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Biomass Production of Water-Meal (*Wolffia globosa*) and Its Chemical Composition and Amino Acid Profiles when Grown with Chemical Fertilizer in an Out-door Polyethylene (PE) Tank Cultivation System

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Abstract

Water-meal (*Wolffia globosa*) was cultivated with NPK (15:15:15) fertilizer under three treatments with four replicates. The treatments consisted of NPK fertilizer applications at three different levels: 16 g/tank (CF1), 26 g/tank (CF2), and 36 g/tank (CF3), which were applied weekly. The water-meal was grown for 35 days, the biomass production was assessed, and the treatment with the highest mass production was harvested in order to determine the chemical composition and the amino acid profiles. The results showed that water-meal cultivated with NPK fertilizer at the rate of 26 g/tank (CF2) had exhibited the highest biomass production. There was a significant difference ($P<0.05$) compared to the CF1 treatment, while no significant difference ($P>0.05$) was found when compared to the CF3 treatment. The water-meal that had been cultivated with the CF2 treatment exhibited high protein content (40.64±2.13%) and rich amino acid profiles. It contained 17 types of amino acid profiles, with 9 essential amino acids (EAA), and 8 non-essential amino acids (NEAA). Glutamic acid was the major amino acid, while leucine and lysine were the most abundant EAA. Our findings indicated that this cultivation system can be used to produce highly nutritious water-meal biomass for human consumption or animal feed.

Introduction

By 2050, the world's population has been projected to reach about 10 billion with an expected 50% increase in food production (Shrestha & Mahat, 2022). The growing population significantly challenges food security, necessitating the provision of adequate food quantity and quality without harming the environment (Liliane & Charles, 2020). The global demand for animal products is projected to increase by 60% to 70% by 2050 (Makkar, 2018). In particular, it is expected that the increasing demand for animal-based proteins will have negative environmental impacts, generate greenhouse gas emissions, and will require more water due to the associated health benefits of consuming meat (Ismail et al., 2020). The increasing demand for animal products and the scarcity of conventional feed ingredients are driving the search for alternative protein sources for animal feed (Chia et al., 2019). Protein supplements are one of the most expensive and limiting feed ingredients (Kim et al., 2019). Fishmeal is a major protein ingredient that is commonly used in the diet of most farm animals. In fact, the largest user of fishmeal is the aquaculture industry sector, which consumes approximately 46% of the total annual fishmeal production (Miles & Chapman, 2006). Fishmeal is considered unsustainable and not eco-friendly (Gokulakrishnan et al., 2023). Presently, this issue has received increasing concern since more and more consumers are turning to vegetarianism or looking for products that are not animal-based (Kurek et al., 2022). Therefore, the discovery of alternative protein sources is essential for sustainable human consumption and animal production.

