

Original Article

Effect of dietary supplementation of Yeast, Garlic and Enzymes on growth performance and economic evaluation in European Eel (*Anguilla anguilla*) fry

Abdelhady M. Moghazy, Abdallah T. Mansour*, Walid M. Fayed, Eglal A. Omar and Tarek M. Srouf

Fish and Animal Production Department, Faculty of Agriculture (Saba Basha), Alexandria University, Egypt.

ABSTRACT

This study was conducted to investigate the effect of feeding three commercial feed additives Tonilissat® (*Saccharomyces cerevisiae*), Garlin® (26% allicin), Enziver® (Multi-enzymes) and different mixtures of them on growth performance, feed utilization, body composition and cost benefit of European eel (*Anguilla anguilla*) fry. A total of 160 European eel fry (3.0 ± 0.2 g/fish) were divided into 8 groups (10 fish/tank in two replicates) for 120 days. Eight dietary treatments were conducted as follow: the first diet considered to be the control without any additives. The second and third diets contain 0.2% of Tonilissat® and Garlin®, respectively. The fourth diet contains 0.1% of Enziver®, The fifth diet contains a mixture of 0.1% Tonilissat® with 0.1% Garlin®, the sixth diet contains a mixture of 0.1% e Tonilissat® with 0.05% Enziver®, the seventh diet contains a mixture of 0.1% Garlin® with 0.05% Enziver®, and the eighth diet contains a mixture of 0.1% Tonilissat®, 0.1% Garlin® and 0.05% Enziver®, respectively. The obtained results indicated that growth performance, feed conversion ratio and nutrient utilization were significantly improved in eels fed diet supplemented with multi-enzymes and different mixtures of feed additives. The survival rate (%) and whole body proximate composition didn't differ significantly among different experimental groups. Meanwhile, the profit index improved significantly with multi-enzyme supplementation and the combinations of yeast, garlic and multi-enzyme increased diets benefits. Therefore, it's reasonable to incorporate exogenous multi-enzymes or combination of yeast, garlic and multi-enzyme in the diets of European eels.

Keyword: Feed additives, Eel (*Anguilla anguilla*), Growth performance, feed utilization, economic evaluation.

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1. INTRODUCTION

Eel are migrant fish found in marine, brackish and fresh waters worldwide (Nandlal, 2005). Eels classified as tropical and subtropical fish, and it includes 19 species distributed throughout the world

(Arai, 2014). Four eel species are commercially important such as European eel (*Anguilla anguilla*), Asian eel, (*A. japonica*), North American eel, (*A. rostrata*) and Australian eel (*A. australis*).

Correspondence:

Abdallah T. Mansour

Fish and Animal Production Department, Faculty of Agriculture (Saba Basha), Alexandria University, Egypt

mail: a_taag@yahoo.com

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The European eel, *A. Anguilla*, has long been an important economic resource for fishermen in the Atlantic and Mediterranean coasts (Melia et al., 2006). Meanwhile, in East-Asian countries more than 90% of *Anguilla* production is based on eel farming, which requires wild-caught glass eels for stocking, as the captive reproduction and raising of larvae to glass eel stage is not commercially viable yet (Shiraishi and Crook, 2015). The eels farming going to grow from extensive to intensive culture in several regions of the world (Genç et al., 2005). Whereas, may much attention for culturing fish with high global market has been paid off. In Egypt, *A. anguilla* is one of the most important and valuable commercial species with a wide market and great customer appeal. *A. anguilla* is distributing along the Nile river specially the north Delta region and costal lagoons (Hamza, 1996). Eels farms will be success in Egypt because of the Egyptian climate is very suitable for eels farming and the production cost in Egypt is lower than the main producer countries (Hamza, 1996). Whereas, the total Egyptian production of eels increased from 466 tons in 2004 to 636 tons in 2017. However, the aquaculture contributes by 5.80% of this production (GAFRD, 2018). Moreover, the Egyptian aquaculture is concentrated on inland farms with the main species culture being tilapia and mullets. Due to the high productivity of the typical culture species which in turn led to an oversupply of the two species with a consequent decline in market prices. Hence, there is interest to investigate new species amenable for culture under the existing conditions as a strategy for diversifying marketing opportunities for the cultured species in local and global markets, thus will be increase eel production (El Shebly et al., 2007).

