POTENTIAL OF DUCKWEED (*Wolffia arrhiza*)-AN INVASIVE AQUATIC PLANT AS FISH FEED IN TILAPIA (*Oreochromis niloticus*) FRY REARING

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ABSTRACT

Duckweed (Wolffia arrhiza) is a tiny aquatic plant, abundant in nutrient rich wetlands which contain up to 43% protein by dry weight and could be used as feed for rearing Tilapia (Oreochromis niloticus) fry. Tilapia fingerlings are in high demand in the development of inland fish production in the country. The aim of this study was to evaluate the potential of duckweed as feed in fry rearing of Tilapia and to utilise this plant in a profitable manner. The trial was carried out at National Aquatic Resources Research & Development Agency, Sri Lanka for a period of 41 days. Six cement tanks (0.75 m^3) were stocked with Tilapia fry (0.0754±0.0441g) according to the stocking density of 500 fry m^{-3}. Fresh duckweed (Dw) and commercial feed (Cf) were provided daily at a rate of 5% of body weight. Fish were sampled weekly and the feed amounts were adjusted according to total biomass in respective tanks, assuming there was no mortality. The pH and temperature were measured daily. Nitrite (NO^{-2}), Nitrate (NO^{-3}) and Total Ammonia Nitrogen (TAN) were measured weekly. Average Daily Growth (ADG), Specific Growth Rate (SGR-W), Condition Factor (CF), Final body weight (BW_{final}), Feed Conversion Ratio (FCR), % survival rates of Tilapia and Toxic Ammonia (NH_3) were determined. The Mean ADG (0.0261±0.0077, 0.0039 ± 0.0020 g day^{-1}), BW_{final} (1.1450±0.7390, 0.2880 ± 0.2070 g) and CF (1.7727±1.18, 2.655±2.06) of Tilapia fed on Cf and Dw were significantly different ($p<0.05$), while SGR-W (6.7324±1.7879, 3.9035±0.4696) and % survival rates (44.6±16.35, 25.5± 6.74) were not significant statistically. The mean temperature, pH, (NO^{-2}), (NO^{-3}) and NH_3 in Cf and Dw provided tanks were not significantly different ($P>0.05$). FCR of Cf and Dw were 1.003±0.6097and 4.2066±0.2462. Wolffia arrhiza could be used as feed for Tilapia fry rearing but the amount Dw provided (5% of body weight) is not sufficient. Further research is needed to determine optimal amounts. The use of W. arrhiza as fish feed would reduce its nuisance value considerably.

Key words: Tilapia fry rearing, Duckweed, aquatic plant

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INTRODUCTION

Tilapia *(Oreochromis niloticus)* is an exotic fish, well established in Sri Lankan water bodies and it is the mainstay of reservoir fisheries in Sri Lanka (Amarasinghe, 1995). Though it is a naturally bred fish species with parental care, fry rearing with special attention on feeding would be much benefited to produce more fingerlings. These fingerlings could be used to stock perennial reservoirs and seasonal tanks to promote inland fisheries and culture based fisheries in the country. However, it is necessary to use a supplementary feed in the seed production of Tilapia, for better results. According to De Silva and Anderson (1995), the cost of feed is the highest recurrent cost of the aquaculture industry, which often ranges from 30% to 60% of the total cost depending on the intensity of the practice. As such, alternative feeds should be used to reduce the cost of feed in the aquaculture industry.

According to Islam *et al.* (2004) a tiny, fragile, free-floating, aquatic plant, Duckweed (Dw) (*Wolffia* spp.) could be used as an alternative fish feed. As cited by Ferdoushi *et al.* (2008), *Lemna minor, L. gibba, Wolffia arrhiza* and *Azolla pinnata* are free floating duckweeds included in Lemnaceae family. Mbagwu *et al.* (1990) have shown that fresh Dw is a good food source for Tilapia, as it contains about 35-45% crude protein (CP) with good amino acid and mineral profiles. Likewise Hassan and Edwards (1992) have shown that Dw is converted efficiently to live weight by certain fish, which include carp and Tilapia. According to Yilmaz *et al.* (2004), Dw occurs naturally in heavily fertilized/nourished wetlands. Mazid *et al.* (1992) has shown that the Dw grows abundantly in warm climatic zones in fresh water with rapid rate of vegetative reproduction *i.e.* double their quantity in three days or less.

