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Impact of Feed Supplemented with Azolla Plant on Growth Performance and Survival Rate of Tilapia in Desert Areas

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ABSTRACT

This study was conducted to investigate the effect of partial and complete replacing artificial fish feed with Azolla in feeding Nile tilapia fish. Five treatments were done and fish were distributed randomly, where the only variable was the percentage of Azolla addition by using ten mesh cages of 0.5 m^3 (75*80*80 cm) of brackish water. Artificial feed was replaced by Azolla as 0%, 25% , 50% ,75% and 100%, in the treatments T_1, T_2, T_3, T_4 and T_5, respectively. The experiment lasted 135 days, and water measurements were taken from salinity, acidity, oxygen and ammonia. The weight of the fish was taken every 15 days, and analyzes were made for data on growth, nutritional transformation, weight, length and feed consumption for fish. The results showed that the mean final weight of individuals decreased significantly ($P<0.05$) in the experimental treatments with increasing Azolla as a supplementary feed. The best treatments were T2, which is the substitution of Azolla by 25%, and there is no difference between it and the treatment T1, which is the control. Therefore, it is recommend to use of Azolla as an inexpensive alternative in feeding fish at a rate of 25% to give the best desired results in terms of length and weight.

***Keywords*:** Azolla plant, Nile tilapia (*Oreochromis niloticus*), Feed Supplemented, Growth Performance, Desert Areas**.**

INTRODUCTION

Fish is a major source of animal protein, so aquaculture is being increased as a major food resource to ensure sustainability for future generations (Ahmad *et al.,* 2021; Osmundsen *et al.,* 2020; Riverter *et al.,* 2020). However, under intensive farming conditions, production and profit from aquaculture are insufficient to obtain a viable aquaculture activity (Nguyen *et al.,* 2021). Therefore, there is a need to increase non-traditional technologies and intensify production using available resources (Hisano *et al.,* 2021).

Aquaculture in North Sinai suffers from a lack of production due to the lack of feed and its high prices as the cost of buying fish feed is very high, so there is a need for a cheap alternative feed that is readily available and environmentally friendly.

Nile tilapia (*Oreochromis niloticus*) are easy to cultivate and can live in tropical climates, and have high economic value (Dawood *et al.,* 2019;d Van Doan *et al.,* 2019). The growth performance in tilapia fed 20% Azolla meal as feed supplemented did not differ significantly from those fed on a diet without Azolla supplemented (Magouz *et al.,* 2020). Saad *et al.* (2021) indicated that Azolla can be used as a feed supplement at the rate of 25-50% in the grass carp diet.

This study aims to replace a percentage of artificial feed by adding the Azolla plant in the diet of tilapia fish in order to reduce the cost and improve the economic condition of the small farmer.

MATERIALS AND METHODS.

1. Study Area and Period

The integrated system for fish and Azolla farming was implemented in the small farm holdings of Ber Al-Abd, from mid-March until the end of July, 2020.

2. Experimental design

Treatments were designed in duplicate. Azolla and fingerlings were cultured in 10 net cages of 0.5 m^3 (75*80*80 cm) sunk into the concrete pond. A total number of 150 live fries of Nile tilapia fish, *Oreochromis niloticus* were used in the experiment (in each net cages, 15 fries were kept randomly). They were obtained from private farm, with an initial average net weight of 10.08 ± 0.09 g. The experiment was carried out by replacing the artificial feed by Azolla plants in feeding in different proportions through five treatments. The experiment was carried out as follows:

T_1: first treatment control (100% artificial feed).

T_2: second treatment,75% of the artificial feed with Azolla plant as supplementary green fodder.

T_3: third treatment, 50% of the artificial feed with Azolla plant as supplementary green fodder.

T_4: fourth treatment, 25% of the artificial feed with Azolla plant as supplementary green fodder.

T_5: fives treatment, 100% of the Azolla plant was used.

Fish was fed on artificial feed of 27% protein, obtained from Skretting Egypt Factory. Fish were fed twice daily by an artificial diet at a rate of 5% of the weight of the fish, for each treatment separately with Azolla.

3. Measurements

3.1 Water quality

The experiment lasted 135 days, and water measurements were taken daily for salinity, pH, oxygen, and ammonia, and the weight of the fish was taken every 15 days.

