Nutrition Value and Growth Ability of Aquatic Weed *Wolffia globosa* as Alternative Feed Sources for Aquaculture System

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**Abstract.** One of the problems of aquaculture is the high cost of feed. Therefore, how to minimize the feed cost is of the main goals. It is necessary to find out natural sources that can be used as alternative feed. *Wolffia globosa* is an aquatic weed that is presumed to be used as an alternative feed source. Study the nutritional content of the *Wolffia* was conducted in June 2019, while the growth ability was conducted both outdoors and indoors in July 2019 at Research Center for Limnology Laboratory, LIPI. The *W. globosa* (percentage in dry weight) contained protein: 45.54%, fat: 5.33%, crude fiber: 9.98%, ash: 20.43%, and Nitrogen Free Extract:19.21%. It also contains 15 types of amino acids as much as 1.51 %/w/w. At the outdoor system culture, the *Wolffia* has daily growth rate/GR (g/day) was 64.62−120.6, specific growth rate/SGR (%/day) was 14.622−15.333, and the daily productivity/P (g/m²/day) was 97.757−176.659. While at the indoor system culture the value of GR (g/day), SGR (%/day), and P (g/m²/day) were 0.675−1.50; 9.889−12.643; and 25.883−64.564 respectively. It is indicated that this *Wolffia* is very potential to be an alternative natural feed source for aquaculture or another purpose and needs further research.

1. Introduction

It is well known that several types of aquatic plants have high potential as natural feed such as those of duckweeds group [1, 2]. This potential could be used to anticipate problems in aquaculture in terms of high feed prices. One of them is looking for potential duckweed species to be used as alternative feed sources. A research about the ability of dry biomass duckweed to substitute up to 50% pellets on Tilapia has been carried out in Brazil with encouraging results that the fish can grow well [3].

The genus *Wolffia*, known by the typical name watermeal, belongs to the Lemnaceae family, also known as duckweed. This duckweed is very small (0.4–1.3 mm), the smallest of the Lemnaceae family, oval-shaped, green with yellow patterns (Figure.1). *Wolffia* lives in stagnant waters and tends to associate with other duckweeds such as *Lemna* to form a thick green layer on the surface of the water. The genus of *Wolffia* has 11 species such as *Wolffia australiana, W. colombiana, W. arrhiza, W. globosa, W. angusta* found among others in New Zealand, America, Sri Lanka, Thailand, Malaysia [4–7]. The *Wolffia* in Indonesia is *Wolffia globosa*, that was found in various fertile open water such as Deli Serdang, North Sumatra, Enrekang, South Sulawesi, and Boyolali, Central Java. However, the information on the *Wolffia* in Indonesia is still rare.

In contrast with a member of Lemnaceae, the genus *Lemna*. *Lemna* has been known that have many functions both as a phytoremediator [8–12], and as an alternative feed ingredient in aquaculture.
such as in the integrated multi-trophic aquaculture (IMTA) technology [12–14]. *Lemna* has also been known to have high protein and other nutrient content and are helpful as feed sources [3, 13, 15]. Based on the information of the duckweed *Lemna*, it is expected that *Wolffia* has a similar ability as a feed source in aquaculture and as a phytoremediator.

Several studies on *Wolffia* have been carried out. The use of duckweed *Wolffia* as an alternative feed for various types of fish has been reported [16], and a try of *W. arrhiza* as a source of feed for Tilapia (*Oreochromis niloticus*) fry has been conducted [6]. The presence of *W. arrhiza* (L.) in an intensive polyculture system seems to improve the quality of meat and organoleptic quality of several test fish species [17]. According to [18], that has also suggested the ability of *W. globosa* as a phytoremediator in eliminating Cadmium content. In Thailand, *W. globosa* flour has also been tested as a substitute for soybean flour for broiler chicken feed ingredients [5]. The nutrient contents of several species of *Wolffia* have been reported [7]. Research on *Wolffia* (*W. globosa*) is not only a source of feed for aquaculture or animal husbandry, but even [19] have developed a rearing system to produce *Wolffia* is a hygienic food source for humans. According to [7] in several countries, that duckweeds also serve as human food like the rootless duckweed *W. globosa* in Thailand is sold in various traditional markets under local names *khai nam*, *kai-pum*, or *kai nhae* (literally meaning: water-eggs). Meanwhile, research on *Wolffia* in Indonesia is still rare.

Based on this information, research is proposed to study the duckweed *Wolffia globosa* in Indonesia. Therefore, it is necessary to research on *Wolffia* among others, to assess the nutritional content and the growth ability of duckweed *W. globosa* in cultivation. This information is very needed when making a policy on using the duckweed as an alternative protein source to be applied in aquaculture or animal husbandry or its use as a phytoremediator. Therefore, this research was conducted to answer these objectives. The results of this study expected to be able to encourage or promote further research on duckweed *W. globosa* in Indonesia.

