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Effect of Poly-Unsaturated Fatty Acids Fortification on Growth Performance, Survival, Fatty Acid Composition and Antioxidant Balance of Meagre, *Argyrosomus regius* Larvae

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Abstract

A total of 120 meagre, *Argyrosomus regius*, larvae (0.37 ± 0.02 g) were assigned to four treatments (three replicates each) to determine the optimum dietary level of PUFAs, at which there will be the best growth performance, feed utilization, fatty acids composition and antioxidant status. The treatments were a control group (fed a basal diet 3% PUFAs of total fatty acids) and another three groups fed PUFAs supplemented diets at levels 4.55, 6% and 7% for 21 days.

The results indicated that increasing dietary PUFAs levels up to 4.5% significantly improved growth and survival compared to the control group. Feed intake, feed conversion ratio and protein utilization were significantly increased with 4.5% PUFAs followed by the control group. The results revealed significant differences in PUFAs among treatments and the best value was reported for the 4.5% PUFAs supplemented diet followed by the control. There is a significant increase in TBARs levels with increasing dietary PUFAs concentration. Meanwhile, the SOD and CAT activities decreased significantly with increasing PUFAs levels. However, TAS decreased significantly with higher PUFAS level (6%) only compared to other treatments. Therefore, the dietary requirements of *A. regius* larvae is 4.5% PUFAs of total fatty acids.

Keywords: PUFAs; *Argyrosomus regius*; Growth; Feed conversion ratio; Fatty acid composition; Antioxidant status

Introduction

The continuous expansion of aquaculture in the Mediterranean region and around the world is urgently needed to mitigate the extensive over-fishing of wild fisheries, the rapid increase in populations and customer awareness (Food and Agriculture Organization [1]. Nevertheless, the development of sustainable aquaculture that depends on incorporating native, emerging fish species must be considered [2]. The immense increase in meagre, *Argyrosomus regius*, production during the last two decades highlights the appearance of a new native aquaculture species in the Mediterranean region [3].

A. regius has potential characteristics suitable for aquaculture diversification, because it adapts easily to captivity, exhibits high growth, an excellent feed conversion rate and tolerates wide ranges of temperature and salinity [4]. Also, the reproduction of *A. regius* was effectively controlled via hormonal induction to provide sufficient quality and quantity for commercial production [5]. Larvae and juveniles have been reared with similar facilities and methodologies used for other marine fish species [6].

In addition, *A. regius* mature at a larger size (>4 kg) than the harvest size avoiding problems of reduced growth associated with maturation [7]. It has a high nutritional value owing to the fact it is a lean fish with low lipid, and high protein quality flesh and long shelf-life [8,9].

Lipids play a crucial role in fish nutrition for the provision of an efficient source of energy than carbohydrates where lipids are readily metabolized by fish (National Research Council, 2011) and represent protein sparing effect [10]. Also, it provides a source of essential fatty acids (EFA) [11]. Namely, the n-3 series HUFAs docosahexaenoic acid (DHA, 22:6n-3) and eicosapentaenoic acid (EPA, 20:5n-3), and the n-6 series HUFA arachidonic acid (20:4n-6) [1]. The HUFAs have important functions in the fish body including controlling and regulating growth performance, survival, immune function, cell membrane integrity, nervous system development and pigmentation [12-14].

However, they are synthesized in very small quantities from their precursors linoleic acid (18:2n-6) and alpha-linolenic acid (18:3n-3) [15], due to the limited activity of D-5 and D-6 desaturases and elongases and are considered essential [16]. Therefore, they must be included in live preys and larvae diets to fulfill the requirements for growth, organ and tissue development and functioning, stress resistance and survival [17].

Meanwhile, these fatty acids are very prone to oxidation and more exposed in formulated diets for marine fish larvae 17]. Moreover, at a physiological level, the oxidative risk is particularly high in the fast-growing larvae, due to the high metabolic rate, oxygen consumption and water content in the larval tissues [18].

The n-3 HUFA requirements have been extensively studied in marine fish larvae and have been reported between 0.3 and 55 g kg^{-1} n-3 HUFA on a dry weight basis [16]). In meager as new aquaculture species, the requirements of PUFAs did not determine yet in larval diet. The recently available study of El-Kertaoui et al. [19] showed the highest growth performance of *A. regius* with 3% dry weight PUFAs.

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Therefore, the aim of the present study was to investigate higher levels of PUFAs for the best growth performance, feed utilization, fatty acids and antioxidants balance.

