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Gut Weed, *Enteromorpha* sp. As a Partial Replacement for Commercial Feed in Nile Tilapia (*Oreochromis niloticus*) Culture

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Abstract: A 42-day experiment was carried out to evaluate the fresh and dried gut weed (*Enteromorpha* sp.) as a direct alternative feeds for monosex Nile tilapia (*Oreochromis niloticus*) comparing with commercial feed. Triplicate groups of fish with initial size of 3.04±0.48 g were stocked in 21 plastic tanks at a rate of 30 fish per tank. The water volume of each plastic tank was 80 liters. Fish were fed the respective diets to satiation twice a day. The results showed that the growth performance of *O. niloticus* in the alternating feeding treatments, 1 day commercial feed and 1 consecutive day fresh or dried gut weed (1CF_1FGW and 1CF_1DGW) were not significantly different from the control group fed solely commercial feed, whereas significantly reduced performance were observed in fish fed the fresh or dried gut weed as single diet. Carcass analysis documented the highest crude lipid content in fish fed commercial feed while protein content has no influenced by the gut weed replacement levels in the diet. Economic analysis suggested that the cost of the combined feeding regimes could be reduced with 41% without hampering the growth of tilapia. These results indicated that fresh and dried gut weed can be used as a feed to partially substitute commercial feed for Nile tilapia *O. niloticus* juvenile.

Key words: Gut Weed · Nile Tilapia · Feed Utilization · Carcass Composition

INTRODUCTION

Globally the aquaculture production is highly dependent on commercial feeds whose basic ingredients are fish meal and fish oil derived from wild harvested whole fish. Currently, its availability is a major concern for its high cost and scarcity of raw materials. In aquaculture, feed cost is the highest proportion and it accounts for more than 50% of the total production costs [1, 2]. Most feed manufactures are using expensive imported fishmeal as a protein source for aqua feeds resulting in high price. Therefore, investigation of cheaper or more readily available alternative protein sources such as soybean meal, seaweed meal or other sources that may reduce the use of fishmeal in feeds is necessary [2,3]. The current trend to intensify fish culture is making imperative the use of alternative protein sources in their diets. Nowadays, a variety of seaweeds are receiving attention as possible alternative protein sources of fish feed especially

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herbivorous fish feed [4, 5]. Like other seaweeds, gut weed *Enteromorpha* spp. has a high nutritional value; it contains 9-14% protein, 2-3.6% lipid, 32-36% ash and n-3 and n-6 fatty acids 10.4 and 10.9 g/100 g of total fatty acid and the protein of this seaweed has a high digestibility up to 98% [5-7]. Hence, gut weed can be used as a direct feed or as ingredient in diets for herbivorous fish [8, 9]. Recent study reported that fresh and dried gut weed can be used as a feed to substitute commercial feed for herbivorous fish such as spotted scat, (*Scatophagus argus*), red tilapia (*Oreochromis* sp.) and giant gourami (*Osphronemus goramy*) juveniles [10]. In Vietnam, recent investigates revealed that gut weed (*Enteromorpha* sp.) belonging to green algae distribute abundantly in the extensive shrimp farms and other brackish water bodies of the Mekong delta [11, 12]. This indicates large quantity of gut weed is available for aquaculture feeds.

Tilapia is the second most farmed fish group worldwide and over the past decade has markedly increased in production, due to their many characteristics conducive to aquaculture conditions as well as to the high marketability and relatively stable market prices [13]. Furthermore, one of the great advantages of tilapia for aquaculture is that they feed on a low strophic level. The members of the genus *Oreochromis* are all omnivores, feeding on algae, aquatic plants, small invertebrates, detritus material [14, 15]. In addition, tilapia can be applied in poly-culture with other species or integrated aquaculture system [13]. They have a good alternative to rearing conditions, are species of high economic and can live in fresh and brackish water [16, 17]. The aim of this study was to assess the effect of commercial feed replacement with fresh or dried gut weed (*Enteromorpha* sp.) as direct feed on growth, feed utilization, body composition and water quality of *O. niloticus*. In this experiment, fresh and dried gut weed were used as a tilapia feed as many fish farmers in Vietnam use it as a substitute of the high priced commercial feed. In Vietnam herbivorous fish are preferred in household-scale culture systems that primarily produce fish for home or local consumption. However, a combination of natural food (macroalgae, aquatic plants) with pellet feed, the production cost can be reduced [12, 18]. However, this work could help them to quantify the maximum use of gut weed in the tilapia feed in order to reduce feed costs.