Plant protein sources are acknowledged as the best sources to replace fish meal (Daniel, 2018) and meat products (Kurek et al., 2022). *Wolffia* spp. (water-meal or duckweed), which belong to the Lemnaceae family (Said et al., 2022), are the smallest flowering plants on Earth (Pagliuso et al., 2022). The fresh plants, which are a traditional food from a third country, have been consumed for more than 25 years in Asia (Myanmar, Laos and Thailand). The Thai common names are Khai-nam, which has been translated to eggs of water-meal, Kai-Pum, and Kai-nhae (European Food Safety Authority (EFSA), 2021). Two species of water-meal, *W. arrhizal* and *W. globosa*, are closely related sister species (Park et al., 2024). The *W. globosa* is an aquatic plant that is native to Southeast Asia, but is invasive in Europe (Vávra et al., 2024). Due to its outstanding nutritional profile, water-meal has emerged as a promising candidate as an alternative protein source. Because it is rich in proteins and carbohydrates and contains beneficial PUFAs, it is an excellent candidate for health-conscious and protein-deficient diets (Boonarsa et al., 2024). Furthermore, it has a cosmopolitan distribution, rapid growth, and a scalability that ranges from household tanks to large lagoons and does not require arable land, which means there is no competition with major crops (Sulaiman et al., 2025). Consequently, water-meal has a high potential for practical applications in human nutrition (Appenroth et al., 2018), in natural feed sources for aquaculture (Said et al., 2022), and in animal feed for sustainable livestock production (Thongthung et al., 2024). Moreover, water-meal can be utilized to produce ethanol, butanol, and biogas (Cui & Cheng, 2015). Previously, inorganic fertilizers and organic fertilizers have been used in the cultivation of water-meal in Thailand (Sricharoen et al., 2001; Rowchai & Somboon, 2007). The cultivation method to produce water-meal depends upon the purpose of the water-meal utilization. Organic fertilizers derived from animal manure have been used to produce water-meal biomass for animal feed (Sricharoen et al., 2001). In terms of inorganic fertilizers, these have been used to grow water-meal for human consumption (Ruekaewma et al., 2015; Rowchai & Somboon, 2007) given that hygienic water-meal is required. Organic fertilizers are relatively low in nutrients, so larger volumes are required to supply sufficient nutrients for plant growth. In addition, they may contain pathogens that are harmful to humans or plants. In contrast, inorganic fertilizers, which are usually immediate and lasting, contain all the necessary nutrients that are directly accessible for plants, so only small amounts are required for productivity (Roba, 2018) and to more rapidly increase the growth rate and overall productivity of the plants (Sharma & Chetani, 2017). Ezeani & Abu (2019) reported that for commercial algae biomass production, inorganic fertilizers (NPK 15:15:15 and NPK 20:20:20) can be relatively cost-effective and are a locally available substitute. However, knowledge about the cultivation of water-meal with NPK fertilizer for biomass production is limited.

The purpose of this study was to discover an efficient water-meal cultivation system to produce water-meal biomass as a plant-based proteins source with a high nutritional profile and sustainable for human consumption or animal feed production. The biomass production of water-meal (*W. globose*) grown with NPK (15:15:15) fertilizer utilizing an outdoor polyethylene (PE) tank cultivation system was evaluated. The parameters of water quality were examined. The chemical composition and amino acid profiles of the treatment, which had produced the highest biomass were analyzed and a simple economic assessment was conducted.

Materials and Methods

Plant Collection and Experimental Site

Water-meal (*Wolffia globosa*) was collected from a local market in the Kantharalak District of Sisaket Province, Thailand. This study was performed in twelve circular polyethylene (PE) tanks, which had approximate diameters of 1 m and heights of 0.4 m (area: 0.79 m^2) and were filled with water to a depth of 20 cm (water volume =157 L). All PE tanks were set up under outdoor conditions and covered with sunshade netting.

Experimental Design and Cultivation

The experiment was performed in a completely randomized design (CRD) in 3 treatments with 4 replicates. NPK fertilizer (15:15:15) was used as the inorganic fertilizer for this study. The treatments included the application of NPK fertilizer at three different levels: 16 g/tank (CF1), 26 g/tank (CF2), and 36 g/tank (CF3). The concentration of NPK fertilizer in each treatment is shown in Table 1. Underground water was used to fill the tank, and it was stored in the tank before use. The NPK fertilizer was completely dissolved in water before it was loaded into the tanks. Water-meal was weighed at 16 g/tank (or at a rate of 20 g/m^2) and was then added into each tank as the initial weight. The water-meal was grown for 35 days. During the trial, the NPK fertilizer was loaded into each tank on a weekly basis, and the underground water was filled to replace the water that had been lost through evaporation.

Water Quality Parameters Analysis

During the experiment, the water quality profiles of each tank were examined daily.

Water samples were collected at early in the morning, between the hours of 6:00 AM and 8:00 AM to eliminate temperature effects (Ushurhe et al., 2024).

