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Effect of weaning time on growth, body composition, fatty acids and digestive enzyme activities of European seabass (*Dicentrarchus labrax*) larvae

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ABSTRACT

The present study addressed to investigate early, 25 days post hatching (dph) and late co-feeding time; 35 dph on European seabass (*Dicentrarchus labrax*) larvae. Initially, larvae were reared from the 5thdph in six glass aquaria, and each aquarium contained 200 larvae.The results revealed higher, but no significant ($P > 0.05$) effect on larval final weight, weight gain, average daily gain and specific growth rate when larvae were co-feed at 35 dph. However, the highest survival rate was noticed then larvae were co-fed from 25 dph. Growth parameters showed significant ($P < 0.05$) effect on final gainsof both total and standard lengthswhen larvae were started co-feeding at 25 dph. The water quality showed no significant ($P > 0.05$) differences between treatments. However, significant ($P < 0.05$) effect was revealed on total culturable bacteria - and bacilli counts in water samples at 45 dph,when larvae were co-fed at 25 dph, but no significant ($P > 0.05$) effect was noticed on *Staphylococcus* counts at 45dph of larvae co-fed from 35 dph. Digestive enzymes analysis revealed that lipase activity was not significantly ($P > 0.05$) affected, however, a significant ($P < 0.05$) increase in amylase activity was revealed when larvae co-fed at 35 dph.

INTRODUCTION

Egypt marine aquaculture is still not well developed due to lack of marine hatcheries and larval aquafeeds. According to FAO (2018), European seabass (*Dicentrarchus labrax*) is one of the most valuable cultured species in Mediterranean countries; however, the specie is not well developed in Egypt (Salem 2014). Egypt marine aquaculture has several challenges before it is a sustainable industry; lack of fry, and fingerlings with good quality are the bottlenecks. In 2016, Egypt had only ninemarine hatcheries (three governmental and six in private sector) (GAFRD 2018). As a consequence, Egyptian marine aquaculture is still depending on fry collected from natural environments. The total collection of wild fry in Egypt in 2016 was 51 million, but only 0.900 million fry were seabream and seabass. Total marine fry

production in Egypt hatcheries in 2016 was 44.224 million, but only 5.686 million were European seabass fry (GAFRD 2018).

Good fry quality is essential for the development of marine fish culture (Saleh *et al*. 2014; Campoverde *et al*. 2017), and the success of high fry production is affected by nutritional quality of the larval diets (e.g. Izquierdo *et al*. 2000; Kolkovski *et al*. 2009; Saleh *et al*. 2014; Campoverde *et al*. 2017). The initial feeding time is an important factor of early weaning success of marine fish larvae and has great effects on growth performance, physiology and nutrition (Curnow *et al*. 2006; Süzer *et al*. 2011; Hebalah 2015; Saleh *et al*. 2014), live foods accounts of 79% of the production costs for seabass larvae up to 45 day post hatching (dph) (Süzer *et al*. 2007). It is generally accepted that the main challenge in fish larval nutrition is to formulate an effective compound diet able to substitute live preys as early as possible during early rearing (Watanabe and Kiron, 1994; Süzer *et al*. 2011; Hebalah 2015). Inert diets must be attractive, adequate to fit larval mouth size and must fulfill the nutritional requirements of the larvae (Kolkovski *et al*. 2009). An overlapping co-feeding period during early feeding which live food is gradually replaced by increasing quantities of formulated microdiets has shown to improve growth and survival of marine fish larvae compared to larvae only fed live food (Curnow *et al*. 2006). A recent study, revealed positive effects of Inve *O. Range* and fish oil based microdiets, and co-feeding time of seabass larvae on growth performance, physiologyand nutrition (Hebalah 2015).

Therefore, the present study, addressed to compare growth performance, water quality, culturable bacterial load in the rearing water, as fish are in an intimate contact with bacteria in their environment, body composition, fatty acids, and digestive lipase - and amylase activities of early and late microdiet co-fed European seabass larvae.

