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EVALUATION OF USING JOJOBA MEAL *(simmondsia chinensis)* SUPPLEMENTED WITH METHIONINE AND BIOGEN® INSTEAD OF FISH MEAL IN THE DIET OF MONO-SEX NILE TILAPIA *(Oreochromis niloticus).*

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SUMMARY

The present study was carried out to evaluate the partial or total replacement of fish meal (FM) by jojoba meal (JM) ,*Simmondsia chinensis*, (as a new plant protein source) at levels of 25 (T_2), 50 (T_3), 75 (T_4) and 100% (T_5) after supplemented with methionine and Biogen® (natural and commercial probiotic) at 0.6 and 2 g/kg diet, respectively, against the control diet 0% (T_1), to study the effects on growth performance, feed and nutrients utilization, carcass composition, organs indices, blood hematological and biochemical parameters, histometric examination of dorsal muscles and economic efficiency of mono-sex Nile tilapia (*O. niloticus)*. Therefore, fish were randomly assigned to five treatments and were fed for 8 weeks as experimental period. Results in the present study indicated that the partially replacement of 25% of FM by supplemented JM (T_2) led to significant ($P \leq 0.05$) increases of all growth performance, nutrients utilization, plasma globulin and mean diameter (µm) of muscular bundles and muscular bundles area per mm^2 of fish dorsal muscles compared with the control fish (T_1). Also, results illustrated that T_2 led to improve feed conversion ratio (FCR) compared with (T_1), significant improvement of the intensity of muscular bundles and connective tissues percentage per mm^2 and the best economic efficiency compared with other dietary treatments. However, the same treatment (T_2) significantly ($P \geq 0.05$) increased protein and energy utilization, dry matter in fish carcass, concentrations of plasma total protein, albumin, albumin/globulin ratio, internal organs indices and economic efficiency compared with (T_1). Increasing replacement of FM by supplemented JM led to significantly ($P \leq 0.05$) decrease in fish growth performance and all organs indices and significantly affected liver functions enzymes (AST and ALT) compared with (T_1). Whereas T_5 recorded worst means of all traits studies compared with the other treatments. Neverless, not significant differences were recorded in hemoglobin and uric acid concentrations among all treatments. Consequently, the present study recommended that using the JM after being supplemented with methionine and Biogen® at 0.6 and 2 g/kg diet respectively, to replace 25% FM in mono-sex *O. niloticus* diet reduced costs of aquafeeds without any adverse effects on the fish, human health, safety of the environment. More scientific research is needed to maximize the commercial benefit from JM by other fish species.

Keywords: *tilapia, fish meal, jojoba mealm, fish physiological nutrition,* Biogen®.

INTRODUCTION

Fish meal is generally considered to represent the "gold standard" dietary protein source for fishes and in aquaculture-formulated diet (FAO, 2004). The future development of aquaculture will be greatly constrained by the increasing costs of fish meal and fish oil. The shortage in global fish meal (FM) production coupled with increased demand and competition for its use in livestock and poultry feeds has further increased FM prices. It is evident, on the long-run, that many developing countries will be unable to depend on FM as a major protein source in aquafeeds. Therefore, several attempts have been made to partially or totally replace FM with less expensive, locally available protein sources (El-Sayed, 1999). In this sense, the partial or total replacement of fish meal and fish oil by raw plant materials has been accomplished in fish with good results in growth performance traits and fish quality (Carter and Hauler, 2000; Mente *et al.*, 2003; Cai *et al.*, 2005 and Palmegiano *et al.*, 2005). Generally, replacing fish meal by plant protein sources in fish nutrition is increasing interest (Cai *et al.*, 2005; Borgeson *et al.*, 2006; Gaber, 2006; Luo *et al.*, 2006; Hernández *et al.*, 2007 and Sarker *et al.*, 2007).

To be a viable alternative feedstuff to fish meal in aquafeeds, a candidate ingredient must possess certain characteristics, including wide availability, competitive price, plus ease of handling, shipping, storage and use in feed production. Furthermore, it must possess certain nutritional characteristics, such as favorable amino acid profile, high nutrient digestibility and reasonable palatability. It is likely that a combination of plant-derived feed ingredients will be required to replace fish meal, and that supplements, such as amino acids, flavorings and possibly exogenous enzymes, will be needed to produce aquafeeds without fish meal that support growth rates necessary for the economic production of farmed fish (Gatlin III *et al.*, 2007).

Jojoba (*Simmondsia chinensis* Link Schneider) is a perennial shrub that grows naturally in the Sonora desert (Mexico) and in the South-West of USA. Jojoba is now cultivated in some countries: Argentina (2.0 kt yr $^{-1}$), Israel (1.1 kt yr $^{-1}$), USA (1.0 kt yr $^{-1}$) and some Mediterranean and African lands are the main seed producers (data are for the campaign 2002–03). Actually, there are 7930 ha in the world planted with Jojoba. Jojoba is being examined for its potential as a crop in many countries around the world with climate and soil conditions similar to those of its native habitat (IJEC., 2004).The rest of jojoba seed, a protein rich meal (32%) (Elliger *et al.*, 1973), can be used as animal feed (Flo *et al.*,1998; Jones and Lewis, 1999 & 2001; Brown, 2003 and Motawe, 2006). Unfortunately, this meal contains approximately 15% of group of toxic glycosides (Plate 1), namely simmondsin, (Van Boven *et al.*, 2000).The simmondsin has been identified as the most responsible food intake inhibition and appetite suppression to rodents, rats, dogs, and chickens (Hawthorne and Butterwick 1998; Flo *et al.*, 1999; Cokelaere *et al.*, 2000; Ham *et al.*, 2000 and Lievens *et al.*, 2003).

