
Inclusion of Fresh Raw Material on Tilapia Fry Diets Growth Under Aquaponics System

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Abstract: This research was conducted in a greenhouse in Bajío Experimental Station of INIFAP to study the effects of inclusion of fresh raw material dietary on growth performance, food conversion rate, percentage of weight gained and the survival rate in Nile Tilapia fry. The diets consisted are as follow: 1) 60% commercial feed + 40% live worm; 2) 50% commercial feed + 40% live worm + 10% alfalfa dry leaf; 3) 60% commercial feed + 40% beetle larvae; 4) 60% commercial food + 40% beetle larvae; 5) 100% commercial feed. The fresh raw material levels positively influenced ($P > 0.05$) the specific growth rate and final weight. The growth parameters studied indicate that diets formulated with ingredients of plant and animal origin were the best diets for tilapia fry during the growth period. These results indicate that the fresh raw material could be incorporated in the diet of Tilapia fry with cost benefit.

Keywords: Feed Commercial, Growth Performance, Ingredients, Tilapia Fry

1. Introduction

Actually, the demand for fish feed has continued to increase as a result of growing global aquaculture production. In the aquaculture production system like the Aquaponics system is a challenge formulate quality fish feeds that minimise production cost, limit environmental impacts and enhance products quality using sources from both plant and animal origins [21].

Nutrition is an important aspect in aquaculture, as most of the production cost is related to the use of commercial food, thus, it is important to have information on other food sources that cover the nutritional requirements of Tilapia [27], especially in the juvenile stage, in order to formulate balanced diets that reduce nitrogen excretion and that are inexpensive such as soybean meal [13], lupins [17] and various oilseeds [21] have been widely explored.

The incorporation of non-conventional protein sources in fish feed at high inclusion level, efforts are geared towards the enhancement of their nutritive value through processing to increase the availability of nutrients and reduce or remove their antinutritional factors [15, 30]. In previous studies it was reported the use of sources from both plant and animal

origins with different level of crude protein such as earthworms (54-64%), insects [6] or certain plants (oleaginous or leguminous crops) might be viable alternatives to reduce food costs without decreasing product quality [32]. However, it has also been reported that the use of sources of vegetable origin can cause anorexia or low food consumption, therefore, it is important to determine the amount of vegetable protein that can substitute animal protein to prevent fish reduce their food intake [14].

Based on the all mentioned above, the aim of this study was to determine the percentage of inclusion of fresh raw material that could improve the growth rate, food conversion rate to benefit the Tilapia fry's growth and survival.

2. Material and Methods

2.1. Experimental Aquaponics System and Fish

The experiment was conducted inside a macro tunnel with plastic cover located at the Bajío Experimental Station-INIFAP (Celaya, Gto., Mexico), with average of $26 \pm 2^\circ\text{C}$

room temperature. The aquaponics system consists: 20 polyethylene rearing tank (80 L) containing each tank 20 juveniles, a pump oxygenator (2.0 mg L⁻¹ of dissolved oxygen) with two outlets, a submersible pump (Aquatech; 1, 500 L h⁻¹) and 2 cylindrical filter blocks (diameter: 0.15 m, height: 0.3 m), and a hydroponic bed of 4.2 m² (25 lettuces per m²). The systems were supplied with water from an overhead 7, 500 L capacity reservoir.

Tilapia fry (*Oreochromis niloticus* x *O. aureus*) with 0.5 ± 0.2g average weight per juvenile g of initial weight and sexed with methyl testosterone (50 mg kg⁻¹ food) [28], were provided by a commercial company (Criaderos de Tilapia Arcoiris, Jerecuaro, GTO, Mexico). Prior to the experiment, the fry went through a period of adaptation to the environment of 10 days, and during that time they were fed with 45% protein commercial pellets (CAMPI) twice a day (9:00 am and 13:00 pm). The design of the experiment was completely randomized with five treatments and four replications and a total of 400 tilapia fry were seeded (0.5 ± 0.2 g average weight per juvenile). Experimental fish were batch-weighed with a top loading balance (RCL-15), one-time week till the end of the experiment.