MATERIALS AND METHODS

Experimental System and Fish: The feeding trial was carried out in the experimental hatchery of the College of Aquaculture and Fisheries, Can Tho University, Vietnam. The fresh or dried gut weeds were used as a direct feed to replace commercial feed in an alternative regime for feeding mono-sex tilapia (*Oreochromis niloticus*) juveniles for 42 days. Feeding regimes were run in triplicate tanks and each day fish were fed either commercial feed or gut weed. Seven treatments comprised (1) single commercial feed daily as a control treatment (CF), (2 and 3) single fresh or dried gut weed everyday (FGW or DGW) and 2 alternative feeding regimes where (4 and 5) 1 day commercial feed and 1 consecutive day fresh or dried gut weed (1CF_1FGW and 1CF_1DGW) and (6 and 7) 2 consecutive days fresh or dried gut weed (1CF_2FGW and 1CF_2DGW).

The juveniles were purchased in Bac Lieu province and visually checked for sign of diseases and parasites. The juveniles were acclimatized for one week in a 1 m^3 tank to get them acquainted with the use of a feeding tray. After acclimation, 30 uniformly sized juveniles with initial individual weight of 3.04±0.48 g were stocked in each tank. Fish were fed two times a day at 8:00 and 17:00 up to satiation.

Experimental Diets: Fresh guts weed *Enteromorpha* spp. was collected from the extensive farm of Bac Lieu province (Vietnam) and cleaned with 5 g L^{-1} saline water to maintain the quality [10]. Dried gut weed was obtained by air-drying a thin layer of biomass till reaching a moisture content of 12-14%. It was stored in the fridge for later use. Both fresh and dried gut weeds were cut with scissor into small pieces (± 2 mm) for feeding the fish. Commercial floating feed (Grobest& I-MEI Company, Dong Nai province, Vietnam).was purchased from the market. The proximate composition of the three experimental diets is presented in Table 1.

Rearing Conditions: The feeding trial was carried out in 80-L plastic tanks which were filled with 60-L water (salinity 5 g L^{-1}). Each tank was provided with a soft continuous aeration. The feeding trays were left in the tanks for 1.5 h. Uneaten gut weed was collected and dried in the sun till constant weight. In case of commercial feed, the number of pellets (per gram) was determined before the feeding and the uneaten pellets were counted after 1.5 h. Every day, about 30% of the tank water was replaced.

Table 1: Proximate composition (% dry matter) of the experimental diets

Nutrients	FGW	DGW	Commercial feed*
Dry matter	10.01 ± 0.06	84.91 ± 1.15	-
Moisture content	89.99 ± 0.06	15.09 ± 1.15	= 11
Crude protein	15.13 ± 3.97	13.77 ± 0.53	= 30
Crude lipid	1.55 ± 0.52	1.92 ± 0.22	= 6
Crude fiber	4.26 ± 1.72	3.72 ± 0.48	= 6
Ash	26.82 ± 2.54	28.17 ± 2.02	= 14
NFE	52.24 ± 6.13	52.42 ± 1.90	-
Calcium	2.10 ± 0.35	1.47 ± 0.68	-
Phosphorus	0.64 ± 0.24	0.72 ± 0.16	-

Values are mean ± standard deviation. FGW: Fresh gut weed, DGW: Dried gut weed, *Formulation from manufacturer.

Sampling: Fish sampling was conducted at 2-weeks interval. A total of 10 fish were randomly taken from each tank for individual weight measurements. Afterwards, the fish were returned to their tank. After six weeks, all fish were counted to calculate the survival and to measure the individual weight. Daily water temperature and pH were recorded at 7:00 and 16:00 h using a thermo-pH meter (YSI 60 Model pH meter, HANNA instruments, Mauritius). The salinity of water was monitored daily using a hand refractometer (Atago, Japan), respectively. Water samples were collected weekly between 9:00 and 10:00 h for determination of nitrite (NO_2) and total ammonia (TAN) following the standard methods [19].

