AquaTechnica 4(2): 75-84 (2022)
ISSN 2737-6095
DOI https://doi.org/10.33936/at.v4i2.4610
https://doi.org/10.5281/zenodo.6845905

Growth and alkaline digestive proteases activity of *Parachromis dovii* larvae fed live prey and formulated diets with different protein sources

Crecimiento y actividad de proteasas alcalinas digestivas de larvas de *Parachromis dovii* alimentadas con presas vivas y dietas formuladas con diferentes fuentes proteicas

Juan B. Ulloa Rojas, Silvia Valverde Chavarría, Mauricio Herrera Campos.
Laboratorio de Acuicultura Continental, Escuela de Ciencias Biológicas, Universidad Nacional, 86-3000 Heredia, Costa Rica
Correspondencia: *Juan B. Ulloa Rojas*, ***E-mail:*** *juan.ulloa.rojas@una.cr*

Artículo original | *Original article*

Keywords
Fish larvae
Protein sources
Enzymatic activity
Feeding

ABSTRACT | *Parachromis dovii* is a carnivorous cichlid fish from Central America, which easily accepts formulated feeds since exogenous feeding starts; however, its larval growth is lower than that obtained using live food. For this reason, the effect of different dietary protein source combinations on growth and alkaline digestive proteases activity were tested in *P. dovii* larvae. The test protein mixtures were prepared using fish, meat and bone (tankage), and poultry by-product meals in different proportions (four experimental diets: D1-D4) and compared to a commercial product (*Artemia* cysts Vitellus) (VD) and *Artemia* nauplii as control (AD). At the end of the trial, larvae fed *Artemia* nauplii showed the best specific growth rate (SGR): 17.6% body weight per day (BW/d), contrary to the Vitellus diet which presented the lowest SGR (12.0% BW/d) (LSD, $p<0.05$). From the protein sources, D3 (with the highest content poultry by-products meal) showed the worst SGR (12.6% BW/d) while the other treatments gave similar values (D1: 13.5%, D2: 13.2% and D4: 13.7% BW/d). Survival was not affected by dietary treatment (over 93%, $p>0.05$). The lowest alkaline digestive protease activity was found six days after hatching (DAH), and it was similar among treatments. The protease activity increased up to 27 DAH, being higher with larvae fed *Artemia* nauplii than with the other treatments. There were no differences in protease activity among the protein mixture diets, nor were there differences when comparing it with the Vitellus, except at 20 DAH.

Palabras clave
Larvas de peces
Crecimiento
Fuentes proteicas
Actividad enzimática

RESUMEN | *Parachromis dovii* es un pez cíclido carnívoro de Centroamérica, el cual acepta fácilmente alimento balanceado desde el momento en que se inicia su alimentación exógena; sin embargo, su crecimiento larvario es menor cuando se usa alimento balanceado que con el alimento vivo. Por esta razón, se evaluó el efecto de diferentes mezclas de proteínas en el alimento sobre el crecimiento y la actividad de las proteasas alcalinas digestivas en larvas de *P. dovii*. Las mezclas de proteínas en el alimento fueron preparadas con harina de pescado, carne y hueso, tortave (subproductos avícolas) (cuatro dietas, D1-D4), y comparadas con un producto comercial (Vitelo de quistes de *Artemia*, VD) y nauplios de *Artemia* como control (AD). Al final del ensayo, las larvas alimentadas con nauplios de *Artemia* presentaron el mejor crecimiento (tasa especifica de crecimiento (TEC): 17.6% peso corporal por día, PC/d) y el menor crecimiento se presentó con el Vitelo (12.0% PC/d) (LSD, $p<0.05$). De las fuentes de proteína, la D3 (con el mayor porcentaje de tortave) mostró la menor TEC, mientras que las otras mezclas proteicas dieron valores similares ((D1: 13,5%, D2: 13,2% and D4: 13,7% PC/d). La sobrevivencia (mayor a 93%) no fue afectada por los tratamientos evaluados (LSD, $p>0.05$). La actividad de las proteasas digestivas alcalinas mas bajo se encontró a los seis días después de la eclosión (DDE), y esta fue similar entre todos los tratamientos. La actividad de las proteasas se incrementó hasta los 27 DDE, siendo más alta con las larvas alimentadas con nauplios de *Artemia* que con los otros tratamientos. No se encontró diferencias en la actividad de las proteasas entre las dietas con mezclas de proteínas, ni tampoco hubo diferencias al compararla con la dieta Vitelo, excepto a los 20 DDE.

INTRODUCTION

The *Parachromis dovii*, a carnivorous freshwater cichlid from Central America, is highly appreciated for sport fishing and as a food source in rural areas (Bussing 2002). According to our observations, this fish species has shown good features for aquaculture, such as easy reproduction in captivity, high resistance to

management and diseases, and early acceptance of formulated feeds (Günther 1996; Quirós *et al.* 2014).