2.2. Diet Formulation

For diet formulation it was considered the minimum of protein (45%) recommended for growing fry supply with sources of fresh raw material. Thus, the crude protein (Kjeldahl nitrogen x 6.25) present in alfalfa, beetle larvae and earthworms was determined with the procedure described by Diaz et al. (2008) and following the instruction of Association of Official Analytical Chemists methods [5] (Table 1). Also, the proportion of crude fat on each ingredient was determined by extraction with hydrophobic solvents, ash was estimated by combustion of the starting material, crude fiber by the gravimetric method and moisture by drying at 105°C for 24. h [5].

Table 1. Composition calculated of four sources of protein given to tilapia fry grown in aquaponics system. Winter, 2014.

Characteristic	Commercial food	Live worm	Dehydrated alfalfa leaf	Beetle larvae
Moisture	10.0*	88.7	5.0	58.1
Crude protein	45.0	39.1	26.2	42.6
Crude fat	8.5	8.4	3.8	18.2
Ash	10.0	18.7	--	2.8
Fiber	4.0	1.14	27.0	--

*Values expressed in percent (%).

With the crude protein data from each ingredient, four diets were formulated with different sources of fresh raw material and percentage of inclusion according to the chemical composition of each ingredient (Table 2). The ingredients were cut to a smaller diameter than 0.51 mm and provided without pelletizing. The control was pelleted commercial diet with 45% of crude protein.

Table 2. Contents of crude protein (CP) in diets evaluated in tilapia fry grown in aquaponic systems. Winter, 2014.

Treat	Ingredient and percentage of inclusion	Crude protein (%)
Diet 1	60% commercial food + 40% live worm.	43
Diet 2	50% commercial food + 40% live worm +10% Dehydrated alfalfa leaf.	41
Diet 3	40% commercial food + 25% live worm +25% Dehydrated alfalfa leaf + 10% beetle larvae.	41.2
Diet 4	60% commercial food + 40% beetle larvae.	44.1
Control	100% commercial food.	45

2.3. Fish Maintenance

The feed rate was calculated based on the 7% of the total biomass and the amount of food was offered twice daily in 2 equal portions at 9:00 am and 13:00 p. m. The quantity of feed was adjusted according to the weekly weight gain and the experiment lasted for 28. days. Water temperature, pH and conductivity were measured using calibrated combined meter (Combo, Hanna). A pond lab oxygen test kit was used to measure dissolved oxygen. Nitrite was monitored using a nitrite test kit, and ammonia was detected using ammonium test kit.

2.4. Measurements and Statistical Analysis

Data obtained from the feeding trials were analyzed using a non-parametric one-way ANOVA and rank mean comparisons by the Kruskal-Wallis test (*P* < 0.05) [23] with the Statistical Analysis System software [35]. Fry growth was determined by the specific growth rate (SGR) with the Fulton index (K) was:

$$K = 100 \left(\frac{W}{L^3} \right) \tag{1}$$

Where, W is corporal weight (g) of fish; L is length (cm) of fish [16].

The feed conversion rate (FCR), percentage of weight gained (GW%) and the survival rate (SR) were calculated as described by Alhadhrami and Yousif (1994) and Cifuentes et al. (2012).

Equations:

$$FCR = \frac{\text{amount of food supplied}}{\text{weight increase of the population}} \tag{2}$$

$$GW\% = \frac{100 (\text{final weight} - \text{initial weight})}{\text{initial weight}} \tag{3}$$

$$SR = \frac{\text{Final number of organisms}}{\text{Inicial number of organisms}} \times 100 \tag{4}$$

The cost incidence was determinate based on the prevailing market price (US\$) of each raw material and the quantity that was required to make the different diets, the cost for 1. kg of each diet was calculated as follow:

$$CI = FCR \times \text{Cost per kg feed} \tag{5}$$

Where, CI is the cost of feed; FCR is the feed conversion rate.

3. Results

3.1. Chemical Analysis of the Ingredients

The results of proximate analysis of the ingredients used in the formulation of diets showed lower protein content in earthworms (39%) and beetle larvae (42.6%), with 6 and 2.4% less than commercial food (45%), while alfalfa leaf had the lowest protein content (Table 2). The moisture content was higher in the ingredients of animal origin. The composition of the live earthworm is similar to the commercial diets, followed by the beetle larva.

