Lecithin-enriched *ARTEMIA* combined with inert diet and its effects on reproduction and digestive enzymes of *Aequidens RIVULATUS*

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Abstract

The present study investigated the effects of soybean lecithin bioencapsulated in adult *ARTEMIA FRANCISCANA* and unenriched *ARTEMIA* in combination with an inert diet on digestive enzymes activity and reproductive perfor- mance in *Aequidens RIVULATUS* (green terror cichlid). Eight hundred and ten fish (3.1 ± 0.2 g) were randomly allotted into glass tank (80 L) and assigned to ten dietary treatments with 5 different levels (0, 25, 50, 75, and 100%) of either lecithin-enriched *ARTEMIA* (EA) or unenriched *ARTEMIA* (UA) over a period of 90 days. The amount of total polar lipid increased from 39.2% in the *unenriched ARTEMIA to 43.7% in the lecithin-enriched ARTEMIA* ($P < .05$). The fish fed with 50% EA had higher total weight, and total length compared with other groups ($P < .05$). The peculiar functions of total alkaline proteases, α-amylase, and lipase in green terror increased in the groups fed with lecithin-enriched *ARTEMIA,* compared to the un-enriched groups ($P < .05$). The highest total alkaline proteases activity was observed in the fish fed with 75% EA treatment. In comparison to the other groups, fish fed 100 and 0% levels of *ARTEMIA* replacement had significantly highest and lowest α-amylase ac- tivity values, respectively. Concerning reproductive performance, the highest average fecundity, egg diameter, egg weight, fertilization, hatching, and larval survival rates, as well as the lowest time between two spawning episodes, were obtained in fish fed 50% EA. In conclusion, this feeding strategy is advisable for a proper nu- tritional management of broodfish of green terror cichlid.

1. Introduction

Ornamental fish industry is a flourishing business in international trade, fisheries, aquaculture, conservation, and poverty reduction in developing countries (Ahmadifard et al., 2018). Considering the high economic value of these fish, and in order to support the sustainability of this activity, the study of cultivation methods of broodstock and larvae seems important in order to avoid the reliance on animals obtained from the natural environment (Firouzbakhsh et al., 2011). There are more than a thousand species of freshwater fish, including 100 fa- milies among the commercial ornamental fish list (Dey, 2016). The green terror cichlid (*A. RIVULATUS)* is a prevalently ornamental fish originating from South America (Yeh et al., 2018). However, there is limited knowledge regarding the nutritional requirements and re- productive characteristics of these fish species. Considering its carnivorous feeding habits, the available nutritional recommendations are based on compound diets containing salmonid and other cichlid species, as these diets can partially meet their nutritional requirements (Firouzbakhsh et al., 2011; Alishahi et al., 2015).

Diet plays an important role in increasing reproductive performance of broodstock and larva quality (Izquierdo et al., 2001). Broodstocks fed live food have usually better performance in relation to those fed inert compound diets since live food is more palatable and easier to digest and generally has higher nutritional value, leading to a positive impact on the development of gonads and an increased reproductive perfor- mance (Langroudi et al., 2009). Success or failure in fish production plans depends on breeding conditions including the provision of sui- table food to ensure the growth and better quality of larvae, juvenile, and broodfish stages (Lim, 2002). Live foods are excellent nutrient sources of fostering both freshwater and marine ornamental fish as well as shrimp species (Lim, 2003). Among different sources of live preys, *ARTEMIA* sp. (nauplii, metanauplii, juveniles, and adults) is the most used live prey in cultured fish and crustaceans. However, due to the high cost of *ARTEMIA* cyst, the infrastructures and laboratories conditions for *ARTEMIA* production and the nutritional value of *ARTEMIA*, it is necessary to find a replacement commercial diet having a stable nutritional value for the development of ornamental fish trade (Sorgeloos et al., 2001; Conceição et al., 2010). Regardless of the advantages of using com- pound feeds, live preys are still commonly used in ornamental fish retailers and commercial fish hatcheries. *ARTEMIA* can transfer some useful nutrients such as vitamins, EFA, phospholipids (PLs) to a target organism due to their non-selective feeding behavior (Agh and Sorgeloos, 2005; Guinot et al., 2013a, b). Moreover, it due to the higher palatability can enhance the ingestion, digestion, and absorption of formulated diet provided under a co-feeding regime (Kolkovski et al., 1997; Øie et al., 2011). Furthermore, other researchers found that the combination of live prey with an inert diet could result in an increase in feed intake and as well as an enhancement of food digestibility (Engrola et al., 2009; Nhu et al., 2010; Anh et al., 2009; Jamali et al., 2018).

Lecithin, as a PLs source, has the potential to augment stress re- sistance as well as growth and survival in fish species (Jafari et al., 2018). The effects of lecithin on the growth of both freshwater and marine fish as well as crustaceans have been studied in European sea- bass *DICENTRARCHUS LABRAX* (Cahu et al., 2003), *Oncorhynchus mykiss* (Azarm et al., 2013), large yellow croaker *LARMICHTHYS CROCEA* (Zhao et al., 2013), white leg shrimp *LITOPENAEUS VANNAMEI* (Niu et al., 2011); Atlantic salmon *SALMO SALAR* L., (Hung et al., 1997), and Japanese blue crab *Portunus TRITUBERCULATUS* (Li et al., 2014). From a biological point of view, as an important part of cell membranes, PLs are vital for a normal cell and function of an organ (Niu et al., 2011) and supply fish with vital fatty acids, choline, and phosphorus, which is required for their development, growth, and reproduction (Jamali et al., 2018). In addition, PLs were reported to reduce the non-polar lipid droplets in the gastrointestinal mucosa (Wold et al., 2007) and act as lipid emulsifiers (Hamza et al., 2008) which improve the digestion and absorption of dietary fatty acids (Niu et al., 2011). Furthermore,

lecithin was shown to have noteworthy effects on the reproductive performance of several species of fish and crustaceans (Wu et al., 2007; Hossen et al., 2014). However, the effects of lecithin on many aspects of reproduction have not been determined since most of the previous nutritional studies have been conducted in aquaculture species with long lifespans. Therefore, in this study, the effects of soybean lecithin bioencapsulated in adult *A. FRANCISCANA* and un-enriched *ARTEMIA* in combination with an inert diet were evaluated in terms of the activity of the main pancreatic digestive enzymes, and reproductive indices in *A. RIVULATUS*, as a valuable species of ornamental fish.

2. Materials and methods

2.1. *ARTEMIA enrichment*

Adult brine shrimps *A. FRANCISCANA* were obtained from Artemia and Aquaculture research (AAR) Institute (Urmia, Iran). Soya lecithin (with 74.4% total phospholipid) was purchased from Monil Global SDN.BHD Company, Malaysia. In this study, we adopted the enrichment method of *ARTEMIA* described in Jamali et al. (2018). Briefly, liposomes (< 50 μm) were prepared with a mixture of lecithin and seawater (1 g/ 10 mL) by means of a digital homogenizer (IKA, Turrax, Germany). A number of adult *ARTEMIA* were transferred to the vessels containing sterile salt water and fasted for 18 h. At this time, two-thirds of the *ARTEMIA* guts were empty. Afterward, 3000 adults *ARTEMIA* were counted and transferred to conical glass containers with a volume of 1 L of seawater at salinity 30 g L^{-1}. Then, 3 mL of the prepared emulsion was added to the enrichment vessel at zero and after 3 h. This procedure was carried out at 27 ℃ for 6 h.

2.2. *EXPERIMENTAL diets AND fish HUSBANDRY*

Green terror cichlids were obtained from an ornamental fish farm in Urmia, Iran. Before beginning the experimental phase of the study, a group of fish (900 fish) was acclimatized with receiving commercial feed (FFT pellets, 2 mm pellet size, BioMar, France) during 7 days. From this group, a total of 810 fish (25 fish per each container) with a mean initial body weight of 3.1 g ± 0.2 (mean ± standard deviation) were randomly distributed into 27 glass aquaria (working volume: 80 L). The experimental groups were fed 5 different levels (0, 25, 50, 75, and 100%) of either lecithin-enriched *ARTEMIA* (EA) or un-enriched *ARTEMIA* (UA). As for pre-test, the dietary intake based on *AD libitum* was achieved through using live adult *ARTEMIA* and commercial food (FFT pellets, 2 mm pellet size, BioMar, France). We found 8% (approximately 12 *ARTEMIA* per gram of fish) and 3% of the body weight for adult *ARTEMIA* (8 mm in size) and commercial food, respectively. These fixed levels of *ARTEMIA* and inert diet were used in our experiments. We in- creased the proportion of Adult *ARTEMIA* in our replacement treatments. Green terror cichlids received live *ARTEMIA* or the commercial diet four times per day. Throughout the experimental period, the following environmental features were measured with a Hach's multi-parameter (Model DR1900-01H and HQ40D portable multi meter): temperature at 26.0 ± 1.5 ℃; dissolved oxygen at 8.10 ± 1.20 mg L^{-1}; pH at 7.6 ± 0.50 units; hardness > 120 mg L^{-1} of $CaCO_3$ with 50% water change per day, and photoperiod was set at 12:12 (light: darkness). At the onset of the study, day 18, 54 and 90, a number of fish were an- esthetized with 200 mg L^{-1} clove powder. Using a caliper and a digital balance, we recorded the total body length (BL, nearest 0.01 mm) and body weight (BW, nearest 0.01 g), respectively.

