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Utilization of Hydroponic Fertilizer for Watermeal Cultivation and an Investigation of the Suitability of the Fresh Watermeal (*Wolffia arrhiza* (L.)) Supplement for Tilapia Rearing

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ABSTRACT

This study investigated the watermeal cultivation using different concentrations of hydroponic fertilizer at 0, 0.5, 1.0, and 1.5 ml/l for 20 days in addition to feeding the tilapia fingerlings with a commercial diet (CD) and fresh watermeal (FWM) at ratios of 100:0, 70:30, 50:50, 30:70 and 0:100 for 90 days. The results revealed that watermeal cultivated at 1.5 ml/l (treatment 4) showed the highest growth, while no significant difference (p>0.05) was found when compared to watermeal cultivated at 1.0 ml/l (treatment 3). Watermeal cultivated with the highest level of hydroponic fertilizer resulted in the highest nitrate content at 61.53±0.34 mg/kg of fresh weight, which is higher than a lower one (treatment 3) by about sixfold. The suitable level of hydroponic fertilizer for watermeal cultivation was 1 ml/l of water. The tilapia fish were reared by CD and FWM in combined feeding. Fish cultivated in the control treatment (100% CD feeding) showed the highest growth with no significant difference (p>0.05) when compared to fish cultivated at 70:30 of CD and FWM feeding. The control treatment had the lowest survival rate at 52.00±17.43%, recording a significant difference (p<0.05) when compared to other treatments. Fish in treatment 2 (70%CD:30%FWM) showed the highest specific growth rate (SGR) as 3.33±0.02 %/day, but no significant difference was detected when compared to 100% CD feeding. Therefore, a combined feeding of 70%CD and 30%FWM was found to be a suitable practice in FWM utilization for rearing the Nile tilapia, *Oreochromis niloticus.*

INTRODUCTION

Watermeal are the smallest aquatic plants in the world; they are grouped into family Araceae and genus *Wolffia*. Watermeal are the flowering plants, rootless, with neither foliage leaf nor stem. They have egg-shaped body that is called fronds and are comparable to the leaf structures. A new plant occurs by asexual budding from each frond. The frond size is one character used to identify duckweed species; watermeal have size around 0.5 – 1.5 mm similar to a sugar crystal or grain of salt. Lentic freshwater surface as swamps, marshes and ponds are watermeal habitats. **(Sricharoen *et al*., 2001; Sricharoen *et al*., 2002; Koschnik *et al*., 2014; Pandey & Verma, 2018)**. A number of 11 species of *Wolffia* have been discovered worldwide **(Ivan & Katya, 2013; Appenroth *et al*., 2018; Pandey & Verma, 2018)**. Two species of *Wolffia* have been recorded in Thailand; namely, *Wolffia globosa* (L.) and *Wolffia arrhiza* (L.) **(Chareontesprasit & Jiwyam, 2001; Rodroil *et al*., 2012; Ruekaewma *et al*., 2015; Damna *et al*., 2017)**.

The *Wolffia* spp. contained 20-30 % protein and high essential amino acids that are necessary for preschool- aged children. Other nutrients included 1-5% fat, 10-20% starch and 25% fiber. Importantly, over 60% of the low fat composition was polyunsaturated fatty acids **(Appenroth *et al*., 2018)**. Khai-nam is Thai local name of this plant and is also called Khai-pum, Khai-nhae and Pum. "Khai" means "egg" and is used to refer to the plant's egg-like appearance. Khai-num is traditionally consumed by villagers in Thailand, Myanmar and Laos **(Bhanthumnavin & Mcgarry, 1971; Suppadit *et al*., 2008; Ruekaewma *et al*., 2015; Appenroth *et al*., 2018)**. In addition, *Walffia* spp. act as a protein source, substituting a soybean meal, which is used as a poultry replacement. Moreover, soybean used in the tilapia diet was replaced with *Wolffia* meal without exceeding 15% of the replaced item **(Chareontesprasit & Jiwyam, 2001; Chantiratikul *et al*., 2010)**. In addition, *Oreochromis niloticus* could feed on 30% dried watermeal combined with 70% commercial diet (25% protein) and could consume 15% of fresh *Wolffia arrhizal* supplement with 85% of formulated diet **(Srichareon *et al.*, 2001; Sirirustananun, 2018)**. Watermeal are also utilized for water quality improvement **(Fujita *et al*., 1999; Suppadit *et al*., 2008; Phadungpran & Wangwibulkit, 2017)**, biotechnology, bioenergy and bioactive purposes **(Tipnee *et* al., 2017; Heenatigala *et al*., 2018; Khvatkov *et al*., 2018; Sela *et al*., 2020)**.