Materials and Methods

Experimental fish and facilities

A total of 120 meagre, *A. regius*, larvae (0.37 ± 0.02 g) were obtained from the coastal waters of Domyata governorate (Egypt). Fish were acclimatized at Fish Nutrition Laboratory, El-Anfoushy, National Institute of Oceanography and Fisheries, Alexandria, Egypt in four circular fibreglass tanks (0.5 m3) for 14 days. During the adaptation period the fish were weaned from live food to artificial diet as follow: fish were fed on minced bloodworm for the first five days then weaned to artificial diet (control diet) by replacing 10% of bloodworm by the diet and gradually increased by10% daily to reach 100% of the control diet at the fifteenth day.

After the acclimatization, the fish were randomly distributed into twelve glass aquaria measuring 100 × 40 × 30 cm (four treatments, each in triplicate) at a stocking density of 10 fish per aquarium.

The daily water exchange rate was 30% and excreta were removed by manual siphoning. The rearing conditions were: temperature (20.10 ± 0.43°C), pH (7.51 ± 0.17), dissolved oxygen (6.52 ± 0.37 mg L^{-1}), salinity (38.97 ± 0.16 g L^{-1}) and a photoperiod regime (8:16 h Light: Dark).

Experimental design and diets

Four isonitrogenous (57%) and isolipidic (16%) experimental diets were formulated in a pellet size of 250-500 µm (Table 1). The control diet was not supplemented with PUFAs and the fish oil (herring) was used at dose of 16% as a source of PUFAS contents to get the final concentration of 3% PUFAs. The other three diets were supplemented with PUFAs source (OMEGA-3, Puritant's pride, USA) at doses of 3, 6 and 9% replacing from fish oil to get the final PUFAs concentration of 4.5, 6 and 7.4.5% of total fatty acids and the final concentration of PUFAs was determined and presented in Table 1.

The used protein sources (squid meal, shrimp meal and soybean meal) were defatted three consecutive times according to Folch et al. [20] with chloroform: methanol: protein source ratio of 3:2:1 to allow a better control of the fatty acid composition of the diets. The diets were prepared in the following manner: the ingredients were ground to a fine powder and carefully mixed with mineral and vitamin premix. In a separate mixture, oils and PUFAs source (OMEGA-3) were added as designed for each treatment and mixed to obtain a homogeneous blend. Then, gelatin was dissolved in warm water and when its temperature was lowered than 35°C, added to the rest of the previously mixed ingredients.

The paste was pelleted by colander with very small holes and dried in an oven at 35°C for 6 h. Pellets were ground and sieved to obtain the desired particle size and kept at -20°C until feeding. Diets were analyzed for proximate composition according to Association of Official Analytical Chemists in 2000 (Table 2). Fish were fed one of the four experimental diets at the apparent satiation, four times a day for three weeks.

Sample collection

After the feeding period, all fish were weight, counted and 2 fish per each aquarium were homogenized for the analysis of thiobarbituric reactive substance (TBARs) and the antioxidant enzymes. The other 2 fish were kept frozen for fatty acids.

The fish samples were minced and homogenized (10% w/v) in ice-cold sucrose buffer (0.25 M) in a Wise Tis HG-15D homogenizer (Daihan Scientific, Bangalore, India). The homogenates were centrifuged (7063 g, 20 min, 4°C) and the resulting supernatants were collected and stored at -80°C.

Measured parameters

Growth and feed utilization: The final body weight (FBW) of each experimental treatment was determined by dividing the total fish weight in each aquarium by the number of fish. Weight gain (WG), Average daily gain (ADG), and Specific growth rate (SGR%), survival (%), feed conversion ratio (FCR), protein efficiency ratio (PER) and protein productive value (PPV%) were calculated using the following equations [21].

Weight gain (g fish-1): WG = W2 – W1

Average daily gain (g $fish^{-1}$ day^{-1}): ADG = W2 – W1/n

Specific growth rate (% day^{-1}): SGR = 100 × (ln W2 - ln W1)/ days

where W1: Initial weight of fish (g); W2: FBW of fish (g); and n=days; ln is the natural log.

Survival rate (%) = 100 × (final number of fish/initial number of fish)

FCR = Feed intake (g)/weight gain (g).

PER = Weight gain (g)/protein intake (g).

