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Response of Nile Tilapia mono-sex (*Oreochromis niloticus*) Fingerlings to Different Sources and Levels of Protein Using Garlic and Onion as Feed Phytophytoadditives

استجابة أصبعيات أسماك البلطى النيلي وحيد الجنس للعلائق المحتوية على مصادر ومستويات مختلفة من البروتين باستخدام الثوم والبصل كإضافات علفية نباتية

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Abstract: Twelve experimental diets containing 35% crude protein and 434.29 Kcal gross energy $100g^{-1}$ were formulated to process three groups of diets according to protein source and level: group 1 (G_1) consists of: 25% fish meal (FM) + 75% soybean meal (SBM); group 2 (G_2): 50% FM + 50% SBM and group 3 (G_3): 75% FM + 25% SBM. Each group included four treatments according to feed additive source and level: a) control diet (without garlic or onion: D_1, D_5, D_9), b) supplemented with 4% garlic (D_2, D_6, D_{10}), c) supplemented with 6% onion (D_3, D_7, D_{11}) and d) supplemented with 10% mixture (4% garlic and 6% onion: D_4, D_8, D_{12}) on dry matter basis as phytoadditives in order to study its effects on growth performance, feed utilization and whole body composition of Nile tilapia (*Oreochromis niloticus*) mono-sex fingerlings. Fish were reared in thirty six glass aquaria for 84 days, triplicate per treatment. Ten fingerlings of tilapia were placed per each aquarium with an average initial weight of 3.12 ± 0.3 g/fish. Fish were fed twice daily (six days a week) at a daily feeding rate of 5 % of live body weight on dry matter basis until the end of the trial. The results illustrated that the best growth performance values (weight gain; average daily gain, ADG; specific growth rate, SGR%) and feed utilization (feed intake, FI; feed conversion ratio, FCR; protein productive value, PPV% and energy utilization, EU) were significantly ($P<0.05$) realized with fingerlings fed G_2 diet followed by that fed G_3 diet and then that fed G_1 diet, respectively regardless of type and level of phytoadditives. On the other hand, addition of 10% mixture of garlic and onion as phytoadditives significantly ($P<0.05$), increased growth performance, feed and nutrient utilization rather than solitary addition of either garlic or onion, regardless of protein source and level which clearly showed with fingerlings fed D_8 diet. Furthermore, no significant differences (P0.05>)

were observed in feed and nutrient utilization between fingerlings fed additive mixture diet (G_{g+o}) and control diet (G_c) in spite of the lowest feed intake recorded for the later group (70.55 g/fish). Taking into account the diet number, which reflects the interacted effect of both protein source and level beside phytoadditives source and level, it was found that fingerlings fed diet D_8 realized the best growth performance followed by diet D_{12}. On the other hand, fingerlings fed D_5 and D_8 realized the higher values for protein efficiency ratio (PER), protein productive value (PPV %), energy utilization (EU %) and the best feed conversion ratio (FCR). Furthermore, the results illustrated also that fingerlings fed onion supplemented diets recorded better growth performance response than those fed supplemented diets or control diets, and fingerlings fed onion and garlic mixture supplemented diets showed better growth performance and feed utilization than any of them in the same protein combination group. It could be concluded that diets consist of 50% FM + 50% SBM with 10% mixture of garlic and onion as phytoadditives in Nile tilapia (*O. niloticus*) mono-sex fingerlings diets, are considered ideal for optimal growth and feed utilization without any adverse effect on fish health.
Keywords: *Dietary phytoadditives, growth performance, tilapia, chemical composition.*

المستخلص: في هذه التجربة، تم تكوين أثنتا عشر عليقة تحتوى على 35 % بروتين خام وكانت تحتوي على ثلاثة مجموعات بروتينية: مجموعة 1): 25 % مسحوق سمك + 75 % فول الصويا . مجموعة 2): 50 % مسحوق سمك + 50 % فول الصويا، مجموعة 3): 75 % مسحوق سمك + 25 % فول الصويا. وقسمت كل مجموعة بروتينية إلى أربعة معاملات تبعاً للإضافات العلفية: مجموعة أ) المجموعة القياسية (بدون إضافة ثوم أو بصل)، مجموعة ب) مجموعة مضاف إليها ثوم بنسبة 4 %، مجموعة ج) مجموعة مضاف إليها بصل بنسبة 6 %، مجموعة د) مجموعة مضاف إليها خليط من الثوم والبصل بنسبة 10 % (4 % ثوم و 6 % بصل على أساس المادة الجافة)، وتمت رعاية الأسماك في 36 حوض زجاجي سعة 100 لتر للحوض بواقع ثلاثة مكررات لكل معاملة لمدة 84 يوم، وتم وضع عشرة أصبعيات من أسماك البلطى النيلي وحيد الجنس بمتوسط وزن 3.12 ± 0.30 جرام /سمكة، وغذيت الأسماك مرتين يوميا (6 أيام في الأسبوع) بمعدل 5 % من وزن جسم الأسماك على أساس الوزن الجاف، عند دراسة تأثير أستخدام المجموعات البروتينية بغض النظر عن تأثير الأضافات العلفية المختلفة، وجدت الدراسة أن أعلى كفاءة في النمو والاستفادة من الغذاء والعناصر الغذائية قد تحققت في أصبعيات أسماك البلطي النيلي وحيد الجنس التي غذيت على المجموعة البروتينية رقم 2 (المحتوية على 50 % مسحوق سمك + 50 % فول الصويا)؛ تليها مجموعة رقم 3 (75 % مسحوق سمك + 25 % فول الصويا)؛ ثم المجموعة رقم 1 (25 % مسحوق سمك + 75 % فول الصويا)، على التوالي. من جهة أخرى، عند دراسة تأثير أستخدام تأثير الأضافات العلفية المختلفة (4 % ثوم – 6 % بصل – 10 % من خليط منهما) بغض النظر عن تأثير المجموعات البروتينية وجدت الدراسة أن أعلى كفاءة في النمو والاستفادة من الغذاء والعناصر الغذائية قد تحققت في أصبعيات أسماك البلطي النيلي وحيد الجنس التي غذيت العلائق المضاف اليها الخليط المكون من الثوم والبصل بنسبة 10 % وكانت الفروق معنوية (ح≤0.05) بينها وبين المعاملات الأخرى. كما أشارت النتائج أيضاً إلى أن الأصبعيات التي غذيت على العلائق المحتوية على البصل حققت استجابة أفضل للنمو والإستفادة من الغذاء عن تلك التي غذيت على الأعلاف المحتوية على الثوم. عند دراسة التداخل بين تأثير المجموعات البروتينية الثلاثة وبين الأضافات العلفية المختلفة، وجدت الدراسة أن أعلى كفاءة في النمو والاستفادة من الغذاء والعناصر الغذائية قد تحققت في تلك الأصبعيات التي غذيت على العليقة 8 المحتوية على (50 % مسحوق سمك + 50 % فول الصويا) مع خليط من الثوم والبصل بنسبة 10 % (4 % ثوم و 6 % بصل على أساس المادة الجافة) ؛ تليها العليقة رقم 12 المحتوية على (75 % مسحوق سمك + 25 % فول الصويا) مع خليط من الثوم والبصل بنسبة 10 % (4 % ثوم و 6 % بصل على أساس المادة الجافة) على التوالي. بصورة عامة، وبناء على النتائج المتحصل عليها، توضح الدراسة بأن مصدر ومستوى بروتين الغذاء بالإضافة إلى نوع ومستوى الإضافات العلفية تؤثر على كفاءة النمو والإستفادة من الغذاء والعناصر الغذائية لأصبعيات أسماك البلطي النيلي وحيد الجنس تحت ظروف المعمل التجريبية. ويمكن إستخلاص أن أقصى نسبة من يمكن إحلالها من مسحوق السمك بمسحوق فول الصويا في أعلاف أصبعيات أسماك البلطي النيلي وحيد الجنس هي 50 %، وأن إستخدام خليط من مسحوق الثوم ومسحوق البصل كإضافات علفية بنسبة 10 % تحسن من كفاءة النمو والاستفادة من الغذاء والعناصر الغذائية.
كلمات مدخلية: *إضافات غذائية، كفاءة النمو، البلطي، التركيب الكيميائي.*

