Egyptian J. Anim. Prod. (1999) 36 (1):71-82

PRODUCTION EVALUATION OF NILE TILAPIA *OREOCHROMIS NILOTICUS* UTILIZED FRESH AND DRIED *AZOLLA PINNATA* IN SEMI-INTENSIVE FISH CULTURE

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SUMMARY

The present study was conducted in 16 outdoor concrete ponds (about 2.5 m^3 water/ pond) to evaluate the growth performance of Nile tilapia *Oreochromis niloticus* fed on fresh and dried *Azolla pinnata* as a partial or full replacement of the control diet throughout six months. Performances studied included growth, feed conversion ratio, protein utilization and body composition for each treatment. Results indicate that the growth and feed utilization of *O.niloticus* were not significantly reduced ($P>0.05$) when Azolla meal was incorporated into the control diet up to 50%. While, fish fed on fresh Azolla alone exhibited extremely poor growth performance ($P<0.05$). Concerning the body composition, protein and lipids contents decreased with increasing levels of Azolla in the diet reaching their lowest values with fresh Azolla alone. However, ash content showed an inverse relationship. Furthermore, fish yield was economically evaluated on the basis of feeding cost, fish marketing size and net return for each treatment.

Keywords: Nile tilapia, Azolla pinnata, fish culture

INTRODUCTION

Azolla is a small aquatic fern, which has a symbiotic relationship with the nitrogen-fixing blue green algae *Anabaena azallae* within its leaf cavities. Azolla doubles its biomass in 3-10 days depending on conditions and easily reaches a standing crop of 37.8 tones/ha fresh weight (2.78 t /ha dry weight) which has been reported by Pullin and Almazan (1983) for *Azolla pinnata* in India. Azolla has been used for centuries in Asia as a green manure, fertilizer for rice fields and a supplement to diets for pigs and poultry, however,little is known on Azolla as a fish food (Pullin and Almazan, 1983). Azolla is consumed by various fish which often prefer it rather than other aquatic plants (Antoino *et al.*, 1986 and Micha *et al.*, 1988). Azolla is a potential source of fish fodder due to its high yield, enriched nutrients and good edibility. Some Azolla strains have well balanced amino acid contents with the

exception of slight methionine, cystein and sometimes lysine deficiencies, and represent good quality food if cultivated in good conditions (Van Hove and Ruelle, 1989). However, few studies have been conducted on the use of *Azolla pinnata* as supplemental or complete feed ingredient for Nile tilapia *Oreochromis niloticus*. Moreover, the results of these studies are inconsistent and ambiguous. For example, Pantastico *et al.* (1986) found that, the fresh *A. pinnata* as supplemental feed enhanced growth of *O.niloticus* fingerlings in cages in Laguna Lake. While, Almazan *et al.* (1986) found that fresh, dried powder or dried pellet form of *A. pinnata* as the only feed caused weight loss in *O.niloticus* fingerlings and adult males. Therefore, more research is needed to settle this subject.

The present study aims to evaluate the biological, chemical and economical values of fresh and dried *Azolla pinnata* when fed to Nile tilapia (*O. niloticus*) at different levels compared to a control diet during six months feeding trial.

MATERIALS AND METHODS

Azolla pinnata was collected from the main irrigated canal at El-Kalubia Governorate, northern of Cairo and transported directly to the Fish Production Unit (FPU), Faculty of Agriculture, Cairo University. The collected material were washed by running water. Some fresh Azolla was cultivated in 4 outdoor rectangular concrete ponds (2.0 x 1.4 x 0.9 m^3) and maintained at the linear phase of its population growth curve according to Van Hove *et al.* (1987). Another amount of fresh Azolla was sun-dried for about three days, grounded finely as Azolla meal. Both of fresh Azolla and Azolla meal were incorporated into the experimental diets (the control diet was made on the basis of crude protein content of Azolla) and its chemical analysis was performed according to AOAC (1990) (Table 1).

Nile tilapia (*O. niloticus)* fingerlings (mean weight 5.0 g $\pm$ 0.1) produced in FPU were stocked in twelve outdoor concrete ponds (about 2.5 m^3 water / pond) at stocking rate of 20 fish per pond. The ponds were supplied with underground fresh water nearby the river Nile. Water was exchanged at the rate of 10 % per pond daily throughout the experimental period from April 1st to September 30, 1998. The experimental diets (Table 1) were given in the morning at 3% of fish biomass. Duplicates ponds were set up for each feeding treatment.