In Thailand, watermeal cultivation began by studying environmental factors affecting its living and cultured testing with algae media (BG-11 media), dried pig manure, dried chicken manure, dried cow dung and N-P-K fertilizer **(Sricharoen *et al*., 2001; Sricharoen *et al*., 2002; Rowchai & Somboon, 2007)**. In the past decade, the rising of watermeal for human consumption has attracted the researchers due to its potential as a healthy food, but the harvested products from natural water and manures are unhygienic. Therefore, the continuing development of culture systems for hygienic production by applying hydroponic fertilizer is necessary for watermeal cultivation **(Kongban, 2014; Ruekaema *et al*., 2015; Damna *et al*., 2017)**. Nonetheless, findings regarding the nitrate content of watermeal and a suitable hydroponic fertilizer for watermeal planting are lacking due to the limited knowledge of a suitable FWM in combined feeding with CD for rearing the tilapia fish. The tilapia is an economically important fish, and the monoculture of male tilapia wins the interest of farmers because males grow faster than females, forming the marketable size. It is worth noting that, sex-reversed tilapia are produced by sex reversal of the tilapia into all males with male sex hormone (methyl testosterone, MT) **(Megbowon & Mojekwu, 2014)**.

The objectives of the present study was to evaluate the usage rates of hydroponic fertilizer for watermeal cultivation, assessing its nitrate content, and hence determine the appropriate FWM level for the tilapia rearing.

MATERIALS AND METHODS

1. First experiment with watermeal cultivation in hydroponic fertilizer

Watermeal preparation and experimental protocols

All experiments were performed at the Aquaculture Unit, Faculty of Agricultural and Industrial Technology, Phetchabun Rajabhat University. Watermeal samples were collected from natural water in Bungkla district, Amphoe Lomsak, Phetchabun, Thailand. Samples were acclimatized for a day in cement ponds of 80 cm diameter with an addition of 20 cm of water without using a fertilizer. The next day, 60 grams of initial wet weight of watermeal cultured were put in three replicates of each hydroponic fertilizer, with a concentration of 0 ml/l (treatment 1; control), 0.5 ml/l (treatment 2), 1.0 ml/l (treatment 3) and 1.5 ml/l (treatment 4), following the methods of **Kongban (2014)**. Watermeal samples were cultured for 20 days for the experiment. Finally, the final wet weight of watermeal in each pond was recorded, and the average daily growth (ADG) and specific growth rate (SGR) were calculated using the equations of **Macchiavello and Bulboa (2014)**. Watermeal samples, before and after the experiment, were cleaned and analyzed for nitrate content using In-house method **(ISO, 1975)**.

ADG (g/day) = (final wet weight (g) – initial wet weight (g))/days

SGR (%/day) = [{ln(final wet weight (g)) – ln(initial wet weight (g))}/days] ×100

2. Second experiment with watermeal supplement for tilapia rearing

Fish preparation

Samples of two months old sex-reversed tilapia from Santipanphar, Bor Vattana district, Amphoe Nongphai, Phetchahbun, Thailand were subjected to study. The fish were acclimatized in 15 cement ponds (80 cm diameter) for one week before the beginning of the experiment, and each pond contained 25 fish. Meanwhile, an amount of CD:FMW (30% protein), with a ratio of 50:50 was applied forming a combined diet. Samples were fed thrice/ day till satiation, and the feeding rates ranged between 6-12% of body weight.

Experimental planning and procedures

This experiment consisted of five treatments and three replicates, and a completely randomized design (CRD) was considered and described by 100:0 (treatment 1; control), 70:30 (treatment 2), 50:50 (treatment 3), 30:70 (treatment 4) and 0:100 (treatment 5) of CD and FWM in combined feeding.