Like other freshwater fish species, *P. dovii* larva contains a great amount of yolk reserves and develops its stomach earlier than marine species, specifically during the transition to exogenous feeding (Lazo *et al.* 2011; Valverde-Chavarría *et al.* 2013); these characteristics, together with their larger size, facilitate the consumption of formulated foods during the weaning period. Despite of its high survival rate during larval phase and weaning time (over 90% from 6 to 30 DAH), its larval growth when fed inert diets has been lower compared to live food (*Artemia* nauplii) (Quirós *et al.* 2014; Valverde *et al.* 2013). To overcome this constraint, it is needed to optimize the formulated feeds to promote a better growth.

Live food has some disadvantages, such as it is more laborious to produce, it has a variable nutrient composition, and it can be a vector for disease (Giguere 2011). For this reason, many studies have pointed out the importance of designing artificial diets to replace it. Also, there is a trend to use alternative protein sources to partially replace fish meal in formulated diets, because of its decreasing availability (Alarcón *et al.* 2001). These protein sources must be highly digestible because larvae still may have an incomplete digestive system and they have a high protein requirement. Further, due to changes in larvae digestive capacity during ontogeny, it is recommended to select adequate feedstuffs to formulate highly digestible diets for different larval stages (Lazo 2000; Martínez-Montaño and Lazo 2012).

Several studies on fish larvae or juveniles have shown that diet composition may affect the growth and digestive enzyme profile during ontogeny (Kamarudin *et al.* 2011; Aguilera *et al.* 2012; Hassantabar *et al.* 2015; Murashita *et al.* 2015). There are some controversial results, however, and most findings indicate that live food supports better growth, survival, and digestive performance than formulated ones. Insufficient digestive enzymes to thrive in compound diets, nutrient leaching, poor digestibility, and palatability could be some of the major factors constraining its performance.

Based on our results (Valverde-Chavarría *et al.* 2016), fish, meat and bone (tankage) and poultry by-products meals gave the best in *in vitro* digestibility. Therefore, these three meals were selected as protein sources to prepare the mixtures to evaluate their feasibility to be used as feed ingredients in pelletized diets for *P. dovii* larvae and their effect on survival, growth, and alkaline proteolytic digestive enzymes.

MATERIALS AND METHODS

Reproduction and larvae

Reproduction and larvae rearing were performed at the hatchery unit of the Escuela de Ciencias Biológicas of Universidad Nacional, Heredia, Costa Rica as described by Quirós *et al.* (2014) and Valverde-Chavarría *et al.* (2016).

Feeds and larvae feeding

Ninety-two larvae were randomly distributed into each of 18 aquaria (11 L each, about 8.4 larvae L^{-1}) of a recirculation system and reared with one of the six feed treatments (three replicates per treatment). Larvae were fed in excess, at the same feeding frequency (9 h, 13 h, and 17 h), from 6 days after hatching (DAH) to 27 DAH, with six feed types: *Artemia* nauplii (AD) (about 250 mL^{-1}), a commercial product designed for shrimp larvae and post larvae composed of *Artemia* cysts yolk platelets (*Artemia* cysts Vitellus standard, BernAqua NV) (VD) and four experimental diets (D1-D4) containing different proportion of protein sources (Table 1). The Vitellus (VD) used in this work had a particle size of 150-400 µm (Giguere, 2011). Before feeding, all feed and feces wastes remaining were removed from the bottom of the aquaria and during feeding, the water flow was stopped for about 30 min to avoid feed losses. The survival was recorded during the feeding process.

The four experimental diets were prepared by using a pelletizer provided with a 2 mm die. The spaghetti-like feed was oven-dried at 60°C for 24 h and then was crushed with a laboratory mill. The different feed

particles used during the larvae phase (powder, 300µ, 600µ and 1 mm diameter) were separated using appropriate sieves. Diets were formulated to contain about 70% of protein sources, of which around 30% was fish meal (except D4 which had 40%). In diets D1 to D3, the remainder 40% were completed with tankage (D2), poultry by-products (D3) or a mixture (D1:20% tankage and 20% poultry by-products). The protein mixtures were formulated accordingly to get isoproteic (42%) and isoenergetic (320 kcal/g) diets, and protein sources were selected based on results of *in vitro* digestibility tests (Valverde-Chavarría *et al.* 2016). However, after proximal analysis, D4 had a slightly lower protein content (38.4%) (Table 1).

Table 1. Feedstuff formulation (% as feed) and proximal composition (%, DM) of the four experimental diets (D1-D4) and Vitellus diet (VD) used for rearing *Parachromis dovii* larvae. D1 to D4 were produced in our laboratory.