As the size of Dw is very tiny, small fish (fry) can easily consume it. As such, newly harvested duckweed plants contain up to 43% protein by dry weight and can be used without further processing as a complete feed for fish (Mbagwu *et al.*, 1990). As cited by Islam *et al.* (2004), Dw has long been recognized as a potential source of protein for animals and studies conducted in various countries of the world, e.g. the former U.S.S.R., U.S.A. and Canada have demonstrated the nutritional benefits of Dw for both livestock and fish. Accordingly, the attempt of this study is to evaluate the efficacy of duckweed (*Wolffia arrhiza*) as fish feed in the fry rearing of Tilapia and reduce the nuisance value of duckweed.

MATERIALS AND METHODS

The trial was carried out at National Aquatic Resources Research & Development Agency (NARA), Colombo, Sri Lanka for a period of 41 days from 23rd August, 2005 to 4th October, 2005. Six rectangular cement tanks (1.6m × 1.0m × 0.5m; 0.75 m^3) were cleaned, washed and filled with tap water up to 45 cm that was the maximum depth of the tanks. After 5 days, Tilapia fry (initial weight 0.0754±0.0441g) were stocked according to a stocking density of 500 fry m^{-3}. Tilapia fry were obtained from the Aquaculture Development Centre in Udawalawe, Sri Lanka.

Two feed types i.e. fresh duckweed (Dw) (*W. arrhiza*) and commercial feed (Cf) were used as feed for Tilapia fry and tested in triplicate in randomly selected tanks. Cf was the control feed and was purchased from Ceylon Aquatech (Pvt.) Limited, Rock House Lane, Colombo-15, Sri Lanka. Dw was cultured in two cement tanks using organic manure, cow dung, and collected using hand nets, washed with tap water and left to drain for 45 min. before being used as feed.

During the experimental period fish were fed twice per day, once in the morning (0930 hrs) and once in the afternoon (1500 hrs) at a daily rate of 5% of body weight. Twenty fish in each tank were sampled weekly to determine average weight of the fish in respective tanks and calculated the total biomass accordingly. The feed amount was adjusted according to the total biomass in respective tanks, assuming there was no mortality.

The water temperature and the pH of the tanks were measured using glass mercury thermometer and the pH meter (Model: GENWAY-3051) daily around 0900 hrs. Nitrite-nitrogen (NO^{-2}), Nitrate-nitrogen (NO^{-3}) and Total Ammonia Nitrogen (TAN) were measured once a week using DR 4000 spectro photometer. Toxic un-ionized ammonia (NH_3) was calculated using the SRAC publication No.463 (Durborow *et al.*, 1997). After 41days of rearing, the fish were harvested, counted and % survivals were determined in each tank, respectively. As such, the final sampling (determine weight and total length of fish respectively) was carried out with 20 fish collected randomly from each tank.

Proximate analysis of Cf was carried out using standard method described in APHA (1985). The moisture content was determined by oven drying a weighed sample in porcelain crucibles at 105 °C for 24 hrs. The total volatile matter lost at this temperature was taken as the moisture content. The Ash content was determined by incinerating the dried samples 1-2 g of flesh overnight in a Muffle furnace at 550 °C. The percentage of protein (N × 6.25) was estimated by semi-micro Kjeldahl digestion, distillation and titration. The % of fat was determined using the chloroform method (Bligh and Dyer, 1959).

The Average Daily Growth (ADG), Specific Growth Rate in weight (SGR-W), Feed Conversion Ratio (FCR), Condition factor (CF) and % survival of the fish in each tank were determined according to the following equations.

$$ADG\ (g/day) = \frac{\text{Final weight of fish- Initial weight of fish}}{\text{Days of rearing}}$$

$$CF = \frac{\text{Total weight of fish(W)}}{\text{Standard length } (L)^3} \times 100 \quad \text{Bagenal, 1978}$$

$$\%\ Survival = \frac{\text{No. of fish harvested}}{\text{No. of fish stocked}} \times 100$$

$$SGR = \frac{Ln_{\text{Final weight}} - Ln_{\text{Initial weight}}}{\text{Experimental duration}} \times 100 \quad \text{Ricker, 1979}$$

$$FCR = \frac{\text{Dry weight of feed given(g)}}{\text{Wet weight gain(g)}} \times 100 \quad \text{Hepher, 1988}$$

Two- Sample t-tests were applied to determine the significance of the calculated indices in two variables. Statistical significance was assessed using a probability level of $P=0.05$.