INTRODUCTION

According to the proposed principles of organic aquaculture feeds, all fish meal (FM) incorporated must be derived from fishery resources certified to be sustainability managed (NOP, 2006), where FM is the protein source traditionally used in aquaculture diets, yet it is a limited resource and is expensive (FAO, 2006). Alternate protein sources can lower the cost of aquaculture diets to reduce the amount of wild fish used as protein, and potentially reduce the nutrient levels in effluent waste. However, for most species, there is a limit to how much FM can be replaced by alternative protein sources without any adverse effects on the fish. Several studies have been done on fresh and marine water fish to investigate their tolerance for alternative protein sources. Soybean meal (SBM) is less expensive than FM, readily available (Hardy, 2006) and considered to be one of the most suitable and stable supply of an alternative ingredient for replacing FM in commercial fish feed industries. In addition, SB is one of the most promising ingredients because of its high protein content, very low carbohydrate and fiber, high digestibility and good amino acid profile (Gatlin *et al*., 2007). Moreover, SB protein has produced encouraging results in diets for different fish species in spite of being limited technically for its amino acid profile and poor palatability (Tacon and Akiyama, 1997). Furthermore, SBM has significantly less phosphorous than FM (NRC, 1993), the feature that makes it has a negative impact on local waterways.

Gabor *et al*. (2010) concluded that the use of phytoadditives in fish feeding has a diverse effects: a) stimulate the appetite as a nutritional additive, b) improving the voluntary intake of a diet as a sensory additive, c) improve the nutritional value of a diet as a zootechnical additive, or d) control the health of fish through direct effects as a coccidiostats and histomonstats. However, phytoadditives have benefits for both fish welfare and environment.

Garlic (*Allium sativum*), member of the *Alliaceae* family, is one of the most popular herbs used worldwide to reduce various risk factors associated with several diseases. It is a rich source of Ca, P, Zn, Fe ; has a high content of carbohydrates and as a consequence a high nutritive value; contains I, Si, S salts, B1, B complex, A, C and F vitamins (Drăgan *et al*., 2008). It has long been considered that garlic (*Allium sativum*) has several beneficial effects for human and animals; exhibiting antimicrobial, antioxidant, and antihypertensive properties (Konjufca *et al*., 1997; Sivam, 2001), and has proved to be hypolipidemic (Sumiyoshi, 1997), antimicrobial (Kumar and Berwal 1998), antihypertensive (Suetsuna, 1998), hepatoprotective and insecticidal (Wang *et al*., 1998). Garlic extract has also been shown to reduce serum cholesterol levels (Bordia *et al*., 1975; Augusti, 1977). The antimicrobial effect of essential oil extracts of three types of onion (green, yellow and red) and garlic was studied by Benkeblia (2003) who found that the strongest antibacterial (inhibitory activity) effect was observed in garlic and red onion. It is also used as immunostimulants and growth promoters for Nile tilapia (Shalaby *et al*., 2006; Ahmed *et al*., 2008; Salah *et al*., 2008; Metwally, 2009). Garlic bulbs (on DM basis) contain 6.1% CP, 0.65% EE, 0.86% CF, 1.48% crude ash and high concentrations of trace minerals (Se), glucosinolates and enzymes (Grela and Klebaniuk, 2007), 17 amino acids, which include lysine, arginine and cysteine (Adetumbi *et al*., 1986; Rees *et al*., 1993; Corzo-Martinez *et al*, 2007). Garlic contains also 0.1–0.36% of a volatile sulfur containing compounds: *Allicin* (responsible for the distinctive odor), diallyl-disulfied, diallyl-trisulfied and others, which are responsible for most of the pharmacological properties of garlic (Silagy and Haw, 1994; Benkeblia, 2003).

Moreover, onion (*Alliumcepa*) is a member of family *Alliaceae*, used as a medicinal plant (antibiotic, antiseptic and anti-infectious) and has hipoglicemiant, antioxidant, antithrombotic, anticholestremia, antiplatelet activity and tonic effects. These pharmacological properties of onion can be ascribed to sulfur compounds which are responsible for the typical odor, flavor and to flavonoids, in particular quercetin which was well known for its anticarcinogenic properties (Deschner *et al*., 1991). Also, onion peel improve male sexual function (Junemann, 2003; Lines and Ono, 2006), where it contains small quantities of sugar, fats and A, C and B

complex vitamins; high content of Mg, K and Cu (Drăgan *et al*., 2008). Simultaneously, it have been considered a digestive material and used to improve the appetite.

The objective of the present study is to investigate the response of Nile tilapia mono-sex (*Oreochromis niloticus*) fingerlings to diets containing different sources and levels of protein using garlic (*Allium sativum*) and onion (*Allium cepa*) as phytoadditives.

MATERIAL AND METHODS

Experimental design

In a 3 × 4 factorial design, three different protein combinations: a) 25% fish meal (FM) + 75% soybean meal (SBM) , b) 50% FM+ 50% SBM and c) 75% FM + 25% SBM were combined with four different additive supplements a) control (without garlic or onion) , b) with 4% garlic; c) with 6% onion and d) with a mixture of garlic and onion (4:6%, respectively) to obtain 12 experimental treatments . Triplicate per treatment were used in this study .The experimental design is shown in Table (1).

Table 1. The experimental design and diets combinations.

Groups	Diet No.	Phytoadditives
G1	D1	without garlic or onion (Control)
	D2	With 4% garlic
	D3	With 6 % onion
	D4	with a mixture of 4 % garlic and 6 %onion
G2	D5	without garlic or onion (Control)
	D6	With 4% garlic
	D7	With 6 % onion
	D8	with a mixture of 4 % garlic and 6 %onion
G3	D9	without garlic or onion (Control)
	D10	With 4% garlic
	D11	With 6 % onion
	D12	with a mixture of 4 % garlic and 6 %onion

G_1: (25% FM + 75% SBM) G_2: (50% FM + 50% SBM)

G_3: (75% FM + 25% SBM)

Fish and culture facility

Fingerlings with an average initial body weight of 3.12 ± 0.3 g/fish were placed randomly in thirty six glass aquaria with dimensions of 100×40×30cm and 100 l water volume/ aquarium; triplicates per treatment were used in this study. Fingerlings were acclimated to the experimental system for 15 days before starting the experiment, fingerlings in each aquarium were reweighed, and their initial weights were recorded. Each aquarium was stocked with ten fingerlings of *O. niloticus*.