After one week of acclimatization, the fish were weighed and fed diet following the plan for 90 days. The fish were fed thrice daily at feeding rates of 6-12 % of body weight till satiation, except for treatment 5 in which feeding rate reached 100% of body weight.

Fish weight was randomly taken every 30 days. At least twice a week, the ponds were cleaned and half of water amount was renewed. Water samples were collected for dissolved total ammonia nitrogen (TAN) analysis using the indophenol blue-hypochlorite method **(APHA, 1998)** including pH, DO and water temperature by using meters. Finally, fish were counted and sampled for proximate analysis **(AOAC, 1995)**. The growth parameters were calculated according to the following equations **(Ariyaratne, 2010)**.

ADG (g/day) = (final weight (g) – initial weight (g))/days of rearing

SGR (%/day) = [{ln(final weight (g)) – ln(initial weight (g))}/days of rearing] ×100

FCR = weight of feed given (g)/ weight gain of fish (g)

Survival rate (%) = (number of fish harvested/ number of fish stocked) ×100

3. Statistical evaluation

The recorded data from each treatment in triplicate were used to find the mean and standard deviation. One way ANOVA and Duncan's multiple range test (DMRT) were used for analyzing the difference of means between treatments at 0.05 of significance level. These procedures were conducted on IBM SPSS statistics version 21.

RESULTS

1. First experiment

Growth of watermeal applied hydroponic fertilizer

After 20 days of experiment, the results showed that the watermeal grown at 1.5 ml/l of fertilizer concentration presented the highest final wet weight, final dry weight, wet weight gain, ADG and SGR, but no significant difference (p>0.05) was detected compared to treatment 3, which used fertilizer at 1.0 ml/l. Interestingly, the control treatment showed negative growth (Table 1).

Table 1. Growth parameters of watermeal cultured at different concentrations of hydroponic fertilizer for 20 days

Growth parameter	Hydroponic fertilizer concentrations			
	0 ml/l	**0.5 ml/l**	**1.0 ml/l**	**1.5 ml/l**
Initial wet weight (g)	60.41±0.19	60.21±0.09	60.28±0.30	60.59±0.22
Final wet weight (g)	44.66±13.15^{c}	690.69±4.91^{b}	876.55±80.0^{a}	947.63±36.29^{a}
Final dry weight (g)	2.12±0.81^{b}	21.09±1.38^{a}	21.68±2.35^{a}	22.89±0.69^{a}
Wet weight gain (g)	-15.74±13.02^{c}	630.48±4.82^{b}	816.26±79.82^{a}	887.03±36.37^{a}
ADG (g/day)	-0.78±0.65^{c}	31.52±0.24^{b}	40.81±3.99^{a}	44.35±1.81^{a}
SGR (%/day)	-1.64±1.38^{c}	12.19±0.02^{b}	13.37±0.45^{ab}	13.74±0.19^{a}

The same superscripts in the row indicate no significant difference (p>0.05).

Nitrate content of watermeal after hydroponic uptake

At the end of the experiment, it was noticed that, the watermeal grown in 1.5 ml/l of fertilizer recorded the highest ($p<0.05$) nitrate content, with a value of 61.53±0.34 mg/kg fresh weight (Table 2).

Table 2. Nitrate content of watermeal cultured at different concentrations of hydroponic fertilizer for 20 days

Parameter	Before experiment	After experiment at differences of hydroponic fertilizer concentrations		
		0.5 ml/l	**1.0 ml/l**	**1.5 ml/l**
Nitrate content (mg/kg)	7.52±1.76^{c}	3.16±0.03^{d}	9.30±0.03^{b}	61.53±0.34^{a}

The same superscripts in the row indicate no significant difference ($p>0.05$).

2. Second experiment

Growth of fish fed FWM

The growth performance of fish fed CD combined with FWM in 30, 60 and 90 days revealed that, the growth of fish in all treatments increased gradually following the experimental time, except for treatment 5 in which fish were fed 100%FWM and presented negative growth at 30- day period (Fig. 1). Nevertheless, the SGR trend decreased slowly as the experimental time increased, while fish fed 70%CD: 30%FWM showed the highest SGR (Fig. 2).

