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EFFECT OF DIET TYPE AND STOCKING DENSITY ON GROWTH PERFORMANCE AND BLOOD PARAMETERS OF THE EGYPTIAN SOLE (*SOLEA AEGYPTIACA* CHABANAUD, 1927)

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Abstract

Two experiments were conducted to evaluate the optimum diet type and stocking density on growth performance, blood parameters and fish body composition of *Solea aegyptiaca* fingerlings. The first experiment was tested four different diet type (unconventional feeds, Sand smelt minced (SSM), Shrimp minced (SM), Housefly maggot (HM)) and (conventional feed, artificial feed (AF)) for 120 days. The results cleared that, unconventional feeds were better than AF on growth performance and feed conversion ratio (FCR). The highest protein content in whole fish body was obtained with fish fed on SSM and SM, while the highest ether extract content was with fish fed on SM and HM. Blood parameters cleared that, improve in the general health status of fish fed on unconventional feeds. The second experiment was tested four different stocking densities 10, 20, 30 and 40 fish/m^3 and continued for 120 days. The results showed that, the low stocking densities at 10 and 20 fish/m^3 were the best in growth performance and FCR. The highest protein content in whole fish body was obtained with fish under stocking density (20 fish/m^3), while ether extract content was not different between the four stocking densities groups. Blood parameters cleared that, fish was on stress status with increasing stocking density.

Keywords: Growth performance, blood parameters, unconventional feeds conventional feed, stocking density, *Solea aegyptiaca*.

INTRODUCTION

Members of the genus *Solea* sp. are recorded among the most important and valuable commercial flatfishes in Egypt and greatly appreciated by consumers of sea products (Gabr *et al.,* 2003). The common sole (*Solea Solea*) is highly appreciated fish by the Egyptians especially in the coastal communities because of its high quality flesh and is one of the commercially important fish in Egypt providing up to 90 million LE annually (Mehanna, 2014). The Egyptian sole (*Solea aegyptiaca*) is the most common species of soles that contributed about 6.5% of the total catch of trawl fishery, forming about 13% of the gross revenue of the trawling (Mehanna, 2007). Kariman (2009) recorded that catch composition of sole species during summer and winter seasons in Lake Qarun were more than 50 and 35%, respectively.

During the last decades, marine fish aquaculture in Egypt has been mostly concentrated on Gilthead sea bream (*Sparus aurata*) and Sea bass (*Dicentrarchus labrax*). The common sole (*Solea solea*) is a species appreciated by the market and is a candidate for rearing in a commercial scale, although on significant productions presently exist in Egypt. Imsland *et al.* (2003), published a review of the culture potential of *S. solea* in comparison with *S. sengalensis* and pointed more information about the commercial husbandry of *S. sengalensis.* Among the most promising candidates are solied flatfishes provided that effective culture methods and strategies for increasing market opportunities can be developed (Agulleiro *et al.,* 2006).

Senegal sole is currently an important focus of research for flatfish aquaculture in Europe particularly in Spain and Portugal. However, before a reliable technology for mass production of Senegal sole can be transferred to the industry, several aspects of its culture still need to be solved and optimized (Agulleiro *et al.,* 2006). The common sole is an esteemed, high priced fish for consumption with a relatively fast production cycle of 1-1.5 years at 20°C (Howell, 1997 and Imsland *et al.*, 2003).

Studies on stomach contents of *Solea sengaleses* showed a dominance of polychaetes (*Hediste diversicolor*) but some Amphipods and Isopods were also identified (Bernardo, 1990). The highest densities of sole were recorded in deep, warm, low salinity area with sediment of fine sand and high abundance of amphipods (Cabral and Costa, 1999). In Lake Qarun, *S. aegyptiaca* feeds mainly on macrobenthos. The polychate (*Neries succinea*) seems to be the most preferred items species for sole, since it formed about 39.9 and 31.1% of its total food items during 1980 and 1995 (El-Shabrawy and Ahmed, 2007). Braber and de Groot (1973) mentioned that, the diet of *Solea solea* from Ebroestuary consisted mainly of polychaetes, crusraceans and mollusks. In contrast, the findings of the above authors (where polychaetes were the most important prey items of sole), crustaceans were the most important prey of *S. solea* in Bardawil lagoon (Sabrah, 2004).

The major animal protein ingredient in aquaculture diets is most often fishmeal because of its nutritional quality. However, the rising cost and its limited availability of fishmeal has lead to investigations of either lowering or replacing the fishmeal content with more economic protein sources of plant and/or animal origin (Hardy, 2006; Gimenez *et al.,* 2009; Gumus and Ikiz, 2009 and Sevgili *et al.,* 2009). The efficiency of various alternative animal protein sources has been evaluated in fish diets, e.g., meat and bone meal (Zhang *et al.,* 2006), poultry by-product meal (Yang *et al.,* 2006; Pine *et al.,* 2008 and Saoud *et al.,* 2008), turkey meal (Muzinic *et al.,* 2006), gambusia meal (Abdelghany, 2003 and Ahmad, 2008), tuna liver meal (Gumus *et al.,* 2009), sand smelt meal (Gumus *et al.,* 2010 and Gumus, 2011), housefly maggot meal (Ogunji *et al.,* 2006, 2008; Aniebo *et al.,* 2009 and Kayode and Jacob, 2012). The need for cheap, protein rich feeds is a universal requirement, relevant to large and small producers alike.

Stocking density is an important parameter in fish culture, not only because it has strong implications on growth performance, but also because it can affect fish welfare (Ellis *et al.,* 2005 and Turnbull *et al.,* 2005) and has an economical impact. Social interactions could be behind the differences observed in growth efficiency of several fish species. For example, gilthead sea bream feeding efficiency has been observed to be affected by ration size, with lower rations leading to increased competition, faster swimming speeds and higher densities under the feeder (Andrew *et al.,* 2004). Similarly, common sole reared at different stocking densities between 0.5 and 12 Kg/m^2 showed a density-dependent growth performance, with productivity maxima at intermediate densities (7.4 Kg/m^2, Schram *et al.,* 2006). On the other hand, growth efficiency of species presenting schooling behavior can be improved rising stocking densities (Gardeur *et al.,* 2001).

The present study aimed to determine the best stocking rate and comparison between unconventional feed (Sand smelt minced, Shrimp minced, Housefly maggot) and conventional feed (Artificial feed) for growth performance, survival rate, feed utilization, internal organs parameters, blood hematological and biochemical parameters and fish body composition of *Solea aegyptiaca* fingerlings.

MATERIAL AND METHODS

Fingerlings- rearing conditions.

The Egyptian sole, *Solea aegyptiaca* fingerlings were obtained from Lake Qaroun, El- Fayoum Governorate. They were obtained during the beginning of December 2014 and maintained at National Institute of Oceanography and Fisheries (NIOF), Shakshouk Fish Research Station, El- Fayoum Governorate. Fish were acclimated to laboratory conditions for 14 days before being randomly distributed into rectangular fiberglass tanks of 1.5 m^3 water capacity. All tanks were provided with continuous aeration. The bottom of each tank was covered by a sand layer about (5-10 cm) for shelter. Fish were held under natural photoperiod condition throughout the trials. Prior to weighing, 15 fish were sacrificed for determination of carcass analysis. At the end of experiment, 5 fish from tanks were randomly taken for the determination of carcass analysis.

The water used in the all trials was obtained from Lake Qaroun. The trials began 14/12/2014 and ended 14/4/2015, (120 days).

Diets formulation and preparation.

All diets were prepared in Fish Rearing Lab., Shakshouk Fish Research Station, El- Fayoum Governorate, Inland Water and Fish Culture Branch, National Institute of Oceanography and Fisheries (NIOF), Egypt.

Artificial diet was formulated based on fish meal as the only animal protein source and a mixture of corn gluten, yellow corn and soybean meal as plant protein sources. Fish oil and sunflower oil were added as the major dietary lipid source to the experimental diet. The diet formulated to be almost containing 50% crude protein, diet was hand made.

Sand smelt and Shrimp were obtained from local market in Shakshouk, El- Fayoum Governorate, as a local ingredients to replace artificial diet. Sand smelt and Shrimp were minced by meat mincer then stored in plastic bags in the freezer (-18°C ±1°C) until used. During fish feeding amount of frozen meat enough 4 days were taken then put in the cooling until fish feeding.