RESULTS AND DISCUSSION

Mean final body weight (BW_{final}), CF and FCR of the fish that was fed on Cf and Dw were significantly different ($P<0.05$), respectively. However, SGR-W and % survivals of the fish in these two feed types were not significantly different ($P>0.05$), respectively (Table-1). Nevertheless Fasakin *et al*. (1999) found that the difference in final weights, specific growth rates, feed conversion of tilapia that were fed on diets containing up to 20% of Dw were not significantly different from those of tilapia that were fed on the control diet.

Average Daily growth (ADG)

The mean ADG of the fish fed on Dw was 0.0039±0.0020 g/day and it was significantly lower than the ADG of the fish fed on Cf (0.0261±0.0077 g/day). It may be due to the insufficient amount of Dw provided for the fish. Accordingly, the provided feed amount (5% of body weight) should be increased up to optimal levels to improve the growth of fish.

Final Body weight (BW_{final})

The BW_{final} of Tilapia fry fed on Cf and Dw were significantly different and lower in the fish fed on Dw than the fish fed on Cf (Table-1). The low growth of Tilapia fry may be due to insufficient amounts of Dw (5% of body weight) that was provided for fish. Nevertheless, polyculture of Tilapia, Common carp (*Cyprinus carpio*) and Mrigal

(*Cirrhinus mrigala*) in mud ponds for food fish culture has recorded fast growth with Dw (Thy *et al.*, 2008). However the SGR-W of the fish was not significantly different in these two feeds. It has shown the suitability of the two feeds provided.

Table-1. Mean values of Average Daily Growth (ADG), Final Body Weight (BW_{final}), Specific Growth Rate in weight (SGR-W), Feed conversion ratio (FCR) and % survival rates of Tilapia (*Oreochromis niloticus*) fry fed on commercial feed (Cf) and duckweed (Dw)

Feed type	ADG ($g\ day^{-1}$)	SGR-W	% Survival	CF	BW_{final} (g)	FCR
Cf	0.0261[a] ±0.0077	6.7324[c] ±1.7879	44.6[d] ±16.35	1.7727[e] ±1.18	1.1450[g] ±0.739	1.0030[j] ±0.6097
DW	0.0039[b] ±0.0020	3.9035[c] ±0.4696	2 5.5[d] ±6.74	2.6550[f] ±2.06	0.2880[h] ±0.207	4.2066[k] ±0.2462

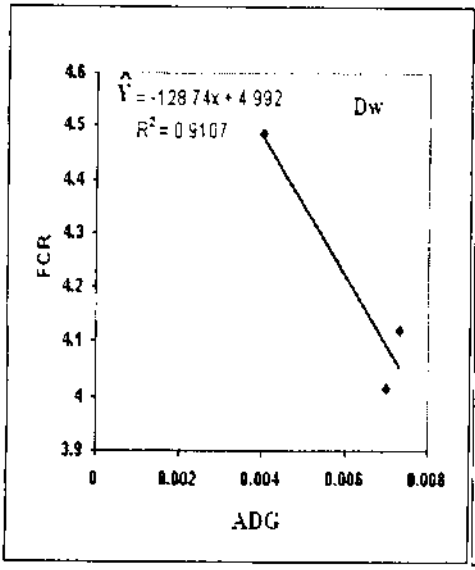
All values over Mean±S.D. Mean with the same letter in the same column is not statistically significant ($P<0.05$)

Condition factor (CF)

CF, which is related to both growth and feeding, was another variable checked in the study. It was significantly different ($p<0.05$) in these two feed types and significantly higher in Dw than in Cf (Table-1). In fisheries science, the CF is used to compare the "condition" "fatness" or wellbeing of fish (Anene, 2005). It has been shown that the wellbeing and health of Tilapia fry that were fed on Dw were significantly higher than that fed on Cf. Accordingly, the use of Dw as feed in fry rearing of Tilapia has shown good indicator for better growth.

Feed conversion ratio (FCR)

The FCR of Dw and Cf with Tilapia fry were significantly different ($p<0.05$) and significantly higher in Dw than the Cf. The Cf is a complete commercial feed that was manufactured to fulfill the nutritional requirement of the fish (Table-1). Accordingly the FCR value should be low. The correlation of ADG and FCR in two feed types has shown negative relationship and the relationship in tanks provided with Dw was very strong ($R^2=0.9107$) (Fig.1). It was shown that Dw supported the growth of Tilapia fry more than Cf. It may be due to the nourishment of Dw.



