Gonad development and sperm characteristics of male silver catfish (Rhamdia quelen) fed diets with different oil sources

Desenvolvimento gonadal e características espermáticas de jundiás machos (Rhamdia quelen) alimentados com dietas com diferentes fontes de óleo

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ABSTRACT
It is widely accepted that broodstock nutrition or enriched diets with certain compounds greatly impacts fish reproductive performance, especially the input of different concentrations of fatty acids (especially polyunsaturated ones). Thus, here we evaluated reproductive variables in male catfish (*Rhamdia quelen*) of 18.45 ± 1.22 g fed with diets containing different lipid sources: fish, canola, sunflower, soybean and olive oils. 300 juvenile *R. quelen* were randomly distributed in 20 net-tanks ($1m^3$) disposed inside a 200 m^2 masonry pond, and the experiment was composing five treatments with four replicates, and were fed during 90 days, with a 3% inclusion level of the oils in the diets. The histological evaluations of the fish testicles were characterized in the final development stage and maturation in all animals, regardless of the offered diet. Spermatic normality, seminal volume, total sperm production, seminal pH and testosterone level did not present significant differences among treatments. Higher sperm concentration was more pronounced in males fed a diet containing sunflower oil. Parameters obtained by computer-assisted sperm analysis (CASA) showed that the poor sperm quality were verify when fish were fed diets containing olive and canola oil. The gonadosomatic, hepatosomatic and viscerosomatic fat indexes of the animals were similar, regardless of the supplied lipid source. In a practical perspective, we concluded that soybean and sunflower oils can be used as replacement to fish oil in diets for male specimens of *R. quelen* while the canola and olive oils should be avoid, because decrease the sperm quality.

Keywords: animal nutrition, native species, energetic displacement, seminal quality.

RESUMO
É amplamente aceito que a nutrição dos reprodutores ou dietas enriquecidas com determinados compostos impactam muito o desempenho reprodutivo dos peixes, especialmente a entrada de diferentes concentrações de ácidos graxos (especialmente os poliinsaturados). Assim, foram avaliadas variáveis reprodutivas em jundiás machos (*Rhamdia quelen*) de 18,45 ± 1,22 g alimentados com dietas contendo diferentes fontes lipídicas: peixes, canola, girassol, soja e azeite de oliva. 300 juvenis de *R. quelen* foram distribuídos aleatoriamente em 20 tanques-rede (1m3) dispostos dentro de uma lagoa de alvenaria de 200 m^2, e o experimento foi composto por cinco tratamentos com quatro repetições, e foram alimentados durante 90 dias, com nível de inclusão de 3% do óleos nas dietas. As avaliações histológicas dos testículos dos peixes foram caracterizadas no estágio final de desenvolvimento e maturação em todos os animais, independente da dieta ofertada. A normalidade espermática, volume seminal, produção total de espermatozoides, pH seminal e nível de testosterona não apresentaram diferenças significativas entre os tratamentos. A maior concentração de espermatozóides foi mais pronunciada em machos alimentados com dieta contendo óleo de girassol. Parâmetros obtidos por análise de esperma assistida por computador (CASA) mostraram que a má qualidade do esperma foi verificada quando os peixes foram alimentados com dietas contendo azeite e óleo de canola. Os índices de gordura gonadossomática,

hepatossomática e viscerossomática dos animais foram semelhantes, independente da fonte lipídica fornecida. Do ponto de vista prático, concluímos que os óleos de soja e girassol podem ser utilizados em substituição ao óleo de peixe em dietas para machos de *R. quelen* enquanto os óleos de canola e de oliva devem ser evitados, pois diminuem a qualidade espermática.

Palavras-chave: nutrição animal, espécies nativas, deslocamento energético, qualidade seminal.

1 INTRODUCTION

The South American catfish (*Rhamdia quelen*) has become a widely studied native species in Brazilian fish farming, due to this species' handling characteristics, which are well adapted to the existing rearing models in the country, and also its omnivorous feeding habits with a tendency to carnivory (Diemer et al., 2012; Borges-Neto et al., 2013), reflecting in greater plasticity and adaptability to different diets.

Due to its reproductive characteristics, such as sexual maturity after the first year of life and apportioned spawning, their reproduction in captivity has be facilitated (Montanha et al., 2011). However, information related to nutritional influences on reproductive processes must be elucidated in orde to promote productive increases of the species in captivity.

