Egyptian Journal of Aquatic Biology & Fisheries
Zoology Department, Faculty of Science,
Ain Shams University, Cairo, Egypt.
ISSN 1110 –6131
Vol. 23(3): 483– 491(2019)
www.ejabf.journals.ekb.eg

Influence of dietary protein level and feed inputs on growth and feeding performance of the Nile tilapia under biofloc conditions

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ARTICLE INFO
Article History:
Received: Aug. 28, 2019
Accepted: Sept. 15, 2019
Online: Sept. 19, 2019

Keywords:
Nile tilapia
crude protein
feeding performance
growth performance
restricted feeding
biofloc culture

ABSTRACT
The current experiment was designed to test the effect of crude protein content (25, 30 and 35% C.P.) as well as restricted feeding in biofloc culture on growth performance of Nile tilapia (*Oreochromis niloticus*) and water quality dynamics. The experiment included five treatments with duplicate tanks per treatment. Three normal feeding treatments and two restricted feeding treatments were adopted in the experiment. Two feed inputs were employed in the experiment: 1) The normal feeding rates which ranged 1.4 to 1.8% of fish biomass on daily basis according to season for the 25, 30 and 35% crude protein treatments, 2) The restricted feeding rate at 80% of the normal feeding rates (1.12 to 1.44% of fish biomass daily) according to season for the 30% and 35% crude protein treatments. Molasses were dissolved in water at dry feed to molasses ratio of 1:1 on a daily basis in order to develop biofloc and nourish heterotrophic bacteria. Starting with average initial weights of 13.5 to 20.3 grams/fish, Nile tilapia juveniles grew to harvest weight of 310.2 to 342.1 grams/fish. Increasing dietary protein from 30% to 35% within the biofloc treatments did not improve final body weight at harvest. Daily weight gains (0.82 g/fish/day) were slightly improved with the 30% crude protein diet compared to those of the 25% crude protein diet ($p<0.05$). Similar daily weight gains were obtained when fish were reared under the 30% restricted protein treatment (0.77 g/fish/day) compared to that of the 30% crude protein treatment. The higher crude protein content in the 35% crude protein treatment did not improve daily weight gain of Nile tilapia compared to the 30% crude protein treatment ($p>0.05$). It is recommended to feed Nile tilapia at crude protein level of 30% in order to obtain acceptable growth with economic returns. Nile tilapia reared under the 30% crude protein treatment had better PER ratio (1.96) and lower feed costs (15.11 L.E. /kilogram fish) at lower feeding rate compared to the 25% crude protein treatment.

INTRODUCTION

Biofloc technology is mainly employed to reduce water consumption required to raise aquatic animals while maintaining good water quality parameters in rearing tanks (Crab *et al*., 2012). Freshwater scarcity is a major problem in the Middle East countries including Egypt. Consequently, the use of biofloc technology conserves its use (Ogello *et al*., 2014). Desert aquaculture depends on pumping expensive water from deep wells, which limit its development. Consequently, the use of biofloc technology optimize the use of fresh water making fish culture more profitable in water scarce areas. Biofloc material rich in protein (i.e. 25-30% crude protein) is simultaneously produced in rearing units, which can spare dietary protein, reducing the cost of fish production.

Correia *et al*. (2014) stated that feed is the major driving force of intensive production systems ; consequently, it is important to optimize its use to improve profitability, maximize growth and minimize potential water quality deterioration. This can be done through optimizing crude protein level used to feed fish in biofloc system.

Azim and Little (2008) reported that biofloc is considered as a natural food for fish. Moreover, biofloc material produced in rearing tanks could be used to reduce protein requirement in feed (Burford*et al*., 2003; Ballester *et al*., 2010). As a result, biofloc technology produce low cost material rich in protein (Crab *et al*., 2007, 2009). In addition, low protein diets produced similar growth performance in shrimp parallel to that of high protein diets (Wasieleasky *et al.,* 2006). Correia *et al*. (2014) indicated that similar PER values were obtained in both low and high protein diets when fed to shrimp.

Dietary protein is the most expensive ingredient in fish feed which is required to sustain normal growth of aquatic animals. The use of rich protein diets above optimum levels, increases operating costs required to raise fish. Optimum protein levels in biofloc systems should be evaluated in terms of growth and feed performance as well as water quality parameters in culture units. Optimum levels of dietary protein not only reduce production costs, but also ameliorate water quality and growing conditions.