3.2. Growth performance and Survival Rate

3.2.1 Daily Growth Rate

$$\textbf{Daily Growth rate (DGR) (g/ day)} = \frac{\textbf{W2} - \textbf{W1}}{\textbf{t2} - \textbf{t1}}$$

Where W_1 = initial weight (g), W_2 = final weight (g)

t_2-t_1 = duration between W_2 and W_1 (days).

3.2.2Weight Gain

$$\textbf{WG (g)} = \textbf{FW} - \textbf{IW (g/fish)}$$

Where IW = initial weight (g)

FW = final weight (g)

3.2.3 Specific Growth Rate (SGR, %/day)

$$\textbf{SGR} (\%) = \frac{\textbf{100} \times [(\textbf{ln final fish weight}) - (\textbf{ln initial fish weight})]}{\textbf{experimental days}}$$

3.2.4 Survival Rate

Mortalities for fishes were recorded every 15 days and the survival rate was calculated.

$$\textbf{Survival rate } (\%) = \frac{\textbf{fish stocked} - \textbf{mortality}}{\textbf{fish stocked}} \times \mathbf{100}$$

3.3 Length-weight relationship

At the end of the study, 100% of the stock was caught and total length (TL) and body weight (BW) of each individual were measured to 0.1 cm and 0.1 g by using rubber ruler and an electronic balance. The length weight relationships were determined according to the allometric equation (Garcia *et al.*, 1989):

$$\mathbf{W = aL^b}$$

Where: W=total weight (g).

L= total length (cm).

a=constant. b= relative growth coefficient of weight and length.

4. Statistical analysis

Different mean values were analyzed using Minitab software version 17.0, Tukey's test was used to compare between samples. One-way ANOVA analyzes were used. Statistical significance was evaluated using a probability level P = 0.05.

RESULTS

1. Water quality

The results indicated that there were no significant differences in water quality between the treatments for water temperature, salinity, and pH. While the values of dissolved oxygen and ammonia concentrations recorded significant differences between most of the treatments, as shown in Table (1).

Table. 1. Water quality of Tilapia experiment under different feeding treatments for 135 days

Parameters	Treatments				
	T1	T2	T3	T4	T5
Salinity	3.17	3.17	3.16	3.17	3.17
Temperature °C	25.02	25.07	25.10	25.01	25.08
Dissolved oxygen ($mg \cdot l^{-1}$)	5.38	5.45	5.49	5.51	5.53
pH	7.71	7.69	7.69	7.70	7.72
Ammonia (mg^{-L})	0.07	0.068	0.06	0.06	0.058

2. Effect of Azolla supplemented on growth performance and survival rate

Data of Fish Final Body Weight, daily growth rate, Weight Gain, Specific Growth R**ate** (SGR) of Nile tilapia fish, *O. niloticus* throughout the experimental period with different feeding percentage of Azolla is shown in Table (2).

Table 2. Growth performance of Tilapia under different feeding treatments for 135 days.

Item/Treatments	T1	T2	T3	T4	T5
Initial Weight	10.2±0.2	10.0±0.16	10.1±0.22	10.0±0.11	10.1±0.08
Fish Final Body Weight (g)	100.6^{a} ±1.3	100.1^{a} ±0.5	72.4^{b} ±1.1	49.0^{c} ±3.5	40.8^{d} ±2.1
Daily Growth Rate	0.7^{a}	0.7^{a}	0.5^{b}	0.3^{c}	0.2^{d}
Weight Gain (g)	90.4^{a}	90.1^{a}	62.4^{b}	39.0^{c}	30.7^{d}
SGR (%/day)	1.7^{a}	1.7^{a}	1.5^{b}	1.2^{c}	1.0^{d}
b-value of the Length-weight relationship	3.1^{ab}	3.2^{a}	2.9^{bc}	2.9^{bc}	2.9^{c}

a,b,c…. etc: means within the same row with different superscripts are significantly different (P<0.05).

2.1. Final Body Weight

The recorded average final fish weight was 100.6, 100.1, 72.4, 49.0 and 40.8 g of treatments T_1, T_2, T_3, T_4 and T_5, respectively (Table 1 and Fig. 1). No significant differences were observed in final body weight between T_2 compared with the control (P>0.05). The obtained results revealed that the average of Nile tilapia fish, *O. niloticus* individuals final weight decreased significantly (P<0.05) in experimental treatments with increasing of percentage of Azolla as a supplementary feed.

