

## **Influence of dietary fatty acid composition on enzymatic activity and digestive histology in silver catfish (*Rhamdia quelen*)**

**Influência da composição em ácidos graxos da dieta na atividade enzimática e histologia digestiva do jundiá (*Rhamdia quelen*)**

**Influencia de la composición de ácidos grasos de la dieta sobre la actividad enzimática y la histología digestiva del jundiá (*Rhamdia quelen*)**

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### **Abstract**

Dietary fatty acids may influence the development of the digestive tract and initial activation of digestive enzymes in fish. The objective of this study was to evaluate the effect of the diet lipid profile on ontogeny and activity of digestive enzymes in silver catfish post larvae fed practical diets. Five diets were tested, replacing broiler liver by soybean protein concentrate (SPC) or fish meal (FM): Standard, 15SPC, 30SPC, 15FM and 30FM. The enzymatic activity of fish was analyzed from 32 Hours after fertilization (Haf). Were analysed acid protease, trypsin, chymotrypsin, lipase, amylase and maltase. The development of the digestive system was analyzed from first feeding up to 28 days by histological techniques. The enzymatic activity presented peaks for the post-larvae fed with the 15SPC diet and fish fed with diet 15FM showed best growth. The development of the digestive system did not suffer damage from the diets offered. Post-larvae fed the 30SPC diet showed reduced development of the digestive tract. The diet 15FM provide good lipid profile for silver catfish post larvae.

**Keywords:** Fish meal; Growth; Larviculture; Soybean protein concentrate.

### **Resumo**

Os ácidos graxos da dieta podem influenciar o desenvolvimento do trato digestório e a ativação inicial das enzimas digestivas em peixes. O objetivo deste estudo foi avaliar o efeito do perfil lipídico da dieta na ontogenia e atividade de enzimas digestivas em pós-larvas de jundiá alimentadas com dietas práticas. Foram testadas cinco dietas, substituindo o fígado de aves por concentrado protéico de soja (CPS) ou farinha de peixe (FP): controle, 15CPS, 30CPS, 15FP e 30FP. A atividade enzimática dos peixes foi analisada a partir de 32 horas após a fertilização. Foram analisadas a protease, tripsina, quimiotripsina, lipase, amilase e maltase. O desenvolvimento histológico foi analisado desde o início da alimentação até 28 dias. A atividade enzimática apresentou picos para as pós-larvas alimentadas com a dieta 15CPS e os peixes alimentados com a dieta 15FP apresentaram melhor crescimento. O desenvolvimento do sistema

digestório não sofreu danos das dietas ofertadas. Pós-larvas alimentadas com a dieta 30CPS mostraram redução do desenvolvimento do trato digestório. A dieta 15FP proporciona bom perfil lipídico para pós-larvas de jundiá.

**Palavras-chave:** Farinha de peixe; Crescimento; Larvicultura; Concentrado proteico de soja.

### Resumen

Los ácidos grasos de la dieta pueden influir en el desarrollo del tracto digestivo y la activación inicial de las enzimas digestivas en el pescado. El objetivo de este estudio fue evaluar el efecto del perfil lipídico de la dieta sobre la ontogenia y la actividad de las enzimas digestivas en postlarvas de jundiá alimentadas con dietas prácticas. Se probaron cinco dietas, reemplazando el hígado de ave por concentrado de proteína de soja (CPS) o harina de pescado (FP): control, 15CPS, 30CPS, 15FP y 30FP. La actividad enzimática de los peces se analizó 32 horas después de la fertilización. Se analizaron proteasa, tripsina, quimotripsina, lipasa, amilasa y maltasa. Se analizó el desarrollo histológico desde el inicio de la alimentación hasta los 28 días. La actividad enzimática mostró picos para las postlarvas alimentadas con la dieta 15CPS y los peces alimentados con la dieta 15FP mostraron un mejor crecimiento. El desarrollo del sistema digestivo no sufrió daños por las dietas ofrecidas. Las postlarvas alimentadas con la dieta 30CPS mostraron un desarrollo reducido del tracto digestivo. La dieta 15FP proporciona un buen perfil lipídico para las postlarvas de jundiá.

**Palabras clave:** Harina de pescado; Crescimento; Larvicultura; Concentrado de proteína de soja.

## 1. Introduction

Digestive enzymes act according to the diet composition and the dietary habits of fish (Mente et al., 2017). The main essential fatty acids that should be present in early fish diets are 22: 6n-3, 20: 5n-3 and 20: 4n-6, and their ratio should be adequate for proper fish development (Sargent et al., 1999; El Kertaoui et al., 2019).

The enzymes involved in the digestion of luminal protein (trypsin, chymotrypsin), lipids (lipases) and carbohydrates (amylases and maltases) are present in larvae before the first feeding (Lazo et al., 2011; Alveal et al., 2019). The development of the digestive tract, the identification of digestive enzymes and analysis of their activities provide valuable information about the nutritional status of larvae and post-larvae of fish (Yúfera & Darias, 2007; Silveira et al., 2013; Alveal et al., 2019). The first gastric glands can be detected a few days or weeks after hatching and their numbers progressively increase by covering the epithelium of the stomach (Zambonino Infante et al., 2008; Alveal et al., 2019).

Fish larvae undergo morphological, anatomical and functional changes during development and the digestive tract is still immature at hatching (Teles et al., 2017). The stomach in some species is not differentiated during the first days of life (Teles et al., 2017). The development of these organs and gastric glands appears at different ages for the various species of fish (Zambonino Infante and Cahu, 2007; Teles et al., 2017; Alveal et al., 2019). In the catfish, the digestive tract is formed 48 hours after hatching at 24°C. At 16Haf, differentiation of the digestive tract is complete, presenting oropharyngeal cavity, esophagus, liver, pancreas, stomach and intestine (Silveira et al., 2013).

Fish meal made from waste of silver catfish (FM) tested in the feeding of juveniles, is considered as an alternative sustainable for this species (Rossato et al., 2018). Fish meal has an adequate percentage of fatty acids that is essential for early fish development (Sargent et al., 1999, Seong et al., 2019). According to Vizcaíno et al. (2014) the rising costs of fish meal worldwide promote great research effort aimed to find alternative and renewable ingredients for aquafeeds. The use of available food waste as an alternative protein source for producing fish feed has been suggested as a means of tackling the problem of sourcing safe and sustainable feed (Mo et al., 2018).

Soy protein concentrate is usually the main source of post larvae of fish feed protein. But it has many anti-nutritional factors (Kumar et al., 2011) that inhibit enzyme activity and low palatability (Tacon and Akiyama, 1997). Consequently, it is one of the major causes of the low survival of post larvae of fish. But SPC is the most abundant raw material compared to animal meal. The soybean meal was tested of Piaia and Radünz Neto (1997) and SPC by Fontinelli and Radünz Neto (2007). But in proportions and quantities that did not favor the development of the animals. For carnivorous fish, some studies revealed that the fish were able to tolerate less than 50% of SPC replacement in the feed (Mohd Faudzi et al., 2018).