MATERIAL AND METHODS

Fish and culture facilities

Newly hatched European seabass larvae; the 5th dph wereobtained from induced spawning of broodstock maintained under controlled conditions at Marine Fish Hatchery, (El-Anfoshy), National Institute of Oceanography and Fisheries (NIOF), Alexandria, Egypt. Triplicate groups of 200 larvae, 5 dph, were stocked in 140-L glass aquaria (with 60 L water). The aquaria was supplied with filtered marine water stored in a 2 m^3 poly phenyl ethylene tank, and waterwas re-filtered using 55-60 µ plankton net before daily partial water exchange of about 30% using siphon hose to a plastic white jar covered partly with 55-60 µ plankton net. This procedure was done to avoid larval escape and disturbance. Larvae were reared in green water usingalgae (*Nanochlorapsis salina*) at density not less than 300.000 cell per ml throughout the experiment. Aeration of the experimental aquaria were carried out using 0.5 horse power electronic air blower connected with an air stone. Light was supplied by fluorescent tubes, with a power of 50-100 lux at water surface. Photoperiods were: from the 5th dph to the 15th dph; 8 hours light: 16 hours darkness, from the 16th dph to the 25th dph; 12 hours light: 12 hours darkness and from the 26th dph to the experiment end, the 45th dph; 16 hours light: 8 hours darkness according to Hebalah (2015).

Microdiet and larval daily feeding regime

An experimental microdiet (SPAROS Lda, Olhão, Portugal) of 100 – 200µ was used in the present study. Ingredients and the chemical compositions of the microdiet

are shown in Table 1. The larval feeding regime from the 5th dph to the 45th dph was carried out as showed in Fig. 1 according to Süzer *et al*. (2011) and Salem *et al*. (2015). Rotifers, *Brachionus plicatilis* and *Brachionu sroutindiformis* were enriched with DHA SELCO® for 4 hours*;* starting with 20 rotifers per ml from the 5th dph. Every day, after daily water exchange the numbers of rotifers were counted, and the numbers were constant at 20 per ml until the 14th dph. At the beginning of cofeeding on *Artemia franciscana* (GSL) naupliienriched with DHA SELCO® starting with 1-2 nauplii, the number of rotifers; 15 rotifers per ml at the 18thdph was reduced to 10 rotifers per ml at the 20th dph.

Table 1: Composition of SPAROS microdiet, calculated and dietary proximate analysis.

Ingredients	**Sparos Co. Proximate analysis**	
	Nutrient	**Measured values**
Plant protein concentrates	Protein (% DM)	69.9
Fish protein hydrolisate	Fat (% feed)	16.5
Fish gelatin	Energy (kJ / g DM)	23.7
Fish oil	Ash (% DM)	6.8
Copepod Oil	Dry matter (% feed)	93.5
DHA-rich oil		
Vitamin premix		
Mineral premix		
Autolysed yeast		
Monocalcium phosphate		
L-Lysine		
Taurine		
Estimated values according to Sparos Co. based on known ingredient composition		
Fatty acids and nutrients	**Estimated values**	**Unit**
20:4 (n-6)	0.1	% DM
20:5 (n-3)	1.0	% DM
22:6 n-3	2.8	% DM
DHA/EPA	2.8	-
EPA/ARA	13.0	-
n-3/n-6	11.0	-
Unsat./Sat.ratio	0.3	-
Total PL	1.9	% DM
Vitamin A	60000	IU Kg^{-1}
Vitamin B1 (Thiamin)	90	mg Kg^{-1}
Vitamin B2 (Riboflavin)	90	mg Kg^{-1}
Vitamin B3	300	mg Kg^{-1}
Vitamin B5	600	mg Kg^{-1}
Vitamin B6 (Piroxin HCL)	60	mg Kg^{-1}
Vitamin B9 (Folic acid)	45	mg Kg^{-1}
Vitamin B12 (Cianocobalamin)	300	mg Kg^{-1}
Vitamin C	2800	mg Kg^{-1}
Vitamin D3 (Colecalciferol)	6000	IU Kg^{-1}
Vitamin E (α Tocoferol)	530	mg Kg^{-1}
Vitamin H (Biotine)	9000	mg Kg^{-1}
Vitamin K3 (Menadione)	75	mg Kg^{-1}
Total P	0.8	% DM
Cu	27	mg Kg^{-1}
Fe	18.0	mg Kg^{-1}
I	1.50	mg Kg^{-1}
Mn	28.8	mg Kg^{-1}
Se	0.030	mg Kg^{-1}
Zn	22.5	mg Kg^{-1}