Experimental diets

Chemical proximate analysis of feed ingredients used in the presents study is presented in Table (2). Twelve experimental diets were formulated to contain 35% crude protein and almost 434.29 Kcal gross energy/100g. According to protein source and level, diets were divided into three groups: G1) 25% FM + 75% SBM, G2) 50% FM+ 50% SBM and G3) 75% FM + 25% SBM. As for phytoadditives, each group was divided into four treatments; a) control diet (without garlic or onion supplementation); b) diet supplemented with garlic c) diet supplemented with onion and d) diet supplemented with a mixture of garlic and onion (4:6%, respectively). Formulation of the tested diets is presented in Table (3). Each ingredient was ground and thoroughly mixed with the other dietary ingredients, vitamins and minerals mixtures. A few drops of oil was added at the same time of mixing with warm water (45°C) which was slowly added until the diets began to clump. SBM was heat treated to reduce the trypsin inhibitor. Diets were processed by a California pellet mill machine and dried for 48 hrs at 70°C in a drying oven. The experimental pellets were soft enough for the fish to take and retain. Processed diet particle size was 0.6 mm in diameter and 2 mm - length. Fish in each aquarium were fed three times daily (six days a week) at a rate of 5 % of body weight for 84 days.

Table 2. Chemical analysis (%) of the ingredients used in the experimental diets (on DM basis).

Ingredient*	DM (%)	CP (%)	EE (%)	CF (%)	Ash (%)	NFE (%)	Gross energy * (Kcal/100g DM)
Fish meal	89.59	63.18	4.10	1.00	17.95	13.77	452.44
Soybean meal	89.66	45.74	7.70	7.30	7.59	31.67	461.68
Yellow corn	89.49	9.49	3.80	3.20	2.64	80.87	422.71
Rice bran	89.00	15.12	3.90	10.60	6.40	63.98	385.88
Garlic meal	94.71	9.61	6.60	18.30	6.47	53.73	338.03
Onion meal	91.31	13.63	2.90	24.18	14.56	14.18	162.84
Active yeast	96.48	43.08	1.60	2.60	5.05	47.67	454.92

* Estimated, NRC, (1993), Using the factor 5.65, 9.45 and 4.11 for crude protein, ether extract and carbohydrate, respectively.

DM: Dry matter CP: Crude protein EE: Ether extract CF: Crude fiber NFE: Nitrogen – free extract (Calculated by differences)

Table 3. Formulation and proximate analysis (%) of experimental diets (on DM basis).

Items	G1				G2				G3			
	D1	D2	D3	D4	D5	D6	D7	D8	D9	D10	D11	D12
Ingredient (% as fed)												
Fishmeal	13.85	13.85	13.85	13.85	27.70	27.70	27.70	27.70	41.93	41.93	41.93	41.93
Soybean meal	46.77	46.77	46.77	46.77	25.89	25.89	25.89	25.89	4.44	4.44	4.44	4.44
Rice bran	10.46	10.46	10.46	10.46	12.80	12.80	12.80	12.80	15.21	15.21	15.21	15.21
Yellow corn meal	20.92	16.92	14.92	10.92	25.61	21.61	19.61	15.61	30.42	26.42	24.42	20.42
Active yeast	3	3	3	3	3	3	3	3	3	3	3	3
Garlic meal	---	4	---	---	---	4	---	---	---	4	---	---
Onion meal	---	0	6	---	---	---	6	---	---	---	6	---
Garlic and onion meal	---	---	---	10	---	---	---	10	---	---	---	10
Yellow corn oil	3	3	3	3	3	3	3	3	3	3	3	3
Vitamin and Mineral premix*	2	2	2	2	2	2	2	2	2	2	2	2
Proximate analysis (% DM)												
Dry matter	94.62	94.25	94.35	94.46	93.48	93.79	93.25	93.80	92.84	94.65	94.62	94.00
Crude protein	35.11	35.01	35.00	35.03	35.26	35.20	35.12	35.18	35.22	35.11	35.03	35.09
Crude fat	5.42	5.53	5.37	5.48	4.65	4.77	4.58	4.65	3.86	3.97	3.80	3.91
Crude fiber	5.41	6.01	7.97	7.10	4.42	5.11	6.88	6.04	3.48	4.29	5.81	5.10
Ash	7.41	7.56	8.25	7.93	8.58	8.92	9.21	9.07	9.41	9.89	10.22	10.13
NFE	46.65	45.89	43.41	44.46	47.09	46.00	44.21	45.06	48.03	46.74	45.14	45.77
Gross energy (kcal/100g)	440.92	438.27	426.51	432.03	436.30	432.62	423.02	427.51	432.48	427.60	418.97	422.93
P/E ratio (mg cp/kcal)	79.63	79.88	82.06	81.08	80.82	81.36	83.02	82.29	81.44	82.11	83.61	82.97

*Each Kg vitamin & mineral mixture premix contained Vitamin A, 4.8 million IU, D3, 0.8 million IU; E, 4 g; K, 0.8 g; B1, 0.4 g ; Riboflavin, 1.6 g; B6, 0.6 g, B12, 4 mg; Pantothenic acid, 4 g; Nicotinic acid,8 g; Folic acid, 0.4 g Biotin, 20 mg , Mn, 22 g; Zn, 22 g; Fe, 12 g; Cu, 4 g; I, 0.4 g, Selenium, 0.4 g and Co, 4.8 mg.

Water quality:

Water quality parameters in the experimental glass aquaria were determined according to the methods of APHA (1992). The concentrations of ammonia, total alkalinity, nitrate, nitrite were determined according to Boyd, (1979). Ammonia and nitrite were measured at weekly intervals, while water temperatures were recorded daily in each aquaria .Also, dissolved oxygen was measured daily by oxygen meter and pH using pH meter.

Measurement of growth:-

Total weight gain, average daily gain, specific growth rate, feed conversion ratio, protein and energy utilization were determined according to Recker (1975) and Castell and Tiews (1980).

Total gain (g/fish) = (WT-WI)

Where:

WT: Final means weight of fish in grams and WI: Initial means weight of fish in grams.

Average daily gain (ADG) (g/fish day^{-1}) = total gain / duration period

Specific growth rate (SGR, % day^{-1}) = 100 × (ln WT - ln WI) / duration period. Where: (ln) is the natural log and (n) is the duration period.

Measurement of feed and nutrient utilization:-

Feed conversion ratio (FCR) = dry matter intake (g) / total gain (g).

Protein efficiency ratio (PER) = total gain (g) / protein intake (g).

Protein productive value (PPV %) = (PT – PI) ×100 / protein intake (g).

Where: PT: Protein content in fish carcass at the end and PI: Protein content at the start.

Energy utilization (EU %) = (ET–EI) ×100/ Energy intake (kcal).

Where: ET: Energy in fish carcass (kcal) at end and EI: Energy in fish carcass (kcal) at start.

Proximate Analysis of Diet and Fish

At the start of the experiment, 20 fish were taken and kept frozen for the chemical analysis. At the end of the experiment, the basal diet and fish samples from each treatment were chemically analyzed according to the standard methods of AOAC (2000). Gross energy (GE) and energy content (Eco) were calculated from (NRC, 1993) as 5.65, 9.45, and 4.11 kcal/g for protein, lipid, and carbohydrates, respectively.

Statistical Analysis

Statistical analyses of growth performance, feed utilization and whole body composition were done using F-test and analysis of variance for treatments difference was performed according to Steel and Torrie (1980). Statistical analysis was done by, ANOVA, F-test, and L.S.D procedures available within the SAS software package 9.0 (2004).