The aim of the present study was to evaluate growth performance and dietary efficiency for Nile tilapia cultured in intensive biofloc system using different crude protein levels and restricted feeding at 80% of normal feeding rates and determine the best biofloc conditions for tilapia growth and feed efficiency.

MATERIALS AND METHODS

The study was conducted at The Fish Culture Research Lab, Faculty of Agriculture, Cairo University, Egypt, during 2017 - 2018. A static outdoor rearing system consisting of ten rectangular concrete tanks (2.2×1.2×1.0 m) were filled with underground water and were used as rearing units for Nile tilapia. Each tank had a water volume of 2.0 m^3, with a constant water depth of 75 cm. Nile tilapia juveniles (*Oreochromis niloticus*) 13.5-20.3 grams/fish were randomly distributed among experimental tanks, with duplicate tanks per treatment. Each tank contained 24 juveniles of Nile tilapia. The experiment duration lasted for 388 days.

Experimental design:

The current experiment was designed to test the effect of crude protein percent (25, 30 and 35% C.P.) of the test diets as well as restricted feeding of Nile tilapia in biofloc culture on growth performance of Nile tilapia and water quality dynamics in concrete tanks. A total of 240 fish were randomly distributed into tanks at the rate of 24 juveniles per tank. Treatments were evenly distributed to tanks and arranged in completely randomized design (CRD). The experiment included five treatments with duplicate tanks per treatment. Two commercial floating feed inputs were employed in the experiment: The first is the normal feeding rates for the 25, 30 and 35% crude protein treatments (three normal feeding treatments) and the second is the restricted feeding rates at 80% of the normal feeding rates for the 30% and 35% crude protein treatments (two restricted feeding treatments). The normal feeding rates depended on stage of growth and season (water temperature) and were applied in the 25%, 30% and 35% crude protein treatments as follows: 1.4% of fish biomass during fall, 1.65 % of fish biomass during spring and 1.8 % of fish biomass during summer, respectively. The two restricted feeding treatments were fed at 1.12%, 1.25 and 1.44% of fish biomass during fall, spring and summer, respectively. Fish were fed once a day, six days a week during the experimental period. Water were 100% renewed once every month in each tank. Artificial aeration continued 24-hours a day using a blower (model HG 100, China – 1.5 horse power). Molasses were added to tank water along with the dry feed with a ratio of 1:1 by weight. Molasses were dissolved in water and sprinkled over water surface in each tank on a daily basis in order to develop biofloc and nourish heterotrophic bacteria.

Growth and feeding performance

Fish in each tank were weighed and counted at the beginning of the experiment and at monthly basis during the experiment. The growth and feed performance parameters were calculated as follows:

Daily weight gain (DWG g/day) = (final body weight – initial body weight)/experimental period (days).

Monthly weight gain (MWG/fish) (g\fish) = (final weight – initial weight) during each month.

Specific growth rate (SGR %)= (In Wt – In W0) ×100/t.

Where Wt: is final weight at time t, W0 initial weight, and t is the duration of experiment in days.

Feed conversion ratio (FCR) = dry weight of used feed (g) / fish weight gain (g).

Protein efficiency ratio (PER) = fish weight gain (g) / amount of protein fed (g).

Feed cost of production (EGP/kg fish) = FCR ×price per kilogram diet.

Water quality parameters

Water temperature and dissolved oxygen were measured using HANNA Instrument dissolved oxygen meter. The pH was measured on site by pH digital meter at early morning (7:00 a.m.) and sunset time (30 minutes before sunset), while pH gain was determined from the difference between early morning and sunset values. Biofloc volume (ml/L) in each tank was measured using the Imhoff cone (one-liter capacity). Visibility was determined using Secchi disk. Nitrite-nitrogen was measured using the diazotizing method and total ammonia concentration (TAN) was measured using the indophenol method employing colorimetric method according to APHA (1995).

Statistical analysis

Growth performance of the cultured fish as well as water quality parameters in culture tanks were subjected to one-way analysis of variance to determine statistical significant differences among treatments. Differences between means were assessed by Duncan multiple range test (Duncan's, 1955). Statistically significant differences were determined by setting the aggregate type I error at 5% ($p<0.05$) for each comparison. This statistical analysis was performed using the software package SPSS for windows, release 8.0 (SPSS, 1997).