2.3. Reproductive PERFORMANCE

Fish reached maturity at the end of the study; therefore, three pairs of fish which were ready to spawn from each experimental replicate were selected, weighed, and transferred to a new glass aquarium (1 mating pair per aquarium) containing 40 L of water similar to their counterparts measured in the nutritional trial stage of the study. Water was obtained from two 300 L tanks that had been aerated *VIA* a central pump for 24 h in order to remove its chlorine. At the aquarium floor, several small rocks were placed for spawning the fish. To prevent fungal infections of eggs, methylene blue (Merck, Germany) was added to each aquarium at concentration of 1 mg L^{-1}. Twenty hours after mating by counting the number of developing eggs (blastopore closing stage), the fertilization rate was determined in line with Woynarovich and Horváth (1980). In brief, a glass tube (30 cm) whose diameter was 30–50% thicker than an egg was filled with eggs. Then, viable and developed and non-viable (white, opaque, and have turbid contents) eggs in the tube were counted (Langroudi et al., 2009).

We determined the hatching rate by counting the total number of newly hatched larvae and unhatched after a lapse of 60 to 72 h. Moreover, 6–7 days later, the number of active swimming larvae was used for computing the larval survival rate considering the initial number of hatched specimens. In addition, 20 larvae per replicate were selected to measure their BW and total length (TL) at hatching and the onset of the active swimming behavior. The pairs of fish were kept in similar conditions for forty days to check the number of spawns. Moreover, at the end of the experiment, three mature and un-spawned fish (these fish were different from those used for studying spawning episodes) from each replicate were anesthetized with clove powder solution (200 mg L^{-1}) and weighed. Gonads were removed and weighted to determine the gonadosomatic index (GS). For each group of fish, the following factors were calculated: the gonadosomatic index (GS, %) = [GW(g) / TW (g)] × 100; relative fecundity (RF) = EN / BW (g); total fecundity (TF) = EG; and average egg weight (EW, g) = EW (g)/ TF; where GW was the gonad weight, BW was the body weight, EN was the number of eggs, and EG was the number of eggs in the spawn. In order to determine egg diameter, a sample of 25 to 30 eggs of ripe females (maturity stage IV) were randomly taken from the females' ovaries and measured under a microscope with an ocular micrometer (Jamali et al., 2016).

2.4. Determination of pancreatic enzymes

2.4.1. Sampling and crude extract preparation

The activities of α-amylase, bile-salt activated lipase, and total al- kaline proteases were measured in order to evaluate the effects ofcommercial diet fasted for 24 h and, then, six fish per treatment were sacrificed with a lethal dose of clove powder (500 mg L−1). The abdominal cavity of the selected fish was cut from the mouth to the anus by means of a scalpel and the whole digestive tract was removed and washed by physiological serum. Dissected digestive tracts until being extracted were stored into 5 mL microtubes at e80C degree (Lemieux et al., 1999). We homo- genized the whole digestive tract in 50 mM Tris-HCl buffer (pH = 7.5) with the help of a homogenizer (Polytron PT, 1300 D model, Kinema- tica, Switzerland) and centrifuged it at 10,000g and 4 °C for 20 min. The supernatant was collected, divided into 0.5 mL microtubes, and stored at −80 °C for further analyses (Chong et al., 2002).

2.4.2. Enzyme assays

Total alkaline protease activity was determined using azocasein 2% in Tris-HCl (pH = 7.5) according to the method presented by Imani et al. (2017). In short, 20 μL of crude homogenate was mixed with 0.5 mL of 2% azocasein (pH = 7.5) and incubated (10 min at 25 °C). Finally, the reaction was stopped by adding 0.5 mL of trichloroacetic acid. The stirred mixture was centrifuged (6500 *g*, 5 min), optical density was noted at λ = 440 nm, and the specific enzyme activity was expressed as unit mg pro- tein^{-1} min^{-1}. We assayed bile-salt activated lipase while subscribing to Iijima et al.'s (1998) method. The mixture of 2-methoxy-ethanol, sodium cholate, *p*-nitrophenyl myristate, and Tris–HCl (pH = 9.0) was used as a substrate. The mixture of substrate and crude homogenate were incubated (15 min at 30 °C) and then the reaction was stopped using acetone/n-heptane (5:2, *v*/v). The stirred mixture was centrifuged at 6000 *g* for 2 min and the optical density was noted at λ = 405 nm. The enzyme activity was reported as μmol n-nitrophenol mg^{-1} $\mathrm{protein}^{-1}$ min^{-1} (one unit). Alpha- amylase activity was measured using the hydrolysis of starch (Imani et al., 2017). The mixture of 1% starch solution (*w*/*v*) and 0.02 M sodium phosphate (pH = 6.9) was used as substrate. Crude enzyme extracts were incubated with substrate for 4 min at 25 °C. The reaction was terminated by adding of di-nitrosalicylic acid solution (1% w/v), boiled for 5 min, and cooled down at room temperature. The specific activity of α-amylase was noted at λ = 540 nm and expressed as micromole maltose mg $\mathrm{protein}^{-1\ \mathrm{min}^{-1}}$ at 25 °C. In assaying the total soluble protein, bovine serum albumin was used as a standard according to the method used by Bradford (1976). All assays were carried out in triplicate (methodological replicates).

2.5. Chemical analysis

Experimental diets (commercial diet, lecithin-enriched and un- enriched *Artemia*) were analyzed for their proximate and lipid class composition according to the methods used by the AOAC (1997) and Olsen and Henderson (1989), respectively. All measurements were performed in methodological triplicate.

2.6. Statistical analyses

All statistical analyses were carried through SPSS (21 version) (IBM Statistics). At first, we verified the normal distribution and homo- geneity of the data with Kolmogorov–Smirnov and Levene's tests, re- spectively. Arcsine transformations were conducted in case of all data and were expressed in terms of percentages. Any significant among experimental groups were elucidate using two-way ANOVA. Tukey's HSD test was used for comparisons if significant differences were de- tected among groups. Statistically significant differences were considered at $P < .05$.

The data of reproductive performance and enzyme activity were subjected to regression analysis

(quadratic) where the *ARTEMIA* level served as the independent variable. The polynomial regression model was used to estimate the appropriateness of *ARTEMIA* level for green terror on reproductive performance (Robbins et al., 1979). Using the Pearson Product Moment, we found the degree of correlation in reproductive performance data among the groups similar to Sigma-Stat. (1995). The data were further analyzed by means of linear regression between the two variables with statistical correlation (Gisbert et al., 2000).

3. Results

3.1. *Diet composition*

Proximate and lipid class compositions of the tested diets are dis- played in Table 1 and Fig. 1. The amounts of crude lipid in the lecithin- enriched and unenriched groups were 19.7% and 16.1%, respectively ($P < .05$). The amount of crude ash declined in the enriched-live prey. As far as dry matter and crude protein content are concerned, there was an insignificant difference between the two groups ($P > .05$). Furthermore, the amount of total polar lipid increased from 39.2% in the unenriched *ARTEMIA* to 43.7% in the lecithin-enriched *ARTEMIA* ($P < .05$), whereas the lowest and highest amounts of polar lipid levels (3.7%) and natural lipids (95.88%) were recorded in the inert diet (see Fig. 1).

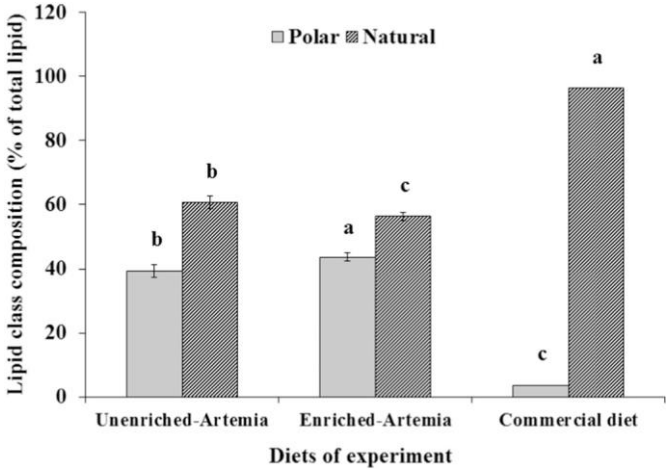

Fig. 1. Lipid class composition of experimental diets (commercial diet, lecithin- enriched, and unenriched adult *A. FRANCISCANA*) (mean ± SD; $n = 3$). Different letters in each same bars indicate significant differences by Tukey's test ($P < .05$).