Therefore, in this study we evaluated percentage of inclusion of this source for post-larvae of silver catfish.

The objective of this study was to evaluate the effect of the diet lipid profile on ontogeny and activity of digestive enzymes in silver catfish post larvae fed practical diets.

## 2. Methodology

They were formulated five isoproteic (44% crude protein) and isolipidic (3900 Kcal of crude energy) diets according to silver catfish requirement: Standard, 15SPC; 30SPC; 15FM and 30FM. The standard diet consisted of fresh poultry liver (25.9% DM) (30%), cane yeast (37%), boiled egg yolk (20%), soy lecithin (2%), defatted rice bran (8%), minerals (1%) and vitamins (2%) (Coldebella et al., 2011). In the other diets, the poultry liver was partially replaced (15%) or totally (30%) by SPC or FM. The diets were prepared by mixing dry ingredients (75 µm). Homogenized with the addition of liver and/or water, until the mixture has sufficient moisture for pelleting in meat grinder. Dried at 40°C for 24 hours in an oven with air recirculation. Grinding and sifting, separating into portions composed of particles of 100-200 µm, 200-400 µm, 400-600 µm, 600-800 µm. Thus obtaining the granulometry suitable for opening the mouth of the post-larvae. Maintained in refrigerator at 4°C and withdrawn only at the time of feeding. The lipid profile (Table 1) of the diets was analyzed by Gas Chromatography (Hartman & Lago, 1973).

The first food was supplied at 108Haf when the larvae began to consume exogenous food, considered post-larvae. The animals were fed every 2 hours (h) between 08h and 20h in amounts exceeding its intake capacity. Housed in a recirculation system composed of 35 experimental units and fed for 28 days. Each unit consists of two plastic containers provided with side screen to prevent the exit of the post-larvae. With individual supply and flow, connected in thermoregulated water recirculation system. 240 post-larvae were used per experimental unit (45 animals per liter). Five treatments were tested with seven replicates. Biometric evaluation was performed on 10 post-larvae per experimental unit for seven days in order to monitor growth. Samples were weighed on a digital scale (0.001 g) without return. The animals were sedated with benzocaine (50 mg L<sup>-1</sup>) (AVMA, 2007), weighed, and measured in order to obtain the Weight of the whole fish (mg).

The analysis of the enzymatic activity was carried out in the Fish Laboratory of the Animal Science Department of the Federal University of Santa Maria. Fertilized eggs (embryos) were obtained by induced reproduction of silver catfishes. At 32 hours after fertilization (Haf), the embryos already showed movement (fully formed larvae), at which point the first collection was performed for enzymatic analysis. Five samples were collected in each period: 32, 40, 48, 60, 72, 84 and 96Haf. After the beginning of the exogenous feeding, five larvae were collected for the experimental unit, totaling 35 samples per treatment during periods - 108, 120, 144, 168, 192, 216, 240, 264 and 312 Haf. Subsequently, two post-larvae were collected per experimental unit (10 post-larvae per treatment) at 360, 408, 456, 528, 600, 696 and 780Haf for enzymatic analysis. Samples were collected in the morning before feeding. The animals were slaughtered by hypothermia so that there was no influence of anesthetics in the sample. Each experimental unit consisted of one replicate, that is, the collected post-larvae were homogenized together forming the sample to be analyzed in quadruplicate. The samples were homogenized with buffer solution pH 7.0 in the proportion of 0.1 mg tissue (whole post-larvae) for each 1 mL of solution and were centrifuged at 3500 RPM for 10 minutes. The supernatants were used as enzymatic source for determination of total body protein activity (Bradford, 1976), acid protease (Hidalgo et al., 1999), trypsin and chymotrypsin (Hummel, 1959), lipase (Albro et al., 1985), amylase (Bernfeld, 1955) and maltase (Corrêa et al., 2007). In Table 1, diet lipid profile was described.

**Table 1:** Lipid profile of the diets used to feed the silver catfish post-larvae.

Fatty acids *	Standard <sup>1</sup>	15SPC	15FM	30SPC	30FM
C14:0	0.44	0.46	0.54	0.42	0.58
C16:0	27.65	26.32	25.78	25.86	25.41
C16:1	3.16	3.29	3.30	3.23	3.35
C18:0	11.92	9.98	9.88	8.75	8.90
C18:1 n-9	37.59	37.39	36.88	37.93	38.23
C18:2 n-6	12.34	15.80	16.20	17.32	16.36
C18:3 n-3	0.44	0.74	0.8	1.02	0.86
C22:1 n-9	0.60	0.51	0.35	0.72	0.73
C20:4 n-6	2.10	2.08	2.30	1.58	1.64
C22:6 n-3	0.44	0.49	0.62	0.48	0.64
ΣSaturated	41.39	38.01	37.49	36.27	36.14
ΣUnsaturated	58.61	61.99	62.51	63.73	63.86
Saturated/Unsaturated	0.71	0.61	0.60	0.57	0.57
ΣMonounsaturated	42.43	41.92	41.36	42.60	43.14
ΣPolyunsaturated	16.18	20.07	21.15	21.13	20.72
Σn-3	0.94	1.34	1.51	1.52	1.57
Σn-6	15.24	18.73	19.64	19.61	19.15
n-6/n-3	16.24	13.93	13.02	12.90	12.18

\* Fatty acids C6: 0; C11: 0; C13: 0; C15: 1 n-5; C17: 1n-7; C18: 2 n-6; C21: 0; C23: 0 and C22: 2 were not detected in the samples. The fatty acids C4: 0; C8: 0; C10: 0; C12: 0; C14: 1n-5; C15: 0; C17: 0; C18: 1 n-9t; C18: 3 n-6; C20: 1 n-9; C20: 2 n-6; C20: 3 n-3; C20: 0; C20: 1; C20: 2; C22: 0; C20: 3 n-6; C24: 0; C24: 1 n-9 and C20: 5 n-3 were found in less than 0.5%. Analysis performed in the NIDAL / UFSM laboratory. Treatments: Standard - liver plus yeast, adapted from Coldebella et al. (2011), 15FM e 30FM – replacing 15 and 30% liver poultry by fish meal (Rossato et al., 2018), 15SPC and 30SPC replacement 15 and 30% of the liver of poultry by soybean protein concentrate. Source: research data.

