases the nutrients and energy.

Moreover, the fermentation process will remodel complex compounds into simpler compounds to easily absorb by the body. On the other hand, cassava leaves have a higher protein content (of 17 to 38%) on a dry weight basis (Latif et al 2020) compared to the fermented cassava (<17%). Between the two treatments, fermented cassava leaves had a higher protein content (2.71%) than fermented duckweeds (0.93%), being suitable for the nutritional needs of loach; thus, it can also support its growth. An increase in protein contents of the fermented duckweeds and fermented cassava leaves after fermentation might be due to the proteolytic activities and to the increased number of microorganisms during fermentation (Adegbehingbe et al 2017). According to Trapsilo et al (2020), enzymes broke the protein into fragments, producing organic acids (which are excreted), by fermentation with lactic acid. Moreover, fermentation process used to increase the feed quality and digestibility by breaking down complex organic compounds, Gunawan et al (2015) used lactic acid bacteria in the fermentation of cassava leaves, with different reaction times, which resulted in increased protein (by 8% higher than without fermentation). The crude protein of cassava leaves using *Lactobacillus* sp. (2.79 to 3.05%) was lower than the levels reported by Tefera et al (2014), who demonstrated that cassava tubers with *S. cerevisiae* has a crude protein content of 0.74 to 4.58%. Therefore, it is higher than the protein content obtained in fermented cassava leaves, which is 2.713%. The difference of might be attributed to the variety of cassava used, agro-ecological conditions and fermentation time.

Sikora et al (2018) showed that the nitrogen content in duckweed may fluctuate from 2 to 6% under a high concentration of nitrogen compounds in water. Additionally, a high concentration of total nitrogen (ammonia) is due to the anaerobic breakdown of proteins, which hinders the anaerobic digestion (Negassa & Fikadu 2021). The nitrogen content of cassava leaves was found to be in the range of 17-39% of its protein content which has been reported by Karuna et al (2022) and other authors. Additionally, there was 3.19% of nitrogen content in the whole cassava leaves which could be used as a nitrogen source for the fermentation. Nevertheless, it is lower than the nitrogen content obtained in fermented cassava leaves (0.086%).

Based on the findings of Fasakin (1999), cyanide contents related to the leaf protein content (LPC) in duckweed ranged from 0.12 mg 100 g^{-1} to 0.15 mg 100 g^{-1}, while in fermented duckweed ranged from 0.0843 mg 100 g^{-1} to 0.0862 mg 100 g^{-1}. Hence, fermentation process can decrease the cyanide contents of a plant. Moreover, the raw duckweeds meal has an LPC of 4.88 mg 100 g^{-1} (Falaye et al 2022). According to the study of Hawashi et al (2019), it was observed in the cassava leaves that an exponential decrease in cyanide content over time can lead to satisfactory detoxification, with cyanide concentration falling to levels lower than 10 ppm after 60 hours of fermentation which provides a safe and healthy food source for fish. Confirming that fermentation is then a very effective process for elimination of endogenous cyanic compounds from cassava roots and leaves obtained by Agbor-Egbe et al (1995) and Kobawila et al (2005). Moreover, fermentation can reduce the HCN level of cassava up to 95% depends on the material and time that influence how much HCN can be reduced (Sudharmono et al 2016). Lactic acid bacteria have role in decreasing the cyanide, it can cause cyanide degradation and increase nutrient digestibility of cassava. Based on the findings of the study, cassava leaves have been fermented for 7 days which can be alternative fish feed. According to Jayanegara et al (2025), lactic acid bacteria fermentation reduces the cyanide content and improves the microbial quality of cassava, supporting the traditional fermentation techniques which enhance food safety through acidification and microbial activity.