RESULTS AND DISCUSSION

Average final weight

Growth performance data of Nile tilapia are shown in Table (1). Starting with average initial individual weights of 13.5 to 20.3 g/fish, fish grew to an average final weight of 310.2 to 342.1 grams. At the end of the culture period, body weights of Nile tilapia differed significantly among treatments (P<0.05). There was no significant difference in final body weight between the 30% and 35% crude protein treatments (340.9-342.1 g/fish) which were significantly higher than that of the 25% crude protein treatment (310.2 g/fish).

Table 1: Growth performance and harvest data of Nile tilapia reared under different dietary protein levels

Parameters	Treatments				
	25%	**30%**	**35%**	**30% Rest.**	**35% Rest.**
Initial weight (g/fish)	13.5 c	17.8 ab	20.3 a	16.9 b	17.9 ab
Initial biomass (g/tank)	443.3 c	587.4 ab	669.9 a	559.9 b	590.7 ab
Final weight (g/fish)	310.2 c	340.9 ab	342.1 a	317.9 bc	317.7 bc
Daily weight gain (g/fish/day)	0.75 b	0.82 a	0.82 a	0.77 ab	0.76 ab
Monthly weight gain (g/fish/month)	22.7 b	24.8 a	24.8 a	23.1 ab	23.0 ab
Stocking rate(number/tank)	24	24	24	24	24
SGR (%)	0.8 a	0.76 ab	0.72 b	0.75 b	0.73 b
Fish harvest (kg/tank)	6.98 a	7.00 a	7.02 a	7.31 a	6.82 a
Fish harvest (kg/m^3)	3.49 a	3.50 a	3.51 a	3.65 a	3.41 a
Survival rate (%)	93.7 a	85.4 a	85.4 a	95.8 a	89.4 a

Increasing dietary protein from 30% to 35% within the biofloc treatments did not improve final body weight at harvest, in both normal feeding and restricted feeding treatments. Within the 30% crude protein treatments, decreasing dietary inputs to 80% in the restricted treatment decreased harvest weight by only 7% compared to that of the 30% treatment.

Xia *et al*. (2015) indicated that reducing dietary protein in fish feed to appropriate level enhanced growth performance and reduced metabolic ammonia. Similarly, the current results suggest that in the presence of biofloc, it is possible to restrict feeding rates, without affecting harvest weight. This indicates that fish can obtain enough nutrition from different sources in the environment including suspended bioflocs.

Daily weight gain

The 30% crude protein diet had significantly (p<0.05) improved daily weight gain to be 0.82 g/fish/day compared to 0.75 g/fish/day which was achieved by 25% crude protein diet.

Consequently, Nile tilapia were able to extract bacterial protein contained in the biofloc material suspended in tank water in the current experiment which enabled tilapia fed the 25% crude protein diet to grow significantly slightly lower (by approximately 8.6%) than those fed with the 30% and 35% crude protein diets in terms of daily weight gain (p<0.05).

Similar daily weight gain patterns were obtained when fish were reared under the 30% restricted protein treatment (0.77 g/fish/day) compared to those of the 25% and 30% crude protein treatments (0.75 and 0.82 g/fish/day, respectively). The use of restricted feeding in fish culture can decrease production costs and enhance water quality in rearing units (Filbrun*et al.*, 2013).

Hargreaves (2006)stated that suspended-growth systems can produce abundant quantities of microbial protein (microbial flocs) which can increase the efficiency of feed utilization. Lowering feed inputs in the current study produced approximately similar growth performance in terms of weight gains.

The higher crude protein content in the 35% crude protein treatment did not improve harvest weight or daily weight gain of Nile tilapia compared to the 30%

crude protein treatment (p>0.05). Consequently, there is no need to employ higher dietary protein in the diets Nile tilapia raised in biofloc systems.

Water quality parameters

Water temperature

Water quality data are presented in Table (2). Early morning average of annual water temperature (at 7:00 a.m.) ranged 24.2-26.7 °C among treatments during the experimental period. Overall averages of annual water temperature at dusk time (30 minutes before dusk) were higher than those of early morning by 1.2-1.5 °C due to the daily heat gain during daylight hours from sunrise to sunset.