Five fish were individually sampled and recorded for their length (0.1 cm) and weight (0.1g) per each pond once every two weeks, and the amount of diet was adjusted for the subsequent two weeks. At the end of experimental period all fishes were harvested, counted and weighed to estimate the following parameters:

Weight gain (g) $= W_F - W_I$

Average daily gain (g) $= W_F - W_I / t$

Percentage of weight gain $= 100 (W_F - W_I) / W_I$

Specific growth rate (SGR) $= \dfrac{Ln (W_F) - Ln (W_I)}{t} \times 100$

W_I = Initial body weight (g / fish)
W_F = Final body weight (g / fish)
t = Experimental period (days)
Condition factor = body weight (g) x 100/ (total length, cm)^3
Feed conversion ratio (F.C.R.) = dry feed intake (g)/weight gain (g)
Feed conversion efficiency (F.C.E.) = weight gain (g) / dry feed intake (g)
Protein efficiency ratio (P.E.R.) = weight gain (g) / protein intake (g)
Protein retained = Final body protein - Initial body protein.
Productive protein value (PPV%) = protein retained x 100
protein intake (g)

Table 1. Composition and chemical analysis of the control diet and experimental diets of *Azolla pinnata* on dry matter basis.

Ingredient	(% crude protein)	%
Control diet (T0) composition		
Yellow corn	(9.6)	50
Soyabean meal	(44.0)	20
Fish meal	(62.0)	15
Rice bran	(12.3)	9
Sunflower meal	(40.0)	5
Vitamin and mineral mixture	-	1
Total	-	100

Experimental diets of Azolla (treatments)

Constituents (%)	T0	T1	T2	T3	T4	T5
Control diet	100	75	50	25	-	-
Azolla meal	-	25	50	75	100	-
Fresh Azolla	-	-	-	-	-	100
Total	100	100	100	100	100	100
Dry Matter (DM)	90.0	90.5	91.0	91.0	92.0	8.0
Crude Protein (CP)	26.0	26.0	26.0	26.0	26.0	26.0
Ether Extract (EE)	5.3	4.9	4.6	4.2	3.9	3.9
Crude Fiber (CF)	4.7	6.4	8.1	9.8	11.5	11.5
Ash Content	6.1	8.3	10.5	12.7	14.9	14.9
Nitrogen Free Extract (NEE)*	57.9	54.4	50.8	47.3	43.7	43.7
Gross Energy (Kcal/Kg diet)**	4283	4105	3933	3756	3584	3584
Protein / Energy ratio (mg/Kcal)	60.7	63.3	66.1	69.2	72.5	72.5

* The values were calculated by the difference.
** Estimated by the values 5.65, 9.4, and 4.0 Kcal GE / g dry matter for CP, EE, and NEF, respectively.

A pooled sample of 10 tilapia fingerlings was chemically analyzed at the beginning of the experiment, while at the termination of the study, a pooled sample of five fish per each pond were analyzed according to AOAC (1990) methods to obtain the initial and final body composition. Analysis of variance (one way ANOVA) and Duncan's Multiple Range Test (1955) were used to determine the significance of differences among treatment means using SAS (1990) Program.

RESULTS AND DISCUSSION

Growth performance and Azolla utilization

In the present study, the chemical composition of *Azolla pinnata* indicates that protein content represented about 26% on dry matter basis. Therefore, the target control diet was incorporated on the base of the same level of protein in Azolla (Table 1). Several investigators have determined the optimum protein requirement for *O. niloticus* fingerlings and the results are not consistent. It was equal to 25% (Wang *et al.*, 1985), 35% (Teshima *et al.*, 1985).

Results presented in Table 2 and Fig 1 revealed that the growth performance and feed utilization efficiency of the Nile tilapia (*O. niloticus)* were significantly reduced with increasing level of Azolla meal in the diet. This reduction was extremely sharp when dry or fresh Azolla were used as the only dietary source. However, the control group was not significantly different ($P>0.05$) compared to T1 and T2 (25 and 50% Azolla meal in the diet, respectively). Similar trend was obtained by Antoino *et al.* (1986), Micha *et al.* (1988) and El-Sayed (1992). They found that incorporation of Azolla into the diets of *O.niloticus* resulted in suppressing fish growth, food conversion efficiency and protein retention. Almazan *et al.* (1986) observed a loss of 24-28% of body weight resulted from feeding Nile tilapia on fresh, solar-dried pellets and powder from Azolla alone. Meanwhile, feeding on pellets with 75% dried Azolla enabled the fish to grow positively, however the growth rates were still significantly lower than those for control group.