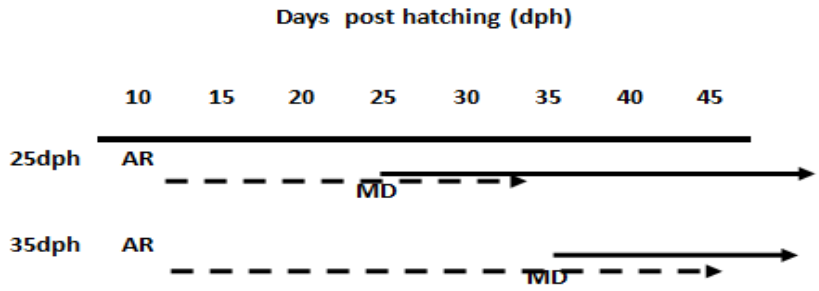

Fig. 1: Early and late weaning of European seabass (*D. labrax*) larvae fed *Artemia* (AR) and microdiet (MD).

From the 20th dph, adding of rotifers was stopped and two *Artemia* nauplii per ml was supplemented until the 25th dph. For the early cofeeding group, 25th dph larvae were co-fed on *Artemia* metanauplii and the microdietfive times daily. From the 26th dph, larvae were co-fed on four DHA SELCO® enriched *Artemia* metanauplii per ml and the microdiet; 15 gram per m^3, until the 28th dph.There after, larvae co-fed on *Artemia* metanauplii enriched with DHA SELCO® with 3 *Artemia* per ml and the microdiet with 30 gram per m^3 until the 31th dph. Thereafter, larvae co-fed on DHA SELCO® enriched *Artemia* metanauplii with 2 *Artemia* per ml and the microdiet; 30 gram/m^3 until the 35thdph. Supplementation of *Artemia* metanauplii was stopped at the 35th dph, and from then; larvae were only fed on the microdiet with 45 gram per m^{3}until the end of the experiment, the 45th dph. For the late cofeeding, from the 35th dph larvae daily co-fed on DHA SELCO® enriched *Artemia* metanauplii with 4 *Artemia* per ml and the microdiet; 15 gram per m^3 in 5 times until the 37th dph. Larvae co-fed on DHA SELCO® enriched *Artemia* metanauplii with 3 *Artemia* per ml and the microdiet with 30 gram per m^3 until the 40th dph, adding *Artemia* metanauplii stopped and larvae fed only on the microdiet with 45 gram per m^3 in 5 times until the end of the experiment; the 45th dph.

Growth performance

Growth rates and feed efficiency of larvae were calculated as follows:

- ✓ Total weight gain (WG g/larvae) = Wf – Wi, where Wi and Wf are initial and final weights (g).
- ✓ Weight average daily gain (WADG mg/day) = Wf - Wi/t, where t is the time of experiment (days).
- ✓ Specific growth rate (%SGR) = 100 (lnWf – lnWi)/t.
- ✓ Feed conversion ratio (FCR) = dry feed intake (g)/larvae live weight gain (g).
- ✓ Protein efficiency ratio (PER) = weigh gain (g)/crude protein intake (g) on dry weight basis.
- ✓ Protein productive value (PPV) = 100 (protein gain (g))/protein fed (g) on dry weight basis.
- ✓ Energy utilization (gross energy) (GE in Kcal) = 100 (final fish body energy (Kcal) – initial larvae body energy (Kcal))/ energy intake (Kcal).

Length of larvae were measured as total length (TL) and standard length (SL) to nearest 0.1 mm using binocular light microscope, Wild Heerbrugg, Switzerland, and the larval length were calculated as follows:

- ✓ Length gain (LG in mm) = Lf – Li.
- ✓ Length average daily gain (LADG in mm d^{-1}) = Lf – Li/t.

- ✓ Length specific growth rate % (LSGR % d^{-1}) = (Lin Lf – Lin Li) 100/t.
- ✓ Length gain % (LG %) = LG/ Li x 100, where Li and Lf are initial and final lengths(mm) and t is the time of experiment (days).

