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Improving co-feeding strategies for Neotropical green terror cichlid (*Aequidens rivulatus*) larvae with lecithin-enriched *Artemia franciscana* nauplii: Effects on survival, growth performance and body composition

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Abstract

The effects of feeding on a commercial diet and lecithin-enriched (EN) Artemia franciscana nauplii for improving co-feeding strategies of Neotropical green terror cichlid (*Aequidens rivulatus*) larvae were conducted. For this purpose, eight groups of fish in triplicates were assigned with two different diets (unenriched Artemia [UN] and EN Artemia) and four feeding regimes (1, 5, 10 and 25 days feeding with UN and EN diets and then a 10% daily replacement Artemia nauplii with commercial diet). The crude lipid (21.4%) and total polar lipid (12.96% of total crud lipid) levels signifi- cantly increased in enriched Artemia nauplii ($p < 0.05$). The highest amount of satu- rated fatty acids (SFA) were in enriched and UN Artemia nauplii (41.74% and 49.64% respectively) but the highest level of monounsaturated fatty acids (MUFA) (25.69%) and polyunsaturated fatty acids (PUFA) (49.11%) were obtained in commercial diet. Growth performance of fish fed 10 EN and 5 EN had significantly higher values of total weight (120.67, 120.31 mg), %WG (584.48, 580.50%) and SGR (7.69, 7.67%) respectively ($p < 0.05$). Nevertheless, fish fed 25 EN had signifi- cantly higher FCE (190.4%), PER (3.95) and NPU (202.5), in comparison with other groups. In terms of body composition, the EN Artemia nauplii led to increased lipid contents in 25 EN, 10 EN and 5 EN treatments. In conclusion, the results of this study revealed that feeding regimes of 10 EN and 5 EN could improves survival and growth performance of Neotropical green terror cichlid, *A. rivulatus* larvae.

KEYWORDS
Artemia nauplii, body composition, green terror cichlid larvae, growth performance, soybean lecithin

INTRODUCTION

Larval feeding plays an important role for the successful culture of fresh or salt water, and ornamental fish and live feeds such as *Arte- mia* nauplii, rotifers, daphnia and copepods are essential to succeed of this stage of fish feeding (Baskerville-Bridges & Kling, 2000; Sorgeloos, Dhert, & Candreva, 2001). Live feeds stimulate the feed- ing of finfish and shellfish larvae through their colour, movement and chemical attractants such as free amino acids and secreted metabolites (Cahu & Infante, 2001; Kolkovski, 2001; Kolkovski, Cur- now, & King, 2004). In addition to, live feeds are easier to digest than commercial foods and help digestion process through providing exogenous enzymes (Sorgeloos et al., 2001). Nonetheless, due to the establishment and the requirements of the laboratory with high costs and the variable nutritional value of live foods (Callan, Jordaan, & Kling, 2003; Langdon, 2003), suggests the need to replace with com- mercial foods that usually have a steady and reliable nutritional value (Baskerville-Bridges & Kling, 2000).

Moreover, the best time to change from live food to commercial foods in ornamental fish is not completely clear. The duration of the feeding period of live feed is very important to achieve higher growth and survival of aquatic larvae. Co-feeding (i.e., combining live feed and artificial feed) in the early stages of larval development, improves nutritional conditions of the larvae and helps larvae to accept the formulated diet when the live feed is withdrawn, as a result, the duration of feeding on live feed is reduced (Rosenlund, Stoss, & Talbot, 1997). Replacement live feed with formulated diets has been reported with varying degrees of success in aquaculture lit- erature, for both marine and freshwater aquatic species (Bonaldo et al., 2011; Engrola et al., 2007, 2009 ; Muguet, Lazo, Conklin, & Piedrahita, 2011).

In a number of strategies, earlier replacement of live feed with commercial foods leads to low growth and survival, and high skeletal deformities of larvae (Cahu & Infante, 2001). One way of increasing the efficiency of the weaning protocol is improving the quality of diets. In aquatic larviculture several enrichment protocols have been used to improving the nutritional value of live foods (Conceição, Yúfera, Makridis, Morais, & Dinis, 2010). The freshly hatched *Artemia* nauplii is most commonly live feed used in larviculture of marine and freshwater species (Conceição et al., 2010; Sorgeloos et al., 2001). Enrichment of *Artemia* nauplii has been reported as well as with nutrients such as vitamin A (Takeuchi et al., 1995), vitamin C, methionine (Monroig, Navarro, Amat, & Hontoria, 2007), essential fatty acids and phospholipids (PLs) (Guinot, Monroig, Hontoria, et al., 2013; Guinot, Monroig, Navarro, et al., 2013).

Lecithin as a dietary PL source can lead to increased survival, growth and stress resistance in fish larvae (Tocher, Bendiksen, Campbell, & Bell, 2008). Several studies have shown the positive effect of soybean lecithin on growth of fish larvae including, Euro- pean seabass *Dicentrarchus labrax* (Gisbert, Villeneuve, Zambonino- Infante, Quazuguel, & Cahu, 2005), Atlantic cod *Gadus morhua* (Wold et al., 2007), pikeperch *Sander lucioperca* (Hamza, Mhetli, Khemis, Cahu, & Kestemont, 2008), large yellow croaker *Larmichthys crocea* (Cai, Feng, Xiang, Mai, & Ai, 2016; Zhao, Ai, Mai, Zuo, & Luo, 2013),

pond loach *Misgurnus anguillicaudatus* (Gao et al., 2014) and Atlantic salmon *Salmo salar* (Taylor et al., 2015). Studies have shown that PL synthesized in the body may not meet the requirements of fish larval during the development stage (Kanazawa, 1993; Tocher et al., 2008). Therefore, the role of PL in stimulating of larval growth is depends on fish ability to synthesis of sufficient PL (Geurden, Bergot, Van Ryckeghem, & Sorgeloos, 1999), or diets require to be supplemented with PL sources. To get this, in this study, the effects of lecithin- enriched (EN) *Artemia franciscana* nauplii were evaluated on growth performance and body composition of green terror cichlid (*Aequidens rivulatus*) larvae, a Neotropical cichlid species that is highly appreciated as an ornamental aquarium fish.