It is widely accepted that broodstock nutrition or enriched diets with certain compounds greatly impacts fish reproductive performance (Izquierdo et al., 2001; Butts et al., 2015; Butts et al., 2019). Lipids are organic molecules of animal and plant origin used as sources of energy and essential fatty acids, which is directly related to fish's productive and reproductive performances, as well as its general metabolism (Navarro et al., 2010). Most fish species preferentially use lipids as an energy supply, which is directed to somatic growth, but also serves as a source of essential fatty acids that are necessary for the development of cell membranes (Sargent et al., 2002).

The influence of plant-origin oils as an ingredient for fish feed occurs in the triggering of hormonal disturbances via pituitary gland activity (Nasopoulou and Zabetakis, 2012; Futawaka et al., 2016) - especially during growth (Kutluyer et al., 2017), due to increases in protein synthesis and lipolysis (Biga and Meyer, 2009). The growth hormone participates in the metabolic activity of lipids, proteins and carbohydrates and acts expressively in reproductive physiological processes (Reinecke et al., 2005; Hoseinifar et al., 2017) as the gametogenesis.

The input of different concentrations of fatty acids (especially polyunsaturated ones) may influence the reproductive performance of fish, by acting in hormonal responses in the function of growth, development and gonadal maturation (Zhang et al., 2013). Furthermore, these elements serve as transporters for liposoluble vitamins and play fundamental roles in biochemical and

hormonal processes, as well as in the equilibrium during substance carriage through cell membranes (Nelson and Cox, 2014).

Plant origin oils may contain significant concentrations of linoleic acid (LA), which are called essential fatty acids - as animals are unable to produce (Bell, 1998). Thus, these oils must be part of fish diets, either by the use of plant ingredients or by supplementation. Olive, canola, soybean and sunflower oils have concentrations of 7.9%, 20.2%, 51.0% and 67.5% of LA of the n-6 series, respectively. However, similarly to oils derived from plant materials, animal-origin oils may also have low concentrations of LA, varying from 0.6% to 10.5% (NRC, 2011). The linolenic acid (LNA) of n-3 series of plant-origin is found in lower concentrations in comparison to LA, in the range of 0.3%, 0.6%, 6.8% and 12% in sunflower, olive, soybean and canola oils, respectively. However, fish oil has LNA concentrations varying from 0.4% to 2.5% (NRC, 2011). Both LA and LNA act as precursors of the synthesis and release of arachidonic, eicosapentaenoic and docosahexaenoic acids, regardless of the lipid sources, which are fundamental for cellular maintenance, especially regarding the animal's reproductive system (Kus and Mancini-Filho, 2010).

The correlation between fatty acids and hormonal levels influences the enzymatic activity of gonadotropins and steroids, which directly relates to processes of maintenance and maturation of germ cells in fish (Zhang et al., 2013; Araújo et al., 2017). Throughout sexual maturation, fatty acids act in spermatogenesis, seen that eicosapentaenoic, arachidonic and docosahexaenoic acids modulate the synthesis of androgens and are essential during gonadal development (Baeza et al., 2015).

During the spermatogenesis process, lipid biomolecules have relevant structural importance in gonadal development and gamete formation. In diets' formulation, one can use different lipid sources with distinct fatty acids composition in diets offered, in order to improve its reproductive capacity. Considering the constant growth of fish farming and the need to increasingly provide juvenile fish, studying the influence of dietary sources in fish reproduction is of great importance (Rocha et al., 2013; Alvarenga et al., 2015). In this sense, here we evaluated the effects of different lipid sources (canola, sunflower, soybean, olive and fish oils) in reproductive variables of *R. quelen*.

2 MATERIAL AND METHODS

2.1 STUDY STRATEGY

Aiming to evaluate the effects of different lipid sources in reproductive variables of *R. quelen*, we fed fish with diest containing canola, sunflower, soybean, olive and fish oils as lipid sources, during 90 days and after sampling fish. We then evaluated testosterone levels, the sperm quality parameters and gonadal histology.

2.2 ANIMALS AND EXPERIMENTAL DESIGN

Three hundred *R. quelen* juveniles (18.45 ± 1.22 g) were distributed in 20 net-tanks (1 m^3) disposed inside a 200 m^2 masonry pond, and the experiment was carried our for 90 days. Throughout this period, water quality variables remained stable: dissolved oxygen 6.0 ± 0.8 mg L^{-1}; pH 6.07 ± 0.2; electrical conductivity 36.1 ± 1.5 µS cm^{-1}; and temperature 23.6 ± 2.1 °C. The adopted experimental procedures were approved by the Ethics Committee on Animal Use of the State University of West Paraná (CEUA/Unioeste n° 05/18).