On the other hand, Santiago *et al.* (1988) found that the growth of Nile tilapia increased and feed ratios improved as the level of the dietary Azolla meal increased. Moreover, the levels of Azolla in the diets did not affect the survival rate. The tested groups in their experiment were fed diets containing 35% crude protein and various levels of Azolla up to 42.5% were included.

It is worthy mentioning in the present study that the survival rate was 95 and 90% for fingerlings *O. niloticus* fed only Azolla meal and fresh Azolla, respectively. While all groups in the other treatments survived (Table 2). The relative growth rate of fish fed fresh Azolla was very little (Figure 1) as a result of high moisture content in fresh Azolla (94%) which represents a water excretory load for fish and leads to consuming high energy to osmoregulation. On the other hand, highly water productivity (bloom) was observed for fresh Azolla (T5) when compared with other treatments. This result indicates that fresh Azolla enriches the aquatic medium as a result of increase primary and secondary production (Phyto- and Zooplankton) which are consumed by the fishes as a natural food.

Fish fed Azolla meal grows faster than those fed fresh Azolla (Figure 1). This result was confirmed by Pullin and Almazan (1983), who reported that dried Azolla could be a useful food even for microphagous tilapias such as *O.niloticus*. In addition, there were no deficiencies with respect to essential amino acids, essential lipids micronutrients. However, the negative effect for high levels of Azolla inclusion on gain performance had been investigated by Buckingham *et al.* (1978). The authors claimed that this effect might be due to the deficiency of specific free amino acids in Azolla (lysine, methionine and histidine), high natural detergent fiber (NDF), high ash content and low digestibility. While, this growth decline owed to deficiency of methionine, cystein and lysine (Van Hove and Ruelle, 1989) or the presence of growth inhibitors (Wery *et al.*, 1987).

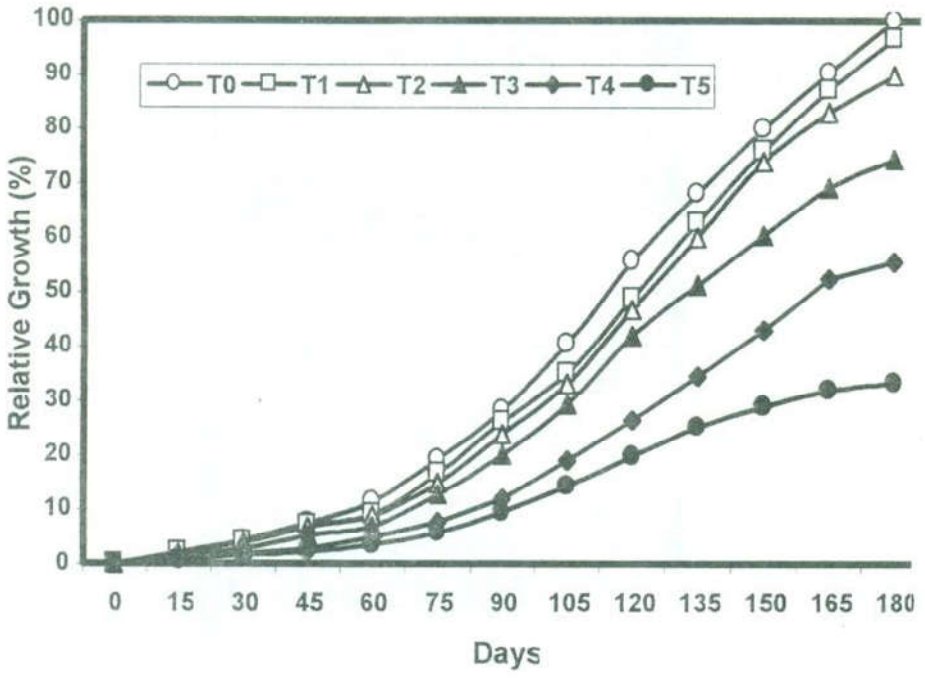

Figure 1. The relative growth rateof fingerlings *O. niloticus* fed diets containing different levels of Azolla as a percentage of control.

