

## EFFECT OF USING TOMATO MEALS (*SOLANUM LYCOPERSICUN. L.*) AND/OR *SPIRULINA ALGAE* (*SPIRULINA PLATENSIS*) AS ALTERNATIVE PROTEIN SOURCE IN GRASS CARP (*CTENOPHARYNGODON IDELLA*) DIETS

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### SUMMARY

The experiment was conducted to partially replace fish meal (FM) by *Spirulina platensis* (Sp) and tomato meal (TM) protein for grass carp diets. The control diet, 100% protein FM was used as T1 and three experimental diets were formulated to replace FM with Tomato meal (25%) T2, Sp at levels of (25%) T3 and Mixture TM (25%) + Sp (25%) T4 (50%). Diets were twice fed daily for nine weeks to triplicate groups 10 fish (initial mean weight  $1.77 \pm 0.11$  g) kept in aquaria. All diets were formulated to be iso-nitrogenous ( $30.33 \pm 0.29\%$  CP) and iso-caloric ( $4467.5 \pm 0.41$  Kcal/kg diet). Fish were fed the tested diets at a rate of 6% of their wet body weight / day, in two equal portions, 6 days a week then gradually reduced to 4 % of the total fish biomass daily. Provided feed was adjusted bi- two weekly according to the changes in body weight. The fish fed T4 diet TM (25%) & Sp (25%) and T3 diet (Sp (25%)) recorded the highest value of feed and nutrient utilization while the fish fed tomato meal 25% (T2) was the lowest value compared with the fish fed control diet (T1). However, there were not significantly of nutrient utilization with experimental diets compared with the control diet (T1). Results indicated that up to 50% of FM can be replaced by algae meal and tomato meal without significantly affected performance, feed utilization and whole body composition of fish in practical diets of Grass carp. Also, mixture meals (as its protein is generally less expensive than fish meal protein) can reduce feed cost and feed cost/kg gain. Under the experimental conditions it seems that the diet contained mixture meal was the most promising diet.

**Keywords:** *Spirulina, meal, tomato meal, Grass carp, performance, feed utilization, body chemical composition and economic evaluation.*

### INTRODUCTION

Diets are one of the major cost variables for most aquaculture species, representing up to 70% of operating costs. Fish meal (FM) is used as a major protein source within most finfish and crustacean diets (Lovell, 1989). Due to the high cost of fish meal and other considerations, there is interest in the partial or total replacement of this ingredient with less expensive plant protein meals without adversely affecting growth and health of culture species (Hardy, 1996).

Tomato is one of the major vegetables of world production; the annual production of tomato in Egypt is 8.1 million tones resulting in production of 20.3% of manufacturing by-product. This by-product is a good source of amino acid energy and crude fiber. In addition, it contains more essential amino acids for animals. Tomato pomace is a cheap source of energy and nutrients than many alternative ingredients with potential biological value by Hoda *et al.* (2013). Tomato wastes were one of the wastages produced in excessive amounts by vegetable processing industries. Meanwhile the residue is a good source of vitamin B1 and a reasonable source of vitamin A and B2, also contained 1.8% leucine, 1.7% lysine and 0.1% methionine El-Kholy *et al.* (2009). El-Dahhar *et al.* (1993) investigated the effect of different levels 10, 20 and 30% of tomato waste (TW) on growth and feed utilization of Nile tilapia fish. The results showed that protein productive value and protein efficiency ratio were improved when 10% of fish meal protein was replaced by (TW), however increasing the level of replacement above 10% decreased body weight gain. Magdy and Saeed (2008) showed that the possibility of replacing 30% of dietary protein by silage dried meal (tomato and potato by-products) in tilapia diets without adverse effect on growth or feed