2.3 EXPERIMENTAL DIETS

A completely randomized design with five treatments and four replicates was used, considering the use of different sources of oils (soybean, sunflower, canola, olive, and fish oil) in the animals' diets. One experimental unit was considered to be one net-tank (1 m^3) containing 15 fish each. Five isoenergetic (3,250 kcal DE/kg of feed) and isoproteic (20.09% DP) diets were prepared with the aforementioned oils at a concentration of 3.0% (Table 1).

Table 1. Composition of experimental diets containing different lipid sources for male catfish (*Rhamdia quelen*)

Ingredients (%)	Lipid Sources (oils)				
	Soybean	Sunflower	Fish	Canola	Olive
Fishmeal	33.30	33.30	33.30	33.30	33.30
Corn	18.89	18.89	18.89	18.89	18.89
Rice Grits	12.65	12.65	12.65	12.65	12.65
Poultry viscera meal	12.55	12.55	12.55	12.55	12.55
Soy bran 45%	10.00	10.00	10.00	10.00	10.00
Wheat bran	8.00	8.00	8.00	8.00	8.00
Soybean oil	3.00	-	-	-	-
Sunflower oil	-	3.00	-	-	-
Fish oil (tilapia)	-	-	3.00	-	-
Canola oil	-	-	-	3.00	-
Olive oil	-	-	-	-	3.00
Premix[1]	1.00	1.00	1.00	1.00	1.00
Common salt	0.30	0.30	0.30	0.30	0.30
Choline chloride	0.10	0.10	0.10	0.10	0.10
Calcium propionate	0.30	0.30	0.30	0.30	0.10
Vitamin C	0.10	0.10	0.10	0.10	0.10
Hydroxytoluene butylate	0.02	0.02	0.02	0.02	0.02
Total	100	100	100	100	100
Nutrients (%)					
Digestible protein[1]	29.09	29.09	29.09	29.09	29.09
Linoleic acid	2.97	2.97	2.97	2.97	2.97
Starch	25.00	25.00	25.00	25.00	25.00
Total arginine	2.67	2.67	2.67	2.67	2.67
Calcium	3.21	3.21	3.21	3.21	3.21
Digestible energy (kcal)[1]	3250	3250	3250	3250	3250
Crude fiber	1.68	1.68	1.68	1.68	1.68
Total phosphorus	1.45	1.45	1.45	1.45	1.45

Fat	9.36	9.36	9.36	9.36	9.36
Total lysine	2.01	2.01	2.01	2.01	2.01
Total methionine	0.71	0.71	0.71	0.71	0.71
Proximate composition (natural matter %)					
Crude energy (kcal kg^{-1})	4540.00	4480.00	4500.00	4480.00	4480.00
Crude Protein	38.43	39.06	39.08	38.28	39.47
Fat	8.60	8.03	8.68	8.79	8.84
Mineral matter	3.84	4.05	3.64	4.23	4.05

[1]Premix composition - warranty levels of the product: vit. A - 500,000 UI; vit. D3 - 250,000 UI; vit. E - 5,000 mg; vit. K3 - 500 mg; vit. B1 - 1,500 mg; vit. B2 - 1,500 mg; vit. B6 - 1,500 mg; vit. B12 - 4,000 mg; folic acid - 500 mg; calcium pantothenate - 4,000 mg; vit. C - 10,000 mg; biotin - 10 mg; inositol - 1,000; nicotinamide - 7,000; choline - 10,000 mg; cobalt - 10 mg; copper - 1,000 mg; iron - 5,000 mg; iodine - 200 mg; manganese - 1,500 mg; selenium - 30 mg; zinc - 9,000 mg.
Values of digestible energy and crude protein (%) estimated for *Rhamdia voulezi* proposed by Freitas et al. (2011).

All ingredients were milled in a hammer mill coupled to a 0.6 mm mesh, blended and extruded (extruder Ex-Micro®, Company Ex-Micro, Ribeirão Preto - São Paulo, Brazil). Subsequently, feeds were dried in a forced ventilation oven (55 °C) for 24h. After drying, all diets received its respective oils - which were manually sprayed and mixed, and were stored in a freezer. The proximate composition of the diets was determined by the methodology proposed by AOAC (2005).

Fish were fed four times a day until apparent satiety throughout the entire experiment. Then, based on the observed external sexual characteristics and semen liberation upon mild abdominal press (Reidel et al., 2010; Diemer et al., 2014), seven males with a mean weight of 118.73 ± 23.48 g and 21.36 ± 4.16 cm of length were randomly selected from each treatment when able to reproduce.