Water quality

Water quality was measured each week at 2 pm using a Hanna® HI9828 Water quality portable electric device.The electric device was gently putted at the bottom of the experimental aquaria away from the airstone and water quality measured; dissolved oxygen (DO; mg/L), pH and salinity as part per thousand (ppt), conductivity, and total dissolved solids (TDS).

Culturable bacterial measurements of the rearing water

Colony forming units (CFU) per ml were measured in water samples from the water source, and the aquaria water; total bacterial count (TBC), *Aeromonas* sp. (ABC), *Vibrio* sp. (VBC), *Staphylococcus* sp. (SBC) and *Bacillus* sp. (BBC). Bacterial cultivation were carried out at the Microbiology Laboratory, Marine Environment Division, NIOF. Serial dilutions of $10^{-2}\pm 10^{-4}$ were made using filtered sterilized sea water. For each water sample; 100 µl was inoculatedon sterile plates and incubated at 30^{o}C for 24 - 72 h. Plates of the five selective media (sea water agar for TBC and BBC, bile salts irgasan brilliant green agar (BSB) for ABC, thiosulfate citrate bile salt sucrose agar (TCBS) for VBC, and mannitol salt agar for SBC) were inoculated with 0.1 ml of the diluted samples, and the different bacterial generawere counted as described elsewhere (Salem *et al*. 2015).

Feeds, nutritional utilizations methods and parameters

Proximate analysis of diet was measured using randomly samples according to the procedures of AOAC (2000) for determination of dry matter (DM%) using a drying oven at 55-60ºC overnight. Ash was determined using ash oven at 650ºC for 6 hours.Crude protein (CP) was measured using the Kjeldahl method, ether extract (EE) by the Soxhlet method, crude fiber (CF) by the Soxhlet method and calculated nitrogen free extract (NFE). Evaluation of proximate analysis were carried out on pooled samples of 40 larvae for DM and ash, but for CP and EE analysis, calorimetric kites using Spectrophotometer according to Biuret method for protein (Gornall *et al*.1949) and an adaptation of the sulphophosphovanilin method for lipid as described by Knight *et al*.(1972) were used. Proximate analysis (% dry weight) of microdiet, and fatty acids composition (%) of total dietary lipids are shown in Table 2.

Fatty acids analysis of larval samples

Pooled samples of six larvae were randomly collected from all treatments and immediately frozen at -20°C until further use. Total lipid contents were analysed according to Bligh and Dyer (1959). Fatty acid methyl esters were analysed using gas liquid chromatography (HP-6890 gas–liquid chromatography) as described by Radwan (1978).

Amylase and lipase analysis

Pooled samples of six European seabass larvae at 45th dph after co-feeding on SPAROS microdiet from day 25th and 35th dph (15-20 larvae per aquarium, depending on age and size) were collected and weighted for enzyme analysis according to Süzer *et al*. (2007). Whole body homogenates were used for enzymatic assay, and the samples were homogenized in five volumes (v/w) of ice-cold distilled

water and reserved in -20ºC freezer until extracted and enzymes measured. Extracts for enzyme assay were obtained after homogenization of larvae (35 mg/ml) in cold 50 mM Tris-HCl buffer, pH 8.0 using the electric homogenizer followed by centrifugation using a centrifuge cooler at 13.500 x *g* for 30 min at 4°C. Separated supernatantswere collected and reserved in -20ºC freezer until measuring amylase and lipase using QCA®, Spain kites using spectrophotometer according to the manufactures instructions.

Table 2: Proximate analysis (% dry weight) of microdiet, and fatty acids composition (%) of total dietary lipids. All values are mean ± S. D.

