Effect of *Wolffia* sp. as Supplemental Feed on Growth Performance and Body Composition of Indian Major Carp, *Labeo rohita* (Hamilton, 1822) Fingerlings

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**Abstract**

The effect of *Wolffia* sp. as supplemental feed on growth performance and the body composition of Indian major carp, *Labeo rohita* fingerlings were examined. The experiments were carried out in the laboratory in triplicates. The commercial feed of tokyu was used as feed for all experimental fish. In addition to tokyu, 10 gram wet weight of *Wolffia* sp. was directly introduced as supplemental feed for all experimental fish except control. Good survival rate was observed in control (95%) and experiments (97%). At the end of experiments, the growth of all *Wolffia* sp. fed *L. rohita* showed significantly higher (p<0.05) than control fish. More significantly different in weight gain, protein efficiency ratio, food conversion ratio, specific growth rate, means daily weight gain and condition factor were also found in *Wolffia* sp. fed *L. rohita* than that of control. The protein content of *Wolffia* sp. fed *L. rohita* was better than that of control fish. All experimental fish gave positively length weight relationships and *L. rohita* consumed *Wolffia* sp. throughout the experimentation was observed. The present study can be
concluded that the use of *Wolffia* sp. improve the nutritional quality of *L. rohita* fingerlings.

**Keywords:** *Wolffia* sp., *Labeo rohita* fingerlings, supplementary feed, growth.

1. **Introduction**

Fish feed are very important and of interest in the economic production of fish by farmers in the world. Each of the fish production countries has produced a variety of local traditional commercial fish diets. If commercial fish diets alone are used in the culture fish species. Though, feeding management plays a critical role in the success of fish culture (Murugesan *et al.*, 2010).

Most commercial fish feed ingredients are costly so a lot of research has been undertaken to find out suitable and cheaper ingredients in fish feed. Fish farmers need plenty and cheaper fish feed than local traditional commercial fish diets. The advantages of live food are superior because of its nutritional value which promotes normal growth and good health. To obtain natural live food for cultured of many herbivorous fish species should be investigated. A number of plants were continuously investigated for their potential in supplementing or replacing fish meal (Amisah *et al.*, 2009).

Aquatic weeds and algae of *Lemma*, water hyacinth, *Hydrilla*, *Spirogyra*, *Wolffia*, *Ipomoea*, *Azolla* and *Elodia* etc. are abundantly found in Mandalay Environrs. It should be considered of these aquatic weeds and algae to use for the supplemental feed of some fish species. The duckweed (Family Lemnaceae), are mainly the rootless *Wolffia arrhiza*, which are able to rapid vegetative reproduction and adaptable to unfavorable
environmental conditions. They also present detoxication ability (Czerpak and Szamrej, 2004).

Protein quality is important characteristic on evaluation of a protein source for animal feed. The protein content of Wolffia sp. dry weight contained 17.94% (Myo Thidar Aye, 2011) and it could be comparable to some protein levels in others: Pithofora oedogonia contained 16.45% and Cladophora sp. contained 15.75% (Aung May Sein, 2007); Spirogyra contained 21.05% (Htay Htay Win, 2007). The Wolffia sp. are usually situated and found as the dominant species of some ponds and lakes of Mandalay Environs. Lemna and Spirodela species form clusters of varying number whereas the Wolffia species remain solitary (Murugesan, Sivasubramanian and Altaff, 2010).

Therefore Wolffia sp. was chosen and used as supplemental feed in this experiment because live feeds play a vital role in the enhancement of fish growth. The research on Wolffia arrhiza used as food and feed additives were used for supplemental feed replacing soybean meal in the diet of Nile Tilapia, Oreochromis niloticus (Chareontesprasit and Jiwyam, 2001). Labeo rohita is also chosen in this experiment because it is one of the economic cultured fish species in Myanmar fish farms.

Thus, the study on the effect of L. rohita fingerlings and Wolffia sp. as supplemental live food was investigated. The production of fish was often been significantly influenced by the quality and quantity of phytoplankton in fish ponds and plants of the Lemnaceae, called duckweeds and water meals has high protein content from 15 to 45%, making it an excellent material for animal, poultry, and fish feed (Padmavathi and Veeraiah, 2009).
This research was carried out to investigate the growth response of *L. rohita* which were fed with the feed using *Wolffia* sp. as supplemental feed under laboratory. Only dietary protein concentration in live food may show significant influence on fish growth and fish performance. The growth of *Wolffia* sp. can be a good resource of proteins and utilized for the production of value-added products especially supplement fish feed.