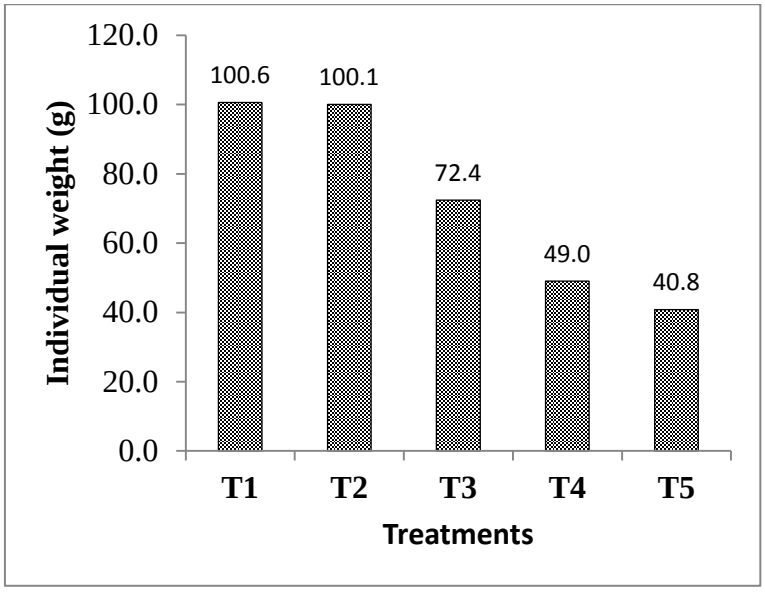

Fig. 1. Final fish weight of tilapia, *O. niloticus* in different treatments with Azolla.

2.2.Daily growth rate

The results showed as that the control treatment and the Azolla feeding treatment with a 25% reduction of the artificial feed gave better growth of daily growth rate than the other

treatments. Treatments (T_3 to T_5) showed a significant decrease in growth at the end of the period compared to the treatments T_1 and T_2 (Fig. 2).

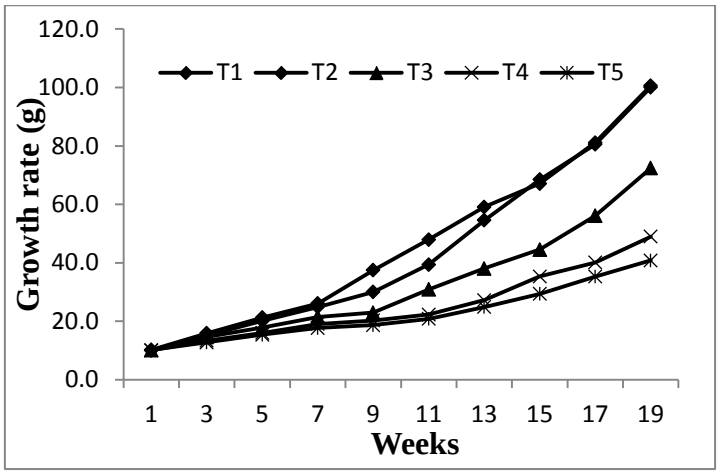

Fig. 2. Change of individual weight of tilapia, *O. niloticus* over experimental period.

2.3. Weight Gain

There were significant differences in the increase in the weight gain of the fish in treatments T_3, T_4 and T_5 compared to T_1 and T_2, (Table 1). Also, there was no significant difference between T_1 and T_2 ($P>0.05$).

2.4. Specific growth rate (SGR, % / day)

The specific growth rate (SGR, % / day) decreased significantly ($P < 0.05$) in the experimental treatments with the increase of Azolla as a supplementary feed, where the results were 1.7, 1.7, 1.5, 1.2 and 1.0 for treatments T_1, T_2, T_3, T_4 and T_5, respectively (Table 1). It was obvious that there were no significant differences between T_1 and T_2.

2.5. Survival rate

Survival rate was not significantly different between fish fed the artificial feed and other treatments (Table 1).

3. Length-weight relationship and growth pattern

Table (3) indicates the length, weight and growth pattern (b value) of a Nile tilapia fish, *O. niloticus* from different treatments. The growth pattern of treatments (T_1 & T_2) was positive allometric (b value >3.0) and there was no-significant difference between them. Also, non-significant difference was recorded between T_3 and T_4 with T_1. The growth pattern of T_3, T_4 and T_5 were negative allometric (b value <3.0). The b-value of the length–weight relationships for tilapia fish was significantly different between treatments (Table 3).