Eels fish rearing have many management problems as acceptance of artificial feed, feed costs (Ghonimy, 2013), escaping behavior and high mortality rate occurs during the weaning to an artificial diet, whereas survival rate to marketable size is only 25-40 % (Larkin, 2000). The commercialization of eel aquaculture still need more study especially the feeding cost, which constitutes 30-70% of total expenditure in fish culture and for this reason, different methods have been essayed to gain benefit from every kind of feed source especially in the developed countries (Yildirim et al., 1999). The overall goal of the aquaculture is to maximize production, increase profitability and reduce production costs (Yildirim et al., 1999). The increasing of commercial aquaculture intensification, encourage the invention of many products as feed additives (microbes, animals, plants and chemicals origin) for aquaculture with varying success rate. Yeast is one of the most used probiotic feed additive in the animal nutrition. It could be improved growth performance and diet utilization (Li and Gatlin, 2003, Abdel-Tawwab et al., 2008, Zhao et al., 2017, Abdel-Aziz et al., 2020 and 2021) antioxidant system and stimulate the immune response (Ortuño et al., 2002); Choudhury et al., 2005 and Vetvicka et al., 2013) of several fish species. Garlic (*Allium sativum*) has had an important dietary and medicinal role for centuries and has long been known to have broad antibacterial properties (Guo et al., 2012). Garlic (*Allium sativum*) also has been reported to produce various beneficial effects, including anti-stress protection, growth promotion, appetite stimulation, immuno-stimulation and anti-microbial properties in finfish and shrimp larvae culture (Aly et al., 2010, Guo et al., 2011 and Millet et al., 2011).

Moreover, the exogenous enzyme supplementation to fish diet specially in larval stage or with plant-based protein diets becomes common practice (Kolkovski et al., 1993, Farhangi and Carter 2007 and Cao et al., 2007). Enzymes supplementation enhance the nutritional value of fish diet via transforming the complex components to absorbable nutrients (Soltan, 2009). They can improve fish growth, feed intake, feed and nutrients utilization and reduce the cost of fish production and the release of nutrients to

2. Materials and Methods

2.1. Experimental fish and culture technique:

The study was carried out at privet fish farm, Kafr El-Sheikh Governorate in cooperation with the Department of Fish and Animal Production at Faculty of Agriculture (Saba Basha), Alexandria University. A total of 160 *A. anguilla* fry (3.0 ± 0.2 g/fish) were obtained from Brollos Lake, Kafr El-Sheikh governorate. The fish caught were transported in a small bucket (30 L capacity) to circular plastic tanks filled with freshwater. The transport acclimation was done immediately after arrival to fish farm by adding water gradually to the buckets contained the caught fish, in order to acclimate it to rearing water temperature. Then eels were acclimated to rearing condition for two weeks prior to start the experiment. The fish was randomly assigned to sixteen plastic tanks (0.2 m^3 water capacity) to represent the eight treatments each in duplicate. The tanks were covered with nylon net (1 mm mesh). Experimental fish were reared in fresh water at a temperature ($27 \pm 1^\circ \text{C}$), pH (8.5 ± 0.2), dissolved oxygen (6 ppm) and a photoperiod of 12 H light: 12 H dark. Water was changed daily at the rate of 25% of the total volume before the first feeding.

the environment (Kolkovski et al., 1993, Debnath et al., 2005, Farhangi and Carter 2007 and Dawood et al., 2014). However, the available literature regarding the application of feed additives in eel culture is very rare. Therefore, this study was conducted to determine the effect of feeding three commercial feed additives Tonilissat[®], Garlin[®], Enziver[®] and different mixtures of them on growth performance, feed utilization, body composition and cost benefit of European Eel (*A. anguilla*) fry.

2.2. Experimental diets and feeding regime:

Eight isonitrogenous 47% protein and isocaloric 502.26 kcal/100gm energy experimental diets were formulated as the following: The first diet without any additives (D₁, control diet). The rest of the seven diets are the basil control diet supplemented with the various feed additives as follow: The second diet contains 0.2% of Tonilissat[®] (D₂), the third diet contains 0.2% of Garlin[®] (D₃), the fourth diet contains 0.1% of Enziver[®] (D₄), The fifth diet contains a mixture of 0.1% Tonilissat[®] with 0.1% Garlin[®] (D₅), the sixth diet contains a mixture of 0.1% Tonilissat[®] with 0.05% Enziver[®] (D₆), the seventh diet contains a mixture of 0.1% Garlin[®] with 0.05% Enziver[®] (D₇), and the eighth diet contains a mixture of 0.1% Tonilissat[®], 0.1% Garlin[®] and 0.05% Enziver[®], respectively (D₈). The experimental diets were prepared by grinding all the ingredients, mixed with vitamins and minerals before adding the respect feed additive dissolved in oil. Warm water (40°C) was added slowly until the diets began clumping. The resultant dough was placed in plastic pages and store frozen at - 20°C until use.

Fish were fed the experimental diets at (6% of their live body weight) at two times a day (10.00 a.m. and 3.00 p.m.) in six days a week. Fish were weighted at every month

intervals and feed amounts were adjusted on the basis of live fish weight.