Diets Components *	D1	D2	D3	D4	VD
Fish meal	31.0	31.0	28.0	40.0	-
Tankage	20.0	40.5	0.0	15.5	-
Poultry by-products meal	20.0	0.0	40.0	15.5	-
Starch	0.0	0.0	20.0	8.5	-
Wheat meal	18.0	10.25	0.0	10.5	-
Fish meal hydrolyzed	2.0	2.0	2.0	2.0	-
Blood meal	0.0	6.0	0.0	0.0	-
Soybean oil	2.0	2.5	2.0	2.0	-
Fish oil	2.0	2.75	2.5	2.0	-
salt	1.0	1.0	1.0	1.0	-
Vitamin premix	2.0	2.0	2.0	2.0	-
Na Alginate	2.0	2.0	2.5	1.0	-
Proximal composition **					
Moisture	8.2	7.93	8.62	14.44	20.46
Crude protein	42.53	41.03	43.07	38.36	47.64
Crude fat	19.58	18.76	13.81	12.54	15.96
Ash	19.30	24.38	13.28	16.08	7.77
NFE	18.59	15.83	29.84	33.02	28.63
kcal/100 g***	319.7	318.4	319.3	318.4	319.0

* Fish meal as tuna meal (48% CP) and fish (tuna) oil from Alimentos Prosalud S.A., Costa Rica; Tankage (42% CP) and blood meal (92% CP) from CoopeMontecillos, Costa Rica; Poultry byproducts (51% CP) from PIPASA (Cargill S.A.), Costa Rica; Starch (cassava) for human consumption; Wheat meal (14% CP) from Molinos de Costa Rica, S.A., Fish (tuna) meal hydrolyzed from own manufacturing, Soybean oil from INOLASA, S.A., Costa Rica; Trout vitamin premix donated by DSM Nutritional Products, S.A., Costa Rica; Na alginate from Sigma Aldrich.
** Proximal composition was determined following the AOAC (1990) standard methods: moisture, crude protein by microKjedahl, crude fat (920.39A), and crude ash (942.05). The NFE was calculated by difference (100-crude protein-crude fat-ash).
*** Calculation based on literature energy coefficients.

Larvae sampling and biometrics

Ten larvae per aquarium were randomly sampled at 6, 13, 20 and 27 DAH before initial feeding and kept for two hours in clear water to allow the emptiness of the digestive tract. Next, they were sacrificed with an overdose of tricaine-methanesulfonate (MS-222) and weighed with an analytical balance (Sartorius 2492 ± 0.1 mg). Finally, larvae were washed with distilled water and kept frozen at -20ºC for further biochemical analysis. The growth in length and weight was calculated by the formula: $SGR = (\ln Wf - \ln Wi / t2 - t1) * 100$ (% BW day^{-1}), where ln is natural logarithm, W_f is final weight (mg), W_i is initial weight (mg), t_2 is final day, t_1 is initial day and BW is body weight.

Preparation of enzymatic extracts and determination of alkaline digestive proteases activity

The enzymatic extracts were prepared according to Alarcón *et al.* (2001). Briefly, the visceral bulk was removed from each larvae in cold conditions by cutting off the tail, head and dorsal part of the body. Samples

from the smallest larva were taken by removing their head and tail. Visceral bulks were homogenized with deionized water, in a proportion of 30 mg tissues/ml water, using a tissue homogenizer (Contes). Next, the homogenized sample was centrifuged at 16 000 *g*, 4°C for 30 min (Hettich Mikro 200). The supernatant was kept at -20°C for further enzymatic analysis.

The measurement of alkaline digestive proteases activity was done according to Walter (1984), incubating the enzymatic extract with 0.5% casein at pH 9.0 and 37 ^{0}C. One unit of enzymatic activity was defined as 1 μg tyrosine released min^{-1} larva^{-1}, using the tyrosine 0.005 molar extinction coefficient. All determinations were done by triplicate and the enzymatic activity was calculated as: $\mathrm{U/larvae} = \frac{\Delta\ \text{absorbance x FD(ml)}}{\text{MEC tyrosine x T(min) x N}}$, where U is enzymatic activity units, Δ absorbance is the increment in absorbance respect to blank at 280 nm, FD is the final volume of enzymatic extract reaction/extract volume, MEC is the tyrosine molar extinction coefficient, T is the incubation time (min) and N is the number of larvae per ml of enzymatic extract.

Statistical analyses

To determine differences in growth and enzymatic activity, the mean values of data were analyzed by applying a one-way ANOVA. The homogeneity of variances and the normal distribution were tested according to the Levene and Shapiro–Wilk tests, respectively. Accordingly, treatment means comparison was done using the LSD test with 95% confidence intervals. When data were not normally distributed, data transformation and Kruskal-Wallis analyses were performed. All analyses were performed using the software STATGRAPHICS CENTURION XVI (StatPoint Technologies Inc., 2010).