At the end of the experiment of 90 days, it was observed that the final wet weight, wet weight gain, ADG and feed conversion ratio (FCR) of fish fed 100%CD showed better results than the fish in other treatments. Yet, there was no significant difference when compared to fish fed 70%CD: 30%FWM. Notably, the survival rate of the control treatment was the lowest, recording value of 52.00±17.43% and showing significant difference ($p<0.05$) when compared to the other treatments. Fish in treatment 2 (70%CD:30%FWM) showed the highest SGR at 3.33±0.02 %/ day, but no significant difference ($p>0.05$) was detected when compared to 100%CD feeding (Table 3).

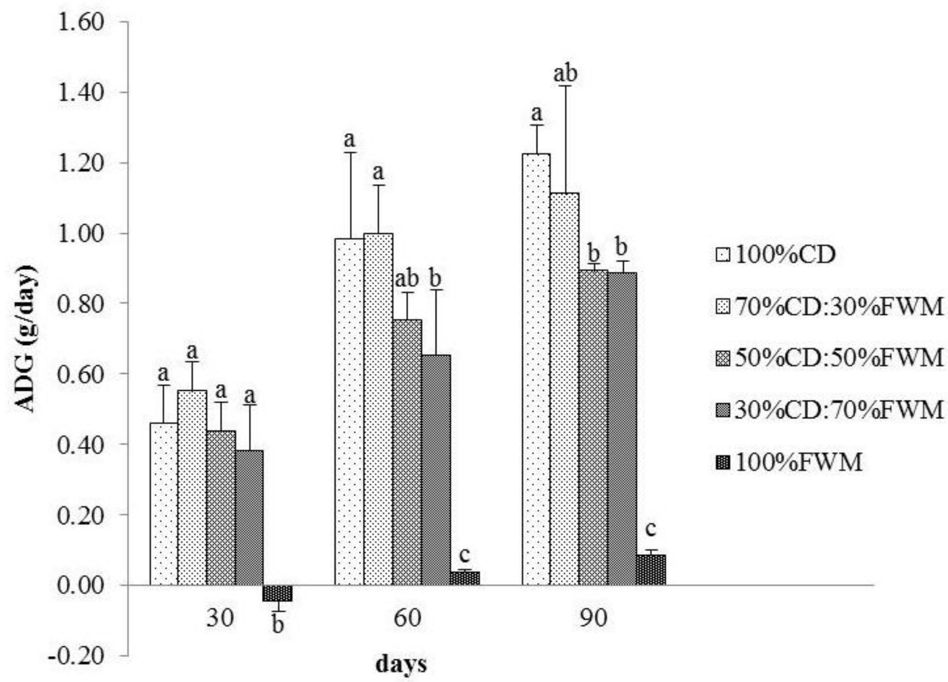

Fig. 1. ADG of fish fed different ratios of CD combined with FWM for 30, 60 and 90 days.

No difference of alphabets above the error bars indicates no significant difference (p>0.05). CD, commercial diet; FWM, fresh watermeal.