Analysis of the development of the digestive system, through histology, was performed through samples of five post-larvae per treatment in each period. Samples of all treatments and periods were collected in biometrics every week. Samples collected every 7 days after starting feeding. In the following periods: 108; 240; 408; and 600Haf. After statistical analysis of the weights, only the post-larvae of the treatments that presented higher and lower weight at the end of the experimental period were chosen. The samples of the treatments with the highest (15FM) and the lowest (30SPC) were analyzed at 21 days. When for the small number of animals due to collections for enzymatic analysis and cases of cannibalism, the experimental units of the animals that consumed the 30SPC diet did not present enough animals and the treatment was terminated. The samples were fixed in Bouin solution for 12 hours and then stored in 70% alcohol until the analyzes were carried out. Later they were dehydrated in ethanol, diaphanized in xylol and embedded in paraffin, according to the methodology described by Carginin-Ferreira and Sarasquete (2008). The histological sections were cut with a thickness of 4 µm in a rotary microton and stained with hematoxylin-eosin. Histological histories of the post-larvae were made through serial cuts for later analysis of the ontogenetic development of the organs of the digestive system (stomach, intestine, pancreas and liver). They were evaluated in light photomicroscope (Zeiss, Primo Star model). All post-larvae collected were euthanized by benzocaine overdose.

The experimental design was completely randomized. Initially, all data obtained were submitted to the normality test of Shapiro-Wilk, considered normal distribution of those data that presented  $p > 0.05$ . Subsequently ANOVA and test of comparison of means for the different components were performed. The minimum level of significance used in the statistical analysis was 5% ( $p < 0.05$ ).

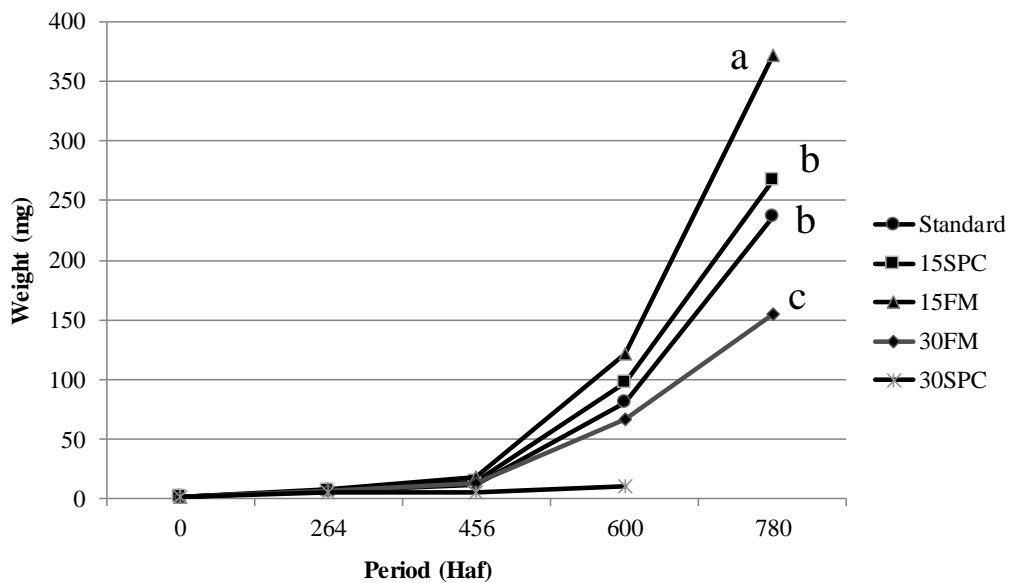
### 3. Results and Discussion

The lipid profile of the diet (Table 1) showed similarities to the percentage of saturated Fatty Acids (AG), saturated / unsaturated AG ratio and n-6 / n-3 ratio for 15SPC and 15FM diets. The 30SPC diet presented the lowest percentage of arachidonic acid (C20:4 n-6). The 15FM diet presented the highest C20:4 n-6 percentage compared to the other diets. It also

presented higher sum of polyunsaturated fatty acids and n-6 summation.

The animals fed the 15FM diet presented higher weight (> 350 mg) than the 30FM treatment (> 150 mg) at the end of the experiment (Figure 1). In this study at 600Haf the post-larvae fed the 30SPC diet did not present a representative number to continue the experiment, due to collections for analysis, cannibalism cases and mortality.

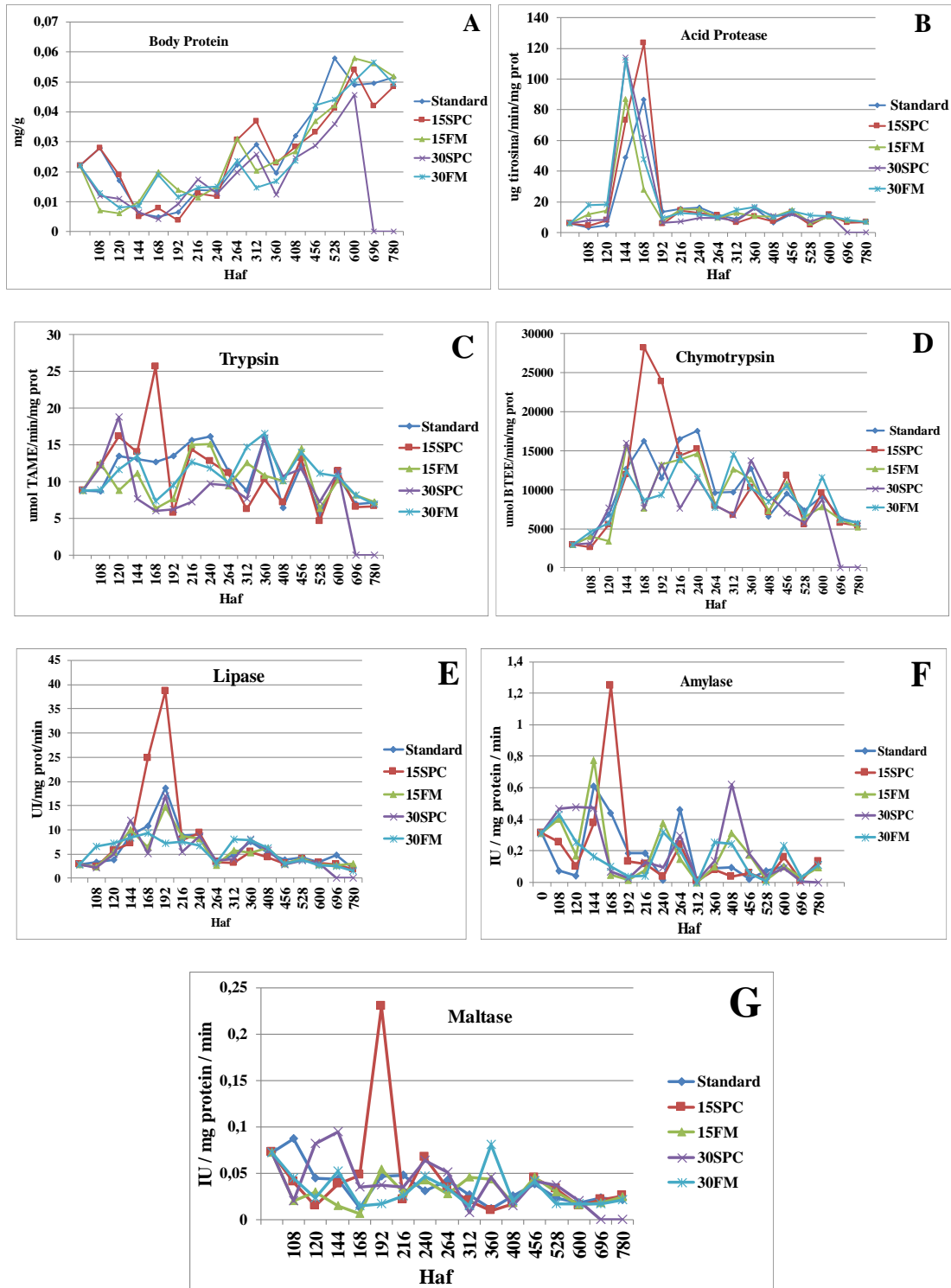
**Figure 1:** Growth curve of silver catfish post-larvae fed diets composed of protein sources of animal and vegetable origin. Different letters represent statistical difference by the Tukey test. Treatments: Standard; 15FM and 30FM, 15 and 30% liver replacement per fish meal (FM) (Rossato et al., 2018), 15SPC and 30SPC replacement 15 and 30% of the liver per soy protein concentrate.