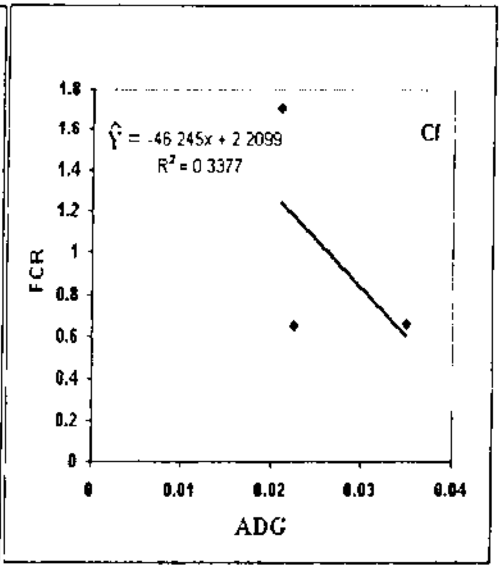


Figure 1. Relationship of feed conversion ratio (FCR) and average daily growth (ADG) of Tilapia *Oreochromis niloticus*) fry fed on duckweed (Dw) and commercial feed (Cf).

Fish survival rate (%)

The % survivals of the fish fed on Cf and Dw were not significantly different (P>0.05). However, the % survival of the fish that was fed on Dw was recorded as 25.5±6.74 which was lesser than the % survival of the fish fed on Cf (44.6±16.35). It could be due to the higher standard deviation of the respective mean values (Table-1). Thy *et al.* (2008) have observed the high % survival (89.6) of Tilapia than Common carp (75) and Mrigal (75) while fed on Dw in polyculture grow out trials.

The correlation between % survival and ADG of the fish in Cf provided tanks was positive and in Dw provided tanks was negative (Fig. 2). It could be due to the insufficient amount of feed that provided as Dw. However, the correlation was not strong in Dw provided tanks as well as Cf provided tanks. Due to the insufficient feed amount that provided Dw as 5% of body weight was not sufficient, the cannibalism could have happened. Katavic *et al.* (1989) have observed the size variation of the fish and food availability are the primary causes of cannibalism. As such cannibalism among tilapia fry and fingerlings has been identified as one of the major problems by small-scale hatchery operators (Pantastico *et al.,* 1988). Accordingly cannibalism may be the cause for the low % survival of Tilapia fry fed on Dw and for the negative relationship between ADG and % survival.

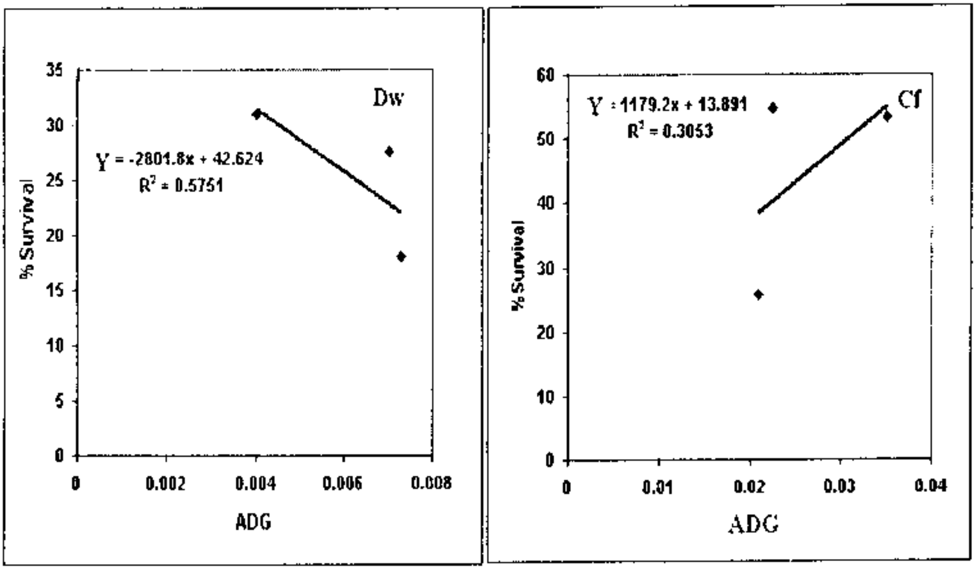


Figure 2. Relationship of % survival and average daily growth (ADG) of *Oreochromis niloticus* fry with commercial feed (Cf) and duckweed (Dw).

Water quality

There were no differences on water quality parameters (pH, water temperature, NO^{-2}, NO^{-3} and TAN (Table-2).Water quality parameters measured over the 41 days rearing period are shown in Table-2. During the experimental period, the values of water temperature were more or less similar in all six tanks for two treatments and fluctuated from 28.0 ºC to 33.0 ºC. It may have therefore affected fish growth similarly in these two feed types.