Table (1): Formulation and proximate composition of basal diet diets (% on DM basis).

Ingredients	%
Fish meal (67%)	50
Soybean meal (47%)	18
Yellow corn	10
Wheat bran	10
Corn oil	5
Fish oil	5
Vitamin & Mineral mix.¹	2
Proximate composition (%) on DM ² basis	
Moisture	10.99
Crude Protein	47.1
Ether extract	15.3
Crude fiber	8.95
Ash	6.37
NFE³	22.28
GE kcal/100g⁴	502.26
P/E ratio⁵	93.89

¹Vitamin and mineral mixture each kg contains: 4800 I.U. Vit. A, 2400 IU cholecalciferol (Vit. D), 40 g Vit. E, 8 g Vit. K, 4.0 g Vit. B12, 4.0 g Vit. B2, 6 g Vit. B6, 4.0 g Pantothenic acid, 8.0 g Nicotinic acid, 400 mg Folic acid, 20 mg Biotin, 200 gm Choline, 4 g Copper, 0.4 g Iodine, 12 g Iron, 22 g Manganese, 22 g Zinc, 0.04 g Selenium. Folic acid, 1.2 mg; niacin, 12 mg; d-calcium pantothenate, 26 mg; pyridoxine. HCl, 6 mg; riboflavin, 7.2 mg; thiamin. HCl, 1.2 mg; sodium chloride (NaCl, 39% Na, 61% Cl), 3077 mg; ferrous sulfate (FeSO₄•7H₂O, 20% Fe), 65 mg; manganese sulfate (MnSO₄, 36% Mn), 89 mg; zinc sulfate (ZnSO₄•7H₂O, 40% Zn), 150 mg; copper sulfate (CuSO₄•5H₂O, 25% Cu), 28 mg; potassium iodide (KI, 24% K, 76% I), 11 mg; Celite AW521 (acid-washed diatomaceous earth silica), 1000 mg.

²DM: dry matter.

³NFE: Nitrogen free extract

⁴GE= Gross energy (kcal/100g DM): Calculated using gross calorific values of 5.65, 9.45 and 4.11 kcal/100g for protein, fat and carbohydrate, respectively NRC, 1993).

⁵P/E ratio: Protein to energy ratio = mg crude protein/Kcal GE.

2.3.Feed additive composition:

1. Tonilistat[®]: Active live yeast, *Saccharomyces cerevisiae*, contain 8×10^9 cells/gram (China Way Corporation, Taiwan).
2. Garlin[®]: Garlic contains 26% allicin loaded on white silica and starch as a carrier in powder form (Jinan Tiantianxiang Co., Ltd., China).
3. Enziver[®]: Exogenous multi-enzymes contain phytase (1000 FTU), xylanase (5000 IU), amylase (7500 IU), pectinase (900 IU), protease (5500 IU), glucanase (800 IU), cellulase (10000 IU) (Zoetis Philippines, Inc., Philippines).

2.4.Measured parameters:

Growth performance:

Mean final body weight (FBW) of each experimental treatment was determined by dividing total fish weight in each tank by the number of fish. Weight gain (WG), specific growth rate (SGR%), Average daily gain (ADG) and survival rate (%) were determined as follow:

Body weight gain (g/fish): $BWG = W_2 - W_1$.

Where; W_1 : Initial weight of fish in grams and W_2 : Final weight of fish in grams.

Average daily gain (g/fish/day): $ADG = \frac{W_2 - W_1}{\text{days}}$. Where; W_1 : Initial weight of fish in grams; W_2 : Final weight of fish in grams, and n =days.

Specific growth rate (%/day): $SGR = 100 \times (\ln W_2 - \ln W_1) / \text{days}$. Where; \ln is the natural log.

Survival rate (%) = $100 \times (\text{final number of fish} / \text{initial number of fish})$.

Feed and nutrient utilization:

Feed intake (g/kg fish) was determined by dividing total feed intake by fish number after exclusion of the part eaten feed by dead fish. Feed conversion ratio (FCR), protein efficiency ratio (PER), protein productive value (PPV), fat retention (FR) and energy retention (ER) were calculated by using the following equations:

$FCR = \text{feed intake (g)} / \text{weight gain (g)}$.

$PER = \text{weight gain (g)} / \text{protein intake (g)}$.

$PPV (\%) = \text{protein gain (g)} / \text{protein intake (g)} \times 100$.

$\text{Energy utilization (EU \%)} = 100 \times (E_T - E_I) / \text{Energy intake (kcal)}$

Where: E_T : Energy in fish carcass (kcal) at the end and E_I : Energy in fish carcass at the start.