3.2. Growth Analysis

The specific growth rate (SGR) determined by the Fulton factor, showed that the relationship between weight and size of the fry was better with D1 and D2, while the D3 and

control was lower (Table 3); this shows that the fish were exposed to periods of stress, which can be attributed to water temperature during the experiment as it was below the optimum threshold of 26-28°C [19]. Cifuentes et al. (2012) and Sánchez-Cardozo et al. (2014) reported that values less than one mean stress on the fish and if the value is higher than one, the fish are in a period of prosperity, thus, suggesting that when Fulton index is as close to the unity, the fish growing conditions are optimal.

Evolution in weight gain show significant differences ($P < 0.05$) among diets for the fry growth (Table 3; Fig.1). The increase of protein content and ingredient in diets did not correlate with fry weight gain (Table 3). The D2 showed better weight gain and it was the best among the other treatments during the 28. days of experiment. The D2 (41% protein) reported a final weight of 1.51 g and a 196% weight gain, slightly better than control and D4 (45 and 44.1% protein, resp.), which reported a 1.40 and 1.26 g final weight, respectively (Table 3).

Table 3. Growth performance and physical indicators of Nile tilapia fry (initial weight of 0.51 ± 0.03 g) grown in aquaponics systems and fed with different ingredients and protein levels.

Treat	SGR	FW (gr)	GW (%)	Size (cm)
Diet 1	$1.80 \pm 0.10a$	$0.88 \pm 0.03b$	$72.5 \pm 4.28c$	$3.33 \pm 0.26a$
Diet 2	$1.55 \pm 0.07a$	$1.51 \pm 0.20a$	$196.1 \pm 5.05a$	$3.50 \pm 0.27a$
Diet 3	$0.72 \pm 0.04c$	$1.10 \pm 0.06ba$	$115.7 \pm 3.92c$	$3.58 \pm 0.23a$
Diet 4	$1.11 \pm 0.04b$	$1.26 \pm 0.06ba$	$147.1 \pm 10.68b$	$3.92 \pm 0.15a$
Control	$0.63 \pm 0.10c$	$1.40 \pm 0.13ba$	$174.5 \pm 3.61ba$	$3.75 \pm 0.17a$

The means \pm standard error within a column, followed by the same letter is not significantly different determined by one-way analysis of variance and Kruskal-Wallis test ($P \leq 0.05$). n=4