Table 1. The concentration of NPK (15:15:15) fertilizer in each treatment

Treatment	Nitrogen (mg/l)	Phosphorous (mg/l)	Potassium (mg/l)
CF1	15.29	15.29	15.29
CF2	24.48	24.84	24.84
CF3	34.39	34.39	34.39

All tanks were measured for pH and temperature (pH meter) and for dissolved oxygen (DO meter; Lutron PDO-520). The total alkalinity (titration method) was determined following the standard method of Boyd (1979), while the total ammonia nitrogen (TAN), nitrite (NO_2), nitrate (NO_3), and orthophosphate were determined by using commercial kits. These water quality parameters were determined every 5 days.

Water-meal Mass Production Analysis

During the experiment, the water-meal in each experimental group was randomly weighed every five days. The water-meal was randomly scooped out for 1 liter of water from the surface and filtered. Then the water-meal was weighed with a digital weight scale at 2 positions, and this process was repeated 3 times. At the end of the experiment, all the water-meal in each treatment was weighed. The evaluations of water-meal biomass production were as follows: 1) weight gain, 2) the total daily growth rate (GR), 3) the specific growth rate (SGR) and daily productivity according to Said et al. (2022), and 4) the relative growth rate (RGR) according Chakrabarti et al. (2018). This was calculated using the following formulas:

Weight gain (g) = (total weight-initial weight)

The total daily growth rate (g/day) = (total weight-initial weight)/duration (days)

The specific growth rate (%) = ((In total weight-In initial weight)/duration (days)) × 100

Daily productivity (g/m^2/day) = total yield/unite area (m^2)/duration (days)

Relative growth rate (g/g/day) = In (fresh total weight at the time of harvest / fresh total weigh at the time of introduction)/time interval in days.

Economic Analysis

The cost of fertilizer and the value of water-meal were calculated based on local market prices. The price of fertilizer was 25 THB per kilogram, while the water-meal was 50 THB per kilogram. Total fertilizer cost (THB/tank) was calculated by multiplying total fertilizer used (kg/tank) with fertilizer price (THB/kg). The estimated production cost (THB/kg) was calculated by total fertilizer cost (THB/tank) divided by final weight (kg/tank). Total gross revenue and the gross profit was calculated according to Nunes & Masagounder (2022). The gross revenue (THB/tank) was calculated by multiplying the water-meal price (THB/kg) with final weight (kg) from each tank. The gross profit (THB/tank) was calculated by the gross revenue subtracted by total fertilizer cost. Benefit cost ratio was calculated by dividing the gross revenue by the total fertilizer cost.

Proximate Composition Analysis

At the end of the experiment, the experimental group with the highest mass production of water-meal was collected for chemical composition and amino acid analysis. Fresh water-meal was harvested and was dried using sun drying until the weight had stabilized. Then the dried water-meal was ground using a fine grinder. The water-meal powder was stored in a plastic bag and kept at 4 °C until required for use. The chemical composition, such as moisture, ash, crude protein, and crude lipids, were analyzed in accordance with the methods of the Official Association of Analytical Chemists (AOAC, 2010). The amino acid content was analyzed at the Research Instrument Center of the laboratory at Khon Kaen University. The amino acid content was determined using an ion-exchange chromatography with a post column derivatization and spectrophotometric detection of ninhydrin reaction products, as well as with the use of an automatic amino acid analyzer (SCION Artemis 6000 Amino Analyzer, Goes, Netherland), which was conducted in accordance with the producer’s standard.

Statistical Analysis

Data processing was conducted with Microsoft Excel 2010 and was statistically analyzed using IBM SPSS Statistics version 29. Then the statistically significant differences between the groups were determined by the analysis of variance (ANOVA) with the least significant differences tested (P<0.05).