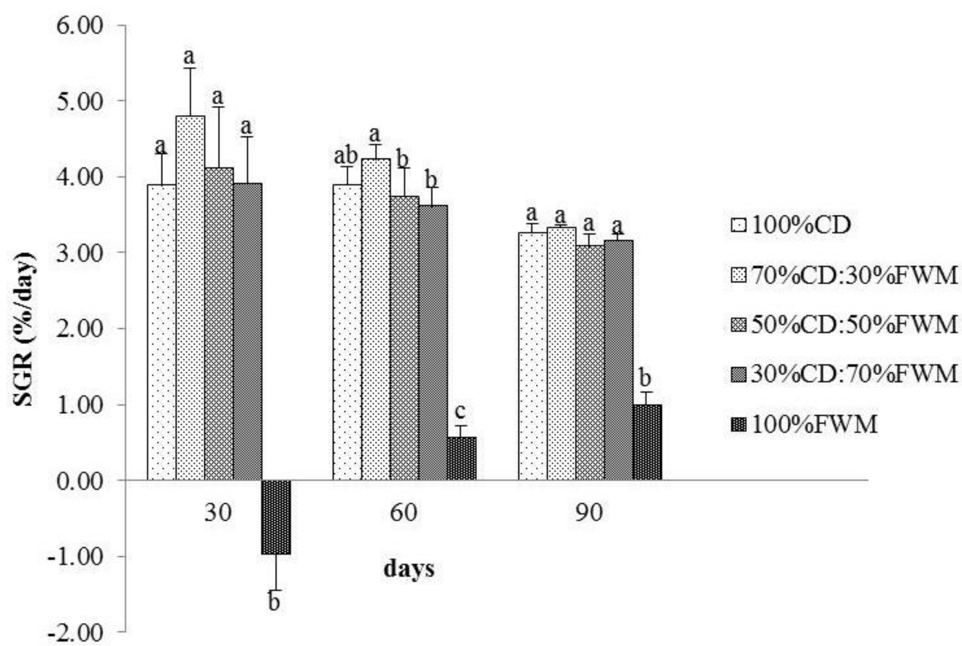

Fig. 2. SGR of fish after fed different ratios of CD combined with FWM for 30, 60 and 90 days.

No difference of alphabets above the error bars indicates no significant difference (p>0.05). CD, commercial diet; FWM, fresh watermeal.

Table 3. Growth parameters of fish fed different ratios of CD and FWM for 90 days

Growth parameter	CD:FWM (%)				
	100:0	**70:30**	**50:50**	**30:70**	**0:100**
Initial wet weight (g)	6.20±0.70	5.23±1.37	5.39±0.82	4.97±0.55	5.35±0.85
Final wet weight (g)	116.34±7.88[a]	105.23±28.88[ab]	85.64±1.72[b]	84.58±3.56[b]	12.97±1.14[c]
Wet weight gain (g)	110.13±7.59[a]	100.00±27.51[ab]	80.24±1.98[b]	79.61±3.26[b]	7.62±1.29[c]
ADG (g/day)	1.22±0.08[a]	1.11±0.30[ab]	0.89±0.02[b]	0.88±0.03[b]	0.08±0.01[c]
SGR (%/day)	3.26±0.11[a]	3.33±0.02[a]	3.08±0.16[a]	3.15±0.09[a]	0.99±0.17[b]
FCR	0.87±0.05[b]	1.29±0.33[b]	1.80±0.04[b]	2.39±0.09[b]	38.11±6.25[a]
Survival rate (%)	52.00±17.43[b]	81.33±20.13[a]	94.66±6.11[a]	93.33±6.11[a]	89.33±10.06[a]

The same superscripts in the row indicate no significant difference (p>0.05). CD, commercial diet; FWM, fresh watermeal.

Nutritional values of FWM and fish meat

The proximate analysis of FWM revealed that, its nutrients consisted of 17.83±0.12%protein, 1.27±0.01%fat, 9.86±0.48%fiber, 14.03±0.05%ash and 3,652.63±4.64 kcal/kg energy. The nutritional values of the fish flesh in each treatment are presented in Table (4). Fish fed 100%CD showed the highest protein content with no significant difference (p>0.05) when compared to 70:30 and 30:70 of CD and FWM feeding.

Table 4. Nutritional values in fish flesh fed different ratios of CD and FWM for 90 days

Analyzed parameter	CD:FWM (%)			
	100:0	**70:30**	**50:50**	**30:70**
Crude protein (%)	85.73±0.44[a]	85.19±0.52[ab]	84.82±0.14[b]	85.17±0.27[ab]
Crude fat (%)	2.08±0.07[c]	2.27±0.04[b]	2.72±0.03[a]	2.35±0.03[b]
Crude fiber (%)	0.02±0.02	0.04±0.03	0.03±0.01	0.02±0.02
Ash (%)	5.08±0.01[c]	5.15±0.03[b]	4.99±0.04[d]	5.22±0.02[a]
Gross energy (kcal/kg)	5,058.50±14.23[a]	5,016.20±8.51[b]	5,008.26±18.78[b]	5,002.50±8.37[b]

The same superscripts in the row indicate no significant difference (p>0.05). CD, commercial diet; FWM, fresh watermeal.

