

EFFECT OF DIETARY PROTEIN LEVELS AND SEX ON GROWTH PERFORMANCE, FEED UTILIZATION AND BODY COMPOSITION OF NILE TILAPIA (*OREOCHROMIS NILOTICUS*) FRY CULTURED IN HAPS.

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(Received 5/9/2017, accepted 24/10/2017)

SUMMARY

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he effect of dietary protein levels (35%, 40% and 45%) on growth, survival, feed utilization and body composition of mono sex and mixed sex of Nile tilapia; *Oreochromis niloticus* L. fry was investigated. Fish were fed frequently to satiation for 7 days a week for 56 days. Results showed that fish growth was significantly affected by protein level. The highest growth performance of fry were obtained with 45% protein diet and the poorest one was obtained with 35% protein diet in both mono and mixed sex Nile tilapia, *Oreochromis niloticus* fry. Mono sex tilapia were reared in hap in earthen pond showed the highest weight gain, specific growth rate, feed conversion ratio, feed efficiency and protein efficiency ratio, while mixed-sex tilapia showed the lowest weight gain, specific growth rate, feed conversion ratio, feed efficiency and protein efficiency ratio. In conclusion, it was found that 45% crude protein had the highest growth performance parameters, feed utilization and economical evaluation for mono sex Nile tilapia (*O. niloticus*) fry under this experimental condition.

Keywords: Nile tilapia fry, growth, protein levels, feed conversion, body composition, economical evaluation, mono sex tilapia.

INTRODUCTION

Tilapias are widely recognized as one of the most important groups of finfish for culture purposes in a wide range of freshwater culture systems, from simple waste-fed fish to intensively stocked and managed culture systems (Fitzsimmons, 2005). Attempts have also been made in some developed countries to grow them in full-strength sea water. In Egypt, the Nile tilapia (*Oreochromis niloticus*) is the major farmed species and constitutes over eighty percent of aquaculture production in the country and it occurs in several rivers, and in natural as well as man-made lakes (FAO, 2011). Tilapia grows and reproduces in a wide range of environmental conditions and tolerates stress induced by handling (Tsadik and Bart, 2007). However, this efficiency of reproduction in tilapia has undesirable consequences. Problems common for many Nile tilapia culture systems are the reduction of growth rates at the onset of sexual maturity and precocious and excessive reproduction, leading to various sizes of small fish production (Lèveque, 2002).

The importance of monosex tilapias has been established in many commercial contexts but there are relatively few published studies comparing the growth performance of androgen treated monosex Nile tilapia with mixed-sex tilapia, under different culture system (Mair *et al.*, 1995). Moreover, sex-specific differences in growth were significant in *O. niloticus* where males grow significantly faster, larger and more uniform in size than females (Bwanika *et al.*, 2007). The desirability of monosex male populations of tilapia is well established for increased production potential and low management requirements (El-Sayed, 2006).

Protein is the main constituent of the fish body thus sufficient dietary supply is needed for optimum growth. Protein is the most expensive macronutrient in fish diet (Pillay, 1990). So, the amount of protein in the diet should be just enough for fish growth where the excess protein in fish diets may be wasteful and cause diets to be unnecessarily expensive (Ahmad, 2000). Reducing feeding costs could be a key factor for successful development of aquaculture. Protein requirements for optimum growth of the fish seem to be affected by numerous factors such as temperature, salinity, fish age and size, etc. (Cowey, 1976). Most studies are confined to fry and young tilapia, although the supplementary feed is used during grow-out phase. Furthermore, understanding the protein requirement during the grow-out period is an important thing in fish culture management. Realization of the optimum protein level for cultured fish would help to reduce the costs and maximize the feed conversion efficiency (Chiu *et al.*, 1987). Increase in yields can result from the development and adoption of new technologies and improved farming operations (Coelli, 1995). Hence, the propagation potentiality of sex-reversed tilapia under various traditional and nontraditional culture practices must be clearly documented. Therefore, the objective of this study was to assess the optimum protein level leading to optimum growth and feed utilization of mono and mixed sex of Nile tilapia (*Oreochromis niloticus* L) fry.

MATERIALS AND METHODS

The experiment: - The experiment was conducted at Ramses private fish farm, Sharkya Governorate, Egypt. The experiment aimed to investigate the effect of protein levels 35, 40, and 45% diet for mono and mixed sex (*O. niloticus*) fry.