utilization parameters and this replacement reduced feed costs/kg diet and feed costs/kg weight gain by 22.43 and 22.02%, respectively. The *Spirulina* alga is rich in protein and vitamins, and can be used to improve the immunity capacity of the animals which consume it. Consumption of *Spirulina* alga also increases the ability to absorb nutrients. When *Spirulina* alga is used as feed for young prawns and fingerlings, the fish exhibit good coloring, as well as maintain a low death rate and a high growth rate Sermwattanakul and Bamrungtham (2000). *Spirulina* is a cyanobacterium that has been commercially cultivated for more than 10 years due to its high nutritional content; e.g. protein, amino acid, vitamin, minerals, essential fatty acid and  $\beta$ - carotene Vonshak (1997). Positive effects of *Spirulina* on growth, feed utilization and stress and disease resistance of cultured fish have been reported in earlier studies. Qureshi and Ali (1996). High protein content of *Spirulina* as well as its well-balanced amino acid profile compared with other plant protein sources makes it as potential fish meal replacer in aquafeed formulation (Hanel et al., 2007). *Spirulina* was reported to replace up to 40% of fish meal protein in tilapia (*Oreochromis mossambicus*) diet Olvera-Novoa et al., 1998) and even higher replacement of fish meal was possible in common carp (*Cyprinus carpio*) (Nandeesha et al., 1998) and Mekong giant catfish (*Pangasianodon gigas*) (Tongsiri et al., 2010).

It has been found that the alga can be used as an alternative source of protein and can also be used to improve the color, flavor and quality of meat. Nowadays, *Spirulina* can be used to establish immune-potentiating functions in carp (Watanuki et al., 2006 and Tongsiri et al., 2010). *Spirulina* contain huge proportions of proteins and carbohydrates; the dried *Spirulina* is an exceptional feed for various aqua cultured organisms (Devdatta, 2014).

The present study aimed to determine the growth performance of Grass carp fingerlings fed diets containing *spirulina* (*Spirulina platensis*) and tomato meals and mixture as a substitute for fish meal protein supplements.

## MATERIALS AND METHODS

The present study was carried out at the Utilization By-products Department, Animal Production Research Institute, Agriculture research Center, Egypt. Sample amount of tomato by-products was provided by Heinz Company, 6 October, Cairo, including skin, seeds and hard tissues were sun-air dried for seven days and stored in bags until use. While *Spirulina platensis* grow on the tomato by-products.

### ***Experimental diets:***

Four experimental diets were formulated where fish meal  $T_1$  (control diet) was replaced by tomato meal( $T_2$ ), *Spirulina platensis* meal ( $T_3$ ) and mixed of tomato and *Spirulina platensis* meals ( $T_4$ ) where it replaced 0, 25, 25 and 50%, respectively. All diets were formulated to be iso-nitrogenous ( $30.33 \pm 0.29\%$  CP) and iso-caloric ( $4467.5 \pm 41$  Kcal/kg diet). Tomato and *Spirulina platensis* meal chemical composition and diets ingredients and its chemical composition are presented in Tables (1 and 2), respectively. Diets were formulated by mixing thoroughly the dry ingredients. The diets was added in water first and mixed thoroughly. All diets were pressed through meat mince (0.5 mm diameter) and sun dried for 3 days. Representative samples of fish were taken at the start and at the termination of the study and frozen at  $-18^{\circ}\text{C}$  for chemical analysis. Chemical analyses of diets and fish were made as described by AOAC (2000). Data collected included growth performance parameters, feed intake, feed utilization and body chemical composition (dry basis, (Richardson et al., 1985). The economic evaluation was calculated by the cost of one kg of feed and weight gain of fish (Table 5).

### ***Experimental fish:***

A total number of 120 Grass carp fingerlings (*Ctenopharyngodon idella*) averaging of an initial average body weight of  $1.77 \pm 0.11$  g (Table 3) were obtained from commercial fish hatchery at El-Hamol Kafr El shiekh Governorate. The fish weighted in bulk at the start of experiment and the fish were adapted for 15 days before starting the experiments which lasted for the actual experimental period 91 days. The fish were stocked at a density of 10 fish / aquarium (70 Liter each) in triplicate via water recirculation system with water rate exchange approximately 10% of total volume per day. The daily ration was divided into two equal portions and was offered handling two times a day at 9.00 and 13.00 hrs at a level of 6% of body weight. The fish in each triplicate were weighed biweekly at the 14<sup>th</sup> day whereas, the feeding was stopped at this day to weighing and the amount of daily diet was adjusted accordingly.