2.4 SAMPLING AND ANALYSIS

Three males from each experimental unit were collected for blood sampling. The animals were anaesthetized with a benzocaine solution (100 mg L^{-1}), as suggested by Gomes et al. (2001), and blood was sampled through a puncture of the caudal vein, with the aid of disposable syringes containing 10% EDTA. Plasma samples were obtained by centrifugation at 2500 rpm for five minutes, and then kept at -80 °C until the analysis. Free testosterone levels were assessed by radioimmunoassay of the solid phase by using commercial kits (Symbiosis diagnostica Ltda), and the interpretation was performed with the aid of an ELISA spectrophotometer.

Breeding males were selected according to external morphological characteristics. Seven animals per treatment were hormonally induced with carp pituitary extract (CHE) in a single intramuscular injection of 2.5 mg CHE kg^{-1}. After 240 hours-degree of induction, extrusion was performed by means of head-caudal abdominal massage for gametes collection. Semen samples of each fish were collected in Falcon tubes (10 mL) for volume measurement and kept in a polystyrene

box containing ice cubes (maintained at approximately 12 °C) until the end of the analyzes (Sanches et al., 2010).

The seminal pH was measured by colourimetric method using Merk® litmus paper (Asturiano et al., 2001). Sperm concentration was assessed from semen dilution in saline-buffered formaldehyde (1:1000) and counted with the aid of a Neubauer hematimetric chamber (Sanches et al., 2011). Sperm normality was also evaluated by fixation in saline-buffered formaldehyde (1:1000 semen: fixator), from 500 µL of semen, which were posteriorly stained with rose Bengal. Using extension slides, 300 sperm cells of each fish were counted and classified as normal or abnormal, according to Streit et al. (2006), with the analyzes being carried out with the aid of a light microscope under 40x lens (CBRA, 1998). Both the volume of released semen (mL) and relative seminal volume (semen released (ml) / fish weight (kg)) were measured. The total of sperm production per male was estimed (semen released (ml) * Sperm concentration (spz mL^{-1})).

Sperm motility evaluations were carried by the computerized method, as suggested by Wilson-Leedy and Ingermann (2007) and Sanches et al. (2013), with the aid of the open-code software IMAGEJ (National Institute of Health, USA). The activation process for fresh semen was performed in Eppendorf tubes (1.5 mL) by using 1 µL of semen and 400 µL of distilled water (25 °C; 0.0 mOsm kg^{-1}). The images were obtained with the aid of a trinocular microscope (Solarist Bel) in a 10x objective, coupled to a Basler camera (model acA640-120uc), connected to a computer (Intel Core i7© CPU 2.4GHz, 8Gb Ram) with a Microsoft Windows 8© operational system.

The videos were captured by the software Basler Pylon Camera Software (baslerweb.com) at 100 fps (658x492 pixels) in *.avi format, edited in the VIRTUALDUB-1.9.0 software (virtualdub.org) and exported as an image sequence in *.jpg format into a specific directory. The corresponding images were opened, edited and compiled by means of the *CASA* plugin (University of California and Howard Hughes Medical Institute, USA). Video processing was performed based on the description of the components needed for the utilization of the CASA application, by means of free software (Wilson-Leedy and Ingermann, 2006), however, the adopted settings were adapted for the species, according to Sanches et al. (2010).

The evaluated sperm parameters were: motility rate (MOT), curvilinear velocity (VCL), average path velocity (VAP), straight line velocit (VSL), straightness (STR), wobble (WOB) and progression (PRG). The analyses were performed during one second (1s) of video (100 images), and in 10 seconds post-activation in three videos for each male specimen, with seven males considered in each treatment as replicates.

After collecting the seminal samples, fish were euthanized with a benzocaine solution (250 mg L^{-1}) and eviscerated for testicles, liver and fat removal, aiming to obtain the gonadosomatic

(GSI), hepatosomatic (HSI) and viscerosomatic fat (VSFI) indexes by means of the calculation (organ weight/total weight of the fish x 100).

Regarding the histological evaluation of the testicles, the organs were fixed in FAA (formaldehyde, alcohol, acetic acid) solution, stored in alcohol 70% up to histological processing. After fixation, the biological material was serially dehydrated with alcohol solutions, clarified in xylene, included and embedded in histological paraffin. Histological sections (5 µm) were obtained in a microtome (Micron 340E - Thermo Scientific) and stained with Hematoxylin-Eosin (HE). Then, slides were analyzed in a light microscope (NIKON Eclipse-50) and classified according to the stage of gonadal maturation (only qualitative evaluation), as suggested by Brown-Peterson et al. (2011) and Quagio-Grassiotto et al. (2013).