Source: Research data.

The activity of protease, trypsin, lipase and maltase enzymes increased from 72Haf (Table 2). The body protein of all treatments remained in similar patterns, with peaks in the different treatments in the periods tested. Acid protease was elevated for animals consuming the 15FM (> 120  $\mu\text{mol}$  tyrosine / min / mg protein), followed by the 30FM and 15FM, the standard showed similar values (> 80). The trypsin activity showed a peak for the 15SPC in the period 168Haf (> 25  $\mu\text{mol}$  TAME / min / mg protein) (Figure 2). The other treatments showed that trypsin activity remained below 20  $\mu\text{mol}$  TAME / min / mg protein. Chymotrypsin activity had a peak for 15SPC in the 168Haf. The activity was higher than 25.000  $\mu\text{mol}$  BTEE / min / mg protein while the other treatments the activity in all periods were below 20.000  $\mu\text{mol}$  BTEE / min / mg protein (Figure 2). The activity of the lipase for 15SPC was increased from the period 168Haf (> 25 IU/min/mg protein) to the period 192Haf (> 35 IU/min/mg protein), afterwards it remained similar to the other treatments (<10 IU/min/mg protein). The activity of the amylase was superior in the treatment 15SPC in relation to the other treatments in the 168Haf period (1.2 IU/min / mg protein). The 30SPC presented increase in the period 408Haf (> 0.6) in relation to the other treatments. Subsequently the amylase activity remained similar in all treatments. The activity of the maltase for 15SPC showed elevation in the 190Haf period (> 0.2 IU/min/mg protein). Later, the activity of this enzyme was maintained for the treatments in the different periods (<0.1 IU/min/ mg protein) (Figure 2).

**Figure 2:** Enzymatic activity of silver catfish post-larvae fed diets composed of protein sources of animal and vegetable origin. Treatments: Standard, adapted from Coldebella et al. 2011 (15, 30, 30, 30, 30 and 30%) of the liver for soybean protein concentrate (SPC). Haf: Hours after fertilization.



Source: Research data.

In the histological sections was observed reduced development of the animals fed the 30SPC (Figure 3A and 3C) in relation to the animals fed the 15FM (Figure 3B and 3D). As a consequence, the development of the organs linked to the digestion of food is similarly lower in the animals fed the 30SPC (Figure 3). In relation to the development of intestinal villi,

no alterations were observed between the different treatments tested. When we compare the treatments, the intestinal villi and stomach crypts are proportionally smaller in the animals that consumed the 30SPC (Figure 3E, 3F and 3G).

The diet presented an adequate profile in fatty acids, especially the 15FM diet, higher values of arachidonic acid (20:4 n-6) and the sum of polyunsaturated fatty acids and n-6. Studies have shown that essential fatty acids (EFAs) such as docosahexaenoic acid (DHA, C22: 6 n-3) and eicosapentaenoic acid (EPA, C20: 5 n-3) are important in the nutrition of fish larvae (Mousavi-Sabet et al., 2013; El Kertaoui et al., 2019). According to the NRC (2011), freshwater fish strictly demand only linoleic acid (LA) and linolenic acid (ALA) fatty acids, unlike marine and stenohaline fish, in which diet it is indispensable to include highly unsaturated fatty acids (Segura et al., 2017). The diet 15FM presented higher values of arachidonic acid (ARA) in the composition. In marine fish larvae, studies have indicated that ARA levels may be important for tolerance to stress, pigmentation, growth and survival (Park et al., 2006). Freshwater fish have a greater ability to convert C18:0 fatty acid to highly unsaturated fatty acids (Asil et al., 2017). Arachidonic acid supplementation in diet can improve growth performance and enhance physiological characteristics of freshwater fish (Ji et al., 2011; Asil et al., 2017). This may have contributed to the greater development of the post-larvae fed the diets that presented the highest percentage of this fatty acid. Fatty acids are essential mainly in this phase of fish life, since they contribute to the formation of membranes and hormones (Izquierdo and Koven, 2011), consequently increasing growth and reducing mortality.

The inclusion of 30SPC in diets for post-larvae of silver catfish inhibits the development of the animals. According to Kumar et al., (2017) total replacement of soybean meal to replace fish meal in practical diets for *Silurus glanis* induced negative influences on growth and feed utilization. But for silver catfish, the inclusion of 15SPC in the diet provided positive performance. The nutritional value of soybeans is limited by the presence of several antinutritional factors such as trypsin and chymotrypsin protease inhibitors that affect the digestion and physiology of animals (Zhou et al., 2017). Soy products may contain non-starch polysaccharides that are not digested by fish, may have detrimental effects on nutrient performance and digestibility and on fish health (Krogdahl et al., 2010, Serra et al., 2019). These adverse effects are associated with the viscous nature of non-starch polysaccharides and their interaction with intestinal epithelium, mucus and microbiota, which result in physiological and inflammatory imbalances (Sinha et al., 2011, Serra et al., 2019). The soybeans, in the form of protein concentrate had less of these factors. These, even in small amounts may have contributed to reduce the development of the animals as observed for those fed on the 30SPC diet.

The fish that consumed the 15SPC presented similar development to that found for the animals that consumed the 15FM. Tolerance regarding the amount of SPC added to feed is closely related to the dietary habits and nutritional requirements of each species (NRC, 2011). From this, that total liver replacement is not advisable, as the inclusion of 30SPC in the diet of catfish post-larvae.