MATERIALS AND METHODS

Artemia nauplii enrichment

Artemia franciscana cysts were obtained from *Artemia* & Aquaculture Research Institute (Urmia University, Urmia, Iran), and hatched at a density of 2 g/L in 1 L conical glass well-illuminated vessels containing seawater with a salinity of 26 ppt, temperature 28°C vigorous aera- tion from the bottom (Sorgeloos, 1980). The source of lecithin was soybean lecithin, a light yellow powder containing 74.42% ± 0.24% total PL (Monil Global SDN.BHD Company, Malaysia) (Table 1). To prepare the enrichment emulsion, 1 g powdered lecithin was mixed in 10 ml seawater (40°C) with a homogenizer (IKA T25 Digital, ultra, Turrax, Germany) for 1 min and then, it was observed by optical microscope to ensure that the lecithin particles were smaller than 30 μm. Afterwards, 200,000 *Artemia* nauplii were counted and trans- ferred to 1-L conical glass vessels containing seawater with a salinity of 30 ppt. Then 2 × 3 per ml of enrichment emulsion at two times (0 and 12 hr) were added to the enrichment vessel. *Artemia* nauplii were enriched with lecithin suspension for 24 hr, at temperate 27°C and vigorous aeration from the bottom to keep oxygen levels above 5– 6 mg/L. After washing *Artemia* nauplii with tap water, enriched *Arte- mia* nauplii were used to feed the green terror cichlids larvae.

Experimental diets and feeding fish larval

The experiment was carried out at the *Artemia* and Aquaculture Institute, Urmia University, Urmia, Iran. To have uniform larvae for experiment, first one pairs of green terror cichlid (*A. rivulatus*) ready to spawn were obtained from local fish farm at Urmia city, Iran and transferred to a glass aquarium containing 40 L of dechlorinated and aerated well water (temperature 26.0 ± 1.5°C, pH 7.6 ± 0.5, DO 8.1 ± 1.2 mg/L and hardness>120 ppm, gentle aeration and 50% daily water renewal for each aquarium). Several pieces of smooth stones for spawning were placed in the bottom of the aquarium. After spawning, to prevent of fungal infections on eggs' surface, methylene blue (1 ppm, Merck, Germany) was added to each aquar- ium. After 60–72 hr, hatching occurred and 6–7 days later 720 lar- vae at a density of 30 specimens per tank were randomly allotted into 24 plastic tanks (working volume: 6 L) filled with well water. At the start of the experiment, all fish were weighted (17.5 mg ± 0.30; *M* ± standard deviation, *SD*) and randomly distributed into eight treatments with three replicates per each dietary treatment. This trial lasted for 25 days and consisted of different diets and feeding regimes: (a) 25

days fed with unenriched *Artemia* nauplii (25 UN); (b) 25 days fed with EN *Artemia* nauplii (25 EN); (c) 10 days fed with UN *Artemia* nauplii, and a 10% daily replacement *Artemia* nauplii with commercial diet (10 UN); (d) 10 days fed with EN *Artemia* nau- plii, and a 10% daily replacement EN *Artemia* nauplii with commercial diet (10 EN); (e) 5 days fed with UN *Artemia* nauplii, and a 10% daily replacement *Artemia* nauplii with commercial diet (5 UN); (f) 5 days fed with EN *Artemia* nauplii, and a 10% daily replacement *Artemia* nauplii with commercial diet (5 EN); (g) 1 day fed with UN *Artemia* nauplii, and a 10% daily replacement *Artemia* nauplii with commercial diet (1 UN); and (h) 1 day fed with EN *Artemia* nauplii, and a 10% daily replacement EN *Artemia* nauplii with commercial diet (1 EN) (Table 2).

TABLE 1 Lipid class composition of soybean lecithin ($M \pm SD$, $n = 3$)

Lipid class (% of total lipid)	Soybean lecithin
PC	32.53 ± 0.70
PEa	16.72 ± 0.21
PSe + PI	16.84 ± 0.34
LPC	1.39 ± 0.14
LPEa	nd
Unknown	6.91 ± 0.44
Total PL	74.42 ± 0.24
Cholesterol	0.97 ± 0.09
Free fatty acids	5.20 ± 0.12
Triacylglycerides	2.46 ± 0.12

Note. LPC: lysophosphatidylcholine; LPEa: lysophosphatidylethanolamine; nd: not detected; PC: phosphatidylcholine; Pea: phosphatidylethanolamine; PL: phospholipid; PI: phosphatidylinositol; PSe: phosphatidylserine.

