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Effect of Micro Particulate Diets and Different Weaning Periods on Survival and Growth of European Sea Bass (*Dicentrarchus labrax)* Larvae

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ABSTRACT

In the present study ***Dicentrarchus labrax*** larvae were weaned through two microparticulate diets; laboratory prepared diet 51.5% protein content (Micro diet; MD) and tested pelleted diet obtained from INVE® Company 56% protein (Commercial diet; CD) at three weaning periods (40, 50 and 55-day post-hatch (dph)) in 2 × 3 factorial experiment. Each treatment was replicated in three tanks. Eighteen conical fiberglass tanks each of 100 liters seawater capacity with density 15 larvae /L and initial weight of average 27.6 mg were stocked. The rearing water of the experiment was designed as a recirculating aquaculture system in a salinity of 37 ppm. The larvae were fed 6 times a day within the time of 8:00 a.m. till 6:00 p.m., at a rate: one meal every two hours. The experiment lasted 84 days. From the outcomes achieved in the present work, it can be decided that the experimental MD differs significantly from the CD in survival percent. The final body weight (FBW) of the larvae weaned at 40 DPH using the MD was better for sea bass hatcheries during the period of weaning and post-weaning stages. Also, growth, survival, and feed utilization of sea bass larvae were improved using the MD on the different weaning periods.

INTRODUCTION

The diets of larvae rearing production is a big challenge in aquaculture. To optimizing nutritional protocols in this bottleneck according to the larval digestive system is critical **(Taylor *et al*., 2020; Laczynska *et al*., 2020).** The mechanism of which marine fish larvae weaned from live feed to commercial feeds commonly starts with finishing larval period. However, this mechanism has been used during the larval stages since the 1980s. The purposes of this mechanism are: to know the degree of the larval acceptance, digest the diet fed and tolerate it to completely change the live prey; to make the larval gut find different specific compounds; and finally, to implement weaning in order to

decrease the larval depending on live feed (**Conceição *et al*., 2010**). Different marketable micro diets have been verified in different relation rations with live pray e.g. rotifers and *Artemia* **(Aristizabal and Suarez 2007**; **Fletcher *et al*., 2007; Engrola 2008, 2010**).

Live feeds possess a sole prospect towards prepared microparticulate diets, specially aiming to acceptability and profitability **(Taylor *et al*., 2020; El-Dakar *et al*., 2020).** It was well known that early co-feeding period sets and enhance the gut for accepting and processing inert diets, allowing earlier weaning with better growth performances than when weaning starts at the end of the larval stage **(Curnow *et al*., 2006; Engrola 2010; El-Dahhar *et al*, 2016 a,b**). Such as the research of **Pauao-Ferreira *et al.* (2001)** represents the successful feeding of small amounts of feed and live bait, thereby minimizing the need for Artemia, which is encouraging more development of micro-diets and the possibility of Full replacment of live feed in the feeding of finfish larvae. **(El-Dahhar *et al*., 2017)** reported that, micro-diet formulation for larval rearing have two main advantages, that the easily available diet is reliable, and the cost advantage eliminates the need for live food farming.

In the last century, obtained results in the laboratory revealed that the time before weaning was greatly reduced. For instance, **Person Le Ruyet *et al*. (1993)** suggested a diet composition sufficient to maintain better growth and survival in the sea bass ***Dicentrarchus labrax*** from the 40th dph, and at the same time it was probably weaned on the day 55th day in most hatcheries . Whereas, in 1997, starting from the 20th day onwards, the sea bass fed with compound feed alone achieved remarkable growth and good survival rate similar to that of live feed **(Zambonino Infante *et al*., 1997).** For the best of authors knowledge, a considerably rare successful studies using MD in early weaning have been reported by**(Cahu and Zambonino-Infante, 1994; Cahu *et al*., 1999; Suzer *et al*., 2007).** However, in the last decade, improvements in extruder feed technology and exploration of new food formulations have elevated the larval survival and quality **(Cahu and Zambonino-Infante, 2001; Kolkovski, 2001).**

The effects of early weaning on E. Sea bass regarding the digestive enzymes, growth performance and survival aspects have been investigated world wide but in Egypt, research on this trend is still very limited. Therefore, the purpose of this work is to study the formula of Micro Diet (MD) that has been manufactured and used in previous studies by the Marine Fish Laboratory (MFL) of the Faculty of Agriculture of Alexandria University in comparison with the well known commercial Diet (CD) INVE® in three different weaning periods on some growth parameters and the survival of sea bass during postlarval period.

MATERIALS AND METHODS

This study was carried out in the National Institute of Oceanography and Fisheries (NIOF), Alexandria, EGYPT in cooperation with the Animal and Fish production Department, Faculty of Agriculture (**Saba Basha**), Alexandria University. The work

aimed to evaluate using a microparticulate diet (MD) vs the INVE® commercial diet using a factorial experiment within three early weaning periods for the larvae of E. sea bass (***Dicentrarchus labrax***) during the larvae rearing period.