RESULTS

Fish Growth and survival

Survival was similar and over 93% for all treatments at the end of the experiment (LSD, p>0.05). At the beginning of the exogenous feeding, the larvae weight was similar for all treatments (D4: 4.1±0.07 mg to D1: 4.32±0.07 mg, p=0.14). After 27 days of culture, fingerlings fed *Artemia* nauplii (AD) had the highest weight (175.42±10.99 mg), followed by those fed D1 (74.54±4.20 mg); the lowest weight was obtained with larvae fed Vitellus (VD) (52.58±2.30 mg) (LSD, p<0.05). The SGR showed the same pattern as the final weight, being higher with *Artemia* (17.64±0.40% BW/d) and lower with Vitellus (12.03±0.41% BW/d). From the protein source mixtures, diet D3, containing a higher proportion of poultry by-products (40%) and a lower proportion of fish meal (28%) showed a slightly lower SGR than the other combinations, being only significantly lower than D1 (with a similar proportion of tankage and poultry by-products, 20%) and D4 (with the highest proportion of fishmeal (40%) and similar proportions of tankage and poultry by-products, 15.5%) (Table 2).

Table 2. Wet weight (mg), specific growth rate (SGR), and survival (%) of *P. dovii* larvae reared with live prey and different protein-mixture diets after 21 days. Means ± standard error (n=30 for weight and n=3 for SGR and survival). Different letters, in the same row, indicate significant differences among diets (p<0.05).

Diets Variable	AD	VD	D1	D2	D3	D4
Initial weight[1]	4.28±0.07a	4.18±0.06a	4.32±0.05a	4.24±0.07a	4.18±0.05a	4.09±0.07a
Final weight	175.42±10.99a	52.58±2.30d	74.54±4.20b	68.09±4.00bc	59.39±3.19cd	72.12±3.52b
SGR (% BW d^{-1})	17.64±0.40a	12.03±0.41d	13.55±0.18 b	13.21±0.34bc	12.63±0.32cd	13.71±0.28b
Survival	93.12±2.02a	94.93±2.02a	96.38±0.36a	96.38±1.45a	97.10±1.81a	95.29±2.02a

[1] Weight at beginning of exogenous feeding (6 DAH).

The cumulative SGR (from 6 to 27 days) of larvae fed *Artemia* nauplii decreased with time, whereas the cumulative SGR of larvae fed formulated and Vitellus diets remained with few changes until the end of the experiment (Figure 1).

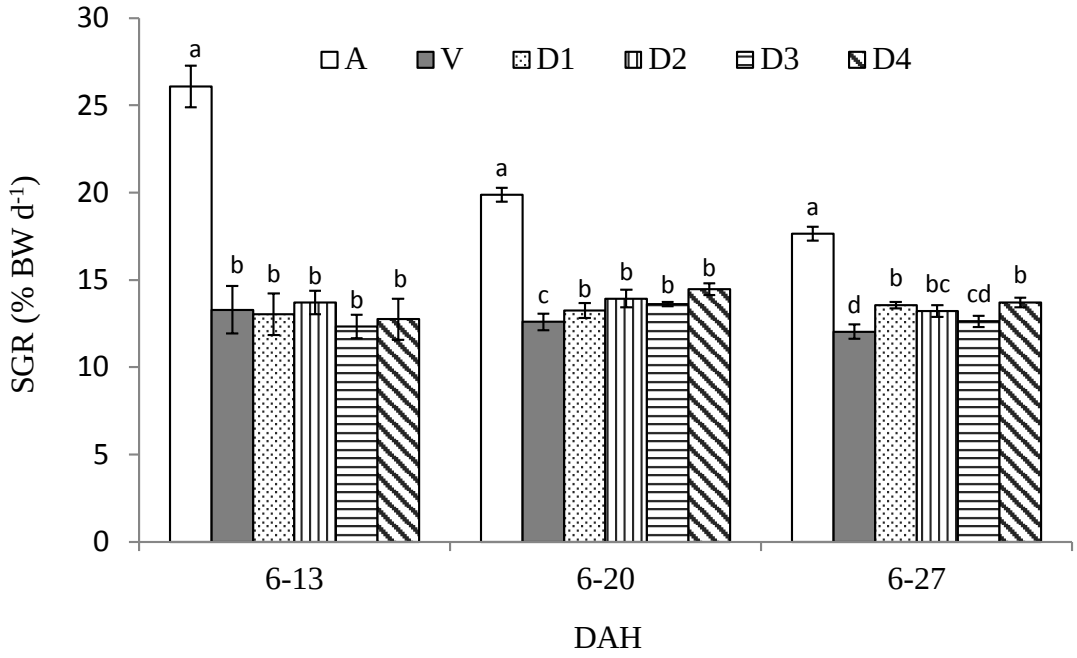

Figure 1. Cumulative specific growth rate (SGR) of *P. dovii* larvae reared with *Artemia* nauplii (AD), Vitellus (VD), and four different protein-mixture diets after seven (6-13), 14 (6-20), and 21 (6-27) days of culture. Means ± standard error (n=3). Different letters, in the same time period, indicate significant differences among diets ($p<0.05$).