The objectives of experiment were to study on the growth of *L. rohita* fingerlings and to determine the body composition of *L. rohita* fingerlings due to the effect of *Wolffia* sp. as supplemental food.

2. Materials and Methods

2.1. Study Area and Period

The study was conducted at the laboratory of Zoology Department, University of Mandalay at Mandalay region. All experiments were carried out from June to November 2011.

2.2. Collection of fish and *Wolffia* sp.

The *Labeo rohita* fingerlings were collected from Thetyetkone Fish Culture Farm, Mandalay. They were brought alive immediately to laboratory in oxygenated plastic bags and taken immediately to the laboratory.

Fresh *Wolffia* sp. were collected from fish ponds at east of the Bawdikone Banyan Pagoda campus (Fig. 1), which were put into the sterile-bottles and taken immediately to the laboratory. On the day of sampling, taxonomic identification was
made on live specimens under light microscope according to the method of Prescott (1968) and Desikachary (1959). They were recorded as photomicrographs by using DP 12 Olympus digital camera.

Then the identified species were placed in glass aquaria (30 cm x 15 cm x 15 cm) containing 5 L tap water and they were kept under the sunlight to culture as supplement feed for experimental fish. The crude protein of *Wolffia* sp. was analysed in Analytical Laboratory Section, Fish Inspection and Quality Control Division Yangon, Ministry of Livestock and Fisheries Department of Fisheries, Myanmar.

![Collection site of *Wolffia* sp. at Bawdikone Banyan Pagoda campus, fish ponds](image)

**Figure 1** Collection site of *Wolffia* sp. at Bawdikone Banyan Pagoda campus, fish ponds

### 2.3 Experimental design and diets

The experiments were carried out in rectangular six glass aquaria (60 cm x 30 cm x 30cm) each containing 40 L tap water and 15 *L. rohita* fingerlings were stocked. Adequate aeration used by air pump and the level of water were maintained throughout
the experimental period. All aquaria water was changed once a week to keep the water in good quality. There were triplicates in each treatment.

At the beginning of experiment, fish from all aquaria were fed with the diet tokyu (10% of initial total body weight) twice a day, during 9-10 am and during 1-2 pm (Fig. 2 A). In addition, 10 ml (wet weight) of Wolffia sp. (smallest flowering plant) was introduced directly as supplemental food for all experimental fish except control during 4-5 hours (pm) (Fig. 2 B and C).

2.4. Growth Parameter

Fish mortality and the parameters of water quality such as temperature, dissolved oxygen (DO) and pH were recorded monthly for all glass aquaria. The initial body weights of all experimental fishes were measured with electric balance (Mettler PE 3600 DeltaRange) and the standard lengths were also measured. Monthly recording was made till the end of the experiments. Survival rate (SR %), Mortality (M %), initial means length (IML), initial means weight (IMW), final means length (FML), final means weight (FMW), weight gain (WG), protein efficiency ratio (PER), food conversion ratio (FCR), specific growth rate (SGR), means daily weight gain (DWG) and condition factor (K) were calculated using the following formula (Amisah, Oteng, and Ofori, 2009). Waste materials including fish faeces from each aquarium were taken out weekly and examined under light microscope. At the end of experiments the body composition of all experimental fishes in each aquarium were examined in Analytical Laboratory Section,
Fish Inspection and Quality Control Division Yangon, Ministry of Livestock and Fisheries Department of Fisheries, Myanmar.

Survival rate (S %) = 100 (final number of fish / initial number of fish)

Mortality (M %) = (N₀ - Nₜ) x 100 % / N₀

(N₀ - Number at the start of the experiment; Nₜ - Number at the end of the experiment)

Weight gain (WG) = BWf - BWi  (BWf: final body weight; BWi: initial body weight)

Protein efficiency ratio (PER) = weight gain (g) / protein fed (g)

Food conversion ratio (FCR) = Total feed consumed by fish (g) / Weight gain by fish (g)

Specific growth rate (SGR) (% g/day) = (Log W₂ - Log W₁) / (T₂ - T₁) x 100

(W₂ = Weight of fish at time T₂ (final); W₁ = Weight of fish at time T₁ (initial))

Means daily weight gain (DWG) (% g/day) = 100 (Wf - Wi) / ∆t

(Wf: final weight; Wi: initial weight; ∆t: duration (days))

Condition factor (K) = W / L^3 (W = Weight of fish; L = Standard length of fish)

2.5. Statistical analyses

Data were analyzed by one way ANOVA (SPSS version 11.5) and was used to compare difference among group means.