Table 3. Data on length and weight of a Tilapia from different treatments.

Treat.	Length groups (cm)	Mean length (cm)	Mean Weight (g)	b-value
T1	14.7 -18.8	17.02±1.2	100.6±22.6	3.1429^{ab}
T2	14.6 -18.5	16.73±1.20	99.92±22.69	3.2168^{a}
T3	11.9 -17.9	14.92±1.77	72.43±27.20	2.9308^{bc}
T4	8 -15.4	13.21±1.84	48.98±18.10	2.8732^{c}
T5	9.1 -15.8	12.59±1.98	40.78±18.79	2.898^{bc}

a,b,c…. etc: means within the same row with different superscripts are significantly different (P<0.05).

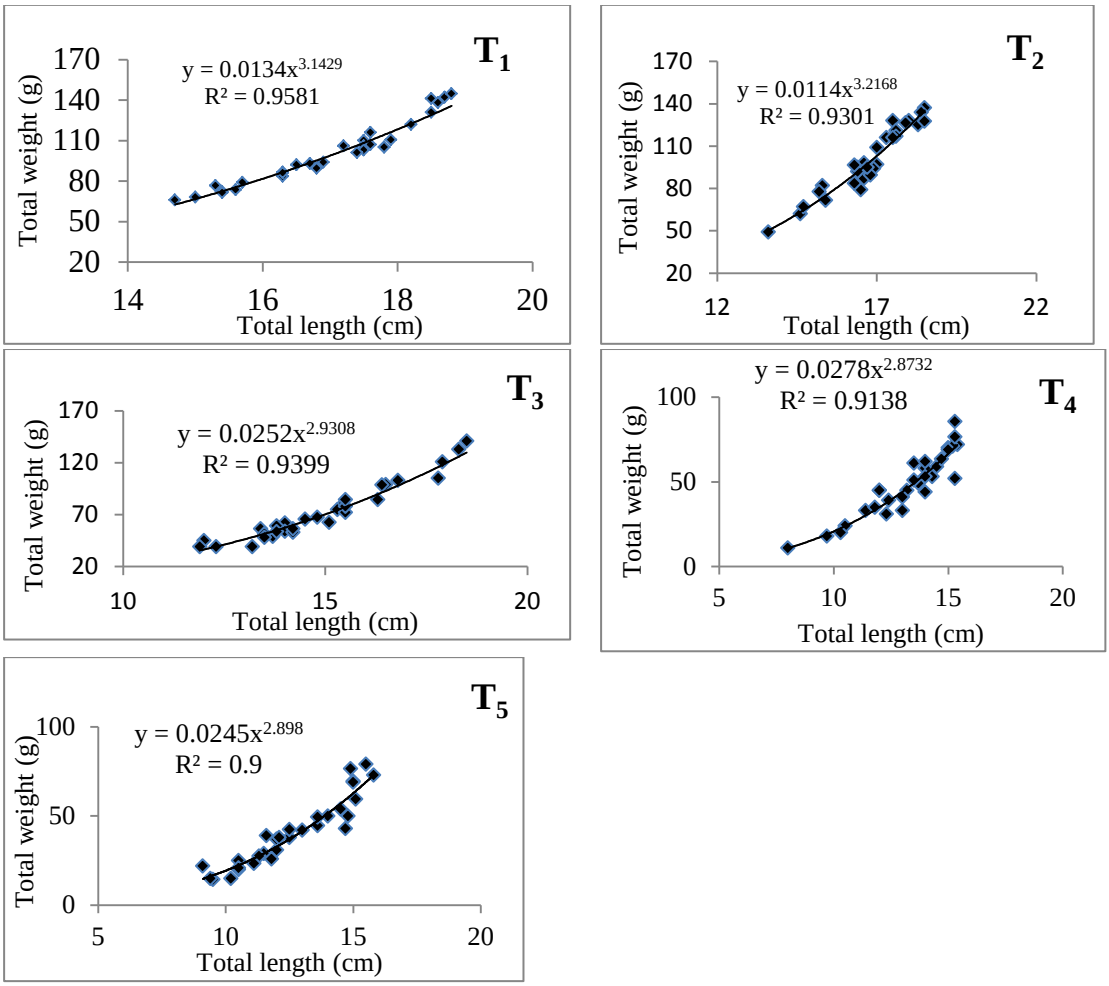

Fig.3 Length-weight relationship of tilapia, *O. niloticus* in fifth treatment

4.Yield

The total average biomass of 15 Tilapia (150 g) were recorded in each cage. At the end of experiment, yield of different treatments were 2.87, 2.9, 2.17, 1.47 and 1.22 kg for treatments T_1, T_2, T_3, T_4 and T_5 respectively (Fig. 4). No significant differences were observed in yield between T_2 with the control and T_1 ($P>0.05$). The results showed a significant ($P < 0.05$) decrease in yield of tilapia by feeding on Azolla plant only, as well as with a 50% reduction in the amount of artificial feed.

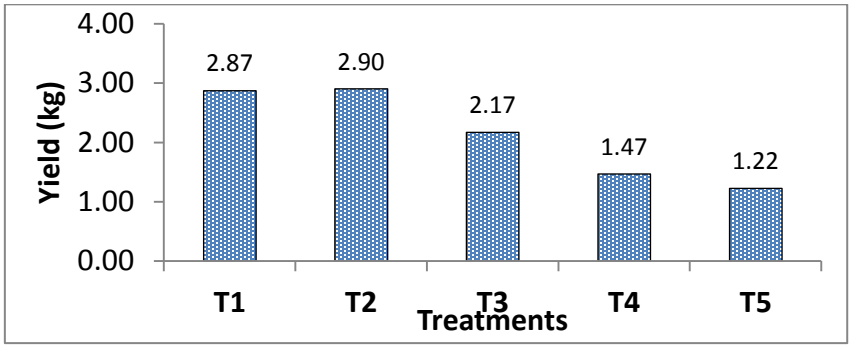

Fig. 4. Yield of different treatments of tilapia, *O. niloticus* at the end of experiment.

5. Economic analysis:

The results indicated a low significance in the net revenue of the Nile tilapia fish *O. niloticus* which was cultured underfed on an artificial diet compared with the net revenue of the fish fed on an artificial diet with Azolla plant, which was affected by feed input cost than other input costs (Table 4).

Table (4). Cost of production and net revenue of the experimental diets of tilapia supplemented with Azolla plant.

Treatments / **Costs/m3**	**T1**	**T2**	**T3**	**T4**	**T5**