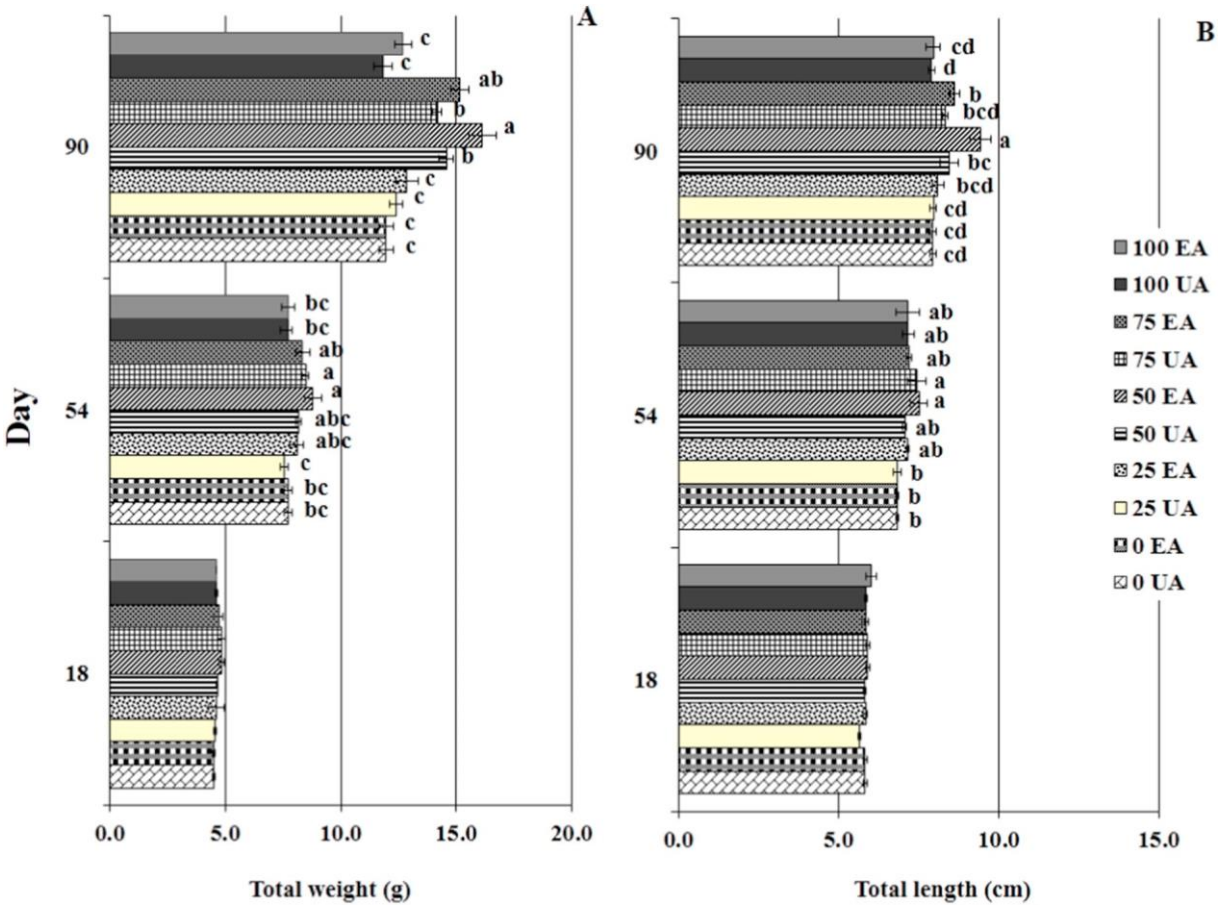

Fig. 2. Total weight (**A**) and total length (**B**) of green terror fed with different dietary ratios of unenriched (UA) and lecithin-enriched (EA) adult *A. FRANCISCANA* for 90 days (mean ± SD; $n = 3$). Different letters in each day indicate significant differences by Tukey's test ($P < .05$).

3.2. *Growth PERFORMANCE PARAMETERS*

According to the results of the two-way ANOVA regarding total weight (P, 0.023; r^2, 0.936) and total length (P, 0.001; r^2, 0.855) at the end of three months were influenced by the interaction of *ARTEMIA* adult enrichment and commercial diet replacement with *ARTEMIA*. There was no significant difference in the green terror total weight recorded be- tween days 0 and 18 ($P > .05$, see Fig. 2 A). At 54 and 90 days, there were significant differences among green terror total weights ($P < .05$). The findings also demonstrated that the total weights in green terror increased in the group fed with lecithin-enriched *ARTEMIA,* and it was found to be significantly higher compared to the un-enriched *ARTEMIA* group ($P < .05$), and 50 EA treatment was significantly higher compared to other treatments ($P < .05$) (Fig. 2 A). There was no sig- nificant difference in the total length of green terror between days 0 and 18 ($P > .05$, Fig. 2 B). At 54 and 90 days, there were significant dif- ferences among green terror total lengths ($P < .05$). The analyses showed that the total length in green terror increased in the group fed lecithin-enriched *ARTEMIA,* and it was significantly higher compared to the un-enriched *ARTEMIA* group ($P < .05$), and 50 EA treatment was significantly higher compared to other treatments ($P < .05$) (Fig. 2 B).

3.3. Activity of pancreatic digestive enzymes

3.3.1. Total alkaline proteases

The two-way ANOVA results in the case of the activity of total al- kaline proteases in different experimental groups are shown in Table 2. The activity of total alkaline proteases was influenced by the interaction between the enrichment of the adult *Artemia* ("enrichment") and the replacement of the commercial diet with *Artemia*. The findings showed that the specific activity of total alkaline proteases in green terror in- creased in the groups fed lecithin-enriched *Artemia*, whereas, in those fed with 75% EA (0.304 ± 0.02 U mg protein^{-1} min^{-1}), it was sig- nificantly higher compared to the other groups (Figs. 3, $P < .05$). Be- sides, the lowest total alkaline proteases activity was observed in the fish fed 25% (0.145 ± 0.01 U mg protein^{-1} min^{-1}) and 0% (0.141 ± 0.01 U mg protein^{-1} min^{-1}) *Artemia* groups (Fig. 3).

3.3.2. Alpha-amylase

The two-way ANOVA results showed that the specific activity of α- amylase activity in green terror was significantly affected by *Artemia* enrichment and replacement factors (see Table 2). Therefore, fish fed 100 and 0% level of *Artemia* replacement had significantly higher (27.08 ± 9.94 U mg protein^{-1} min^{-1}) and lower (17.16 ± 1.28 U mg protein^{-1} min^{-1}) α-amylase activity values, respectively, compared to the other groups ($P < .05$, Fig. 4 A). Besides, the highest α-amylase activity was obtained in the enriched group (Fig. 4 B).

3.3.3. Bile-salt activated lipase

Based on two-way ANOVA results in Table 2, the lipase enzyme was only affected by the enrichment of the adult *Artemia*. There were no significant differences in bile-salt activated lipase among replacement treatments ($P > .05$) (Fig. 4 C). Nevertheless, the enrichment factor had significant effects on bile-salt activated lipase (Fig. 4 D).

3.3.4. Relationship between digestive enzymes activity and Artemia replacement

The α-amylase activity was quadratically related to the increase of *Artemia* percentage in both lecithin-enriched and unenriched dietary treatments. These activity values generally increased with *Artemia* re- placement up to 100%. Based on PNR analysis, the maximum activity was observed in 100% replacement of lecithin-enriched *Artemia* and 86% replacement of unenriched *Artemia* (Fig. 5 A). However, the re- lationship between bile-salt activated lipase and unenriched and en- riched *Artemia* replacement percentages were not statistically sig- nificant (Fig. 5 B).

The relationship between alkaline proteases activity and levels of *Artemia* replacement was not statistically significant in the unenriched- *Artemia* groups. Based on the PNR analysis, the maximum total alkaline proteases activity was observed in 85% lecithin-enriched *Artemia* (Fig. 5 C).

3.4. Reproductive performance

3.4.1. Reproductive indices in adult fish

The two-way ANOVA results of the variables related to the re- productive performance of green terror in different experimental groups are shown in Table 3. Gonadosomatic index, absolute fecundity, relative fecundity, and egg weight were influenced by the interaction between adult *Artemia* enrichment and commercial diet replacement with *Artemia*, whereas the other factors were only impressed by one dietary factor ("replacement" or "enrichment").

The highest and lowest values for GSI were observed in broodfish in 50% of the EA group (5.8% in females and 0.56% in males) and 100% of the UA group (1.25% in for females and 0.07% in males), respec- tively

(Fig. 6).

The best results of relative and absolute fecundity values were ob- tained in those groups co-fed EA and UA and the inert commercial diet, regardless of the combination of live prey and the inert diet (Table 4). The administration of only UA resulted in the lowest relative fecundity and absolute fecundity values, whereas the rest of the treatments (EA and CD) showed intermediate values with regard to those feeding re- gimes containing *ARTEMIA* and the CD ($P < .05$).