Fish and Experimental Design

The E. sea bass fish larvae obtained from the Marine Finfish Hatchery of the General Authority of Fish Resources Development (GAFRD) in Alexandria. The larval of initial weight averaged 27.6 ± 1.3 mg were transferred to eighteen experimental fiberglass tanks of 100 liter each at the rate of 150 larvae / tank. A 2 × 3 factorial experiment was conducted to examine two diets; MD and CD and three weaning periods (WP; 40, 50 and 55 DPH) in arrangement for their main effects and interaction on survival and growth of E. sea bass larvae. Diets were our artificial micro diet (MD) (was reported by **El-Dahhar *et al.*,(2017**) in comparison with the commercial diet (CD INVE®) and the WP (40, 50 and 55 DPH). Experiment was designed to replicate each treatment in three tanks.

Culture Condition

Tanks were filled with sea water with salinity 37 ± 1 ppt, temperature 16 ± 2°C, pH 7.8 ± 0.3 and ammonia 0.007 mg L-1. Photoperiod was 10h light: 14h dark were maintained during the experimental period with continuous artificial aeration. Tanks of the experiment were designed as recirculating aquaculture system. Larvae were fed 6 times daily every two hours using the two diets.

Diets formation and preparation:

The commercial diet (CD) INVE® (THAILAND, batch no:120-005251-A00) ingredients were fish meal, wheat gluten, wheat starch, fish oil, animal marine plankton meal, yeasts, dried algae, plant fatty acids, magnesium sulfate, magnesium oxide and mono sodium phosphate, While the present experimental micro diet (MD) was formulated from commercial ingredients (fish meal, poultry eggs, powdered milk, fish oil, zymogen®, Carboxy- Methyl- Cellulose, ascorbic acids, vitamin and minerals mixture. Ingredients of our diet and chemical analysis of the current diet MD and the CD used to feed larvae during the experimental period are shown in Table (1).

The MD ingredients were milled through screen (0.1 mm holes diameter) before mixing into the diets. Mixture was homogenized in a food grinder model 5 NFGA (Kitchen Aid St. Joseph, MI 49085 USA). Five percent fish oil was emulsified using 0.7% phosphatidyl choline (lecithin) according to **El-Dahhar and El-Shazly (1993).** Emulsified oil with the boiled eggs in 300 ml boiling water were then mixed for five min. in a food mixer and added to the mixture in the Kitchen Aid and homogenized for extra 5 min. Fish oil emulsification, zymogen® addition, to the micro diet, For pelting the mixture, it was passed through the meat grinder and air dried in the Lab condition and

milled through several screens to perform several particle sizes. The experimental larvae were fed using live feed (Rotifer and *Artemia*) and either MD or CD at the following schedule stated at 27.6 mg, 6 times daily every two hours as tabulated in Table (2). The two artificial diets (CD and MD) and three weaning periods (40, 50 and 55 DPH) were evaluated in a 2 × 3 factorial arrangement for their main effects and interaction on survival and growth of E. sea bass. Each treatment was replicated in three tanks.

Table (1): Composition of the micro diet (MD) and Chemical analysis of MD and commercial diet (CD) used during the experimental period to feed European sea bass (Dicentrarchus labrax).

Ingredients:	**%**	
	MD	**CD**
Fish meal	36.5	
Fish oil	5.0	
Powdered Milk	27.0	
Poultry Egg	23.0	
Zymogen	4.0	
CMC**	3.0	
Vit. & Min. Mix*	1.0	
Ascorbic acid	0.5	
Chemical analysis		
Moisture	6.08	8.12
Crude protein	51.40	56.00
Crude Fat	22.78	13.00
Crude Ash	8.50	10.00
Crude Ash non- soluble in HCl	2.70	2.40
Ca	1.50	1.30
P	1.40	1.20
Fiber	0.02	1.00
NFE [1]	7.80	12.88
HUFA	28.89	40.00
DHA/EPA	6.84	2.00

Vitamin and minerals mixture of the micro diet / 2.5 kg premix: vitamin A, 12 million IU; vitamin D3 2g; vitamin E, 10g; vitamin K, 2g; vitamin B1, 1g; vitamin B6, 1.5 g; Vitamin B12, 10mg; Vitamin B2, 4g; pantothenic acid, 10g; Nicotinic acid, 20 g; Folic acid, 1000mg; Biotin, 50mg; Choline chloride, 500g;I, 1g; Iron, 30g; Mn, 55g; Zn, 55g; Selenium, 1g. (Phaezer Co.).

Vitamin and minerals mixture of the commercial diet, 2.5 kg premix: vitamin A, 20000 IU; vitamin D3 2500 IU; vitamin E, 700 mg; Vitamin C, 2000 mg; I, 5 mg; Mn, 50 mg; Zn, 50 mg; Selenium, 0.3 mg; Cu, 6 mg; Co, 1 mg; Ethoxyquin, 120 mg; BHA, 30 mg; Propyl Gallate, 30 mg.

[1] *NFE: Nitrogen free extract.*

Table (2): The larval weaning protocol from live food (*Artemia*) from 30 DPH until 55 DPH for sea bass showing (microalgae, rotifers, *Artemia* and micro-diet densities (individual / ml).