![Figure 1. Wolffia globosa](image_url)

### 2. Materials and Methods

#### 2.1. Proximate and Amino Acid Analysis

*Wolffia globosa* used for proximate analysis is taken from the outdoor growth experimental culture. The proximate and amino acid content analysis was conducted at the Laboratory of Research Center for Biological Resources and Biotechnology, LPPM, Bogor Agriculture University (IPB). Those consists of the content of moisture, ash, protein, lipid, crude fiber, and Nitrogen Free Extract. The proximate analysis method referred to AOAC and SNI [20, 21], while the analysis of amino acid used high-performance liquid chromatography HPLC Post-Column derivatization method [22], with reference to 15 types amino acids standards. The sample was brought to the laboratory with a chilled condition.
2.2. Growth ability trial of Wolffia globosa

Research of W. globosa growth was conducted at the Laboratory of Research Center for Limnology, LIPI, Cibinong, Bogor, Indonesia. The research was conducted by two different methods. One test was carried out outdoors and the other was carried out indoors. The rearing media used was a commercial hydroponic fertilizer obtained from an agricultural shop in Bogor. It is packed in 2 separate plastic wraps (A and B mix). The stock medium was prepared by diluting 5 g of A mix and 5 g of B mix with 1 L tap water and then applied for Wolffia culture a concentration of 1 mL/L water. Hydroponic fertilizer A contains: (5Ca(NO$_3$)$_2$2NH$_4$NO$_3$,10H$_2$O: 1.176 g/L, KNO$_3$: 0.616 g/L, and Fe-EDTA: 0.038 g/L) mL while the B contains: (KH$_2$PO$_4$: 0.335 g/L, (NH$_4$)$_2$SO$_4$: 0.122 g/L, K$_2$SO$_4$: 0.036g/L, MgSO$_4$: 0.79 g/L, CuSO$_4$: 0.0004 g/L, ZnSO$_4$: 0.0015 g/L, H$_3$BO$_3$: 0.004 g/L, MnSO$_4$.H$_2$O: 0.008 g/L, (NH$_4$)$_7$MoO$_{24}$: 0.0001 g/L). The Wolffia was taken from the culture laboratory at RC for Limnology, LIPI. Several parameters of water quality i.e., temperature (ºC), dissolved oxygen/DO (mg/L), pH (Table 5), were monitored every two days by Water Quality Checker (WQC) equipment [Toshiba-Japan].

2.2.1. Growth ability of W. globosa at outdoor experiment.
The experiment was conducted using a fiber tube measuring 150 cm x 85 cm x 35 cm. The tub is cleaned and then dried. The experimental tube was then filled with 250 L of water as the medium. A total of 125 mL of fertilizer A and 125 mL of fertilizer B were mixed and then added into a tube filled with water and stirred until evenly distributed. A total of 300 g of Wolffia was spread into the tube. Harvesting is done after five days of culture. The resulting Wolffia was then harvested and weighed (W1). A portion of the first harvest is stocked back into the rearing tank and then gathered again on day 5 or day 11, counting from the start of stocking (Table 3) (Figure 2)

2.2.2. Growth ability of W. globosa at indoor experiment.
The experiment was conducted with two replications using an aquarium measuring 31.5 x 18.5 x 24.5 cm and placed in a greenhouse (a room with translucent polycarbonate roof and insect net walls). The aquarium was cleaned and then filled with 10 L of water. The water was added 10 mL of hydroponic fertilizer (5 mL of fertilizer A and 5 mL of fertilizer B). After the media was ready, every 5 grams of Wolffia was stocked into the aquarium. The calculation of growth was carried out for two periods. The first period was carried out on the 7th day since stocking (for six days of culture). Wolffia was harvested using a scoop net and then swung out to remove some of the water. Wolffia yields were then weighed (W1). The harvested Wolffia then stocked back into the aquarium. After the next four days the resulting Wolffia were harvested and re-weighed as in the first period (Table 4) (Figure 3)

2.2.3. Data analysis and observation parameters.
All data were analyzed descriptively for the experiments were conducted using replication, the data was displayed by the mean value. Observation parameters of the Wolffia growth consist of the total daily growth rate (GR), specific growth rate (SGR) were determined following this equations and daily productivty (P) per unit area per day is the total of yields (g) per unit area (m$^2$) per unit time (day) [23–26].