Price of fingerlings (LE)	7.5	7.5	7.5	7.5	7.5
Feeding(kg)	2.2	1.6	1.1	0.54	0
Cost of feed (LE)	32.4	24.2	16.2	8.2	0
Add. Cost (LE)	10.0	12.0	13.0	14.0	15.0
Total Cost /m^3	**52.1**	**45.3**	**37.8**	**30.2**	**22.5**
Income					
Fish yield/m^3	2.87	2.90	2.17	1.47	1.22
Price	30.0	30.0	27.0	25	21
Income (LE/kg/m^3)	**86.2**	**87.1**	**58.7**	**36.7**	**25.7**
Net Rev.	**34.2^a**	**41.8^b**	**20.9^c**	**6.5^d**	**3.2^f**

a,b,c…. etc: means within the same row with different superscripts are significantly different ($P<0.05$).

DISCUSSION

The current water parameters (salinity, temperature, PH value, and dissolved oxygen mg/L) were suitable for culturing Nile tilapia fish *O. niloticus* as recorded by Abdelhamid, (2009). Also, the survival rate of fish has not been affected by water parameters or Azolla diet. Moreover, the results clearly showed that the growth of fish decrease with increasing fresh Azolla plants in diet for fish fed in T3 and T4 (20% and 25% artificial feed of the required amount with fresh Azolla, respectively) . There was significant difference ($P<0.05$) between growth rate of fish in T3 and T4 compared with those in T1 and T2. Also, the growth of fish (T_5), where the artificial feed was completely replaced by the Azolla plant, was highly decreased. Almazan *et al.*(1986) and Maity and Patra (2008) reported that Azolla seems to be good replacer of protein from expensive sources, however, increasing dried Azolla meal incorporated in the diet for Nile tilapia (*Oreochromis niloticus*) will decrease fish growth performance and food conversion with worsening FCR value, and they concluded that, 10-45% of Azolla inclusion level can be incorporated in the diet for Tilapia species.