	Period DPH	**25**	**30**	**40**	**50**	**55**
Micro algae (Cell / ml)		300,000 decreased to 0			0	0
Sea bass	Rotifer	10 decreased to 0 / ml		0 / ml	0 / ml	0 / ml
	Artemia	1.5 increased to 7 / ml		Gradually decreased to 0 ml^{-1} for treatments 40, 50 and 55		
	MD	MD Started at 30 DPH		Starter 150 -250 µ, 250 -350 µ and 350 450 µ		
	CD	CD Started at 30 DPH		Starter 200 -300 µ, 200 -400µ and 300 500 µ		

Analytical methods:

Water quality parameters; temperature, ºC and dissolved oxygen, mg / L, using Oxygen meter (Model 5946-55) Hannan® instrument Woonsocket RI USA; they were 5.9 ± 0.8 ppm and 24 ± 0.5C respectively. Salinities, ppt were confirmed using burette titration for chloride against standard 0.014N silver nitrate; pH and total ammonia nitrogen and unionized ammonia, (mg /L) were measured in the Environmental Laboratory, and in the Central Laboratory of the National Institute of Oceanography and Fisheries (NIOF) using Spectro photometer (YSI 9300) USA.

Experimental data of the present study were subjected to statistical analysis according to **Snedecor and Cochran,(1967).** Using 2 × 3 factorial ANOVA with two factors (weaning diets and weaning periods) each in three replicates. Two experiments were applied to detect the significant differences among treatments according to the model:

$$Y_{ijk} = \mu + \alpha_i + \beta_j + (\alpha\beta)_{ij} + e_{ijk}$$

where $i = 1, ..., a$; $j = 1, ..., b$; $k = 1, ..., n$.

The differences within each experiment were tested using LSD ($P \leq 0.05$)

Dry weight and moisture content

Samples of fry were collected, and then the external water was removed by placing them on top of paper towels. The samples were immediately transferred to a pre-weighed glass pan and weighed to the nearest ±0.0001 g on microbalance for wet weight determination, then frozen. Samples were dried at 60°C overnight. Moisture content was calculated by subtracting sample dry weight from its wet weight, dividing by wet and multiplying by 100. The samples were kept frozen until further analysis**.**

Proximate analysis of the feeds were performed by standard methods for determination of the moisture, dry matter, crude protein, crude fat, ash and nitrogen free extract levels **(AOAC, 1990)**, at the feeding Laboratory of the Fish, Faculty of Agriculture (Saba Basha) Alexandria University.

Evaluation of survival and growth

Dead fish were collected and counted daily to determine mortality. Ten larvae were collected from the experimental larval batch at the beginning of the experiment to determine their initial body weight (IBW). At the end of the experiment within each replicate, ten larvae were collected to measure final body weight (FBW) of sea bass. Specific growth rate (SGR) for each replicate within each treatment was determined for the larval growth rate determination according to (**Hopkins, 1992)**. Formula $SGR = 100 \times \frac{Ln\ FTL - Ln\ ITL}{days\ No.}$ where FTL and ITL were final and initial total length (mm), respectively. Growth parameters, Feed and nutrients utilization were estimated according to the following equation:

Total weight gain (g/fish) = W_t - W_0

Where: W_0: initial mean weight of fish in grams; W_t: final mean weight of fish in grams

Average daily gain (ADG) (mg/fish/day) = W_t - W_0/ N

Where: N: duration period

Specific growth rate (SGR) (%/ day) = 100 × (ln W_t - ln W_0)/ days

Where: ln: natural logarithm

Feed Intake (FI): This is the amount of feed given or supplied during the experimental period.

Feed conversion ratio (FCR) = dry matter intake (g)/body weight gain (g)

Where: Body weight gain = (W_t - W_0)

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

Protein productive value (PPV %) = 100 × (P_t - P_0)/protein intake (g)

Where: P_0: protein content in fish carcass at the beginning of the experiment; P_t: protein content in fish carcass at the end of the experiment

Energy Retention (%) **=** 100 × (E_t - E_{0-}) / Energy intake (Kcal)

Where: E_0: energy content in fish carcass (Kcal) at the start

E_t: energy content in fish carcass (Kcal) at the end

Lipid extraction:

With a sufficient amount of water, 2-20 g of the sample (micro diet) were weighed into a 250 ml centrifuge bottle so that the total amount of water together with 40 ml of methanol and 20 ml of chloroform reaches 16 ml. Soak for 2 minutes; add 20 ml of chloroform and soak for 30 seconds; add 20 ml of water and soak for 30 seconds. Centrifuge the mixture for 10 minutes at 2000-2500 rpm. The lower chloroform layer was drawn out and filtered through coarse filter paper into a dry weighing flask. According to **(Pearson's 1981),** chloroform is evaporated to dryness.