**Table (1). Proximate analysis of feed ingredients on DM basis %.**

Ingredients	Crude protein	Ether extract	Crude fiber	Moisture	Ash	NFE	( <sup>1</sup> GE) Kcal/kg
Fish meal	65.00	5.71	1.02	7.1	14.87	13.4	4748
Soybean meal	43.12	1.49	7.19	8.85	6.23	41.97	4539
Yellow corn	7.21	4.02	2.67	11.19	1.27	84.83	4231
Wheat bran	13.73	3.35	11.62	10.78	7.11	64.19	4222
Corn Gluten	60.42	2.04	1.36	9.45	1.28	34.90	5031
<i>Spirulina</i> (SP)	56.00	5.50	9.00	3.50	7.50	22.00	4614
Tomato meal	22.54	8.50	38.00	6.05	4.50	26.46	4652

<sup>1</sup>Gross energy (GE Kcal/kg) was 5.65 kcal/g for protein; 9.45 kcal/g for lipid; 4.00 kcal/g for crude fiber & 4.10 kcal/g for carbohydrates Jobling, (1983).

**Table (2). Formulations and proximate composition of the tested diets.**

Item	Treatments*			
	T1	T2	T3	T4
<b>Feed Ingredients, %:</b>				
Yellow corn	35..0	26.91	34.61	26.53
Soybean meal	30.0	30.0	30.0	30.0
Corn Gluten	6.0	7.0	6.0	7.0
Wheat bran	8.0	8.0	8.0	8.0
Fish meal, 65%CP	15	11.25	11.25	7.49
Tomato meal	-	10.84	-	10.84
<i>Spirul.</i> Meal	-	-	4.14	4.14
Vegetable oil	4	4	4	4
Vitamins minerals **	1.50	1.50	1.50	1.50
DI calcium phosphate	0.50	0.50	0.50	0.50
Total	100	100	100	100
<b>Proximate chemical composition::</b>				
DM, %	91.97	92.51	92.09	92.30
CP, %	30.49	30.20	30.35	30.29
EE, %	6.13	6.37	5.30	5.40
CF, %	5.82	8.77	5.78	9.82
Ash, %	7.82	7.12	8.20	7.40
NFE, %	49.74	46.54	50.37	74.09
GE kcal/kg***	4483	4488	4447	4452
Ca, %	0.93	0.72	0.73	0.62
TP, %	0.55	0.45	0.45	0.40

\*Percentage is from the diet; T<sub>1</sub>: control, T<sub>2</sub>: 25% tomato, T<sub>3</sub>:25% *Spirulina*, T<sub>4</sub>:50% Mix. (25 %tomato &25% *Spirulina*), other abbreviations are as footnoted in Table (1).

\*\* Each 1Kg contains vitamin: B1,1.4g; B2 ,0.8g; B6, 3.8g, B12,4.2g; pantothenic acid, 7g; nicotinic acid, 400mg; folic acid, 25g; biotin, 150g ;choline chloride, 5g ; A, 5000 000, I. U; D3, 1000 000, I. U;4g; K,0.5g; copper, 0.5g; iodine, 10g; manganese, 20g and Zinc, 0.07g.

\*\*\* Gross energy was 5.65 kcal/g for protein; 9.45 kcal/g for lipid; 4.00 kcal/g for crude fiber & 4.10 kcal/g for carbohydrates (Jobling, 1983).

**Table (3). Growth performance parameters of Grass carp as affected by the based diet.**

Item	Initial weight (g/fish)	Final weight (g/fish)	Weight gain (g/fish)	SGR(%day)
Control diet (T <sub>1</sub> )	1.75 ±.04	8.26 ±0.58	6.51±0.54	1.70 <sup>b</sup> ±0.52
Tomato (25%) (T <sub>2</sub> )	1.76 ±0.09	8.21 ±.59	6.45±0.50	1.69 <sup>b</sup> ±0.02
Spirulina sp(25%).(T <sub>3</sub> )	1.83 ±0.08	9.34 ±0.66	7.51±0.57	1.79 <sup>b</sup> ±0.03
Mix (50%) (T <sub>4</sub> ) (25%tomat. &25%Spiru.)	1.72 ±0.10	10.20 ±0.83	8.48±0. 73	1.95 <sup>a</sup> ±0.03

<sup>a</sup>and <sup>b</sup> Means with different superscripts in the same column within each item differ significantly ( $P<0.001$  level).