Housefly maggots produced on poultry droppings and foods wastes. Fifteen kilogram of poultry droppings and foods wastes were mixed together and spread on three wood box (40 cm length, 40 cm width and 10 cm height) to a thickness of 7 cm to constitute the substrate. The odor of fresh poultry droppings and foods wastes, fermenting substrate attracted flies, which later laid eggs on it. The eggs hatched into larvae within two days and were allowed 48 hours to develop further. The mature maggots were harvested. Housefly maggots were caught from wood box by using tweezers then stored in plastic bags in the freezer (-18°C ±1°C) until used. Moisture in wood box were maintained high all time during housefly maggot production period.

The first experiment: Effect of diet type.

The first experiment was conducted to investigate the effect of diet type (Sand smelt minced (SSM), Shrimp minced (SM), Housefly maggot (HM) and artificial diet (AF)) on growth performance, feed utilization, internal organs parameters, body chemical composition, blood hematological and biochemical parameters and survival rate of *Solea aegyptiaca* fingerlings. *Solea aegyptiaca* fingerlings were healthy, free from parasites with an average initial weight of 15.54±0.06g. Fish were randomly distributed and stocked at 15 fingerlings/tank in 8 fiberglass tanks with a volume of (1.5m^3). The experimental treatments were duplicated. The amount of feed was biweekly adjusted according to the changes in body weight throughout the experimental period (120 days), and rate of mortality was recorded, and feed consumption was recorded daily. The experimental diets (SSM, SM, HM and AF) are present in Table (1) and Table (2). SSM, SM, HM were fresh without any additives during feeding. Feed was offered by hand (on dry weight basis) twice meals/day (9:30 and 16:30 h) at 2% of body weight daily, fish were fed 6 days/week. The average water quality criteria of all treatments are present in Table (3). About 30% of water tanks was changed twice every day. Zooplankton in water of all treatments were count during experimental period (Table 4).

The second experiment: Effect of stocking density.

The second experiment was conducted to investigate the effect of stocking densities (10, 20, 30 and 40/m^3) on growth performance, feed utilization, internal organs parameters, body chemical composition, blood hematological and biochemical parameters and survival rate of *Solea aegyptiaca* fingerlings. *Solea aegyptiaca* fingerlings were healthy, free from parasites with an average initial weight of 18.48±0.08g. Fish were randomly distributed in 8 fiberglass tanks with a volume of (1.5m^3) and fed on artificial diet. The experimental treatments were duplicated. The amount of feed was biweekly adjusted according to the changes in body weight throughout the experimental period (120 days), and rate of mortality was recorded, and feed consumption was recorded daily. The artificial diet is present in Table (1). Feed was offered by hand twice meals/day (9:30 and 16:30 h) at 3% of body weight daily, fish were fed 6 days/week. The average water quality criteria of all treatments are present in Table (5). About 30% of water tanks was changed twice every day.

Zooplankton in water of all treatments were count during experimental period (Table 6).

Table 1. Composition of the artificial diet used in the fingerlings experiment.

Ingredients %	Artificial diet
Fish meal, (CP 72%)	56
yellow corn, (CP 10%)	19
Corn glutin, (CP 60%)	7
Soybean, (CP 48%)	8
Fish oil	3
Sunflower oil	4
Starch	2
vit. & Min. *	1

* Vitamins and minerals mixture each 3 Kg of mixture contains: 12000 00 IU Vit. A, 3000 00 IU Vit. D3, 700 mg Vit. E, 500 mg Vit. K3, 500 mg Vit. B1, 200 mg Vit. B2, 600 mg Vit. B6, 3 mg Vit. B12, 450 mg Vit. C, 3000 mg Niacin, 3000 mg Methionine, 10000 mg Cholin chloride, 300 mg Folic acid, 6 mg Biotin, 670 mg Panthonic acid, 3000 mg Magnesium sulphat, 3000 mg Copper sulphat, 10000 mg Iron sulphat, 1800 mg Zinc sulphat, 300 mg Cobalt sulphat.

Table 2. Chemical composition of feeds used in the fingerlings experiment.

Items	SSM	SM	HM	AF
Moisture, %	75.80	74.07	74.43	9.31
Crude protein, CP %	68.56	64.66	58.60	50.26
Ether extract, EE %	16.74	9.97	15.82	13.83
Crude fiber, CF %	- -	- -	- -	1.21
Ash, %	13.50	24.35	24.18	6.55
Nitrogen free extract. NFE, % *	- -	- -	- -	18.84
GE, kcal/g**	5.291	4.462	4.659	4.842

* Calculated by differences . ** Calculated according to NRC, 1993.

Table 3. Average values of water quality parameters under effect of diet type of *Solea aegyptiaca* fingerlings during experimental period.

Parameters	**Diet type**			
	SSM	**SM**	**HM**	**AF**
Temperature, °C	15.22	15.33	15.36	15.33
pH	8.25	8.21	8.24	8.21
Salinity, ‰	31.79	31.75	31.75	31.79
Dissolved oxygen, mg/l	8.8	7.9	8.1	8.6
Total ammonia, mg/l	1.01	1.39	1.38	1.75
Un-ionized ammonia, mg/l	0.039	0.045	0.054	0.068
Nitrite, mg/l	0.36	0.52	0.34	0.184
Nitrate, mg/l	0.63	0.89	0.52	0.419

Table 4. Zooplankton count and species in experimental treatments under effect of diet type of *Solea aegyptiaca* fingerlings (No. of zooplankton/ liter).

Species	**Diet type**			
	SSM	**SM**	**HM**	**AF**
Brchionus plicatilis	125	67	90	632
Nauplis larvae	352	402	347	741
***Fevelli sp*.**	184	40	720	866
Cirripedia larvae	3	1	0	6
Annelida larvae	5	1	0	1
Mesochra heldti	3	2	1	2
Total count zooplankton	672	513	1158	2248

Table 5. Average values of water quality parameters under effect of stocking density of *Solea aegyptiaca* fingerlings during experimental period.

Parameters	Stocking density (fish/m^3)			
	10	**20**	**30**	**40**
Temperature, °C	15.41	15.37	15.41	15.42
pH	8.21	8.18	8.05	8.02
Salinity, ‰	31.79	31.79	31.75	31.79
Dissolved oxygen, mg/l	8.1	8.8	7.3	8.2
Total ammonia, mg/l	2.09	2.45	3.34	4.25
Un-ionized ammonia, mg/l	0.082	0.096	0.105	0.134
Nitrite, mg/l	0.152	0.092	0.066	0.061
Nitrate, mg/l	0.236	0.145	0.109	0.103

Table 6. Zooplankton count and species in experimental treatments under effect of stocking density of *Solea aegyptiaca* fingerlings (No. of zooplankton/ liter).

Species	Stocking density (fish/m^3)			
	10	**20**	**30**	**40**
Brchionus plicatilis	500	875	140	47
Nauplis larvae	53	120	43	222
Fevelli sp	91	42	62	343
Cirripedia larvae	0	1	0	5
Annelida larvae	1	1	0	0
Mesochra heldti	3	15	1	0
Total count zooplankton	648	1054	246	617

Parameters measurements

At the end of the experiment, growth performance, survival rate, feed utilization and internal organs parameters were calculated as follows:

- Weight gain (g) = final weight, g - initial weight, g.
- Average daily gain (g) = average weight gain, g/ experimental period, day.
- Specific growth rate (SGR, %) = [(ln final weight -ln initial weight)/ period in days] × 100, where ln is the natural log.
- Condition factor (g/cm^3) = (wet weight)/ (total length^3) ×100.

- Feed conversion ratio (FCR) = feed intake, g/ weight gain, g.
- Protein efficiency ratio (PER) = weight gain, g/ protein intake, g.
- Protein productive value (PPV, %) =(retained protein, g/ protein intake, g) × 100.
- Energy efficiency ratio (EER) = weight gain, g/ energy intake, Kcal.
- Energy productive value (EPV, %) = (retained energy, Kcal/ energy intake, Kcal) × 100.
- Survival rate,% =(number of fish at end/ number of fish at start) × 100.
- Hepatosomatic index (HSI, %) = (liver weight)/ (fish weight) × 100.
- Viscerosomatic index (VSI,%) = (viscera weight/ body weight) × 100.
- Relative intestinal length (RIL) = (intestinal length, cm)/ (total length, cm).