Fatty acids composition: Fatty acid methyl esters for diets were obtained by transmethylation of crude lipids as described by Christie [21]. Briefly, total lipids were extracted in chloroform: methanol (2:1, v: v) using the method of Folch et al. [20]. The reaction was conducted in dark conditions under nitrogen atmosphere for 16 h at 50°C. Afterwards, fatty acid methyl esters were extracted with hexane: diethyl ether (1:1, v/v) and purified by adsorption chromatography on NH2 Sep-pack cartridges at Waters S.A., Massachussets, USA. Fatty acid methyl esters were separated by HP (Hewlett Packard-6890 GC, Germany) in a Super colvax-10-fused silica capillary column (length: 30 mm, internal diameter: 0.32 mm; Supelco, Bellefonte, USA) using helium as a carrier gas. Column temperature was 180°C for the first 10 min, increasing to 215°C at a rate of 2.5°C min^{-1} and then held at 215°C for 10 min.

Antioxidant parameters: Thiobarbituric acid reactive substances (TBARS) were measured in wet tissue homogenates at 532 nm. Briefly, 37.5 ml of wet tissue homogenate were added to 1000 ml of trichloroacetic acid (TCA 24.5%, El-gomhoria Co., Egypt), mixed well and centrifuged (1075g, 20 min). 600 µl of the supernatant were added to 300 µl of 2-thioxodihydropyrimidine-4, 6 (TBA, 0.7%, Sigma, USA). The mixture was boiled for 20 min and cooled to room temperature. The lipid peroxides or malondialdehyde (MDA) in the samples reacted with TBA to form MDA: TBA adducts (1:2), generating red fluorescence. An extinction coefficient of 156000 M1 Cm1 was used for the calculation [22].

Catalase activity (CAT, U g^{-1} tissue) was measured according to Luck [23]. Briefly, 10 ml tissue homogenate sample was added to 1.25 ml of freshly prepared buffer containing 50 µl of H_2O_2 10 ml Na-K-phosphate buffer (0.15 M, pH 7, El-gomhoria Co., Egypt). The absorbance was recorded after 20 s (A1) and after 80 s (A2) of incubation at 240 nm against air. The CAT value calculated as A1-A2/0.0008. Superoxide dismutase (SOD; U g-1 tissue) activity was evaluated according to Misra and Fridovich [24]. Briefly, 20 µl of tissue homogenate was added

to 940 µl sodium carbonate buffer (pH 10.2, 0.05 M, El-gomhoria Co., Egypt) and 40 µl epinephrine (30 mmol l-1 dissolved by adding 30 µl of HCL, Sigma, USA). The inhibition of epinephrine auto-oxidation in the alkaline medium to adrenochrome was recorded after 30 and 90 seconds at 480 nm. A control was prepared as 960 µl sodium carbonate buffer and 40 µl epinephrine.

$$\text{The percent of inhibition } (\%) = 100 - \left((\Delta A \text{ control} - \Delta A \text{ sample} / \Delta A \text{ control}) \times 100 \right).$$

SOD activity in tissue homogenate (U g-1 tissue) = % inhibition × 3.75.

Total antioxidant status (TAS) was assayed using the method [25]. Briefly, the TAS was evaluated as the reaction of antioxidants in 200 µL of tissue homogenate and 500 µL of hydrogen peroxide (H_2O_2, 0.3%, El-gomhoria Co., Egypt), at 37°C for a 10 min incubation period. The antioxidants in the sample eliminated some of the H_2O_2 provided. The residual H_2O_2 was determined calorimetrically by an enzymatic reaction which involved the conversion of 3, 5, dichloro-2-hydroxy benzensulphonate (Sigma, USA) into a colored product assessed at 505 nm.

Statistical analysis

All data were tested for homogeneity by Levene's tests and the normality was checked by Shapiro-Wilk test. The normally distributed data and log transformed non-normal data were treated using one-way ANOVA by SPSS (Standard Version 17.0 SPSS Inc. Chicago, Illinois). When F values were significant (P≤ 0.05) duncan's multiple range test was used to compare the differences between means [26].

Results

Growth performance and survival

Growth performance of A. regius, fed different levels of (PUFAs) showed a significant increasing of FBW, WG and ADG of fish fed 4.5% PUFAs followed by the control group. Meanwhile, the highest two level of PUFAs (6 and 7%) significantly decreased growth performance than the control group (Table 3).

Figure 1 presenting the SGR values in response to dietary PUFAs in order to define requirements [27]. The Polynomial regression seems here the best procedure and lets to define the requirement at 4.33% PUFAs of total fatty acids. No significant difference (P< 0.05) was observed in survival percent among dietary PUFAs treatments (Table 3).

Feed and protein utilization

Feed intake was increased significantly with 4.5% PUFAS compared to the highest two doses of PUFAs and followed by the control group (Table 3). Statistical analysis indicated that there were no significant differences in FCR% and PER%. Meanwhile, FCR and PER tended to improve in fish fed 4.5% PUFAs.