In addition, egg weight was significantly impressed by the interac- tion between the enrichment and replacement percentages ($P < .05$). In particular, the heaviest eggs were found in the broodfish fed with 50% EA, 75% UA, and 75% EA feeding regimes, whereas the smallest ones belonged to 100% CD and 25% UA groups. The other feeding regimes resulted in intermediate egg weight values in the above-men- tioned dietary treatments.

Hatching and larval survival rates, as well as the time between two spawning episodes, were significantly affected by the "replacement" factor (Table 3; $P < .05$). In addition, the hatching rate was affected by the "enrichment" factor (Table 3; $P < .05$). The best hatching and larval survival rates were found in the 50% replacement group in each of UA and EA (Table 5). The shortest time between two spawning episodes was observed in broodfish in the 50 and 75% replacement factor groups, whereas the longest interval was observed in 0 and 100% replacement factors groups (Table 5; $P < .05$).

Although fertilization rates and egg diameter were not affected by the "enrichment" factor (Table 5, $P > .05$), the egg diameter was in- fluenced by the "replacement" factor. The highest eggs in term of size were found in the broodfish fed with 50% CD replaced by each of UA and EA, whereas the smallest ones were those in 0% UA and 0% EA (100% CD) groups.

3.4.2. *Reproductive indices in LARVAE*

The two-way ANOVA results of larval TL and BW at hatching and 170 h after hatching (hah) in different experimental groups are shown in Table 6. All parameters were influenced by the interaction between "enrichment" and "replacement" factors. In particular, larval TL and BW at hatching and 170 hah in different dietary experimental groups are shown in Table 7. Larval TL and BW at hatching were lowest (3.1 mm and 6.6 mg) and highest (4.7 mm and 9.4 mg) for those fed 100% CD and 50% EA, respectively. At 170 hah, the lowest and highest total length and weight were recorded in the 100% CD and 50% EA groups, respectively.

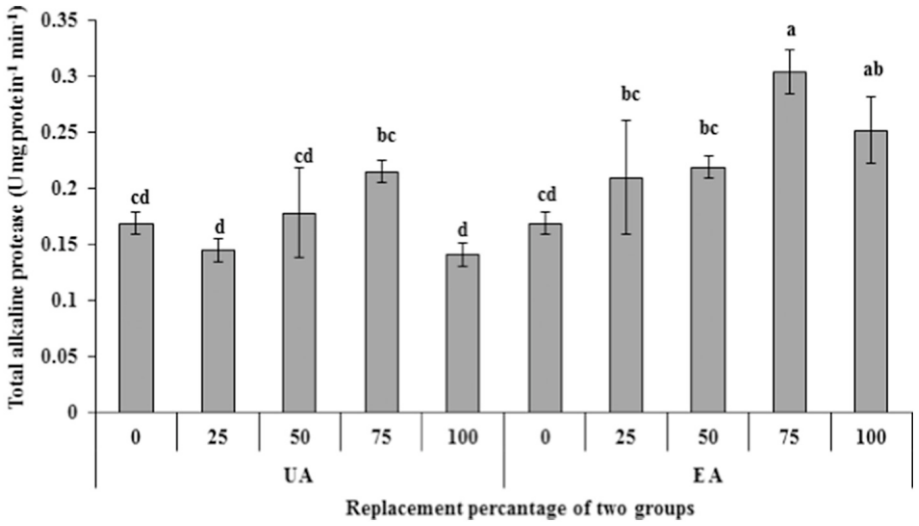

Fig. 3. Total alkaline proteases activity of green terror fed with different dietary ratios of unenriched (UA) and lecithin-enriched (EA) adult *A. FRANCISCANA* for 90 days (mean ± SD; $n = 3$). Different letters in bars indicate significant differences by Tukey's test ($P < .05$).

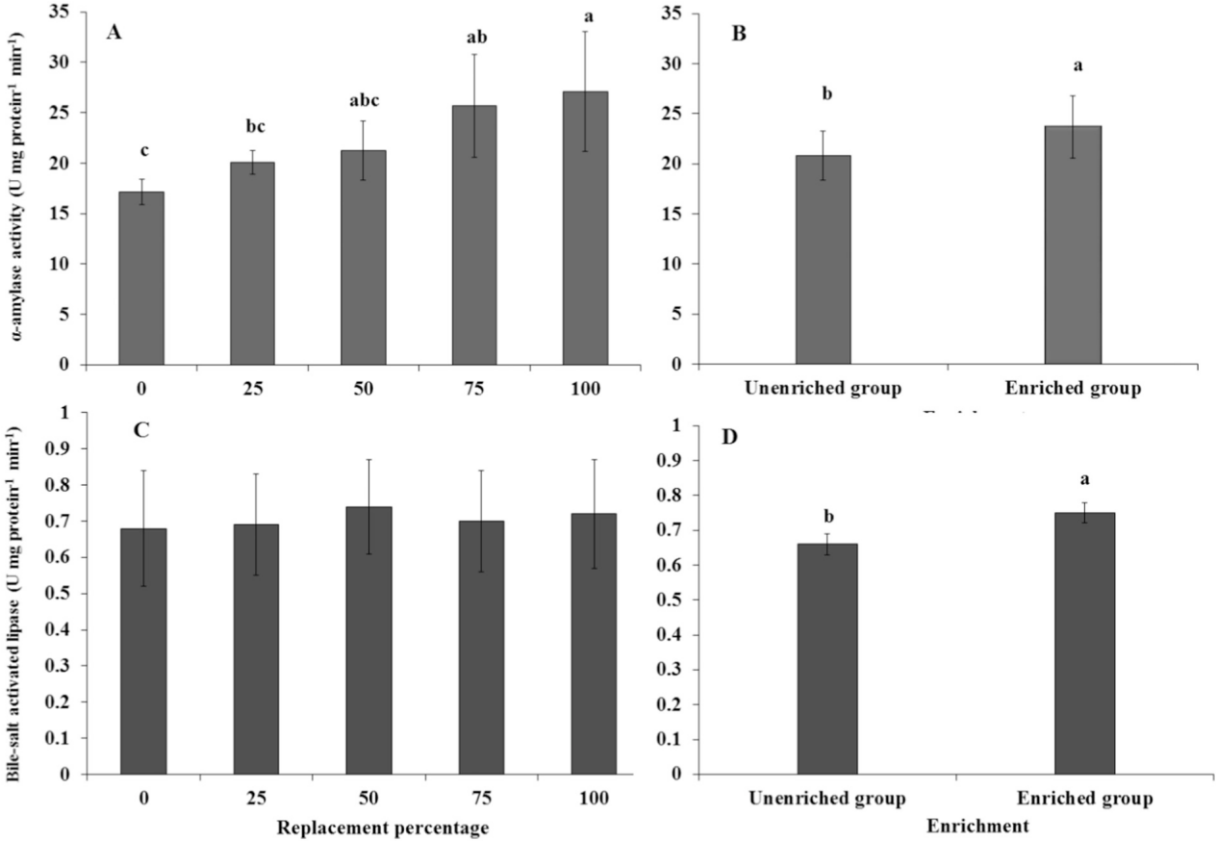

Fig. 4. α-amylase activity (A and B) and Bile-salt activated lipase (C and D) of green terror fed with different dietary in replacement (A and C) and enrichment (B and D) groups. Different letters in bars indicate significant differences by Tukey's test ($P < .05$).