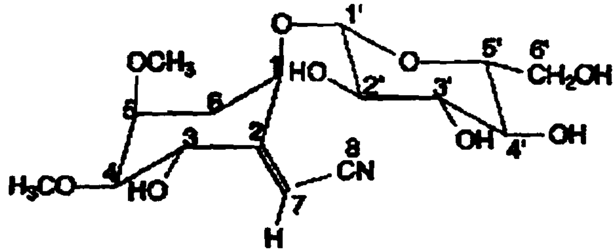


Plate (1): Structure of simmondsin

The Egyptian Natural Oil Company introduced jojoba plant in Egypt. It has been planted in areas near Ismailia Governorate with promising crop production. As the plant requirements for water in volume and quality is very moderate, it is expected that it will be propagated in the Egyptian deserts. Jojoba meal, as a by-product of jojoba seeds, is a promising feedstuff after being detoxified (Motawe, 2006).

Accordingly, the present study aims to evaluate the replacement of partial or total replacement (0, 25, 50, 75 and 100%) of fish meal by jojoba meal (*Simmondsia chinensis*) as a new plant protein source, after being supplemented with methionine and Biogen® (as a natural and commercial probiotic) at 0.6 and 2 g/kg diet respectively, and studying its effects on growth performance, feed and nutrients utilization, carcass composition of fish, organs indices, blood hematological and biochemical parameters, histometric examination of dorsal muscles and economic efficiency of mono-sex Nile tilapia (*Oreochromis niloticus*) for 8 weeks.

MATERIALS AND METHODS

Fish and experimental management:

This study was conducted at the Fish laboratory research, Animal Production Department, Faculty of Agriculture, Mansoura University, Dakahlia Governorate. Nile tilapia were stocked in a rearing tank for two week preconditioning period during which time they were offered the basal diet. a total of 70 size sorted fish (16-17g) that appeared healthy were stocked at 7 fish/glass aquarium (90 x 40 x 50 cm). Two replicate aquaria were randomly assigned to the five dietary treatments (D_1 to D_5). Each aquarium was supplied with 108 l dechlorinated tap water and an air stone connected with electric compressor. The replacement of the aquaria water was done partially every day to re-new the tap water and to remove the wastes. Light was controlled by a timer to provide a 14h light: 10h dark as a daily photoperiod.

Dietary treatments:

Five experimental diets were formulated (D_1 – D_5). Jojoba meal (29% CP) partially or totally (0, 25, 50, 75 and 100%) replaced fish meal. Methionine and Biogen® were added at the rate of 0.6 and 2g/Kg containing J M, respectively. Their ingredients and chemical composition are shown in Table (1).

All the dietary ingredients and additives were purchased from the local market. The ingredients were milled and mixed well. The Biogen® was added to increase the palatability of feed, promote against simmondsin, identified as the most responsible food intake inhibition and appetite suppression. Methionine was also added (0.6g/kg diet, 3.21

% dietary protein, 28%) crude protein in diet for *O. niloticus*, according to (Fayed, 1997 and Shehatta, 1997) since it is the limiting amino acid in jojoba seed meal and extracted whole seed jojoba meal was methionine (Motawe, 2006). All, ingredients and additives were milled and mixed, then pressed by manufacturing machine (pellets size 1mm). During the experimental period (8 weeks), the fish were fed the previous diets at a rate of 3% of the live body weight daily, six days a week and twice daily, at 8 a.m. and 2 p.m. The amount of food was adjusted bi-weekly based on the actual body weight changes.

Experimental procedures:

Water quality parameters measured weekly included temperature (via a thermometer), pH (using Jenway Ltd., Model 350-pH-meter) and dissolved oxygen (using Jenway Ltd., Model 970- dissolved oxygen meter). At the end of the experiment, the remained fish were sampled from each aquarium and kept frozen for chemical analysis. The chemical analyses of the basal diet and whole fish body were carried out according to the AOAC (2000).

Table (1): Ingredients and chemical composition of experimental diets.

Item	D_1	D_2	D_3	D_4	D_5
Ingredients (g)*					
Fish meal	220	165	110	55	0.00
Jojoba meal	0.00	55	110	165	220
Soybean meal	285	340	392	442	490
Yellow corn	195	180	158	138	100
Wheat bran	200	160	130	100	90
Corn oil	30	30	30	30	30
*Vit. and Min. premix	20	20	20	20	20
Molasses	50	50	50	50	50
Total (g)	1000	1000	1000	1000	1000
Additives					
Methionine (g/kg diet)	0.00	0.6	0.6	0.6	0.6
Biogen® (g/kg diet)	0.00	2	2	2	2
Chemical composition (% DM basis):					
Dry matter (DM)	90.42	90.88	91.19	90.91	90.84
Crude protein (CP)	30.25	30.14	30.20	30.19	30.11
Ether extracts (EE)	4.75	4.89	3.88	3.75	4.40
Ash ()	12.45	11.94	12.48	12.42	11.07
Carbohydrates	52.55	53.03	53.44	53.64	54.42
**Gross energy (GE) (Kcal/100 g DM)	431.43	434.10	426.60	426.13	435.03
***Protein/energy (P/E) ratio (mg CP/KcalGE)	70.12	70.81	70.79	70.85	69.21

*Each 3 kg premix contains: Vit. A, 12000,000 IU; Vit. D_3, 3000,000 IU; Vit. E, 10,000 mg; Vit. K_3, 3000 mg; Vit. B_1 200 mg; Vit. B_2, 5000 mg; Vit. B_6, 3000 mg; Vit. B_{12}, 15 mg; Biotin, 50 mg; Folic acid 1000 mg; Nicotinic acid 35000 mg; Pantothenic acid 10,000 mg; Mn 80g; Cu 8.8g; Zn 70 g; Fe 35 g; I 1g; Co 0.15g and Se 0.3g.