Proximate analysis (%)	**Mean**	**±**	**S. D**
Mostire	13.10	±	0.42
Dry matter	86.90	±	0.42
Crude protein	57.28	±	0.59
Ether extract	16.50	±	0.26
Ash	0.37	±	0.02
Crude fiber	10.10	±	0.66
Nitrogen free extract	2.66	±	0.60
Dietry fatty acid:			
C14:0	4.32	±	1.12
C15:0	0.39	±	0.55
C16:0	17.75	±	1.23
C17:0	0.22	±	0.07
C18:0	3.95	±	0.62
C20:0	1.92	±	1.53
TSFA	28.56	±	0.97
C15:1	0.38	±	0.05
C16:1	6.79	±	0.75
C20:1	0.15	±	0.03
C22:1	2.20	±	1.79
TMUSFA	22.18	±	4.38
C18:2c	13.83	±	1.12
C20:4	0.49	±	0.69
C22:2	2.57	±	0.19
T (n-6)	16.89	±	2.00
C18:3	0.11	±	0.00
C20:3	3.02	±	0.96
C20:5	9.93	±	0.87
C22:6	19.31	±	7.44
T (n-3)	32.37	±	7.34

Nitrogen free extract (%) = 100 – (CP + EE + CF + Ash). TSFA: Total saturated fatty acids (%), TMUSFA: Total monounsaturated fatty acids (%), T (n-3): Total omega 3 unsaturated fatty acids (%), T (n-6): Total omega 6 unsaturated fatty acids (%).

Statistical analysis

All data were also subjected to a one-way analysis of variance (ANOVA) at a 95% confidence limit, means compared by Duncan's test ($P < 0.05$) (Duncan1955) using a SPSS software (SPSS for Windows 16; SPSS Inc., Chicago, IL, USA).

RESULTS AND DISCUSSION

The effect of microdiets have been investigated by Curnow *et al*. (2006) on barramundi (*Lates calcarifer* Bloch) larvae, Saleh *et al*. (2014) on gilthead sea bream (*S. aurata*) larvae, and in European seabass larval studies (Hebalah 2015; the present study).

Results of the present study showed that starting co-feeding at 25th dph and 35th dph of the SPAROS microdiet significantly ($P < 0.05$) affected some of water quality parameters (Table 3). Larvae co-fed with SPAROS microdiet at 35th dph showed significant ($P < 0.05$) improvement in DO% and DO mg/l at 29th and 37th dph of the rearing water. The pH values at 37th and 45th dph revealed significant ($P < 0.05$) effects when larvae were co-fed on SPAROS microdiets at 35th dph. The salinity (mg/l) and conductivity (mS/cm)at 13dph larval aquaria showed that significant ($P < 0.05$) differences were revealed by SPAROS microdiets at 35th dph. TDS (ppm) at 7dph showed that significant ($P < 0.05$) different results were achieved by SPAROS microdiets treatments at 35th dph co-feeding start time. The co-feeding start time is an important factor of early weaned marine fish larvae success, and has significant effects on larvae growth, physiology and nutrition (Süzer *et al*. 2007; Hebalah 2015; Saleh *et al*. 2014; Campoverde *et al*. 2017).

Table 3: Effect of microdiet and cofeeding time on rearing water of European seabass (*D. labrax*) larvae. All values are mean ± S.D.

Treatment	7 dph		29 dph		37 dph		45 dph	
	25 dph	**35 dph**	**25 dph**	**35 dph**	**25 dph**	**35 dph**	**25 dph**	**35 dph**
DO	81.00	82.33	84.43^{b}	85.90^{a}	79.33^{b}	80.87^{a}	79.93	81.00
	±1.00	±0.58	± 0.60	± 0.96	±0.76	±0.23	±0.12	±1.00
DO mg/L	6.50	6.48	6.66^{b}	6.83^{a}	5.97^{b}	6.11^{a}	6.02	6.14
	±0	±0.03	±0.04	±0.08	±0.04	±0.05	±0.05	±0.17
pH	8.33^{b}	8.40^{a}	8.46	8.49	8.15^{b}	8.39^{a}	8.09^{b}	8.29^{a}
	±0.03	±0.04	±0.18	±0.21	±0.05	±0.02	±0.05	±0.02
Sppt mg/L	34.82	35.49	36.41	36.48	36.53	36.88	36.77	36.92
	±0.13	±0.01	±0.75	±0.29	± 0.42	± 0.29	± 0.09	± 0.10
Cond	51.88	52.63	54.80	54.93	55.40	55.46	50.91	56.47
	±0.94	±0.24	±1.05	±0.40	± 0.65	± 0.39	± 7.71	± 0.09
TDS	25.38^{b}	25.75^{a}	27.47	27.46	27.72	27.72	27.65	27.55
	±0.16	±0.21	±0.53	±0.20	± 0.30	± 0.19	± 0.05	± 0.06
Temp	18.82	18.46	17.62	17.27	17.76	17.45	19.76	19.59
	±0.32	±0.23	±0.52	±0.44	± 0.32	± 0.45	± 0.53	± 0.69