DISCUSSION

Watermeal cultured in a gradual increase of hydroponic fertilizer levels showed increasing nitrate content due to the nitrogen content of duckweed that depends on the nitrogen and phosphorus in water **(Food and Agriculture Organization of United Nations, 1997; Hasan & Chakarbarti, 2009)**. This finding corresponds with the result of **Davlamynck *et al.* (2020)** who studied the effect of different growth medium on nitrate accumulation in *Lemna minor*. Their result showed that nitrate accumulation in duckweed increased with the increase in water nutrients. With respect to the present study, although the nitrate content of the samples in all treatments were at low levels, which poses no danger to consumers, the decreased nitrate accumulation with watermeal cultured at 1.0 ml/l of hydroponic fertilizer was about six times of the highest level. Besides, watermeal planting in 1.0 ml/l showed no growth difference when compared to the application of 1.5 ml/l of fertilizer. Therefore, the most suitable hydroponic fertilizer for watermeal cultivation is 1.0 ml/l.

In the second experiment, fish in all treatments obviously showed incremental growth following the experimental time, but fish fed 100%FWM (treatment 5) grew slowly and experienced negative growth at 30 days of experiment because the nutrients and mono-feeding of FWM were insufficient for growth. This explanation was similar to that of **Srichareon *et al*. (2001)** who presented wet weight gain reduction when the fry tilapia fish were fed FWM, recording more decline at 42 and 56 days of experiment. In addition, the present result coincides with that of **Ariyaratne (2010)** who confirmed that the fry tilapia fed 100%FWM for 41 days showed the lowest final wet weight, ADG and SGR. Moreover, **El-Sayed Saleh (2020)** postulated that, the hybrid red tilapia fed only fresh macro algae (*Enteromorpha flaxusa*) exhibited lower growth than fish fed artificial diet. The current experiment showed that, the SGR of fish trended to decline with experimental time due to the SGR of fish that decreases with the increase in body weight because the ingestion and fish metabolic rate reverse with body weight **(Jobling, 1994)**. In the present study, the SGR was 3.08 – 3.33 %/ day, a result which is similar to that of **Tavares *et al*. (2008)**. The previous authors addressed dried duckweed and commercial diet on the growth performance of the tilapia fingerlings and deduced that, their SGR values were 3.02, 3.30 and 3.72 %/ day when fed dried duckweed alone, 50%feed: 50%dried duckweed and commercial feed, respectively. Remarkably, the values of the present SGR fill below those of **Chareontesprasit and Jiwyam (2001)** who evaluated *wolffia* meal replacing soybean meal in formulated diet of the Nile tilapia. They recorded SGR values of 3.83 – 4.25 %/day. This could be explained by the high moisture of FWM, since in case of having the same weight of FWM and dried watermeal (wolffia meal), the dried meal would contribute more energy and nutrients. The survival rate of fish fed 100%CD was the lowest at 52%. This finding corresponds with the results of **Chareontesprasit and Jiwyam (2001)** and **Ariyaratne (2010)**. They assessed the survival rates at 58.8% and 44.6% of fish fed unadded *wolffia* meal diet and 100% commercial diet, respectively. **Chareontesprasit and Jiwyam (2001)** commented that the increased *wolffia* meal levels in formulated feed could increase the survival rates of fish. Likewise, protein replacement by duckweed meal (*L. minor*) in formulated diet contributed higher survival rate than fish fed control diet **(Anthonius *et al*., 2018)**. Regarding water qualities of treatment 1-4 in which CD was added, the pH ranged from 7.78 – 7.89, DO ranged from 3.93 – 4.45 mg/l, water temperature was 27.44 – 32.18 ºC and ammonia nitrogen was 0.056 – 0.067 mg/l. These indicators were solely for fish living **(Svobodova *et al*., 1993)**.

In this study, hydroponic fertilizer level at 1.0 ml/l was suitabile for watermeal planting, noting that the biological fertilizer is an interested option for organic watermeal cultivation. Although fish cultivated with 100%CD presented the highest growth, it showed a low survival rate, and the growth performance showed no difference when compared to 70%CD: 30%FWM feeding. For 90 days of rearing, the cost of 100%CD feeding was 52.76 baht/kg of the tilapia production. If fed with 70%CD: 30%FWM, then the diet cost would be 47.22 baht/kg; hence, the presented cost saving would be approximately 10.50% of the complete pellet feeding. Therefore, feeding of 70%CD combined with 30%FWM is determined to be the best choice for the tilapia fingerlings rearing.

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