®Biogen® is a natural non-antibiotic feed supplement comprised of allicin (aged garlic extract) not less than 0.247 m mole/g, *Bacillus subtilis* nato (6 x 10^7 cells/g), high unit hydrolytic enzymes not less than 3690 units/g (amylotic, lipolytic, prolytic and cell separating enzymes), germanium (Ginseng 41.98 ppm of Ge. element) and organic selenium. It has bactericidal effects and increases the palatability of feed, promotes the secretion of digestive fluids and stimulates the appetite (China Way Corporation, 1999).

**GE (Kcal/100 g DM) = CP x 5.64 + EE x 9.44 + Carbohydrates x 4.11 (Macdonald *et al.*, 1973).

***P/E ratio (mg protein/Kcal gross energy) = CP/GE x 1000

Body weight of individual fish was measured biweekly to adjust feed inputs to calculate growth performance and feed utilization (Abdelhamid, 2000). At the end of the experiment, the liver, spleen, kidneys and gonads were removed and weighed individually to calculate the liver, spleen, kidneys and gonads indices i.e. hepato-somatic index (HSI) (Jangaard *et al.*, 1967), Spleeno-somatic index (SSI), Kidney-somatic index (KSI) (Alabaster and Lioyd, 1982) and Gonado-somatic index (GSI) (Tseng and Chan, 1982).

At the end of the experiment, blood samples from the fish of the different groups were collected from the caudal peduncle. Adequate amounts of whole blood in small plastic vials containing heparin were used for the determination of hemoglobin (Hb) by using commercial kits (Diamond Diagnostic, Egypt). Other blood samples were collected and then centrifuged at 3500 rpm for 15 min to obtain blood plasma for determination of total protein (Gornall *et al.*, 1949), albumin (Weichsebum, 1946), globulin by difference (Doumas and Biggs, 1977), uric acid (Barham, 1972), aspartate aminotransferase (AST) and alanine aminotransferase (ALT) (Varley, 1976) using a spectrophotometer (model 5010, Germany) and commercial kits. As well as, at the end of the experiment all fish were sacrificed and fish dorsal muscles were sampled and fixed in 10% neutralized formalin solution to histological examination (Pearse, 1968).

The collected data were subjected to statistical analysis using general linear models procedure adapted by SAS (1997) for users guide, with a one-way ANOVA. Means were statistically compared for the significance ($p \leq 0.05$) using multiple range test Duncan (1955).

RESULTS AND DISCUSSION

Rearing water quality:

Daily mean values for water temperature ranged between 24 and 28°C, pH values 6.55 – 7.60 and dissolved oxygen 5.50 – 8.80 mg/l. All tested water quality criteria in the present study were suitable for rearing mono-sex Nile tilapia *(O. niloticus)* fingerlings as cited by Abdelhamid (2000) and Abd El-Hakim *et al.* (2002).

Growth performance:

Results in Table (2) indicated that the partial replacement of 25% of fish meal (FM) by supplemented jojoba meal (JM) with both additives of methionine and Biogen® at 0.6 and 2 g/kg diet respectively, (T_2) led to significant increases ($P \leq 0.05$) in final weight, average weight gain (AWG), relative growth rate (RGR), average daily gain (ADG) and specific growth rate (SGR) of mono-sex *O. niloticus* compared with the control group and other groups. but no significant ($P \geq 0.05$) differences were detected in survival rate. These positive effects may be related to the better nutritive values of JM supplemented with methionine and Biogen® at levels of 0.6 and 2 g/kg diet, respectively. All above growth performance parameters significantly ($P \leq 0.05$) decreased by increasing replacement of FM by supplemented JM of mono-sex *O. niloticus* diet. The negative effects on growth performance associated with increasing replacement of FM by JM, might be due to the increase in glycosides, namely simmondsin, in the diets.

These findings are in agreement with those of Vermauti *et al.* (1998) who demonstrated the potential use of jojoba meal, which is currently considered a waste product for broiler breeder pullets. Moreover, recently Abdel-Warith (2008) reported that the mono-sex Nile tilapia can be fed plant protein sources (soybeans) to replace 50% of total dietary protein or 61% of FM in the diets without deleterious effects on growth or on mortality and the overall health appearance of fish was normal during an experimental period of 12 weeks.

Table (2): Effects of partial or total replacement of fish meal by jojoba meal on growth performance of mono-sex *O. niloticus* (Means and ± SE).