2.5 STATISTICS

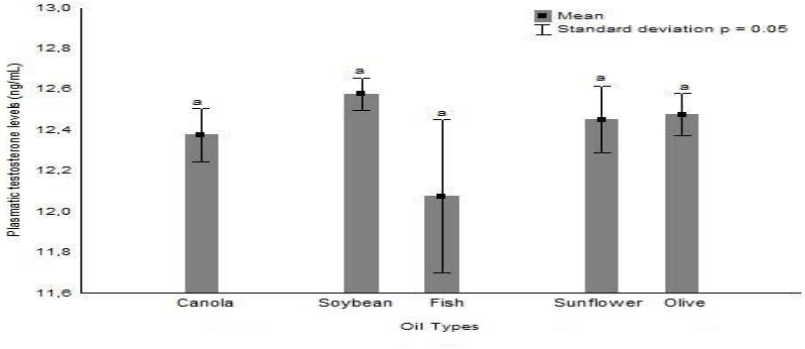
All obtained data were submitted to a variance analysis (*one-way* ANOVA), followed by the multiple comparison test of Tukey at a 5% significance level, with the aid of the software Statistica 7.0®. The basic statistical assumptions were checked in the residues as suggested by Myers (1990) and Quinn and Keough (2002).

3 RESULTS

3.1 FREE TESTORTERONE LEVELS

Plasma testosterone levels were not influenced ($p>0.05$) in fish that were fed diets containing different lipid sources (Figure 1).

Figure 1. Free plasma testosterone levels of male Rhamdia quelen fed diets containing different lipid sources. Data were expressed as the mean ± standard deviation. No differences were detected according Tukey's test

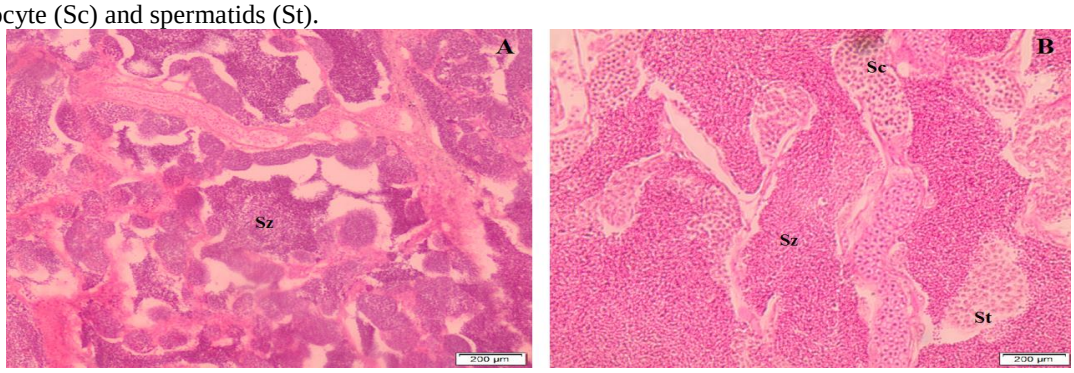


3.2 HISTOLOGICAL ANALYSIS

Histological evaluations of the fish testicles were performed at the final developmental stage and maturation in all animals, regardless of the offered diet. Sperm cells were visualized within the

organ's duct, and the testicular lobes were enlarged with irregular shape, presenting few spermatids in the peripheral area (Figure 2).

Figure 2. Histological sections of the testicles of catfish (*Rhamdia quelen*) fed diets containing different lipid sources. All catfish showed the same stage of gonadal maturation. A and B: testicles a containing sperms (Sz); primary spermatocyte (Sc) and spermatids (St).



3.3 SEMINAL AND SPERMATIC CHARACTERIZATION

Fish that were fed diets containing sunflower oil presented higher sperm cell concentration ($p < 0.05$) (Table 2). However, to normality of sperm cells, volume semen released, relative volume of semen, total sperm production and seminal pH were not influenced ($p > 0.05$) by the tested types of oil included in the experimental diets, which presented mean values of 95.85 ± 1.61%, 3.24 ± 2.27 mL, 28.03 ± 19.07 mL kg^{-1}, $6.71x10^{10}$ ± $4.63x10^{10}$ sperm mL^{-1}; 7.0 ± 0.0 and 12.38 ± 0.35 ng mL^{-1}, respectively.