3. Results

Growth performance and nutrient digestibility of Labeo rohita fingerlings fed with the experimental diets were shown in Table 1. The body Compositions of L. rohita fingerlings fed with the experimental diets was shown in Table 2. Moisture and crude protein of Wolffia sp. was shown in Table 3.
Table 1 Growth and nutrient digestibility of *Labeo rohita* fingerlings

| Parameter | Control | Experiment |
|-----------|---------|------------|
|           | (Tokyu) | (Tokyu + *Wolffia* sp.) |
| M%        | 5%      | 3%         |
| IML (cm)  | 2.62 ± 1.85 | 9.83 ± 1.72 |
| IMW (g)   | 1.92 ± 0.96  | 6.76 ± 3.24  |
| FML (cm)  | 11.56 ± 2.62 | 14.49 ± 1.47 |
| FMW (g)   | 9.75 ± 2.89  | 26.48 ± 6.48  |
| PER       | 1.43 ± 0.73  | 17.86 ± 13.63 |
| FCR (tokyu) | 0.44 ± 0.19  | 2.26 ± 2.65  |
| FCR (*Wolffia* sp.) | - | 0.72 ± 0.85 |
| NWG       | 4.85 ± 2.66  | 9.08 ± 7.4   |
| SGR       | 0.69 ± 0.15  | 0.79 ± 0.4   |
| DWG       | 5.34 ± 0.95  | 8.34 ± 3.33  |
| K         | 2.22 ± 2.54  | 1.07 ± 0.47  |

SR% = survival rate; M% = motility; IML = initial means length; IMW = initial means weight; FML = final means length; FMW = final means weight; PER = protein efficiency ratio; FCR = food conversion ratio; WG = weight gain; SGR = specific growth rate; DWG = means daily weight gain; K = condition factor
Table 2 The body compositions of *Labeo rohita* fingerlings

| Proximate Analysis (%) | Control fish tissue | Experiment |
|------------------------|----------------------|------------|
|                        | Wet tissue           | Dry tissue | Wet tissue | Dry tissue |
| Moisture               | 80.49                | 4.0        | 70.4       | 3.5        |
| Crude Protein          | 11.59                | 41.29      | 16.9       | 60.2       |
| Crude Fat              | 13.0                 | 10.14      | 3.5        | 6.5        |
| Total Ash              | 15.43                | 23.86      | 4.5        | 12.5       |

Table 3 The moisture and crude protein of *Wolffia* sp.

| Type of Sample | Analyzed Sample | Proximate Analysis (%) | Moisture | Crude Protein |
|----------------|-----------------|------------------------|----------|---------------|
| *Wolffia* sp.  | Wet             | 94.62                  | 3.06     |
|                | dry             | 11.68                  | 17.94    |

At the end of experiments, faeces were observed to contain the incompletely undigested parts of *Wolffia* sp. especially cell wall and detritus (Fig. 2 D and E). The growth of *L. rohita* fingerlings in the *Wolffia* sp. supplemented food showed significantly higher (*p*<0.05) than controls. Comparative growth of all experimental fish was shown in Fig. 3 A. The length weight relationship was observed $R^2=0.9228$ in control fish and *Wolffia* fed fish was $R^2=0.724$ at the end of experiment (Fig. 3 B and C).

During the experiments, water temperature was observed within 20-22°C in the morning and 25-28°C in the evening, dissolved oxygen (DO) was 3 mg/L-5.8 mg/L and pH was within 7.8-5.
Figure 2 (A) Experimental diets of Tokyu; (B) Experimental diets of *Wolffia* sp. (fresh); (C) Experimental diets of *Wolffia* sp. (x400); (D and E) Portion of *Wolffia* sp. (fat arrows) and detritus (thin arrows) in the fish faeces.
4. Discussion

At the end of experiments good survival rate (SR\%) and low mortality (M\%) were observed in all experimental fish of *Labeo rohita* fingerlings. The final means length (FML) and final means weight (FMW) of 14.49 ± 1.47 cm and 26.48 ± 6.48 g was recorded in *Wolffia* sp. fed *L. rohita* fingerlings and 11.56 ± 2.62 cm and 9.75 ± 2.89 g in control fish. The FML and FMW of *Wolffia* fed *L. rohita* fingerlings were significantly \( p<0.05 \) higher than control fish.