Growth Performance Parameters: The following equations were used to determine growth and feed utilization:

- Weight gain (g) = Final weight - Initial weight
- Daily weight gain (DWG) = (final weight -initial weight)/rearing period (day)
- Specific growth rate (SGR) = [(ln final weight - ln initial weight)/Days of rearing] × 100
- Total feed intake = [(Total feed supplied (DW) - Total feed remaining (DW)/(initial number of fish + final number of fish)/2] ×100
- Feeding rate = [Feed intake (gDW fish^{-1} day^{-1})/(initial number of fish + final number of fish)/2] ×100
- Feed conversion ratio (FCR) = Feed intake (dry weight)/ Weight gain (wet weight)
- Protein efficiency ratio (PER) = weight gain (WW)/protein intake (g).
- Survival (%) = [Final number of fish/Initial number of fish] × 100
- Biochemical analysis: At the end of the feeding trial, all fishes were fasted for 24 h prior to final sampling. A pooled sample of 10 randomly selected fish from each replicate tank was collected and stored at -20°C for analysis. The ingredients, test diets and fish carcass were analyzed for moisture, crude protein, total lipid and ash, in triplicate, according to APHA [20]. Nitrogen-free extract (NFE) was estimated on a dry weight basis by subtracting the percentages of crude protein, lipids, crude fiber and ash from 100%.

Statistical Analysis: All data were analyzed using SPSS, Version 16.0. All data were checked for normality before analyses. Only percent data had to be arcsine-transformed before analysis. Variations between dietary treatments were compared by one-way ANOVA. If the effects were significant, ANOVA was followed by the Tukey HSD post-hoc test for unplanned multiple comparison of means. Significant differences were considered at $P<0.05$.

RESULTS

Water Quality Parameters: The water quality parameters across all treatments were: water temperature 26.7-30.4°C; DO 7.6-8.2 mg L^{-1}; pH 6.95-7.32 and salinity 4.93-5.12 g L^{-1}. The effects of dietary treatment and its interaction with time on water temperature, pH, NO_2 and TAN concentrations are presented in Table 2. Dietary treatment, sampling time and their interaction had significant effect on water pH, NO_2 and TAN concentrations. Sampling time had significant effect on water temperature but dietary treatment and the interaction of dietary treatment and sampling time had no effect on water temperature.

The mean concentration of NO_2 and TAN in all treatments tended to increase with the culture period in which the highest concentration of NO_2 and TAN were observed in the control group, received solely commercial feed (CF) everyday, followed by the group fed alternative1 day commercial feed and 1 consecutive day fresh or dried gut weed (1CF_1FGW and 1CF_1DGW) and 2 consecutive days fresh or dried gut weed (1CF_2FGW and 1CF_2DGW),while the lowest values of NO_2 and TAN were found in the group fed single gut weed (GW) (Figs. 1 and 2). These results indicated that fish can maintain better water quality in case of combined feeding of commercial feed with gut weed than single commercial feed.

Table 2: Effects of treatment and sampling time on measured water quality parameters based on one-way repeated measure ANOVA

	Significance (P value)		
Variable	Treatment	Time	Treatment × Time
Temperature (°C)	NS	**	NS
pH	**	**	**
Dissolved Oxygen (mg L^{-1})	**	**	*
Nitrite nitrogen (mg L^{-1})	**	**	**
Total ammonia nitrogen (mgL^{-1})	**	**	**

* P ≤ 0.05; ** $P < 0.001$; ns, not significant.

Table 3: Fish growth performance and feed utilization in different dietary treatments