SGR, specific growth rate, (%/d) = 100 ( $\ln$  final weight –  $\ln$  initial weight)/period in days NB, no mortalities were detected throughout the study and fish was in good conditions.

### **Preparation of algae:**

The experimental algae were planted at Agricultural Microbial Department, Soils, water and Environment Research Institute (SWERI) Agricultural Research Center (ARC) Giza, Egypt, (*S. platensis* strain LEB-18 was cultivated). The tanks of *Spirulina* are protected from UV radiation by the use of a transparent film. When the concentration reached 0.50 g L<sup>-1</sup> (maximum growth), the biomass was separated by filtration, washed and dried at 50°C for 5 h and stored under refrigeration . *Spirulina* biomass value was calculated through Optical Density (OD) measurements using a spectrophotometer (UV/VIS to 670 nm) and a calibration curve of OD against dry weight (g L<sup>-1</sup>) of *Spirulina* biomass. After harvesting, the biomass was crushed into a ball mill (88 µm) for analyses. (Zarrouk, 1966).

### **Growth performance and feed utilization parameters:**

Growth response parameters were calculated according to Cho and Kaushik (1985) as follows: SGR (specific growth rate) = 100 (ln final weight - ln initial weight)/No of days. Where: ln is the natural log FCR (feed conversion ratio) = total dry feed intake (g)/total wet weight gain (g). PER (protein efficiency ratio) = wet weight gain (g)/protein intake (g). Feed intake = total dry feed fed (g/fish) (Richardson *et al.*, 1985). FER (feed efficiency ratio) = wet weight gain (g)/dry feed intake (g). Total gain (g/fish) = Final weight (g) - Initial weight (g). Average daily gain (ADG) (g/fish/day) = total gain/duration period in day; Protein productive value (PPV %) = (P<sub>T</sub> - P<sub>I</sub>) × 100/protein intake (g) Where: P<sub>T</sub>: Protein content in fish carcass at the end and P<sub>I</sub>: Protein content at the start. Energy utilization (EU %) = (E<sub>T</sub>-E<sub>I</sub>) × 100 / Energy intake (kcal). Where: E<sub>T</sub>: Energy in fish carcass (kcal) at the end and E<sub>I</sub>: Energy in fish carcass at the start.

### **Proximate analysis of diet and fish:**

Chemical proximate analysis of feed ingredients is shown in Table (1). The chemical analysis of ingredients, diets and fish samples were analyzed according to AOAC (2000) methods. Gross energy (GE) contents of the experimental diets and fish samples were calculated by multiplying CP x 5.65 ± fat x 9.45 ± Carbohydrate x 4 (Jobling, 1983). At the start of the experiment, 25 fish were sampled and stored at -20°C for analysis of whole body composition. After the final weighing (91 days), ten fish per aquarium were withdrawn and frozen at -20°C till analysis.

### **Water quality:**

Water temperature was maintained at 24 ± 1 °C. Rates of water flow ammonia 0.1-0.3 mg l<sup>-1</sup>, and pH 6.8-8.2. No critical values were detected for nitrite (NO<sub>2</sub>) and nitrate (NO<sub>3</sub>) radicals. Analytical methods were done according to American Public and adjusted to maintain oxygen saturation above 80%. The water quality parameters in the system were monitored every other day and the ranges were: dissolved oxygen 6.5-8.0 mg l<sup>-1</sup>, total (APHA, 1992). The pH and water temperature values were determined by digital temperature and pH meter. Dissolved oxygen was monitored by using Oxy meter, Jan way model 9071.

### **Statistical analysis:**

Biological data obtained from the treatments were subjected to statistical evaluation using one-way analysis of variance (ANOVA) of the general liner model (GLM) using SAS (2006) statistical package. Duncan's multiple range test (Duncan, 1955) was used to test the significance (P<0.001) of differences among means. Collected data were subjected to statistical analysis using one-way-analysis of variance according to Snedecor and Cochran (1980) uses the following mathematical model:

$$Y_{ij} = \mu + T_i + e_{ij}$$

Where: Y<sub>ij</sub> is the parameter under analysis,  $\mu$  is the overall mean, T<sub>i</sub> is the effect due to treatment and e<sub>ij</sub> is the experimental error.