Methylation of lipid:

5 ml of methanolic sulfuric acid (1 ml of concentrated sulfuric acid and 100 ml of methanol) and 2 ml of benzene were added to a test tube weighing 50 mg of lipids. Close the test tube and place it in a 19°C water bath for one hour. Then half Cool, add 8 ml of water and 5 ml of petroleum ether and shake vigorously, and separate the ether layer in a drying tube. Evaporate to dryness **(Radwan 1978).**

Analysis of lipid:

Analysis was carried out by gas chromatography (GC) using Hp (Hewlett Packard) 6890 GC equipped with flam ionization detector (FID) under the following conditions:

An analytical glass column DB- 32 (50%-Cyanopropyl- methylpolysiloxane), 30m, 0.32mm ID, 0.25 µm film thickness. Detector temperatures 240˚c, injector temperature 220˚c, injection volume 3 µl, split ratio 50:1 and carrier gas: nitrogen, gas flow: 1 ml/min. Fatty acid compositions as % of dry weight in total lipids of the formulated diet (MD) compared with (CD) were shown in Table (3).

Table (3): Fatty acid (% dry weight) composition in total lipids of Micro diets (MD) and (CD).

FA %	MD	CD
C14:0 (Myristic acid)	3.62	4.11
C16:0 (palmitic acid)	23.03	24.3
C17:0 (heptadecanoic acid)	2.02	1.33
C20:0 (Arachidic acid)	0.66	0.218
Total Saturated	29.32	29.958
C16:1 (Palmitoleic acid)	4.89	6.00
C17:1 (Heptadecanoic acid)	0.71	0.65
C18:1t (Oleic acid)	8.69	2.70
C18:1c (Oleic acid)	28.46	33.01
Total Monounsaturated	42.75	42.36
C18:2 (Linoleic acid)	2.93	3.02
c20:3 (Mead acid)	0.25	0.28
c22:2 (Docosadienoic acid)	0.69	0.72
Σn-6	3.87	4.02
C18:3α (Alpha-Linolenic acid)	0.48	0.34
c18:3ϐ (Gama-Linolenic acid)	1.26	1.34
c20:5 (ecozapentaenoic acid) EPA	5.49	5.53
c22:6 (docosahexaenoic acid) DHA	12.28	13.2
Σn-3	19.91	20.41
n-3/n-6	5.14	5.8
DHA/EPA	2.34	2.39
Total Polyunsaturated	23.78	24.43

RESULTS

Water parameters qualification

Data of Water parameters measurments were monitored at a week intervals and were summarized in Table (4). The results showed that the average temperature was 16 – 17 ^{o}C, pH 7.8 – 7.9, salinity 34 - 37 ppt, dissolved oxygen (DO) (more than5.5 ppm) i.e.higher than 80%, and unionized ammonia (NH3) less than 0.007 mg/L and indicated no significant differences between treatments .Besides,They were within the acceptable range for marine fish larvae.

Table (4): Water quality parameters; temperature (T ^{o}C), salinity (PPT), pH, dissolved oxygen (DO), and total ammonia nitrogen (TAN), in the period of European sea bass *Dicentrarchus labrax* larvae during the study.

Parameter	Temperature	Salinity	Dissolved Oxygen	NH_3	pH
Weekly	^{o}C	ppt	mg\L	mg\L	
1 W	16.6 ± 0.5	36 ± 0.08	6.4 ± 0.04	0.002	7.8 ± 0.1
2 W	16.2 ± 0.5	36 ± 0.08	6.7 ± 0.04	0.007	7.9 ± 0.1
3 W	15.4 ± 0.5	34 ± 0.08	6.2 ± 0.04	0.001	7.7 ± 0.1
4 W	15.3 ± 0.5	37 ± 0.08	7.1 ± 0.04	0.002	7.8 ± 0.1
5 W	16.4 ± 0.5	35 ± 0.08	6.6 ± 0.04	0.002	7.8 ± 0.1
6 W	16.6± 0.5	35 ± 0.08	5.8 ± 0.04	0.008	7.9 ± 0.1
7 W	17.3 ± 0.5	36 ± 0.08	5.5 ± 0.04	0.007	7.7 ± 0.1
8 W	15.4 ± 0.5	34 ± 0.08	6.2 ± 0.04	0.001	7.7 ± 0.1
9W	16.3 ± 0.5	35 ± 0.08	6.4 ± 0.04	0.002	7.8 ± 0.1
10W	17.3 ± 0.5	36 ± 0.08	5.5 ± 0.04	0.007	7.7 ± 0.1
11W	16.9 ± 0.5	35 ± 0.08	5.9 ± 0.04	0.008	7.8 ± 0.1
12W	17.5 ± 0.5	37 ± 0.08	5.9 ± 0.04	0.002	7.9 ± 0.1

Survival and Growth

Growth parameters measurements as previously terminated as S % , (FBW mg / fish), (SGR % / d), FC and (FCR) of E. sea bass larvae fed the MD or CD at the three weaning periods 40,50 or 55 DPH during this experiment were shown in Table (5). Percent survival of the larvae weaned using CD at the weaning periods (40,50 and 55 DPH) increased significantly from 87.11 ± 0.94 % found with the group fed CD to 90.22 ± 1.02 % for the group fed MD ($P < 0.05$). But it did not affect significantly by the weaning period 40, 50 or 55 DPH ($P > 0.05$). However, interaction between the two factors (diets and time of weaning) is significant ($P <0.05$). The best significant survival rate (92.00 ± 0.001 %) was found with the group fed MD at 55 dph weaning period, while the least (86.67 ± 1.89 %) was found with groups fed CD at 40 and 50 dph weaning periods. Final body weight (FBW) at the main effect of using different diet (MD and CD) was not significant ($P < 0.05$).While weaning period affected FBW significantly ($P < 0.05$). It was higher at the group weaned after 40 dph having the value of 279.6 ± 3.5 mg, than 272.9 ± 1.6 and 272.0 ± 4.4 mg observed with 50 and 55 dph respectively. FBW have the best significant value 285.3 ± 0.5 mg with the group fed the MD at weaning