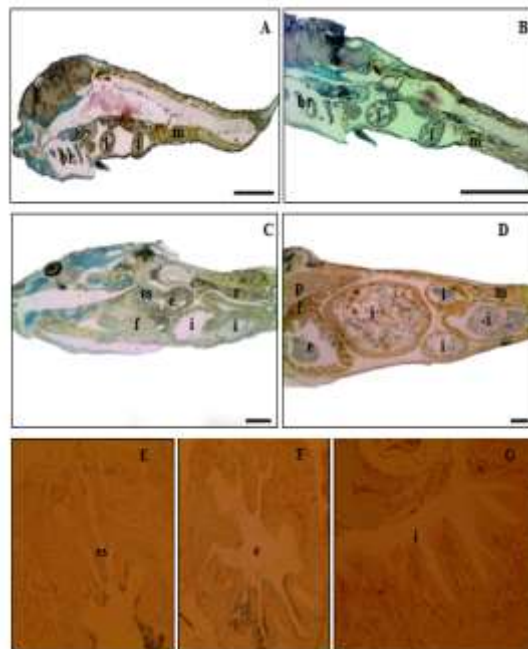
The activity of the main enzymes (acid protease, trypsin and chymotrypsin) at 32Haf were reduced. The enzymatic activity in larvae was low up to 32Haf. Then the sharp rise and fall to new 96Haf, when the food transition end. This behavior was also observed by Silveira et al. (2013). The yolk is the source of energy for the larvae to develop, and at the same time a substrate for the differentiation and growth of new structures (Portella et al., 2012). This is the first food to be metabolized with the help of existing enzymes, the main digestive enzymes produced by the pancreas being trypsin, amylase and lipase that can be detected even before opening the mouth (Gao et al., 2016; Cui et al., 2017). The activity of the acid protease had a gradual increase from 32Haf to 166Haf (Table 2).

**Table 2:** Enzymatic activity of silver catfish larvae, before onset of exogenous feeding.

Haf	Protein	Acid Protease	Trypsin	Chymotrypsin	Lipase	Amylase	Maltase
32	0.054 ± 0.01	1.56 ± 0.8	5.86 ± 0.8	3484.98 ± 627.4	1.35 ± 0.4	0.03 ± 0.04	0.043 ± 0.00
40	0.062 ± 0.02	1.52 ± 0.9	8.21 ± 5.1	5633.07 ± 1655.2	1.03 ± 0.4	0.23 ± 0.1	0.015 ± 0.02
48	0.040 ± 0.01	2.88 ± 2.1	10.39 ± 3.9	4470.54 ± 1086.3	1.32 ± 0.2	0.11 ± 0.07	0.006 ± 0.01
60	0.055 ± 0.02	1.96 ± 1.1	11.76 ± 7.0	3488.64 ± 395.0	1.35 ± 0.3	0.04 ± 0.08	0.009 ± 0.01
72	0.020 ± 0.01	3.27 ± 1.8	12.53 ± 3.1	4067.53 ± 676.5	3.29 ± 0.7	0.09 ± 0.09	0.046 ± 0.06
84	0.017 ± 0.01	3.77 ± 1.6	10.07 ± 2.6	3818.88 ± 866.4	4.32 ± 0.6	0.37 ± 0.19	0.047 ± 0.05
96	0.022 ± 0.00	5.87 ± 3.3	8.78 ± 1.6	2990.23 ± 273.3	2.87 ± 1.0	0.31 ± 0.23	0.072 ± 0.08

Values expressed as mean ± standard error of the mean. \* Total body protein (mg / g), acid protease (µg tyrosine / min / mg prot), trypsin (umolTAME / min / mg prot), chymotrypsin (umol BTEE / min / mg prot), lipase (min), amylase (IU / mg protein / min), maltase (IU / mg protein / min). Haf: hours after fertilization. Source: research data.

**Figure 3:** Histological sections of silver catfish post-larvae fed diets composed of protein sources of animal and vegetable origin. Exemplary post-larvae of silver catfish fed diets 30SPC (A, C) and 15FM (B, D) in time (240, 528 Haf) in longitudinal serial sectioning. E, F and G representative histological sections of the esophagus, stomach and intestine. (br) gills; (e) stomach; (es) esophagus; (f) liver; (i) intestine; (m) muscle; (p) pancreas; (r) kidney. Scale: 1 mm. Color: Vanderbilt modified.



Source: Research data.

For *Salminus brasiliensis* larvae, acid protease activity was detected on the third day after hatching and then increased up to the seventh day, which indicates functionality of the stomach (Vega-Orellana, Fracalossi & Sugai, 2006). The trypsin activity remained linear up to 72Haf and from this began to reduce. According to Conceição, Aragão and Rønnestad, (2011) is considered the key factor in the activation of pancreatic enzymes. For *Salminus brasiliensis* larvae, the activity of intestinal



proteinase (trypsin and chymotrypsin) were detected 12 h after hatching before the start of exogenous feeding (Vega-Orellana, Fracalossi & Sugai, 2006). The activity of trypsin and chymotrypsin decreased from 72Haf and the activity of the acid protease, amylase and maltase increased until the beginning of exogenous feeding. Trypsin and chymotrypsin in newly hatched larvae are involved in the cleavage of proteins contained in the yolk (Gisbert et al., 2008) and are the first enzymes to act more actively on the same. Lipase increased from 40Haf to 84Haf. This enzyme is linked to the digestion of calf lipids contained in vitellogenin which are broken down by lipases and contribute to the development of the embryo (Mommsen & Korsgaard, 2008). It is an enzyme of pancreatic origin that acts in the intestine acting on the lipids present in the chyme, releasing fatty acids and glycerol, which play important energetic, structural and hormonal functions in the organism (Moura et al., 2012).

After the beginning of the exogenous feeding (108Hpf), a peak of enzymatic activity occurred and later it stabilized. This peak occurred mainly for acid protease for all diets, two hours after the first feeding. For the other enzymes the peak occurred more markedly for the 15SPC diet (trypsin, chymotrypsin and amylase at 168Hpf, lipase and maltase at 192Hpf). This behavior occurred as a way of adaptation of the post-larvae to the diet so that there was an improvement of the digestibility and absorption of the nutrients. Also observed by Mitra et al. (2017) that concludes that these fluctuations of the enzymatic activities in featherback larvae reflect the ability of the fish to adapt with the diet during ontogenetic shift. According to Silveira et al. (2013), pepsin activity was low up to 32Haf, increasing and decreasing in the transition to exogenous feed. In the post-larvae that received the 30SPC diet, trypsin activity decreased from 120 to 312Haf. The same behavior observed for the amylase that at 144Haf had expressive decrease with recovery at 216Haf. The reduced enzymatic activity in the initial period of development may have contributed to the low growth presented by the animals that consumed this diet. Afterwards all the enzymes have stabilized, which may indicate an adaptation of the post-larvae to the food provided. For most fish species, digestive capacity becomes more efficient after hatching, when there is an abundance of gastric glands, pepsin-like enzymes and hydrochloric acid, which perform the first hydrolysis of proteins, thus facilitating the subsequent action of the enzymes alkaline, such as trypsin and chymotrypsin (Portella et al., 2014).