2.5. Diets and whole body proximate chemical composition:

The chemical composition of fish and diet samples were assessed according to procedures of (AOAC 2010). Dry matter was determined after drying the samples in an oven (105°C) for 24 h. Ash was

measured following incineration at 550°C for 12 h. Crude protein was determined by the micro-Kjeldhal method, with $N\% \times 6.25$ (using a Kjeltech autoanalyzer, Model VELP Scientifica, UDK 127), and crude fat was assessed by Soxhlet extraction (Model VELP Scientifica, SER 148) with diethyl ether (40-60°C). Crude fiber was determined after 5% sulfuric acid and 5% sodium hydroxide digestion for 15 min. then the residues dried and ashed. Nitrogen free extract calculated using the following equation: $NFE = 100 - (\text{crude protein} + \text{ether extract} + \text{crude fiber} + \text{ash})$. Table (1).

2.6. Economic evaluation:

Economic evaluation of the experimental diets has been calculated by evaluating the feed cost in Egyptian pound (LE) needed to produce 1 kg of live weight of fish.

$\text{Feed costs/kg weight gain (Incidence cost)} = FCR \times \text{costs of kg feed}$.

$\text{Profit index} = \text{value of fish} / \text{cost of feed consumed}$

2.7. Statistical analysis:

Data were expressed as mean \pm SE values. The data were analyzed by one-way analysis of variance (ANOVA) followed by TUKEY test multiple comparison tests for the means ($P < 0.05$). Statistical analyses were performed using Rapid publication-ready MS-Word tables for one-way ANOVA (Assaad et al., 2014).

enzymes (D₄) and the combination of garlic and multi-enzymes (D₇) or yeast, garlic and multi-enzymes (D₈) significantly increased than fish fed the control diet (non-supplemented diets). Whereas, the final weight of these treatments outperforms the control group by 14%, 21% and 30%, respectively. The survival rate (%) in the present study ranged between 55-60%

3. RESULTS

3.1. Growth performance and survival:

The growth performance parameters and survival (%) of European eel (*A. anguilla*) fry which fed on diets supplemented with different feed additives of yeast, garlic and multi-enzymes are shown in Table (2). The results showed that the growth performance (FBW, gain, ADG and SGR) of eel fed diets supplemented with multi-

without any significant differences among different groups. Moreover, the total length was increased significantly with enzyme mixture supplementation (D₄) and different combinations of enzyme, garlic and yeast (D₅-D₈) than the control and other treatments

(Table,3). Meanwhile, condition factor increased significantly with diet supplemented the mixture of the three supplementations (D₈: enzyme, garlic and yeast) than other experimental diets (Table, 3).

Table (2): Effect of dietary supplementation of yeast, garlic and enzymes on growth performance and survival (%) European eel (*Anguilla anguilla*) fry.

Items ¹	Experimental diets ²							
	D ₁	D ₂	D ₃	D ₄	D ₅	D ₆	D ₇	D ₈
IBW (g/fish)	3.01 ± 0.005	3.01 ± 0.005	3.01 ± 0.005	3.01 ± 0.005	3.01 ± 0.005	3.01 ± 0.005	3.01 ± 0.005	3.01 ± 0.005
FBW (g/fish)	4.86 ^{de} ± 0.042	4.78 ^e ± 0.005	4.70 ^e ± 0.209	5.45 ^{bc} ± 0.079	5.38 ^{bcd} ± 0.105	5.22 ^{ce} ± 0.0165	5.88 ^{ab} ± 0.0685	6.33 ^a ± 0.108
BWG (g/fish)	1.84 ^{de} ± 0.037	1.76 ^e ± 0.01	1.68 ^e ± 0.214	2.43 ^{bc} ± 0.084	2.36 ^{bcd} ± 0.10	2.2 ^{ce} ± 0.0215	2.87 ^{ab} ± 0.0735	3.31 ^a ± 0.113
ADG (g/fish/day)	0.015 ^{de} ± 0.0003	0.015 ^e ± 0.0001	0.014 ^e ± 0.002	0.02 ^{bc} ± 0.0001	0.02 ^{bcd} ± 0.0008	0.02 ^{ce} ± 0.0002	0.024 ^{ab} ± 0.0006	0.03 ^a ± 0.0009
SGR (%/day)	0.397 ^{de} ± 0.006	0.383 ^e ± 0.0023	0.369 ^e ± 0.04	0.493 ^{bc} ± 0.014	0.482 ^{bcd} ± 0.015	0.457 ^{ce} ± 0.004	0.557 ^{ab} ± 0.01	0.618 ^a ± 0.016
Survival (%)	60 ± 0.00	55 ± 5.00	55 ± 5.00	55 ± 5.00	60 ± 0.00	60 ± 0.00	60 ± 0.00	60 ± 0.00

Means in the same row without a common superscript letter differ significantly ($P < 0.05$).