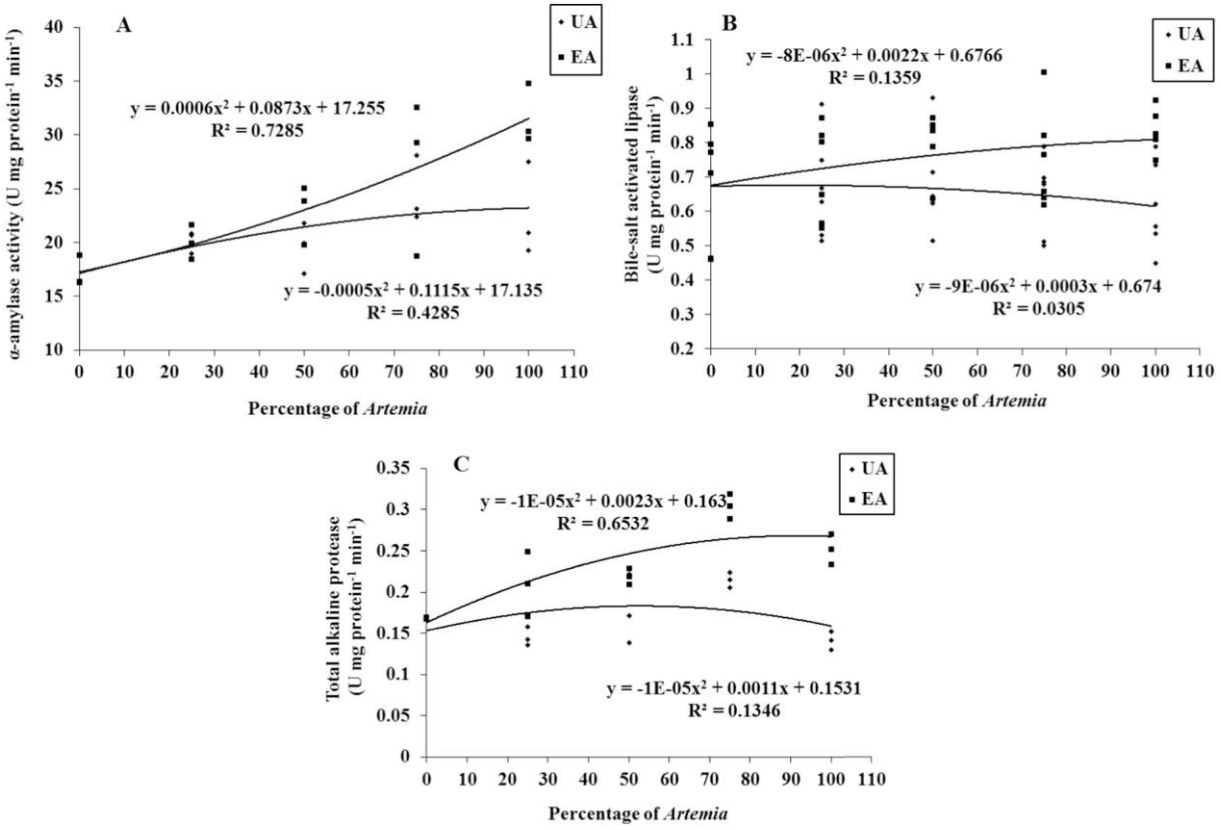

Fig. 5. Polynomial regression analysis of α-amylase activity (A), bile-salt activated lipase activity (B), and total alkaline proteases activity (C) in groups fed with lecithin-enriched (EA, ■) and unenriched (UA, ◆) treatments.

3.4.3. Relationships between reproductive indices

A positive and significant correlation was detected between gona- dosomatic index with absolute fecundity, relative fecundity, egg dia- meter, and egg weight; absolute fecundity with egg diameter and egg weight; relative fecundity with egg diameter and egg weight; and egg diameter with egg weight values of the experimental fish in lecithin- enriched groups (Table 8). However, in unenriched (UA) groups only a positive and significant correlation was found between gonadosomatic index with egg diameter as well as egg diameter with egg weight values (Table 8). The relationships between other indices were not statistically significant in the unenriched (UA) *A. francisCana* treatments (Table 8). A positive and significant correlation was detected between hatching and larval survival rates of experimental fish in both un- enriched and enriched groups. Also, a correlation between larval body weight, total length, and egg diameter was obtained from that those feeding both unenriched and lecithin-enriched *A. francisCana* (Figs. 7). Based on the PNR analysis and considering different feeding regimes and reproductive indices such as absolute fecundity, relative fecundity, fertilization and hatching rate it was suggested thatthe optimal levels of lecithin-enriched *Artemia* were 54, 53, 54, 56, and 56% (average value = 54.6 ± 1.3%), whereas, for the unenriched *Artemia*, they were 46, 46, 56, 50, and 53% (average value = 50.2 ± 4.4%) (Table 9).

4. Discussion

In this study, the use of soybean lecithin bioencapsulated in adult *A. FRANCISCANA* and its combination with an inert commercial diet improved the pancreatic digestive enzymes, and reproductive performances of terror cichlid. This feeding strategy (combination of a commercial diet with *ARTEMIA*) is commonly used in ornamental fish hatcheries, although authors considered that there is room for the nutritional improvement of diets, especially in terms of PL content. As *ARTEMIA* has non-selective and continuous feeding behavior, its nutritional value may be modu- lated in order to fit the nutritional requirements of the desired fish species (Dhert, 1991; Lim, 2003). In the current study, the crude lipid content in adult *ARTEMIA* increased by 22.36%, whereas its PL content increased by 11.47%. Similar results were reported in liposomes-en- riched *ARTEMIA* (Monroig et al., 2003, Guinot et al., 2013b) with 12.8, 53.44% and 21.6% increase in crude lipid and PL, although the mag- nitude of the increase can be depended on the stage of development *AND* different strain of *ARTEMIA*.

In the current study, the administration of PL-enriched *ARTEMIA* in combination with a commercial diet, in 50% EA groups, resulted in 34.17% increase in growth performance, in comparison with the 0% in the replacement group. These results may be attributed to the fact that

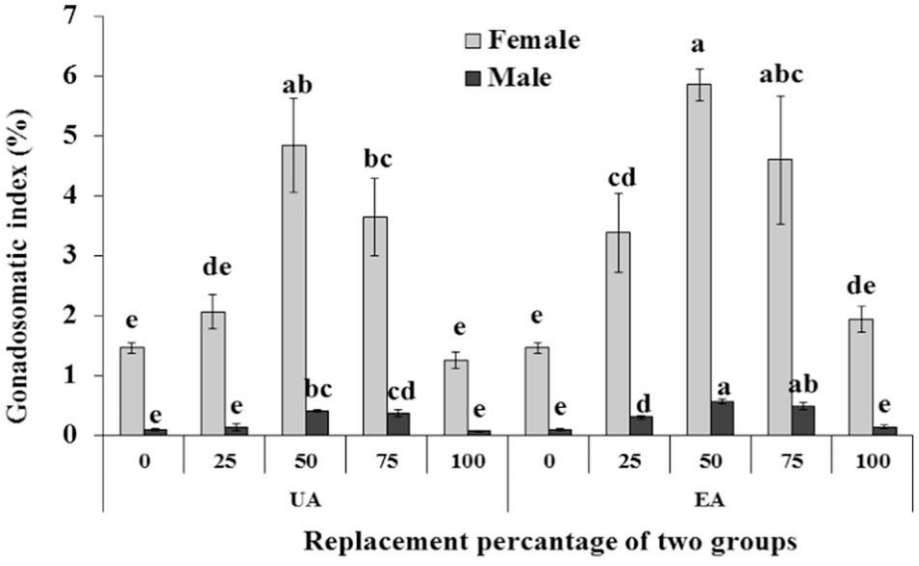

Fig. 6. Gonadosomatic index (GSI) of green terror (male and female) fed with different dietary ratios of unenriched (UA) and lecithin-enriched (EA) adults *A. FRANCISCANA* for 90 days (mean ± SD; $n = 3$). Different letters in each same bars indicate significant differences by Tukey's test ($P < .05$).

crude lipid and PL levels increased in enriched *ARTEMIA* compared to the commercial diet. In addition, feeding behavior was modified in terror cichlid when enriched *ARTEMIA* was offered. As the fish were growing, their food intake rate enhanced in comparison to those in the 0% re- placement group (the data is not shown). These results may be due to the higher palatability of this type of live prey (Øie et al., 2011) and attractant effect of phosphatidylcholine that was previously reported by and Harada et al. (1987) and Koven et al. (1993) in seriola (*SERIOLA LALANDI*) and gilthead sea bream (*SPARUS AURATA*), respectively. Further- more, Jamali et al. (2018) found that the combination of *ARTEMIA* with an inert diet could result in increased feed intake as well as an en- hancement of food digestibility. However, feeding terror cichlid with just enriched or unenriched *ARTEMIA* did not result in the best growth performance. In Senegalese sole (*SOLEA SENEGALENSIS*) and cobia (*RACHY- centron CANADUM*) larvae (Engrola et al., 2009; Nhu et al., 2010; Mai et al., 2009) and freshwater prawn *MACROBRACHIUM rosenbergii* (Anh et al., 2009), the co-fed with *ARTEMIA* and a micro-diet had the best effects on growth performance. Moreover, it was reported that dietary PLs may promote somatic growth by supplying energy, enhancing the efficiency of lipid utilization by their emulsification and digestion,increasing lipid transport between organs, and supplying phosphati- dylcholine with a growth-promoting effect (Kasper and Brown, 2003; Geurden et al., 1998; Shields et al., 1999; Tocher et al., 2008; Zhu et al., 2018).

Previous studies using lecithin as lipid emulsifiers in the aquafeeds improved lipid emulsification and enhanced lipid digestion and ab- sorption (see the review in Tocher et al., 2008). It was reported that some species of fish only at larval and early juvenile stages may have a limited capacity to biosynthesize of PLs (Saleh et al., 2013). It is no- teworthy that older fish have not been investigated in this respect. As the authors postulated, the requirement for intact phospholipids in fish feeding is extremely low levels of PLs (Tocher et al., 2008).