The decline in specific activity of both enzymes throughout the larval development of the catfish could be explained by the normal increase in tissue proteins (Zambonino Infante et al., 2008; Mitra et al., 2017). Reflecting anatomical and physiological changes in fish post-larvae, but not corresponding to the reduction in the amount of digestive enzymes (Zambonino Infante et al., 2008). As well as the progressive transformation of larval alkaline digestion, characterized primarily by pancreatic proteases, such as trypsin and chymotrypsin, to a juvenile acid digestion mode (Babaei et al., 2011; Mitra et al., 2017).

The development of the digestive system occurred in the same way in the 15FM and 30SPC diet animals. Slower development was observed in smaller animals due to the lower digestibility of SPC in relation to fish meal (FM) confirmed in studies carried out by Hien et al. (2017). According to Drew et al. (2007) the protein quality of the ingredient depends fundamentally on its digestibility. In the 15FM diet, the addition of fish meal contributed to improve the palatability of the rations. According to Liu et al. (2012), the animal meal significantly improves palatability. Diets with total liver replacement by SPC, fish did not develop as much as those fed the diet with partial replacement (15SPC). Low consumption, low palatability and cases of cannibalism were observed. In addition to the reduced amount of antinutritional compounds (Fontinelli & Radünz Neto, 2007; Sá et al., 2013) present in SPC, they may interfere in the general development of post-larvae. The post-larvae fed the 30SPC diet showed reduced development in relation to the animals that consumed the other diets. The histopathological analysis of the post-larvae independent of the different diets, did not reveal morphological alterations in the tissues and internal organs. No inflammatory process was observed in the individuals of both diets. Intestinal villi were normal. According to Lazo et al. (2011), the major histological changes in the intestinal mucosa include decreased enterocyte height and number and size of epithelial folds. Further studies should be conducted to further clarify these effects.

## 4. Conclusion

The results of the present study show that silver catfish consumed the 15FM diet, presented highest amount of fatty acids C20:4 n-6 and C22:6 n-3, lower incidence of peaks in the production of digestive enzymes.

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## References

- Albro, P. W., Hall, R. D., Corbett, J. T., & Schroeder, J. (1985). Activation of nonspecific lipase (EC3.1.1.) by bile salts. *Biochimica et Biophysica Acta*, 835, 477-490. [https://doi.org/10.1016/0005-2760\(85\)90117-1](https://doi.org/10.1016/0005-2760(85)90117-1)
- Alveal, K., Silva, A., Lohrmann, K. B., & Viana, M. T. (2019). Morphofunctional characterization of the digestive system in the palm ruff larvae, *Serirolella violacea* under culture conditions. *Aquaculture*, 501, 51-61. <https://doi.org/10.1016/j.aquaculture.2018.10.020>
- Asil, S. M., Kenari, A. A., Miyanji, G. R., & Van Der Kraak, G. (2017). The influence of dietary arachidonic acid on growth, reproductive performance, and fatty acid composition of ovary, egg and larvae in an anabantid model fish, Blue gourami (*Trichopodus trichopterus*; Pallas, 1770). *Aquaculture*, 476, 8-18. <https://doi.org/10.1016/j.aquaculture.2017.03.048>
- American Veterinary Medical Association (AVMA) (2007). *Guidelines on Euthanasia (Formerly Report of the AVMA Panel on Euthanasia)*, American Veterinary Medical Association (AVMA), United States of America.
- Babaei, S. S., Abedian Kenari, A., Nazari, R., & Gisbert, E. (2011). Developmental changes of digestive enzymes in Persian sturgeon *Acipenser persicus* during larval ontogeny. *Aquaculture*, 318, 138-144. <https://doi.org/10.1016/j.aquaculture.2011.04.032>
- Bernfeld, P. (1955). Amylases  $\alpha$  e  $\beta$ : colorimetric assay methods. In: Colowick, S.P.; Kaplan, N.O. *Methods in Enzymology*, Academic Press
- Bradford, M. M. A. (1976). A rapid and sensitive method for the quantification of microgram quantities of protein utilizing the principle of protein-dye binding. *Analytical Biochemistry*, 72, 248-254. [https://doi.org/10.1016/0003-2697\(76\)90527-3](https://doi.org/10.1016/0003-2697(76)90527-3)
- Cargnin-Ferreira, E., & Sarasquete Reiriz, C. (2008). *Histofisiología de moluscos bivalvos marinos*. CSIC, 94p.
- Coldebella, I. J., Radünz Neto, J., Mallmann, C. A., Veiverberg, C. A., Bergamin, G. T., Pedron, F. A., Ferreira, D., & Barcellos, L. J. G. (2011). The effects of different protein levels in the diet on reproductive indexes of *Rhamdia quelen* females. *Aquaculture*, 312, 137-144. <https://doi.org/10.1016/j.aquaculture.2010.12.021>
- Conceição, L., Aragão, C., & Rønnestad, I. Proteins. (2011). In: Holt, G. J. *Larval Fish Nutrition*, 3, 88-120
- Corrêa, C. F., Aguiar, L. H., Lundstedt, L. M., & Moraes, G. (2007). Responses of digestive enzymes of tambaqui *Colossoma macropomum* to dietary cornstarch changes and metabolic inferences. *Comparative Biochemistry and Physiology*, 147, 857-862. <https://doi.org/10.1016/j.cbpa.2006.12.045>
- Cui, K., Cheng, D., Ma, Z., Qin, J.G., Jiang, S., Sun, D., & Ma, S. (2017). Ontogenetic development of digestive enzymes in larval and juvenile crimson snapper *Lutjanus erythropterus* (Bloch 1790). *Aquaculture Research*, 48, 4533-4544. <https://doi.org/10.1111/are.13278>
- Drew, M. D., Borgeson, T. L., & Thiessen, D. L. (2007). A review of processing of feed ingredients to enhance diet digestibility in finfish. *Animal Feed Science and Technology*, 138, 118-136. <https://doi.org/10.1016/j.anifeeds.2007.06.019>
- Mohd Faudzi, N., Yong, A. S. K., Shapawi, R., Senoo, S., Biswas, A., & Takii, K. (2018). Soy protein concentrate as an alternative in replacement of fish meal in the feeds of hybrid grouper, brown-marbled grouper (*Epinephelus fuscoguttatus*) × giant grouper (*Epinephelus lanceolatus*) juvenile. *Aquaculture Research*, 49, 431-441. <https://doi.org/10.1111/are.13474>
- Fontinelli, E., & Radünz Neto, J. (2007). Efeito do concentrado proteico de soja em rações, com e sem suplementação em aminoácidos, para pós-larvas de jundiá (*Rhamdia quelen*). *Revista Brasileira de Agrociência*, 13, 225-229.
- Gao, X. Q., Liu, Z. F., Guan, C. T., Huang, B., Lei, J. L., Li, J., Guo, Z., Wang, Y., & Hong, L. (2016). Developmental changes in digestive enzyme activity in American shad, *Alosa sapidissima*, during early ontogeny. *Fish Physiology and Biochemistry*, 43, 397-409. <https://doi.org/10.1007/s10695-016-0295-2>
- Gisbert, E., Giménez, G., Fernández, I., Kotzamanis, Y., & Estévez, A. (2008). Development of digestive enzymes in common dentex *Dentex dentex* during early ontogeny. *Aquaculture*, 287, 381-387. <https://doi.org/10.1016/j.aquaculture.2008.10.039>
- Hartman, L., & Lago, B. C. (1973). A rapid preparation of fatty methyl esters from lipids. *Laboratory Practice*, 22, 475-477.
- Hidalgo, M. C., Urea, E., & Sanz, A. (1999). Comparative study of digestive enzymes in fish with different nutritional habits: Proteolytic and amylase activities. *Aquaculture*, 170, 267-283. [https://doi.org/10.1016/S0044-8486\(98\)00413-X](https://doi.org/10.1016/S0044-8486(98)00413-X)