Parameters	CF	FGW	DGW	1CF_1FGW	1CF_1DGW	1CF_2FGW	1CF_2DGW
Growth parameters							
Initial body weight (g)	3.04±0.48	3.04±0.48	3.04±0.48	3.04±0.48	3.04±0.48	3.04±0.48	3.04±0.48
Final body weight (g)	12.35±0.64^{c}	5.87±0.07^{a}	5.92±0.24^{a}	9.86±0.28^{bc}	10.07±0.48^{c}	8.79±0.03^{b}	9.45±0.25^{b}
weight gain (g)	8.68±0.64^{c}	2.83±0.07^{a}	2.80±0.24^{a}	6.82±0.28^{bc}	7.03±0.48^{c}	5.75±0.03^{b}	6.41±0.25^{b}
SGR (%day^{-1})	3.30±0.10^{c}	1.53±0.03^{a}	1.50±0.08^{a}	2.74±0.10^{b}	2.82±0.12^{bc}	2.49±0.02^{b}	2.63±0.03^{b}
DWG (g day^{-1})	0.22±0.05^{bc}	0.07±0.03^{a}	0.07±0.03^{a}	0.16±0.05^{b}	0.17±0.04^{b}	0.14±0.04^{b}	0.15±0.06^{b}
Survival (%)	84.3±2.3^{a}	81.0±1.7^{a}	83.7±2.8^{a}	83.3±3.5^{a}	82.0±1.7^{a}	85.7±2.3^{a}	84.3±2.3^{a}
Feed Utilization							
Total feed intake (g fish^{-1})	11.98 ± 0.29^{bc}	12.98 ± 0.34^{c}	13.05 ± 0.55^{c}	11.69 ± 0.15^{bc}	12.40 ± 0.09^{c}	9.44 ± 0.08^{a}	10.18 ± 0.37^{ab}
Feeding rate (% BW day^{-1})	3.71 ± 0.17^{a}	5.94 ± 0.36^{c}	6.0 ± 0.13^{c}	4.31 ± 0.10^{ab}	4.51 ± 0.15^{b}	3.80 ± 0.05^{ab}	3.87 ± 0.49^{ab}
FCR	1.29 ± 0.09^{a}	4.61 ± 0.49^{b}	4.73 ± 0.55^{b}-	1.71 ± 0.05^{a}	1.77 ± 0.12^{a}	1.64 ± 0.02^{a}	1.59 ± 0.19^{a}
PER	2.59 ± 0.18^{c}	1.44 ± 0.16^{a}	1.55 ± 0.19^{a}	2.58 ± 0.08^{c}	2.56 ± 0.18^{c}	2.03 ± 0.14^{b}	2.31 ± 0.40^{b}

Values in the same row with no superscript in common are different significantly ($P > 0.05$). SGR, Specific growth rate; DWG, Daily weight gain; FCR, food conversion ratio; PER, protein efficiency ratio.

Table 4: Carcass proximate compositions (% dry weight basis) of tilapia fed commercial feed and fresh/dried gut weed for 42 days experimentation

Parameters	CF	FGW	DGW	1CF_1FGW	1CF_1DGW	1CF_2FGW	1CF_2DGW
Moisture content	78.70 ±0.24^{a}	79.89 ±0.02^{d}	80.13±0.35^{d}	79.21±0.30^{b}	79.25±1.02^{b}	79.70±0.79^{c}	79.17±0.62^{b}
Crude protein	62.71±1.12^{a}	60.70 ±1.68^{a}	60.66±1.30^{a}	61.48±0.47^{a}	61.37±0.93^{a}	60.44±2.25^{a}	62.01±1.39^{a}
Crude lipid	7.52±0.43^{d}	5.46 ±0.24^{a}	5.45±0.43^{a}	6.49±0.98^{c}	5.69±0.74^{ab}	6.06±1.18^{bc}	5.91±0.59^{b}
Ash	5.91±0.15^{a}	6.52±0.21^{b}	6.47±0.45^{b}	6.80±0.18^{c}	6.28±0.76^{ab}	6.70±0.80^{c}	6.50±0.43^{b}

Values are mean ± standard deviation. Mean values in each row bearing same superscripts are not significantly different *(P>0.05)*.

Table 5: The expenditure of commercial feed combined with fresh and dried gut weeds to replace in diets of tilapia

Treatment	Quantity of commercial feed (CF) used for fish growth (kg CF/kg fish)	CF cost for fish growth (USD/kg)	Reduction ratio compared to control treatment (%)
CF (control)	1.43 ± 0.10	1.10 ± 0.08	_
1CF_1FGW	0.83 ± 0.03	0.64 ± 0.02	-41.88 ± 2.14
1CF_1DGW	0.84 ± 0.06	0.65 ± 0.05	-41.09 ± 4.23
1CF_2FGW	0.77 ± 0.02	0.59 ± 0.05	-46.49 ± 1.46
1CF_2DGW	0.73 ± 0.07	0.56 ± 0.05	-49.41 ± 5.13
FGW	_	_	_
DGW	_	_	_