¹ IBW: initial body weight, FBW: final body weight, BWG: body weight gain, ADG: average daily gain, SGR: specific growth rate.

² D₁: control diet; D₂: supplemented with 0.20% of Tonilissat[®]; D₃: supplemented with 0.20% of Garlin[®]; D₄: supplemented with 0.1% of Enziver[®]; D₅: supplemented with mixture of 0.1% of Tonilissat[®] with 0.1% of Garlin[®]; D₆: supplemented with mixture of 0.1% of the Tonilissat[®] with 0.05% of Enziver[®]; D₇: supplemented with 0.1% of Garlin[®] with 0.05% of Enziver[®]; D₈: supplemented with mixture of 0.1% of Tonilissat[®] with 0.1% of Garlin[®] with 0.05% of Enziver[®] (D₈).

Table (3): Effect of dietary supplementation of yeast, garlic and enzymes on length and condition factor European eel (*Anguilla anguilla*) fry.

Items	Experimental diets							
	D ₁	D ₂	D ₃	D ₄	D ₅	D ₆	D ₇	D ₈
Initial length (cm/fish)	12.73 ± 0.18	12.44 ± 0.45	12.06 ± 0.17	12.17 ± 0.05	12.11 ± 0.24	12.05 ± 0.27	12.68 ± 0.23	12.80 ± 0.10
Final length (cm/fish)	14.05 ^d ± 0.05	14.10 ^d ± 0.00	14.00 ^d ± 0.10	14.45 ^c ± 0.05	14.40 ^c ± 0.10	14.30 ^c ± 0.00	14.85 ^b ± 0.05	15.15 ^a ± 0.05
Length gain (cm/fish)	1.33 ± 0.13	1.67 ± 0.45	1.94 ± 0.07	2.28 ± 0.10	2.30 ± 0.14	2.25 ± 0.27	2.18 ± 0.18	2.35 ± 0.15
Condition Factor (K value)	0.18 ^b ± 0.00	0.17 ^b ± 0.00	0.18 ^b ± 0.00	0.18 ^b ± 0.00	0.18 ^b ± 0.00	0.18 ^b ± 0.00	0.18 ^b ± 0.00	0.20 ^a ± 0.01

Means in the same row without a common superscript letter differ significantly ($P < 0.05$).

3.2. Feed and nutrient utilization:

Results of feed and nutrient utilization in terms of feed intake, FCR, PER, PPV and EU of fish fed diets supplemented with different feed additives were presented in Table (4). The results indicated that feed intake was significantly increased with different combinations of feed additives (D₆-D₈) which reflect the increasing of diet acceptability. Moreover, feed additives (yeast, garlic and enzyme) exhibited a significant effect on all estimated feed and nutrient utilization traits.

The FCR and PER were significantly improved with enzyme mixture supplementation (D₄) and the different combination of yeast and garlic (D₅) garlic and enzyme (D₇) or yeast, garlic and multi-enzymes (D₈). Furthermore, the PPV, energy gain and energy utilization were significantly increased with the combination of the three additives (D₈; yeast, garlic and multi-enzymes) compared to the control and other supplemented diets.

Table (4): Effect of dietary supplementation of yeast, garlic and enzymes on feed and nutrients utilization of European eel (*Anguilla anguilla*) fry.

Items	Experimental diets							
	D ₁	D ₂	D ₃	D ₄	D ₅	D ₆	D ₇	D ₈
FI (gm/fish)	11.59 ^b ± 0.19	12.56 ^a ± 0.55	11.48 ^b ± 0.30	12.33 ^{ab} ± 0.41	12.18 ^{ab} ± 0.01	12.78 ^a ± 0.11	12.74 ^a ± 0.02	13.17 ^a ± 0.13
FCR (g)	6.19 ^{ab} ± 0.09	6.88 ^a ± 0.39	6.70 ^a ± 1.16	4.97 ^{cde} ± 0.04	4.97 ^{cde} ± 0.13	5.71 ^{abc} ± 0.07	4.38 ^{cd} ± 0.09	3.54 ^d ± 0.03
PER (g)	0.35 ^c ± 0.00	0.31 ^c ± 0.02	0.33 ^c ± 0.06	0.43 ^{ab} ± 0.01	0.43 ^{ab} ± 0.01	0.38 ^{bc} ± 0.01	0.49 ^b ± 0.01	0.60 ^a ± 0.01
PPV (%)	6.07 ^{bcde} ± 0.53	4.29 ^e ± 0.25	5.12 ^{de} ± 0.81	7.31 ^{bc} ± 0.53	7.11 ^{bcd} ± 0.19	5.76 ^{cde} ± 1.23	8.13 ^{ab} ± 0.04	9.88 ^a ± 0.44
EU (%)	5.96 ^{bcde} ± 0.45	4.45 ^d ± 0.26	5.18 ^{cd} ± 0.65	6.88 ^{bc} ± 0.35	6.60 ^{bcd} ± 0.14	5.51 ^{cde} ± 0.92	7.50 ^b ± 0.06	9.21 ^a ± 0.23

Means in the same row without a common superscript letter differ significantly ($P < 0.05$).