- Hien, T. T. T., Phu, T. M., Tu, T. L. C., Tien, N. V., Duc, P. M., & Bengtson, D. A. (2017). Effects of replacing fish meal with soya protein concentrate on growth, feed efficiency and digestibility in diets for snakehead, *Channa striata*. *Aquaculture Research*, 48, 3174-3181. <https://doi.org/10.1111/are.13147>
- Hummel, B. C. W. (1959). A modified spectrophotometric determination of chymotrypsin, trypsin and thrombin. *Canadian Journal of Biochemistry and Physiology*, 37, 1393-1399. <https://doi.org/10.1139/o59-157>
- Izquierdo, M., & Koven, W. (2011). *Lipids*. In: Holt, I. *Larval Fish Nutrition*. Wiley Online Library. 448 p.
- Ji, H., Li, J., & Liu, P. (2011). Regulation of growth performance and lipid metabolism by dietary n-3 highly unsaturated fatty acids in juvenile grass carp, *Ctenopharyngodon idellus*. *Comparative Biochemistry and Physiology Part B: Biochemistry and Molecular Biology*, 159, 49-56. <https://doi.org/10.1016/j.cbpb.2011.01.009>
- Krogdahl, A., Penn, M., Torsen, J., Refstie, S. & Bakke, A. M. (2010). Important antinutrients in plant feedstuffs for aquaculture: an update on recent findings regarding responses in salmonids. *Aquaculture Research*, 41, 333-344. <https://doi.org/10.1111/j.1365-2109.2009.02426.x>
- Kumar, V., Sinha, A. K., Makkar, H. P. S., Boeck, G. D., & Becker, K. (2011). Phytate and phytase in fish nutrition. *Journal of Animal Physiology and Animal Nutrition*, 96, 335-364. <https://doi.org/10.1111/j.1439-0396.2011.01169.x>
- Kumar, S., Sándor Zs, J., Nagy, Z., Fazekas, G., Havasi, M., Sinha, A. K., Boeck, G., & Gál, D. (2017). Potential of processed animal protein versus soybean meal to replace fish meal in practical diets for European catfish (*Silurus glanis*): growth response and liver gene expression. *Aquaculture Nutrition*, 23, 1179-1189. <https://doi.org/10.1111/anu.12487>
- Lazo, J. P., Darias, M. J., & Gisbert, E. (2011). Ontogeny of the digestive tract. In: Holt, I. *Larval Fish Nutrition*. Wiley Online Library. 448 p.
- Liu, X., Ye, J., Wang, K., Kong, J., Yang, W., & Zhou, L. (2012). Partial replacement of fish meal with peanut meal in practical diets for the Pacific white shrimp, *Litopenaeus vannamei*. *Aquaculture Research*, 43, 745-755. <https://doi.org/10.1111/j.1365-2109.2011.02883.x>
- Mente, E., Solovyev, M. M., Vlahos, N., Rotllant, G., & Gisbert, E. (2017). Digestive Enzyme Activity during Initial Ontogeny and after Feeding Diets with Different Protein Sources in Zebra Cichlid, *Archocentrus nigrofasciatus*. *Journal of the World Aquaculture Society*, 48, 831-848. <https://doi.org/10.1111/jwas.12381>
- Mitra, A., Mukhopadhyay, P. K., & Homechaudhuri, S. (2017). Profile of Digestive Enzymes Activity During Early Development of Featherback *Chitala chitala* (Hamilton, 1822). *Proceedings of the Zoological Society*, 70,141-149. <https://doi.org/10.1007/s12595-016-0169-8>
- Mo, W. Y., Man, Y. B., & Wong, M. H. (2018). Use of food waste, fish waste and food processing waste for China's aquaculture industry: Needs and challenge. *Science of the Total Environment*, 613, 635-643. <https://doi.org/10.1016/j.scitotenv.2017.08.321>
- Mommsen, T., & Korsgaard, B. (2008). Vitellogenesis. In: Rocha, M. J., Arukwe, A., Kapoor, B. G. *Fish Reproduction*. Science Publishers, Enfield, N.H., 113-169.
- Moura, G. S., Oliveira, M. G. A., & Lanna, E. A. T. (2012). Desempenho e atividade de lipase em tilápias do Nilo. *Archivos de Zootecnia*, 61, 367-374. <http://dx.doi.org/10.4321/S0004-05922012000300005>
- Mousavi-Sabet, H., Ghasemnezhad, H., & Petrescu-Ma, I. V. (2013). Effects of diet containing enriched Artemia with unsaturated fatty acids and vitamin C on growth, survival and stress resistance of swordtail *Xiphophorus hellerii* fry. *Poeciliid Research*, 3, 14-21.
- NRC - National Research Council. (2011). *Nutrient requirements of fish and shrimp*. National Academies Press, 376 p.
- El Kertaoui, N., Lund, I., Assogba, H., Domínguez, D., Izquierdo, M. S., Baekelandt, S., Cornet, V., Mandiki, S.N.M., Montero, D., & Kestemont, P. (2019). Key nutritional factors and interactions during larval development of pikeperch (*Sander lucioperca*). *Scientific Reports*, 9, 7074. <https://doi.org/10.1038/s41598-019-43491-1>
- Park, H. G., Puvanendran, V., Kellett, A., Parrish, C. C., & Brown, J. A. (2006). Effect of enriched rotifers on growth, survival, and composition of larval Atlantic cod (*Gadus morhua*). *Journal of Marine Science*, 63, 285-295. <https://doi.org/10.1016/j.icesjms.2005.10.011>
- Piaia, R., & Radünz Neto, J. (1997). Avaliação de diferentes fontes protéicas sobre o desempenho inicial de larvas do jundiá *Rhamdia quelen*. *Ciência Rural*, 27, 319-323. <http://dx.doi.org/10.1590/S0103-84781997000200025>.
- Portella, M. C., Leitão, N. J., Takata, R., & Lopes, T. S. (2012). Alimentação e nutrição de larvas. In: *NUTRIAQUA: nutrição e alimentação de espécies de interesse para a aquicultura brasileira*, 9, 185-216.
- Portella, M. C., Jomori, R. K., Leitão, N. J., Menossi, O. C. C., Freitas, T. M., Kojima, J. T., Lopes, T. S., Clavijo-Ayala, J. A., & Carneiro, D. J. (2014). Larval development of indigenous South American freshwater fish species, with particular reference to pacu (*Piaractus mesopotamicus*). *Aquaculture*, 432, 402-417. <https://doi.org/10.1016/j.aquaculture.2014.04.032>
- Rossato, S., Maschio, D., Martinelli, S. G., Nunes, L. M. D. C., Radünz Neto, J., & Lazzari, R. (2018). Fish meal obtained from the processing of *Rhamdia quelen*: An alternative protein source. *Boletim do Instituto de Pesca*, 44, 1361-1372. <https://doi.org/10.20950/16782305.2018.44.4.350>
- Sá, M. V. C., Sabry-Neto, H., & Nunes, A. J. P. (2013). Dietary concentration of marine oil affects replacement of fish meal by soy protein concentrate in practical diets for the white shrimp, *Litopenaeus vannamei*. *Aquaculture Nutrition*, 19, 199-210. <https://doi.org/10.1111/j.1365-2095.2012.00954.x>
- Sargent, J., McEvoy, L., Estevez, A., Bell, G., Bell, M., Henderson, J., & Tocher, D. (1999). Lipid nutrition of marine fish during early development: current status and future directions. *Aquaculture*, 179, 217-229. [https://doi.org/10.1016/S0044-8486\(99\)00191-X](https://doi.org/10.1016/S0044-8486(99)00191-X)
- Segura, J. G., Campanharo, J. C., Oliveira, K. R. B., Natori, M. M., Medeiros, A. C. L., & Viegas, E. M. M. (2017). Relação 18:3n3/18:2n6 sobre a digestibilidade de ácidos graxos em pacu. *Boletim do Instituto de Pesca*, 43, 222-230. <https://doi.org/10.20950/1678-2305.2017v43n2p222>