RESULTS AND DISCUSSION

Water quality

All water quality parameters tested throughout the experimental period revealed that all parameters were within the permissible levels for optimum Nile tilapia growth. Determined water quality parameters averages in the experimental glass aquaria were temperature (28 ± 0.5°C), dissolved oxygen (6.7 ± 0.4 mg l^{-1}), total ammonia (0.08 ± 0.01 mg l^{-1}), nitrite (0.06 ± 0.01 mg l^{-1}), total alkalinity (165 ± 35 mg l^{-1}), chlorides (573 ± 110 mg l^{-1}) and pH (8.4 ± 0.11). These results are in accordance with finding of Abdel-Hakim *et al*.,(2008) working with Mono sex Nile tilapia.

Expermental diets

The proximate chemical analysis (%) of FM, SBM, yellow corn, rice bran, garlic meal, onion meal, and active yeast are shown in Table (2).

The composition and proximate analysis (%) of the twelve experimental diets used in the present experiment are shown in Table (3). The experimental diets were almost isonitrogenous (35.24 ±0.11) % and isocaloric (434.29±2.60 Kcal 100g^{-1}) .The mean value of protein to energy ratio was (88.63±0.23) mg protein /kcal gross energy.

Growth performance

The effects of different replacement levels of fish meal (FM) by soybean meal (SBM) using garlic (*A.sativum*) and onion (*A.cepa)* as phytoadditives on growth performance of Nile tilapia (*O.niloticus*) fingerlings are summarized in Table (4) and Figs. (3).

Concerning protein source combination and level, the result in the present study (Fig.1) illustrated that fingerlings fed G2 diets grew as

Table 4. Effect of using three protein sources combinations and four treatments as feed phytoadditives on growth performance parameter of Nile tilapia *(O. niloticus)* fingerlings.

Treatment			Body weight		Total weight gain (g/fish)	ADG (g/fish/day)	SGR (%/day)
			Initial (g/fish)	Final (g/fish)			
Protein sources combinations (PSC)							
G1			3.13[a]	27.83[c]	24.72[c]	0.22[c]	1.95[c]
G2			3.13[a]	38.98[a]	35.85[a]	0.32[a]	2.26[a]
G3			3.13[a]	35.17[b]	32.04[b]	0.29[b]	2.16[a]
Feed phytoadditives (FA)							
Without garlic or onion		(C)	3.13[a]	29.48[d]	26.35[d]	0.23[c]	2.00[c]
With 4% garlic		(G)	3.12[a]	32.79[c]	29.69[c]	0.27[b]	2.10[b]
With 6% onion		(O)	3.13[a]	35.41[b]	32.29[b]	0.29[b]	2.16[b]
With 10 % garlic and onion		(G+O)	3.12[a]	38.28[a]	35.15[a]	0.31[a]	2.23[a]
PSC * FA							
G1	Control	D1	3.14	24.67	21.53	0.19	1.85
	+ 4% garlic	D2	3.12	26.84	23.79	0.21	1.92
	+ 6% onion	D3	3.12	28.66	25.54	0.23	1.98
	+10% mixture	D4	3.12	31.16	28.04	0.25	2.06
G2	Control	D5	3.12	32.14	29.02	0.26	2.09
	+ 4% garlic	D6	3.13	37.29	34.16	0.31	2.25
	+ 6% onion	D7	3.13	41.46	38.33	0.34	2.31
	+10% mixture	D8	3.12	45.04	41.90	0.37	2.38
G3	Control	D9	3.14	31.63	28.49	0.25	2.07
	+ 4% garlic	D10	3.12	34.26	31.14	0.28	2.14
	+ 6% onion	D11	3.13	36.13	33.00	0.29	2.18
	+10% mixture	D12	3.14	38.65	35.51	0.32	2.24
L.S.D (P<0.05)*			0.518	0.169	0.121	0.045	0.106

G1: (25% FM + 75% SBM) G2: (50% FM + 50 % SBM) G3: (75% FM + 25% SBM)

*Mean in the same column bearing different superscript are significantly different at (P<0.05).

ADG = Average daily gain (g/fish day-1) SGR= Specific growth rate (% day-1)

well as or better than the other groups (G3 and G1) and differences were significant (P<0.05) between the three groups. It was observed that increasing SBM up to 75% in G1 diets decreased fish growth performance to the lowest values. Similar results have been reported by Mbahinzireki *et al*. (2001) who replaced SBM instead of FM; Soltan (2005) who replaced canola seed meal instead of FM in Nile tilapia diets. In contrast, Tacon *et al*. (1983) reported that growth of Nile tilapia was improved with increasing SBM inclusion level instead of FM up to 75%, while Khan *et al*. (2003) successfully replaced 100% of FM with SBM in *Labeo rohita* diets and Kasper *et al*. (2007) up to 47.6% in yellow perch (*Perca flavescens*) diets without affecting the feed consumption, weight gain, feed efficiency or survival of the fish. In that respect, Sullivan (2008) reported that lysine (EAA) content appeared to be lower in SBM than in FM and as SBM substitution increased, lysine decreased. He added that the importance of Lysine as a limiting amino acid for growth and its role as a necessary building block for all protein in the body; plays a major role in calcium absorption; building muscle protein; and the body's production of hormones, enzymes, and antibodies, may explain the cause of decreasing occurred in growth performance and feed utilization resulted from increasing

SBM substitution level more than 50%.

Taking into account phytoadditives formula, addition of garlic only in the present study slightly increased growth performance, while the addition of onion only obtained better performances. In agreement, Zaki and El-Ebiary (2003) found that 3 g dry garlic/kg diet is recommended as growth promotors for mono-sex Nile tilapia favorable growth. In addition, Abou-Zeid (2002) and Aly *et al*. (2008) found that garlic improved the growth performance for Nile tilapia and similar results were reported by El-Saidy and Gaber (1997); Metwally (2009) and Abd El-Hamid (2010) when fed Nile tilapia on diets contained garlic meals. Moreover, Shalaby *et al*. (2006) recorded the highest growth performance of Nile tilapia fed on 3% garlic, while Diab *et al*. (2002) recommended 2.5%. On the contrary, Salah *et al*. (2008) found no significant differences (P>0.05) in Nile tilapia growth when supplemented diet included 10 and 20 g garlic / kg feed, however several studies (Gomes *et al*., 1993 on rainbow trout; Degani *et al*., 1997 on hybrid tilapia; Goddard and Mclean, 2001; Khattab, 2001 and Shalaby *et al*., 2006 on Nile Tilapia) concluded that apparent protein digestibility was improved with increasing levels of garlic in fish diet.

The highest final weight, weight gain, ADG and SGR% (38.28, 35.15, 0.31 and 2.23%, respectively) obtained with fish fed mixture of garlic and onion included diets (D4, D8 and D12) in the present study as shown in Fig. (2). In concordance with the present study, Al-Salahy (2002) found that onion and garlic dietary administration for *Clarias lazera* caused a rise in liver free amino-acids; meanwhile, the garlic fed fish presented a rise in muscle free amino-acid levels and according to enhanced muscle uptake of free amino-acids may enhance protein synthesis.

Concerning protein source combination and phytoadditives, fish fed diets D4, D8 and D12 (included 50% FM + 50% SBM and mixture of 4% G and 6% O) grew significantly (P<0.05) better than that fed control diet. On the other side, fish fed D8, recorded the highest growth rather than other treatments as shown in Fig. (3). In another words, fingerlings fed D7 and D8 in the second group (G2) recorded the best values for growth performance. Moreover, increasing SBM replacement level up to 50% with garlic, onion or mixture of both led to increase growth performance, meanwhile increasing SBM replacement level up to 75% in G3 diets (D9, D10, D11 and D12) decreased growth performance significantly (P<0.05) than G2 diets (D5, D6, D7 and D8) in spite of being better than G1 diets (D1, D2, D3 and D4). In addition, results found that fingerlings fed D8 and D12 diets grew as well or better than other treatments. Similar results have been reported by Abd El-Hamid (2010); El-Saidy *et al*. (1999) and Zaki and El-Ebiary (2003).