Green terror cichlids larvae were fed ad libitum six times per day during the whole experimental period (25 days). Water conditions were as follows: temperature 26.0 ± 1.5°C, pH 7.6 ± 0.5, DO 8.1 ± 1.2 mg/L and hardness>120 ppm, gentle aeration and 50% daily water renewal for each aquarium. At the beginning and at the end of the experiment, all fish were measured for body length (BL) and body weight (BW) to the nearest 0.01 mm and 0.001 g using a digital balance and caliper respectively. Prior to their measurement, fish were gently anesthetized with clove powder (100 mg/L, 1,040 plants, Shiraz, Iran). The following growth parameters and survival were calculated using standard formulae: daily weight gain (DWG, g/ day) = (BWf−BWi)/T; specific growth rate (SGR, %) = (Ln BWf−Ln BWi)/T × 100; feed conversion ratio (FCR) = FI/WG; feed conversion efficiency (FCE, %) = (WG/FI) × 100; protein efficiency ratio (PER) = WG/PI, lipid efficiency ratio (LER) = WG/LI, net protein utilization (NPU) = (FPG/PI) × 100 and survival rate (SR, %) = number of fish at 25th day/number of fish at zero day × 100,where BWf was the final BW, BWi was the initial BW, T was the number of days that the trial lasted, FI was the food intake, PI was the protein intake, LI was the lipid intake, FPG was the fish protein gain and WG was the BW gain. For treatments that were used the *Artemia* nauplii, for calcula- tion food intake, 15 min after feeding, uneaten *Artemia* and com- pound diet were collected from the bottom of the aquarium and subtracted from the among of given feed.

TABLE 2 Summary of feeding regime for green terror cichlid (*Aequidens rivulatus*) larvae fed different dietary ratios of commercial diet (CD), unenriched (UN) and lecithin-enriched (EN) *Artemia franciscana* nauplii for 25 days

Treatments	Feeding regimes		
25 UN	25 days UN		
25 EN	25 days EN		
10 UN	10 days UN	10 days UN + CD	5 days CD
10 EN	10 days EN	10 days EN + CD	5 days CD
5 UN	5 days UN	10 days UN + CD	10 days CD
5 EN	5 days EN	10 days EN + CD	10 days CD
1 UN	1 day UN	10 days UN + CD	14 days CD
1 EN	1 day EN	10 days EN + CD	14 days CD

Chemical analysis

The proximate composition of experimental diets was analyzed according to the methods of the AOAC (1997). Dry matter contents of fish larvae and experimental diets were determined after oven- drying to constant weight at 105°C. Protein contents of fish larvae and diets were measured by the Kjeldahl method using an auto Kjel- dahl system. Lipid contents of fish larvae and diets were measured by ether extraction. Ash contents of fish larvae and diets were determined using a muffle furnace at 550°C for 8–10 hr.

Fatty acids of diets were analyzed according to the methods of Agh, Jasour, and Noori (2014). Briefly, total lipids from the experi- mental diets were extracted by sample homogenization in chloro- form/methanol (2:1, v/v). Then, methyl esters were prepared by transmethylation using methanolic KOH and n-heptane according to ISO5509 method (1978) with minor modification (Agh et al., 2014). The fatty acids composition of diets were determined using an auto sampler gas chromatography (GC, Agilent technologies 7890 N, USA), equipped with a flame ionization detector (FID) and a cyano- propyl- phenyl capillary column (DB-225MS, 30 m × 0.250 mm ID ×0.25 μm Film thickness, USA). Identification of the fatty acids was performed by comparing their retention time with those of an external commercial standard mixture (GLC-68d, Nu-Chek Prep., MN, USA) according to Agh et al., (2014).

The PL profile of the experimental diets were analyzed using Densitometer GS900 calibrated (Bio Rad, Germany) according to the methods of the Olsen and Henderson (1989). In summary, the silica gel plates were initially placed into hexane:diethyl ether (1:1 v/v) and then, the top 1 cm of the silica layer was lined up. The plate was activated at 160°C for 30 min. The lipid sample was added as a spot to the plate using a glass micro-syringe. All steps were done at room temperature. The samples were first treated for the emergence of polar lipids using methyl acetate: isopropanol: chloroform: methanol as the solvent system. After evaporation of the solvents under oven, the plates were dried in a vacuum desiccator for 30 min. Afterwards the plate was transferred to new solution (hexane:diethyl ether:gla-

cial acetic acid) for emergence of neutral lipids. Separated lipid classes were detected by spraying the plate with 3% cupper acetate in phosphoric acid, followed by charring at 160°C for 20 min. Quantitation was performed on a Shimadzu dual wavelength TLC scanner. All measurements were performed in triplicate.

Statistical analyses

Statistical analyses were conducted by IBM Statistics (21 version) software. Initially, data were checked for normality and homogeneity by using Shapiro–Wilk and Levene's test respectively. Data from different dietary treatments were compared by means of a two-way ANOVA and differences between the means were assessed by Tukey's post-hoc test. Statistically significant differences were considered at the 0.05 level.

RESULTS

Diets composition

Proximate, lipid class and fatty acid composition of the tested com- mercial diet and, UN and EN *A. franciscana* nauplii are shown in Tables 3–4. *Artemia* nauplii enrichment with soybean lecithin significantly increased the amount of crude lipid from 17.6% in the UN *Artemia* to 21.4% in the EN *Artemia* ($p < 0.05$). There was no significant difference in dry matter, crude protein and ash content between enriched and UN *Artemia* ($p > 0.05$). The highest amount of dry matter (92%), and the lowest crude lipid (9.5%) and crude fibre (10.50%) were in commercial diet. On the other, the enrich- ment of *Artemia* with soybean lecithin resulted in a significant increasing phosphatidylcholine (6.74%) and phosphatidylserine + phosphatidylinositol (1.88%). In addition to, *Artemia* enrich- ment with soybean lecithin significantly increased the amount of triglyceride (53.35%) and decreased the amount of free fatty acid (15.22%). Although the highest amount of triglyceride (76.13%) and the lowest amount of free fatty acid (6.68%) were observed in commercial diet.