Table (5): Mean ± standard error (SE) of survival, final body weight (FBW), specific growth rate (SGR), feed consumption, and feed conversion ratio (FCR) of E. sea bass (*Dicentrarchus labrax*) (27.6 mg initial BW) fed two weaning diets (MD and CD) at three weaning periods (40, 50 and 55 DPH).

Weaning Diets	Weaning Periods	Survival	Final body Weight	SGR	Feed consumption	Feed conversion ratio
	DPH	%	(mg/fish)	% /d	(mg/fish)	FCR
MD	40	90.67 ± 1.89 ^{ab}	285.3± 0.5 ^{a}	5.85± 0.0021 ^{a}	540.9 ± 8.4	1.97± 0.033 ^{c}
	50	88.00 ±3.27 ^{b}	272.7± 2.9 ^{bc}	5.77± 0.0133 ^{b}	541.0 ± 13.0	2.06± 0.055 ^{b}
	55	92.00 ± 0.00 ^{a}	268.6 ± 10.0 ^{c}	5.74± 0.047 ^{b}	560.0 ± 23.1	2.16± 0.031 ^{a}
CD	40	86.67±1.89 ^{b}	274.0 ± 3.6 ^{bc}	5.78± 0.017 ^{b}	543.9 ± 2.1	2.06± 0.014 ^{b}
	50	86.67±1.89 ^{b}	273.0 ± 3.6 ^{bc}	5.77± 0.016 ^{b}	539.1 ± 4.6	2.05± 0.003 ^{b}
	55	88.00 ±3.27 ^{b}	275.3 ± 6.1 ^{b}	5.79± 0.028 ^{b}	537.7 ± 4.1	2.03± 0.045 ^{bc}
Significant level		0.05	0.05	0.05	NS	0.05
Pooled means						
MD		90.22 ± 1.02 ^{g}	275.6 ± 3.5 ^{g}	5.79± 0.023 ^{g}	267.60± 3.48	2.06± 0.03
CD		87.11 ± 0.94 ^{h}	274.11± 1.8 ^{g}	5.78±0.011 ^{g}	263.41±1.71	2.05±0.15
Significant level		0.05	0.05	0.05	NS	NS
	40	88.66 ±1.34 ^{x}	279.7 ± 3.0 ^{x}	5.82 ± 0.019 ^{x}	268.97 ± 2.97 ^{x}	2.02± 0.029 ^{y}
	50	87.00 ±1. 34 ^{x}	272.9 ± 1.6 ^{y}	5.77± 0.010 ^{y}	261.99 ± 1.58 ^{y}	2.06± 0.027 ^{xy}
	55	90.00±1.49 ^{x}	272.0 ± 4. 4 ^{y}	5.76± 0.028 ^{y}	261.29 ± 4.34 ^{y}	2.10 ± 0.043 ^{x}
Significant level		0.05	0.05	0.05	0.05	0.05

Mean in the same column not sharing the same super script are significantly different $P < 0.05$

period 40 dph, but the least value 268.6 ± 10.0 mg was found with the group fed MD at 55 dph ($P < 0.05$). The same trend was found with SGR at both the main effect and the interaction. No significant difference was found at the main effect of the weaning diets MD and CD with the values of 5.79 ± 0.023 and 5.78 ± 0.011 %/d ($P < 0.05$) respectively. However, the effect of the weaning period 40, 50 and 55 dph was significant with the higher value 5.82 ± 0.019 %/d found with 40 dph than 5.77 ± 0.01 and 5.76 ± 0.028 %/d found with the larvae weaned at 50 and 55 dph respectively ($P < 0.05$). Interaction observed that the higher significant SGR value 5.85 ± 0.021 %/d was found with the group weaned at 40 dph using MD ($P < 0.05$). Insignificant effect was found between MD and CD in feed consumption, weight gain and FCR data ($P > 0.05$). Also, the main effect of weaning period and interaction of feed consumption were insignificant ($P > 0.05$) as shown in Table (6). The 40 dph weaning period affected significantly weight gain and FCR at the main effect of the weaning period with the values of (268 97± 2.97 mg and 2.02 ± 0.029) respectively ($P < 0.05$). It also affected the interaction of them significantly getting the higher values (274.5 ± 0.6 mg and 1.97 ± 0.033) for weight gain and FCR with the group fed MD at 40 dph and the least values of (257.9 ± 10.0 mg and 2.16 ± 0.031) for them with the group fed MD at 55 dph respectively ($P < 0.05$).