Feed and nutrient utilization

Regardless of phytoadditives, table (5) and Fig. (4) illustrated the effect of protein source combination on feed and nutrients utilization. Results revealed that differences were significant (P<0.05) between groups in feed and nutrients utilization. On the other hand, FI, PPV% and EU% were significantly (P<0.05) increased with increasing SBM substitution level up to 50% while FCR decreased and PER does not changed.

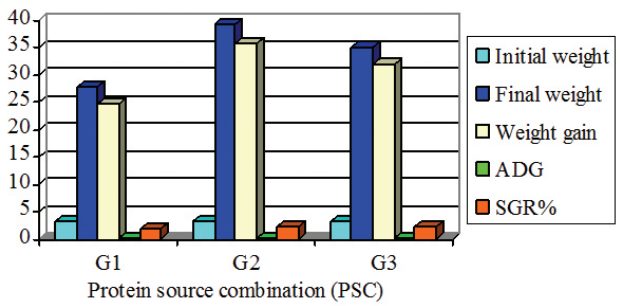

Fig. 1. Effect protein sources combinations on growth performance.

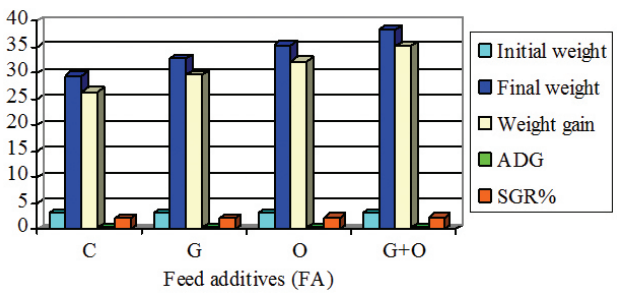

Fig. 2. Effect of phytoadditives combinations on growth performance.

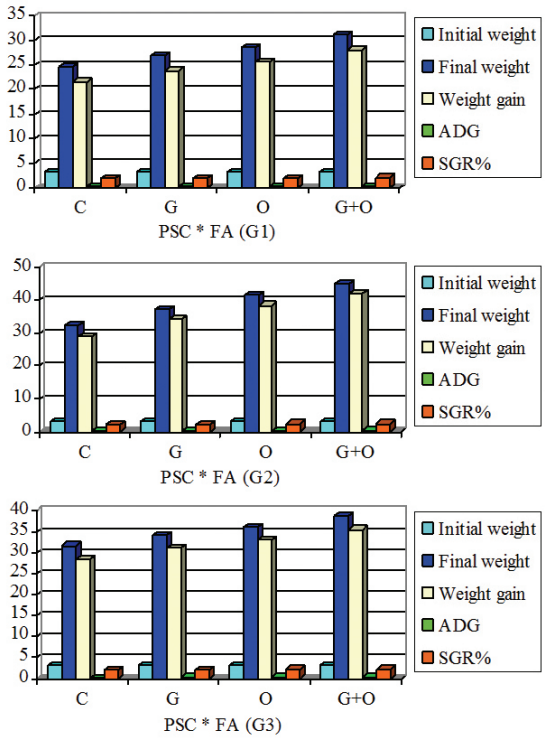

Fig. 3. Effect of protein sources and phytoadditives combinations (PSC*FA) on growth performance.

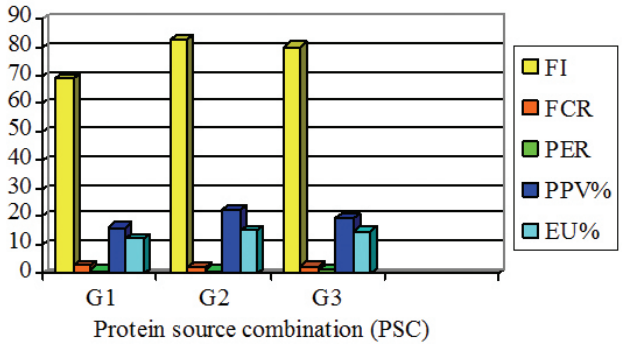

Fig. 4. Effect of protein sources and combinations (PSC) on feed and energy utilization.

Regardless protein sources combinations, the effect of phytoadditives on feed and nutrients utilization clearly showed (Table 5 and Fig. 5) that FI and PER of Nile tilapia fingerlings increased significantly ($P<0.05$) with addition of garlic, onion and mixture of both in ascending order, while FCR decreased in the same order. Similar results have been reported that feed intake increased with increasing *A.sativum* levels, while feed conversion ratio decreased (El-Saidy and Gaber,

Table 5. Effect of using protein sources and phytoadditives combinations on feed and nutrient utilization of Nile tilapia *(O. niloticus)* fingerlings diets.

Treatment			Feed utilization		Protein utilization		Energy utilization (%)
			FI[1] (gfish)	FCR[2]	PER[3]	PPV %[4]	
Protein sources combinations (PSC)							
G1			68.95[c]	2.81[a]	1.02[b]	16.35[c]	12.51[c]
G2			82.78[a]	2.33[c]	1.02[b]	22.32[a]	15.05[a]
G3			80.27[b]	2.51[b]	1.14[a]	19.47[b]	14.77[b]
Feed phytoadditives (FA)							
Without garlic or onion	(C)		70.55 [d]	2.62[a]	1.16[a]	20.26[b]	13.67[c]
With 4% garlic	(G)		77.04 [c]	2.62[a]	1.09[b]	18.55[c]	13.93[b]
With 6% onion	(O)		78.39 [b]	2.55[b]	1.08[b]	17.93[d]	13.70[c]
with With 10 % garlic and onion	(G+O)		83.34 [a]	2.40[c]	1.18[a]	20.77[a]	15.15[a]
PSC * FA							
G1	Control	D1	66.19	3.08	1.09	18.35	13.51
	+ 4% garlic	D2	67.19	2.85	1.00	15.96	12.27
	+ 6% onion	D3	68.77	2.68	0.93	13.72	10.93
	+10% mixture	D4	73.32	2.62	1.06	17.36	13.35
G2	Control	D5	72.15	2.21	1.29	23.69	12.51
	+ 4% garlic	D6	83.24	2.44	1.17	20.89	15.20
	+ 6% onion	D7	84.59	2.49	1.15	20.29	15.13
	+10% mixture	D8	91.12	2.17	1.30	24.41	17.35
G3	Control	D9	73.32	2.57	1.11	18.74	14.59
	+ 4% garlic	D10	80.38	2.56	1.10	18.79	14.31
	+ 6% onion	D11	81.82	2.48	1.15	19.79	15.04
	+10% mixture	D12	85.57	2.41	1.18	20.54	15.15
L.S.D ($P<0.05$)*			0.125	0.097	0.089	0.461	0.061

G_1: (25% FM + 75% SBM) G_2: (50% FM + 50 % SBM) G_3: (75% FM + 25% SBM) *Mean in the same column bearing different superscript are significantly different at ($P< 0.05$). 1 Feed intake (g/fish) 2 Feed conversion ratio 3 Protein efficiency ratio 4 Protein productive value

1997; Shalaby *et al*., 2006; Zaki and El-Ebiary, 2003; Aly *et al*., 2008 and Abd El-Hamid, 2010).