Whole-body fatty acid composition

The supplementation with PUFAs significantly decreased MUFA levels than the control and the best values were recorded with control followed by (5% PUFA). The results revealed significant differences in PUFA among treatments; the best value was reported for the (5% PUFAs) diet, followed by the control (Table 4).

Thiobarbituric acid-reactive substances and antioxidant enzymes

Results indicated that there was a significant increase in TBARs concentration with increasing dietary PUFAs levels represented in Figure 2. Meanwhile, the SOD and CAT activities decreased significantly with increasing PUFAS levels. However, TAS decreased significantly only with higher PUFAS level (6.5%) compared to other treatments.

Discussion

Meagre, *A. regius*, is a new emerging species for marine finfish aquaculture and as such lack information exists as to its growth and nutrient requirements. The determination of the optimum level of PUFAs for *A. regius* larvae for best growth performance, survival, feed utilization and oxidative stress responses of *A. regius* was the main goal of the present work.

The current findings showed that *A. regius* larvae fed 4.5% PUFAs had the highest significant growth performance compared to the other treatments. Meanwhile, the highest two levels of PUFAs (6.0 and 7.0%) significantly decreased growth performance than 4.5% and the control group (3%). The FCR and protein utilization were improved with increasing HUFAs fortification level-up to 4.5% and deteriorated with higher levels.

These results are supported by the findings which showed that supplementation with 3% EPA and DHA increased the weight gain and feed conversion efficiency of juvenile black seabream, *Acanthopagrus schlegeli*. Also, Nevejan et al. [28] showed that dietary supplementation of 20% to 40% lipid emulsion rich in HUFA led to a significant higher growth rate of scallop larvae, *Argopecten purpuratus*. Rezek et al. [29] showed that growth (body length and body weight) was higher in larvae of black seabass, Centropristis striata, fed preys enriched with 10% DHA against 0%. Also, clownfish, *Amphiprion ocellaris*, fed with a live prey enriched with DHA had better growth performance compared to control group (non-enriched live prey) [30].

Recently, Campoverde and Estevez [31] demonstrated that *A. regius* larvae required 12-15% HUFAs in live prey enrichment for optimum larval growth. Also, Carvalho et al. [32] recommended

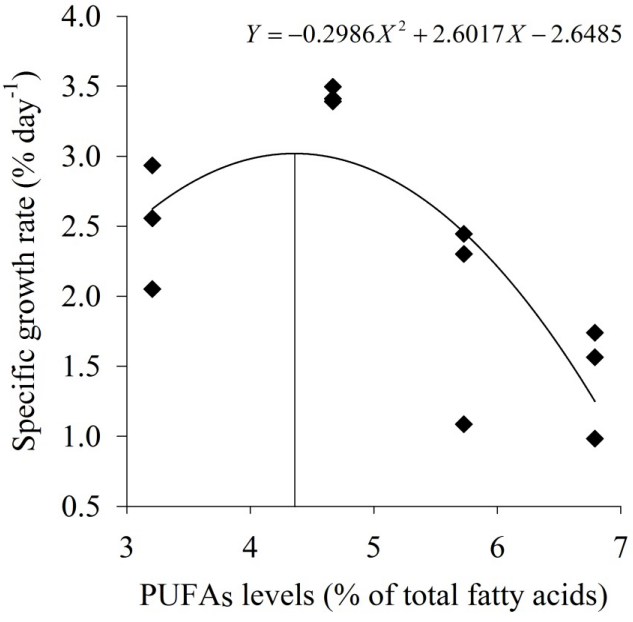

Figure 1: Polynomial regression of specific growth rate (% day-1) and dietary PUFAs levels of meagre, *A. regius* fry.