SGR = Specific growth rate FW = Final weight WG = Weight gain

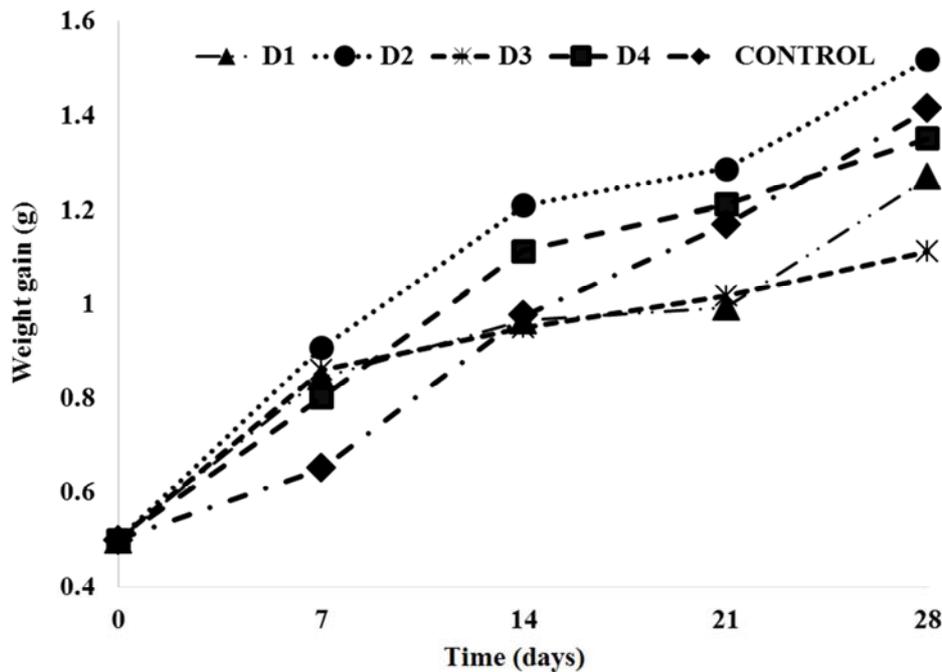


Figure 1. Final weight gain (g) of fry fed with five diets in aquaponics system for 28. days. Polynomial model for D1: $y = 0.0E-07x^3 - 0.01x^2 + 0.08x + 0.26$ ($n = 4, P < 0.001, R^2 = 0.86$). D2: $y = 2.60E-04x^3 - 7.96E-03x^2 + 1.12E-02x + 2.55E-0.1$ ($n = 4, P < 0.001, R^2 = 0.95$). D3: $y = 2.07E-04x^3 - 6.94E-03x^2 + 9.49E-02x + 2.55E-0.1$ ($n = 4, P < 0.001, R^2 = 0.89$). D4: $y = 2.26E-04x^3 - 7.35E-03x^2 + 1.03E-02x + 2.55E-0.1$ ($n = 4, P < 0.001, R^2 = 0.92$). Control: $y = 2.36E-04x^3 - 7.45E-03x^2 + 1.07E-02x + 2.55E-0.1$ ($n = 4, P < 0.001, R^2 = 0.95$). "x" as days of growth; "y" as weight gain (g).

Fry size did not show significant differences ($P < 0.05\%$), the fry showed similar behavior in all diets and they reached 3.3 to 3.9 cm in length (Table 3).

The food conversion ratio (FCR) showed significant differences ($P < 0.05\%$), the D3 with 60% substitution of the commercial feed for live earthworms (25%) plus alfalfa leaf (25%) and larva (10%), showed the best ratio of food consumed-weight gain compared to the other diets, including the control, while the D2 showed the lowest food conversion (Table 4).

Table 4. Feed and survival indicators in fry fed for 28. days with five diets with different protein inclusion percentage. Winter, 2014.

Treat	FCR	SR	Cost kg (\$)*
Diet 1	2.5 ± 0.20ba	90.0 ± 1.82ba	26.7
Diet 2	2.0 ± 0.11b	95.2 ± 1.29ba	21.8
Diet 3	2.7 ± 0.02a	95.2 ± 1.19ba	28.1
Diet 4	2.5 ± 0.16ba	85.0 ± 3.84b	24.7
Control	2.1 ± 0.10ba	95.3 ± 1.76a	28.9

The means ± standard error within a column, followed by the same letter is not significantly different determined by one-way analysis of variance and Kruskal-Wallis test ($P \leq 0.05$). n=4

FCR = Feed conversion rate SR = Survival rate * Mexican currency

The survival was 95% in D2, D3 and control, which was slightly better than D1 (90%) and D4 (85%), showing that the inclusion of fresh raw material is likely to cause fry health risks, as the physicochemical parameters are altered by physicochemical changes in the water.

3.3. Cost analysis

The cost in developing diets showed a cost per kg of US \$ 1.11 for D2, while the cost of commercial feed (control) was US \$ 1.47 (Table 4). The addition of fresh raw material such as earthworm or beetle larvae, dehydrated vegetable raw material such as alfalfa up to 40% of the ration, showed a behavior similar to the control and it helps reduce the cost of food.

4. Discussion

Various sources of plant and animal origin have been evaluated as a partial or complete substitutes of protein in Tilapia diets [14, 24, 1]. Previous studies have shown that a proper balance of protein in food ingredients may increase metabolic energy and thus improve fish growth [38, 34, 2], which in this study it was considered with different inclusion percentages by ingredient of plant or animal origin, and covering the nutritional requirements of the fry. Likewise, it has been reported that levels above 40% for tilapia fry protein does not confer any particular advantage in terms of growth [39], whereas Gunasekara *et al.* (1995) observed that feeding tilapia with protein levels of 32 and 40% with ingredients of plant origin stimulate growth and accelerate maturation in comparison to the food with 10, 17 and 25% crude protein levels.