TABLE 3 Proximate composition of commercial diet, unenriched (UN) and lecithin-enriched (EN) *Artemia franciscana* nauplii ($M \pm SD$, $n = 3$)

	Diets		
Composition	Unenriched *Artemia* nauplii	Lecithin-enriched *Artemia* nauplii	Commercial diet
Dry matter (%)	13.6 ± 0.9^{b}	12.8 ± 0.4^{b}	92.0 ± 2^{a}
Crude protein (% DW)	50.1 ± 2.0	48.2 ± 1.8	48.0 ± 1.5
Crude lipid (% DW)	17.6 ± 1.3^{b}	21.4 ± 1.4^{a}	9.5 ± 0.5^{c}
Ash (% DW)	13.6 ± 1.3^{a}	11.9 ± 1.7^{a}	10.5 ± 1^{b}

Note. Different letters on the each column indicate significant difference by Tukey's test ($p < 0.05$).

Based on Figure 1, enrichment of *Artemia* with soybean lecithin resulted in a significant increase in total polar lipid levels (12.96% of total crud lipid), whereas the lowest polar lipid levels were observed in commercial diet (4.12% of total crud lipid). There was no signifi- cant difference in natural lipid between enriched and UN *Artemia* nauplii ($p > 0.05$). Although the highest amount of natural lipids were observed in commercial diet (95.88%).

Fatty acid composition of the tested commercial diet and, UN and EN *A. franciscana* nauplii are shown in Tables 5. The highest amount of saturated fatty acids (SFA) was in enriched and UN *Artemia* (41.74% and 49.64% respectively) and the lowest of SFA was in commercial diet (23.14%). The highest amount of monounsaturated fatty acids (MUFA) (25.69%), polyunsaturated fatty acids (PUFA) (49.11%) and n-6 levels (34.25%) were in commercial diet. There was no significant difference in n-3 levels between commercial diet, enriched and UN *Artemia* ($p > 0.05$).

Survival and growth performance of fish

Two-way ANOVA results of growth performance of different experimental groups were shown in Table 6. All parameters were influ- enced by the interaction of enrichment of *Artemia* nauplii (Enrichment) and duration of feeding with *Artemia* nauplii (Feeding regime).

TABLE 4 Lipid class composition of commercial diet, unenriched (UN) and lecithin-enriched (EN) *Artemia franciscana* nauplii ($M \pm SD$, $n = 3$)

Lipid class (% of total lipid)	Unenriched *Artemia* nauplii	Lecithin-enriched *Artemia* nauplii	Commercial diet
PC	5.59 ± 0.47^{b}	6.74 ± 0.04^{a}	3.01 ± 0.21^{c}
PEa	4.69 ± 0.17^{a}	4.30 ± 0.14^{a}	0.60 ± 0.01^{b}
PSe + PI	0.75 ± 0.21^{b}	1.88 ± 0.06^{a}	0.50 ± 0.12^{c}
Cholesterol	5.94 ± 0.12	5.08 ± 0.11	6.11 ± 0.01
Free fatty acids	37.35 ± 2.26^{a}	15.22 ± 0.40^{b}	7.68 ± 0.34^{c}
Triacylglycerides	32.29 ± 1.23^{c}	53.35 ± 0.16^{b}	76.13 ± 1.06^{a}

Note. Different letters on the each column indicate significant difference by Tukey's test ($p < 0.05$).
LPC: lysophosphatidylcholine; LPEa: lysophosphatidylethanolamine; nd: not detected; PC: phosphatidylcholine; Pea: phosphatidylethanolamine; PI: phosphatidylinositol; PSe: phosphatidylserine.

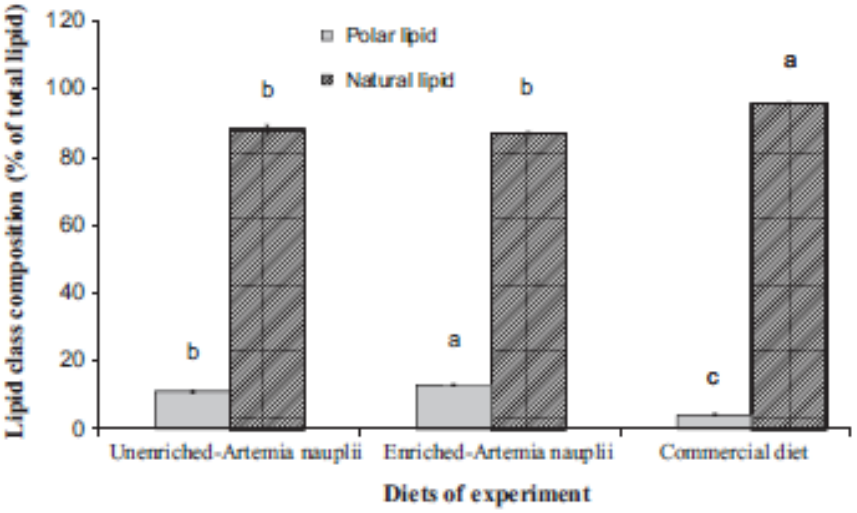


FIGURE 1 Lipid class composition of commercial diet, unenriched (UN) and lecithin-enriched (EN) *Artemia franciscana* nauplii ($M \pm SD$, $n = 3$)