Table 2: Water quality parameters of Nile tilapia reared under different dietary protein levels

Parameters	Treatments				
	25%	**30%**	**35%**	**30% Rest.**	**35% Rest.**
Oxygen concentration (mg/l)	4.3 a	4.2 a	4.6 a	4.9 a	4.7 a
Water temperature (°c)					
Temp. a.m.	25.3 a	24.6 a	24.7 a	24.2 a	24.3 a
Temp. p.m.	26.7 a	26.1 a	25.7 a	25.4 a	25.5 a
Temp. difference	1.4 a	1.5 a	1.0 a	1.2 a	1.2 a
pH reading					
pH a.m. (units)	7.7 a	7.7 a	7.6 a	7.8 a	7.7 a
pH p.m. (units)	7.6 a	7.6 a	7.7 a	7.8 a	7.7 a
pH difference	-0.1 a	-0.1 a	0.1a	0.0 a	0.0 a
Biofloc volume (ml/l)	20.5 a	19.1 a	25.3 a	17.4 a	13.5 a
Secchi disk (cm)	7.7 a	11.2 a	10.4 a	10.1 a	9.5 a
Nitrite (NO_2-N, mg/l)	0.4 a	0.2 a	0.1 a	0.2 a	0.2 a
Ammonia (TAN, mg/l)	0.0 a	0.0 a	0.0 a	0.0 a	0.0 a

Dissolved Oxygen

The current experiment reflected a pattern where oxygen concentrations at dusk ranged between 4.2-4.9 mg/l, with no significant differences among treatments. Dissolved oxygen concentrations were slightly higher in the restricted feed treatments due to the lower feed input and its effect on reduced community respiration rates. The current rate of feed inputs induced a very low visibility depth (i.e. Secchi readings), but without oxygen depletion problems due to artificial aeration.

Ammonia and nitrite

Ammonia concentrations in water in the rearing tanks were near zero, indicating that bacterial activities were very efficient in absorbing metabolic ammonia secreted by fish and/or transforming ammonia to nitrite. Hargreaves (2006) stated that biofloc represents only a temporary packaging of nutrients because nutrients are released upon decomposition of dead bacteria. Averages of nitrite concentrations were relatively low, and ranged from 0.1 to 0.4 mg\L among treatments, with no significant differences among treatments. Nitrite-nitrogen concentrations were within acceptable limits. The concentrations of TAN and NO_2-N of water in the biofloc tanks were lower with adding molasses during the culture period. This is in agreement with Hari and Madhusoodana (2003) and Asaduzzaman *et al.* (2008) who found out that the use of carbon sources in the biofloc rearing units reduced total ammonia and nitrite concentrations in the rearing environment. TAN concentrations remained near zero during the experiment although fish should have excreted more than 3.0 mg TAN/L/day. Similarly, nitrite concentrations ranged from 0.1 to 0.4 mg/L among treatments indicating that ammonia oxidizing bacteria worked well in all biofloc treatments. Jatoba*et al*. (2014) indicated that heterotrophic and nitrifying bacteria work simultaneously in water of the biofloc units, where ammonia and nitrite ions are transformed into harmless nitrate during nitrification.

Biofloc volume and secchi disk visibilities

All treatments had shallower photic zone depths (7.7 – 11.2 cm) than normal photic zone depth (30 cm, Boyd, 1990) indicating dense suspension of bacteria. Biofloc volumes (BFV) were nearly similar within and among all treatments over time ($p>0.05$). The decrease of daily feed input from 100% to 80% did not affect biofloc volume in culture tanks due to the renewal of water at the end of each month during the experimental period. During a preliminary experiment, it was observed that biofloc volume often exceeds 80 mL/L without water renewal (Elnady *et al.*, 2015). Consequently, the design of the current experiment included monthly renewal of 100% of the water in each tank in order to reduce excessive biofloc volumes in culture tanks.

Early morning and dusk pH

Early morning and dusk pH values in water as well as its concentration during daytime hours in rearing tanks are presented in Table (2). Average pH gains (pH difference between sunset and morning) during daylight hours were near zero, indicating the lack of photosynthesis in all treatments during daytime hours. Differences between pH values at early morning and dusk time were low and ranged between -0.1 to 0.1 units.