Table 2. Seminal and spermatic variables of Rhamdia quelen fed with diets containing different lipid sources

Variables*	Lipid sources				
	Soybean	Sunflower	Fish	Canola	Olive
Final body weight	140.70±24.30	117.93±17.44	131.24±29.57	118.57±26.22	125.93±32.00
Volume of released semen (mL)	3.47±0.45^{ab}	2.28±0.66^{a}	6.00±2.29^{b}	5.50±1.80^{ab}	3.28±0.63^{ab}
Relative volume (mL kg^{-1})	2.57±0.56^{a}	2.01±0.27^{a}	4.54±1.45^{b}	5.02±1.03^{b}	2.75±0.19^{a}
Sperm concentration ($x10^{9}$ SPZ mL^{-1})	1.34±0.13^{ab}	1.88±0.02^{c}	1.19±0.04^{a}	1.28±0.15^{ab}	1.50±0.10^{b}

*Means within the same line with distinct letters indicate significant effect ($p < 0.05$) for each experimental diet, according to the Tukey's test. SPZ: spermatozoa

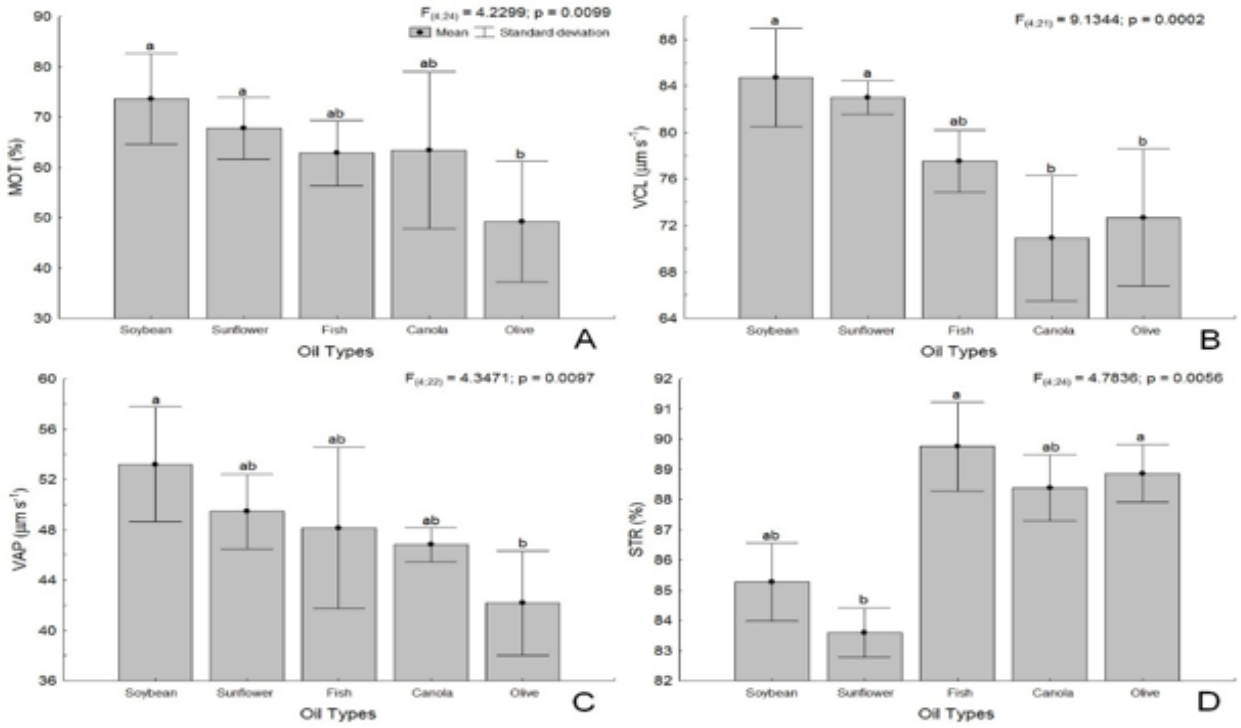
3.4 SPERM MOTILITY

With an exception of straightness, all spermatic parameters were higher in animals fed diets containing soybean oil, whilst the lowest values were observed in animals fed olive and canola oils-supplemented diets (Figure 3).

The motility rate was lower ($p < 0.05$) in fish fed diets containing olive oil, despite the fact that it did not differ from fish fed canola and fish oil (Figure 3A). A significant effect ($p < 0.05$) was

observed in curvilinear speed, with an increase in the sperm cells of fish fed diets containing soybean and sunflower oil, being statistically similar to fish oil (Figure 3B). The average path velocity was lower ($p < 0.05$) in fish fed diets containing olive oil, and similar when sunflower, canola and fish oil were included (Figure 3C). Concerning the straightness - parameter that indicates the percentage of straight movement of sperm cells - the lowest value ($p < 0.05$) was observed in fish fed diets containing sunflower and soy oil (Figure 3D). Straight line velocity, wobble and progression were not influenced ($p > 0.05$) by diets containing different types of oil, and presented the following mean values: 41.17 ± 5.25 µm s^{-1}, 62.25 ± 5.80%, and 1773.19 ± 221.91 µm, respectively.