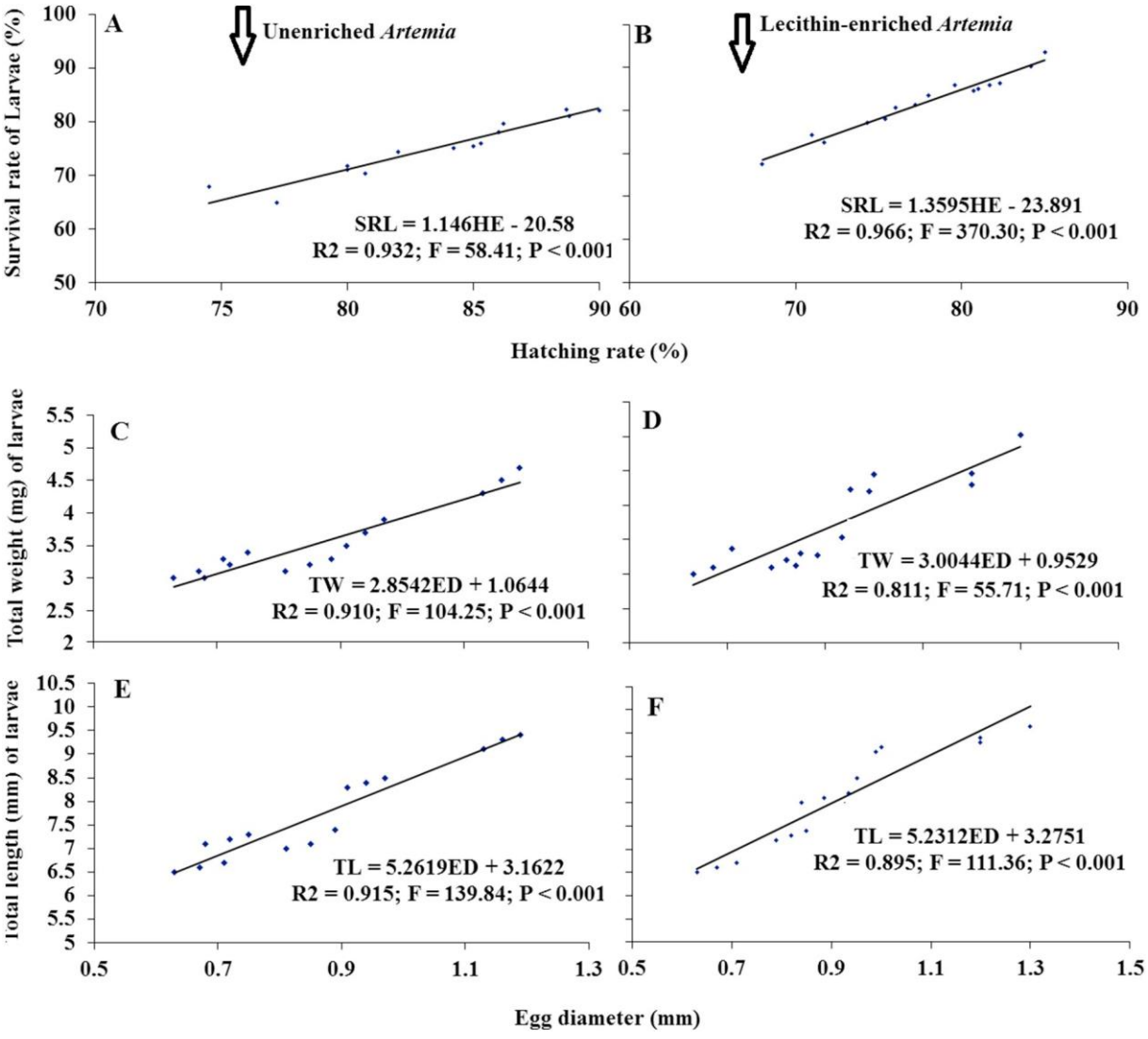

Fig. 7. Linear regression equations and the relationship between hatching rate and larval survival rate (A: unenriched and B: lecithin-enriched group); total weight of larvae and egg diameter (C: unenriched and D: lecithin-enriched group); total length of larvae and egg diameter (E: unenriched and F: lecithin-enriched group) in green terror fed with different dietary ratios of unenriched and lecithin-enriched adults *A. FRANCISCANA.*

Considering the main pancreatic digestive enzymes, the results varied depending on the enzyme considered. The changes in α-amylase activity were significantly correlated with the level of *ARTEMIA* inclusion in the feeding regime in green terror cichlid. In particular, the highest activity of α-amylase was observed in both groups fed unenriched- and lecithin-enriched *ARTEMIA*. As previous authors have reported, these results may be attributed to different carbohydrate content and source in live prey with regard to the inert diet (Ma et al., 2005; Gisbert et al., 2009). Regarding the bile-salt activated lipase, the enrichment factor played a significant role; however, it increased bile-salt activated lipase negligibly among the experimental treatments. These results may be attributed to 22.36% increase in the crude lipid in the enriched *ARTEMIA* compared to the un-enriched *ARTEMIA*, as the synthesis of this lipolytic enzyme is regulated by lipid classes (chain length and degree of sa- turation) and levels (Morais et al., 2004). Based on Fig. 1, the highest and lowest values of total alkaline protease activities may not be at- tributed to the different dietary proteins, as unenriched and enriched adult *ARTEMIA* contain similar level and quality of crude protein (50–51%). In particular, the above-mentioned different levels of total alkaline proteases may be linked to the effects of dietary fatty acids and phospholipid levels, especially lysophospholipids that act as emulsifiers in the intestine of fish, on the synthesis and secretion of pancreatic enzymes (Infante and Cahu, 1999; Gisbert et al., 2005; Rønnestad et al., 2014). In line with our study, Adel et al. (2017) reported that the use of soybean lecithin in the common carp (*Cyprinus CARPIO*) diet increased the activity of lipase (5.4%), amylase (6.8%), and protease (27%) en- zymes. The researchers reported that lecithin usually increases the se- cretion of enzymes, especially pancreatic enzymes, for digestion. This increase in the secretion of enzymes can improve the absorption of food by aquatic animals (Bakke-McKellep et al., 2000). In our study, the *ARTEMIA* encapsulated with soybean lecithin increased protease activity by $>$ 79%, and helped to enhance amylase and lipase activities ap- proximately by 13% in both when compared to the un-enriched *Ar- TEMIA*. Additionally, Gisbert et al. (2005) found that that the increased lipase activity in the fish fed with lecithin-based diet could be due to their increased capacity of using neutral lipids through phospholipids in fish.

In addition, unenriched and lecithin-enriched adult *ARTEMIA* com- bined with an inert diet was also beneficial in terms of reproductive performance. On-grown *ARTEMIA* appears to contain lipid compounds such as polyunsaturated fatty acids and peptides similar to sexual hormones that, in broodstock diets, can induce enhanced sexual ma- turity and, consequently, increased reproductive performance of aquatic animals (Gandy et al., 2007). In this study, reproductive para- meters improved after incorporating the enriched or unenriched adult *ARTEMIA* into the feeding regime of green terror cichlid. With regard to the incorporation of the unenriched *ARTEMIA,* the positive effects have been reported in shrimp (*L. VANNAMEI*) broodstocks (Naessens et al., 1997), *CARASSIUS AURATUS, Pterophyllum leopoldi* broodstocks (Tamaru and Ako, 2003), and L. *VANNAMEI* broodstock (Wouters et al., 2002). They suggested that on-grown *ARTEMIA* acted as a transferor for essential nutrients that were able to increase the reproductive performance in term of the fertilization rates, the number of spawning events, female's fecundity, sperm counts, and spermatophore weight. In line with our work, the positive effects of adult *ARTEMIA* in diets on reproductive performance of ornamental fish broodstocks has been well-documented in the golden corydoras catfish, Corydoras aeneus (Tamaru et al., 2000), the goldfish, Carassius auratus (Tamaru and Ako, 2003), the severum cichlid Cichlasoma severum (Langroudi et al., 2009), and angelfish Pterophyllum scalare (Langroudi et al., 2009).

There is no data about whether lecithin-enriched *ARTEMIA* was used in feeding regimes. Nonetheless, the impact of lecithin in diets on the reproductive indices of aquatic organisms are well documented (Bray et al., 1990; Cahu et al., 1994; Tocher et al., 2008; Sui et al., 2009). In

this study, the lecithin-enriched *ARTEMIA* increased the reproductive performance of green terror in term of fecundity, egg diameter, egg weight, hatching rates, and larval survival. In addition, dietary PLs have resulted in decreased interval between the two spawning events and increased larval size in body weight and total length at hatching and 170 hah. Although there is limited information on the effects of dietary PL on fish reproductive performance, in different shrimp species, si- milar results were observed (Cahu et al., 1994; Sui et al., 2009). The above-mentioned results may be attributed to the improvement of egg quality through vitellogenesis, as PLs may enhance lipid mobilization from the liver into the ovaries (Wu et al., 2007; Sui et al., 2009) and promote larval development due to a more efficient use of triacylgly- cerides at early larval stages (Tocher et al., 2008). Sui et al. (2009) and Alava et al. (1993) reported that in *Eriocheir sinensis* and *MARSUPENAEUS JAPONICUS* fed phospholipid affected lipid mobilization from the hepa- topancreas to the ovary. This could be a plausible explanation for the higher rate of GSI in our study. As shown in our findings, males and females fed 50 EA had 460% and 300% GSI, respectively, which were higher than those in the 0% replacement group. As pointed out by Cheng et al. (1998) and Wen et al. (2001), it seems that the dietary phospholipids are transported to the developing ovaries. In the same vein, Teshima and Kanazawa (1980) concluded that the triglycerides converted to phospholipids could enter the ovary. Furthermore, other studies showed that triglycerides can be considered as an energy sup- plier for embryo development (Kaitaranta and Ackman, 1981). There- fore, co-feeding with commercial food containing high triglyceride source (73.44%) and lecithin-enriched *ARTEMIA* containing high phos- pholipid source (43.72%) can improve the reproductive performance of green terror cichlid through increasing the transfer process of neutral lipids (triglyceride) by phospholipids.