The daily total growth rate (GR) is calculated based on the following equation:

\[
\text{Daily GR (g/day)} = \frac{W_t - W_0}{t}
\]  
(1)

The specific growth rate (SGR) is calculated using the following equation

\[
\text{SGR (%) } = \left( \frac{\ln W_t - \ln W_0}{t} \right) \times 100\%
\]  
(2)
where \( W_t \) is *Wolffia* biomass at time \( t \), \( W_0 \) is *Wolffia* initial biomass, and \( t \) is length of cultivation (days)

![Image](image1.png)

**Figure 2.** The outdoor experiment of *Wolffia globosa* growth (a) stocking and (b) harvesting

![Image](image2.png)

**Figure 3.** The indoor experiment of *Wolffia globosa* growth (a) stocking and (b) harvesting

### 3. Results and Discussion

#### 3.1. Nutrient value

From the calculations based on dry weight, it shows that the *W. globosa* in this study has a high protein content of 45.041±4.374% (Table 1). This protein content is higher than the *W. arrhiza*, which of 28.6% dry weight [17], *W. globosa* in Thailand, which of 29.6% dry weight [5]. According to [7] has reported that 11 species of *Wolffia* have various protein content of about 20–30% freeze-dry weight. When compared to the protein content of duckweed *Lemma*, it is also higher than duckweed *Lemma* reared in nutrient-rich media, which can achieve 38.86% [15, 16] and [14, 27] have reported that the protein content of minute duckweed (*Lemma*) was 32.9–38.10% dry weight.

The ash, fat, crude fiber, and nitrogen-free extract content of this *Wolffia* were 20.432±5.279%, 5.333±0.794%, 9.981±0.223%, and 19.213±10.223%, respectively. The ash content is remarkably higher while the fat content is significantly lower than of *W. arrhiza* that was 9.1% [17], duckweed *Lemma* (9.732±1.98%), but the crude fiber content remarkably a bit higher than the *Lemma* (8.747±1.517)% [14] but lower than *W. arrhiza* of 15.4% [17].

| Table 1. The Nutrient Value of *Wolffia globosa* |
|-----------------------------------------------|
| Contain | Moisture (%) | Ash | Fat | Protein | Crude fiber | Nitrogen Free Extract |
| Average | 94.94 | 20.432 | 5.333 | 45.041 | 9.981 | 19.213 |
| Sd | 0.042 | 5.279 | 0.794 | 4.374 | 0.223 | 10.223 |
Table 2. The Amino Acid Composition of *Wolffia globosa*

| Category | Amino Acids contain (% w/w) | %  |
|----------|-----------------------------|----|
| EAA      | Methionine                  | 0.02|
|          | Threonine                   | 0.07|
|          | Histidine                   | 0.04|
|          | I-leucine                   | 0.08|
|          | Leucine                     | 0.15|
|          | Lysine                      | 0.12|
|          | Valine                      | 0.1 |
|          | Phenylalanine               | 0.1 |
|          | Arginine                    | 0.11|
| EAA Total|                             | 0.79| 52.318|
| NEAA     | Glutamic acid               | 0.19|
|          | Aspartic acid               | 0.18|
|          | Alanine                     | 0.11|
|          | Glycine                     | 0.09|
|          | Serine                      | 0.08|
|          | Tyrosine                    | 0.07|
| NEAA Total|                             | 0.72| 47.682|
| Amino Acid Total |                 | 1.51| 100  |

Amount of amino acids (15 types) from this *W. globosa*, which are 1.51 % w/w which consists of 52.318% are nine types of Essential Amino Acid (EAA), and 47.682% of them are six types of Non-Essential Amino Acid (NEAA) (Table 2). Essential Amino Acids cannot be synthesized in the human body, so they must be met through food intake. Therefore, we must consume food or supplements to get them. At the same time, NEAA can be synthesized by the body. EAA is needed for the balance of the human diet, especially, cysteine + methionine, threonine, phenylalanine, tyrosine, lysine, and leucine, are necessary for human nutrition [28–30].

These results are indicated that the *W. globosa* in Indonesia has the potential to be a source of protein and Amino Acid. These potentials could be very helpful for aquaculture such as IMTA system like the duckweed *Lemma* application. Duckweed *Wolffia* could also be developed as a source of protein for animal feed, such as in the farming of broiler chicken [5]. It is also may even be developed for human food as in Thailand [7].

In general, it can be concluded that some duckweed contains high protein, as well as the Amino Acids composition is almost the same as animal protein [3]. According to [31], when the duckweed is reared in an optimal growth condition, it can be source of various minerals, such as Phosphor (P) and Kalium (K), some pigments like xanthophylls and carotenes. As stated by [7] that all *Wolffia* species contain macro and microelements, carotenoids, and Vitamin E.