Experimental unit:-

Fish were stocked in 250 fry /m³ hapa (2x 5.00 x 1m) randomly divided into six equal experimental groups (250 fry /replicate, three replicate hapas) in earthen ponds . The hapa were supplied all day with air blowers and located in earthen pond sized (acre). Water temperature was maintained at (28 ±1°C). The part of water hapa was exchanged with freshwater 10%/day.

Experimental Fish:

Four thousand and five hundred fry monosex and mixed sex of Nile tilapia (*O. niloticus*) with average initial body weight of 0.02 ± 0.01 g were obtained from Ramses Fish Hatchery, Sharkya Governorate, Egypt. Fish were homogenous in body weights and apparently healthy. Fish were acclimated to farm conditions for 2 weeks before the experiment started.

Experimental Diets:-

Three diets were formulated from practical ingredients (Table 1) where the protein levels were 35, 40, 45% respectively and gross energy 450 Kcal/100g. The experimental diets were prepared by individually weighing of each component thoroughly mixing the mineral, vitamins and additives with corn or wheat bran. This mixture was added to the components together with oil. Water was added until the mixture became suitable for making fine granules. The wet mixture was passed through CBM granule machine with fin 0.2 mm diameter. The produced pellets were dried at room temperature then kept until experimental start. The composition and proximate analysis of the experimental diets are presented in (Table 1). The fish were hand-fed to satiation 15 times /day throughout the experimental period 56 days.

Experimental Methodology

At the beginning and at the end of experiment 5 fish sample was taken to determine chemical analysis of body composition according to AOAC, 1995. The tested diets were analyzed for crud protein (CP %), ether extract (EE %), crude fiber (CF %), ash (%) and moisture. While whole body composition of fish samples were also analyzed except crud fiber (CF %) according to the procedures described by standard AOAC (1995). The nitrogen free-extract (NFE %) was calculated by differences. Fish were weighed every two weeks and the average of 4 weeks was determined by UWE MJW-300 balance to the nearest 0.01g. All the analytical parameters were based on the dry matter (DM) contents. Feeding rates were monitored closely and adjusted accordingly upon observation of satiation point . the fish were hand fed to satiation 15 times/ day throughout the experimental period 56 days.

Table (1). Composition and proximate analysis of experimental diets.

Ingredient	Diets (% protein)		
	35	40	45
Fish meal (70 %CP)	10.00	10.00	16.00
Meat and bone meal	5.00	10.00	10.00
Corn	19.0	7.0	–
Wheat flour	10.00	11.0	13.0
Wheat bran	3.0	–	–
Soya meal (44 %CP)	47.00	56.00	57.00
Fish oil	2.00	2.00	2.00
Limestone	2.0	2.0	--
Fish vitamin and mineral mix ¹	2.0	2.00	2.00
Proximate chemical composition (%)			
Dry matter	89.2	89.4	89.4
Crude protein	35.0	40.0	45.0
Crude fat	3.96	4.0	4.0
Ash	8.74	9.1	9.1
NFE ²	52.30	46.90	41.90
GE(kcal/100g) ³	449.60	456.09	464.78

1) Each Kg vitamin & mineral mixture premix contained :Vitamin A, 4.8 million IU; D3, 0.8 million IU; E, 4 g; K, 0.8 g; B1, 0.4 g; Riboflavin, 1.6 g; B6, 0.6 g ; B12, 4 mg; Pantothenic acid, 4 g; Nicotinic acid, 8 g; Folic acid, 0.4 g; Biotin,20 mg ;choline chloride 200 mg ; Mn, 22 g; Zn, 22 g; Fe, 12 g; Cu, 4 g; I, 0.4 g ; Selenium, 0.4 g and Co, 4.8 mg.

2) Nitrogen Free Extract = 100 – (%Protein + %Fat + %Fiber + %Ash).

3) Gross Energy based on protein (5.65 Kcal/g), fat (9.45 Kcal/g) and carbohydrate (4.11Kcal/g). According to (NRC, 2011).