Table 2. Growth performance parameters (mean ± standard deviation) of fingerlings Nile tilapia (*O. niloticus*) fed diets containing various levels of Azolla

Growth performance	Experimental diets of Azolla					
	T0 (Control)	T1	T2	T3	T4	T5
Initial weight (g/fish)	5 a ± 0.17	5 a ± 0.13	5 a ± 0.15	5 a ± 0.12	5 a ± 0.13	5 a ± 0.14
Final weight (g/fish)	190 a ± 3.52	184 a ± 3.90	172 a ± 3.61	140 b ± 3.14	95 c ± 3.09	60 d ± 2.10
Final total length (cm/fish)	21.0 a ± 0.91	20.8 a ± 0.78	20.5 a ± 0.83	19.2 b ± 0.99	17.0 b ± 0.97	15.0 c ± 0.80
Condition factor (Kc)	2.05 a ± 0.27	2.04 a ± 0.30	2.00 a ± 0.25	1.98 b ± 0.22	1.93 c ± 0.16	1.78 d ± 0.10
Weight gain (g/fish)	185 a ± 3.18	179 a ± 3.26	167 a ± 3.17	135 b ± 3.05	90 c ± 2.94	55 d ± 2.11
Daily gain (g/fish/day)	1.03 a ± 0.09	0.99 a ± 0.10	0.93 a ± 0.07	0.75 b ± 0.06	0.50 c ± 0.06	0.31 d ± 0.05
Percentage of weight gain	3700 a ± 32	3580 a ± 29	3340 b ± 27	2700 c ± 22	1800 d ± 20	1100 e ± 16
Growth as % of control	100.0	96.7	90.3	73.0	48.6	29.7
Specific growth rate (SGR)	2.01 a ± 0.16	2.00 a ± 0.15	1.97 a ± 0.12	1.85 b ± 0.11	1.64 c ± 0.09	1.38 d ± 0.06
Feed conversion ratio	1.97 c ± 0.20	1.97 c ± 0.24	1.98 c ± 0.13	2.10 b ± 0.39	2.63 b ± 0.42	3.20 a ± 0.53
Feed conversion efficiency	0.51a ± 0.05	0.51 a ± 0.05	0. 51 a ± 0.04	0.48 b ± 0.03	0.38 b ± 0.02	0.31 c ± 0.01
Protein efficiency ratio	1.96 a ± 0.41	1.96 a ± 0.35	1.94 a ± 0.29	1.83 b ± 0.22	1.46 c ± 0.14	1.10 d ± 0.08
Survival rate (%)	100	100	100	100	95	90
Fish yield (Kg/m^2)	1.480 a ± 0.13	1.432 a ± 0.12	1.336 a ± 0.10	1.080 b ± 0.09	0.684 d ± 0.07	0.396 d ± 0.05

Means with the same letter in the same row are not significantly different (P>0.05).

Body composition

Body composition was significantly affected (P<0.05) by the level of Azolla in the diet (Table 3). Fish fed on fresh Azolla (T5) had significantly higher (P< 0.05) moisture content than the other treatments. However, T1, T2, T3, and T4 exhibited slight increases in moisture content when compared with T0 (the control treatment) and these increases were not significant (P>0.05). Both protein and lipid contents were negatively correlated to Azolla level in the diets (P<0.05), while ash content showed a positive correlation (P<0.05). On the other hand, the differences between T0, T1 and T2 were not significant (P>0.05) for each of protein, lipid and ash, respectively. The present results are in agreement with those of El-Sayed and Abdel Aziz (1990) and El-Sayed (1992). On the other hand, Micha *et al.* (1988) found that fresh Azolla in the diets for *O. niloticus* and *T. rendalli* fingerlings increased the water and ash contents and reduced drastically the lipid content of body tissues for both species of fish whereas crude protein content was not affected.

Regarding protein utilization, the analysis of variance (ANOVA) indicated that, feeding the control diet significantly (P<0.05) improved the protein retention (PR) and protein productive value (PPV%) compared with T3, T4 and T5. However, T1, T2 and T0 (control) were not significantly different (P> 0.05) regarding PR and PPV%. Meanwhile the present results are supported by Micha *et al.* (1988), who found that the apparent net protein utilization and protein efficiency ratio were decreased with increasing the dietary Azolla content for *O. niloticus*. This decrease was attributed to the lack of methionine, lysine and histidine and low digestibility (Buckingham *et al.*, 1978). Moreover, *A. pinnata* is low in tryptophan and threonine compared to the requirements for the same amino acids reported for *O. mossambicus* (Jauncey *et al.*,1983) and *O. niloticus* (Santiago, 1985). Other essential amino acids may actually be limiting when correlated for their biological availability.