TABLE 5 Fatty acid composition of commercial diet, unenriched (UN) and lecithin-enriched (EN) *Artemia franciscana* nauplii ($M \pm SD$, $n = 3$)

Fatty acid (% area of total fatty acids)	Commercial diet	Unenriched *Artemia* nauplii	Lecithin-enriched *Artemia* nauplii
C14:0	1.14 ± 0.23	0.99 ± 0.67	0.94 ± 0.52
C14:1n5	0.23 ± 0.07^{b}	2.91 ± 0.91^{a}	0.25 ± 0.11^{b}
C16:0	16.58 ± 4.52^{a}	6.13 ± 3.77^{b}	7.57 ± 3.8^{b}
C16:1n7	1.61 ± 0.37^{b}	3.96 ± 1.11^{a}	0.71 ± 0.43^{b}
C18:0	5.12 ± 2.21	3.92 ± 0.11	3.85 ± 1.5
C18:1n7	21.59 ± 8.41^{a}	11.92 ± 0.1^{b}	9.16 ± 4.33^{b}
C18:1n9	2.02 ± 0.27	2.77 ± 1.41	4.03 ± 2.16
C18:2n6cis	33.76 ± 6.85^{a}	5.72 ± 3.27^{b}	7.51 ± 3.31^{b}
C18:3n3	6.79 ± 1.45^{b}	13.76 ± 7.22^{a}	14.37 ± 6.19^{a}
C20:0	0.11 ± 0.05^{a}	0.19 ± 0.06^{a}	0^{b}
C20:1n9	0.24 ± 0.06^{a}	0^{b}	0^{b}
C20:2n6	0.08 ± 0.03^{a}	0^{b}	0^{b}
C20:4n6	0.41 ± 0.12^{b}	0^{c}	1.92 ± 0.58^{a}
C20:5n3	2.13 ± 0.44	4.05 ± 1.73	3.71 ± 2.29
C22:0	0.19 ± 0.04^{b}	30.5 ± 13.13^{a}	37.28 ± 18.51^{a}
C22:6n3	5.94 ± 1.81^{a}	0^{b}	0^{b}
ΣSFA	23.14 ± 4.11^{b}	41.74 ± 8.21^{a}	49.64 ± 6.38^{a}
ΣMUFA	25.69 ± 2.41^{a}	18.05 ± 2.81^{b}	10.12 ± 1.45^{c}
ΣPUFA	49.11 ± 3.35^{a}	23.54 ± 5.71^{b}	27.52 ± 4.23^{b}
Σ*n*−6	34.25 ± 4.17^{a}	5.72 ± 3.27^{c}	9.43 ± 2.05^{b}
Σ*n*−3	14.86 ± 3.47	17.81 ± 5.21	18.08 ± 4.61

Note. Different letters on the each row indicate significant difference by Tukey's test ($p < 0.05$).

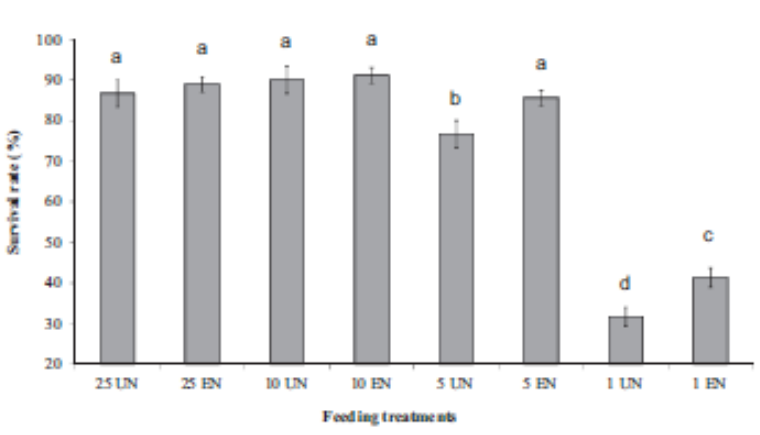


FIGURE 2 Survival rate (SR) (%) of green terror cichlid (*Aequidens rivulatus*) larvae fed unenriched (UN) and lecithin-enriched (EN) *Artemia franciscana* nauplii for 1–25 days ($M \pm SD$, $n = 3$)

TABLE 6 Two-way ANOVA output for growth and nutritional feeding regime of different experimental groups at the end of the experiment (*p*-values)

Parameters	Feeding regime	Enrichment	Feeding regime × enrichment	r^2
BW_f	0.001	0.001	0.001	0.956
TL_f	0.001	0.001	0.004	0.870
SGR	0.001	0.001	0.001	0.951
WG	0.001	0.001	0.001	0.947
SR	0.001	0.001	0.001	0.973

Note. BW_f: body weight final; SGR: specific growth rate; SR: survival rate; TL_f: total length final; WG: weight gain.

Larval SRs in each different trial (UN and enriched) are shown in Figure 2. The SR was significantly affected by the co-feeding strate- gies and decreased by reducing the feeding period of *Artemis* nauplii in both UN and enriched groups. In UN group, the higher SR was sig- nificantly observed in treatments 25 UN (86.67%) and 10 UN (90%) compared to other UN groups. Besides, lowest SR (31.67%) was obtained in 1 UN group. However, enrichment of *Artemia* nauplii with soybean lecithin led to increased SR and fish fed 25 EN (88.89%), 10 EN (91.11%) and 5 EN (85.56%) had significantly higher SRs.