Chemical composition of sea bass fish:

As shown in table (6), the body composition of *D.labrax* larvae did not differ significantly (P > 0.05) among the main effects of both factors and the interaction between them.

Table (6): Mean ± standard error (SE) of Chemical composition of the larval body (moisture, crude protein and crude lipids) of E. sea bass (*Dicentrarchus labrax*) (27.6 mg initial BW) fed two weaning diets (MD and CD) at three weaning periods (40 , 50 and 55 DPH).

Weaning Diets initial	Weaning Periods	Moisture	Protein	Lipid
	DPH	%	%	%
MD	40	74.20± 0.06	16.30 ± 0.06	6.73± 0.09
	50	74.25± 0.03	16.03± 0.18	6.77± 0.17
	55	74.09± 0.06	15.87± 0.34	6.61± 0.25
CD	40	74.11± 0.09	16.07± 0.13	6.51± 0.09
	50	74.14± 0.03	15.93± 0.13	6.74± 0.15
	55	74.07± 0.09	15.93± 0.15	6.50± 0.09
Significant level		NS	NS	NS
		Pooled Means		
MD		74.18±0.036	16.06±0.13	6.70± 0.099
CD		74.10±0.040	15.98±0.07	6.58± 0.071
Significant level		NS	NS	NS
	40	74.15±0.054	16.18±0.09	6.61± 0.081
	50	74.19±0.032	15.9±0.11	6.75± 0.111
	55	74.08±0.054	15.9±0.18	6.55± 0.131
Significant level		NS	NS	NS

Growth performance

The results of (PPV), (ER) and (PER) of *D.labrax* larvae fed the two weaning diets (MD and CD) at three weaning periods (40, 50 and 55 DPH) at end of the experiment were recorded in Figure (1). ER at the main effect of using different diet (MD and CD) increased significantly from 13.48± 0.12 % found with the group fed CD to 14.10 ± 0.32 % for the group fed MD (P < 0.05) the results were summarized in Figure (1) .While weaning periods affected (ER) insignificantly (P > 0.05). ER have the best significant value 14.97 ± 0.25 with the group fed the MD at weaning period 40 dph, but the least value 13.20 ±0. 36 were found with the group fed MD at 55 dph (P < 0.05). The same trend was found with PPV at the main effect, while the interaction between the two factors (weaning diets and weaning period) is significant (P <0.01). The best significant PPV (14.95 ± 0.28 %) was found with the group fed MD at 40 dph weaning period, while

the least (13.11 ± 0.34%) was found with group fed MD at 55 dph weaning period ($P < 0.01$). Also, the main effect of the diets affected PER significantly ($P < 0.01$). It decreased from 0.92 ± 0.023 at the group fed MD to 0.82 ± 0.009 at the group fed CD. While the main effect of the weaning period was not significant ($P > 0.05$). The interaction between the two factors (weaning diets and periods) was significant with the best value of 1.00 ± 0.016 % found with the group fed MD at 40 dph weaning period and the least 0.87 ± 0.029 % found with groups fed MD at 55 weaning period ($P < 0.05$).