Feed conversion and protein efficiency ratio

Feeding performance data are shown in Table (3). Among the dietary treatments, Nile tilapia had slightly higher FCR during spring and summer seasons (1.87 - 2.34) compared to the overall FCR values obtained during warm seasons of the whole experiment (1.51-2.23). The deterioration in feed conversion ratios were due to the slow growth rate of older Nile tilapia during spring and summer seasons which was negatively affected by older growth stage. This indicated that Nile tilapia were more efficient in transforming dietary protein into flesh during early stages of growth compared to those of older fish.

Table 3: Feeding performance and feed cost of Nile tilapia reared under different dietary protein levels

Parameters	Treatments				
	25%	**30%**	**35%**	**30% Rest.**	**35% Rest.**
Overall FCR	2.23 a	1.7 bc	1.51 c	1.82 b	1.87 b
FCR (spring and summer seasons)	2.34 a	2.05 a	1.94 a	1.87 a	2.09 a
Overall PER	1.8 a	1.96 a	1.90 a	1.84 a	1.52 b
Feed cost (L.E./kg fish)	19.02 a	15.11 b	15.86 b	16.20 b	19.73 a

Among the dietary treatments, Nile tilapia had higher FCR with the 25% crude protein treatment (2.23) compared to all FCR values obtained by other treatments (1.51-1.87). The deterioration in feed conversion ratios in the 25% crude protein treatment was due to the slightly slow growth rate of Nile tilapia which was negatively affected by lower protein content. The 30% and 35% crude protein treatments produced slightly better feed conversion (1.51-1.7) compared to all restricted treatments.

The protein efficiency ratios (PER) were better in all treatments (1.8-1.96) compared to those of the 35% restricted treatment (1.52). PER values were better in the 25% and 30% crude protein treatments than those obtained in the restricted 35% crude protein treatment. The deterioration in PER ratio in the restricted (35%) crude protein treatment may be due to the lower efficiency of protein transformation when high protein diet was used.

Yang *et al*. (2002) reported that PERs and Net Protein Utilization (NPU) values were deteriorated when high dietary protein was used in fish diets. Since carbohydrate did not effectively spare protein (Kim *et al*., 1991).

PERs were significantly deteriorated in the restricted (35%) protein treatment. Yang *et al*. (2002) reported that dietary protein can positively enhance fish growth rate to a certain limit, beyond which reduced growth was observed.

Hargreaves and Tucker (2003) indicated that overfeeding can impair growth and feeding performances in cultured fish due to the metabolic waste products resulting from overfeeding. Moreover, when restricted feeding was employed both feeding performance and water quality were ameliorated (Cho and Lovell, 2002). Amirkolaie (2011) pointed out that carbohydrates and fat can spare protein in fish feed, and suitable protein content in fish feed can ameliorate its use by cultured fish.

Similar fish yields per tank were obtained in all treatments (6.82-7.31 kg\tank) during the current experiment (p>0.05). However, the restricted treatment (30% crude protein) had more economical yield (7.31 kg/tank). Heterotrophic bacteria absorb toxic ammonia excreted in the biofloc medium as well as provide biofloc material rich in protein for aquatic animals such as tilapia and shrimp, leading to better growth and feeding performances of cultured organisms (Burford*et al.*, 2002 and Azim and Little, 2008).

Nile tilapia reared under the 30% normal feeding treatment had better PER ratio (1.96) and lower feed costs (15.11 L.E. /kg fish) compared to the 25% crude protein treatment.

Ballester *et al*. (2010) indicated that protein content of feed could be lowered by 10% when shrimp are reared in biofloc medium without compromising growth rate of cultured animals.

Feed costs

The results of the current study demonstrated that the biofloc system is more economical in desert aquaculture in terms of water consumption needed to produce fish under limited water availability and high pumping costs.

When Juveniles of Nile tilapia were reared in the biofloc tanks, the amount of daily feed inputs can be reduced without affecting the production costs, indicating that biofloc could contribute to the nutrition and physiological health of Nile tilapia.

Feed costs for producing one kilogram of Nile tilapia were better for the 30% treatment (15.11 L.E./kg), which was similar to the restricted 30% and normal feeding 35% crude protein treatments (15.86 – 16.2 L.E./kg). Feed costs required to produce one kilogram of fish were higher in the restricted 35% crude protein treatment and the 25% treatment. The high feed cost primarily resulted from the high cost of higher protein content as well as the inferior PER ratio obtained when protein input was reduced.