- Serra, C. R., Almeida, E. M., Guerreiro, I., Santos, R., Merrifield, D. L., Tavares, F., Oliva-teles, A. & Enes, P. (2019). Selection of carbohydrate-active probiotics from the gut of carnivorous fish fed plant-based diets. *Scientific Reports*, 9, 6384. <https://doi.org/10.1038/s41598-019-42716-7>
- Seong, T., Matsutani, H., Haga, Y., Kitagima, R., & Satoh, S. (2019). First step of non-fish meal, non-fish oil diet development for red seabream, (*Pagrus major*), with plant protein sources and microalgae *Schizochytrium sp.* *Aquaculture Research*, 50, 2460-2468. <https://doi.org/10.1111/are.14199>
- Silveira, J., Silva, C.P., Cargnin-Ferreira, E., Alexandre, D., Elias, M.A., & Fracalossi, D. M. (2013). Freshwater catfish jundiá (*Rhamdia quelen*) larvae are prepared to digest inert feed at the exogenous feeding onset: physiological and histological assessments. *Fish Physiology and Biochemistry*, 39, 1581-1590. <https://doi.org/10.1007/s10695-013-9810-x>
- Sinha, A. K., Kumar, V., Makkar, H. P. S., De Boeck, G. & Becker, K. (2011) Non-starch polysaccharides and their role in fish nutrition – A review. *Food Chemistry*, 127, 1409–1426. <https://doi.org/10.1016/j.foodchem.2011.02.042>
- Tacon, A. G. J., & Akiyama, D. M. (1997). Feed ingredients. In L. R. D'Abramo, D. E. Conklin, & D. M. Akiyama (Eds.), *Advances in World aquaculture 6: Crustacean nutrition* (pp. 411–472). Baton Rouge, LA: World Aquaculture Society.
- Teles, A.O., Salas-Leiva, J., Alvarez-González, C. A., Gisbert, E., Ibarra-Castro, L., Urbiola, J. C. P., & Tovar-Ramírez, D. (2017). Histological study of the gastrointestinal tract in longfin yellowtail (*Seriola rivoliana*) larvae. *Fish Physiology and Biochemistry*, 43, 1613-1628. <https://doi.org/10.1007/s10695-017-0397-5>
- Vega-Orellana, O. M., Fracalossi, D.M., & Sugai, J. K. (2006). Dourado (*Salminus brasiliensis*) larviculture: Weaning and ontogenetic development of digestive proteinases. *Aquaculture*, 252, 484-493. <https://doi.org/10.1016/j.aquaculture.2005.07.002>
- Vizcaíno, A. J., López, G., Sáez, M.I., Jiménez, J.A., Barros, A., Hidalgo, L., Camacho-Rodríguez, J., Martínez, T. F., Cerón-García, M. C., & Alarcón, F. J. (2014). Effects of the microalga *Scenedesmus almeriensis* as fishmeal alternative in diets for gilthead sea bream, *Sparus aurata*, juveniles. *Aquaculture*, 431, 34-43. <https://doi.org/10.1016/j.aquaculture.2014.05.010>
- Yúfera, M., & Darias, M. J. (2007). The onset of exogenous feeding in marine fish larvae. *Aquaculture*, 268, 53-63. <https://doi.org/10.1016/j.aquaculture.2007.04.050>
- Zambonino Infante, J. L., Gisbert, E., Sarasquete, C., Navarro, I., Gutiérrez, J., & Cahu, C. (2008). Ontogeny and physiology of the digestive system of marine fish larvae. *Feeding and digestive functions of fishes*, 281-348.
- Zambonino Infante, J. L., & Cahu, C. L. (2007). Dietary modulation of some digestive enzymes and metabolic processes in developing marine fish: Applications to diet formulation. *Aquaculture*, 268, 98-105.
- Zhou, Z., Ringø, E., Olsen, R. E., & Song, S. K. (2017). Dietary effects of soybean products on gut microbiota and immunity of aquatic animals: A review. *Aquaculture Nutrition*, 4(1):644-665.