FI: feed intake; FCR: feed conversion ratio; PER: protein efficiency ratio; PPV: protein productive value, EU: Energy utilization.

3.3. Whole body composition:

Whole body compositions of European eel at the end of the feeding trial are presented in Table (5). The proximate composition of fish body for all fish groups fed supplemented

diets with yeast, garlic and multi-enzymes revealed that no significantly ($P > 0.05$) differences were observed in dry matter, protein, total lipid and ash contents among all treatments.

Table (5): Effect of dietary supplementation of yeast, garlic and enzymes on whole-body proximate chemical composition (on a wet weight basis) of European eel (*Anguilla anguilla*) fry.

Items	Experimental diets							
	D ₁	D ₂	D ₃	D ₄	D ₅	D ₆	D ₇	D ₈
Dry matter (%)	27.70 ± 1.04	25.70 ± 0.01	26.80 ± 0.39	27.00 ± 0.72	26.70 ± 0.01	26.20 ± 2.09	26.70 ± 0.41	26.30 ± 0.25
Protein (%)	19.50 ± 0.71	18.00 ± 0.02	18.70 ± 0.27	19.00 ± 0.52	18.80 ± 0.01	18.40 ± 1.53	18.70 ± 0.23	18.30 ± 0.32
Lipid (%)	6.47 ± 0.23	6.10 ± 0.024	6.30 ± 0.122	6.29 ± 0.046	6.20 ± 0.028	6.09 ± 0.39	6.28 ± 0.11	6.23 ± 0.01
Ash (%)	1.76 ± 0.11	1.56 ± 0.01	1.79 ± 0.02	1.76 ± 0.16	1.73 ± 0.02	1.76 ± 0.17	1.78 ± 0.08	1.73 ± 0.06
Carcass energy (Kcal/100gm)	617.11 ± 1.03	620.04 ± 0.67	615.65 ± 1.37	615.84 ± 4.01	615.88 ± 0.75	614.42 ± 2.13	615.86 ± 1.00	617.0 ± 0.56

Means in the same row without a common superscript letter differ significantly ($P < 0.05$).

3.4. Economic evaluation

Calculations of economic efficiency of the tested diets based on the cost of feed, costs of one Kg gain in weight and its ratio with the control group are shown in Table (6). The results showed that feed cost per kg gain (L.E) significantly decreased with the

combination of garlic and enzyme (D₇) and yeast, garlic and enzyme (D₈). Meanwhile, the profit index improved significantly with enzyme supplementation (D₄) and the combination of yeast and garlic (D₅), garlic and enzyme (D₇), and yeast garlic and enzyme (D₈).

Table (6): Effect of dietary supplementation of yeast, garlic and enzymes on cost-profit analysis of European eel (*Anguilla anguilla*) fry.

Items	Experimental diets							
	D ₁	D ₂	D ₃	D ₄	D ₅	D ₆	D ₇	D ₈
Cost (L.E/kg diet)¹	16.59	16.59	16.69	16.69	16.75	16.72	16.72	16.74
FCR	6.185	6.875	6.695	4.965	4.97	5.71	4.38	3.535
Feed cost / Kg gain	102.62	114.73	112.06	83.03	83.17	95.42	73.35	59.42
Relative to control	100.00	111.80	109.21	80.91	81.05	83.17	65.46	71.56
Changes in feed cost / kg gain	0.00	-11.80	-9.21	19.09	18.95	16.83	34.54	28.44
Fish price (L.E / kg diet)	120	120	120	120	120	120	120	120
Profit index	1.17	1.05	1.10	1.45	1.44	1.26	1.64	2.02
Changes in profit index	0.00	-27.50	-25.37	6.650	6.44	7.69	22.95	55.76

¹Cost of 1 kg ingredients used were 23 L.E for fish meal, 6.25 L.E for soybean meal, 3.50 L.E for yellow corn, 3.50 L.E for wheat bran, 14 L.E for oil, 8 L.E for Vit. and Min., 45 L.E for yeast, 55 L.E for garlic powder and 120 L.E enzymes. Egypt Feed Ingredients Price at the start of 2016.