Water quality analysis.

Water temperature and pH were measured daily by Combined meter (pH/ EC/ TDS/ temperature, Mi 805). Salinity was measured daily by Refractometer (VITAL Sine SR-6, China). Dissolved oxygen (DO) concentration was determined titrimetrically according to the modified Winkler, full-bottle technique (Method 360.2; EPA, 1983). Water ammonia, nitrite and nitrate were determined by using Spectrophotometer model (LKB Bichrom UV visible spectrophotometer) according to the method described by APHA (1992). To determine un-ionized ammonia concentration, multiply total ammonia concentration by the percentage which is closest to the observed temperature and pH of the water sample (Swann, 1997).

Enumeration and identification of zooplankton in water.

Zooplankton samples were collected using zooplankton net. The sample were fixed immediately using formaldehyde solution (4-7%), two ml of Rose Bengal stain (0.5 %) was added after fixation. The samples were examined under a binocular research microscope. The organisms were identified and counted on the counting try with magnification varying from 100X to 400X. Planktonic organisms were identified, classified and described according to description and keys constructed by Ruttner-Kolisko (1974); Campbell (1982); Abdel-Malek *et al.* (1993) and Parveen and Mola (2013).

Blood hematological and biochemical parameters.

Blood samples were obtained from fish at the end of experimental period. Seven fish per tank were randomly chosen for blood analyses. At the end of the experiment, blood samples were collected from the fish caudal vein by a sterile syringe containing EDTA as an anticoagulant. Blood samples were held on ice until all samples were collected. Hematology and serum biochemistry analyses were performed within 6 h of blood collection. Haemoglobin (Hb), hematocrit (HCt%), red blood cells (RBCs) and white blood cells (WBCs) measurements were determined in whole blood by using Mindray cell counter Auto-analyzer BC-2800.

The collected blood samples were centrifuged at 5000 rpm for 5 min at 25°C to get sera for the following analyses: Serum glucose and cholesterol were measured using commercial kits (Spinreact – spain) by Mindray BA-88A spectrophotometer. Serum total protein was determined using commercial kits (Human GmbH – 65205 Wiesbaden – Germany) by Mindray BA-88A spectrophotometer. Serum aspartate aminotransferase (AST) and alanine aminotransferase (ALT) activities were determined by kientic enzymatic using commercial kits (Human GmbH – 65205 Wiesbaden – Germany) by Mindray BA-88A spectrophotometer.

Chemical analysis.

Diets used and fish body composition were analyzed for their proximate composition in triplicates following the methods described by AOAC (2000). Gross energy was calculated according to (NRC, 1993) for formulated diets the factors 5.5, 9.08 and 4.1 Kcal/g for protein, fat and carbohydrates, respectively, for fish 5.5 and 9.5 Kcal/g for protein and fat, respectively (Viola *et al*., 1981).

Statistical analysis.

The data were analyzed by one-way ANOVA and significant differences were determined by Duncan Waller Multiple Range Test at 5% level using SPSS Statistical Package Program (SPSS, 2008) 17, released version.

RESULTS

The first experiment:

Effect of diet type on growth performance and survival rate.

Results of growth performance parameters and survival rate of fish fed on the different feed are shown in Table (7). There was no significant difference in the initial body weight of the fish among treatments. Survival rate was within the range 90-100% without any statistical difference ($P \leq 0.05$) between treatments. Concerning growth performance parameters (Table 7) the highest final weights of sole fish ($P \leq 0.05$) were recorded with fed on SM, HM and SSM groups followed in a significant decreasing order by fish fed on AF, however differences in final weights among SM, HM and SSM groups were insignificant ($P \leq 0.05$). The same trend was observed with total weight gain, daily gain and specific growth rate, where SM, HM and SSM groups recorded the highest values followed in a significant ($P < 0.05$) decreasing order by AF. As shown in the same table, groups fed on SM, HM and SSM recorded significantly ($P \leq 0.05$) higher values of condition factor compared to the fish fed on AF group. The results indicated that the three former groups (SM, HM and SSM) grow better in weight under experimental conditions.

Effect of diet type on feed utilization.

As shown in Table (8). The results showed that significant differences ($P \leq 0.05$) were obtained in all feed utilization parameters between treatments. The highest feed offered was observed with fish fed on SM, while there was insignificant difference among the other experimental groups. The best FCR (lowest) was recorded with fish fed on HM, with insignificant differences between (SM, HM and SSM). The worst FCR was recorded with fish fed on AF. PER and EER values obtained for fish fed HM were relatively highest than those for the other feeds. PPV values were highest with fish fed

HM with insignificant differences between (SM, HM and SAM), but EPV values were highest with fish fed SM.

Table 7. Effect of diet type on growth performance and survival rate of *Solea aegyptiaca*.

Items	Diet type				SED*
	SSM	SM	HM	AF	
Initial aveg. Weight, g/fish	15.48	15.61	15.57	15.57	0.152
Final aveg. Weight, g/fish	35.95[a]	37.86[a]	37.17[a]	25.93[b]	0.970
Total weight gain, g/fish	20.47[a]	22.25[a]	21.61[a]	10.36[b]	0.823
Average daily weight gain, g/fish/day	0.17[a]	0.19[a]	0.18[a]	0.09[b]	0.007
Specific growth rate (SGR), %/day	0.70[a]	0.74[a]	0.73[a]	0.42[b]	0.021
Condition factor, g/cm^3	0.84[a]	0.89[a]	0.84[a]	0.58[b]	0.060
Survival rate, %	93.34	100	100	90	5.268

[a-b]Average in the same row having different superscripts are differ significantly (P≤0.05). * SED is the standard error of difference

Table 8. Effect of diet type on feed utilization of *Solea aegyptiaca*.

Items	Diet type				SED*
	SSM	SM	HM	AF	
Offered feed, g/fish/period	43.80[b]	48.10[a]	44.60[b]	43.45[b]	0.548
FCR, g feed/g gain	2.14[b]	2.17[b]	2.07[b]	4.23[a]	0.246
Protein utilization					
PER	0.68[b]	0.72[b]	0.83[a]	0.47[c]	0.029
PPV,%	50.37[a]	51.23[a]	52.53[a]	31.99[b]	2.443
Energy utilization					
EER	0.09[b]	0.10[a]	0.10[a]	0.05[c]	0.003
EPV,%	38.79[c]	53.33[a]	46.03[b]	19.10[d]	1.673

[a-d]Average in the same row having different superscripts are differ significantly (P≤0.05). * SED is the standard error of difference

Effect of diet type on internal organs parameters.

As shown in Table (9). The results showed that insignificant differences (P≤0.05) were obtained in viscerosomatic index (VSI) parameter between treatments and Lake Qaroun fish samples. Hepatosomatic index (HSI) was not significantly different (P≤0.05) between fish fed on SSM, SM, HM and Lake Qaroun fish, but was significantly greater than fish fed on AF.

Relative intestinal length (RIL) was significantly different (P≤0.05) between treatments and Lake Qaroun fish samples, and the highest value was with fish fed on HM.

Table 9. Effect of diet type on internal organs parameters of *Solea aegyptiaca*.

Items	LQS**	Diet type				SED*
		SSM	SM	HM	AF	
Viscerosomatic index, %	4.66	4.41	5.89	4.42	4.04	1.475
Hepatosomatic index, %	1.10[a]	1.18[a]	1.20[a]	0.97[a]	0.56[b]	0.111
Relative intestinal length	1.28[ab]	1.12[b]	1.10[b]	1.40[a]	1.25[ab]	0.089

[a-ab]Average in the same row having different superscripts are differ significantly (P≤0.05). * SED is the standard error of difference. ** LQS - Lake Qaroun sample.

Effect of diet type on blood hematological and biochemical parameters.