According to the results more weight gain (WG) of 9.08 ± 7.4, specific growth rate (SGR) of 0.79 ± 0.4 and daily weight gain (DWG) of 8.34 ± 3.33 were found in *L. rohita* fingerlings fed with the diets *Wolffia* than in control fish WG of 4.85 ± 2.66, SGR of 0.69 ± 0.15 and DWG of 5.34 ± 0.95. Agreed with Chareontesprasit and Jiwam (2001), reported that some aquatic plants have been commonly used as supplementary feeds, e.g. high protein *Azolla, Lemna* whilst *Wolffia*, a floating aquatic plant with high protein contents could possibly do the same.

In *Wolffia* fed *L. rohita* fingerlings were found PER of 17.86 ± 13.63 and FCR of tokyu (2.26 ± 2.65) and *Wolffia* sp. (0.72 ± 0.85). In control fish was also found PER of 1.43 ± 0.73 and FCR of tokyu was 0.44 ± 0.19. This result gave the well PER and lowest FCR were found in control fish and in diet *Wolffia* sp. fed *L. rohita* fingerlings. Therefore it may be assumed that experimental fish can be easily consumed of the *Wolffia* sp. as tokyu. *Wolffiaarrhiza* is favorite food of fish, therefore it is beneficial to pisciculture especially when they are young (Parmar and Patel, 2010).
The body compositions of *Wolffia* sp. fed *L. rohita* fingerlings gave crude protein of 16.9% (wet) and 60.2% (dry), crude fat of 3.5% (wet) and 6.5% (dry), total ash of 4.5% (wet) and 12.5% (dry), moisture of 70.4% (wet) and 3.5% (dry). In the tissue of control fish also gave crude protein of 11.59% (wet) and 41.29% (dry), crude fat of 13.0% (wet) and 10.14% (dry), total ash of 15.43% (wet) and 23.86% (dry), moisture of 80.49% (wet) and 4.0% (dry).

According to the results, body composition of *L. rohita* fingerlings was recorded more crude protein, lower moisture, crude fat and total ash were found in *Wolffia* fed fish than that of control fish. The experimental diet of *Wolffia* sp. contained crude protein content and moisture of 3.06% and 95.87% in wet and 17.94% and 11.68% in dry.

The condition factors K value of 1.07±0.47 was found in *Wolffia* fed *L. rohita* fingerlings and that was 2.22±2.54 in control fish. The K value was not significantly difference between *Wolffia* sp. supplemented food fed *L. rohita* fingerlings and control fish. In fisheries science, the condition factor is used to compare the “condition” “fitness” or wellbeing of fish (Anene, 2005). Length weight relationship of *L. rohita* fingerlings was positively correlated with $R^2$ values of 0.724 for diets *Wolffia* fed fish and control fish was 0.9225. During the experimental period, the values of water temperature, DO and pH were more or less similar in all aquaria.

The faeces of *Wolffia* sp. fed fish expect control and the gills of death *L. rohita* were also examined. Faeces were observed to contain the incompletely undigested parts of *Wolffia* sp. especially cell wall and detritus (Fig. 3D and E). Dead fish were suffered from *Dactylogyrus* sp. infection on gills filaments because of these parasites were
attached in almost all gills filaments. Pieces of indigestible cell wall and detritus of *Wolffia* sp. was occurred in the faeces of experimental fish except control. This may be difficult to digest.

![Graph A](image.png)

![Graph B](image.png)

\[ y = 1.0714x - 1.3427 \]

\[ R^2 = 0.9225 \]
Figure 3 A Comparative growth of *L. rohita* fingerlings during the experiment

B Length weight relationship of *Labeo rohita* fingerlings in control fish

C Length weight relationship of diet *Wolffia* fed *Labeo rohita* fingerlings

During the experiment, more growth was found as well as non-polluted the water quality in all aquaria. Therefore *Wolffia* sp. could be used not only supplement feed but also aquatic plant in fish culture ponds. In addition the aquatic plant of *Wolffia* sp. is occurred annually found in many ponds and lakes. Fish, like other organisms, required food (energy) in order to grow, survive and reproduce, the food items (source of energy) in aquatic habitat are in the form of plankton, periphyton, nuston, benthose, nekton and plants are available throughout the year (Narejo *et al.*, 2010).