Considering feeding regimes, in UN treatments, fish fed 10 UN had significantly higher values of BWf, WG % and SGR compared to other UN groups. Besides that, larvae fed 1 UN diet showed signifi- cantly lower TLf than the other treatments (Table 7, $p < 0.05$). The enrichment of *Artemia* nauplii with soybean lecithin led to increased somatic growth in all treatments (Table 7). Fish fed 10 EN and 5 EN diets had significantly higher BWf, WG % and SGR, compared to other groups ($p < 0.05$) (Table 7).

Nutritional performance of fish larvae

Two-way ANOVA results of nutritional performance of different experimental groups were shown in Table 8. All parameters were influenced by the interaction of enrichment of *Artemia* nauplii (Enrichment) and duration of feeding with *Artemia* nauplii (Feeding regime).

Considering feeding regimes, in UN groups, fish fed 25 UN had significantly higher values of FCE, PER and NPU compared to other UN groups. Besides that, larvae fed 1 UN diet showed significantly lower FCE, PER and NPU than the other treatments (Table 9, $p < 0.05$). The enrichment of *Artemia* nauplii with soybean lecithin led to increase the above-mentioned variables in all treatments (Table 9). Fish fed 25 EN had significantly higher FCE, PER and NPU, compared to other groups ($p < 0.05$). In addition to, feed con- version ratio (FCR) significantly increased by reducing the feeding period duration of *Artemis* nauplii ($p < 0.05$). Fish fed 1 UN had sig- nificantly higher FCR value compared to other UN groups. Besides, FCR decreased with the enrichment of *Artemia* nauplii, and the lowest FCR was observed in 25 EN treatments (Table 9).

Body composition of fish larvae

According to Table 10, only lipid contents of body components were affected by interaction of enrichment of *Artemia* nauplii (Enrichment) and feeding period with *Artemia* nauplii (Feeding regime).

Regarding feeding regimes containing EN *Artemia* nauplii, fish larvae fed 10 EN and 25 EN diets showed significantly higher body lipid content in comparison to other groups (Figure 3). The lowest body lipid content was significantly obtained in fish fed 10 UN ($p < 0.05$) and the rest of dietary regimes showed intermedi- ate values in comparison to the above-mentioned groups. How- ever there was no significant difference in dry matter, crude protein and ash content between feeding strategies or UN and EN *Artemia* nauplii ($p > 0.05$, Table 11).

TABLE 7 Growth performance of different experimental groups at the end of the experiment (*p*-values).

	Growth performances			
Treatments	BW_f (mg/fish)	TL_f (cm)	SGR (%/day)	WG (%)
25 UN	90.5 ± 0.7^{d}	20.05 ± 0.1^{c}	6.54 ± 0.01^{d}	412.7 ± 11.9^{d}
25 EN	101.0 ± 2.6^{c}	20.20 ± 0.3^{bc}	6.99 ± 0.10^{c}	473.9 ± 15.0^{c}
10 UN	112.7 ± 2.1^{b}	20.63 ± 0.2^{ab}	7.43 ± 0.07^{b}	540.1 ± 11.8^{b}
10 EN	120.7 ± 4.7^{a}	20.93 ± 0.1^{a}	7.69 ± 0.19^{a}	584.5 ± 32.2^{a}
5 UN	93.3 ± 1.0^{d}	20.16 ± 0.2^{bc}	6.61 ± 0.10^{d}	421.5 ± 13.5^{d}
5 EN	120.3 ± 1.5^{a}	20.96 ± 0.2^{a}	7.67 ± 0.07^{a}	580.5 ± 12.3^{a}
1 UN	84.7 ± 2.5^{e}	18.70 ± 0.2^{d}	6.27 ± 0.13^{e}	379.3 ± 15.5^{e}
1 EN	101.1 ± 4.2^{c}	20.15 ± 0.8^{bc}	6.99 ± 0.17^{c}	473.9 ± 24.1^{c}

Note. Different letters on the each column indicate significant difference by Tukey's test ($p < 0.05$).

TABLE 8 Two-way ANOVA output for feed efficiency parameters of different experimental groups at the end of the experiment (*p*- values).

Parameters	Feeding regime	Enrichment	Feeding regime × enrichment	r^2
FCR	0.001	0.001	0.012	0.990
FCE	0.001	0.001	0.001	0.992
PER	0.001	0.001	0.001	0.995
LER	0.001	0.173	0.002	0.954
NPU	0.001	0.001	0.001	0.988

Note. FCE: feed conversion efficiency; FCR: feed conversion ratio; LER: lipid efficiency ratio; NPU: net protein utilization; PER: protein efficiency ratio.

TABLE 9 Feed efficiency parameters of convict cichlid fed different experimental diets.

Treatments	Nutritional performance				
	FCR	FCE (%)	PER	LER	NPU
25 UN	0.66 ± 0.02^{f}	150.4 ± 4.32^{b}	3.01 ± 0.09^{b}	8.54 ± 0.24^{a}	147.8 ± 5.52^{b}
25 EN	0.53 ± 0.02^{g}	190.4 ± 7.47^{a}	3.95 ± 0.15^{a}	7.89 ± 0.31^{a}	202.5 ± 9.09^{a}
10 UN	0.99 ± 0.01^{e}	100.9 ± 0.91^{c}	2.05 ± 0.02^{d}	6.51 ± 0.06^{b}	106.9 ± 2.73^{d}
10 EN	0.89 ± 0.02^{e}	112.4 ± 2.72^{c}	2.34 ± 0.06^{c}	6.24 ± 0.15^{b}	112.2 ± 3.87^{c}
5 UN	1.40 ± 0.04^{c}	71.3 ± 2.27^{e}	1.47 ± 0.05^{e}	5.70 ± 0.18^{c}	74.6 ± 2.70^{e}
5 EN	1.13 ± 0.05^{d}	88.2 ± 4.28^{d}	1.83 ± 0.09^{d}	5.88 ± 0.28^{bc}	96.7 ± 4.85^{d}
1 UN	1.73 ± 0.08^{a}	57.9 ± 2.86^{f}	1.21 ± 0.06^{f}	5.79 ± 0.29^{c}	60.1 ± 3.03^{f}
1 EN	1.53 ± 0.02^{b}	65.3 ± 1.09^{ef}	1.36 ± 0.02^{ef}	6.53 ± 0.11^{b}	70.7 ± 1.83^{e}

Note. Different letters on the each column indicate significant difference by Tukey's test ($p < 0.05$).