3.2. Growth ability of *W. globosa*

In both experiments, the *Wolffia* were able to grow (Table 3 and Table 4). At the outdoor system culture, the duckweed *Wolffia* has a daily growth rate/GR (g/day) was 64.62−120.6, specific growth rate/SGR (%/day) was 14.622−15.333, and the daily productivity/P (g/m²/day) was 97.757–176.659. While at the indoor experiment, the value of GR (g/day), SGR (%/day), and P (g/m²/day) of the duckweed *W. globosa* were 0.675–1.50; 9.889–12.643; and 25.883–64.564, respectively.
Table 3. Initial weight (W0), final weight (W1), time (day), daily growth rate (GR) (g/day), specific growth rate (SGR) (%/day), and daily Productivity (g/m²/day) at outdoor culture of W. globosa the period I and II

| Period | W0 (g) | W1 (g) | time (day) | W1-W0 (g) | GR (g/day) | SGR (%/day) | Productivity (g/m²/day) |
|--------|--------|--------|------------|-----------|------------|-------------|------------------------|
| I      | 300    | 623.2  | 5          | 323.2     | 64.64      | 14.622      | 97.757                 |
| II     | 523.2  | 1126.2 | 5          | 603.0     | 120.60     | 15.333      | 176.659                |

The value of GR, SGR, and P of both experiments showed that cultivation in the second period was higher than in the first period. In addition, it has been clear that the outdoor experiment of all the observed components was higher than of the indoor investigation. Both experiments used the same concentration of fertilizer (1 mg/L). The overall conditions of several water quality parameters were the same (Table 5) and could support the growth of the Wolffia. Several different components of both experiments were the materials and sizes of containers, placements, and stocking density. The outdoor experiment used a stocking density of 300−532.2 g of 250 L media (1.2−2.13 g/L media), while the stocking density of the indoor experiment was 5–9.05 g in 10 L (0.5–0.905 g/L media). In addition, the outdoors experiment without shade from the sun, but it’s unfortunately, this research was not equipped with light intensity data. The results of [31] the productivity of Lemna minor cultivation at higher light intensity was more elevated than of lower light intensity. Light is one of the main limiting factors for plant growth[32]. Some of these components suggested were suspected to affect the growth rate and productivity of duckweed Wolffia. According to [33] that the production of biomass or other components produced by duckweed depends on the strain of duckweed, environmental conditions of growth, and stage of development of the duckweed.

Table 4. Mean values (±Sd) of initial weight (W0), final weight (W1), time (day), daily growth rate (GR) (g/day), specific growth rate (SGR) (%/day), and daily Productivity (g/m²/day) the indoor culture of W. globosa the period I and II

| Period | W0 (g) | W1 (g) | time (day) | W1-W0 (g) | GR (g/hari) | SGR (%/hari) | Productivity (g/m²/hari) |
|--------|--------|--------|------------|-----------|-------------|--------------|--------------------------|
| I      | 5      | 9.05   | 6          | 4.05      | 0.675       | 9.889        | 25.883                  |
| Sd     | 0      | 0.071  | 0          | 0.071     | 0.012       | 0.130        | 0.202                   |
| II     | 9.05   | 15.05  | 4          | 6.0       | 1.50        | 12.643       | 64.564                  |
| Sd     | 0.071  | 1.626  | 0          | 1.697     | 0.424       | 2.902        | 6.977                   |

Table 5. Conditions of Some Water Quality Parameters

| Experiment | Temperature(°C) | pH     | DO (mg/L) |
|------------|-----------------|--------|-----------|
| Indoor     | 25.59±0.55      | 7.69±0.17| 4.84±0.50 |
| Outdoor    | 25.75±0.30      | 7.65±0.21| 5.05±0.49 |

Overall, the results of this study are relatively good. Wolffia have a good growth rate, and various Wolffia species have different daily growth rates were 5.4−30 g/week or 0.77−4.29 g/day equivalent (in dry weight) [7]. Several reports indicated that duckweed production in general is 39.1−105.9 tons/ha/year or equivalent to 10.71−29.014 g/m²/day [34]. The duckweed Lemna has the SGR 17.57
(3.15−31.43) %/day and productivity as much as 48.19 (35.00−72.92) g/m²/day [17] and [10] reported that growth rate of Lemma that reared used Saguling Reservoir water was 12−24 %/day (wet weight). The results of this study indicated that W. globosa are able to grow well, both in indoor and outdoor conditions.

This research is initial research on duckweed W. globosa in Indonesia. The results of this study are expected to stimulate the various further studies and application of duckweed W. globosa in Indonesia

4. Conclusion
Duckweed Wolffia globosa in Indonesia is excellent in nutrition content, a very high protein, and high ash as well as the Amino Acids content. For this reason, the Wolffia could be a good source of protein as an alternative natural feed in aquaculture, animal husbandry, or even for humans.

The duckweed Wolffia globosa can grow well in indoor or outdoor conditions. The ability to growing (specific growth rate, daily growth rate, and daily productivity) at outdoor conditions was higher than at indoor conditions. Further research of duckweed Wolffia globosa in Indonesia is needed to get more information and to optimize its application.

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