Results of blood hematological and biochemical parameters of fish fed on the different feed are shown in Table (10). The results showed that significant differences (P≤0.05) were obtained in all blood hematological and biochemical parameters between treatments. Red blood cells (RBCs) counts and hematocrit (HCt) values were highest with fish fed on AF, but were significantly greater than RBCs and HCt in the other feeds and Lake Qaroun fish samples. Hemoglobin (Hb) values were not significantly different from each other (P≤0.05) in fish fed on SSM, AF and Lake Qaroun fish sample, but they were significantly higher than Hb of fish fed on HM and SM. The highest white blood cells (WBCs) counts were with Lake Qaroun fish samples, but the lowest WBCs counts were with fish fed on SM. Total protein values were not significantly different from each other (P≤0.05) in fish fed on different feeds (relatively highest with fish fed SM), but were significantly less than total protein in Lake Qaroun fish samples. Cholesterol value of Lake Qaroun fish samples was significantly greater than cholesterol of fish fed on the different feeds. Glucose values were highest with fish fed on AF and SSM, but glucose values were lowest with Lake Qaroun fish samples. Aspartate aminotransferase (AST) and alanine aminotransferase (ALT) activities were significantly different from each other (P≤0.05) in the serum of fish from all treatments. Showing maximum activity in fish fed on SSM and SM for AST, but maximum activity in Lake Qaroun fish samples for ALT, and decreasing in the other treatments.

Table 10. Effect of diet type on blood hematological and biochemical parameters of *Solea aegyptiaca*.

Items	LQS **	Diet type				SED*
		SSM	SM	HM	AF	
RBCs, $\times 10^{12}$/l	0.83^{bc}	1.10^{ab}	0.65^{c}	0.69^{c}	1.25^{a}	0.130
Hb, g/dl	2.90^{a}	2.80^{a}	1.50^{b}	1.80^{b}	3.09^{a}	0.293
HCt, %	10.6^{bc}	11.3^{ab}	5.6^{d}	7.5^{cd}	14.2^{a}	1.348
WBCs, $\times 10^{9}$/l	25.5^{a}	14.7^{c}	4.1^{e}	8.3^{d}	20.0^{b}	1.511
Serum total protein, g/dl	3.0^{a}	1.7^{b}	2.0^{b}	1.8^{b}	1.5^{b}	0.329
Serum cholesterol, mg/dl	246^{a}	135^{c}	189^{b}	148^{c}	84^{d}	6.663
Serum glucose, mg/dl	24.5^{d}	43.5^{a}	30.0^{c}	34.4^{b}	44.9^{a}	1.604
Serum AST, U/l	150^{d}	468^{a}	468^{a}	443^{b}	268^{c}	7.694
Serum ALT, U/l	276^{a}	139^{b}	108^{c}	94^{d}	108^{c}	5.404

$^{a-d}$Average in the same row having different superscripts are differ significantly ($P \leq 0.05$). * SED is the standard error of difference. ** LQS - Lake Qaroun sample.

Effect of diet type on fish body chemical composition.

Body chemical composition and energy content of the Egyptian sole at the beginning and at the end of the expriement are shown in Table (11). The results showed that significant differences ($P \leq 0.05$) were obtained in moisture, crude protein (CP), ether extract (EE), ash and gross energy (GE) of body composition at the end of the experimental period. At the end of the experiment, moisture content of whole fish was not significantly different among samples obtained from fish fed on SSM, HM and AF. However, fish in these treatments contained high body moisture than fish fed on SM and at initial fish samples. The protein proportion in the body of the fish was not significantly different ($P \leq 0.05$) among samples obtained from fish fed on SSM and SM, but was significantly greater than protein proportion in the other feeds and initial fish samples. EE proportion of initial fish was significantly greater than EE proportion of fish fed on the other feeds. The ash proportion in the body of the fish was not significantly different ($P \leq 0.05$) among samples obtained from fish fed on HM and AF, but was significantly greater than ash proportion in the other feeds and initial fish samples.

Table 11. Effect of diet type on fish body chemical composition and energy content (on DM basis) of *Solea aegyptiaca*.

Items	Start	Diet type				SED*
		SSM	SM	HM	AF	
Moisture, %	73.65[c]	77.10[a]	74.99[b]	77.44[a]	77.50[a]	0.484
CP, %	65.63[bc]	70.34[a]	69.13[a]	64.42[c]	66.35[b]	0.547
EE, %	19.69[a]	10.80[c]	15.88[b]	14.70[b]	12.56[c]	0.829
Ash, %	13.96[c]	17.56[b]	14.40[c]	19.48[a]	19.50[a]	0.305
GE, Kcal/g	5.48[a]	4.89[c]	5.31[b]	4.94[c]	4.84[c]	0.051

[a-c]Average in the same row having different superscripts are differ significantly ($P \leq 0.05$). * SED is the standard error of difference

The second experiment:

Effect of stocking density on growth performance and survival rate.

Results of growth performance and survival rate are shown in Table (12). There was no significant difference in the initial body weight of the fish among treatments. Survival rate was within the range 85-90% without any statistical difference ($P \leq 0.05$) between treatments. The results showed that significant differences ($P \leq 0.05$) were obtained in all growth performance parameters between treatments except the condition factor. Concerning growth performance parameters (Table 12) the highest final weights of sole fish ($P \leq 0.05$) were recorded by stocking densities 10 and 20 fish/m^3 groups followed in a significant decreasing order by stocking densities 30 and 40 fish/m^3, respectively. However differences in final weights among stocking densities 10 and 20 fish/m^3 groups were insignificant. The same trend was observed with total gain, daily gain and specific growth rate. The results indicated that the best growth for sole fish was obtained at stocking densities 10 and 20 fish/m^3. Growth performance parameters such as final weight, total gain, daily gain, SGR were decreased with increased stocking density under experimental conditions.

Table 12. Effect of stocking density on growth performance and survival rate of *Solea aegyptiaca*.

Items	Stocking density (fish/m^3)				SED*
	10	20	30	40	
Initial aveg. Weight, g/fish	18.56	18.40	18.41	18.41	0.243
Final aveg. Weight, g/fish	26.49^a	26.13^a	24.20^{ab}	22.79^b	0.831
Total weight gain, g/fish	7.93^a	7.73^a	5.80^b	4.39^b	0.616
Average daily weight gain, g/fish /day	0.07^a	0.06^a	0.05^b	0.04^b	0.005
Specific growth rate (SGR), %/day	0.30^a	0.29^a	0.23^b	0.18^c	0.017
Condition factor, g/cm^3	0.56	0.53	0.61	0.54	0.043
Survival rate, %	90.00	88.34	85.56	85.00	2.991

$^{a-c}$Average in the same row having different superscripts are differ significantly (P≤0.05). * SED is the standard error of difference

Effect of stocking density on feed utilization.

As shown in Table (13). The results showed that significant differences (P≤0.05) were obtained in all feed utilization parameters between treatments. Feed offered values were highest with stocking densities 10 and 20 fish/m^3. The best FCR (lowest) was recorded with stocking densities 10 and 20 fish/m^3, while the worst FCR was recorded with stocking density 40 fish/m^3. PER values were highest with stocking densities 10 and 20 fish/m^3, The same trend was observed with PPV and EER, but EPV values were highest with stocking density 10 fish/m^3.

Table (13). Effect of stocking density on feed utilization of *Solea aegyptiaca*.

Items	Stocking density (fish/m^3)				SED*
	10	20	30	40	
Offered feed, g/fish/period	55.90^a	54.95^a	51.55^{ab}	50.00^b	1.529
FCR, g feed/g gain	7.10^c	7.12^c	8.91^b	11.41^a	0.428
Protein utilization					
PER	0.28^a	0.28^a	0.22^b	0.17^c	0.015
PPV,%	21.00^a	18.14^a	13.57^b	10.74^b	1.587
Energy utilization					
EER	0.03^a	0.03^a	0.02^b	0.02^b	0.002
EPV,%	12.09^a	9.45^b	6.98^c	3.99^d	0.725

$^{a-d}$Average in the same row having different superscripts are differ significantly (P≤0.05). * SED is the standard error of difference

Effect of stocking density on internal organs parameters.

As shown in Table (14). The results showed that insignificant differences ($P \leq 0.05$) were obtained in viscerosomatic index (VSI), hepatosomatic index (HSI) and relative intestinal length (RIL) parameters between treatments.

Table 14. Effect of stocking density on internal organs parameters of *Solea aegyptiaca*.

Items	Stocking density (fish/m^3)				SED*
	10	20	30	40	
Viscerosomatic index,%	3.25	3.41	4.37	4.15	0.835
Hepatosomatic index,%	0.65	0.59	0.64	0.57	0.230
Relative intestinal length	1.09	1.15	1.13	1.07	0.077

* SED is the standard error of difference.

Effect of stocking density on blood hematological and biochemical parameters.