Results

Water-Meal Biomass Production

The mass production of *W. globosa* was cultivated with NPK fertilizer (15:15:15) at three different levels for 35 days. The results indicated that the water-meal cultivated with NPK fertilizer at a rate of 26 g/tank/week (CF2) had achieved the highest final weight, weight gain, growth rate, specific growth rate, daily productivity, and relative growth rate. There was a significant difference

($P<0.05$) compared to water-meal cultivated with NPK fertilizer at a rate of 16 g/tank (CF1), while there was no significant difference ($P>0.05$) with water-meal cultivated with NPK fertilizer at a rate of 36 g/tank (CF3) (Table 2). The biomass production of water-meal is shown in Figure 1. The results showed that the water-meal cultivated with NPK fertilizer at a rate of 26 g/tank had the highest production of biomass with maximum biomass production with 37.68 g/L, followed by the CF3 and CF1 treatments, in which the maximum biomasses were 32.95 g/L and 30.65 g/L, respectively.

Economics

A simple economic was calculated and indicated that the water-meal cultivated with NPK fertilizer at a rate of 36 g/tank (CF3) had the highest ($P<0.05$) estimated production cost per kilogram (7.21±0.63), followed by CF2 (4.86±0.35) and CF1 (3.73±0.49) treatments. The results showed that the water-meal cultivated with NPK fertilizer at a rate of 26 g/tank/week had achieved the highest ($P<0.05$) gross revenue and the highest ($P>0.05$) gross profit. However, the water-meal cultivated with NPK fertilizer at a rate of 16 g/tank/week (CF1) gave the highest ($P<0.05$) benefit cost ratio.

Chemical Composition and Amino Acid Profiles

The water-meal, which was cultivated with NPK fertilizer at a rate of 26 g/tank, had provided the highest biomass production. It was collected in order to analyze the chemical composition and amino acid profiles based on dry weight. The results showed that the moisture, ash, crude protein, and crude fat of water-meal were 9.01±0.05%, 18.95±0.10%, 40.64±2.13% and 3.44±0.10% respectively. The total amount of amino

Table 2. Biomass production and economic analysis of water-meal cultivated NPK fertilizers (15:15:15) at different levels for 35 days (mean±SD)

	Treatment			
Parameters	CF1	CF2	CF3	P-value
Initial weight (g)	16.00±0.00	16.00±0.00	16.00±0.00	-
Final weight (g)	542.40±67.97[b]	671.21±45.78[a]	627.50±56.24[ab]	0.032
Weight gain (g)	526.40±67.97[b]	655.21±46.78[a]	611.50±56.24[ab]	0.032
Daily growth rate (g/day)	15.04±1.94[b]	18.72±1.34[a]	17.47±1.60[ab]	0.032
SGR (%/day)	10.05±0.36[b]	10.67±0.20[a]	10.47±0.25[ab]	0.034
RGR (g/g/day)	0.100±0.003[b]	0.106±0.002[a]	0.105±0.002[ab]	0.034
Productivity (g/m^2/day)	19.62±2.46[b]	24.27±1.69[a]	22.69±2.03[ab]	0.032
Total Fertilizer used (kg/tank)	0.08	0.13	0.18	-
Total Fertilizer cost (THB/tank)	2.00	3.25	4.50	-
The estimated production cost (THB/kg)	3.73±0.49[c]	4.86±0.35[b]	7.21±0.63[a]	<0.001
The gross revenue (THB/tank)	27.12±3.39[b]	33.56±2.34[a]	31.38±2.81[ab]	0.032
The gross profit (THB/tank)	25.12±3.40	30.31±2.34	26.88±2.81	0.082
Benefit cost ratio (B/C)	13.56±1.70[a]	10.33±0.72[b]	6.97±0.62[c]	<0.001

Data in the same row with different letters are significant difference ($P<0.05$) among treatments.