1kg commercial feed = 16,000 VND = 0.77 USD (1 USD = 20,800 VND)

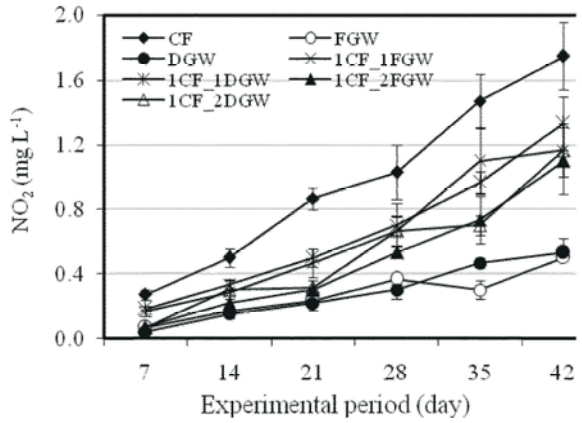

Fig. 1: NO_2 in different treatments during the experimental period (Mean concentration ± standard error)

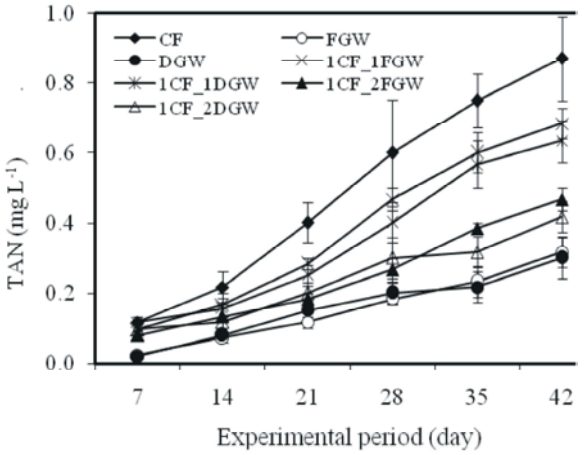

Fig. 2: TAN in different treatments during the experimental period (Mean concentration ± standard error)

Growth Performance: Effects of different dietary treatments on growth performance of tilapia juveniles are given in Table 3. The survival was not affected by dietary treatments. The highest mean final body weight (FBW) and specific growth rate (SGR) were found in fish fed the commercial feed whereas, the lowest FBW and SGR were observed in treatments feeding fresh and dried gut weed as single feeds. Alternative feeding with commercial feed and fresh or dried gut weed (1CF_1FGW, 1CF_2FGW and 1CF_2DGW) resulted in intermediate growth performances except feeding with 1 day commercial feed and 1 consecutive day dried gut weed (1CF_1DGW). There was no significant difference between treatment CF and treatment 1CF_1DGW on growth performances of tilapia.

Feed Utilization: Feeding gut weed as a single diet, both fresh or dried, led to significantly higher FCR than feeding commercial feed as a single diet (CF) or alternative feeding treatments of commercial feed and gut weed (1CF_1FGW, 1CF_1DGW, 1CF_2DGW and 1CF_2FGW). Treatments 1CF_1FGW, 1CF_1DGW, 1CF_2DGW and 1CF_2FGW showed statistically similar FCR of tilapia juveniles (Table 3). The protein efficiency ratio (PER) of tilapia juveniles in treatments CF, 1CF_1FGW and 1CF_1DGW were significantly ($P<0.05$) higher than in treatments CF, 1CF_2DGW and 1CF_2FGW followed by treatments FGW and DGW. The protein efficiency ratio (PER) of tilapia juveniles in treatment CF, 1CF_1FGW and 1CF_1DGW were statistically similar ($P>0.05$).

Carcass Composition: Fish fed different experimental diets showed a significant change in their carcass composition except for protein (Table 4). The highest moisture content in tilapia carcass was observed in treatments FGW and DGW, while lowest was observed in treatments CF. An opposite result was observed in case of the lipid content of tilapia carcass. The ash contents of tilapia carcass in treatments 1CF_1FGW and 1CF_2FGW were significantly higher than in treatments FGW, DGW, 1CF_1DGW and 1CF_2DGW. The lowest ash content of tilapia carcass was observed in treatment CF.