Results of blood hematological and biochemical parameters of fish under different stocking densities are shown in Table (15). The results showed that significant differences ($P \leq 0.05$) were obtained in all blood hematological and biochemical parameters between treatments except the total protein. RBCs counts value was highest with stocking densities 30 and 40 fish/m^3, but were significantly greater than RBCs in the other stocking densities. Hb and HCt values were highest with stocking density 40 fish/m^3. The highest WBCs counts was with stocking density 40 fish/m^3, but the lowest WBCs counts was with stocking densities 10 and 20 fish/m^3. Total protein values were not significantly different ($P \leq 0.05$) between stocking densities. The highest cholesterol and glucose values were with stocking density 40 fish/m^3, but cholesterol and glucose values were lowest with stocking density 10 fish/m^3. AST and ALT activities were significantly different from each other ($P \leq 0.05$) in the serum of fish from all treatments. Showing maximum activity in fish under stocking densities 30 and 40 fish/m^3 for AST, but maximum activity for ALT under stocking density 40 fish/m^3.

Table 15. Effect of stocking density on blood hematological and biochemical parameters of *Solea aegyptiaca*.

Items	Stocking density (fish/m^3)				SED*
	10	20	30	40	
RBCs, ×10^{12}/l	1.41^c	1.71^b	1.94^a	2.02^a	0.032
Hb, g/dl	3.6^b	4.1^{ab}	4.5^{ab}	4.9^a	0.430
HCt, %	16.1^b	20.1^{ab}	21.3^{ab}	21.7^a	1.880
WBCs, ×10^9/l	37.0^b	38.8^b	44.0^{ab}	48.0^a	3.057
Serum total protein, g/dl	2.1	1.9	1.7	1.7	0.339
Serum cholesterol, mg/dl	95^b	107^{ab}	107^{ab}	116^a	5.099
Serum glucose, mg/dl	17.6^d	21.9^c	31.8^b	44.2^a	1.336
Serum AST, U/l	360^b	379^b	456^a	463^a	7.211
Serum ALT, U/l	127^c	135^c	174^b	195^a	3.937

$^{a-c}$Average in the same row having different superscripts are differ significantly (P≤0.05). * SED is the standard error of difference.

Effect of stocking density on fish body chemical composition.

Body chemical composition and energy content of the Egyptian sole at the beginning and at the end of the expriement are shown in Table (16). The results showed that significant differences (P≤0.05) were obtained in moisture, CP, EE, ash and GE of body composition at the end of the experimental period. The highest moisture content was with stocking density 20 fish/m^3. The highest protein content was with stocking density 10 fish/m^3, but the lowest protein content was with stocking density 30 fish/m^3. The EE proportion in the body of the fish was not significantly different (P≤0.05) among samples obtained from fish under stocking densities 10, 20, 30 and 40 fish/m^3, but was significantly less than EE proportion in initial fish samples. The ash proportion in the body of the fish was not significantly different (P≤0.05) among samples obtained from fish under stocking densities 20, 30 and 40 fish/m^3, but was significantly greater than ash proportion in fish under stocking density 10 fish/m^3 and initial fish samples.

Table 16. Effect of stocking density on fish body chemical composition and energy content (on DM basis) of *Solea aegyptiaca*.

Items	Start	Stocking density (fish/m^3)				SED*
		10	20	30	40	
Moisture, %	73.65^{d}	75.31^{c}	78.28^{a}	76.24^{bc}	77.05^{ab}	0.581
CP, %	65.63^{b}	68.31^{a}	65.38^{bc}	64.45^{c}	64.85^{bc}	0.451
EE, %	19.69^{a}	13.90^{b}	12.92^{b}	14.15^{b}	13.50^{b}	0.818
Ash, %	13.96^{c}	16.70^{b}	19.80^{a}	19.71^{a}	19.85^{a}	0.537
GE, Kcal/g	5.48^{a}	5.08^{b}	4.83^{c}	4.89^{c}	4.85^{c}	0.061

$^{a-c}$Average in the same row having different superscripts are differ significantly ($P \leq 0.05$). * SED is the standard error of difference

DISCUSSION

In the present trials, un-ionized ammonia concentration ranged from 0.039 to 0.134 mg/l. For salmonid fishes, it is recommended that the concentration of un-ionized ammonia not exceed 0.0125 to 0.02 mg/l to maintain health of the fish, however, the toxic concentrations of un-ionized ammonia (NH3) for trout are about 0.32 mg/l for Rainbow trout, but 1.50-3.10 for channel catfish (Boyd, 1990). Thus, assuming un-ionized ammonia within the tolerance levels for *Solea aegyptiaca*, but increasing un-ionized ammonia may cause deterioration in water quality leading to stressful conditions.

Effect of diet type.

In the present study, four different feeds (SSM, SM, HM and AF) were tested for *Solea aegyptiaca* fingerlings. The highest growth performance of sole fish were recorded with fed on SM, HM and SSM groups followed in a significant decreasing order by fish fed on AF. These results similar with the findings of Abou-Zied (2015) who reported that, effect of diet type (Poultry offal, Factory by product and Artificial feed) on the growth of African catfish (*Clarias gariepinus*) and found that the best growth performance was achieved at fish fed on Poultry offal. These results in this study may be due to high protein content in SM (64.66% CP), HM (58.60% CP) and SSM (68.56% CP) compared to AF (50.26% CP), similarly with the results of Rodiles *et al.* (2012) studied the effect of dietary protein level (36, 46, 56 and 67% CP) on growth of *Solea senegalensis* juvenile and found that the best growth performance was achieved at fish fed on diets containing 46 and 56% of CP. Also, Yones and Abdel-Hakim (2011) studied the effect of dietary protein level (40, 45, 50 and 55% CP) on growth of *Solea aegyptiaca* juvenile and found that the best growth performance was achieved at fish fed on diets containing 50 and 55% of CP. As well as reported by Rema *et al.* (2008), who defined the optimum CP level for *Solea senegalensis* at 60%. Fish growth depends on numerous factors and is not always correlated with dietary protein level (Kohla *et al.,* 1992). Thus, fish digestive capacity is often studied to optimize the proportion of macronutrients in the diet (Eusebio and Coloso 2002; Debnath *et al.,* 2007 and Gonzalez-Felix *et al.,* 2010). The digestive physiology of *S. senegalensis* is characterized by a residual gastric phase, which contributes modestly to the global digestive process (Saenz de Rodriganez *et al.,* 2005, 2011), followed by an alkaline phase in a long multiple *S*-shaped intestine, where most protein digestion occurs (Yufera and Darias, 2007).

Several studies have investigated growth performances of fish species at fish fed on Sand smelt and Housefly maggot. Gumus *et al*. (2010) and Gumus (2011) suggests that Sand smelt meal can replace up to 75% of the fish meal in diets for Nile tilapia and Carp fry, respectively. Ogunji *et al.* (2006) suggests that Housefly maggot meal can completely replace fish meal in the diet of Tilapia *Oreochromis niloticus* fingerling and can meet the nutrient requirements of this species. Ogunji *et al.* (2008) recommend the suitability of Housefly maggot meal in diets for Nile tilapia fingerling. Aniebo *et al.* (2009) concluded that maggot meal is a viable alternative protein source to fish meal in the diet of African catfish.

The condition factor is a good indicator of fish health (George *et al.,* 1985 and Ni *et al.,* 2014). In the present study, sole fed on SM, HM and SSM recorded significantly higher values of condition factor compared to the fed on AF group. Improved in the condition factor can probably be attributed to high protein level in feed offered (Saoud *et al.,* 2007). Survival rate is a key indicator of health status (Moradyan *et al.,* 2012). In this study, survival rate was within the range 90-100% without any statistical difference between treatments, the highest survival rate of sole fish was recorded with fish fed on SM and HM (100%) could be attributed to sufficient food supply, continuous aeration of water (Al-Herbi and Siddiqui, 2000) and prevention of accumulation of unconsumed food/fecal matter (Shubha and Reddy, 2011).

In the present study, the highest feed offered was observed in fish fed on SM, The best FCR (lowest) was recorded with fish fed on HM, with no differences between (SM, HM and SSM). The worst FCR was recorded with fish fed on AF. These results similar with the findings of Abou-Zied (2015) who reported that, the feed intake of Poultry offal fed fish was superior compared to those fed on artificial feed. But, FCR was better with artificial feed compared to Poultry offal for African catfish. Also, Yones and Abdel-Hakim (2011) who reported that, the best FCR observed with fish fed on diets containing 50 and 55% of CP, but feed intake was no differences between dietary protein level (40, 45, 50 and 55% CP) of *Solea aegyptiaca* juvenile.