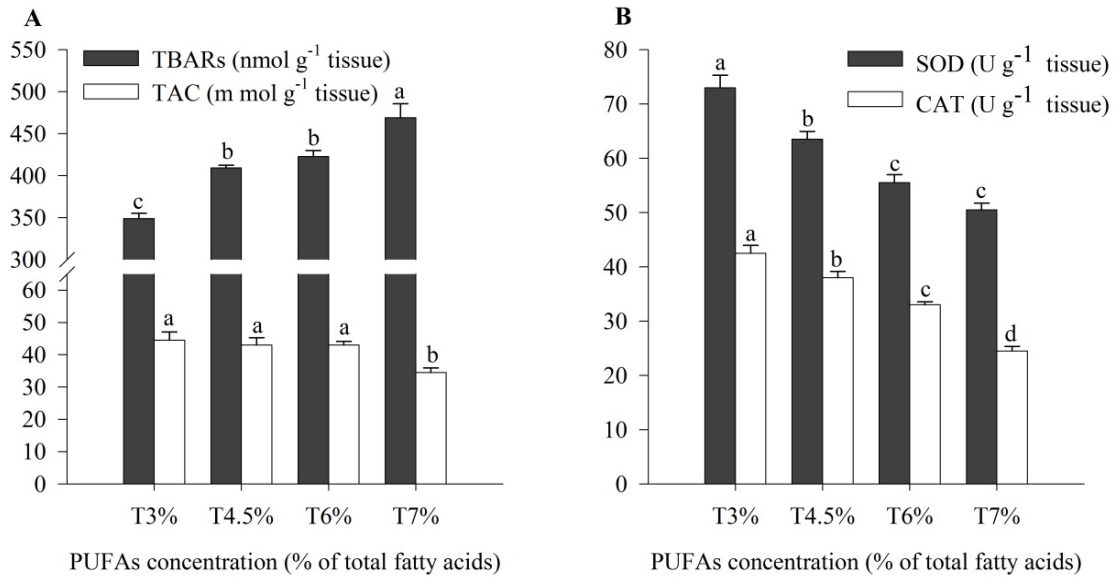

Figure 2: Anti-oxidant status of (A) thiobarbituric acid reactive substance (TBARS) and total antioxidant capacity (TAC), and (B) catalayse (CAT) and super oxide dismutase (SOD) of meagre, *A. regius,* larvae fed diets supplemented with different levels of PUFAs for three weeks. Different letters indicate significant difference between diet groups (p<0.05).

Ingredients (g kg^{-1} diet)	Experimental diets (PUFAs %)			
	$T_{3\%}$	$T_{4.5\%}$	$T_{6\%}$	$T_{7\%}$
Defatted Squid meal	62	62	62	62
Defatted Shrimp meal	3	3	3	3
Defatted Soybean meal	5	5	5	5
Herring oil	16	13	10	7
Gelatin	5	5	5	5
Soy lecithin	4.4	4.4	4.4	4.4
Coline cholirde	0.5	0.5	0.5	0.5
Dicalcium phosphate	0.1	0.1	0.1	0.1
PUFAs[1]	0	3	6	9
Premix[2]	4	4	4	4
Chemical composition				
Dry matter (%)	90.18	90.11	90.09	90.07
Crude protein (%)	57.97	56.87	58.24	57.82
Crude fat (%)	15.87	16.22	16.39	16.22
Fiber (%)	2.04	2.08	2.07	2.06
Ash (%)	13.02	12.48	12.97	13.27
Nitrogen free extract (%)[3]	11.1	12.35	10.33	10.63
GE (MJ kg^{-1} diet^{-1}) [4]	21.79	21.92	21.93	21.94
P/E ratio (mg CP kJ^{-1} GE g diet^{-1})[5]	117.1	116.23	116.13	116.04

[1]OMEGA-3: contain EPA: 416.67 mg ml^{-1} and DHA: 162.67, Puritant's pride, USA.
[2]Vitamin and mineral Premix Composition:- Each 3 kg contains : Vit A (12, 000000 IU.), Vit D (900000 IU), Vit E (10000 mg,) Vit K3 (1000 mg,) Vit B1 (1000 mg), Vit B2 (4000 mg), Vit B6 (1000 mg), Vit B12 (10 mg), Choline chloride (25000 mg), Folic acid (1000 mg), Biotin (25 mg), Pantothenic acid (8000 mg), nicotinic acid (20000 mg), Magnesium sulphate (20000 mg), Copper sulphate (5000 mg), Iron sulphate (250000 mg), Zinc sulphate (4000 mg), Cobalt sulphate (100 mg), manganese (40000 mg), iodine (500 mg), selenium (100 mg), Carrier calcium carbonate up to 3000 mg – produced by AGRE -VET for manufacturing vitamins and feed additives, Egypt.
[3]NFE: Nitrogen free extract calculated using the following equation: NFE = 100-(Crude protein + ether extract + crude fiber + ash).
[4]GE: Gross energy calculated on the basis of 23.6, 39.4 and 17.2 k joule gross energy g^{-1} protein, ether extract and NFE, respectively **(National Research Council, 1993)**
[5]P/E ratio: Protein energy ratio (mg crude protein kJ^{-1} gross energy) = CP/GE × 1000.

Table 1: Ingredients and proximate composition (%) of the experimental diets.

increasing HUFAs level in *A. regius* larvae diet up to 3% for significant improvement of growth compared to 0.4% HUFAs level. Tocher [33] reported that young meagre requirement of n-3 long chain (LC) PUFA were estimated to be at least 2.0% DM of the diet for best growth and FCR.