Values in the same row within the subtales with different letters are significantly different at $P = 0.05$.
DO: Dissolved oxygen (%), Do mg/L: Dissolved Oxygen in mg/L, pH: Aciditiy and alkalinity parameter, Sppt: Salinity in part per thousand, Cond: conductivity in Ms/cm, TDS: Total dissolved solids in part per million; Temp: Temperature in ºC.

As fish are in continuous contact with a wide variety of microorganisms; evaluations of bacteria genera in the rearing water is of importance to investigate with regard to the presence of non-pathogenic and pathogenic bacteria (e.g. Farzanfar 2006; Yeong 2008; Das *et al*. 2017). The effect of co-feeding time of the SPAROS microdiet significantly ($P < 0.05$) decreased CFU/ml of the rearing water (Table 4). Culturable *Aeromonas* sp. was not detected in water samples and *Vibrio* sp. was only detected at 25th dph, while *Staphylococcus* sp. was detected in the water source, and in the larval rearing water at 45th dph. Statistical significance ($P<0.05$) of *Bacillus* sp. counts were observed in water samples at 45th dph,when larvae were co-fed on SPAROS microdietfrom 25th dph. These results are different to that revealed by

Hebalah (2015) showing that microdiets and co-feeding start time significantly ($p > 0.05$) affected culturable counts of; total, *Aeromonas* -, *Staphylococcus* -, *Vibrio*- and *Bacillus* sp. in rearing water of European seabass larvae.

Table 4: Effects of microdiet and cofeeding time on culturable bacterial counts of rearing water of early weaned European seabass larvae. All bacterial counts are expressed as CFU/ml (mean) ± S.D.

Treatment	dph	Water source	Co-feeding start time	
			25 dph	35 dph
TBC	25dph	166.67 ± 35.12	34.67 ± 5.69	34.67 ± 5.69
	35dph		123.33 ± 40.41	20.33 ± 6.11
	45dph		28.33[a] ± 4.16	10.00[b] ± 2.00
BBC	25dph	20.33 ± 5.13	4.67 ± 0.58	4.67 ± 0.58
	35dph		15.33 ± 3.21	5.00
	45dph		12.67[a] ± 2.08	0.00[b]
SBC	45dph	13.00 ± 4.00	14.00 ± 3.00	19.33 ± 7.09
VBC	25dph	0.00	5.33 ± 1.53	5.33 ± 1.53

Values in the same row with different letters are significantly different at $P = 0.05$.
CFU: Colony forming unit, TBC: Total bacterial count, BBC: Count of *Bacillus* sp., SBC: Count of *Staphylococcus* sp., VBC: Count of *Vibrio* sp. *Aeromonas* sp. was not detected in water samples.

Süzer *et al*. (2007) reported that early weaning strongly affected growth and survival rate, in contrast to the present study where no statistical significant variations were observed in weight growth and survival rate when larvae were early or lately started co-fed (Table 5). However, growth rates of total and standard length; final in mm, gain in mm, average daily gain in mm/day, specific growth rate in %/day and gain% were significantly ($P < 0.05$) affected when larvae were started co-feed on SPAROS microdiet at 25th dph (Table 5). Growth and development in larval groups weaned at 15th dph were inferior to the other groups, and our results are in accordance to the results reported by Cahuand Zambonino Infante (1994) and Person Le Ruyet *et al*. (1993).