Economic Analysis: A simple economic analysis was performed to estimate the cost reduction of feed using gut weed compared to commercial feed (Table 5). The economic analysis suggests that the feed cost is being reduced with 42% when applying the alternative 1 day commercial feed and 1 consecutive day fresh or dried gut weed (1CF_1FGW) diet. However, in the alternating regime of 1 day commercial feed and 2 consecutive day fresh gut weed (1CF_2FGW), a 49% reduction was obtained of the feed cost but the performance of the fish was negatively affected. For the economic analysis, the cost of commercial feed was based on the whole sale market price of Can Tho city, Vietnam.

DISCUSSION

Water quality parameters experienced during the experimental period were normal and remained within ranges allowing for high growth rate for tropical fish culture [21-23]. However, the culture water in tanks contained commercial diets (CF) found greater NO_2 and

TAN concentration compared to seaweed contained tanks over the experimental period. This result indicated that the water quality of rearing system can be improved by alternative feeding with gut weed. However, in the present study, because of the relatively high rate of water exchange (about 30% day^{-1}), the NO_2 and TAN in tanks with commercial feed were diluted. Therefore, no harmful effect to the experimental fish was observed as indicated by high survival of tilapia juvenile.

The equal survival ($P>0.05$) of tilapia juvenile in all dietary treatments was in agreement with the study of Rahman and Meyer [23] who observed similar survival of fish fed diet with seaweed and without seaweed.. However, this result is not consistent in all cases, for example, [8] reported higher survival of *Siganus canaliculatus* fed the fresh *Enteromorpha* compared to commercial diet as feed. In the present study, tilapia fed fresh or dried gut weed only and alternative1 day commercial feed and 2 consecutive days fresh or dried gut weed showed significantly reduced overall growth performance and feed utilization compare to tilapia fed the commercial diet. However, tilapia fed alternative1 day commercial feed and 1 consecutive day fresh or dried gut weed showed similar growth performance and feed utilization to tilapia feed the commercial feed. These result clearly indicated that gut weed can be used 1 day after using 1 day commercial feed without affecting growth and feed utilization of tilapia. There is no previous study comparing the effects of feeding 1 day gut weed after feeding 1 day commercial diet on tilapia growth performance and feed utilization. However, there are many studies that evaluated replacing fish meal with plant ingredients on growth performance of tilapia. Published literatures indicated that in fish feed; fish meal can be replaced with plant ingredient up to 75% based on fishes and plant ingredient. Herbivorous fish like tilapia generally accept plant originated ingredients better than animal originated ingredients in their diet. For example, Swain and Padhi [24] reported that tilapia grew better fed diet replacing 75% fish meal with okra meal (a by- product of soybean meal) than fed diet containing 100% fish meal.

In the present study, the PER values of fish ranged between 1.44 to 2.59 which are in agreement with the values (1.48 to 2.31) obtained by Valente *et al.* [4] in case of European sea bass (*Dicentrarchus labrax*) juveniles fed different seaweeds as dietary ingredient. In all feeding trials, fish fed FGW and DGW or combination of both showed significantly lower ($P \leq 0.05$) lipid content compared to fish fed commercial diet. These results coincide with El-Saidy and Ibrahim *et al.* [25, 26] who reported fat content were significantly decreased with increasing *Azolla* meal percentage in the diets and commercial feed showed highest level of fat content in fish. In the present study, ash contents in tilapia body were significantly higher in treatments containing dietary seaweed than in treatment without dietary seaweed.

The economic comparison of feed cost indicated that increasing level of fresh and dried gut weed in alternative feeding treatments, commercial feed used for fish growth was reduced leading to significantly reduction of feed cost. Compare to commercial feed, treatments 1CF_2DGW and 1CF_2DGW reduced more feed cost than treatments 1CF_1DGW and 1CF_1DGW However, the feed utilization and growth performance of tilapia in treatments 1CF_2DGW and 1CF_2DGW were significantly lower than the feed utilization and growth performance of tilapia in treatments 1CF_1DGW and 1CF_1DGW. The result of this study clearly demonstrated that fresh or dried gut weed can be apply as a tilapia feed 1 day after applying 1 day commercial feed to maintain better water quality and reduce feed costs without affecting feed utilization and growth of tilapia.

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