Feed conversion ratio: -

The feed conversion ratio (FCR) is expressed as the proportion of dry food fed required per unit live weight gain of fish.

$$\text{FCR} = \text{Feed intake (g/fish)} / \text{wet weight gain (g)}$$

Feed Intake (FI):- Amount of consumed feed per period

$$\text{FI} = \text{Total feed intake in hapa/ Number of fish}$$

$$\text{Protein Efficiency Ratio (PER)} = \text{weight gain (g)} / \text{CP intake (g)}$$

$$\text{Feed Efficiency (FE)} = \text{Fed intake(g)} / \text{wet weight gain (g)}$$

Growth performance parameters: -

The growth performance parameters were calculated according to the following equations.

$$\text{Weight gain (WG)} = \text{final weight (g)} - \text{initial weight (g)}$$

The specific growth rate (SGR, %/d) was calculated by the following equation.

$$\text{SGR} = \{ \text{Ln final mean body weight} - \text{Ln initial mean body weight} / \text{time intervals (days)} \} \times 100.$$

Survival rate (SR, %): -

$$\text{SR (\%)} = (\text{Number of fish survived at the end of the experiment} / \text{Number of fish stocked at the start of the experiment}) \times 100$$

Feed and protein utilization parameters:-

Feed and protein utilization parameters are calculated according to the following equations.

Water quality parameters: -

Water temperature and dissolved oxygen were measured by metteler Toledo, model 128.s/No1242. Other water quality including pH and ammonia were measured every two days by pH meter (Orion model 720 A,s/no 13062) and ammonia meter by Hanna ammonia meter. The averages of water quality parameters are presented in Table (2).

Economical Evaluation

The cost of feed to produce unit biomass of fish was estimated by a simple economic analysis. The estimation was based on local retail sale market price of all the dietary ingredients at the time of the study (2016).

Cost /kg diet (LE) = Cost per Kg diet .

Consumed feed to produce 1kg fish (kg) = Feed intake per fish per period/ final weight per fish (Kg)

Feed cost per kg fresh fish (LE) =Step 1X step 2

Relative % of feed cost/ kg fish =Respective figures for step 3/ highest figure in this step

Feed cost /1Kg gain (LE) =Feed intake per Kg gain X step 1

Relative % of feed cost of Kg gain =Respective figures for step 5/ highest figure in this step

Cost of 1 kg ingredients used were 9.90, 11.30, and 12.75. L.E. for 35, 40 and 45% crude protein respectively for Egypt Feed Ingredients Price.

Statistical analysis:

Data was statistically analyzed by analysis of variance as one-way ANOVA using the general linear model procedure of Statistical Analysis System (SAS Institute, 1998). Differences among means were evaluated using Duncan's Multiple Range Test (Duncan, 1955).

RESULTS AND DISCUSSION

Water quality

Table (2) outlined water quality criteria of monosex and mixed sex as effected by protein levels which reared in hapa in earthen ponds. Water temperature and other water quality parameter measurements are given in Table (2). As no apparent difference in water quality parameters were found among the treatments because all hapas in the same water in earthen ponds, the data for each experiment for the whole experimental period were pooled. Water quality was found to be within the acceptable range for tilapia growth (Stickney, 1979). The water quality data for dissolved oxygen, temperature, pH and ammonia measured during the study period were all within the optimum range for rearing *O. niloticus* (Xu *et al.*, 2005; Azaza *et al.*, 2008).

Table (2). Average physico-chemical parameters of water quality throughout experimental period 56 days (Means ± SE).

Parameter	Temperature	Dissolved Oxygen	Ammonia	pH
Measurement range	28 ±1°C	5.7 ± 0.5 mg/l	0.007-0.0028 mg/l	7.7± 0.7

Growth performance and feed utilization

Table (3) presents the final results for the effect of protein levels on growth performance, feed utilization and survival rate for mono and mixed sex of Nile tilapia fry. The highest growth performance parameters (body weight, body weight gain, specific growth rate) were recorded in group for monsex fry fed on diet contains 45% crude protein. The lowest growth performance parameters were recorded for group of mixed sex Nile tilapia fry fed the diet contains 35% crude protein. The highest feed utilization

parameters (PER and feed efficiency ratio) were recorded in group of mono sex fry fed diet containing 45% crude protein and the lowest group of mixed sex fry fed on diet contains 35% crude protein. The favorite FCR was recorded in monosex fry fed diet containing 45% crude protein and the worst FCR for group of mixed sex fry fed on 35% crude protein.