The observed decrease in the pH of the samples is a desirable development because growths of many gram-negative and acid sensitive food borne pathogens are inhibited at low pH (Adegbehingbe et al 2017). According to the study of Kobawila et al (2005), the decrease in pH during the fermentation of cassava leaves results from the production of organic acids by lactic acid bacteria, which constitute the dominant microflora. The pH of fresh cassava decreased to 6.5 as total organic acid of 0.07% increased during fermentation period (Adewusi et al 1999). Based on the study of Febriani et al (2023), the pH during fermentation process relates to the degree of acidity of the medium that will determine the activity of microorganisms. When it is too low (acidic) or high (basic) pH, it triggers the microbial cell death. Generally, a pH ranging from 3-6 is a good growth for the microbes. The effect of the breakdown of organic materials is due to fermentation process that produces carbon dioxide and heat. Hence, the pH decreases since microorganism breakdowns carbohydrate during the fermentation process of cassava (Sudharmono et al 2016). It will keep decreasing during fermentation due to activities of microorganism same with the study of Tivana et al (2007). According to Steinkraus (1992), some of the lactobacilli and pediococci have a pH of 3.5 before inhibiting their own growth. Similarly, Gunawan et al (2015) indicated that the pH condition of *L. plantarum* and *S. cerevisiae* decreases from 5.6 to 3.9 and from 6.6 to 3.9 respectively during the fermentation temperature studied at 30°C. In the present study, using *Lactobacillus* sp. for fermentation ranges from 3.42-3.57 pH. Based on the study of Tefera et al (2014), the mean pH of fermented cassava leaves was 4.95 utilizing single starter cultures wherein it indicates that cassava fermentation is significant in the reduction of pH. Additionally, *L. plantarum* produces lactic acid which can reduce the pH that can inhibit contamination of pathogenic microorganisms (Abubakar et al 2022). Contrarily, in the study of Flibert et al (2021), fermented cassava dough varies from 6.05 to 4.21. Based on the study of Tefera et al (2014), a sample fermented with *L. plantarum* and *L. mesenterodes* has the highest pH reduction from 6.68 to 3.70. According to Wafula et al (2023), the ability of the bacteria to acidify during fermentation process is reflected in the pH production.

The total titratable acidity of fermented duckweeds and cassava leaves during the fermentation period could be due to the ability of the fermenting organisms to secrete acids while utilizing the available nutrients for their metabolic activities. According to Adegbehingbe et al (2017), this might also be due to the presence bacteria which degrade carbohydrate, resulting in acidification. Based on the study of Flibert et al (2021), the level of acidity in fermented cassava ranged from 0.5 to 4.8 for 7 days of fermentation, while in the present study it ranged from 1.78 to 1.99.

The temperature of fermented duckweeds and cassava leaves was found to increase slightly because of the heat that is being generated as a result of exothermic reactions mediated by microbial enzymes, during the fermentation. The reported observations in Adegbehingbe et al (2017) and Onyimba et al (2009) were similar, related to the fermentation of spent sorghum grains. According to Tivana et al (2007), high ambient temperature makes local peasants use a shady place during the day, to create a lower temperature for fungal growth in cassava.

The main bacteria associated with cassava fermentation in the study is lactic acid bacteria. According to the study of Flores-Miranda et al (2014a), the lactic acid bacteria are widely used as starter cultures for the production of fermented foods. Additionally, bacteria are an important source of high-quality protein with up to 70%. The higher microbial counts in ground sample could be a result of grinding treatment which increases the surface area of the sample, making nutrients more readily available for the fermenting microorganisms' growth, according to Adegbehingbe et al (2017). Based on the results, fermented duckweeds have a higher microbial count than fermented cassava leaves. Lactic acid bacteria (LAB) type bacteria produce enzymes which degrade the substrate during the fermentation process (Trapsilo et al 2020). During the fermentation of cassava leaves, *L. plantarum* bacteria reach a density of 10^{14} cells m^{-1} within 14 days. Based on the findings of Tivana et al (2007), the total lactic acid bacteria increased from 10^4 to 10^6 cfu g^{-1} while in the current study, fermented cassava leaves have a LAB count between 2.0 x 10^8 and 5.8 x 10^8 cfu g^{-1}.

To our knowledge, this is the first study that evaluated the palatability of fermented duckweeds and fermented cassava leaves for pond loach at adult stage. Based on the current study, fermented duckweeds have the potential diet with the highest recorded consumed feed. According to Mustofa et al (2022), the protein content of fermented duckweed was estimated at 29.86 and 35.43% which indicates a high protein content of the feed diet. Likewise, since adult loach were used, the present study suggests a juvenile stage and longer rearing trial to qualify the fermented duckweeds as an effective loach co-feed component as a feed diet to determine long-term adaptability and performance of the said species to the ingredients.

Conclusions. The lactic acid fermentation has comparable effect on cassava leaves and duckweed in terms of ash, crude, protein, nitrogen, and cyanide content. The physical parameters of fermented cassava leaves and duckweeds, in terms of pH, are relatively correlated, while the temperatures are inversely correlated. Fermented duckweeds have a higher microbial count than fermented cassava leaves, for a duration of 7 days of fermentation. Moreover, fermented duckweeds had a higher amount of consumed diet by *M. anguillicaudatus* during the 2 days of palatability testing.

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Conflict of interests. The authors declare that there is no conflict of interest.

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