Among all diets tested, the overall performance of the final weight gain was found to be lower than that reported by other authors [21]. Gómez-Ponce *et al.* (2011) after six months of experiments reached a daily weight gain of 0.47 g in fry of 1.0 g initial weight. Moreno *et al.* (2000) after evaluating in tilapia hybrids the nutritional effect of three diets made from commercial food mixed in different proportions with orange peel flour, achieved the best weight gain with a diet of 80% commercial food added with 20% orange peel flour. In other studies, with tilapia fry grown during 100 days, a daily weight gain of 1.6 to 2.8 g of tilapia with initial weight of 0.1 and 0.8 g were reported [36] and 2.6 g gain weight for tilapia with 0.09 g of initial weight [22]. Some other authors report higher growth rates, between 2.6 and 5.0 g, for fish from 3.7 to 4.0 g [38]. On the contrary, Anderson *et al.* (1984) obtained lower values, between 1.5 and 2.5 g, for 2.0 g fish. The results of this study corroborate those reported by Luna-Figueroa *et al.* (2010) who observed a rate of absolute growth of 0.042 g day⁻¹ for creole tilapia fry in captivity, and comparing the addition of live food versus commercial feed during 60. days. The discrepancy in results reported by various authors is probably due to the different eco-environmental and physicochemical conditions, fish density, protein content, maximum feed rate offered, under the assumption that these factors are crucial for growth as they are related to the algae and microorganism's growth dynamic, which in commercial production systems represent up to 60% of the food supply [7]. Similar results were reported by Ruiz *et al.* (2009) when evaluating the development of tilapia fed with a commercial concentrate with 45% crude protein for six weeks to obtain a size between 3.4 and 4.0 cm and weight gain of 0.6 ± 0.1 g, which are results below the ones obtained in this study.

The results obtained in this study on rate conversion were better than those reported by Poot-Delgado *et al.* (2009) who evaluated three commercial feed and they found a conversion rate of 1.16 to 1.18, also their results were consistent with those mentioned by Corella *et al.* (2002) who included 20% flour of dehydrated coffee pulp in the commercial diet obtaining a 1.8 TCA without affecting the weight gain-food consumed ratio. Meanwhile, Chareontespravit and Jiwyam (2001) observed that the inclusion of Wolffia flour partially replacing 15% of the commercial feed results in values of 2.0, without affecting the growth indicators, whereas El-Sayed (1994) obtained values of 2.69 by using protein obtained from Spirulina flour, and he also reported that when replacing 100% of the commercial feed by Spirulina maxima flour in diets for Pacific goldfish (*Rhabdosargus sarba*), it does not meet the nutritional requirements, regarding the essential amino acids supply, necessary for the proper development of this species. In studies previous it was reported an increase of 0.1 g day⁻¹ with the inclusion of chironomidae larvae (nonbiting midges' larvae) at a rate of 90% and the rest with rice flour, resulting in an economical benefit for small producers. The results show that the addition of living and/or processed protein sources should be

less than 50%, since its efficiency is determined by the degree of acceptance, food appearance (size and shape), texture and attraction (taste and odor) that affect the level of consumption, because if this percentage is exceeded it can cause unfavorable results in the development and growth of tilapia fry.

For other hand, the evaluation of parameters of water quality in each tank showed a pH between 7.1 and 7.6 on average; a maximum temperature of 22°C and 11°C as the minimum, dissolved oxygen between 5.8 and 6.5 mg L⁻¹, while N-NO₃⁻ (30-100 mg L⁻¹) and N-NH⁺ (0.6-1.0 mg L⁻¹) values, were below the maximum permitted levels [19]. The results showed that the protein content on diets should be low (<40%) in order to keep the amount of nitrogen released into water as ammonium below the tolerance limit [37]. On the other hand, the difference in mortality among treatments was possibly due to the handling when the morpho-metric samplings were carried out, because the record showed death after sampling; besides, the use of live protein in large quantities for fry feeding is not recommended because of the danger of toxicity, which could only then be inhibited by heat treatment which would raise its cost.

In conclusion the Tilapia tolerates different feed rates with different sources of fresh raw material without having adverse effect on its development except in the juvenile stage. The results show that the presence of fresh raw material into the fry diet could be incorporated in the diet of fry growth with cost benefit. This is the first study on feed diets in tilapia's fry growth under aquaponics system.

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