Treat.	Body weight (g/fish)			RGR	ADG	SGR	SR
	Initial weight	Final weight	AWG				
T_1	16.56	38.04 ab	21.45 ab	129.35 ab	369.65 ab	1.43a	92.85
	±0.16	±1.44	±1.35	±6.85	±22.75	±0.05	±7.15
T_2	16.43	38.84 a	22.4 a	136.40 a	386.30 a	1.48a	92.85
	±0.00	±3.44	±3.40	±20.90	±59.20	±0.15	±7.15
T_3	16.43	31.09 bc	14.65 bc	89.30 bc	252.95 bc	1.09 ab	92.85
	±0.00	±1.12	±1.15	±6.79	±19.25	±0.06	±7.15
T_4	16.43	27.38 c	10.95 c	66.67 c	188.85 c	0.88 b	85.70
	±0.00	±1.16	±1.16	±7.07	±20.05	±0.07	±14.30
T_5	16.57	26.19 c	9.59 c	58.14 c	165.80 c	0.78 b	71.40
	±0.14	±1.89	±2.00	±12.76	±35.00	±0.14	±14.30

a-c: Means in the same column having different small letters are significantly different ($P \leq 0.05$).
AWG = Average weight gain (g/fish) RGR= Relative growth rate
ADG = Average daily gain (mg/fish/day) SGR = Specific growth rate (%/d)
SR = Survival rate (%)

Feed and nutrients utilization:

There were no significant differences ($P \geq 0.05$) in feed intake (FI) and feed conversion ratio (FCR) between all fish treatments. The protein productive value (PPV), protein efficiency ratio (PER) and energy utilization (EU) were significantly ($P \leq 0.05$) influenced by the dietary treatments. In general nutrient retention decreased as the level of inclusion increases. The best results recorded for feed and nutrients utilization were those of T_2 and the worst results were observed by increasing replacement of FM by supplemented JM (Table 3).

In this respect, Olvera-Novoa *et al.* (1997) reported that the best growth rate and feed utilization were observed with 20-30% replacement of fish meal with cowpea protein concentrate, while the protein efficiency was best at 40% inclusion level. Moreover, Borgeson *et al.* (2006) reported that feeding complex mixtures of plant proteins diets significantly improved all growth parameters with the exception of average daily feed intake indicating that tilapia will eat both simple and complex mixtures of plant proteins diets. Recently, Abdel-Warith (2008) reported that the mono-sex Nile tilapia can be fed plant protein sources (soybeans) to replace 50% of total dietary protein or 61% of FM in the diets without any adverse effects on feed and nutrients utilization parameters and the overall health appearance of fish.

Table (3): Effects of partial or total replacement of fish meal by jojoba meal on feed and nutrients utilization by mono-sex *O. niloticus* (Means and ± SE).

Treat.	FI g/fish	FCR	Protein utilization		EU %
			PPV	PER %	
T_1	31.00	1.45	41.00ab	2.28a	18.55ab
	±0.70	±0.06	±0.93	±0.09	±0.61
T_2	30.90	1.40	55.76a	2.40a	26.22a
	±1.30	±0.16	±5.75	±0.27	±2.40
T_3	29.15	2.00	36.21b	1.68ab	17.03b
	±1.75	±0.27	±6.26	±0.23	±3.48
T_4	29.35	2.72	34.80b	1.24b	16.05b
	±0.65	±0.35	±2.69	±0.16	±1.98
T_5	26.60	2.94	28.33b	1.22b	11.61b
	±1.80	±0.81	±4.76	±0.34	±1.64

a-b: Means in the same column having different small letters are significantly different ($P \leq 0.05$).
FI = Feed intake (g/fish) FCR = Feed conversion ratio
PER = Protein efficiency ratio EU = Energy utilization (%)
PPV = Protein productive value (%)

Chemical analyses of the experimental fish:

Results in Table (4) showed that no significant differences ($P \geq 0.05$) were recorded in carcass composition of the whole body (crude protein, ether extract, ash and energy content), of mono-sex *O. niloticus* in all treatments. While, dry matter of fish carcass significantly ($P \leq 0.05$) increased in T_2 fish group compared with treatments T_1,T_3 and T_5 except T_4 whereas, this increase was not significant ($P \geq 0.05$) compared with T_2.

In the same trend, Belal and AL-Dosari (1999) reported that salicornia meal (annual salt-marsh plant in arid regions), can replace up to 40% of the fish meal in *0. niloticus* feeds without affecting growth or body composition. In addition, Hussein *et al.* (2001) found that replacement of fish meal by canola meal had no significant ($P>0.05$) effect on fish body composition.

Table (4): Effects of partial or total replacement of fish meal by jojoba meal on carcass composition of mono-sex *O. niloticus* (Means and ± SE).

Treat.	DM %	% On Dry matter basis			
		CP %	EE %	Ash %	EC(Kcal/100 g)
At the start of the experiment					
	17.10	65.97	11.63	22.40	481.86
At the end of the experiment					
T_1	20.59 b	69.94	11.85	18.21	506.35
	± 0.08	0.07	0.31	±0.24	2.55
T_2	24.79 a	70.80	12.70	16.51	519.20
	± 0.59	0.05	0.56	±0.62	5.60
T_3	21.82 b	70.19	13.51	16.31	523.40
	±0.97	0.39	0.44	±0.05	1.90
T_4	24.38 a	70.27	13.03	16.71	519.30
	± 0.05	0.26	1.51	±1.24	12.70
T_5	20.65 b	71.75	12.38	15.89	521.45
	± 0.69	1.16	0.87	±0.30	1.65

a-b: Means in the same column having different small letters are significantly different ($P \leq 0.05$).
DM = Dry matter (%) CP = Crude protein (%)
EE = Ether extract (%) EC = Energy content (Kcal/100 g), calculated according to Macdonald *et al.* (1973).