The nitrate levels in Cf provided and Dw provided tanks were 1.214±0.765 and 1.679±0.649, respectively. According to Durborow *et al.* (1997) and Floyd and Watson, (2005) nitrate is not toxic to fish except at extremely high levels, and can be considered harmless and it is used by plants, including algae, for food. As cited by Boyd (1992), concentration of nitrite as low in 0.5 mg l^{-1} was toxic to certain cold water fish. However, the levels of nitrite in this trial were 0.0043±0.0029 mg l^{-1} in Cf provided tanks and 0.0114±0.0101 mg l^{-1} in Dw provided tanks and these are very low levels than the toxic levels.

Ammonia is the major end product in the breakdown of proteins in fish (Durborow *et al.* 1997). The toxic Ammonia (NH_3) levels in Dw provided tanks (0.0403±0.0307 mg l^{-1}) was lower than Cf provided tanks (0.0587±0.0596 mg l^{-1}) and not significantly different (P>0.05) (Table-2). As such the amount of NH_3 in Dw provided tanks were lower than the NH_3 in Cf provided tanks throughout the rearing period (Fig. 3). According to Pompa and Masser (1999) the NH_3 levels in these NH_3 tanks were not lethal (>2 mg l^{-1}), sub-lethal (>3 mg l^{-1}) or depress food consumption of the fish (<0.08 mg l^{-1}). According to Durborow *et al.* (1997) 0.6 mg/l are capable of killing fish and 0.06 mg/l can cause gill and kidney damages. However, the toxic NH_3 levels in Cf provided tanks and Dw provided tanks were not reached this level. The toxic NH_3 could be produced in fish tanks in two ways. (1) through the deteriorating of remaining feed and (2) through the excreta of fish. Toxic ammonia in Cf provided tanks were produced in these two ways but in Dw provided tanks were produced through the fish excreta only, because the Dw did not deteriorate, as it is a live aquatic plant. Accordingly the water quality in Dw provided tanks would be more favourable than in the Cf provided tanks for Tilapia fry rearing. This is one of the advantages in feeding fish with Dw.

Table-2. Water quality parameters measured over the 41 days rearing period of Tilapia (*Oreochromis niloticus*) fry with commercial feed (Cf) and duckweed (Dw).

Feed type	Water quality parameters				
	pH	Water Temp °C	Nitrite-nitrogen (NO^{-2}) (mg l^{-1})	Nitrate-nitrogen (NO^{-3}) (mg l^{-1})	Toxic Ammonia (NH_3) (mg l^{-1})
Cf	8.56^{a} ±0.5361	29.26^{b} ±1.3936	0.0043^{d} ±0.0029	1.214^{e} ±0.765	0.0587^{f} ±0.0596
Dw	8.72^{a} ±0.6206	29.26^{b} ±1.3936	0.0114^{d} ±0.0101	1.679^{e} ±0.649	0.0403^{f} ±0.0307

All values over Mean ±S.D. Mean with the same letter in the same column is not statistically significant (P<0.05).

Proximate composition and nutritional value

The Cf used in this trial has provided 32.5±1.13 % of crude protein (Table-3). The Dw is rich in crude protein and it will provide 43% of crude protein in dry weight (Yilmaz *et al.*, 2004). However, the estimated dietary protein requirement for maximal growth of juvenile Tilapia is 30 % (NRC, 1993). This amount of protein is provided through the fishmeal in Cf which is expensive. Nevertheless, Dw could provide this amount of protein without any cost and could be obtained from the paddy fields as it is an invasive plant for paddy farming. Accordingly, use of Dw as fish feed is much benefited for Tilapia fry rearing as well as this intervention will result in the management of duckweed.