## **RESULTS AND DISCUSSION**

### **Growth performance:**

Results of growth performance of Grass carp as affected by the dietary treatments are presented in Table (3). Results revealed that the initial weights of the experimental fish ranged between 1.72 and 1.83 g with insignificant (P<0.001) differences among the treatment groups. The highest final weight (10.20

g/fish) was shown with T<sub>4</sub> which contained (25% tomat & 25% *Spiru.*) compared to the other treatment groups. The lowest final weight (8.21g/fish) was shown with T<sub>2</sub> which contained (25% tomato). Whereas the fish fed of T<sub>2</sub> had insignificantly lower values of SGR than fish fed the all diets except T<sub>4</sub> which had higher ( $P<0.001$ ) significant values as compared with the control group (T<sub>1</sub>), T<sub>2</sub> and T<sub>3</sub>. The highest final weight gain (WG g/fish) was observed in T<sub>4</sub> followed by T<sub>3</sub>, T<sub>1</sub> and T<sub>2</sub> with insignificantly differences (8.48, 7.51, 6.51, 6.45 g/fish, respectively). Belay *et al.* (1996) and Hayashi *et al.* (1998) concluded that feeding *Spirulina* to fish could improve their survival and growth rate. Moreover, Nandeesha *et al.* (2001) recommended that, it was suitable to use *Spirulina* as a protein supplement source for fish. The present results are in agreement with those of Tongtsiri *et al.* (2010) who found that *Spirulina* may be used as a supplement at 5 and 10% of fish meal for the Catfish. These levels could improve the growth performance, specific growth rate and pigment of the Catfish. Ungsethaphand *et al.* (2012) showed that up to 20% of *Spirulina* can be substituted for fishmeal in a fishmeal-based diet for hybrid red tilapia without any adverse effect on fish growth. Sirakov *et al.* (2012) showed that the weight gain, condition factor and average daily growth of rainbow trout fed with 10% *Spirulina spp.* were higher than those from the group fed with feed without algae supplement, but the differences were not statistically proven ( $p>0.05$ ). A study conducted with Red Tilapia demonstrated that a 10-15% replacement of crushed fish as a protein source with *Spirulina* algae led to an increase of average daily growth rate and specific growth (Promya and Chitmanat (2011).