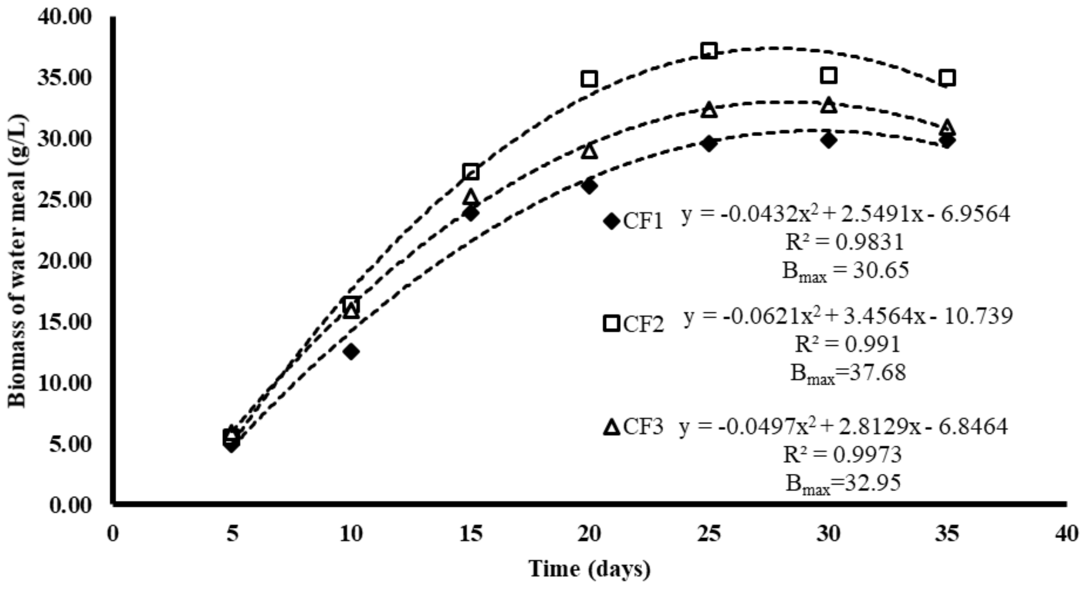

Figure 1. Biomass production of water-meal cultivated with NPK fertilizers (15:15:15) at different levels for 35 days.

acid was 30.7191 mg/100 mg, which contained 17 types of amino acid profiles and consisted of 13.8544 mg/ 100 mg with 9 types of Essential Amino Acids (EAA) and 16.8647 mg/100 mg with 8 types of Non-Essential Amino Acids (NEAA) (Table 3).

Water Quality

The quality of the water in the experimental tanks was examined, and the results are shown in Table 4. The mean value of water quality parameters showed that the values for temperature, dissolved oxygen, nitrate (NO_3), and orthophosphate (PO_4) had ranged from 30.88-31.23°C, 7.00-74.30 mgL^{-1}, 45.83-54.17 mgL^{-1}, and 0.25-0.44 mgL^{-1}, respectively. Moreover, none of the mean values had been significantly different among the treatments (P>0.05). The values of pH, total alkalinity, nitrite (NO_2), and total ammonia nitrogen had ranged from 6.72-7.61, 16.43-26.00 mgL^{-1}, 0.54-0.89 mgL^{-1}, and 3.86-4.89 mgL^{-1} respectively. These figures indicated that among the treatments, the mean values had been significantly different (P<0.05). The present study indicated that the act of increasing the NPK fertilizer concentration had increased the total alkalinity and had decreased pH value. Compared to other treatments, the rate of the 16 g/tank had exhibited the highest nitrite, while the rates of the 26 and 36 g/tank had shown a higher total ammonia nitrogen than that of the 16 g/tank.