4. Discussion

The feeding of eel on artificial diets is the bottleneck of these species culture. Whereas, glass eels started a fasting stage during metamorphosis from *leptocephalus* larvae to glass eel, then started to be fed on small prey including copepods, *polychaetes* (bristle worms), *oligochaetes* (smooth worms), amphipods and aquatic insect larvae (Anon, 2000). After catching the adaptation of eels to artificial diets is a challenge and cause high mortality (Larkin, 2000). Moreover, as eel a carnivore fish with protein requirement ranged between (45 and 47 %) (Satoh, 2002), the feeding cost is very expensive which sharply affect the total production cost (Watene, 2003). Therefore, the improvement of growth and feed utilization is a prime of importance to reduce production cost and more commercialize the culture of these species. The results of the present study revealed that growth performance and feed

utilization of eels fed diets supplemented with exogenous multi-enzymes or the different combinations of yeast, garlic and multi-enzymes significantly higher than the control group. In accordance, Yildirim and Turan (2010) reported that African Catfish, *Clarias gariepinus*, fed diets supplemented with exogenous enzyme (containing fungal xylanase, β -glucanase, pentosanase, β -amilase, fungal β -glucanase, hemicellulase, pectinase, cellulase, cellulase) had significantly higher growth rate, FCR, PER and PPV in dose dependent manner without any changes in survival rate. Moreover, presence of pancreatic digestive enzyme in the micro-diet of sea bream, *Sparus auratus*, enhanced its assimilability by 30% (Kolkovski et al., 1993). Also, Dawood et al. (2014) reported that exogenous digestive enzymes improved growth, feed utilization of rabbitfish, *Siganus rivulatus*.

Effect of dietary supplementation of Yeast, Garlic and Enzymes on growth performance and economic evaluation in European Eel (*Anguilla anguilla*) fry

The positive effects of exogenous enzymes may be attributed to the stimulation of own animal production such as amylases, proteases and lipases to improve the digestion of starch, protein and lipid, respectively (Lin et al., 2007 and Zhou et al., 2009). Also, Enzymes supplementation enhances the nutritional value of fish diet via transforming the complex components to absorbable nutrients (Soltan, 2009). Moreover, there are synergistic effects of yeast, garlic and multi-enzymes supplementation on growth and feed utilization. These findings are in agreement with Tewary and Patra (2011) who found a superior growth performance in terms of weight gain percentage and SGR in *Labeo rohita* fed dietary supplemented with 5% *S. cerevisiae* compared to the control group. Tolan, (2006) reported that increasing total gain of Nile tilapia, *Oreochromis niloticus* by increasing level of dry yeast supplementation from 1 up to 3 g/kg diet. Moreover, Diab et al. (2002) stated that dietary dried yeast fed for Nile tilapia, *O. niloticus* from 1% up to 5% recorded high average body weight compared to fish fed the control group (without yeast). The utilization of dried yeast may effectively improve growth (Craig et al., 2006) and non-specific immune responses (Paulsen et al., 2003) in a variety of fish species. In the line with the obtained results, Abdel-Tawwab et al. (2008) showed that yeast supplementation improved FCR and increased feed utilization of Nile tilapia, *O. niloticus*. Also, Lara-Flores et al. (2003) supported these results where the authors demonstrated that yeast is an appropriate growth-stimulating additive in *O. niloticus* practical diet. The better growth performance with yeast supplemented diets may be due to the improvement of nutrients digestibility (De-Schrijver and Ollevier, 2000 and El-Dakar et al., 2007) and accelerate the digestive system maturation (Waché et al.,

2006). Also, Tovar-Ramirez et al. (2002) recorded an increase in the digestive enzyme activities of amylase, trypsin and lipase in seabass (*Dicentrarchus labrax*) using live yeast. Moreover, yeast reduce the presence of potentially pathogenic bacteria by competitive exclusion and causes intestinal microbial balance of the host organism and confer various beneficial effects include immunostimulation and enhance disease resistance, and antioxidant system (Gatlin et al. 2006, Ortuño et al. 2002, Choudhury et al. 2005 and Vetvicka et al. 2013). Buts et al. (1994) reported that yeast may release spermine and spermidine in the digestive tract, which playing fundamental role in proliferating, fast growth and regenerating tissue (Peulen et al., 2002). It is therefore possible that spermine and spermidine production by yeasts may explain at least partly the effect observed on fish growth and feed utilization. Regarding the improving effects of garlic co-supplementation with yeast and multi-enzymes on growth and feed utilization. The present results agree with the findings of Sasmal et al. (2005) who found that weight gain and FCR of *Carassius auratus* improved significantly with feeding diet supplemented with 1 g garlic/100 g feed. Moreover, Guo et al. (2012) showed a significant increase in weight gain and feed efficiency in grouper fed supplemented diets with 1.3 garlic powder. Similarly, Diab et al. (2008) reported a significantly increased in weight gain of Nile tilapia (*O. niloticus*) after feeding with a 1% garlic. Shalaby et al., (2006) and Yones et al, (2019) also demonstrated that dietary 3% garlic powder enhanced growth, feed intake, SGR, FCR and PER in Nile tilapia. High growth and digestibility were also observed in tilapia fed for 60 days with a diet containing garlic (Xie et al., 2009).