The improving of growth performance with increasing HUFAs level up to 4.5% in the present study may be due to the vital role of HUFA in fish metabolism [12] and conferring vitality to larvae whereas, HUFA being important structural components in the phospholipids of cellular membranes [1] and its presence is necessary for normal physiological

Parameters	Experimental diets (PUFAs %)			
	$T_{3\%}$	$T_{4.5\%}$	$T_{6\%}$	$T_{7\%}$
Saturated fatty acids				
C14:0	11.1	10.1	11.1	9.4
C15:0	0.3	0.5	0.6	0.6
C16:0	31.9	29	30.8	27.5
C17:0	0.9	1.1	1.1	1.1
C18:0	7.2	6.7	7	6.3
Monounsaturated fatty acids				
C14:1	1.2	1.3	1.9	1
C15:1	0.4	0.4	0.2	0.2
C16:1	14.3	13.4	11.3	12.7
C18:1ω9c	16.9	15.7	15.7	15.7
C20:1	0.5	0.4	0.6	0.3
Polyunsaturated fatty acids				
C18:2ω6c	0.5	0.49	0.38	0.26
C18:3ω3	0.14	0.11	0.09	0.06
C20:4ω6	0.09	0.08	0.06	0.04
C20:5ω3	1.1	2.23	3.25	4.28
C22:6ω3	1.37	1.76	1.96	2.15
SFA	51.4	47.4	50.6	44.9
UFA	36.51	35.87	35.43	36.69
MUFA	33.3	31.2	29.7	29.9
PUFAs	3.21	4.67	5.73	6.79
PUFAs-ω6	0.6	0.56	0.435	0.31
PUFAs-ω3	2.61	4.1	5.29	6.49
EPA/ARA	1.24	0.79	0.6	0.5
EPA/DHA	0.81	1.26	1.66	1.99

Table 2: Fatty acid composition of the experimental diets in the first experiment (% total fatty acids).

Parameters	PUFAs level (% of total fatty acids)			
	$T_{3\%}$	$T_{4.5\%}$	$T_{6\%}$	$T_{7\%}$
Final weight (g/fish)	0.65 ± 0.02^{b}	0.77 ± 0.01^{a}	0.56 ± 0.03^{c}	0.49 ± 0.03^{c}
Weight Gain (g/fish)	0.26 ± 0.03^{b}	0.39 ± 0.01^{a}	0.19 ± 0.04^{bc}	0.12 ± 0.01^{c}
Average daily gain (g/fish/day)	0.166 ± 0.01^{a}	0.190 ± 0.03^{a}	0.120 ± 0.02^{b}	0.064 ± 0.01^{c}
Survival (%)	66.66 ± 18.55	56.66 ± 14.52	50.00 ± 11.54	53.33 ± 12.01
Feed intake (g/fish)	0.54 ± 0.09^{ab}	0.69 ± 0.05^{a}	0.40 ± 0.003^{b}	0.37 ± 0.004^{b}
Feed conversion ratio (g)	2.14 ± 0.55	1.75 ± 0.17	2.57 ± 1.03	3.23 ± 0.93
Protein efficiency ratio (g)	0.83 ± 0.19	0.92 ± 0.10	0.81 ± 0.24	0.57 ± 0.14

Values superscripted by different alphabets within the same raw are significantly different ($p<0.05$).
[1]Gross energy calculated on the basis of 23.6, 39.4 and 17.2 k joule gross energy/g protein, ether extract and NFE, respectively **(National Research Council, 1993).**

Table 3: Growth and feed utilization of meagre, *A. regius* larvae fed diets supplemented with different levels of PUFAS (%) for three weeks.

functions of cell membrane including permeability and different membrane receptors, carriers and enzyme functions [34].

Moreover, HUFAs administration may improve larval development through the presence of better-structured mitochondria, coenzymes and precursors of bioactive molecules with a central position in the metabolism [1]; Also, LC-PUFA can function as an energy reserve through fatty acid α-oxidation in mitochondria [35].

Furthermore, LC-PUFA plays specific functional roles as important regulators of metabolism either as themselves or their derivatives. Regulation can be extracellular through LC-PUFA derivatives including eicosanoids or intracellular as ligands for transcription factors that control gene expression [36] whereas, Betancor et al. [18] reported a positive relationship between the dietary level of DHA and the expression of the insulin-like growth factor 1 (IGF-1) gene in European seabass (Dicentrarchus labrax). Whereas, the incorporation of DHA in the larval tissue affects intercellular interaction, receptor expression, nutrient transport and signal transduction, all of which affect cell growth [1]. These interactions stimulate the synthesis and action of the IGF-1, that promote cell proliferation and differentiation [37].