Blood hematological and biochemical parameters:

No significant ($P \geq 0.05$) differences were recorded in both of hemoglobin (g/dl) and uric acid (mg/dl) in all fish treatments. However, significant differences ($P \leq 0.05$) were observed for the liver function enzymes, namely aspartate aminotransferase (AST) and alanine aminotransferase (ALT), which were increased significantly ($P \leq 0.05$) increased by increasing the replacement of FM by supplemented JM. However, the worst results of liver enzymes (AST and ALT) functions were found in T_5 (Table 5). From the other side, total protein (g/dl), albumin (g/dl) and albumin/globulin ratio of fish group fed diet containing the 25 and 75% replacement of FM by supplemented JM were increased without significant differences compared with the control (T_1) and with significant ($P \leq 0.05$) increases compared with other treatments, which increased by increasing JM. But, the globulin (g/dl) of fish fed diets containing 25% and 50% replacement of FM by supplemented JM (T_2 and T_3) respectively, were significantly ($P \leq 0.05$) increased compared with the other treatments (Table 5). This indicated that no negative effects were recorded on blood parameters of when FM was partially replaced by supplemented JM which may be due to supplementation with both of methionine and Biogen® at 0.6 and 2 g/kg diet respectively. But there were some negative effects in blood parameters of total replacement of FM by supplemented JM. These adverse effects of total replacement of FM by supplemented JM may be related to the naturally occurring of toxic glycosides (namely simmondsin) and also may be related to the exposure time of fish for the experimental diet (8 weeks).

In the same topic, Davies *et al.* (1990) found that only 15% rapeseed meal could effectively replace fish meal/soy bean meal in *O. mossambicus* diets, while higher levels resulted in poor growth and feed efficiency, due to the high content of glucosinolate antinutrient in rapeseed. Similarly, Hussein *et al.* (2001) reported that canola meal significantly ($P<0.01$) increased hemoglobin concentration and serum total protein, globulin, triglycerides, AST, ALT and thyroid hormones (T_3 and T_4). They also concluded that diets containing up to 60% canola meal supplemented with G4 yeast strain had no adverse effects on growth performance, body composition or blood constituents of *O. niloticus.*

Internal organs indices:

There were no significant differences in all internal organs indices of fish group fed diet contain 25, 50 and 75% replacement of FM by supplemented JM (T_2, T_3 and T_4 respectively), compared with the control (T_1) as shown in Table 6. However, total replacement of FM by supplemented JM (T_5) led to significant ($P \leq 0.05$) decreases in all internal organs indices compared with the control treatment (T_1). There were significant ($P \leq 0.05$) decreases in all internal organs indices specially in HSI treatment (T_5), whereas, liver in this treatment appeared paled or yellowish and profligated which may be related to the significant ($P \leq 0.05$) increases in the activity of liver enzymes (AST and ALT) as shown in Table 5. Also, these alterations in liver may be related with naturally occurring of toxic glycosides namely simmondsin in the experimental diets.

Theses findings are in agreement with those of Abdel-Warith (2008) who revealed that there were significant ($P<0.01$) decreases of hepatosomatic index (HSI) in fish fed diets contained different sources of soybean meal as partial replacement of fish meal compared with the control group (100% fish meal) for *O. niloticus* fingerlings. Also, the same author indicated that the viscera-somatic index (VSI) did not reflect any trend in tilapia samples at the end of the study. In addition, Hussein *et al.* (2001) reported that there was significant (P<0.01) increase in HSI with the increasing dietary canola meal in the diets of *O. niloticus*.

Table (5): Effects of partial or total replacement of fish meal by supplemented jojoba meal on blood hematological and biochemical parameters of mono-sex *O. niloticus* (Means and ± SE).

Item	T_1	T_2	T_3	T_4	T_5
Hb g/dl	4.79 ±0.06	4.86 ±0.09	4.67 ±0.09	4.73 ±0.07	4.64 ±0.08
AST U/L	19.50b ±1.50	16.50b ±1.50	19.50b ±1.50	23.00b ±2.00	38.00a ±4.00
ALT U/L	18.00ab ±2.00	16.50b ±3.50	19.50ab ±3.50	21.50ab ±1.50	28.00a ±2.00
Uric acid mg/dl	7.04 ±0.32	6.91 ±0.25	7.11 ±0.26	7.13 ±0.37	7.40 ±0.48
Total protein g/dl	8.04a ±0.19	8.90a ±0.10	8.05a ±0.31	7.02b ±0.22	5.58c ±0.30
Albumin g/dl	5.13a ±0.26	5.31a ±0.36	4.44ab ±0.44	4.01bc ±0.09	3.18c ±0.21
Globulin g/dl	2.91bc ±0.08	3.59a ±0.26	3.61a ±0.13	3.01b ±0.13	2.41c ±0.09
*AL/GL ratio	1.77a ±0.14	1.49ab ±0.21	1.24b ±0.17	1.33ab ±0.03	1.32ab ±0.03

a-c: Means in the same row having different small letters are significantly different (P ≤ 0.05).
Hb = Hemoglobin (g/dl) AST= Aspartate aminotransferase
ALT= Alanine aminotransferase * Albumin / Globulin ratio = Albumin/Globulin

Table (6): Effects of partial or total replacement of fish meal by supplemented jojoba meal on internal organs indices of mono-sex *O. niloticus* (Means and ± SE).