Table 3. The chemical composition and amino acid profiles of water-meal cultivated with NPK fertilizers (15:15:15) at 26 g/tank for 35 days

Chemical composition	Content (% dry weight)
Protein	40.6428±2.1324
Lipids	3.4420±0.0990
Moisture	9.0102±0.0452
Ash	18.9462±0.1030
Amino acids contain	mg/100 mg
Essential Amino Acids (EAA)	
Methionine	0.1835±0.0146
Threonine	1.5946±0.0347
Histidine	0.3962±0.0046
Isoleucine	1.0975±0.0873
Leucine	2.9255±0.1539
Lysine	1.9121±0.0417
Valine	1.6895±0.0120
Phenylalanine	1.7618±0.1209
Total EAA	11.5606±0.4605
Non-essential amino acids (NEAA)	
Arginine	1.9372±0.0440
Aspartic acid	3.6885±0.0804
Serine	1.6683±0.0267
Glutamic acid	4.0375±0.0833
Glycine	1.9708±0.0449
Alanine	2.3668±0.0277
Cysteine	0.0684±0.0087
Tyrosine	1.1652±0.0564
Proline	1.6747±0.0069
Total NEAA	18.5773±0.3616
Total Amino acid	30.1379±0.8222

Table 4. Water quality parameters determined in tanks during water-meal cultivated with NPK fertilizers (15:15:15) at different levels for 35 days.

Parameters	CF1		CF2		CF3	
	Rang	Mean±SE	Rang	Mean±SE	Rang	Mean±SE
Temperature (°C)	31.05-31.39	31.23±0.16	31.00-31.13	31.05±0.05	30.88-31.17	31.04±0.12
pH	6.98-7.15	7.61±0.08^{a}	6.73-6.88	7.30±0.05^{b}	6.72-6.75	7.09±0.02^{c}
Dissolved oxygen (mgL^{-1})	7.00-7.26	7.40±0.15	7.00-7.31	7.22±0.08	7.03-7.34	7.20±0.16
Total alkalinity (mgL^{-1})	16.43-20.43	19.36±2.04^{c}	20.00-25.00	22.57±2.27^{b}	25.57-26.57	26.00±0.42^{a}
Nitrite (NO_2) (mgL^{-1})	0.68-0.89	0.79±0.12^{a}	0.50-0.61	0.56±0.05^{b}	0.54-0.64	0.58±0.05^{b}
Nitrate (NO_3) (mgL^{-1})	45.83-62.50	50.00±8.33	45.83-50.00	46.88±2.08	45.83-62.50	54.17±6.80
Total ammonia nitrogen (TAN) (mgL^{-1})	3.86-4.14	4.07±0.14^{b}	4.57-5.14	4.86±0.23^{a}	4.29-5.71	4.89±0.66^{a}
Orthophosphate (PO_4) (mgL^{-1})	0.25-0.50	0.36±0.11	0.32-0.50	0.39±0.08	0.39-0.49	0.44±0.04

Data in the same row with different letters are significant difference (p<0.05) among treatments. Values are mean±SE and rage of the water quality parameter average.

Discussion

During the trial, the water temperature varied between 30.88°C - 31.23°C, which is a suitable temperature for *W. globosa* cultivation. The optimal temperature for growing water-meal (*W. arriza*), which ranges from 26°C - 34°C, was reported by Soda et al. (2013). This was confirmed by Kumar et al. (2022), who reported that the water temperature in the range of 31.21°C - 31.59°C was suitable for the growth of *W. globosa*. The present study indicated that increasing the NPK fertilizer dosages (16, 26, and 36 g/tank) had influenced to water quality by increasing the total alkalinity and the total ammonia nitrogen, while decreasing the pH and nitrite (NO_2) values. Asuquo & Essienibok (2014) reported that the total hardness and total alkalinity due to the concentration of NPK (15:15:15) fertilizer had increased. The application of the inorganic fertilizer (urea + super phosphate) had caused a greater reduction in pH, while causing a greater increase in free CO_2, total alkalinity, hardness, TAN, nitrite, and nitrate content, which was also observed by Das et al. (2005). However, the present study indicated that the pH value and total ammonia nitrogen had significantly ranged from 6.72-7.61 and that the total ammonia nitrogen had ranged from 3.86-5.71 (mgL^{-1}), which are in the optimal ranges for the *W. globosa* growth. Caicedo et al. (2000) reported that the concentration of ammonium and pH values may severely reduce duckweed (*Spirodela polyrrhiza*) growth. The total ammonia concentration in duckweed ponds should be below 50 (mgL^{-1}), while the pH should be maintained below 8. It has been confirmed that the optimal pH value for the growth of duckweed is around 7.