The survival (%) in the present study did not differ significantly, however, there are a decreasing trend in survival percent 66% in the control to 50-53% in high PUFA levels. The negative effect of increasing HUFAs level over 4.5% in case of growth performance and survival in the present study could be attributed to the increasing of oxidative stress (Figure 2) as discussed below. In accordance, Betancor et al. reported that increasing PUFAs over dietary requirements for D. labrax markedly reduced larval survival, growth and increased the incidence of muscular lesions. Also, gilthead seabream, Sparus aurata, juveniles have reduced growth at 6.0% HUFA on dry matter basis [38].

The evaluation of whole-body fatty acids composition has an economic importance for the customers and physiological importance. Whereas, fatty acids are the primary constituents of polar and neutral lipids, specifically phospholipids and triacylglycerols, respectively.

Parameters	PUFAS concentration (% of total fatty acids)			
	T3%	T4.5%	T6%	T7%
Saturated fatty acids				
C14:0	7.20 ± 0.20^{b}	7.70 ± 0.10^{b}	8.55 ± 0.25^{a}	7.90 ± 0.10^{ab}
C15:0	3.75 ± 0.05	3.85 ± 0.05	4.65 ± 0.05	4.90 ± 0.50
C16:0	23.85 ± 0.35^{a}	17.75 ± 0.15^{bc}	16.35 ± 0.35^{c}	19.00 ± 1.00^{b}
C17:0	0.75 ± 0.15	0.55 ± 0.05	0.45 ± 0.05	0.42 ± 0.02
C18:0	12.30 ± 0.10	12.40 ± 0.20	12.20 ± 0.50	12.20 ± 0.50
Monounsaturated fatty acids				
C14:1	6.20 ± 0.10	6.80 ± 0.10	7.65 ± 0.15	7.40 ± 0.50
C15:1	6.45 ± 0.15^{a}	4.85 ± 0.15^{c}	5.70 ± 0.00^{b}	5.20 ± 0.10^{c}
C16:1	4.65 ± 0.25^{a}	2.90 ± 0.10^{b}	3.05 ± 0.05^{b}	4.30 ± 0.10^{a}
C18:1ω9c	18.15 ± 0.05^{b}	17.75 ± 0.15^{c}	18.40 ± 0.10^{b}	19.50 ± 0.00^{a}
C20:1	1.60 ± 0.10^{a}	1.55 ± 0.15^{a}	0.70 ± 0.00^{b}	1.60 ± 0.10^{a}
Polyunsaturated fatty acids				
C18:2ω6c	2.70 ± 0.10^{b}	3.75 ± 0.15^{a}	1.60 ± 0.00^{d}	2.25 ± 0.05^{c}
C18:3ω3	0.45 ± 0.05^{c}	8.35 ± 0.15^{a}	8.10 ± 0.00^{a}	4.75 ± 0.15^{b}
C20:4ω6	0.45 ± 0.15	0.35 ± 0.15	0.35 ± 0.05	0.35 ± 0.04
C20:5ω3	2.65 ± 0.05^{c}	3.90 ± 0.10^{a}	3.10 ± 0.10^{b}	1.75 ± 0.05^{d}
C22:6ω3	3.90 ± 0.10^{b}	4.90 ± 0.10^{a}	4.60 ± 0.10^{a}	3.40 ± 0.10^{c}
SFA	47.85 ± 0.05^{d}	52.90 ± 0.00^{c}	55.30 ± 0.00^{b}	57.10 ± 0.00^{a}
UFA	47.20 ± 0.10^{a}	47.10 ± 0.00^{a}	44.70 ± 0.00^{b}	42.90 ± 0.00^{c}
MUFAs	37.05 ± 0.15^{a}	35.30 ± 0.00^{b}	35.00 ± 0.00^{c}	33.10 ± 0.00^{d}
PUFAs	10.15 ± 0.05^{b}	11.80 ± 0.00^{a}	9.70 ± 0.00^{d}	9.80 ± 0.00^{c}
PUFAs-ω9	18.15 ± 0.05^{b}	17.90 ± 0.00^{c}	18.50 ± 0.00^{a}	16.50 ± 0.00^{d}
PUFAs-ω6	3.15 ± 0.05^{c}	3.60 ± 0.00^{a}	1.60 ± 0.00^{d}	3.50 ± 0.00^{b}
PUFAs-ω3	7.00 ± 0.10^{b}	8.20 ± 0.00^{a}	8.10 ± 0.00^{a}	6.30 ± 0.00^{c}
EPA/ARA	1.47 ± 0.07	13.80 ± 6.20	10.00 ± 0.00	5.48 ± 0.00
EPA/DHA	0.68 ± 0.03^{b}	0.80 ± 0.00^{a}	0.67 ± 0.00^{b}	0.49 ± 0.00^{c}
Values superscripted by different alphabets within the same raw are significantly different (P< 0.05)				

Table 4: Whole-body fatty acid composition of meagre, *A. regius*, larvae fed the experimental diets with graded levels of PUFAS (% total fatty acids) for three weeks.