Alkaline digestive proteases activity

Enzymatic activity was detected from the beginning of exogenous feeding at six DAH and increased until the end of the experiment for all treatments. From 13 to 27 DAH, the larvae fed *Artemia* nauplii showed the highest activity ($p=0.07$), being about twice the activity of the other treatments at the end of the larval period. In general, the protein mixtures tested and the Vitellus diet did not affect significantly the alkaline enzymatic activity ($p>0.05$) all fluctuating between 0.23 to 7.03 UA/larvae (Table 3).

Table 3. Alkaline enzymatic activity (UA/larvae) of digestive proteases of *P. dovii* reared with *Artemia* nauplii, Vitellus and different protein mixtures diets for 21 days. Means ± standard error (n=3). Different letters, in the same row, indicate significant differences among diets ($p<0.05$). [1] Means are different at $p<0.10$ ($p=0.07$).

DAH	A	V	D1	D2	D3	D4
6	0.23±0.04a	0.25±0.04a	0.27±0.04a	0.23±0.06a	0.25±0.06a	0.25±0.04a
13	2.76±0.81a	0.92±0.06b	0.87±0.12b	1.30±0.28b	1.27±0.17b	1.28±0.29b
20	5.24±0.63a	2.88±0.43b	4.61±1.23a	4.37±0.06a	4.08±0.85ab	3.77±1.00ab
27[1]	15.47±5.14a	6.63±0.55b	7.03±0.75b	5.50±1.39b	5.59±1.13b	6.08±1.09b

DISCUSSION

Growth and survival

In this study, some yolk reserves were still observed in the digestive tract of *P. dovii* larvae a few days after exogenous feeding began, agreeing with Quirós *et al.* (2014), and probably this finding could affect positively the transition to exogenous feeding. According to Kolkovski (2001), most freshwater fish larvae are larger than marine ones and well developed at hatching, and possess large yolk reserves, even after exogenous feeding, that could improve the success of the transition to exogenous feeding and be advantageous for any delay in transition. This could be explained by the high survival rate found with *P. dovii* larvae (over 93%). It is hypothesized that these high survival rates may be an indication that larvae could accept the formulated diets (as observed during the trial) and probably have a well-developed digestive system, as it was

macroscopically observed, to provide enough enzymes to digest non-living food as stated by Valverde-Chavarría *et al.* (2013).

The final weight found in larvae fed *Artemia* nauplii is higher than that reported by Valverde-Chavarría *et al.* (2016) (175.4 vs 164.0 mg); apparently, the lower density used in our experiment (8,4 vs 23 larvae L^{-1}) could be one reason explaining this difference. Similar results were reported for pikeperch (Szkudlarek and Zakes 2007) and kutum larvae (Hassantabar *et al.* 2015) who found better larvae final weights at lower culture densities. Additionally, our SGR was like that reported by Quirós *et al.* (2014) for *P. dovii* larvae (at 16 DAH) at similar densities (10.3/L) and sampling period. Other factors contributing to this difference may be the quality of food (*Artemia* nauplii) and broodstock.

The present study showed that *Artemia* nauplii gave the best performance, agreeing with Quirós *et al.* (2014). As found with other freshwater or anadromous fish species, larvae fed inert diets showed lower SGR's than those fed *Artemia* nauplii. For instance, African catfish larvae (*Clarias gariepinus*) had a lower SGR with a commercial diet than with decapsulated *Artemia* or *Daphnia* (Olurin and Oluwo 2010)¸catfish larvae (*Mystus nemurus*) had lower SGR when fed on a formulated diet or a combination (live-inert diet) (Kamarudin *et al.* 2011); larvae of African catfish (*Heterobranchus bidorsalis*) reared with two formulated feeds grew slower than those reared with nauplii, although survival was higher with the beef brain diet (Alla *et al.* 2011); *Acipenser persicus* larvae also obtained a lower growth and survival when fed only a formulated diet than *Artemia* nauplii or a combined diet (Noori *et al.* 2012). It appears that food intake stimulation, higher protein digestibility and content, better amino acid profile, and the auto-hydrolysis of nauplii also favored the best growth found (Alla *et al.* 2011; Noori *et al.* 2012). In contrast, Hassantabar *et al.* (2015) found a significantly higher growth rate in kutum larvae fed an artificial diet compared to *Artemia* nauplii fed group, although the survival was lower. Apparently, their growth rate was compromised by a higher swimming activity for finding nauplii. Aguilera *et al.* (2012) also found better growth rates in larvae of tropical gar (*Atractosteus tropicus*) fed with formulated diets. Drossou *et al.* (2006) found similar survival and growth rates for *Oreochromis niloticus* fed *Artemia* nauplii and formulated diets. Feeding behavior, mouth opening, size at the later larval stage of these species, and *Artemia* nauplii size could interfere with these results. From the latter results, it may be concluded that more research is needed to improve the quality and availability of different feedstuffs for larvae diets; considering that live feeds such as *Artemia* cysts actually are less available and have an increasing price.