Hepatosomatic index (HSI) is related to the nutritional state and growth rate of the fish (Luckenbach *et al.,* 2007 and Yones and Abdel-Hakim, 2011). In the present study, the results showed that not differences were obtained in viscerosomatic index (VSI), while relative intestinal length (RIL) was differences, and the highest value was obtained with fish fed on HM. HSI was not differences between fish fed on SSM, SM and HM, with highest values than fish fed on AF. These results similar with the findings of Yones and Abdel-Hakim (2011) who reported that, HSI not influenced by the level of dietary CP for *Solea aegyptiaca*. Also, Rodiles *et al*. (2012) who reported that, VSI was not influenced by the level of dietary CP (36, 46, 56 and 67% CP) for *Solea senegalensis*, but contrary results in RIL whereas was not influenced. The highest HSI was observed with fish fed on SM, SSM and HM, it is good indicator on nutritional status of fish.

From the results in this study note that the best growth performance was obtained with fish fed SM, HM and SSM diets. This performance was superior to those of AF fed fish possibly due to high protein content, high quality animal protein and total digestible dry matter in SM, HM and SSM compared to AF. SSM is animal protein ingredients are good sources of amino acids and fatty acids with high protein content, all essential amino acids in Sand smelt fish were almost of the same profile of the commercial imported fish meal, higher in theronine as essentional amino acid compared to commercial fish meal according to (Yones and Abdel-Hakim, 2011). Also, SM is animal protein ingredients are good sources of amino acids with high protein content according to (El-Sherif, 2001). Also, HM is animal protein ingredients are high protein content (Sogbesan *et al.*, 2006), good sources of amino acids (Ogunji *et al.*, 2006), fatty acids (Hwangbo *et al.*, 2009), minerals (Fasakin *et al.*, 2003).

In addition, sole fish usually lives on sandy and muddy seabeds. They mainly hunt for feed at night and feed on worms, mollusks, small fishes and crustaceans (Picton and Morrow, 2010). Fish do not ingested the feed offered during the day and may be part of them dissolves in water and the rest falls on the bottom of the tank, which fermented and increasing proportion of ammonia cansing detriment of growth and survival rate under nutrition on artificial feed Under such conditions, it is expected that larvae continue to swim actively for search of feed and expend extra energy that may result in reduced growth. On the contrary, fish feeding on SSM, SM and HM, they stay long time under water without decomposed, making it more susceptible to feed them and easily dominated by individual fish, affecting positively on the growth and survival rate. Moreover, Salas-Leiton *et al.* (2008) and Ali *et al.* (2003), the optimization of the metabolic rate, e.g. reducing locomotory activity and promoting feed ingestion, would have contributed to increase the proportion of the energy available for growth.

In the present study, the highest protein content in whole fish body was obtained with fish fed on SSM and SM, while the highest EE content was with fish fed on SM and HM. These results disagree with the findings of Yones and Abdel-Hakim (2011) found that, not differences in composition of whole fish body in terms of dry matter, crude protein, crude lipid and ash contents by different dietary protein level for *Solea aegyptiaca*. According to Abou-Zied (2015) found that, the highest protein content was with fish fed on artificial feed compared to Poultry offal for African catfish, while the highest EE with fish fed on Poultry offal.

The hematological and biochemical parameters of the fishes are affected by several factors such as species, environmental, response of condition, dietary, age, maturation and nutrition (Ross and Ross, 1999; Regost *et al.*, 2001 and Azarin *et al.,* 2012), temperature, ecological habitat, food selection and mode of life (Francesco *et al.,* 2012), Ammonia, nitrite, culture density and the culture systems could influence the values obtained (Hrubec *et al.,* 1996, 1997). Other possible contributing factors might be differences in sampling procedure, sample processing or the time of sampling (Chen *et al.,* 2003). Other reports have suggested that differences could result from different blood collecting procedures (Hunn and Greer, 1991 and Congleton and La Voie, 2001). Another possible factor is stress in the fish population. The fish under social stress may have significant changes in blood chemistry without showing signs of disease. These fish cannot be distinguished from healthy fish, but contribute to variation within a population (Chen *et al.,* 2003). Sakomoto *et al.* (2001) have proposed that variations in blood parameters among fish could be affected by other variables such as the sampling technique, the capturing method, the condition of captivity and the analysis techniques.

Fish haematology plays an important role in fish culture because of its importance in monitoring the health condition of fish (Hrubec *et al.,* 2000). In this study, RBCs counts, Hb and HCt values were highest with fish fed on AF, it is indicator on activity swimmer for search on feed. Present opinion is supported by Kuzminova *et al.* (2014) recorded that, *Trachurus mediterraneus* is pelagic fast swimmer, which requires high concentration of erythrocytes, red blood cells, which elevate the oxygen consumption in the blood. In addition, the high erythrocyte number was associated with fast movement, predaceous nature and high activity (Rambhaskar and Srinivasa-Rao, 1986). In this study, the lowest Hb was with fish fed on SM, low haemoglobin value was associated with the low active fishes (Satheeshkumar *et al.,* 2011). The major functions of WBCs are to fight infection, defend the body by phagocytosis against invasion by foreign organisms and to produce or at least transport and distribute antibodies in immune response (Nasir and Al-Sraji, 2013), in this study, the lowest WBCs counts was with fish fed on SM then HM, it is good indicator on health status of fish. Present opinion is supported by Andrews (1988) and Al-Shawi and Al-Zaidy (2009) recorded that, the low number of WBCs counts this interpreted to improvement of fish health state and improve the efficiency of the immune system. The increase in WBCs of fish was suggested to indicate alteration in defense mechanism against the action of the highly toxic and exposed to high risk of infection as previously reported by (Haggag *et al.*, 1999; Zaghloul, 2001; Mazon *et al*., 2002; Zaghloul *et al.,* 2005, 2007 and Nasir and Al-Sraji, 2013). These results of hematological parameters confirm aforementioned interpretation for growth performance and survival rate of sole fish.

Biochemical analysis can provide valuable information for monitoring the health and condition of fishes. Moreover, analysis of serum constituents has showed useful information in detection and diagnosis of metabolic disturbances and disease in fishes (Xiaoyun *et al.,* 2009 and Azarin *et al.,* 2012). The levels of total protein, cholesterol are considered to be major indices of the health status of teleosts (Xiaoyun *et al.,* 2009) and as indicator of nutritional status (Yousefian *et al.,* 2010). In this work, total protein and cholesterol had a positive relationship with weight. Similarly, Azarin *et al.* (2012) recorded that, weight and length had a positive relationship with total protein and cholesterol for persian sturgeon, *Acipenser persicus*. Also, Nasir and Al-Sraji (2013) reported that, significant increases in plasma cholesterol levels were recorded in carp fed the high protein and fat diets. Hill (1982) reported that cholesterol concentrations increase as the fish size increased. Glucose in serum is a major metabolite of carbohydrate metabolism (Artacho *et al.,* 2007), an increase in the plasma glucose of teleosts was believed to be caused by a wide range of environmental stressors (such as hypoxic environment, starvation and captivity) (Xiaoyun *et al.,* 2009). In this study, the highest glucose in serum was with fish fed on AF, it is indicator on stress status of fish. Whilst, the highest glucose in serum was with fish fed on SSM, it is possible that handling stress of the fish caused a surge in blood glucose as described in *Siganus rivulatus* (Abou-Daoud *et al.,* 2014). Increase of ALT and AST enzyme activity correlated with fish growth and differences in size, and may be related to changes in physiological status due to sexual maturation, feeding behavior and diet composition (Kuzminova *et al.,* 2014). The rise of hepatic ALT and AST observed in *Solea aegyptiaca* fed high protein diets may reflect the use of excess carbon backbones from amino acids to supply energetic demands as described in *Rhamdia quelen* (Melo *et al.*, 2006). Likewise, high protein/ carbohydrate ratios in the feeding of *Sparus aurata* bring about ALT and AST to increase in the liver (Meton *et al.,* 1999). Similar responses were observed in *Oncorhynchus mykiss* for ALT (Sanchez-Muros *et al.,* 1998) and Kim and Lee (2009) for juvenile tiger puffer (*Takifugu rubripes*). Abou-Daoud *et al.* (2014) revealed that, ALT activity appears to increase with increasing dietary protein for *Siganus rivulatus*. The rise in the hepatic activity of protein-metabolizing enzymes when fish were fed high protein diets may denote use of excess dietary amino acids for growth as well as substrate for gluconeogenesis, particularly for ALT activities (Melo *et al.,* (2006). The high contents of dietary protein and the consequent low levels of carbohydrates, increased transaminations, deamination and nitrogenous metabolites as described in *Rhamdia quelen* (Melo *et al.,* (2006). In spite of all enzyme activities were increased with the dietary protein, AST and ALT were respectively more demanded. The enzymes AST is fully involved in amino acid metabolism; however, carbon residues directly from glycolysis can also supply ALT (Melo *et al.,* (2006). The increase in AST and ALT denotes catabolism of amino acids and is associated with increased nitrogenous excretion. The augment of protein breakdown in fish, resulting in increase of plasma ammonia concentration, was previously observed in *Bidyanus bidyanus* and *Dicentrarchus labrax* (Yang *et al.,* 2002 and Peres and Oliva-Teles, 2001). Excess of ammonia is promptly excreted through the gills (Van Waarde *et al.,* 1983). In some fish species, particularly under adverse environmental conditions when ammonia excretion is impaired, urea excretion is triggered (Wood *et al.*, 1995 and Saha and Ratha, 1998). High levels of environmental ammonia should contribute to plasma ammonia increased. Therefore, the increase of ammonia were certainly due to the increase of protein catabolism (Melo *et al.*, (2006). The increase of ALT and AST suggests protein catabolism at high protein levels in the diet.