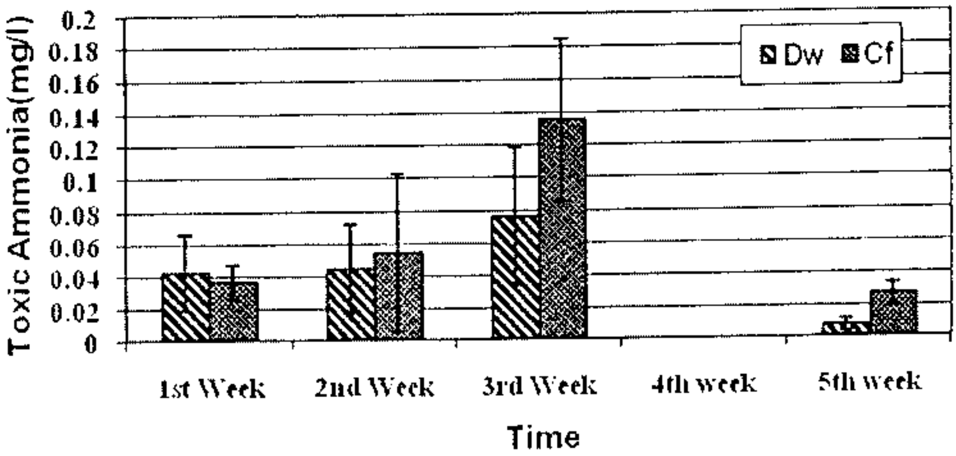


Figure 3. The toxic Ammonia (NH_3) levels in duckweed (Dw) and commercial feed (Cf) provided tanks.

Table-3. Ingredient composition and proximate composition of commercial feed (Cf) used in Tilapia fry rearing trials (APHA, 1985).

Ingredient composition	Proximate composition (%)	
Fish products	Dry matter	90.23±0.17
Other animal by-products	Moisture	9.77±0.17
Cereals & Cereal by-product	Ash	8.81±0.14
Oilseed meals	Protein	32.5±1.13
Fish oil	Fat	8.64±0.09
Minerals	Carbohydrate	49.97±1.64
Vitamins		
Permitted additives		

According to Hilman and Culley (1978), Dw as a natural protein source has a better array of essential amino acids (EAA) than most other vegetable proteins and more closely resembles animal protein including high concentration of Lysine (10.9% in dry weight) (Table-4). It is higher than the lysine requirement (1.3g/kg) of Nile Tilapia (Santiago and Lovel, 1988). According to Palavesam *et al.* (2008) Lysine is one among the ten indispensable amino acids that are required in dietary protein and it is the most limiting amino acid in plant proteins and the most critical amino acid in fish feed. Accordingly, use of Dw as fish feed in Tilapia fry rearing is much benefited.

Table-4. Proximate and amino acid compositions of duck weed (adopted from Yilmaz *et al.*, 2004) and amino acid requirement of juvenile Tilapia (*Oreochromis niloticus*) (NRC, 1993).

Proximate composition	g $100g^{-1}$ dry weight	Amino acid requirement of juvenile Tilapia (NRC, 1993)
Crude protein	**18.38**	
Lipid	2.32	
Ash	23.71	
Amino acids:		
Aspartic acid	1.096	
Serine	0.471	
Glutamic acid	1.216	
Glycine	0.438	
Alanine	0.747	
Tyrosine	0.287	
Essential Amino Acids		
Arginine		1.18
Histidine	0.224	0.48
Isoleucine	0.518	0.87
Leucine	0.696	0.95
Lysine	10.900	1.43
Methionine	0.179	0.75
Phenylalanine	0.623	1.05
Threonine	0.494	1.05
Tryptophan		0.28
Valine	0.646	0.78

Yilmaz *et al.* (2004) have reported that Dw has no anti-nutrients as the most plant-derived nutrient sources like soybean meal, rapeseed meal, lupin seed meal, pea seed meal, sunflower oil cake, cottonseed meal, leucaena leaf meal contain a wide variety of anti-nutritional substances that affect the growth and health of fish. Therefore, Dw provides an easy, practical and cheaper fish feed as it requires no processing to destroy any anti-nutrients. Furthermore, according to Leng *et al.* (1995) Dw grown in nutrient-rich water has a high concentration of trace minerals, Potassium (K), Phosphorus (P) and pigments, particularly carotene and xanthophylls which make Dw especially valuable as a dietary supplement for fish. According to Chaturvedi *et al.* (2003) compared with most other plants, Dw leaves contain little fibre (5% in dry matter for cultivated plants) and little or no indigestible material even for monogastric animals.

However, Dw is an unwanted invasive aquatic plant for paddy cultivation in Sri Lanka. In the meantime it is nutritious feed with no cost for fish farmer. To optimize the use of natural resources, the integration of paddy cultivation and fish culture is an ideal strategy. Then more attention should be paid on the integration of rice farming and fish culture with Dw in Sri Lanka.

The aquatic plant *Wolffia arrhiza* could be used as supplementary feed for Tilapia fry rearing. Nevertheless, the amount provided (5% of body weight) was not sufficient. Further investigation, therefore, is necessary to determine the optimal amounts of Dw needed for the rearing of fry of Tilapia.

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