The highest averages of final body weight, weight was found in monosex followed by mixed sex groups (Table 3). Increase in individual growth of Nile tilapia during monosex culture was observed in different studies (Mair *et al.*, 1995; Dan and Little, 2000; Little *et al.*, 2003). Faster growth of monosex tilapia has been related to the lack of energy expenditure in egg production and mouth brooding by females and lower energy expenditure on courtship by males (Dan and Little, 2000; Tran-Duy *et al.*, 2008).

Mono sex fry gained the highest final body weight and this can be attributed to the anabolic effect of hormone to induce sex reversal in farmed tilapias. In this respect, some studies reported that the anabolic effect of hormone showed an increase in individual growth of tilapia (Mair *et al.*, 1995 and Dan and Little, 2000). Other studies reported that the higher mean weights could be attributed to the improvement of food conversion efficiency of sex-reversed fry of *Oreochromis niloticus* (Dang *et al.*, 2013). Increase in individual growth of Nile tilapia during monosex culture was observed in different studies (Dan and Little, 2000; Little *et al.*, 2003). Al Hafedh (1999) found that, SGR of Nile tilapia was significantly increased as diet protein level increased from 25-45% (with increment of 5%). Also, Ogunji and Wirth (2000) with the same fish specie found that, SGR increased with increasing dietary protein level from 7.3 to 44.24%.

Feed intake increased significantly with increasing dietary protein level only ($P < 0.01$), in agreement with Khattab *et al.*, (2000). Generally FI was significantly lower ($P < 0.05$) in monosex than mixed sex (Table 3). The obtained results for male monosex fish means that these fish used less feed to gain more weight when compared with the mixed sex fry (Table 3). These results are in close agreement with Hossain *et al.*, (2005) who found FCR of 1.58 and 1.64 for male monosex and mixed sex *O. niloticus*, respectively, when fed on formulated diet. Siddik *et al.* (2014) also reported a FCR of 1.71 and 1.78 for male monosex and mixed sex, respectively. Al Hafedh (1999) came to the same results that FCR of Nile tilapia was improved with increasing dietary protein level.

As described in Table (3), averages PER were significantly ($P < 0.05$) increased with increasing protein levels in monosex fry, and were found to be negatively correlated with dietary protein level. Dabrowski (1979) found that the relationship between dietary protein and PER differs from species to species. Jauncey (1982) and De Silva *et al.*, (1989) reported that FCR and PER decreasing with increasing dietary protein content. Siddiqui *et al.*, (1988) found that, PER decreased with increasing diet protein level from 20 to 50% for Nile tilapia. Also, El-Ebiary (1994) found that, increasing dietary protein level up to 35% and feeding rate up to 4% decreased the values of nutrients (protein and energy) utilization in Nile tilapia and its hybrid. In many fish including tilapia, it has been reported that the protein requirement of the fish decreased with increasing size (El-Saidy and Gaber 2005). There was a general decrease in FCR and increase in PER for monosex fish than the mixed-sex ones. Such observation may be related to the fact that FCR decreases while PER increases with increased feeding rate (Pechsiri and Yakupitiyage, 2005).

In general, survival rate was high for all experimental groups (Table 3). There were no significant differences ($P > 0.05$) between and within experimental groups of mono and mixedsex of Nile tilapia (*O. niloticus*) fry fed the experimental diets. The survival rates were 96, 96, 98, 96, 96 and 96% for T1, T2, T3, T4, T5 and T6, respectively. El-Sayed and Teshima (1992) demonstrated that *O. niloticus* fry require 45% protein and 400 kcal/100 g for maximum growth when reared under laboratory conditions, in agreement with the present study.

These variations appear to be the result of different experimental conditions, which include fish species, size and age of fish, stocking density, protein quality, hygiene and environmental conditions, particularly temperature, which has been found to influence dietary protein requirement in tilapia (Jauncey and Ross, 1982) and in some other fish species (Wilson, 1989). The ratio of protein to energy in the diet has also been reported to influence the growth and feed conversion efficiency in *O. niloticus* fry (El-Sayed and Teshima, 1992).