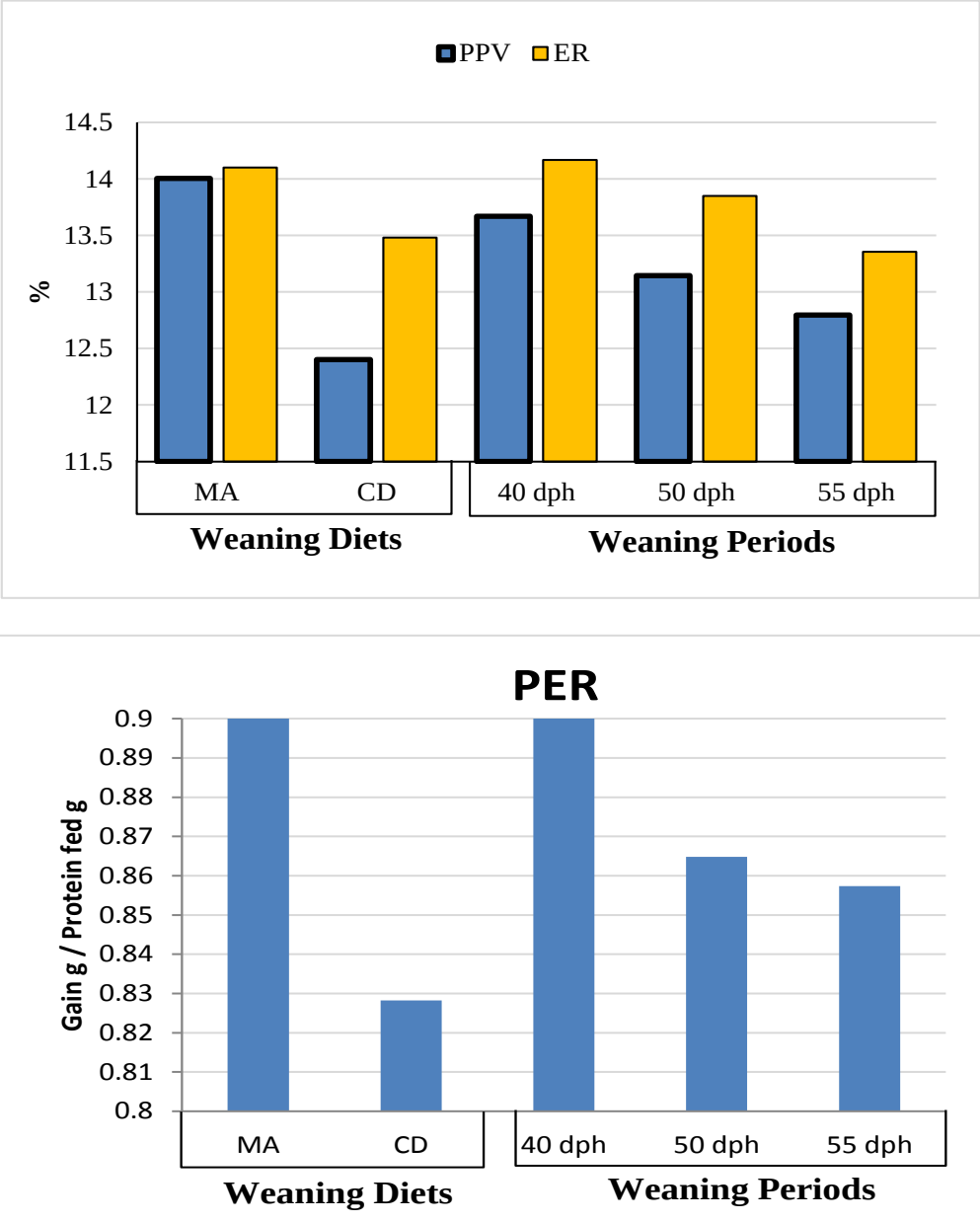

Fig. 1: Protein productive value (PPV), Energy retention (ER) and Protein efficiency ratio (PER) of European Sea bass (27.6 mg initial BW) fed two weaning diets (MD and CD) at three weaning periods (40, 50 and 55 DPH).

DISCUSSION

Water quality measurments were assessed in the present study at weekly intervals and cleared that temperature, salinity, dissolved oxygen (DO) saturation, and unionized ammonia were in acceptable range when agreed with the suitable limits for marine fish larvae by finding of **Moretti, *et al*., (2005),Munday, *et al*. (2009) and El-Dahhar, *et al* ., (2017)**. For example,water temperature for sea bass larvae during the present work ranged from 16 – 17ºC. It was recorded in the weaning period under intensive conditions water temperature for sea bass larvae should not be less than 16.5 ºC and the lethal limit of water temperature is 4 ºC (**Colloca and Cerasi, 2005**). Also, pH was ranged between (7.8 – 7.9). These data were within the time pH fluctuation values of the best range of (pH 6.5-8.5) for sea bass culture as described by **Szymanski and Patterson, (2003)**. Similarly, dissolved oxygen (DO) saturation was more than 5.4 ppm and (NH3) was less than 0.007 mg/L as suggested for efficient rearing by **Pavlidis and Mylonas,(2011**) and statedthat (DO) levels should be maintained above 4.0 mg/ L, and not more than 5.2 mg /L whereas the preferred range of TAN should be less than 1.4 ppm for excellent growth ,improving feeding behavior and larvae appetite of the sea bass **(Person-Le Ruyet, 1989**).

During the present work the average percent survival of the larvae weaned using CD and MD at the three weaning periods 40 and 50, and 55 dph was ranged between (88.00 -90.67 %). These results coincided with the findings of **(Suzer *et al* 2007, Suzer *etal*., 2011)**.Whereas , larvae weaned using our MD get a better significant survival (90.22 %) and the larvae weaned by the CD get an average survival 87.11 % ($P<0.05$) The results of this treatments agree with **El- Dahhar *et al*. (2017**).

Pantazis *et al*.(2014) revealed that no significance ($P > 0.05$) was detected in between the survival percent (at average of 10% of the initial population)of gilthead sea bream (***Sparus aurata* L**.) within treatments of early weaning in the different protocols even with mixing of two varied early-weaning MDs and a late-weaning diet. In a previous work we explored similar results in weaning European Sea bass using the same MD and CD; larvae weaned by our MD get significantly improved percent survival and larval growth performances than CD ($P < 0.01$) **(El-Dahhar *et al*., 2016 a,b)**. Also, in their study, they pointed out that the final population percent of European sea bass was between 82.6% and 85.6%, and there was no significant difference between the early weaning period at (42, 47, 52 and 57 DPH) **(p> 0.05).**Accordingly**, (El-Dahhar *et al*., 2017)** stated that final survivos rate of G. Sea bream achieved 45.77 % without significant differences between the early weaning periods (42, 52 DPH) ($p > 0.05$).

The survival rate of this experiment was closely matching the international statistical results recorded for the Egyptian hatcheries. In Egypt, The produced survived population result is the best way to evaluate the efficiency of fish hatchery in general, especially marine fish hatcheries.