Treat.	HSI	KSI	SSI	GSI
T_1	2.39 ab ±0.15	0.69 a ±0.07	0.33 b ±0.02	2.06 a ±0.08
T_2	2.51 ab ±0.14	0.62 ab ±0.04	0.34 b ±0.01	2.13 a ±0.13
T_3	2.66 a ±0.01	0.62 ab ±0.01	0.34 b ±0.01	1.84 ab ±0.06
T_4	2.44 ab ±0.12	0.72 a ±0.02	0.36 ab ±0.03	1.80 ab ±0.08
T_5	2.13 b ±0.18	0.55 b ±0.02	0.41 a ±0.01	1.61 b ±0.09

a - b: Means in the same column having different small letters are significantly different (P ≤ 0.05).
HSI % = Hepato somatic index. KSI % = Kidney somatic index.
SSI % = Spleeno somatic index. GSI % = Gonado somatic index.

Histometric examination of fish dorsal muscles:

Results concerning characteristics of the dorsal muscle of fish in different experimental groups (Table 7 and Plates 2 - 6) show that mean diameter of muscular bundles of the muscle significantly ($P \leq 0.05$) increased in fish group fed diet containing on partial replacement of 25% FM by supplemented JM (T_2) and decreased in T_5, while it did not differ in T_3 and T_4 as compared with the control group (T_1). The observed change in mean diameter of the bundles of T_2 and T_5 was mainly related to significant ($P \leq 0.05$) change in largest diameter of the bundles rather than in smallest diameter, reflecting insignificant differences among treatments in shape of the bundles (smallest/largest ratio). In spite of the significant differences in mean diameter of the bundles, no significant differences were found among all treatments and the control group (T_1) in intensity of the bundles, muscular bundles area occupied by bundles/mm^2 or by interstitial connective tissue in between bundles.

It is of interests to note that the highest mean diameter of bundles of fish in T_2 was associated with its higher growth performance, increasing feed and nutrients utilization, better carcass composition and improving blood parameters as compared to the control treatment (T_1) and other treatments. In addition, T_5 treatment showed the lowest values in above parameters among all treatments. The present results are in accordance with those reported by Abdelhamid *et al.* (2004) who found that the *O. niloticus* group fed diet containing 1 kg betafin®/ton and 600 ml biopolym/ton was the best treatment among all treatments of the muscular bundles, total surface area occupied by the muscular bundles/mm^2.

Table (7): Effects of partial or total replacement of fish meal by jojoba meal on histometric characteristics of the dorsal muscles of mono-sex *O. niloticus* (Means and ± SE).

Item	T_1	T_2	T_3	T_4	T_5
Smallest diameter (µm)	10.00ab ±1.22	12.80a ±1.46	12.60a ±0.68	10.20ab ±0.97	8.20b ±0.58
Largest diameter (µm)	15.60b ±0.98	19.80a ±1.43	17.40ab ±1.03	17.00ab ±2.10	10.80c ±0.37
Mean diameter (µm)	13.00b ±0.84	16.40a ±0.93	15.40ab ±0.68	14.00ab ±0.89	9.80c ±0.49
Smallest/largest ratio	0.65 ±0.08	0.66 ±0.08	0.73 ±0.06	0.65 ±0.11	0.76 ±0.05
Intensity of muscular bundles/mm^2	5466.67ab ±933.33	3600.00b ±0.00	3600.00b ±0.00	3600.00b ±0.00	7600.00a ±1200
% of muscular bundles area* / mm^2	72.30ab ±0.00	91.60a ±0.00	72.30ab ±0.00	52.00b ±12.81	53.63b ±13.48
% of connective tissue/ mm^2**	27.70ab ±0.00	8.40b ±0.00	27.70ab ±0.00	48.00a ±12.81	46.37a ±13.48

a-c: Means in the same row having different small letters are significantly different ($P \leq 0.05$).

* % of muscular bundles area / mm^2 = ([3.14 X (mean diameter/2)^2] X Intensity of muscular bundles/mm^2) X 100, whereas: the muscular bundles were considered as approximately circularity shape.

** % of connective tissue / mm^2 = (1- muscular bundles area, mm^2) X 100

Economic efficiency:

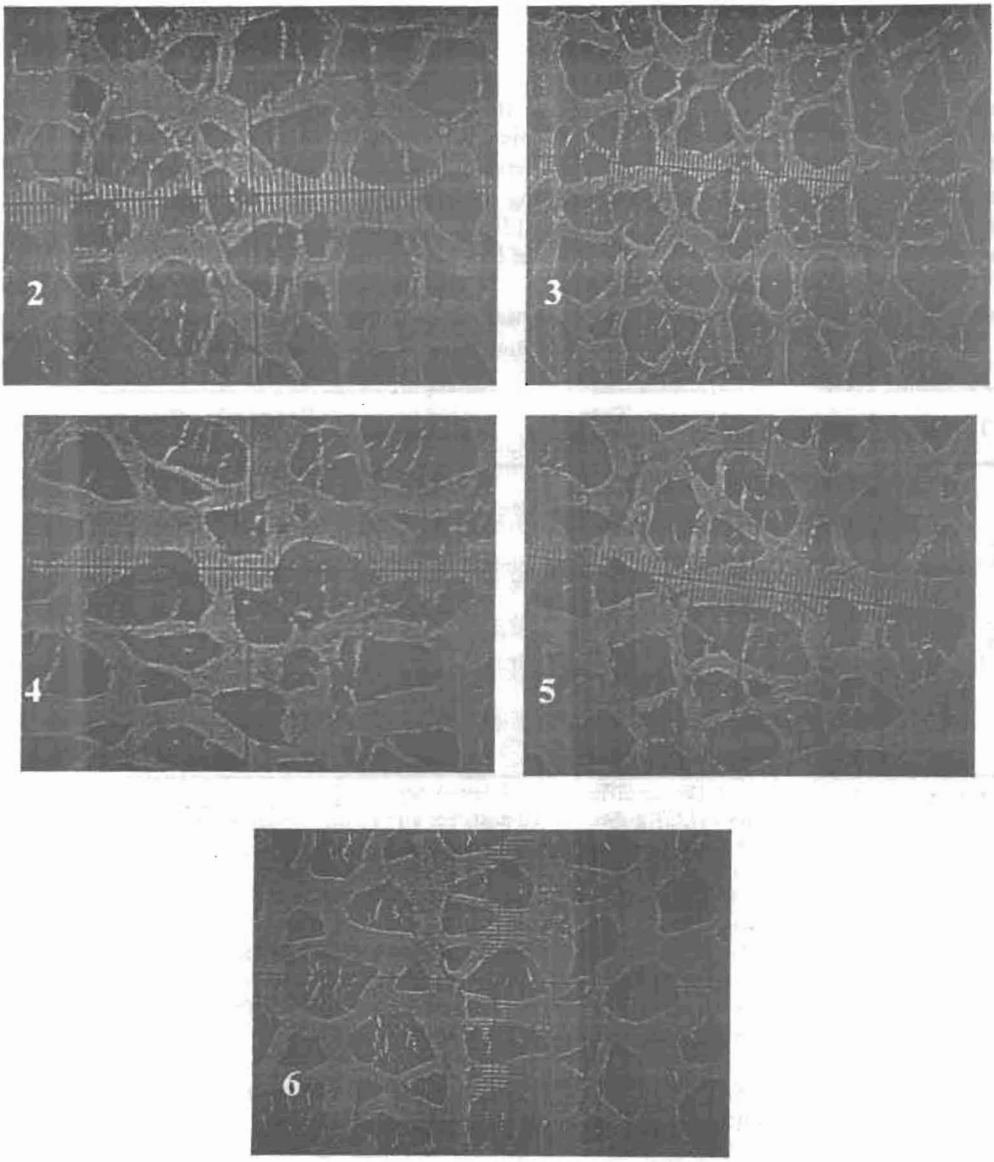
Results in Table (8) indicated that there were no significant differences between fish group fed diet containing 25% FM replaced by supplemented JM (T_2) compared with the control group (T_1) in economic parameters (total outputs, total costs, net return and economic efficiency). However, these economic parameters for T_2 were significantly ($P \leq 0.05$) better compared with the other treatments. From an economic point of view, T_2 group had the best economic efficiency among all other groups, since this group had the best growth performance, feed and nutrients utilization, carcass composition of fish, blood parameters and the best histological structure of fish dorsal muscles.

Table (8): Economic efficiency (%) of partial or total replacement of fish meal by supplemented jojoba meal of mono-sex *O. niloticus* diets (Means and ± SE).

Treat.	Total feed costs[1]	Total outputs[2]	Net return[3]	Economic efficiency[4] (%)
T_1	0.11 a ±0.00	0.56 a ±0.08	0.46 a ±0.08	449.40 a ±30.20
T_2	0.10 a ±0.00	0.58 a ±0.04	0.48 a ±0.04	485.95 a ±9.95
T_3	0.09 b ±0.01	0.39 ab ±0.06	0.30 ab ±0.06	404.90 ab ±48.30
T_4	0.08 b ±0.01	0.27 b ±0.07	0.20 b ±0.08	310.00 bc ±51.60
T_5	0.06 c ±0.01	0.19 b ±0.01	0.13 b ±0.01	254.45 c ±13.55

a - c: Means in the same column having different small letters are significantly different ($P \leq 0.05$).
1- Total feed costs per treatment (LE/Kg diet) = feed costs per one kg diet X feed intake
2- Total outputs per treatment (LE/Kg) = fish price X total fish production*
* Total fish production per treatment = final number of fish X fish weight gain
3- Net return per treatment (LE) = total outputs – total feed costs
4- Economic efficiency per treatment (%) = (net return/ total feed costs) X 100
The price of 1 kg ingredient used was 7.00 LE for fish meal, 0.75 LE for jojoba meal, 2.50 LE for soybean meal, 1.50 LE for yellow corn, 1.50 LE for wheat bran, 7.50 LE for corn oil, 7.00 LE for vit. and min. premix, 1.50 LE for molasses, 26.00 L.E for methionine and 45.00 LE for Biogen® according to local market price at the time of study in Egypt (2007)

In conclusion, the results of the present study indicate the usefulness of replacing of 25% of FM by supplemented JM with both of methionine and Biogen® at 0.6 and 2 g/kg diet respectively, (T_2) in the diets of mono-sex *O. niloticus* to overcome on the high international and local increasing in FM price and achieving the best growth with the low costs without any adverse effects of the fish culture, human health and safety of the environment. In addition, the present study indicated that this new source of plant protein is promising ingredient in aquafeeds. Further scientific efforts are required to maximize the commercial benefit from it.