Effect of protein level on body composition

Results of body composition of each mono and mixed sex Nile tilapia fry fed diets containing 35, 40 and 45% crude protein are summarized in Table (4). Moisture, protein and lipid contents in whole fish

body were affected by fish sex and dietary protein level. The interaction of both factors affects body chemical analysis. Generally, the moisture content in monosex tilapia was significantly lower ($P < 0.05$) than the mixed-sex fish. But, Killian and Kohler (1991) recorded significant increase in moisture content in 17 α -Methytestosterone treated Coho salmon. The body protein content was directly proportionally affected by protein levels ($P < 0.05$). Fish fed 35% protein diet had lower content of protein and higher content of lipid than fish fed 40% or 45% protein diets. These results are similar to that obtained by Wee and Tuan (1988) and Al Hafedh (1999). Ash content in whole fish body was unaffected by dietary protein levels at all both fish sex of fish fry. The body protein percentage on a wet weight basis is mainly affected by the body weight in salmonids (Shearer, 1994). Similar observations have been noticed in Nile tilapia also where body protein content increases with increasing wet weight (Abdelghany and Mohammed, 2002). This explains the higher protein content of the monosex fish than the corresponding mixed-sex fish. Ash content was unaffected by dietary protein level but affected by fish size. Khattab et al., (2000) reported that ash content was unaffected by protein level in Nile tilapia collected from Abbassa fishponds.

Table (3). Effect of protein levels on growth performance, feed utilization and survival rate of mono and mixed sex Nile tilapia (*O. niloticus*) fry throughout the experimental period (56 days).

Parameter	Mono sex			Mixed sex		
	T1 (35)	T2 (40)	T3 (45)	T4 (35)	T5(40)	T6 (45)
Average Initial weight(g)	0.2 ± 0.01	0.2 ±0.01	0.2 ± 0.01	0.2 ±0.01	0.2 ±0.14	0.2 ± 0.01
Average Final weight (g).	4.80 ±0.10 ^e	5.80 ±0.10 ^c	10.20 ±0.10 ^a	4.60 ±0.10 ^e	5.10 ±0.10 ^d	9.60 ±0.10 ^b
Average Weight gain (g)	4.60 ±0.40 ^e	5.60 ±0.60 ^c	10.00 ±0.40 ^a	4.40 ±0.20 ^e	4.90 ±0.40 ^d	9.40 ±0.20 ^b
Specific Growth Rate (SGR) (%/day)	5.68 ±0.10 ^e	6.01 ±0.10 ^c	7.02 ±0.10 ^a	5.60 ±0.10 ^e	5.78 ±0.10 ^d	6.91 ±0.10 ^b
Feed Intake (FI) (g)	7.30 ±1.0f	7.70 ±0.10 ^d	10.50 ±0.10 ^b	9.80 ±0.10 ^c	8.70 ±0.10 ^c	11.50 ±0.10 ^a
Feed Conversion Ratio (FCR)(g/g)	1.59 ±0.10 ^e	1.37 ±0.10 ^e	1.05 ±0.10 ^e	2.23 ±0.10 ^e	1.77 ±0.10 ^e	1.22± 0.10 ^e
Feed Efficiency (FE)(g/g)	0.63 ±0.10 ^d	0.73 ±0.10 ^c	0.95± 0.10 ^a	0.45 ±0.10 ^e	0.56 ±0.10 ^e	0.82 ±0.10 ^b
protein efficiency Ratio (PER)	8.36 ±0.10 ^b	10.37 ±0.10 ^b	21.7 ±0.10 ^a	5.64 ±0.10 ^d	7.00 ±0.10 ^c	17.40 ±0.10 ^b
Survival rate %	96.00 ±0.10 ^l	96.00 ±0.10	98.00 ±0.10	96.00 ±0.10	96.00 ±0.10	96.00 ±0.10

* Means in the same row having the same superscript letter are not significantly different ($P > 0.05$).

Table (4). Effect of protein levels on body composition ((% on wet basis Mean ± SE) of monosex and mixed sex of Nile tilapia (*O. niloticus*) fry throughout the experimental period (56 days).