Duckweed converts substantial amounts of fertilizer into plant biomass, and the nutrient removal rate is directly proportional to the growth rate (Journey et al., 1993). Duckweed growth is measured as the relative growth rate (RGR) per day (Romano & Aronne, 2021). The difference in the RGR was most likely caused by different growth conditions and the concentration of nutrients in the medium, as well as the ratio between nitrate-N and ammonium-N (Petersen et al., 2021). The present study showed that in all the treatments, the RGR had ranged from 0.101±0.003 to 0.106±0.001 g/g/day. This was lower than the range reported by Sree et al. (2015), in which the RGR of *W. globosa* from different origins had ranged from 0.155 to 0.559 g/g/day when cultivated with Schenk-Hildebrandt medium. Both the nitrogen (N) and the phosphorus (P) in the growth medium significantly influence the biomass production and nutritional composition of duckweed (*Lemna minor*) (Ullah et al., 2022). The findings from this study indicated that the water-meal, which had been cultivated with NPK fertilizer concentration at the lowest concentration (16 g/tank or 15.29 mg/L of NPK fertilizer), had decreased biomass production compared to the higher NPK fertilizer concentrations (26 g/tank or 24.48 mg/L of NPK fertilizer and 36 g/tank or 34.39 mg/L of NPK fertilizer). Stadtlander et al. (2022) reported that the optimum TAN concentration is important for operators of duckweed (*Spirodela polyrhiza* and *Landoltia punctate*) slurry systems. Reduced biomass production in the lowest slurry concentration could be a sign of N limitation, while reduced biomass production in the highest slurry concentration could result from increased NH_3 concentrations and could have potential toxicity effects on duckweed. Duckweed (*Lamna minor*) cultivated with high chicken manure concentration (1:8 dilution) led to a nearly complete die-off the duckweed population, while a low concentration of chicken manure fertilization (1:16) resulted in acceptable growth and high crude protein content (Stadtlander et al., 2023). Chicken manure contains high amounts of nitrogen, phosphorus and potassium (Manogaran et al., 2022). Dani et al. (2021) reported that giving a high dose of chicken manure causes the nutrient balance to be disrupted so that plant growth has decreased. Furthermore, Kumar et al (2023) suggested that using an excessive amount of fertilizer should be avoided while growing *W. globosa* in order to obtain the highest quantities (DPPH free radical scavenging activity and total carotenoid content) and to minimize adverse impacts on the quality.

The water-meal cultivated with NPK fertilizer at 26 g/tank was harvested to evaluate the chemical composition and amino acid profiles. Previously, Appenroth et al. (2018) stated that the total protein content of the genus *Wolffia* varied between 20-30% of the freeze-dried weight and that the fat content was between 1-5%. One of the trade-offs for gaining high protein and lipid content is the conditions of cultivation (Sembada & Faizal, 2022). Duckweed that has been grown in water with 10-30 mg/NH_3-N/L has a high protein content (around 40%) of high biological value (Hillman & Culley, 1978). Our results showed that water-meal, which had been cultivated with NPK (15:15:15) fertilizer at 24.48 mg N/L, had shown crude protein and crude lipid contents of 40.64±2.13% and 3.44±0.10% of dry weight, respectively. These results were similar to results from growing *W. arriza* under aquaculture conditions in an outdoor cement pond with hydroponic electrical conductivity (EC) of 0.5 ms/cm. Under those conditions, the protein and lipid contents were 41.81±3.40% and 1.99±0.08%, respectively (Prosridee et al., 2023). In this study, the protein content of water-meal was found to be higher than the *W. arriza* that had been cultivated with 6.648 kg of anaerobically fermented cattle manure and in media that had been maintained at 32 mg N/L. Under those conditions, the crude protein had been determined to be 31.25% (Chowdhury et al., 2000). However, in this study, it was found that the protein and lipid content had been lower than the *W. arriza*, which had been cultivated with 1/5 of modified nitrogen-free Hoagland medium with 7.15 mmol/L urea and had been cultivated under artificial conditions of a photonflux density of 150-160 $\mu molm^{-2}s^{-}$