Table 3. Gross body composition of experimental fish at the beginning and end of the experiment (calculated as % on dry weight basis) and protein utilization of *O. niloticus* fed with the experimental diets containing different levels of Azolla

Treatments	Body composition (%)				Protein utilization	
	Moisture	Protein	Lipid	Ash	retained (g)	PPV %
Initial :	70.2	55.4	21.9	22.0	-	-
Final :						
T0 (control)	73.6^{b}	61.9^{a}	25.0^{a}	12.7^{c}	30.22^{a}	31.05^{a}
T1	74.0^{b}	62.0^{a}	24.4^{a}	13.0^{a}	28.84^{a}	30.60^{a}
T2	74.3^{b}	61.7^{a}	23.5^{a}	14.7^{c}	26.45^{a}	29.87^{a}
T3	73.9^{b}	60.7^{b}	21.6^{b}	17.5^{b}	21.35^{b}	27.93^{b}
T4	74.1^{b}	58.0^{c}	18.5^{c}	23.2^{a}	13.45^{c}	20.70^{c}
T5	77.0^{a}	56.3^{d}	17.7^{d}	25.0^{a}	6.94^{d}	13.90^{d}

Means in the same column having the same superscript are not significantly different (P>0.05).

Economic evaluation

The data given in Table 4 revealed that the cost was affected by the feed conversion ratio and the price of one ton of each experimental diet. The production cost of one ton fish gain was the highest and the most expensive with feeding the control diet. This cost decreased gradually with increasing level of Azolla meal in the diet. Collins and Delmendo, (1979) reported that the feeding costs represent about 50% of the total costs in fish production provided that all other costs are constant.

Fresh Azolla was the cheapest in feeding cost to produce one ton of fish gain followed by dried Azolla (Azolla meal), but the marketing fish size was the lowest as a result of extremely poor growth performance. On the other hand, fish size was not significantly affected ($P>0.05$) when Azolla meal was incorporated up to 50% in the diet. It was also obvious from Table (4) that relative return for fish production per Feddan reached its highest (117.35%) value using 25% Azolla meal in the diet followed by 50% Azolla (110.84%), then the value sharply decreased with increasing level of Azolla. The same trend was also observed for the marketing income, which depends on the average fish size.

Table 4. Economical analysis for production of *O. niloticus* fed diets containing different levels of Azolla

Item	Experimental diets of Azolla					
	T0	T1	T2	T3	T4	T5
Production one ton fish yield:						
Average fish size (No. of fish / Kg)	5.26	5.43	5.81	7.14	10.53	16.67
1- Consumed diet (Ton)	1.967	1.977	1.984	2.100	2.631	3.204
2. Cost per ton diet (L.E)	900	763	625	488	350	175
3. Feeding cost (L.E)	1770	1508	1240	1025	921	561
4. Total costs (L.E)	3540	3016	2480	2050	1842	1122
5.Fish marketing price (L.E/ton)	6000	6000	5500	5000	4000	3000
6. Net return (L.E)	2460	2948	3020	2950	2158	1878
For fish production / Feddan*:						
1. Fish yield (ton)	5.920	5.728	5.344	4.320	2.736	1.584
2. Consumed diet (ton)	11.645	11.324	10.602	9.072	7.198	5.075
3. Feeding cost (L.E)	10480	8640	6626	4427	2519	888
4. Total costs (L.E)	20960	17280	13252	8854	5038	1776
5. Marketing income (L.E)	35520	34368	29392	21600	10944	4752
6. Net return (L.E)	14560	17088	16140	12746	5906	2976
7. Monthly return (L.E)	2427	2848	2690	2124	984	496
8.Relative return to the control (%)	100.00	117.35	110.84	87.52	40.54	20.44

* Feddan = 0.42 hectare, and the actual aquaculture area for Feddan is about 4000m^2.

Fish yield (ton) = fish yield (Kg/m^3) x 4000 x 10^{-3}

Total cost (L.E.) = 2 x feeding cost (L.E.)

Net return (L.E.) = marketing income (L.E.) – total cost (L.E.)