Taylor *et al*. (2020) evaluated varied weaning times from Artemia to MD through receiving Artemia feeding, or deprived from Artemia to MD at three different time age durations. For example in ***Gymnocorymbus ternetzi*** fed to 33 dph, the survival rate at 13 dph was higher (20.6±1.8%) and higher than that of live Artemia weaning time started in the period between (18 -23) dph (13.6±1.5%), A and MD resulted in the lowest survival rate. These results indicated that it can be weaned before gastric differentiation and proving that in this species, Artemia can be less dependent on larval feed.

However, researchers stated that earlier inclusion of dry feed may have adverse effect on fish growth performace and survival % **(Andrade *et al*. 2012 and Laczynska *et al*., 2020**) as fish may loss the cabability to assimilate a formulated diet **(Cahu and Zambonino-Infante 2001**; **Ma *et al*. 2014). Person-Le Ruyet,(1989)** pointed out that when small marine fish larvae are weaned, it is still difficult to feed them directly into composite pellets. However, when combined with live prey or provide a pre-feeding period for live prey, good results can be obtained. The effect size is about 2-3 mg. The same author explained this situation and explained it by "a reasonable intake of micro-diet, because the acceptability and visual inspection of micro-diet can be improved by using feed activators and increase good perceptual contrast. The micro-diet formulated for the earlier larval weaning of larvae must meet all the nutritional requirements of the larvae to improve the nutrition of the larvae, thereby increasing the development and survival of the larvae after weaning.

Laczynska *et al*.(2020) reported that Sterlet larvae should be fed with *Artemia* nauplii at 7–15 dph because fish achieved a higher wet BW, TL and a well developed liver and digestive tract. In the present study the obtained regular body weight values showed that the final growth rate at after 40 days of weaning reached the best (279. 7 mg/fish), and the lowest weaned at 55 days (272.0 mg/fish) and that was a significantly different at ($P \leq 0.05$). **El-Dahhar *et al.* (2016b)** found the same significance in terms of FBW, (WG), (ADG) and (SGR, %) for sea bass weaned with MD obtained in 42 and 57 DPH at different weaning periods ($P < 0.05$). In addition, the results of this study are consistent with the approaches of **(Suzer *et al*., 2007; 2011; Laczynska *et al*., 2020)** who believed that MD inclusion can be started after 25 dph. The 15-day group (dph) of D. labrax larvae culture had the lowest growth and survival rates, while the 25-day group (dph) had the highest. They also recommended 35 (DAH) as the optimal weaning age for sea bass because the glandular stomach is fully developed and the number of digestive glands were increased. Similarly, **Cahu and Zambonino-Infante (1994)** suggested that weaning on the 20th day after hatching stopped or delayed the development of larvae, for example the appearance of pancreatic secretory function, leading to a decline in growth rate. Therefore, as confirmed by this study, the weaning process starting after the 25th day after hatching will not negatively affect the larval growth. More earlier, **(Person-Le Ruyet, 1989)** found that most marine fish larvae are active feeders, and MD may be well accepted quite immediately.

Formulated food should not be used to substitute Artemia for daily use suddenly, but should start with mixed feeding, by decreasing daily live food and gradually increasing formula feed, till proceed as it is completely replaced **(Portella and Dabrowski 2008; Laczynska *et al*., 2020).** In this combined feeding method was longly known as co-feeding strategy, the growth rate may be similar or better than that of Artemia feeding alone **(Person Le Ruyet *et al*., 1993).**As previously reported by **Taylor *et al*. 2020**, who pointed out that, MD is appropriate for larval growth , should have high platability, and have high stability in seawater, and it easily classify them as Suitable size, can be used in automatic feeders.

MD of the present study made to have a wide graded size category started from 60 µm to 1000 µm as follows: (60-150, 150-250, 250-350, 350-450, 450-700 and 700-100 µm). These sizes are suitable for the larvae during the period of weaning; each species accept a range from the above mentioned with a limited particle size matched the mouth size. Sea bass in the period of larvae rearing accept the particle size of the MD from 60 to 450 µm; the larval size is very small.

Preparing a diet to replace live food and coordinating the consumption of ***Sparus aurata*** larvae are closely related to digestive enzyme activities **(Süzer *et al*., 2011).** However those authors suggests that weaning can be undergone in the formation of abnormal and functional stomachs, as described in the Asian sea bass ***(Lates calcarifer)*** **by (Walford and Lam, 1993),** in Atlantic Cod ***Gadus Morhua***, in the early introduction of MD may lead to relatively Low growth performance due to enzyme activity by **(Baskerville-Bridges and Kling, 2000)** and in Haddock ***Melanogrammus aeglefinus*** by **(Hamlin and Kling, 2001).**

CONCLUSION

From the results obtained in the present work, it can be concluded that the experimental MD used for weaning sea bass larvae was differed significantly with the CD in survival percent,FBWas well as in feed utilization for the larvae weaned at 40 dph suggesting the MD was better for using in sea bass marine hatcheries during the period of weaning and post-weaning stages. Moreover, inspite its lower protein content ,the growth, survival and of sea bass larvae were improved using the MD on the different weaning period confirming the need to HUFA represeting in omega 3 fatty acids more than protein reqiurement which was considerable in MD in this larval period.

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