Furthermore, Nya and Austin (2009) reported that growth, FCR and PER of rainbow trout (*Oncorhynchus mykiss*) enhanced via feeding diets containing 0.5 and 1.0% garlic powder. Beneficial health properties of garlic are attributed to organosulphur compounds, particularly allicin, which an important criterion for evaluating the quality of commercial garlic varieties (Rose *et al.*, 2005). The biological activity of these agents was attributed to either antioxidant activity or thiol-disulfide exchange Rabinkov *et al.* (1998). Moreover, Nya *et al.* (2010) reported that the mode of action of allicin may well include the inhibition of cysteine protease, the scavenging, and trapping of free radicals (hydroxyl, superoxide anions and hydrogen peroxide) and the initiation of the inhibition of thiol-containing protein in the cells of pathogens. The survival percent ranged between 55-60% in the present study which is higher than the recorded percent of 25-40% during weaning to an artificial diet of European eel (Larkin, 2000). However, the effect of individual supplementation of yeast and garlic didn't show any improvement in growth performance and feed utilization. This may be in contrast to the most available literature in other species [Abdel-Tawwab *et al.* (2008); Lara-Flores *et al.* (2003); De-Schrijver and Ollevier, 2000); Gatlin *et al.*, (2006); Sasmal *et al.*, (2005); Guo *et al.*, (2012); Diab *et al.* (2002); Diab *et al.* (2008) and Shalaby *et al.* (2006)]. These differences may be related to the low level of garlic supplementation in the present study or the ability of yeast to colonize the intestinal wall of eels. However, there are lacks of information on this species of fish. More investigations should be conducted for more explanation of the microbial colonization in eels gut and the physiological changes caused

by different feed additives. The whole-body proximate chemical composition of different treated groups didn't differ significantly with different dietary supplementations (yeast, garlic and multi-enzymes) in the present study. In accordance, Zehra and Khan (2016) reported that the crude protein of rainbow trout flesh didn't differ with increasing supplementation of yeast RNA extract. Similarly, Li *et al.* (2007) showed that supplementing diets of red drum, *Sciaenops ocellatus*, juvenile with a yeast purified nucleotide mixture have insignificant effect on body composition. Also, Lara-Flores *et al.* (2003); EL-Haroun *et al.* (2006) and Ghosh *et al.* (2008) reported that probiotics supplementation couldn't significantly affect the body composition of different fish species and do not affect strongly tissue synthesis. Moreover, Diab *et al.* (2002) didn't found significant changes in fish body composition caused by different garlic levels. Abdelhamid *et al.* (2002) and Khattab *et al.* (2004), they found that inclusion of Biogen[®] in the diet increased fish protein content.

On the other hand, protein content was increased in African catfish fed diet supplemented with 0.75 g/kg enzyme complex (Yildirim and Turan, 2010). Also, (Oz'orio, *et al.*, 2012) reported that the body composition of tilapia was affected by supplementing the diet with yeast; hence there was a reduction in body protein. In addition, the effects of administration of probiotic (*Bacillus subtilis*) in the diet on body composition showed that this probiotic improved the fat content of the carcass (Ghosh *et al.* 2008).

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The economic benefit of using yeast, garlic and multi-enzymes supplementation in individual form or in combination clearly showed that the combination of these feed additives reduced the cost of production for one kg fish weight gain and increase the profit index by 55.76% when the eels fed the three additives at the same time compared to the control. These findings agree with Dawood et al. (2014) who reported that exogenous digestive enzymes supplementation improve the economic benefit of rabbitfish, *Siganus rivulatus*, production. El-Haroun et al. (2006) reported an increase of profit index of Nile tilapia fed diets supplemented with probiotic (biogin®) in dose dependent manner. Also, El-Dakar et al. (2007) reported that dietary probiotic/prebiotic appears to reduce feed cost per unit growth of Spinefoot rabbitfish, *Siganus rivulatus*.

5. CONCLUSION

In conclusion the present research revealed that dietary supplements of different feed additives (yeast, garlic and enzyme) in combinations improved growth performance and feed utilization of European eel, *A. anguilla*, fry. However, exogenous multi-enzymes is the only feed additive significantly improved the performance of European eel compared to yeast, garlic and the control group. Also, the co-supplementation of the three feed additives can reduce feed cost per unit growth of European eel.

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