Monthly return (L.E.) = net return (L.E.) / culture period (6 months)

In conclusion, the present study recommended that fresh *A. pinnata* could be used as a supplementary feed, while the dried Azolla could be used as a dietary component up to level of 50% for *O.niloticus* to obtain a reasonable fish production with economical income return.

ACKNOWLEDGEMENT

I would like to thank Dr. F. M. Ghazal, Professor of Agriculture Microbiology, Soils, Water & Environment Institute, Giza, Egypt, for his help in revising the manuscript.

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تقييم انتاج أسماك البلطى النيلى المغذاة علي الأزولا بيناتا الطازجة والجافة فـــى الاستزراع السمكى الشبه مكثف

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تم اجراء البحث فى ١٦ حوض خرسانى مستطيل (حجم الحوض حوالى ٢,٥ م^{٣}) بوحدة إنتاج الأسماك بكلية الزراعة _ جامعة القاهرة بغرض دراسة إمكانية الإستفادة من نباتات الأزولا بيناتا الطازجة أو المجففة (مسحوق الأزولا) كعليقة جزئية أو كلية لتسمين أصبعيات أسماك البلطى النيلى. تم تحليل عينة من أصبعيات البلطى (١٠ سمكات) وعينة من نباتات الأزولا قبل البدأ فى التجربة ووجد أن الأزولا تحتوى علـى ٢٦ % بروتين خام على أساس المادة الجافة وبناءا على ذلك تم إعداد العليقة الكنترول بـها نفـس المحتـوى مـن البروتين وتم تجفيف كمية من نباتات الأزولا وطحنها لتكوين المعاملات الغذائية التاليـة (بمعـدل تكـرار حوضين لكل معاملة) :

(١) ١٠٠ % عليقة كنترول + صفر % مسحوق الأزولا.
(٢) ٧٥ % عليقة كنترول + ٢٥ % مسحوق الأزولا.
(٣) ٥٠ % عليقة كنترول + ٥٠ % مسحوق الأزولا.
(٤) ٢٥ % عليقة كنترول + ٧٥ % مسحوق الأزولا.
(٥) صفر % عليقة كنترول + ١٠٠ % مسحوق الأزولا.
(٦) صفر % عليقة كنترول + ١٠٠ % أزولا طازجة.

تم استزراع نباتات الأزولا الطازجة فى ٤ أحواض بينما أصبعيات البلطى النيلى (متوسط وزن السـمكة ٥جم) فى ١٢ حوض بكثافة استزراع ٨ اصبعيات للمتر المكعب. وكان معدل التغذية ٣ % (على أسـاس الوزن الجاف للعليقة) من وزن الكتلة الحية للأسماك مع ضبط هذة الكمية كل أسبوعين حيث تم أخـذ عينـة عشوائية مكونة من ٥ أسماك من كل حوض مرة كل أسبوعين وقياس أطوال وأوزان كل سـمكة بـها ثـم إعادتها للحوض. واستمرت التجربة ٦ شهور بداية من أول أبريل حتى نهاية سبتمبر ١٩٩٨ . ومن الدراسـة وجد أن معدلات النمو ونسبة التحويل الغذائى وكفاءة الاستفادة من البروتين للأسماك لم تنخفـض معنويـا ($P>0.05$) عند زيادة مستوى الأزولا فى العليقة حتى ٥٠ % بينما حدث انخفاض معنوى فى النمـو عنـد زيادة النسبة عن ذلك. أما الأسماك المغذاة على الأزولا الطازجة فقـط كـان معـدل نموهـا بطيئـا جـدا

($P<0.05$). ولدراسة تأثير المعاملات على مكونات الجسم الرئيسية تم التحليل الكيميائى لخمس سمكات من كل حوض عند نهاية التجربة ووجد أن محتوى كل من البروتين والليبيدات أنخفض عند زيادة مستويات الأزولا فى العليقة بينما محتوى الرماد أظهر علاقة عكسية. أما فى حالة التغذية على الأزولا الطازجة بمفردها فقد نتج عنها أقل قيم لمحتوى كل من البروتين والليبيدات وأعلى قيمة لمحتوى الرماد فى جسم الأسماك. كما تناولت الدراسة أيضا التقييم الأقتصادى للأنتاج السمكى لكل معاملة على أساس كمية العليقة المستهلكة، تكاليف التغذية، متوسط حجم السمكة، سعر تسويق الأسماك و العائد الصافى لانتاج واحد طن من الأسماك أو لاستزراع فدان عند كل معاملة.