#### **Feed and nutrient utilization:**

The effects of replacing fish meal with tomato and *Spirulina* meals on feed intake, feed conversion ratio (FCR), protein efficiency ratio (PER) protein productive Value (PPV%) and energy utilization (%) of Grass carp fingerlings are illustrated in Table (4). The fish fed T<sub>4</sub> diet (25% tomat.& 25%*Spiru.*) followed by T<sub>3</sub> diet (25% *Spiru. meal*) recorded the highest values of feed and nutrient utilization while the fish fed 25% Tomato meal (T<sub>2</sub>) recorded the lowest values. However, there were no significantly ( $P>0.001$ ) of nutrient utilization with experimental diets compared to the control diet (T<sub>1</sub>). The lowest values of T<sub>2</sub> may be due to that plant protein as a sole source of protein in low protein diet contains phytic acid and other anti nutritional factors whereas, phytic acid inhibits activities of some digestive enzymes such as pepsin, trypsin and alpha-amylase (Liener, 1994). The present results are in confirmation of the data received from Pókniak (2010), who found that 5% of the spirulina meal can be incorporated in the feed for rainbow trout fry without a significant affect to their productive performance (body weight, feed intake, feed conversion and specific growth rate) or the mortality of the fry. Similar data were received in a research conducted to investigate the replacement of fish meal in the feed for *Pangasianodon gigas* Chevy with *Spirulina meal* at 0, 15, 30 and 100% (Ahmadzadenia *et al.*, 2011). Watanabe *et al.* (1990) reported that, *Spirulina* diet improved the growth rates for striped jack, *Pseudocaranx dentex* at 5% supplementation in the feed. These results may be attributed to that the dietary inclusion of *Spirulina* leads to improve the protein digestibility. Also, Ayyappan (1992) founded that 10% *Spirulina* diet improved SGR and WG for carp fry using six different species of carp, hybrid catfish and *O. niloticus*. These results may possibly due to the improved feed intake and nutrient digestibility. Moreover, *Spirulina* contains several nutrients especially vitamins and minerals that may help in fish growth promotion. The present results agree with those found by Belay *et al.* (1996), Hayashi *et al.* (1998), Hirahashi *et al.* (2002) who reported that feeding *Spirulina* to fish and poultry improved survival and growth rates. Moreover Dawah *et al.*, (2002) found that feed conversion ratio and PER were better when fish were maintained on artificial diets with 10% and 20% dried algae. In addition, Hayashi *et al.* (1998) and Abu-Zead (2001) found that the protein efficiency ratio ranged from 1.1 to 1.7 for Nile tilapia and common carp fed on diets containing aquatic plant and algae. The present results are also in agreement with those of Saad (1998) who concluded that the feed conversion ratio was the best in tilapia groups fed diet contained 10% tomato waste meal, while the addition of 5.63% tomato waste meal to the diet of carp improved their feed conversion ratio specially when contained soybean meal. On the other hand, the results go quite different with those of Al-Shamma *et al.* (1997) who used tomato waste to replace maize at 0,6,12 or 18% levels in common carp fish diet. They revealed no difference in specific growth rate or feed conversion ratio between fish fed the control diet and those fed the experimental diets containing 6 and 12% tomato waste. Hassan (2004) observed that increasing the replacing level of soybean by tomato by product meal from 0 to 10% in carp diet significantly improved the FCR, PER but the higher replacing level (20 to 50%) not significantly adverse the FCR. Shams El-Din and El-Kader (1997), evaluated the chemical and biological values of tomato seeds and total tomato processing waste meal and found that they contain linoleic acid which was the major fatty acid in both tested meal oils, followed by oleic acid. Meanwhile, the explanation of these results may contribute to the amino acids content of tomato waste being methionine and lysine which are deficient in most feedstuffs but were not limiting in tomato wastes. The same authours, reported that valine, not methionine or lysine, was the

second limiting amino acid in the defatted tomato seed meal and threonine and isoleucine were the first and second limiting amino acids in the defatted total tomato processing waste meal. Research conducted by Duncan and Klesius (1996) found that *Spirulina* alga was a good source of protein for animal feed, as well as containing high amounts of vitamins and minerals. Besides this, the cellular structure of *Spirulina* alga is easily digestible and does not contain cellulose. Different levels of *Spirulina* alga can be mixed with feeds according to the eating behaviors of the fish and differing abilities to digest the protein from plant sources. *Spirulina* algae contain carotenoids, that improving health and increasing the ability to fight off infections through the reduction of stress levels (Nakono *et al.*, 2003). The results from Tongsiri *et al.* (2010) experiment indicate that 5% dried *Spirulina* could be used to replace fishmeal and it yielded the highest weight and average daily gain/day while in this research the best replacement of fishmeal was with 10% *Spirulina* and reduced the mortality of experimentally infected *O. niloticus* and these results may be attributed to the inhibition of growth of different microorganisms by Carvacrol and thymol Conner (1993). The results of *Spirulina* meal in immune enhancing properties may be due to the fact that antibodies produced through the feeding (Phromkunthong *et al.*, 2007). The results, obtained show clearly that the control diet was the highest diet to produce unit gain and the cost of producing fish gain increased according to mixture of tomato and *Spirulina* meals ( $T_4$ ) in the diet.

**Table (4). Feed utilization parameters of Grass carp as affected by the based diets.**

Item	FI	FCR	PER	PPV	EU
Control diet ( $T_1$ )	16.81 <sup>b</sup> ±0.50	2.61 <sup>ab</sup> ±0.14	1.29 <sup>ab</sup> ±0.07	19.05 <sup>a</sup> ±1.48	14.95 <sup>a</sup> ±1.30
Tomato 25% ( $T_2$ )	17.30 <sup>ab</sup> ±0.49	2.70 <sup>a</sup> ±0.13	1.24 <sup>b</sup> ±0.06	18.02 <sup>a</sup> ±1.73	14.63 <sup>a</sup> ±1.04
Spirulina sp.25%( $T_3$ )	17.12 <sup>ab</sup> ±0.50	2.30 <sup>ab</sup> ±0.11	1.46 <sup>a</sup> ±0.07	20.22 <sup>a</sup> ±1.42	15.57 <sup>a</sup> ±0.96
Mix 50% ( $T_4$ ) (tomato 25% & Spiru.25%)	19.00 <sup>a</sup> ±0.88	2.26 <sup>b</sup> ±0.09	1.48 <sup>a</sup> ±0.06	21.50 <sup>a</sup> ±1.41	17.60 <sup>a</sup> ±0.86

<sup>a, b and c</sup> Means with different superscripts in the same column within each item differ significantly ( $P<0.001$  level).