Figure 3. Sperm parameters of catfish (*Rhamdia quelen*) fed diets containing different lipid sources (Oil Types). A: motility rates (MOT); B: curvilinear velocity (VCL); C: average path velocity (VAP); D: straightness (STR). Different letters in bars represent p<0,05 according Tukey's test.



In regard of the somatic indexes, these were not statistically different among treatments ($p > 0.05$), with mean values of 4.06 ± 1.74% of GSI; 2.54 ± 0.75% of VSFI; and 1.77 ± 0.57% of HSI.

4 DISCUSSION

Here we show that the reproductive performance of *Rhamdia quelen* males can be affectd by the dietary lipid sources. In fact, in an application perspective, we found that soybean and

sunflower oils can be used as replacement to fish oil in diets for male *R. quelen* and the canola and olive oils should be avoid, because decrease the sperm quality.

We observed that the interference on spermatic parameters of fish occurred both in the regulation and composition of the seminal plasma, according to the presence of distinct lipid sources used in the experimental diets. The plasma composition of fish contains organic substances such as triglycerides (Borges et al., 2005), which might enhance the viability of sperm cells due to its use as energy source, from its production to the moment of release (Aitken and Baker, 2004). It may also aid in the protection of sperm cells against deleterious effects, as it supposedly provides an antioxidant action in the cells (Lahnsteiner et al., 2009). Thus, we hypothesized that this interference occurs due to different lipid composition of the lipid sources, specially regarding the fatty acid composition.

Generally, freshwater fish fed diets containing lipid sources rich in fatty acids have the ability of converting linoleic acid into arachidonic acid, linolenic into eicosapentaenoic acid and finally to docosahexaenoic acid, by means of elongation and desaturation processes via enzymatic activities (Turchini et al., 2006). Among the main components of sperm membrane phospholipids, the presence of Eicosapentaenoic and docosahexaenoic fatty acids facilitates the quantity and mobility of the released sperm (Henrotte et al., 2010; Butts et al., 2015; Silva et al. 2016). In this sense, low fish sperm performance can be related to lower levels of fatty acids of the n-3 series on this diet (Butts et al., 2015) together with the antinutritional factors (Rickard and Thompson, 1997).

Diets for *R. quelen* breeders must include lipid sources that contains highly unsaturated fatty acids (Vargas-Anido, 2013), and in case this source is of plant origin, the adequate utilization of fatty acids by the fish occurs from 30 days of feeding onwards (Higuchi et al., 2013). This species can benefit from its omnivorous feeding habit, as according to Vargas et al. (2008), it was possible to observe the presence of highly unsaturated fatty acids in the animals body composition, showing the capacity of desaturation and elongation during biosynthesis when fish were fed diets containing plant-origin oils. However, a greater effectiveness of fatty acid desaturation occurs in fatty acids of the n-3 series, due to the activity of the Δ6-desaturase enzyme (Izquierdo, 2005; Perini et al., 2010).

After ingestion, digestion and absorption, the fatty acids are carried through the bloodstream by specific lipoproteins and made available during gonadal maturation, for the maintenance of both functional and structural integrity of the germ cells (Cejas et al., 2004). During this process, the poly-unsaturated acids (arachidonic, eicosapentaenoic and docosahexaenoic) are fundamental for the animal's reproductive development (Izquierdo et al., 2001). Specifically for the catfish, Vargas and Bessonart (2007) observed that under natural environmental conditions, the concentration of arachidonic acid is higher at the beginning of gonadal maturation, whilst at the end of this process,

increases in the concentration of both eicosapentaenoic and docosahexaenoic acids might be verified.

The linolenic acid is also important for the fish's reproductive physiology, and can modulate steroid hormone responses that acts in growth, development and maturation of gonads (Rennie et al., 2005; Zhang et al., 2013). The fatty acids to be included in the breeders' diet, such as from canola oil, presents up to 12% of linolenic acid in its composition, which may contribute to low sperm quality when they exceed ideal levels for specific physiological processes for each species. In contrast, soybean and olive oils have about 6.8 and 0.6% of linolenic acid, while sunflower oil does not contain this acid (NRC, 2011).