Thus it could be considered as a supplement feeding material for fish as a direct feeding stuff or could possibly be substituted in place of commercial feed. Furthermore, the market price of commercial feed could be relatively high and so other sources of high protein contents from aquatic plants should be substituting. As such, supplement alternative feeds should be used to reduce the cost of feed in the aquaculture industry.
Therefore the aquatic plant *Wolffia* sp. could be used as supplementary feed for *L. rohita* fingerlings because *Wolffia* sp. could provide this amount of protein without any cost and could be easily obtained from the fish ponds.

5. Conclusion

The result found that the supplemental feed of *Wolffia* sp. was suitable feed for *L. rohita* fingerlings. According to the present study, *Wolffia* sp. should be used in improving the nutritional quality of practical feed of fish because more growth and good body composition gave in *L. rohita* fingerlings. It was readily accepted that the *Wolffia* sp. were observed to consume feed throughout the experimentation. On the basis of this investigation, it was concluded that *Wolffia* sp. could be considered as a feeding material for fish either as a direct feeding stuff or a source of protein for any formulated rations and it could possibly be substituted in any other formulated or commercial fish feed.

Acknowledgements

I want to thank Pro-Rectors, Mandalay University of Distance Education, for permission to read this research paper. I greatly thank to Professor and Head, Department of Zoology, Mandalay University of Distance Education, for her suggestion and encouragement to research topic and critical reading of this paper. Finally I owed my special thanks to Dr Mie Mie Sein and Myo Thidar Aye for all-around energize of my research works.

References

Amisah, S., Oteng, M. A. and Ofori, J. K. (2009) Growth performance of the African
catfish, *Clarias gariepinus*, fed varying inclusion levels of *Leucaena leucocephala* leaf meal. *Journal of Applied Science Environment Management*, 13 (1), 21-26.

Anene, A. (2005) Condition factor of four Cichlid species of a man-made Lake in Imo State, South-eastern Nigeria, Turk. *Journal of Fisheries and Aquatic Science*, 5, 43-47.

Aye, M. T. (2012) *Growth performance and body composition of local breed of rohu Labeo rohita (Hamilton, 1822).* (Doctoral thesis, Department of Zoology, University of Mandalay) pp. 55.

Ayoade, A. A. (2011) Length-Weight Relationship and Diet of African Carp *Labeo ogunensis* (Boulenger, 1910) in Asejire Lake Southwestern Nigeria. *Journal of Fisheries and Aquatic Science*, 6(4), 472-478.

Chareontesprasit, N. and Jiwyam, W. (2001) An Evaluation of Wolffia Meal (*Wolffia arrhiza*) in Replacing Soybean Meal in Some Formulated Rations of Nile Tilapia (*Oreochromis niloticus* L). *Pakistan Journal of Biological Sciences*, 4 (5), 618-620.

Czerpak, R., Piotrowska, A., and Krotke, A. (2004) Biochemical activity of auxins in difference of their structures in *Wolffia arrhiza* (L. Wimm.) *Acta Societatis Botanicorum Poloniae*, 73 (4), 269-275.
Murugesan, S., Sivasubramanian, V. and Altaff, K. (2010) Nutritional evaluation and culture of freshwater live food organisms on *Catla catla*. *Journal of algal biomass utilization*, 1 (3): 82-103

Narejo, N.T., Dars, B.A. and Achakzai, G.D. (2010) Preparation of low-cost fish feed for the culture of *Labeo rohita* (Hamilton) in glass aquaria. *Sindh Univ. Res. Jour. (Sci. Ser.)* 42 (2): 07-10.

Padmavathi, P. and Veeraiah, K. (2009) Studies on the influence of *Microcystis aeruginosa* on the ecology and fish production of carp culture ponds. *African Journal of Biotechnology*, 8 (9): 1911-1918 pp.

Parmar, A. J. and Patal, N. K. (2010) Study of Aquatic Angiospermic Plants of Patan District. *Life Sciences Leaflets*, 3 (54-68): 0976-1098 pp.

Sein, A. M. (2007) *Studies on cyanobacteria, algae and fish growth with reference to pond fertilization*. (Doctoral thesis, Department of Zoology, University of Mandalay) pp. 89

Win, H. H. (2007) *Growth stimulation in Anabas testudineus (Bloch, 1792)* by incorporating with Spirulina in routine diet and the study of survival rates under the laboratory condition. (Doctoral thesis, Department of Zoology, University of Mandalay) pp. 90