Curnow *et al*. (2006) used three weaning protocols and two commercial microparticulate diets, Gemma and Proton, in a study withbarramundi larvae. The Gemma diet improved growth and survival rate, and reduced cannibalism. In a later study, Hebalah (2015) used INVE Orange and fish meal based microdiets in a European seabasslarval study, where co-feeding started at 25th dph and 35th dph. The highest growth rate was revealed when larvae started co-fed at 35dph with a FO microdiet, but no significant difference was noticed.Furthermore, no significant ($P > 0.05$) difference in survival rate was revealed. The larval length showed that significant ($P < 0.05$) effects were achieved when larvae started co-fed on Orange microdiet at 35th dph. In the present study, larval feed utilization; FCR, PER and PPV were significantly ($P < 0.05$) affected when SPAROS was co-fed at 35th dph, but GE was not significantly ($P > 0.05$) affected (Table 5).

Table 5: Effect of microdietcofeeding time onlength, wet weight growth performance and feed efficiency of European seabasslarvae. All values are mean ± S.D.

Growth parameters	**Co-feeding start time**			
	25 dph		**35 dph**	
Initial weight (mg)	3.40	± 0.00	3.40	± 0.00
Final weight (mg)	79.67	± 0.58	80.33	± 1.53
Weight gain (mg)	76.27	± 0.58	76.93	± 1.53
Average daily gain (mg/d)	1.69	± 0.01	1.71	± 0.03
Specific growth rate (%/d)	9.63	± 0.02	9.65	± 0.04
Survival rate (%)	60.83	± 3.82	56.67	± 0.58
Initial total length (mm)	4.4	± 0.25	4.4	± 0.24
Final total length (mm)	16.02[a]	± 2.39	13.78[b]	± 1.57
Final standard length (mm)	13.60[a]	± 1.78	11.91[b]	± 1.20
Total length gain (mm)	11.62[a]	± 2.30	9.38[b]	± 1.49
Standard length gain (mm)	9.20[a]	± 1.71	7.51[b]	± 1.16
Total length average daily gain (mm/d)	0.29[a]	± 0.06	0.23[b]	± 0.04
Standard length average daily gain (mm/d)	0.23[a]	± 0.04	0.19[b]	± 0.03
Total length specific growth rate (%/d)	2.64[a]	± 0.22	2.42[b]	± 0.17
Standard length specific growth rate (% /d)	2.39[a]	± 0.20	2.18[b]	± 0.17
Total length gain Percentage (%)	264.10[a]	± 51.93	213.23[b]	± 31.84
Standard length gain Percentage (mm)	209.23[a]	± 39.49	171.06[b]	± 26.43
Feed conversion ratio	0.64[b]	± 0.00	0.30[a]	± 0.01
Protein efficiency ratio (gm/gm)	2.74[b]	± 0.03	5.74[a]	± 0.13
Protein productive value (%)	26.45[b]	± 5.29	53.38[a]	± 24.56
Gross energy (Kcal)	2926.41	± 70.42	3328.93	± 106.82

Values in the same row with different letters are significantly different at $P = 0.05$.

The results of the present study are in accordance to the significant ($P < 0.05$) improvement in FCR was achieved in larvae started co-fed on FO and Orange microdiets at 35th dph (Hebalah, 2015). Larvae started co-fed on FO microdiet at 35th dph showed significant ($P < 0.05$) improvement in PER and larvae started co-fed on Orange microdiet at 35th dph revealed significant ($P < 0.05$) increase in PPV, but when larvae were started co-fed on Orange microdiet at 35th dph, no significant ($P>0.05$) in GE was noticed (Hebalah 2015). The larval body compositions; MO, DM, CP, EE, Ash and most fatty acids composition were not significantly ($P > 0.05$) affectedby early or late co-feeding while C15:0, DHA C22:6 and total omega 3 unsaturated fatty acids were significantly ($P < 0.05$) affected by late than early co-feeding (Table 6).

These non-significant differences by co-feeding on SPAROS microdiet at 25th dph and 35th dph revealed in the present study are in accordance with the results of Hebalah (2015) feeding Orange and FO microdiets to seabass larvae co-fed at 25th dph and 35th dph. Activities of different digestive enzymes have been reported in numerous finfish studies (e.g. Bakke *et al*. 2011; Hoseinifar *et al*. 2017). Lipase and amylase activities in European seabass larvae at 45th dph after co-feeding on SPAROS microdiet from 25th and 35th dph. Lipase activity was 39.60 ± 22.00 and 13.20 ± 0.00 U/L for 45th dph larvae started co-feeding on microdiets from day 25 and 35 post hatching respectively and was not significantly ($P>0.05$) affected. However, a significant ($P < 0.05$) increase in amylase activity was revealed in larvae startedco-feeding on SPAROS microdiet at 35dph and the activity was 326.00 ± 78.00 and 588.00 ± 312.00U/L for 45th dph larvae started co-feeding on microdiets from day 25 and 35 post hatching respectively. Hebalah (2015) revealed significant ($P < 0.05$) lipase activity when larvae were co-fed on a FO microdiet at 35th dph, while significant ($P < 0.05$) improved amylase activity was achieved when larvae were started co-fed on Orange microdiet at 25th dph.