Content	Mono sex			Mixed sex		
	T1(35%)	T2(40%)	T3(45%)	T4(35%)	T5(40%)	T6(45%)
Moisture	71.6 ± 0.14 ^b	71.0 ± 0.56 ^b	70.46 ± 0.04 ^c	72.30 ± 1.5 ^a	72.20 ± 0.9 ^a	71.15 ± 1.4 ^b
Crude protein	15.44 ± 0.10 ^d	16.20 ± 0.12 ^c	17.10 ± 0.14 ^a	14.40± 0.14 ^e	15.00± 0.14 ^d	16.40± 0.14 ^b
Crude Lipid	6.86± 0.15 ^a	6.60 ± 0.27 ^b	6.24 ± 0.21 ^c	7.20± 0.15 ^a	6.60 ± 0.27 ^b	6.25 ± 0.21 ^c
Ash	6.10± 0.15	6.20. ± 0.15	6.20± 0.15	6.10± 0.15	6.20. ± 0.15	6.20± 0.15

* Means in the same row having the same superscript letter are not significantly different ($P > 0.05$).

Economical evaluation

The cost of all topics involved in the present investigation were constant, therefore recalculation of the economical evaluation of the tested diets based on the cost of feed , cost of one Kg weight gain and the relative of feed cost for the biggest group comparable to the other fish groups (Table 5). Feed costs (L.E) were the highest for T3 and T6 (45% crude protein) and generally decreased with decreasing crude protein levels in the diets (Table 5). The lowest feed cost /1Kg gain (13.39 LE) in T3 and relative % of feed cost of Kg gain (60.64%) and the highest feed cost/kg gain was recorded in T5 (22.08L.E) in and relative % of feed cost of Kg gain (100%). The reduction of feed cost/Kg was easily observed for the feed cost per Kg weight gain which decreased in mono sex than mixed sex. These results indicate that T3 (45% crude protein) for mono sex Nile tilapia (*O. niloticus*) fry is the best in terms of economic analysis as noted in (Table 5).

CONCLUSION

It was found that 45% crude protein had the highest growth performance parameters, feed utilization and economic analysis for monosex Nile tilapia (*O. niloticus*) fry under this experimental condition.

Table (5). Effect of protein levels economical evaluation of mono and mixed sex of Nile tilapia (*O. niloticus*) fry throughout the experimental period (56 days).

Parameter	Mono-Sex			Mixed Sex		
	T1 (35%)	T2 (40%)	T3 (45%)	T4 (35%)	T5(40%)	T6 (45%)
Cost of Kg diet	9.9	11.3	12.75	9.9	11.3	12.75
Feed intake (FI) (g)	7.30	7.70	10.50	9.80	8.70	11.50
Feed cost/ kg fish	72.27	87.01	133.87	97.02	98.31	146.62
Relative to feed cost %	49.00	59.00	91.00	66.00	67.00	100
(FCR)(g/g)	1.59	1.37	1.05	2.23	1.77	1.22
Feed cost /1Kg gain(L.E)	15.74	15.48	13.39	22.07	22.08	15.55
Relative % of feed cost/ kg gain fish	71.28	70.10	60.64	89.25	100	70.42

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تأثير مستويات البروتين والجنس على أداء النمو والاستفادة من الغذاء وتركيب الجسم ليرقات البلطي النيلي المستزرعة في شباك

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تأثير مستوى بروتين العليقة (35،40،45%) على النمو وكفاءة الاستفادة من الغذاء وتركيب الجسم ليرقات البلطي النيلي وحيد وخليط الجنس. تم تغذية الاسماك لدرجة الشبع 7 أيام في الاسبوع لمدة 56 يوما. أظهرت النتائج ان نمو الاسماك تأثر معنويا بمستوى البروتين والحصول على اعلى معدل نمو عند تغذية اليرقات على عليقة تحتوى على 45% بروتين خام و اقل معدل نمو عند التغذية على عليقة تحتوى على 35% بروتين خام لكل من يرقات البلطي وحيد وخليط الجنس. أظهرت النتائج أن البلطي وحيد الجنس المستزرع في شباك بالأحواض الترابية أعطت أعلى زيادة في الوزن و معدل النمو النسبي و معامل التحويل الغذائي وكفاءة الاستفادة من الغذاء والبروتين و اقل تكلفة غذاء بينما البلطي خليط الجنس لوحظ اقل في معدل النمو ومعدل النمو النسبي و معامل التحويل الغذائي وكفاءة الاستفادة من الغذاء والبروتين.

والخلاصة أن تغذية يرقات البلطي النيلي وحيد الجنس على عليقة تحتوى على 45% بروتين خام اعطى اعلى معدل نمو وكفاءة الاستفادة من الغذاء واكثر كفاءة اقتصادية