TABLE 10 Two-way ANOVA results for proximate composition of convict cichlid fed different experimental diets.

Composition	Feeding regime	Enrichment	Feeding regime × enrichment	r^2
Lipid	0.002	0.008	0.03	0.802
Dry matter	0.679	0.322	0.825	0.096
Ash	0.985	0.230	0.680	0.089
Protein	0.859	0.850	0.901	0.021

DISCUSSION

Enrichment and feeding regime had effect on survival, growth per- formance and body composition of green terror cichlid larvae at 25th day. By the end of the experiment, larvae fed with UN *Artemia* nauplii for 1 day (treatment 1 UN) were significantly smaller (weight and length) and had lower SGR than those recorded in fish fed the other diets. The results of this study demonstrate that co-feeding EN *A. franciscana* nauplii with a commercial diet in 10 EN and 5 EN treatments will produce better quality fish at weaning. However, fish fed 25 EN had significantly higher values of feed efficiency com- pared to other groups. Also, regarding to feeding regimes fish larvae fed with *Artemia* nauplii for 25 and 10 days had high SR than those fed with *Artemia* nauplii for 1 and 5 days. Similar observations have also been reported for *Paralichthys dentatus* (Bengtson, 1999), *Solea senegalensis* (Ribeiro, Zambonino-Infante, Cahu, & Dinis, 2002), *Par- alichthys olivaceus* (Teshima, Koshio, Ishikawa, Alam, & Hernandez, 2004), *Danio rerio* (Carvalho, Araújo, & Santos, 2006), *Paralichthys lethostigma* (Faulk & Holt, 2009), *Pterophyllum scalare* (Herath & Ata- paththu, 2013) and *S. Lucioperca* (Ljubobratović et al., 2015).

TABLE 11 Proximate composition of green terror cichlid larvae fed unenriched (UN) and lecithin-enriched (EN) *Artemia franciscana* nauplii for 1–25 days ($M \pm SD$, $n = 3$)

Experimental groups	Proximate composition		
	Dry matter (%)	Crude protein (% DW)	Ash (%DW)
Feeding regime			
25	39.79 ± 0.98	62.72 ± 2.31	16.79 ± 1.21
10	39.29 ± 0.76	61.99 ± 2.58	16.72 ± 0.43
5	39.88 ± 0.24	62.77 ± 3.77	16.91 ± 1.03
1	40.04 ± 0.96	62.91 ± 2.41	16.89 ± 0.37
Enrichment			
UN group	39.51 ± 0.61	62.68 ± 1.65	17.09 ± 0.79
EN group	39.99 ± 0.92	62.52 ± 0.95	16.56 ± 0.54

Note. Different letters on the each column indicate significant difference by Tukey's test ($p < 0.05$).

The poor growth performance of fish larvae in groups that used long period time of commercial food could be due to poor ingestion by fish, low digestibility, low nutritional value and leaching nutrients (Lazo, Dinis, Holt, Faulk, & Arnold, 2000). Meanwhile, the absence of active stomach and inadequate digestive enzymes of larvae have been reported as a cause of the failure of commercial foods to live food, wherever the exogenous enzymes in the digestion of live prey can be useful (Muguet et al., 2011). Besides that, Kolkovski, Tandler, and Izquierdo (1997) noted that *Artemia* nauplii through chemical and visual stimulants had effect on the ingestion and metabolization of formulated foods during co-feeding. Larvae is directed towards the food through chemical stimulants that is related to secreted metabolites of *Artemia* nauplii, and besides that the visual stimulation of larvae is related to the movement of *Artemia* nauplii, which enables the larvae to find the food and increases the success of early feeding.

On the other hand, the nutritional value of *Artemia* can be improved with enrichment. In this study, to improve the nutritional quality, the nauplii of *Artemia* were well-enriched with soybean lecithin for feeding of green terror cichlid larvae. Formerly, the effect of EN *Artemia* nauplii has not been evaluated in fish, especially orna- mental fish. Although there are several studies on the effect of enriched *Artemia* nauplii with fatty acids on *Latris lineata* (Bransden, Battaglene, Morehead, Dunstan, & Nichols, 2005), *Sparus aurata* (Monroig et al., 2006), *Hippoglossus hippoglossus* (Hamre & Harboe, 2008), *S. senegalensis* (Boglino et al., 2012), *S. lucioperca* (Lund, Skov, & Hansen, 2012), *Pagrus pagrus* (Watanabe et al., 2016), *Argyrosomus regius* (Campoverdea & Estevez, 2017). Our results showed that growth and nutritional performance of green terror cichlid larvae increased with enrichment of *Artemia* nauplii, so the group fed 10 EN and 5 EN had significantly higher total weight, WG %, SGR and fish fed 25 EN had significantly higher FCE, PER and NPU, compared to other groups. Nevertheless, the comparison of the present study with the results of those studies is a little complicated.