Through the current experiment, no significant differences appeared between the control treatment and the second treatment in which azolla was replaced by 25% in the feed, in which the average final weight and growth were ideal and was not affected by the replacement, as in the study of Abdo *et al* (2011) who concluded that Azolla could be combined with a percentage of up to 20% in diets without adverse effects on growth in Nile tilapia.

The body weight of fish fed on Azolla groups 75 and 100% significantly decreased in comparison with the control. These results may be due to a decrease protein in Azolla (21%) protein. Ibrahim *et al* (2007) indicated that the Azolla meal can be put (dried granules) suitable as a protein food supplement for tilapia to a maximum of 31.8%. Also, Magouz *et al.* (2020) emphasized that diet of fish including 20-25% of Azolla has no negative effects on growth, feeding efficiency, immune response, or health status of the fish.

The present results, record significant differences between fish in different treatments in their "b" value of the relationship between length and in weight. The best "b" value in the length-weight relationship was recorded with the first treatment (control), while with increasing fresh Azolla incorporated in the diet for Nile tilapia (*O. niloticus*) decreased fish growth performance. Both *O. niloticus* in T_1 and T_2 showed positive allometric growth with 'b' value of 3.14 and 3.21, respectively. According to Riedel *et al.* (2007), this implies that it becomes relatively broader and fatter with the increase in length. On the other side, T_3, T_4 and T_5 reported a negative allometric growth for *O. niloticus* with 'b' value of 2.93, 2.87 and 2.89, respectively. According to the current results, a variation of the allometry coefficient (b) was observed for all treatments.

Economically, the study investigated the feasibility of incorporating Azolla plant with the artificial diets of tilapia, and measures of profitability were determined. The study concluded the incorporation of Azolla plant in fish diets may lower the cost of diets as the cost of feed contributes to the increase in the variable cost of fish production. The current results align with the results of Abu *et al.* (2010) and Jimoh *et al.* (2012) who concluded that the use of cheaper and fewer feed ingredients can achieve a higher profit margin.

Conclusion:

The present results indicated that the Azolla plant tolerates groundwater with relative salinity and can be replaced in fish feed to provide 25% of the artificial feed and gives the same results when using the 100% artificial feed.

Recommendation:

Planting Azolla under desert conditions and using it in fish farms and including it in fish feed to give the desired results in terms of fish weight. replacing this plant (Azolla) by

25% from artificial feed in order to reduce the costs of industrial feed and increase the income of the small farmer.

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تأثير الاحلال لنبات الأزولا كعلف تكميلي مع الأعلاف على أداء نمو البلطي في المناطق الصحراوية

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المستخلص

أجريت هذه الدراسة لمعرفة تأثير الاستبدال الجزئي والكامل لعلف الأسماك المصنعة بالأزولا في تغذية أسماك البلطي النيلي. أجريت خمس معاملات وزعت الأسماك بشكل عشوائي حيث كان المتغير الوحيد هو نسبة إضافة الأزولا باستخدام عشرة أقفاص شبكية بحجم ٠.٥ م ٣ (٧٥ * ٨٠ * ٨٠ سم) مزوده بالمياه قليلة الملوحة. تم استبدال نسبة الأعلاف الصناعية بالأزولا بنسبة ٠٪ ، ٢٥٪ ، ٥٠٪ ، ٧٥٪ ، ١٠٠٪ من العلف الصناعي لـ T1 و T2 و T3 و T4 و T5 على التوالي. استغرقت التجربة ١٣٥ يومًا ، وأخذت قياسات المياه من الملوحة والحموضة والأكسجين والأمونيا. تم أخذ وزن السمكة كل ١٥ يومًا ، وأجريت التحليلات لبيانات النمو والتحول الغذائي والوزن والطول للأسماك. أظهرت النتائج أن متوسط الوزن النهائي للأفراد انخفض معنويا في المعاملات التجريبية مع زيادة الأزولا كعلف تكميلي. أفضل المعالجات كانت T2 وهي استبدال الأزولا بنسبة ٢٥٪ ولا فرق بينها وبين المعاملة T1 وهي الضابطة. لذلك توصي الدراسة بإضافة الأزولا كبديل غير مكلف في تغذية الأسماك بمعدل ٢٥٪ من الاعلاف المصنعة لإعطاء أفضل النتائج المرجوة من حيث النمو والانتاج .

الكلمات المفتاحية: نبات الأزولا ، البلطي النيلي (*Oreochromis niloticus*) ، العلف المكمل ، أداء النمو ، المناطق الصحراوية.