Taking into account SBM replacement level and phytoadditives, results in table (5) and Fig.(6) revealed that feed utilization parameters of fingerlings fed diet D8 were significantly (P<0.05) higher than other treatments. In another words, increasing SBM replacement level up to 50% with either garlic or onion or mixture of both led to increase feed utilization. Meanwhile, increasing SBM replacement level up to 75% in G3 diets (D9, D10, D11 and D12) decreased feed utilization significantly (P<0.05) than G2 diets (D5, D6, D7 and D8) in spite of being better than G1 diets (D1, D2, D3 and D4). Moreover, increasing SBM replacement level recorded best results with mixture of garlic and onion more than each phytoadditive alone. The present results are in agreement with the finding of Mbahinzireki *et al*. (2001) and Soltan (2005).

Carcass composition of fish

The chemical composition parameters of whole body of Nile tilapia *O.niloticus* fingerlings are summarized in Table (6). Regardless of phytoadditives, the effect of protein source combination on dry matter (DM %), crude protein (CP %) was significant (P<0.05) when SBM inclusion level increased up to 50%, while EE% and ash% decreased and no significant (P>0.05) differences were observed in energy content (kcal $100g^{-1}$). It was observed also that DM% and CP% were decreased, while EE% and ash% were increased significantly (P<0.05) with increasing SBM inclusion level up to 75%. At the same time, no significant (P>0.05) differences were noticed in energy content among all groups.

Regardless protein sources combinations, the effect of phytoadditives on chemical composition of harvested fish indicated that DM%, CP%, and energy content were significantly (P<0.05) increased with using garlic or onion or both mixture, where the mixture recorded the superior values while control diet recorded the inferior values. On the other hand, EE% and ash% were significantly (P<0.05) decreased with using garlic or onion or both mixture, where the control diet recorded the superior values while phytoadditives mixture diet recorded the inferior values.

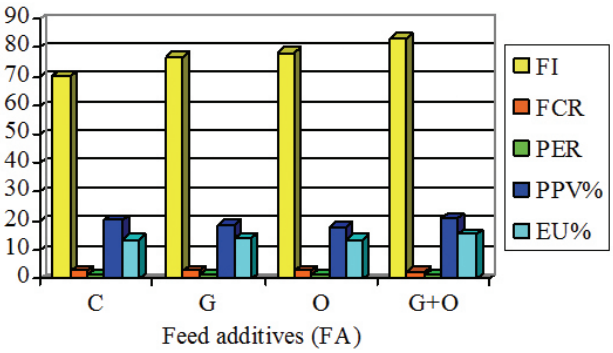

Fig. 5. Effect of phytoadditives combinations (FA) on feed and energy utilization.

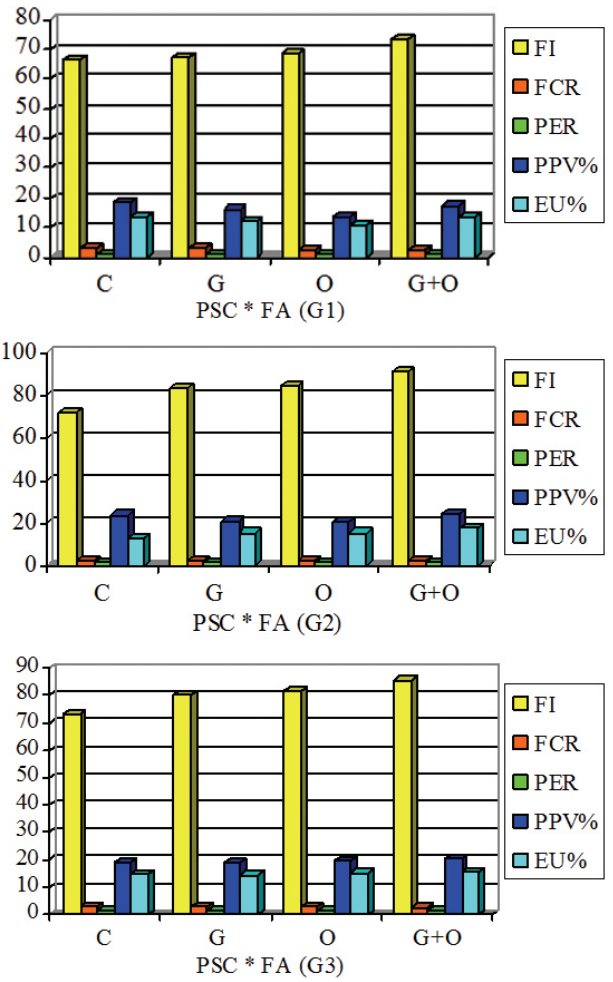

Fig. 6. Effect of protein sources and phytoadditives combinations (PSC * FA) on feed and energy utilization.

Concerning the effect of interaction between protein source combination and phytoadditives on chemical composition of the experimental fish, DM% and CP% recorded the highest values, while EE% and ash% recorded the lowest values with fish fed D8. On the other hand, D1 recorded the

Table 6. Effect of using protein sources combinations and feed phytoadditives on body composition (% dry weight) of Nile tilapia *(O.niloticus)* fingerlings.

Treatment			DM %	% on DM basis			Energy content (kcal/100g)
				CP	EE	Ash	
At the start:			23.67	54.17	17.34	28.49	469.21
At the end:							
Protein sources combinations (PSC)							
G1			26.55^{c}	58.54^{c}	23.80^{a}	17.66^{a}	554.54^{a}
G2			28.51^{a}	62.25^{a}	21.54^{c}	16.21^{c}	554.45^{a}
G3			27.67^{b}	60.53^{b}	22.59^{b}	16.88^{b}	553.84^{a}
Feed phytoadditives (FA)							
Without garlic or onion		(C)	26.84 ^{d}	59.80^{d}	23.02^{a}	17.18^{a}	553.27^{b}
With 4% garlic		(G)	27.52 ^{c}	60.12^{c}	22.81^{b}	17.06^{b}	554.43^{b}
With 6% onion		(O)	27.86 ^{b}	60.68^{b}	22.50^{c}	16.83^{c}	554.60^{a}
with	With 10 % garlic and onion	(G+O)	28.08 ^{a}	61.15^{a}	22.45^{d}	16.59^{d}	554.60^{a}
PSC * FA							
G1	Control	D1	25.12	57.76	24.30	17.94	551.16
	+ 4% garlic	D2	26.70	58.20	23.97	17.83	554.52
	+ 6% onion	D3	27.12	58.88	23.61	17.51	554.96
	+10% mixture	D4	27.26	59.32	23.32	17.36	554.71
G2	Control	D5	27.87	61.86	21.76	16.38	554.30
	+ 4% garlic	D6	28.17	61.05	21.64	16.31	554.24
	+ 6% onion	D7	28.77	62.41	21.43	16.16	554.29
	+10% mixture	D8	29.22	62.67	21.34	15.98	554.91
G3	Control	D9	27.53	59.79	23.00	17.21	554.34
	+ 4% garlic	D10	27.69	60.11	22.83	17.04	554.54
	+ 6% onion	D11	27.70	60.75	22.45	16.81	554.56
	+10% mixture	D12	27.76	61.47	22.08	16.44	554.72
L.S.D(P<0.05)*			0.119	0.094	0.127	0.113	2.221

G1: (25% FM + 75% SBM) G2: (50% FM + 50 % SBM) G3: (75% FM + 25% SBM)

*Mean in the same column bearing different superscript are significantly different at (P<0.05).