[1] in a full light period. Under those conditions, the protein content had been more than 50% of the dry weight and the lipid content had been 6.07±0.30% of the dry weight (Hu et al., 2022). Moreover, the *W. globosa* that had been cultivated with Modified Hutner's medium contained crude protein and fat at 48.2% and 9.6%, respectively (Ruekaewma et al., 2015).

Protein quality was assessed based on the amino acids profile. Duckweed protein has a better array of essential amino acids than most vegetable proteins and more closely resembles animal protein (Hillman & Culley, 1978). Duckweed is a good candidate for nutritious and safe meat protein substitutes for humans, given that the amino acid composition of water-meal meets the World Health Organization (WHO) recommendations (Baek et al., 2021). Species of the genus, *Wolffia*, have high nutritional value for human consumption (Appenroth et al., 2018) and are a promising alternative protein source, which has significant health-promoting properties and nutrient profile (Boonarsa et al., 2024). The present study showed that a total of 17 types of amino acid profile had been detected consisting of 9 types of EAA and 8 types of NEAA. This study showed a similar amino acid profile for *W. globosa* to that, which had been previously reported by Dhamaratana et al. (2025), who reported a higher amino acid profile than was reported by Said et al. (2022), who stated that *W. globosa* contains 15 types of amino acids. *W. globosa* is rich in essential amino acids, including leucine, valine, and phenylalanine, which was reported by Siriwat et al. (2023). The present study revealed that glutamic acid is a major NEAA (4.0964 mg/100 mg), followed by aspartic acid (3.7453 mg/100 mg), while leucine and lysine are the most abundant EAA in water-meal, with contents of 3.0343 mg/100 mg and 1.7416 mg/100 mg, respectively. This study showed that the amino acid compositions of *W. globose* had been similar to findings from the investigation by Dhamaratana et al. (2025).

An economic analysis revealed that the cultivation of water-meal biomass using NPK (15:15:15) fertilizer at 16-36 g/tank yielded low production cost, estimated at approximately 3.73 to 7.21 THB/kg (or 0.11 to 0.22 USD/kg). This cost is considerably lower than those reported for other alternative protein source. For instance, the production cost of microalgae range from 1.6 to 8.1 USD/kg, depending on the scale (Alavianghavanini et al., 2024), while the cost of producing mycoprotein is approximately 3.55 USD/kg (D'Almeida & de Albuquerque, 2025). These findings suggest that water meal biomass produced through this cultivation system present a cost effective alternative protein source, potentially its viability for large-scale applications.

Conclusions

Water-meal (*W. globosa*), which had been cultivated with NPK (15:15:15) fertilizer at 26 g/tank/week (or 24.48 mg/L each of NPK fertilizer), was determined to be acceptable for the production of water-meal biomass, which provides high protein (up to 40%), rich amino acids, and yielded the highest gross revenue and gross profit. The water-meal biomass cultivated with NPK (15:15:15) fertilizer provides a low-cost production method and can serve as a sustainable plant-based protein source for both human consumption and animal feed.

Ethical Statement

No ethical approval required.

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Author Contribution

Jittima Munkit: Conceptualization, Investigation, Formal analysis, Writing-original draft preparation

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Wirat Jiwyam: Conceptualization, Methodology and Validation.

Conflict of Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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