Effect of stocking density

Growth is usually inversely correlated with stocking density of fish in culture (Sanchez *et al.*, 2010). Stocking densities is a major factor in aquaculture influencing growth, welfare, and health (Montero *et al.*, 1999; Alcorn *et al.*, 2003 and Ellis *et al.*, 2005). In the present study, four different stocking densities (10, 20, 30, 40 fish/m^3) were tested for *Solea aegyptiaca* fingerlings. The highest final weights of sole fish were recorded with stocking densities 10 and 20 fish/m^3, survival rate was within the range 85-90% without any statistical difference, growth performance parameters and survival rate were decreased with increased stocking density under experimental conditions. These results agreed with the findings of Sanchez *et al.* (2010) who studied the effect of stocking density (8.6 and 26.6 Kg/m^2) on the growth of Senegalese sole (*Solea senegalensis*) for 195 days, and found that fish reared under 26.6 Kg/m^2 showed poor or no growth. Also, Schram *et al.* (2006) studied the effect of stocking density (0.5, 1.1, 5.1, 7.4, 10.2 and 12 kg/m^2) on the growth of Dover sole for 55 days, and found that the specific growth rate significantly decreased with increasing stocking density. They added mortality increased significantly with increasing stocking density. Moreover, Howell (1998) reported decreasing growth and increasing size variation for Dover Sole when stocking density increased from 5% to 131% bottom coverage. In addition, Ni *et al.* (2014) studied the effect of stocking density (3.7, 6.9 and 9.3 Kg/m^3) on the growth of Amur sturgeon (*Acipenser schrenckii*) for 60 days, and found that the growth under 9.3 Kg/m^3 group were significantly lower than in the 3.7 and 6.9 Kg/m^3 groups. On the contrary, Salas-Leiton *et al.* (2008)

assaying four stocking densities between 2 and 30 kg/m^2 with Senegalese sole did not find any significant differences in biomass production or growth rates. Also, Salas-Leiton *et al.* (2010) studied the effect of stocking density (7 and 30 Kg/m^2) on the growth of Senegalese sole (*Solea senegalensis*) juveniles for 60 days, and no differences in SGR were found between densities. Moreover, Salas-Leiton *et al.* (2011) studied the effect of stocking density (6.20 and 30.97 Kg/m^2) on the growth of Senegalese sole (*Solea senegalensis*) for 60 days, and soles stocked at high density showed an increased growth compared to individuals kept at low density. As well as, Herrera *et al.* (2009) found that no significant differences in growth for wedge sole (*Dicologlossa cuneata*) at different stocking densities.

Several studies have investigated growth performances of flatfish species in relation to stocking density. No effect of stocking density on growth was found for juvenile winter flounder (*Pseudopleuronectus americanus*) up to 350% bottom coverage (Fairchild and Howell, 2001), summer flounder (*Paralichthys dentatus*) up to 466% bottom coverage (King *et al.,* 1998), yellowtail flounder between 30% and 120% bottom coverage (Boyce *et al.,* 1999) and turbot (*Scopthalmus maximus*) at stocking densities up to 200% bottom coverage (Howell, 1998 and Irwin *et al.,* 1999) or at densities between 0.25 and 68 kg/m^2 (Martinez-Tapia and Fernandez-Pato, 1991). In contrast, a negative effect of stocking density on growth has been reported for Atlantic halibut (*Hipploglossus hippoglossus*) above 100% bottom coverage (Bjoernsson, 1994), California halibut (*Paralichthys californicus*) (Merino *et al.,* 2007), Atlantic halibut (*Hippoglossus hippoglossus*) (Kristiansen *et al.,* 2004), turbot (*Psetta maxima*) (Irwin *et al.,* 1999). However, it is difficult to compare the results of the various authors since some studies were performed with larval fish while others used juveniles and yet others used adults. it is impossible to compare results among fishes of different age groups. Furthermore, some researchers report densities as biomass per unit volume, again making comparisons difficult.

Stocking density had effect on survival rate on high density group. It is notable that mortality could be due to positive interactions between stressful factors that threatened fish health (Barton and Iwama, 1991). In this study, survival rate was within the range 85-90% without any statistical difference between treatments, with relatively decreased with increasing stocking density. Similarly, Ni *et al.* (2014) found that, the mortality was not affected by stocking density for Amur sturgeon (*Acipenser schrenckii*). Also, many studies have not found any significant effects of density on survival (Mazzola *et al.*, 2000; Gomes *et al.*, 2006 and Rafatnezhad *et al.*, 2008). Several authors report that high stocking density increases fish susceptibility to disease (Pickering and Pottinger, 1989; Mazur and Iwama, 1993 and Fairchild and Howell, 2001). Higher mortality at higher stocking density has been found for Winter flounder (*Pseudopleuronectus americanus*) (Fairchild and Howell, 2001), ayu (*Plecoglossus altivelis*) (Iguchi *et al.*, 2003) and seabream (*Sparus auratus*) (Montero *et al.*, 1999). In contrast to these findings, Martinez-Tapia and Fernandez-Pato (1991) found higher survival rate for turbot (*Scopthalmus maximus*) at higher stocking density.

In the present study, total and un-ionized ammonia concentrations increased with increasing stocking density. Similar to our results, Kebus *et al.* (1992) reported that in Rainbow trout (*Oncorhynchus mykiss*) culture, at high stocking density the water quality deteriorated. Also, Shi *et al.* (2006) found that high stocking density could deteriorate water quality. Moreover, Moradyan *et al.* (2012) reported that increasing densities may cause deterioration in water quality leading to stressful conditions. In addition, Ni *et al.* (2014) found that the water quality parameters deteriorated with increasing stocking density suggesting that the decrease in growth at high stocking density is in part caused by deterioration in water quality.

In the present study, feed offered values were highest with stocking densities 10 and 20 fish/m^3. The best FCR (lowest) was recorded with stocking densities 10 and 20 fish/m^3, while the worst FCR was recorded with stocking density 40 fish/m^3. These results disagree with the findings of Ni *et al.* (2014)

found that, FCR was not affected by stocking density for Amur sturgeon (*Acipenser schrenckii*). Also contrary, results have been described in sea bass and brill (*Scophthalmus rhombus*) (Sammouth *et al.,* 2009 and Herrera *et al.,* 2012) found that, FCR was not affected by stocking density. HSI is related to the nutritional state and growth rate of the fish (Luckenbach *et al.,* 2007). In the present study, the results showed that not differences were obtained in VSI, and HSI with relatively decreased with increasing stocking density in HSI. Similarly, Ni *et al.* (2014) found that, HSI decreased with increasing stocking density for Amur sturgeon (*Acipenser schrenckii*) but unlike with VSI was decreased with increasing stocking density. Previous studies reported that HSI was significantly lower at high stocking density (Leatherland and Cho 1985). This reduction was probably due to a worse nutritional state and an increased lipid mobilization at high stocking density (Ni *et al.,* 2014).