FI, feed intake in g/fish; FCR, feed conversion ratio= FI/ weight gain, g/g; PER, protein efficiency ratio = weight gain/ CP intake, g/g; %PPV, protein productive value = 100(body protein at the end – body protein at the start) protein intake; %EU, energy utilization = 100(body energy at the end – body energy at the start)/energy intake.

#### Proximate analysis of fish whole-body:

The effect of replacement of fish meal by tomato and *Spirulina meal* as protein sources on whole fish body composition of Grass carp is presented in Table (5). The highest ( $P<0.001$ ) whole fish body protein and ash content (53.03 and 9.29%) were obtained for fish fed the  $T_3$  (25% *Spirulina meal*) diet and the lowest values ( $P<0.001$ ) were obtained for fish fed ( $T_1$ ) control diet (50.58 and 7.90%) respectively. From the all aforementioned results, it seemed that mixture of the second tested Tomato and *Spirulina meal* has a synergistic effect on fish growth and decrease the degradation of protein in tissues by free radical with addition natural antioxidants like mixture Tomato and *Spirulina meal* increase the mineral absorption by increasing mineral availability and increase activity of natural antioxidants and make them water soluble with addition of sulphate in mixture (Hayam, *et al.*, 2010). The whole body lipid and Gross energy contents were increased with fed fish the  $T_2$  (25% tomato) (35.39% and 6464.86 kcal/kg) followed by  $T_4$  (50% mixture tomato and *Spirulina meal*) (35.02% and 6404.09 kcal/kg), respectively with significant differences ( $P<0.001$ ). The increase in the whole body lipid and gross energy contents with tomato and *Spirulina meal* sources as good as that in fish meal and could be related to a dietary imbalance between saturated and unsaturated fatty acids as a consequence of the high ratio of saturated fatty acids presented in the ingredients.

**Table (5). Whole body composition of Grass carp as affected by the based diets.**

Item	DM (%)	CP (%)	EE (%)	Ash (%)	GE*(kcal/kg)
Control ( $T_1$ )	25.74 <sup>a</sup> ±0.57	50.58 <sup>b</sup> ±0.45	29.78 <sup>c</sup> ±0.49	7.90 <sup>a</sup> ±0.47	6126.69 <sup>b</sup> ±14.99
TM 25% ( $T_2$ )	24.89 <sup>a</sup> ±0.48	51.43 <sup>b</sup> ±0.42	35.39 <sup>a</sup> ±0.42	8.37 <sup>a</sup> ±0.41	6464.86 <sup>a</sup> ±32.20
Sp 25%( $T_3$ )	23.23 <sup>b</sup> ±0.48	53.03 <sup>a</sup> ±0.31	33.67 <sup>b</sup> ±0.40	9.29 <sup>a</sup> ±0.32	6321.58 <sup>a</sup> ±29.40
Mix 50% ( $T_4$ )	25.05 <sup>a</sup> ±0.48	51.14 <sup>b</sup> ±0.50	35.02 <sup>ab</sup> ±0.57	8.27 <sup>a</sup> ±0.44	6404.09 <sup>a</sup> ±39.91

<sup>b and c</sup> Means with different superscripts in the same column within each item differ significantly (5% level).

\* Gross energy was 5.65 kcal/g for protein; 9.45 kcal/g for lipid; 4.00 kcal/g for crude fiber & 4.10 kcal/g for carbohydrates (Jobling, 1983).