From the protein sources tested, it could be concluded that diets with tankage inclusion provided the best results in terms of final weight and growth rate. This result agrees with Valverde-Chavarría *et al.* (2016), who found the higher digestibility with this protein source. Mente *et al.* (2017) also reported that fish meal may be partially substituted or used in combination with tankage meal in diets for zebra cichlid (*Archocentrus nigrofasciatus*). The best results obtained with a diet containing more fish meal could be explained by the fact that fish meal contains food stimulators that may promote food intake and enzymes synthesis (Murashita *et al.* 2015).

The worst growth obtained with the Vitellus diet may be attributed to its particle size (standard: 150-400 µm) that could interfere with feed intake, as seen after the feeding period finished. Larvae could get a lower energetic reward due to the high energy expenditure in capturing such small particles. Vitellus is used for feeding shrimp larvae and postlarvae in replacement of *Artemia* nauplii and no records of its use to feed fish larvae were found. Giguere (2011) reported that the maximum substitution rate of Vitellus for newly hatched *Artemia* was 84.33% in the larval culture of *Litopenaeus vannamei* from Z3/M1-PL10, without resulting in significantly different survival and stress test mortalities, although the growth was lower. Giguere (2011) argued that these positive results are due to the small and homogeneous particle size, nutritional composition, and high digestibility of the *Artemia* yolk platelets that the Vitellus is composed of, and to the availability of the feed in the water column because of its neutral buoyancy.

Alkaline digestive proteases activity

At first feeding, protein digestion depends mainly on alkaline proteases, although the stomach is present and functional, levels of pepsin-like enzymes are lower than alkaline proteases (Valverde-Chavarría *et al.* 2013).

The presence of alkaline proteolytic activity (at low levels) at 6 DAH, before the beginning of exogenous feeding, has been also observed in other freshwater and anadromous fish species, such as *Clarias gariepinus* (García-Ortega *et al.* 2000), *Oreochromis niloticus* (Tengjaroenkul *et al.* 2002), *O. mossambicus* (Lo and Weng 2006), *Cichlasoma urophthalmus* (López-Ramírez *et al.* 2011), *Petenia splendida* (Uscanga-Martínez *et al.* 2011), *Acipenser persicus* (Noori *et al.* 2012), *Atractosteus tropicus* (Aguilera *et al.* 2012) and *Cichlasoma trimaculatum* (Toledo-Solís *et al.* 2015).

The total alkaline proteolytic activity increased with age, from 6 DAH (begins of exogenous feeding) until the end of the larval stage (about 27 DAH), agreeing with Quirós *et al.* (2014) and Valverde-Chavarría *et al.* (2016). This increment may be attributed to the morphological changes observed in the larvae at later stages, such as maturation and size increment of the digestive tract (Valverde-Chavarría *et al.* 2013). The sharp increment at the end of the larvae stage (about 27 DAH) may be related to the complete development of the digestive system and its bigger size.

The higher total alkaline enzyme activity in *P. dovii* larvae fed *Artemia* nauplii may be explained by higher food consumption and to their bigger size. Also, the consumption of *Artemia* nauplii may increase the hormones production which in turn can improve gastric activation in fish larvae (Kolkovski *et al.* 1997).

The proteolytic activity of larvae fed Vitellus was low and not significantly different from all protein mixtures tested. From protein mixtures, the similar enzyme activity determined suggests that the proportions used did not affect the activity of proteolytic alkaline enzymes of *P. dovii* larvae, probably because of the high digestibility of all protein sources used (Valverde-Chavarría *et al.* 2016). Drossou *et al.* (2006) found high levels of trypsin activity in combination with high mortality and poor growth due to insufficient food quality.

Freshwater fish larvae, such as *Mystus nemurus* showed the highest enzyme activity when fed a combination diet (live and inert food) compared to only life or inert diets as well as a higher growth rate (Kamarudin *et al.* 2011). They suggest that protein structure, digestibility, exogenous enzymes, and food intake stimulation by *Artemia* nauplii, and higher nutrient concentration from an inert diet, could play an important role. A higher trypsin and chymotrypsin activity was found with *Rutilus kutum* larvae fed *Artemia* nauplii compared to an artificial feed (Hassantabar *et al.* 2015). Aguilera *et al.* (2012) also determined an effect of diet on alkaline digestive enzyme activity in tropical gar larvae, which was higher using formulated diets. It appears that formulation and composition of the diet, type of food, and processing could affect these findings.