Triacylglerols are depot fats while phospholipids are integral components of bio membranes. The degree of unsaturation of the fatty acids maintains the structural and functional integrity of cell membranes [39]. Phospholipids contain higher quantities of PUFAs, and lower levels of monounsaturated compared to the neutral fraction [40]. This tendency confirmed in the present study and was also observed in *S. aurata* [41].

Similar results were obtained in larvae of Senegal sole, *Solea senegalensis* by Ma et al. [42] who showed that EPA and DHA in muscle, liver and adipocytes in juvenile of *A. schlegeli* were markedly increased and saturated, and monounsaturated fatty acid decreased by diet fortification with EPA and DHA. Also, Villalta [43] showed that saturated FA in tissue was all decrease with the increase of dietary DHA and EPA. The fatty acid composition of fish tissue reflects that of the diet on which they have been reared [44] whereas, fatty acid composition of fish body is dependent on the interaction of diet with endogenous metabolism. Recently, *A. regius* proved that to have active Δ6 desaturases and Elovl5, but their activities being insufficient to produce DHA and EPA from PUFA precursors to sustain fast growth [45].

The DHA was readily deposited in fish tissues by 30% of total lipids in fish fed the highest dietary contents [46]. But, because the elevation of dietary DHA markedly increases peroxidation risks, the higher *D. labrax* mortalities found in fish fed increased DHA levels suggested the proliferation of free radicals derived from this fatty acid and the formation of toxic oxidized compounds. Indeed, increased DHA multiplies two, four and eight times the oxidation potential of the diet in comparison with the same increase in arachidonic, linolenic or linoleic acid, respectively [47]. Also, the higher dietary concentration of a fatty acid in the diet, generally associated with the lower deposition, which implies increased dietary concentrations result in increased oxidation [48].

The antioxidant balance of fish fed different dietary levels of PUFAs in the present study revealed that TBARs levels significantly increased and SOD, CAT and total antioxidant activities significantly decreased with increasing dietary levels of PUFAS. In accordance, the majority of bibliography seems to be in agreement with our data such as [18,49]. They reported that the increasing of dietary PUFAs increased the free radical levels and depleted the antioxidant enzyme systems. The susceptibility of PUFAS to oxidation is linearly proportional to the degree of unsaturation (National Research Council, 2011). In this work the diet supplementation with PUFAs at 3% showed a low degree of oxidative stress in *A. regius* larvae compared with fish fed 7% PUFAs.

Oxidation of PUFAs produces compounds such as fatty acid hydroxyperoxides, fatty acid hydroxides, aldehydes and hydrocarbons, several of them being toxic, binding to proteins amino groups, nucleic acid and phospholipid bases and damaging membrane lipids, proteins and DNA [50]. Thus, lipid peroxidation is highly deleterious in fish, resulting in damage to cellular and sub-cellular membranes [51], structural proteins and different fish tissues and organs [52]. Also, the high dietary DHA levels associated with the presence of ceroid pigment within hepatocytes, which has been also found to be related to an imbalance between anti- and pro-oxidants [53].

Moreover, the increase of oxidative stress with increasing

dietary PUFAS may also due to the stimulation of DHA-containing phospholipids for very active Na+K+ ATPase [54] and Ca^{2+} ATPase in muscle [55]. This increased metabolism probably results in a greater consumption of oxygen, being these phospholipids important substrates for damage by the free radicals [56].

In addition, it is possible that larval marine fish may not have sufficiently well-developed antioxidant capability, as many of their biological and physiological systems are poorly developed [57]. Thus, a too high elevation of dietary PUFAS, may easily cause an oxidative imbalance in this very young fish.

Conclusion

The increasing of PUFAs supplementation level over 3% improved growth performance and FCR to reach the peak at level of 4.5% PUFAs, then the growth decline with higher PUFAs levels. Thus, the requirement of *A. regius* PUFAs is 4.5% (4.33% according to polynomial regression according to SGR) which is higher than the level recorded by [58]. Moreover, increasing dietary supplementation of PUFAs subsequently increase oxidative stress in marine larvae which necessitate antioxidant supplementation in larval diets.

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