As far as profitability of tilapia production in biofloc tanks is one of the major concerns of the fish farmers, the 30% crude protein treatment had the most profitable regime to be used in tilapia production during warm season when water temperature are optimal for growth in biofloc systems.

CONCLUSION

The observed results in the current study indicated that it is possible to use restricted feeding when fish are raised in biofloc tanks. The water quality parameters during the current study were within optimal ranges for Nile tilapia growth. Consequently, it is recommended to use lower protein diets at 30% crude protein in order to obtain higher profitability under biofloc systems. The 30% crude protein diet is supposed to be used for Nile tilapia production under biofloc environment during warm seasons.

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ARABIC SUMMARY

تأثير مستوى بروتين العليقة و معدل التغذية على أداء النمو و التغذية للبلطى النيلى تحت ظروف البيوفلوك

أمانى عبدالعال جلال١ ، محمد النادى أحمد محمد٢ ،محمد على إبراهيم سالم٢ ، نيفين السيد متولى١
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تم تصميم هذه التجربة لإختبار تأثير مستوى بروتين العليقة (٢٥، ٣٠ و ٣٥% بروتين خام) بالإضافة إلى إستخدام التغذية المحدودة فى نظام البيوفلوك فيما يتعلق بأداء النمو للبلطى النيلى و ديناميكية جودة مياه الإستزراع. و قد شملت التجربة على ٥ معاملات بمعدل تكرارين لكل معاملة حيث إستخدمت معدلات تغذية كالتالى:

١) معدل التغذية الطبيعى فى معاملات ٢٥، ٣٠ ، ٣٥% بروتين خام.
٢) معدل التغذية المحدودة بواقع ٨٠% من معدل التغذية الطبيعية فى معاملات ٣٠% و ٣٥% بروتين خام فقط.

و قد أضيف المولاس المذاب فى الماء بنسبة ١:١ من كمية العلف الجاف المطبق يومياً بغرض تكوين البيوفلوك و تغذية البكتريا غير ذاتية التغذية. بدأت التجربة بإصباعيات من البلطى النيلى متوسط وزنها ١٣,٥ إلى ٢٠,٣ جرام / السمكة. و قد نمت إصباعيات البلطى النيلى إلى وزن حصاد٣١٠,٢ إلى ٣٤٢,١ جرام / السمكة وقت الحصاد. و عند زيادة المحتوى البروتينى للعليقة من ٣٠% إلى ٣٥% داخل معاملات البيوفلوك لم يؤدى ذلك إلى زيادة الوزن النهائى للسمكة عند الحصاد. و عند تطبيق معاملة ٣٠% بروتين خام كانت هناك زيادة طفيفة فى معدل النمو اليومى فى الوزن (٠,٨٢جرام / السمكة/ اليوم) بالمقارنة بتطبيق العليقة المحتوية على ٢٥% بروتين خام ($p>0.05$). و عند رعاية الأسماك بإستخدام التغذية المحدودة ٣٠% بروتين خام تم الحصول على نمو يومى فى الوزن (٠,٧٧ جرام/ السمكة/ اليوم) مشابه لمعدل النمو اليومى فى الوزن فى معاملات ٢٥% ، ٣٠% بروتين خام (٠,٧٥ – ٠,٨٢جرام / السمكة/ اليوم، على التوالى). وعند إستخدام محتوى بروتين خام مرتفع فى معاملة ٣٥% بروتين خام لم يؤدى هذا إلى تحسين النمو اليومى فى الوزن للبلطى النيلى بالمقارنة بالمعاملات الاخرى ($p>0.05$). و توصى التجربة بتغذية البلطى النيلى عند مستوى بروتين خام ٣٠% لكى نحصل على نمو مقبول و عائدات إقتصادية. و عند رعاية البلطى النيلى فى معاملة ٣٠% بروتين خام أدى هذا إلى الحصول على نسب كفاءة بروتين مرتفعة (١,٩٦) مع إنخفاض تكاليف التغذية (١٥,١١ جنية مصرى لكل كيلو جرام أسماك) بالمقارنةً بالمعاملات الأخرى.