Plates. (2 to 6): Showing cross–section of muscular bundles and interstitial connective tissue of the dorsal muscles of mono-sex Nile tilapia in the 1st, 2nd, 3rd, 4th and 5th treatments respectively. (X 280 E&H stains)

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تقييم استخدام مسحوق الجوجوبا المدعم بالميثيونين و البيوجين بدلاً من مسحوق السمك فى عليقة البلطى النيلى وحيد الجنس

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اجريت هذه الدراسة بغرض تقييم استخدام مسحوق نبات الجوجوبا كمكون جديد فى علائق أسماك البلطى النيلى وحيد الجنس و ذلك بالإحلال التدريجى الجزىء او الكلى لهذا المكون الجديد فى علائق هذه الأسماك و الذى تم تدعيمه بإضافة كل من الحمض الأمينى الميثيونين و البيوجين (بروبيوتك طبيعى تجارى) بتركيزات ٠.٦ ، ٢ جرام/كجم علف على التوالى بدلاً من مسحوق السمك. لذلك وزعت الأسماك عشوائياً و غذيت لمدة ثمانى أسابيع على خمس علائق الأولى هى عليقه المجموعة الضابطة (١٠٠٪ مسحوق سمك) ، اما الثانية و الثالثة و الرابعة فتم فيهم الإحلال التدريجى الجزىء لمسحوق الجوجوبا المدعم كالآتى ٢٥ ، ٥٠ ، ٧٥٪ بدلاً من مسحوق السمك على التوالى و كان الإحلال الكلى لمسحوق الجوجوبا المدعم (١٠٠٪ مسحوق الجوجوبا) بدلاً من مسحوق السمك فى المجموعة الخامسة، حيث تم دراسة تأثير تلك المعاملات على اداء نمو الأسماك، كفاءة الاستفادة من الغذاء و العناصر الغذائية، و التركيب الكيماوى لجسم الأسماك، دلائل الأعضاء الداخلية، قياسات الدم، التركيب النسيجى لعضلات الأسماك وتحديد مدى الكفاءة الاقتصادية للعلائق المختبرة. و و أشارت النتائج المتحصل عليها إلى ان الإحلال الجزىء لمسحوق الجوجوبا بنسبة ٢٥٪ بدلاً من مسحوق السمك (المعامله الثانية) ادت إلى زيادة معنوية فى اداء النمو للأسماك ، كفاءة الاستفادة من العناصر الغذائية و كذا جلوبيولين بلازما الدم ، فى قياسات أقطار عضلات الأسماك و مساحة الحزم العضلية مقارنة بالمجموعة الضابطة ،أيضاً أظهرت النتائج أن المعاملة الثانية ادت كذلك إلى تحسن فى معامل التحويل الغذائى مقارنة بالمجموعة الضابطة ،وكذا حدوث تحسن معنوى فى كثافة الحزم العضلية و نسبة تواجد النسيج الضام لكل مم٢ و من الناحية الاقتصادية حققت المعاملة الثانية افضل كفاءة اقتصادية مقارنة بالمعاملات الأخرى. بينما ادت نفس المعاملة (الثانية) إلى زيادة غير معنوية لكل من الاستفادة من كل من البروتين و الطاقة ، المادة الجافة لجسم الأسماك ، و كذا تركيز كل من البروتين الكلى و الألبيومين و النسبة بين الألبيومين و الجلوبيولين ، و أيضاً لدلائل الأعضاء الداخلية ، و كذا من حيث الكفاءة الاقتصادية مقارنة بالمجموعة الضابطة. و من ناحية اخرى بزيادة نسبة الإحلال أدى ذلك إلى حدوث انخفاض كل من اداء النمو للأسماك و دلائل الأعضاء الداخلية معنوياً ، بينما كانت هناك زيادة معنوية فى انزيمات وظائف الكبد (AST and ALT) حيث كانت أسوء معاملة الخامسة (الإحلال الكلى) مقارنة بالمجموعة الضابطة و كذا كان أسوء معامل تحويل غذائى لنفس المعاملة بين كل المعاملات الأخرى، بينما لم تسجل أى فروق معنوية بين كل المعاملات فى تركيز كل من الهيموجلوبين و حمض اليوريك.

لذا توصى الدراسة الحالية باستخدام مسحوق الجوجوبا (كمصدر جديد للبروتين النباتى) المدعم بكل من الحمض الأمينى الميثيونين ومادة البيوجين (بروبيوتك طبيعى تجارى) بتركيز ٠.٦ ، ٢ جرام/كجم علف على التوالى بالإحلال الجزىء بنسبة ٢٥٪ بدلاً من مسحوق السمك فى علائق أسماك البلطى النيلى وحيد الجنس لتقليل تكلفة الأعلاف الخاصة بالأسماك بدون اى تأثيرات سيئة على كل من حيوية الأسماك و صحة الإنسان و سلامة البيئة مع الحاجة للمزيد من الأبحاث العلمية لتعظيم الاستفادة التجارية من استخدام هذا المكون العلفى فى علائق الأنواع السمكية المختلفة.