Based on survival results, this preliminary study showed that formulated feeds may be acceptable for raising of *P. dovii* larvae. After the larvae phase, the juveniles were fed for several weeks with the remainder diets without any macroscopic adverse effects. Nevertheless, it is necessary to improve the formulation, feedstuff digestibility, texture, and palatability of inert diets, to achieve better results in terms of acceptance, digestive performance, and especially, larval growth.

Declaration of interest

The authors declare no conflict of interest. The authors also are the responsible for the content of this research article.

Declaration of animal welfare

The authors declare the implementation and use of national rules for management and care of animals.

Acknowledgements

This research was supported by the projects SIA 0021-07 "PS Tecnología Acuicultural" and SIA 0315-10 "Desarrollo de una dieta inerte para larvas del guapote lagunero *Parachromis dovii* (Pisces Cichlidae) formulada de acuerdo con su capacidad digestiva y requerimientos proteicos", Escuela de Ciencias Biológicas, Universidad Nacional. To Cristian Fonseca R., from Laboratorio de Control de Calidad de Productos Pesqueros, Estación de Biología Marina, Universidad Nacional, for the ingredient proximal analyses.

REFERENCES

Aguilera, C., Mendoza, R., Iracheta, I., Márquez, G. (2012). Digestive enzymatic activity on tropical gar (*Atractosteus tropicus*) larvae fed different diets. *Fish Physiology and Biochemistry*, 38:6779-691.

Alarcón, F., García-Carreño, F., Navarrete del Toro, M. (2001). Effect of plant protease inhibitors on digestive proteases in two fish species, *Lutjanus argentiventris* and *L. novemfasciatus*. *Fish Physiology and Biochemistry*, 24:179-189.

Alla, Y., Célestin, B., Célestin, A., Tidiani, K. (2011). Effect of three diets on growth and survival rates of African catfish *Heterobranchus bidorsalis* larvae. *The Israeli Journal of Aquaculture-Bamidgeh*, 63:1-8.

AOAC (Association of Official Analytical Chemists). 1990. Official Methods of Analysis of the Association of Official Analytical Chemists. *Food Composition; Additives; Natural Contaminants*. Ed. by K. Helrich. 15th edition. Virginia, USA. 1298 p.

Bussing, W. (2002). Peces de las Aguas Continentales de Costa Rica. Editorial de la Universidad de Costa Rica. San José, Costa Rica.

Drossou, A., Ueberschär, B., Rosenthal, H., Herzig, K. (2006). Ontogenic development of the proteolytic digestion activities in larvae of *Oreochromis niloticus* fed with different diets. *Aquaculture*, 256:479-488.

García-Ortega, A., Verreth, J., Segner, H. (2000). Post-prandial protease activity in the digestive tract of African catfish *Clarias gariepinus* larvae fed decapsulated cysts of *Artemia*. *Fish Physiology and Biochemistry*, 22:237-244.

Giguere, M.A. (2011). The economics of partial *Artemia* replacement using two commercially available feed in diets of *Litopenaeus vannamei* from Z3/M1 – PL10. Master's tesis, University of Miami, Florida, USA.

Günther, J. (1996). Crecimiento del guapote lagunero (*Cichlasoma dovii*) en régimen de cultivo intensivo en estanques y su dependencia de la densidad. *UNICIENCIA*, 13:13-19.

Hassantabar, F., Fereidoumi, A., Ouraji, H., Babaei, S., Jafarpour, A. (2015). Comparison of growth and digestive enzymes activity of kutum (*Rutilus kutum*) during ontogeny fed with live prey and artificial feed. *Aquaculture International*, 23:597-612.

Kamarudin, M., Otoi, S., Saad, R. (2011). Changes in growth, survival and digestive enzyme activities of Asian redtail catfish, *Mystus nemurus*, larvae fed on different diets. *African Journal of Biotechnology*, 10:4484-4493.

Kolkovski, S. (2001). Digestive enzymes in fish larvae and juveniles-implications and applications to formulated diets. *Aquaculture*, 200:181-201.

Kolkovski, S., Koven, W., Tandler, A. (1997). The mode of action of *Artemia* in enhancing utilization of microdiet by gilthead seabream *Sparus aurata* larvae. *Aquaculture*, 155:193-205.

Lazo, J. (2000). Conocimiento actual y nuevas perspectivas en el desarrollo de dietas para larvas de peces marinos. *Avances en Nutrición Acuícola V*. Mérida, Yucatán, México (noviembre 2000).

Lazo, J., Darias, M., Gisbert, E. (2011). Ontogeny of the digestive tract. In: Holt G (ed). *Larval fish nutrition*. John Wiley and Sons. London, United Kingdom. pp: 1-46.

Lo, M., Weng, C. (2006). Developmental regulation of gastric pepsin and pancreatic serine protease in larvae of the euryhaline *Oreochromis mossambicus*. *Aquaculture*, 261:1403-1412.