Calculated based on 5.65, 9.45 and 4.11 (kcal g-1) of protein, lipid and carbohydrate, respectively.

lowest values of DM% and CP% and the highest values of EE% and ash%. In addition, it was observed that D4, D8 and D12 recorded the highest values of DM%, CP% and energy content, while EE% and ash% were inferior between treatments. Meanwhile, using garlic and onion mixture in each SBM combination group realized the higher values in DM%, CP% and energy content, while the lowest in EE% and ash%. In concordance with the present study, results of El-Saidy and Gaber (1997), Zaki and El-Ebiary (2003), Shalaby *et al*. (2006), Mohamed *et al*., (2007), Eid and Mohamed (2008), and Abd El-Hamid (2010) support the previous results; while in contrast, Diab *et al*. (2002) reported that there were no significant changes in fish body composition caused due to addition of different garlic levels.

CONCLUSION

Phytoadditives represent alternative solutions to thrive aquaculture feeds as growth promoters. It could be concluded that protein source combination and phytoadditive

inclusion levels affect growth performance, feed utilization and chemical composition of Nile tilapia (*Oreochromis niloticus*) fingerling under laboratory conditions. More clearly, the maximum percentage of FM could be replaced by SBM is 50% in Nile tilapia (*Oreochromis niloticus*) mono-sex fingerling diets, with mixture of phytoadditives compose of 4% garlic and 6% onion, to improve growth performance and feed utilization. At the same time, further researches including commercial cost and benefit analysis are necessary before phytoadditives can be used on large scale aquaculture.

REFERENCES

Abdel-Hakim, NF, Lashin, ME, Al-Azab, A, and **Nazmi, HM** (2008) Effect of replacing soybean meal protein by other plant protein sources on growth performance and economical efficiency of mono sex Nile tilapia (*O.niloticus*) cultured in tanks. *8th International Symposium on Tilapia in Aquaculture*.P.739-755.Academy Press, Washington DC, USA.

Abd El-Hamid, HMB (2010) *Physiological and nutritional studies on improving growth of Nile tilapia (O. niloticus) fry using some medicinal plants as feed phytoadditives*. MSc. Thesis .Faculty of agri, Kafrelsheikh University, Egypt.

Abou-Zeid, SM (2002) *The effect of some medical plant on reproductive and productive performance of Nile tilapia fish*. Cairo University, Faculty of Agriculture, 2002. 212p. [Ph. D. Thesis]

Adetumbi M, Javor GT, and **Lau, BH** (1986) *Allium sativum* (garlic) inhibits lipid synthesis by Candida albicans. *Antimicrobial Agents and Chemotherapy* **30**: 499–501.

Ahmed, SD, Salah, MA, George, J, Yasser, AH, and **Mohamed, FM** (2008) Effect of garlic, black seed and Biogen as immunostimulants on the growth and survival of Nile tilapia, Oreochromis niloticus (Teleostei: Cichlidae), and their response to artificial infection with Pseudomonas fluorescens; *African Journal of Aquatic Science* **33**(1): 63-68.

Aly, SM, Nashwa, MA, and **Mohamed, MF** (2008) Effect of Garlic on the survival, Growth, Resistance and Quality of *Oreochromis niloticus*. *8th International Symposium on Tilapia in Aquaculture* 277-296.

Al-Salahy, MB (2002) Some physiological studies on the effect of onion and garlic juices on the fish, *Clarias lazera*; *Fish Physiology and Biochemistry* **27**: 129–142, 2002.

AOAC (2000) Association of Official Analytical Chemists, *Official methods of analysis*, 17th Ed. Washington, DC.,USA.

APHA (American Public Health Association) (1992) Standard methods for the examination of water and wastewater. 18th ed. APHA, Washington, DC.

Augusti K. T. (1977) Hypocholesterolaemic effect of garlic, *Allium sativum*, Linn. *Indian J. Exp. Biol*. **15:** 489–490.

Benkeblia, N. (2003) Antimicrobial activity of essential oil extracts of various onions (*Allium cepa*) and garlic (*Allium sativum*); 2003 Swiss Society of Food Science and Technology. *Published by Elsevier Science Ltd*.

Bordia A, Bansal, HC, Arora, SK, and **Singh, SV** (1975) Effect of essential oils of garlic and onion on alimentary hyperlipemia. *Atherosclerosis* **21**: 15–19.

Boyd, CE (1979) "Water quality in warm water fish ponds." Ed. Claude E. Boyd, Third Ed., 1984, Pub. Auburn Univ., Agric. Exp. Station, AID/Dsan G.G., 0039 **pp**. 359.

Castell, JD, and **Tiewes, K** (1980) Report of the EIFAC, IUNS and ICES Working Group on the standardization of Methodology in Fish Research, Hamburg, FRG,212-23 March, 1979. IFAC Tech. **Pap**. (3) 24.

Corzo-Martinez, M, Corzo, N, and **Villamiel, M,** (2007) Biological properties of onions and garlic. *Trends in Food Science & Technology*, **18**, 609-625.

Degani, G, Viola, S, and **Yehuda, Y** (1997) Apparent digestibility of protein and carbohydrate in feed ingredients for adult tilapia (*Oreochromis aureus* X *O. niloticus*). *Isr. J. Aquac*. **49**: 115-23.

Deschner, EE, Ruperto, J, Wong, G, and **Newmark, HL** (1991) Quercetin and rutin as inhibitors of azoxymethanol-induced colonic neoplasia. *Carcinogenesis* **12(7):** 1193-1196.

Diab, AS, El-Nagar, GO, and **Abd-El-Hady, YM** (2002) Evaluation of *Nigella sativa* L (black seeds; baraka), *Allium sativum* (garlic) and BIOGEN as feed additives on growth performance and immunostimulants of *O. niloticus* fingerlings. *Suez Canal Vet. Med. J.* pp 745-775.

Drăgan, S, Gergen, I, and **Socaciu, C** (2008) Alimentaţia funcţională cu componente bioactive naturale în sindromul metabolic; Ed. Eurostampa, Timişoara. pp 200-202,160-161, 314.

Eid, A, and **Mohamed, KA** (2008) Effect of using probiotic as growth promoters in commercial diets for mono-sex Nile tilapia (*O.niloticus*) fingerlings.*8th International Symposium on tilapia in Egypt* .

El-Saidy, DMS, Gaber, MMA, and **Magouz, FL** (1999) Growth response of Nile tilapia fry *(Oreochromis niloticus)* Fed diet containing different leaves of energy. *Egypt. J.Aquat. Biol. & Fish.* **3**(3):157-178.

El-Saidy, DM, and **Gaber, MM** (1997) Effect of different levels of dry garlic meal supplemented to the diets on growth performances,survival and body composition of Nile tilapia ,Oreochromis niloticus fingerlings .*Annuals of Agric.Sci., Moshtor*, **35** (3): 1197-1209.

FAO (2006) State of World Aquaculture. FAO fisheries Technical Paper. No. 500.

Gabor, EF, Şara, A, and **Barbu, A** (2010) The Effects of Some Phytoadditives on Growth, Health and Meat Quality on Different Species of Fish. *Animal Science and Biotechnologies* **43(1):**61-65

Gatlin, DM III, Barrows, FT, Braown, P, Dabrowski, K, Gaylord, TG, Hardy, RW, Herman, E, Hu, G, Krogdahl, A, Nelson, R, Overturf, K, Rust, M, Sealy, W, Skonberg, D, Souza, EJ, Stone, D, Wilson, R, and **Wurtele, E**, (2007) Expanding the utilization of sustainable plant products in aquafeeds: a review. *Aquaculture Research* **38**: 551- 579.