In this study, the lower growth performance of *Solea aegyptiaca* exhibited at higher stocking density could have been caused by energy expenditure because of intense antagonistic behavioral interaction, competition for food and living space and increased stress. It has been reported that high stocking density of *Solea solea* and *Solea senegalensis* might lead to potentially stressful conditions associated to high densities which eventually leads to impaired fish growth, the increase of the differences in size within a stock of fish cultured in the same tank is usually associated to the onset of hierarchies, due to competition for food items, space or other resources (Schram *et al.,* 2006 and Sanchez *et al.,* 2010). To verify this, Costas *et al.* (2008) and Salas-Leiton *et al.* (2008, 2010) for Senegalese sole, Herrera *et al.* (2009) for wedge sole, Montero *et al.* (1999) for Sea bream, Barcellos *et al.* (1999) for Nile tilapia reported that, plasma cortisol concentration increased at high densities, an indication of chronic stress attributable to social stress. Moradyan *et al.* (2012) concluded that the high number of individual/m^3 which reduced the ability of fish to see and access food is another possible reason for the low growth and high feeding conversion ratio of fish in high stocking density, maximum increase in fish biomass was found at the lowest stocking density and decreased with increasing density because of better feed utilization and unstressed conditions at low stocking levels. In general, these studies suggest that fish grown at low density perform better than at higher densities.

In the present study, body moisture and protein content was different between the four stocking density groups while ether extract content was not different. Ash content was significantly higher in the 20, 30 and 40 fish/m^3 groups than in the 10 fish/m^3 group. Similarly, Ni *et al.* (2014) found that, ash content was significantly higher in the 6.9 and 9.3 Kg/m^3 groups than in the 3.7 Kg/m^3 group, in contrast, body moisture content was not different between the three stocking density groups, stocking density also did not affect the crude protein content, total lipid was lower in the 6.9 and 9.3 Kg/m^3 groups than in the 3.7 Kg/m^3 group for Amur sturgeon (*Acipenser schrenckii*). In contrast, Piccolo *et al.* (2008) found that the moisture, crude protein and ash contents of sole muscle were not influenced by stocking densities while the lipid content was higher in the low density group. In gilthead sea bream (*Sparus aurata*), lipid contents was significantly influenced by stocking density density (Montero *et al.*, 1999).

Higher stocking densities and poor water quality are chronic stressors commonly encountered by fish (De Oliveira *et al.*, 2012). Oxygen consumption by fish is generally afected by elevated nitrite and ammonia levels (Tilak *et al.*, 2005; Datta *et al.*, 2005 and Remen *et al.*, 2008) in culture system and can lead to physiological imbalances in fish (Iwama *et al.*, 2000 and Randall and Tsui, 2002). In this study haemoglobin values were highest with stocking density 40 fish/m^3. Similarly, Ni *et al.* (2014) found that, haemoglobin values in the 9.3 and 6.9 Kg/m^3 groups were higher than in the 3.7 Kg/m^3 group for Amur sturgeon (*Acipenser schrenckii*). Also observed by Barcellos *et al.* (2004) in jundia (*Rhamdia quelen*) subjected to 10 days crowding stress. Dicu *et al.* (2013) reported that the haemoglobin level in juvenile stellate sturgeon increased with increasing stocking density. This response may be related to the increase in energy demand imposed by high stocking density (Ni *et al.*, 2014). In this trial, cholesterol levels were found to decrease with increasing stocking density. The decreased trends of cholesterol in our trial are similar to with was observed in Senegalese sole, *Solea senegalensis* (Costas *et al.*, 2011). Kpundeh *et al.* (2013) reported that, cholesterol levels were found to decrease with increasing stocking density for Tilapia Juveniles (*Oreochromis niloticus*). Also, Ni *et al.* (2014) the lowest serum total cholesterol concentration was found in the high stocking density for Amur sturgeon (*Acipenser schrenckii*). In this study glucose levels increased with increasing stocking densities. Similarly, Montero *et al.* (1999) glucose levels increased at high density for *Sparus aurata*. In contrast, Ni *et al.* (2014) differences between stocking densities were not detected for glucose for Amur sturgeon (*Acipenser schrenckii*). Also, Rafatnezhad *et al.* (2008) found that stocking density had no significant effect on glucose concentration in great sturgeon juveniles. European sea bass (*Dicentrarchus labrax*) subjected to diferent stocking densities showed decreased levels in serum protein (Svobodova *et al.*, 2006). Also, Ruane *et al.* (2002) reported marked decrease in plasma total protein level in common carp (*Cyprinus carpio*) when held at high density in confinement. Moreover, Biswas *et al.* (2006) reported decreased plasma total protein in red sea bream after short term handling stress, the above findings are analogous to the current findings in this study. In this study, increases in AST and ALT activities could have resulted from the long stay in chronic ammonia set in by both fish wastes and uneaten feed (Mona and Hegazi, 2011). AST and ALT activity levels can be use to assess finfish response to toxins, malnutrition, disease and other stress related factors. In this study, serum AST and ALT activities were found to increase gradually with increased stocking density and conform to the findings of Chen *et al.* (2011) and Zhang *et al.* (2007) who subjected Half-smooth tongue sole (*Cynoglossus semilaevis*) and common carp (*Cyprinus carpio*) to high density stressors respectively. Contrary, Ni *et al.* (2014) no significant differences were observed in the activity of these enzymes for Amur sturgeon (*Acipenser schrenckii*). Other studies have shown that, water quality problems including high ammonia level, induced oxidative stress in: brain and gills of mudskipper, *Boleophthalmus boddarti* (Ching *et al.,* 2009) and liver of Nile tilapia, *Oreochromis niloticus* (Hegazi *et al.,* 2010).

In conclusion, from the results of the present study, unconventional feeds (SSM, SM, HM) were better than AF on growth performance. The low stocking density at 10 and 20 fish/m^3 were the best in growth performance under experimental conditions. So, it is recommended to *Solea aegyptiaca* cultured with feeding on HM or SSM or SM and under stocking density 10 or 20 fish/m^3.

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تأثير نوع العليقة وكثافة التخزين على أداء النمو ومقاييس الدم لأسماك موسى المصرية

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الملخـص العربــى

أجريت هذه الدراسة لتقييم نوع العليقة والكثافة على كل من مظاهر النمو ومقاييس الدم والتركيب الكيميائي للجسم لأسماك موسى المصرية (*Solea aegyptiaca*). التجربة الأولى أختبرت أربعة أنواع من العلائق (مفروم البساريا، مفروم الجمبري، ديدان (يرقات) حشرة الذبابة المنزلية، عليقة صناعية) وأوضحت النتائج أن الأغذية غير التقليدية مثل مفروم البساريا، مفروم الجمبري، ديدان (يرقات) حشرة الذبابة المنزلية تفوقت على الغذاء التقليدي (العليقة الصناعية) في مقاييس النمو ومعدل تحويل الغذاء وحسنت من الحالة الصحية للأسماك. وأن أعلى محتوى لبروتين جسم الأسماك سجل مع التغذية على مفروم البساريا ومفروم الجمبري بينما سجل أعلى محتوى لدهن الجسم مع التغذية على مفروم الجمبري و ديدان الذباب. التجربة الثانية أختبرت أربعة كثافات مختلفة (١٠، ٢٠، ٣٠، ٤٠ سمكة/ م^{٣}) وأوضحت النتائج أن الكثافات الأقل (١٠، ٢٠ سمكة/ م^{٣}) تفوقت على الكثافات الأعلى (٣٠، ٤٠ سمكة/ م^{٣}) في مقاييس النمو ومعدل تحويل الغذاء وأن أعلى محتوى لبروتين جسم الأسماك سجل تحت كثافة ٢٠ سمكة/ م^{٣} بينما محتوى الجسم من الدهن كان لا يوجد إختلافات معنوية بين الأربع كثافات. وأوضحت مقاييس الدم أن الأسماك تقع في حالة إجهاد مع زيادة الكثافات.