5. Conclusion

The current study revealed that feeding strategy (combination of *ARTEMIA* with a commercial diet) improved the pancreatic digestive enzymes, and reproductive performances of green terror cichlid. Moreover, the administration of lecithin-enriched *ARTEMIA* in combina- tion with a commercial diet had positive effect on above factors. Based on PNR analysis of reproductive performance, the feeding regimes of green terror from 55.8 to 52.5% and 54.6 to 50.2%, respectively, of lecithin-enriched and unenriched *ARTEMIA* in combination with an inert diet could be recommended. Moreover, based on PNR analysis, the activity of α-amylase and total alkaline proteases was related to un- enriched and enriched *ARTEMIA* replacement percentages. On the other hands relationship between bile-salt activated lipase and unenriched and enriched *ARTEMIA* replacement percentages were not significant. This feeding strategy can be applied successfully in this freshwater or- namental fish culture.

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Table 1. Lipid class analysis of soybean lecithin (mean ± SD; n = 3).

Lipid class (% of total lipids)	Soybean lecithin
PC	32.5 ± 0.7
Pea	16.7 ± 0.2
PSe + PI	16.8 ± 0.3
LPC	1.4 ± 0.1
LPEa	nd
Unknown	6.9 ± 0.4
Total phospholipid	74.4 ± 0.2
Chl	0.97 ± 0.09
FFA	5.20 ± 0.12
TAG	2.46 ± 0.12

Abbreviation: nd, not detected; PC, Phosphatidylcholine; PEa, Phosphatidylethanolamine; PSe, Phosphatidylserine; PI, Phosphatidylinositol; LPC, Lysophosphatidylcholine; LPEa, Lysophosphatidylethanolamine; Chl, Cholesterol; FFA, Free fatty acids; TAG, Triacylglycerides

Table 2. Proximate composition of experimental diets (commercial diet, lecithin-enriched and unenriched *A. franciscana* adults) (mean ± SD; n = 3).

	Diets		
Composition	Unenriched *A. franciscana*	Lecithin-enriched *A. franciscana*	Commercial diet*
Dry matter (%)	12.1 ± 0.8^{b}	10.6 ± 0.9^{b}	91.0 ± 2.5^{a}
Crude protein (% DW)	51.1 ± 1.9	50.2 ± 2.6	48.0 ± 2.0
Crude lipid (% DW)	16.0 ± 2.3^{b}	19.7 ±1.1^{a}	13.0 ± 3.0^{c}
Ash (%DW)	20.7 ± 1.5^{a}	17.0 ± 3.2^{b}	11.0 ± 1.5^{c}

*The trade mark of commercial food was BioMar, France

Table 3. Lipid class composition of experimental diets (commercial diet, lecithin-enriched and unenriched *A. franciscana* adults) (mean ± SD; n = 3).

	Diets		
Lipid class (% of total lipids)	Unenriched *A. franciscana*	Lecithin-enriched *A. franciscana*	Commercial diet
PC	17.2 ± 1.8[a]	18.1 ± 0.5[a]	3.2 ± 0.3[b]
Pea	10.8 ± 1.2[b]	13.9 ± 0.6[a]	nd
PSe + PI	7.6 ± 0.2[a]	7.3 ± 0.1[a]	0.5 ± 0.0[b]
LPC	2.7 ± 0.0[a]	1.2 ± 0.1[b]	nd
LPEa	2.7±0.3	2.5 ± 0.6	nd
Unknown	nd	Nd	nd
Total phospholipid	39.2± 2.0[b]	43.7 ± 1.3[a]	3.7 ± 0.1[c]
Chl	23.8 ± 1.0[a]	19.4 ± 0.6[b]	7.1 ± 0.15[c]
FFA	21.9 ± 1.4[a]	20.9 ± 0.2[a]	5.6 ± 0.1[b]
TAG	7.8 ± 1.3[c]	9.8 ± 0.9[b]	73.4 ±0.3[a]

Abbreviations: nd, not detected; PC, Phosphatidylcholine; PEa, Phosphatidylethanolamine; PSe, Phosphatidylserine; PI, Phosphatidylinositol; LPC, Lysophosphatidylcholine; LPEa, Lysophosphatidylethanolamine; Chl, Cholesterol; FFA, Free fatty acids; TAG, Triacylglycerides.

Table 4. Two-way ANOVA output for growth of green terror cichlid (*A. rivulatus*) fed different experimental groups at the end of the experiment (p-values).

Parameters	Replacement regime	Enrichment	Replacement regime × Enrichment	r^2
BW_f	0.001	0.001	0.023	0.936
TL_f	0.001	0.001	0.001	0.855
SGR	0.001	0.001	0.001	0.895
WG	0.001	0.001	0.001	0.897

BW_f, body weight final; TL_f, total length final; SGR, specific growth rate; WG, weight gain

Table 5. Survival and growth performance parameters of green terror cichlid (*Aequidens rivulatus*) fed different dietary ratios of commercial diet (CD), unenriched (UA) and lecithin-enriched (EA) *A. franciscana* for 90 days (mean ± SD; n = 3).

Experimental groups		Growth parameters				
Enrichment	Replacement regime	BW_f	TL_f (cm)	SGR (% day^{-1})	WG (%)	SR (%)
UA	0	12.0 ± 0.3c	7.9 ± 0.1cd	1.51 ± 0.05d	290.6 ± 17.2d	92.2 ± 1.9
	25	12.4 ± 0.2c	7.9 ± 0.1cd	1.54 ± 0.04d	300.2 ±14.3d	93.3 ± 3.3
	50	14.6 ± 0.3b	8.5 ± 0.2bc	1.76 ± 0.02ab	385.6 ± 10.7ab	96.7 ± 3.3
	75	14.2 ± 0.2b	8.3 ± 0.0bcd	1.69 ± 0.02bc	357.0 ± 6.7bc	93.3 ± 3.3
	100	11.8 ± 0.4c	7.9 ± 0.1d	1.49 ± 0.03d	283.5 ± 9.08d	92.2 ± 1.9
EA	0	12.0 ± 0.3c	7.9 ± 0.1cd	1.51 ± 0.05d	290.6 ± 17.2d	92.2 ± 1.9
	25	12.9 ± 0.4c	8.1 ± 0.1bcd	1.61 ± 0.06cd	324.4 ± 21.7cd	94.4 ± 1.9
	50	16.1 ± 0.5a	9.4 ± 0.3a	1.86 ± 0.05a	432.1 ± 24.7a	95.6 ± 1.9
	75	15.2 ± 0.4ab	8.6 ± 0.0b	1.76 ± 0.03ab	386.4 ± 15.1ab	93.3 ± 3.3
	100	12.7 ± 0.3c	7.9 ± 0.2cd	1.58 ± 0.05cd	314.4 ± 19.67cd	94.4 ± 1.9

Different letters on the each column indicate significant difference by Tukey's test ($P < 0.05$). *Abbreviations*: BW_f, final body weight; TL_f, final total length; SGR, specific growth rate; WG, weight gain; SR, survival rate.

Table 6. Polynomial regression (PNR) analysis of BW_f, TL_f, SGR and WG and suggested percentage of *Artemia* replacement.

Groups		Investigated factors[1]			
		BW_f	TL_f	SGR	WG
Lecithin-enriched *Artemia*	P, r^2	0.001,0.748	0.004, 0.601	0.001, 0.743	0.001, 0.723
	F	17.794	9.021	17.377	15.642
	A	11.447	7.764	1.478	275.370
	b_1,b_2	0.140, -0.001	0.046,-0.0004	0.011, -0.0001	4.62, -0.0418
	Suggested of *Artemia*	54%	57%	57%	55%
Unenriched-*Artemia*	P, r^2	0.001, 0.708	0.017, 0.491	0.001, 0.671	0.001, 0.667
	F	14.578	5.786	12.245	12.012
	A	11.527	7.844	1.472	274.699
	b_1,b_2	0.099, -0.001	0.019, -0.0002	0.009, -0.0000	3.377, -0.0321
	Suggested of *Artemia*	55%	47%	55%	53%

Abbreviations for this table are similar to those presented in Table 4.

Table 7. Two-way ANOVA output for activity of pancreatic digestive enzymes of green terror cichlid (*A. rivulatus*) fed different experimental groups at the end of the experiment (p-values).