Goddard, JS, and **Mclean, E** (2001) Acid-insoluble ash as an inert reference material for digestibility studies in tilapia, *Oreochromis aureus*. *Aquaculture* **194(2):** 93-98.

Gomes, EF, Corraze, G, and **Kaushik, SJ** (1993) Effect of dietary incorporation of a coextruded plant protein (rapeseed and peas) on growth, nutrient utilization and muscle fatty acid composition of rainbow trout (*Oncorhynchus mykiss*). *Aquaculture* **113**: 339-353.

Grela, RE, and **Klebaniuk**, **R** (2007) Chemical composition of garlic preparation and its utilization in piglet diets. *Medycyna Wet.* **63 (7):** 792- 795.

Hardy, RW (2006) Worldwide Fish meal production outlook and the use of alternative protein meals for aquaculture. In: *VIII International Symposium on Aquaculture Nutrition*, Nov 15- 17, Universidad Autonoma de Leon, Monterry, Leon, Mexico.

Junemann, KP, (2003) How effective are PDE-5 inhibitors?. *Urol. A* **42**: 553-558.

Kasper, CS, Watkins, BA, and **Brown, PB** (2007) Evaluation of two soybean meals fed to yellow perch (*Perca flavescens)*. *Aquaculture Nutrition* **13**: 431-438.

Khan, MA, Jafri, AK, Chadha, NK, and **Usmani, N**, (2003) Growth and body composition of rohu (*Labeo rohita*) fed diets containing oilseed meals: partial or total replacement of fish meal with soybean meal. *Aquaculture Nutrition* **9(6)**: 391-396.

Khattab, YA (2001) Effect of substituting black seed cake (*Nigella sativa* L.) for soybean meal in diets of Nile tilapia (*O. niloticus* L.) on growth performance and nutrients utilization. *Egypt J. Aquat. Biol. & Fish.* **5(2)**: 31-46.

Konjufca, VH, Pesti, GM, and **Bakalli, RI** (1997) Modulation of cholesterol levels in broiler meat by dietary garlic and copper. *Poultry Science* **76(9)**: 1264-1271.

Kumar, M, and **Berwal, JS** (1998) Sensitivity of food pathogens to garlic (*Allium sativum* L.). *J. Appl. Microbiol.* **84**: 213–215.

Lines, TC, and **Ono, M** (2006) FRS 1000, an extract of red onion peel, strongly inhibits phosphodiesterase 5A (PDE 5A). *Phytomedicine* **13(4)**: 236-239.

Mbahinzireki, GB, Dabrowski, K. Lee, KJ, El-Saidy, D, and **Wisner, ER** (2001) Growth, feed utilization and body composition of tilapia (*Oreochromis sp.*) fed with cottonseed meal-based diets in a recirculating system. *Aquaculture Nutrition* **7**: 189-200.

Metwally, MAA (2009) Effects of Garlic (*Allium*

sativum) on Some Antioxidant Activities in Tilapia Nilotica (*Oreochromis niloticus*); *World Journal of Fish and Marine Sciences* **1(1)**: 56-64.

Mohamed, KA, Abdel Fattah, B, and **Eid, AMS** (2007) Evaluation of Using Some Feed Phytoadditives on Growth Performance and Feed Utilization of Mono-sex Nile Tilapia (*O. niloticus*) Fingerlings. *Agricultural Research Journal, Suez Canal Univ*. **7 (3)**:49-54.

NOP, National Organic Program (U.S. Department of Agriculture) (2006). Impact of Harvey v. Johanns and Restoring the NOP to pre-Lawsuit Status A Report to Congress, Interim Final.

NRC (1993) Nutrient Requirements of Warm Water Fishes and Shellfishes. National Research Council, (NRC), NATIONAL ACADEMY PRESS Washington, D.C.

Recker, WE (1975) Computation and interpretation of biological statistics of fish population. *Fish Res. Board Can. Bull*. **191**: 1-382

Rees, LP, Minney, SF, Plummer, NT, Slater, JH, and **Skyrme, DA** (1993) A quantitative assessment of the antimicrobial activity of garlic (*Allium sativum*). *World J Microbiol iotechnol* **9**:303–307.

Salah, MA, Nashwa, MAA, and **Mohamed FM** (2008) Effect of garlic on the survival, growth, resistance and quality of *Oreochromis niloticus*; *8th International Symposium on Tilapia in Aquaculture 2008*.

Shalaby, AM, Khattab, YA, and **Abdel Rahman, AM** (2006) Effects of garlic (*Allium sativum*) and Chloramphenicol on growth performance, physiological parameters and survival of Nile Tilapia *(Oreochromis niloticus*). *J. Venom. Anim. Toxins incl. Trop. Dis*. **12(2)**: 172-201. Original paper - ISSN 1678-9199.

Silagy, CS, and **Haw, N** (1994) Garlic and Cholesterol. *The Journal of the Royal College of Physicians* **28(1)**: 39-45.

Sivam, GP (2001) Recent advances on the nutritional effects associated with the use of garlic as supplement. *Am. Soc. Nutr. Sci*. 1106 -1108.

Soltan, MA (2005) Potential of using raw and processed canola seed meal as alternative fish meal protein source in diets for Nile tilapia *O. niloticus*. *Egyptain J.Nutrition and feeds* **8(1)**Special Issue:1111-1128.

Steel, RG, and **Torrie, JH** (1980) *Principles and procedures of statistics*. 2nd ed. McGraw Hill, New York, U.S.A.

Suetsuna, K (1998) Isolation and characterization of angiotensin Iconverting enzyme inhibitor dipeptides derived from *Allium sativum* (garlic). *J. Nutr. Biochem*. **9**: 415–419.

Sullivan, KB (2008) *Replacement of Fish Meal by alternative protein sources in Diets for Juvenile Black Bass*. University of North Carolina Wilmington, Department of Biology and Marine Biology, M.Sc. Thesis, **75p**.

Sumiyoshi, H (1997) New pharmacological activities of garlic and its constituents (Review). *Folia Pharmacological Japonica* 110 Suppl. **1**: 93 – 97.

Tacon, AGJ, and **Akiyama, DM** (1997) Feed ingredients. In: Crustacean Nutrition. (ed. by L. R. D'Abramo, D.E. Conklin and D.M. Akiyama) *World Aquaculture Society*, Baton Rouge, LA USA.

Tacon, AGJ, Jauncey, K, Falaye, A, Pantha, M, MacGowan, I, and **Stafford, EA,** (1983) The use of meat and bone meal, hydrolyzed feather meal and soybean meal in practical fry and fingerling diets for Oreochromis niloticus. *In Proc. 1st. lntl, Symp. on Tilapia in Aquaculture*, Fishelson, J. and Yaron, Z. (eds). *Tel Aviv Univ. Press*, Israel, **pp**. 356-365.

Wang, BH, Zuel, KA, Rahaman, K, and **Billington, D** (1998) Protective effects of aged garlic extract against bromobenzene toxicity to precision cut rat liver slices. *Toxicology*, **126(3)**: 213–222.

Zaki, A. M. and **El-Ebiary, E.H.** (2003) Effect of incorporation of onion and garlic into diets on growth performance and body composition of mono-sex Nile tilapia (*Oreochromis niloticus*). *Egypt.J.Aquat.Biol.and Fish*. **7(1)**:113-126.