**Economical efficiency:**

The cost of one kg fed to produce one kg fish gains (Table 6) are 11.03, 9.56, 8.40 and 7.69 L.E for T<sub>1</sub>, T<sub>2</sub>, T<sub>3</sub> and T<sub>4</sub>, respectively. It, should be mentioned that the feeding cost represent about 50% of the total costs in fish production provided that, all other costs are constant (Collins and Delmendo, 1979). However; the reduction of producing one ton fish diet are dependent on the replacement percentage of tomato and *Spirulina* meals in the diet.

**Table (6). Feed cost (L.E) for production one kg weight gain of Grass carp as affected by the based diets.**

Item	T1	T2	T3	T4
Cost/ton feed(L.E)	3816	3563	3687	3434
Change in feed cost	100	93.37	96.62	89.99
Cost of kg fish gain (L.E)	11.03	9.56	8.40	7.69
Percentage change in feed cost to produce one kg fish gain	100	86.76	76.16	69.72

*Local market price (L.E /ton) for feed ingredients used for formulating the experimental diets when the experiment was started (2014); 3700 L.E, soybean meal = 6000 L.E, fish meal L.E, yellow corn= 1800 L.E, wheat bran = 1650 L.E, corn gluten= 8500LE, corn oil = 7500L.E, Tomato meal = 300 LE and Spirulina sp. = 2500 LE. vitamin and minerals mix = 14000 L.E, Di calcium phosphate = 4800LE*

## CONCLUSION

The present study revealed that mixture meal has good protein quality comparable to fish meal protein. In conclusion, it is possible to replace 50% of fish meal protein by the mixture of *Spirulina* algae and tomato meals in feeding of Grass carp fingerlings. When adding an amount of algae (4.14%) and Tomato meal (10.84%) of the dry feed weight, it will increase growth rate, protein, feed utilization and survival rates, as well as improving the cost/performance ratio of the fish feed of the Grass carp.

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## تأثير استخدام مسحوق طحلب اسبيرولينا والطماطم كبديل بروتيني في علائق أصبعيات أسماك مبروك الحشاش وحيد الجنس ذكور

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اجريت هذه الدراسة لمعرفة اثر الاستبدال الجزئي لبروتين مسحوق الطحالب ومسحوق الطماطم كمصدادر بديل للبروتين في علائق مبروك الحشاش وزعت العلائق المختبره والتي تتكون من علائقه اساسيه التي تحتوى 100% مسحوق السمك (T<sub>1</sub>) وتلذ علائق تجريبية اخرى تم استبدال مسحوق السمك بمسحوق الطماطم 25% (T<sub>2</sub>) ومسحوق الطحالب 25% (T<sub>3</sub>) وخلط من مسحوق السمك ومسحوق الطحالب 50% (T<sub>4</sub>) على اربعه مجامي من الاسماك وتم توزيع الاسماك عشوائيا في أربع مجموعات بمعدل 10 سلمكة/ حوض وكان متوسط وزن الاسماك في بداية التجربة 1.77 جم ووزعت عشوائيا على العلائق (3) مجموعات/ علائقه . وتم تكوين علائق تحتوى على 33.30% بروتين خام ، 4467.5 كيلو كالوري طاقة كلية / كجم عليقته. غذيت الاسماك علي العلائق التجريبية لمدة 6 ايام في الاسبوع بمعدل 6% من وزن الجسم الحي على مرتين يوميا 9 صباحا و 2 مساء وخفضت تدريجيا الى 4% من وزن الجسم الحي في اليوم. (وتم تغذية الاسماك لمدة 91 يوم)

اووضحت النتائج ان اعلى معدل اداء نمو وكفاءة اقتصادية كان عند T<sub>4</sub> 50% (مخلوط 25% الطحالب 25% الطماطم ) ثم العلائق T<sub>3</sub> 25% مسحوق طحالب بينما الاسماك المغذاه علي مسحوق الطماطم T<sub>2</sub> سجلت اقل معدل نمو بالمقارنة بالعليقه الكنترول T<sub>1</sub> على التوالى.

اما سبق يتضح أنه من الممكن إستبدال بروتين مسحوق السمك جزئيا حتى 50% باستخدام بروتين (مخلوط الطحالب والطماطم ) في علائق إصبعيات أسماك مبروك الحشاش وحيد الجنس ذكور دون أي تأثيرات عكسية على النمو والأداء والإستفادة من الغذاء ومكونات الجسم للأسماك ، والتقييم الاقتصادي