Table 6. Effect of microdiet and cofeeding time onproximate composition (%) and total fatty acids contents (%) of European seabasslarvae. All values are mean ± S.D.

Composition (%)	**Co-feeding start time**					
	25 dph			**35 dph**		
Moisture	78.36	±	3.38	78.14	±	3.21
Dry matter	21.64	±	3.38	21.86	±	3.21
Crude protein	54.39	±	1.42	54.20	±	3.35
Ether extract	21.11	±	7.45	25.36	±	11.22
Ash	9.91	±	2.58	9.91	±	2.58
Body fatty acid profile:						
C13:0	2.14	±	1.15	1.59	±	0.52
C14:0	8.55	±	1.03	7.71	±	0.86
C15:0	5.20[b]	±	0.03	5.59[a]	±	0.12
C16:0	48.16	±	0.91	46.14	±	1.38
C17:0	1.64	±	0.71	2.55	±	0.00
C18:0	24.28	±	1.44	24.21	±	1.05
C23:0	0.00	±	0.00	0.02	±	0.02
TSFA	89.97	±	1.97	87.79	±	0.93
C14:1	1.32	±	0.34	0.81	±	0.03
C15:1	3.37	±	0.08	3.47	±	0.03
C16:1	1.53	±	0.18	1.67	±	0.10
C17:1	0.15	±	0.15	0.00	±	0.00
C18:1	0.00	±	0.00	0.72	±	0.72
TMUSFA	6.36	±	0.29	6.67	±	0.62
C18:2	2.36	±	1.16	2.23	±	1.45
C20:4	0.54	±	0.54	1.05	±	0.09
T (n-6)	2.90	±	1.70	3.28	±	1.36
C18:3	0.42	±	0.31	0.62	±	0.07
C22:6	0.34[b]	±	0.34	1.61[a]	±	0.14
T (n-3)	0.76[b]	±	0.02	2.23[a]	±	0.21

Values in the same row with different letters are significantly different at $P = 0.05$.
TSFA: Total saturated fatty acids (%), TMUSFA: Total monounsaturated fatty acids (%), T (n-3): Total omega 3 unsaturated fatty acids (%), T (n-6): Total omega 6 unsaturated fatty acids (%).

CONCLUSIONS AND FURTHER INVESTIGATIONS

The present study showed that European seabass larvae at 45 dph after co-feeding on SPAROS microdiet from day 25 and 35 post hatching; larval length was significantly ($P < 0.05$) affected when larvae were started co-feed on SPAROS microdiet at 25th dph. However, no statistical significant variations were observed in larval weight and survival rate. The larval body and fatty acids composition was not significantly ($P > 0.05$) affected when larvae were early or late co-fed. A significant ($P < 0.05$) increase in amylase activity was revealed in larvae starting co-feeding on SPAROS microdiet at 35th dph, but lipase activity was not significantly ($P > 0.05$) affected.

In future studies of European seabass larvae, early co-feeding of microdiets from day 25 post hatching in combination with supplement of probiotics to microdiets merits investigations. Probiotics used in aquaculture produce a wide range of digestive enzymes (e.g. amylase, protease and lipase) (El-Haroun *et al*. 2006; Ray *et al*. 2012; Das *et al*. 2017), and their supplements have positive effect on digestive enzyme activities, even though contradictory effects are reported (Hoseinifar *et al*. 2017). In addition, probiotics revealed positive health effects (New-Fyzul *et al*. 2014; Ibrahem 2015; Dawood and Koshio, 2016; Banerjee and Ray, 2017).

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