Parameters	Replacement regime	Enrichment	Replacement × Enrichment	r^2
α-amylase	0.001	0.026	0.148	0.585
Bile-salt activated lipase	0.800	0.008	0.389	0.072
Total alkaline proteases	0.001	0.001	0.004	0.828

Table 8. Two-way ANOVA output for reproductive of green terror cichlid (*A. rivulatus*) fed different experimental groups at the end of the experiment (p-values).

Parameters	Replacement regime	Enrichment	Replacement × Enrichment	r^2
GSI_f	0.001	0.001	0.024	0.907
GSI_m	0.001	0.001	0.007	0.955
AF	0.001	0.001	0.012	0.877
RF	0.001	0.001	0.026	0.845
EW	0.001	0.148	0.046	0.858
ED	0.001	0.180	0.421	0.815
F	0.056	0.160	0.898	0.153
TS	0.006	0.073	0.402	0.390
H	0.001	0.039	0.826	0.455
SRL	0.002	0.077	0.911	0.403

GSI_f, Gonadosomatic index of female; GSI_m, Gonadosomatic index of male; AF, Absolute fecundity; RF, relative fecundity; ED, egg diameter; EW, egg weight; TS, time between two spawning; F, Fertilization; H, hatching; SRL, larval survival

Table 9. Absolute fecundity (AF), relative fecundity (RF) and egg weight (EW) of green terror cichlid fed different dietary ratios of commercial diet (CD), unenriched (UA) and lecithin-enriched (EA) *A. franciscana* for 90 days (mean ± SD; n = 3).

Experimental groups		Reproductive factors		
Enrichment	Replacement regime	AF (egg number)	RF (egg number g^{-1})	EW (mg)
UA	0	215 ± 35bc	16.9 ± 2.9b	0.81 ± 0.11d
	25	300 ± 20ab	24.2 ± 3.7a	0.88 ± 0.08d
	50	325 ± 25ab	25.4 ± 3.7a	1.89 ± 0.18a
	75	373 ± 55ab	28.6 ± 2.3a	1.37 ± 0.17bc
	100	100 ± 32d	8.5 ± 2.5c	1.20 ± 0.15bcd
EA	0	215 ± 35bc	16.9 ± 2.9b	0.81 ± 0.11d
	25	410 ± 45a	30.3 ± 3.5a	1.54 ± 0.17ab
	50	410 ± 30a	31.6 ± 1.9a	1.95 ± 0.22a
	75	340 ± 40ab	26.1 ± 1.7a	1.29 ± 0.22bcd
	100	253 ± 15bc	19.4 ± 2.8b	1.00 ± 0.13cd

Different letters on the each column indicated significant difference by Tukey's test ($P < 0.05$)

Table 10. Fertilization (F, %), hatching (H, %) and larval survival rates (SRL, %), egg diameter (ED), and time between two spawning (TS) of green terror cichlid fed different dietary ratios of commercial diet (CD), unenriched (UA) and lecithin-enriched (EA) *A. franciscana* for 90 days (mean ± SD; n = 3).

Experimental groups		Reproductive factors				
		F (%)	H (%)	SRL (%)	TS (days)	ED (mm)
Replacement regime	0	89.67 ± 3.51	80.67 ± 3.51c	70 ± 5c	14 ± 0.89a	0.67 ± 0.08d
	25	90.83 ± 2.86	86.67 ± 3.76abc	78 ± 4.60abc	12.5 ± 1.04b	0.79 ± 0.1cd
	50	94.33 ± 3.44	90.67±3.01a	84 ± 5.55a	11.5 ± 1.14b	1.18 ± 0.09a
	75	94.83 ± 2.92	88.83 ± 3.63ab	80.5 ± 5.79ab	11.5 ± 1.43b	0.96 ± 0.06b
	100	93 ± 3.85	82.50 ± 2.92bc	73.83 ± 6.43bc	13.3 ± 1.73ab	0.83 ± 0.07bc
Enrichment	UN group	91.8 ± 3.14	84.27 ± 5.03b	75.4 ± 7.03	12.98 ± 1.27	0.87 ± 0.19
	EN group	93.6 ± 3.79	87.47 ± 5.41a	79.13 ± 6.88	12.14 ± 1.74	0.91 ± 0.2

Different letters on the each column indicated significant difference by Tukey's test ($P < 0.05$)

Table 11. Linear regression and correlationship values between reproductive performance parameters of green terror cichlid fed different dietary ratios of commercial diet (CD), unenriched (UA) and lecithin-enriched (EA) *A. franciscana.*

	Parameter	R^2	F	*P*
Lecithin-enriched *Artemia*	GSI × AF	0.928	38.09	0.009
	GSI × RF	0.953	60.93	0.004
	GSI × ED	0.937	44.45	0.007
	GSI × EW	0.989	266.88	0.001
	AF × ED	0.805	12.42	0.039
	AF × EW	0.882	22.35	0.018
	RF × ED	0.840	15.70	0.029
	RF × EW	0.916	32.69	0.011
	ED × EW	0.975	1543.03	0.001
Unenriched- *Artemia*	GSI × AF	0.588	4.29	0.130
	GSI × RF	0.574	4.04	0.138
	GSI × ED	0.777	10.46	0.048
	GSI × EW	0.758	9.42	0.055
	AF × ED	0.155	0.551	0.512
	AF × EW	0.134	0.465	0.544
	RF × ED	0.145	0.510	0.527
	RF × EW	0.125	0.427	0.560
	ED × EW	0.998	116.17	0.002

Abbreviations for this table are similar to those presented in Table 6 and 7.

Table 12. Polynomial regression (PNR) analysis of AF, RF, FE, HE and SRL and suggested percentage of *Artemia* replacement.

Groups		Investigated factors[1]				
		AF	RF	FE	HE	SRL
Lecithin-enriched *Artemia*	P, r^2	0.035,0.965	0.020, 0.981	0.013, 0.987	0.009, 0.991	0.006, 0.994
	F	27.67	48.47	76.54	116.41	177.13
	a	205.83	16.33	89.89	80.38	70.26
	b_1,b_2	7.83, -0.072	0.574,-0.005	0.259, -0.002	0.428, -0.004	0.553, -.005
	Suggested of *Artemia*	54%	53%	54%	56%	56%
Unenriched-*Artemia*	P, r^2	0.203, 0.801	0.176, 0.823	0.015, 0.985	0.022, 0.978	0.067, 0.933
	F	3.92	4.67	66.99	45.25	14.02
	a	195.09	15.66	89.59	274.699	69.78
	b_1,b_2	7.29, -0.079	0.554, -0.006	0.135, -0.001	3.377, -0.0321	0.414, -0.004
	Suggested of *Artemia*	46%	46%	56%	50%	53%

Abbreviations for this table are similar to those presented in Table 6 and 7.

Table 13. Two-way ANOVA output for larval TL and BW at hatching and 170 hours after hatching of green terror cichlid (*A. rivulatus*) fed different experimental groups at the end of the experiment (p-values).

Parameters	Replacement regime	Enrichment	Replacement × Enrichment	r^2
BW_0	0.001	0.001	0.001	0.959
TL_0	0.001	0.001	0.001	0.974
BW_{170}	0.001	0.001	0.041	0.942
TL_{170}	0.001	0.001	0.006	0.980

BW_0, body weight at hatching time; TL_f, total length at hatching time; BW_{170}, body weight at 170 hours after hatching; TL_f, total length at 170 hours after hatching.

Table 14. Total length (TL) and body weight (BW) of newly hatched and 170-hours larvae of green terror cichlid fed different dietary ratios of commercial diet (CD), unenriched (UA) and lecithin-enriched (EA) *Artemia franciscana* for 90 days (mean ± SD; n = 3).

Experimental groups		Newly hatched larvae		170-hours larvae	
Enrichment	Replacement regime	TL (mm)	BW (mg)	TL (mm)	BW (mg)
UA	0	3.1 ± 0.1 e	6.6 ± 0.2d	5.7 ± 0.1e	9.7 ± 0.2e
	25	3.2 ± 0.1de	7.2 ± 0.2c	6.2 ± 0.2cd	10.3 ± 0.3de
	50	4.5 ± 0.1a	9.3 ± 0.1a	7.4 ± 0.1ab	13.4 ± 0.2a
	75	3.7 ± 0.1c	8.4 ± 0.1b	6.6 ± 0.2c	11.5 ± 0.2c
	100	3.2 ± 0.1de	7.1 ± 0.1c	6.2 ± 0.1d	10.2 ± 0.2de
EA	0	3.1 ± 0.1 e	6.6 ± 0.2d	5.7 ± 0.1e	9.7 ± 0.2e
	25	3.5 ± 0.1cd	8.1 ± 0.2b	6.5 ± 0.2cd	11.2 ± 0.2c
	50	4.7 ± 0.1a	9.4 ± 0.2a	7.6 ± 0.2a	13.7 ± 0.2a
	75	4.2 ± 0.2b	9.1 ± 0.1a	7.1 ± 0.1b	12.1 ± 0.1b
	100	3.2 ± 0.1de	7.3 ± 0.1c	6.5 ± 0.1d	10.6 ± 0.1d

Different letters on the each column indicated significant difference by Tukey's test ($P < 0.05$)