López-Ramírez, G., Cuenca-Soria, C. Álvarez-González, C., Tovar-Ramírez, D., Ortiz-Galindo, J. L., Perales-García, N., Márquez-Couturier, G., Arias-Rodríguez, L., Indy, J.R., Contreras-Sánchez, W. M., Gisbert, E., Moyano, F. J. (2011). Development of digestive enzymes in larvae of Mayan cichlid *Cichlasoma urophthalmus*. *Fish Physiology and Biochemistry*, 37:197-208.

Martínez-Montaño, E., Lazo, J. (2012). *In vitro* protein digestibility of dietary ingredients throughout ontogeny of California halibut, *Paralichtys californicus*, larvae. *Journal of the World Aquaculture Society*, 43:51–62.

Mente, E., Solovyev, M., Vlahos, N., Rotllant, G. and Gisbert, E. (2017). Digestive enzyme activity during ontogeny and after feeding diets with different protein sources in zebra cichlid, *Archocentrus nigrofasciatus*. *Journal of the World Aquaculture Society*, 48:831-848.

Murashita, K., Fukada, H., Takahashi, N., Hosomi, N., Matsunari, H., Furuita, H., Oku, H., Yamamoto, T. (2015). Effect of feed ingredients on digestive enzyme secretion in fish. *Bulletin of Fisheries Research Agency*, 40:69-74.

Noori, F., Van Stappen, G., Sorgeloos, P. (2012). Preliminary study on the activity of protease enzymes in Persian sturgeon (*Acipenser persicus* Borodin, 1897) larvae in response to different diets: effects on growth and survival. *Aquaculture Research*, 43:198-207.

Olurin, K., Oluwo, A. (2010). Growth and survival of African catfish (*Clarias gariepinus*) larvae fed decapsulated *Artemia*, live *Daphnia*, or commercial starter diet. *The Israeli Journal of Aquaculture-Bamidgeh*, 62:50-55.

Quirós, J., Valverde, S., Ulloa, J. (2014). The proteolytic digestive activity and growth during ontogeny of *Parachromis dovii* larvae (Pisces: Cichlidae) using two feeding protocols. *Fish Physiology and Biochemistry*, 40:1253–1261.

Szkudlarek, M., Zakęś, Z. (2007). Effect of stocking density on survival and growth performance of pikeperch, *Sander lucioperca* (L.), larvae under controlled conditions. *Aquaculture International*, 15:67–81.

Tengjaroenkul, B., Smith, B., Smith, S., Chatreewongsin, U. (2002). Ontogenic development of the intestinal enzymes of cultured Nile tilapia, *Oreochromis niloticus L*. *Aquaculture*, 211:241-251.

Toledo-Solís, F., Uscanga-Martínez, A., Guerrero-Zárate, R., Márquez-Couturier, G., Martínez-García, R., Camarillo-Coop, S., Perales-García, N., Rodríguez-Valencia, W., Gómez-Gómez, M., Álvarez-González,

C. (2015). Change on digestive enzymes during initial ontogeny in the three-spot cichlid *Cichlasoma trimaculatum*. *Fish Physiology and Biochemistry*, 41:267-279.

Uscanga-Martínez, A., Moyano-López, F., Álvarez-González, C., Perales-García, N. (2011). Aplicaciones a la mejora de la utilización nutritiva del alimento en cíclidos cultivados en México. *Avances en Nutrición Acuícola XI*. Monterrey, México (noviembre 2011).

Valverde-Chavarría, S., Álvarez-González, C. A., Brais-Medina, M., Calvo-Elizondo, E., Ulloa-Rojas, J. B. (2016). *In vitro* digestibility and proteases inhibitory effect of several feedstuffs for *Parachromis dovii* juveniles and *P. dovii* hybrid larvae. *Fish Physiology and Biochemistry*, 42:1767-1776.

Valverde-Chavarría, S., Álvarez-González, C., Ulloa-Rojas, J., Frías-Quintana, C.A., Guerrero-Zárate, R., Quirós-Orlich, J.R., Brais-Medina, M., Calvo-Elizondo, E., Alvarado-Guzmán, L. (2013). Ontogenia del sistema digestivo del guapote lagunero *Parachromis dovii* durante el periodo larval y selección de ingredientes para su alimentación. *Avances en Nutrición Acuícola XII*. Universidad Autónoma de Nuevo León, San Nicolás de los Garza, Nuevo León, México (noviembre 2013).

Walter, H. (1984). Proteinases: methods with hemoglobin, casein and azocoll as substrates. In: Bergmeyern, H. (Ed). *Methods of Enzymatic Analysis*. Vol. V. Verlag Chemic Weinheim, Germany. pp: 270-277.

Recibido: 20-04-2022
Aprobado: 22-06-2022
Versión final: 11-07-2022