The importance of using of PLs in fish artificial diets on growth performance has been

well-documented in *Oncorhynchus mykiss* (Azarm, Abedian-Kenari, & Hedayati, 2013; Daprà et al., 2011), *Portunus trituberculatus* (Li et al., 2014), *S. salar* (De Santis, Taylor, Marti- nez-Rubio, Boltana, & Tocher, 2015; Taylor et al., 2015), *Lates calcarifer* (Salini, Wade, Bourne, & Turchini, Glencross, 2016) *L. crocea* (Cai et al., 2016; Feng, Cai, Zuo, Mai, & Ai, 2017). It is stated that PLs with an increasing ratio of brush border enzymes to cytoso- lic enzyme enhance the larval growth of *G. morhua* (Hamza et al., 2008; Wold et al., 2007). Also, Poston (1991) indicated that soybean lecithin increased the palatability of diets in *O. mykiss*.

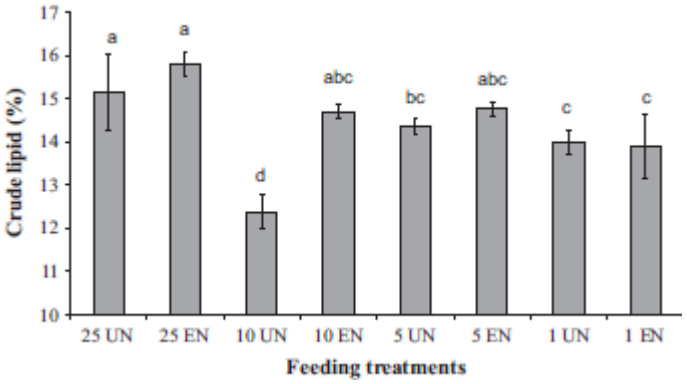
Under current live prey enrichment conditions, the crude lipid content in *Artemia* nauplii was increased from 17.6% to 21.4%, whereas its PLs content was increased by *ca*. 17%, increasing from 11.04% to 12.96% of total lipids. Similar results were reported when enriching *Artemia* nauplii with liposomes (Guinot, Monroig, Navarro, et al., 2013; Monroig et al., 2003), although magnitude of increase in crude lipid and PLs contents varied depending on *Artemia* strain and stage of development considered. In this experi- ment, the highest amount of MUFA (25.69%), PUFA (49.11%) and n-6 levels (34.25%) were in commercial diet. The higher growth of fish larvae in treatments 10 EN and 5 EN can be due to providing of fish nutrition needs through enrichment of *Artemia* nauplii (higher PLs) and essential fatty acids by commercial diet. Although, studies on EFA with flatfish larvae have shown that sufficient amount of n-3 HUFA levels in the diet (up to 3.5%), let to good growth and survival in fish larvae (Dickey-Collas & Geffen, 1992; Izquierdo, Arakawa, Takeuchi, Haroun, & Watanabe, 1992; Minkoff, 1987).

The studies shown that lecithin as a PL source have an effect on larval stage through providing inositol, choline and energy for growth of fish and improve the absorption of nutrient (Coutteau, Geurden, Camara, Bergot, & Sorgeloos, 1997; Geurden, Marion, Charlon, Coutteau, & Bergot, 1998; Hadas, Koven, Sklan, & Tandler, 2003), and lipoprotein synthesis in digestive system (Fontagne, Geurden, Escaffre, & Bergot, 1998; Geurden et al., 1998). Thus, PL-supplemented diets could be used directly or indirectly for maintaining development and sufficient growth performance (Zhao et al., 2013). In the present study, enrichment of *Artemia* nauplii with lecithin increased the amount of lipid content of green terror cichlid larvae. In agreement with our stud- ies, Geurden et al. (1999), Gao et al. (2014) and Cai et al. (2016) found that diet PL significantly increased lipid content of common carp (*Cyprinus carpio*), Dojo loach (*M. anguillicaudatus*) and large yellow croaker (*L. crocea*) larvae. It has been mentioned that PLs through synthesis and secretion of lipoproteins play an important role in the trans- fer of lipids from the intestinal enterocytes to the tissues of the body (Fontagne et al., 1998). Poston (1991) reported that lecithin signifi- cantly increased the amount of total lipid in the body of *O. mykiss* and *S. salar* fry. Researchers have shown PLs are involved in the synthesis of lipoproteins and thus increase the transfer of lipid from intestinal enterocytes to other parts of the body (Olsen, Myklebust, Kaino, & Ringø, 1999; Salhi, Hernández-Cruz, Bessonart, Izquierdo, & Fernández-Palacios, 1999).

In conclusion, the results of this study showed that the green terror cichlid larvae fed with *Artemia* nauplii for 10 days had the highest weight gain, length gain and SR. Our results showed that nutritional

and growth performance of green terror cichlid larvae increased with enrichment of *Artemia* nauplii, so the group fed 25 EN, 10 EN, 5 EN and 1 EN had significantly higher total weight, total length, SGR, FCR, FCE, PER and NPU, compared to 25 UN, 10 UN, 5 UN and 1 UN groups. In addition to, the results demonstrated that this species was able to accept commercial food earlier with using the EN *Artemia* nauplii.

FIGURE 3 Crude lipid (%) of green terror cichlid larvae fed unenriched (UN) and lecithin-enriched (EN) *Artemia franciscana* nauplii for 1–25 days ($M \pm SD$, $n = 3$)



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