The n-6 fatty acids are precursors of several eicosanoids that may influence reproductive and growth processes, as well as vitellogenesis in fish (Sargent et al., 2002; Garcia et al., 2012) as it can lead to a greater use as energy source (Suárez-Mahecha et al., 2002). The oils that have a higher amount of fatty acids of the n-3 series contribute to the progression of maturation of the fish gonads from the induction and cell growth in the final stages of gametogenesis (Silva et al., 2016) which has been observed in fish fed diets containing soybean oil in this study. Thus, high levels of fatty acids allow changes in the proportion and type of acids in the composition of phospholipids, and also in the action of cellular chemical signals, leading to energetic targeting to different metabolic pathways that drive hormonal processes for the animal's development.

In this line, the sperm concentration was higher when sunflower oil was used, which may be influenced by endocrine processes related to metabolic characteristics of the species in function of the used energy sources (Bombardelli et al., 2006; Zhang et al., 2013). Eicosapentaenoic, docosahexaenoic and arachidonic acids may act in the modulation of androgen synthesis in the final stage of maturation in fish, thus influencing in the process of spermatogenesis, by regulating the production of steroids (Baeza et al., 2015), and consequently acting in the spermatic metabolism and fertilization capacity of fish (Navarro et al., 2010). Sunflower oil possibly provided the catfish with greater viability of sperm cell formation, due to the directing of lipids to the sperm cell membrane, generating a protective effect to peroxidation processes, thus maintaining its cell membrane structure and permeability intact (Da Costa e Streit Jr., 2019). Such protective influence occurs due to the presence of 68.2% of linoleic acid in the sunflower oil, besides being a source of unsaturated fatty acids of the n-6 series, which leads to the modulation of other essential acids and regulation of the cellular function (Flagella et al., 2002). In Addition, the sunflower oil has a relevant amount of flavonoids, important substances, such as antioxidant, allelopathic function and enzyme inhibition (Blum, Ramoni and Balbi, 2016), favoring sperm viability, as we observed in our study in motility activity, velocity and displacement of sperm.

Fish fed diets containing soybean oil benefited from the high concentration of n-3 series fatty acids, according to Silva et al. (2016) together with treatment for hormonal induction promoted stimuli in the development of advanced follicular stages in fish fed diets containing higher levels of n-3 series fatty acids. It is noteworthy that soybean oil has a good amino acid profile when compared to other oils and still has a greater facility for eliminating thermolabile antinutritional factors during its processing (Mohanta et al., 2007). The observed reduction on the straightness of sperm cells of fish fed soybean and sunflower oils, is a general indicative of good sperm quality for catfish, seen that less rectilinear movements provide higher sperm quality, which may consequently improve the fertilization capacity of these cells (Sanches et al., 2013b; Sanches et al., 2015).

Plant-origin oils (especially soybean) may allow greater oxygen transport due to its high concentration and availability of iron (NRC, 2011), an important molecule for the synthesis of the heme group via blood tissue, serving as a means for the transportation of electrons among proteins. This is a relevant fact regarding the metabolic process of absorption and assimilation of nutrients in body tissues (Nelson and Cox, 2014), which is also directed to the development of germ cells.

According to Bozkurt et al. (2008) and Lahnsteiner et al. (2009), the availability of lipids and fatty acids to cells during spermatogenesis, might positively influence the sperm cells in the germ cell storage throughout the seminiferous tubule lumen immobility, at the cell regeneration stage and yet in the generated mobility. The dietary intake containing of feed containing an adequate relation of n-3/n-6 fatty acids that make up the lipid sources of plant oils, and factors such as unsaturation degree and the proportion of poly-unsaturated fatty acids (PUFA) in fish diets, may be related to changes in the hormonal metabolism, favoring reproductive performance by the improvement of sperm quality and structural integrity of tests (Cabrita et al., 2014), seen that normal sperm cells present greater proportions of PUFA in its composition (Bobe and Labbé, 2010).

Despite of vegetable oils are oftentimes the best for fish breeding processes when compared to fish oil, this was not observed in our experiments, as canola and olive oils worsen sperm quality. These reinforcing that the use of alternative sources in the aquaculture, due to the global shortage of fish oil, should be used only after careful tests, and for previously defined objectives.

The decrese in sperm quality verified in fish fed with diest containing canola and olive oil diets (Fig. 3) reinforces our hypothesis that the reproductive performance of fish are related to the balance in the ratio of fatty acids in the diets. In fact, the canola and olive oils have a low content of polyunsaturated fatty acids in the n-3 series (Pennington, 2002). The content of polyunsaturated fatty acids in the n-3 series (Pennington, 2002) and its relationship with other nutrients such as vitamins and minerals (Mazzocchi et al., 2021), probably imply the formation of phospholipids, especially phosphatidylcholine, which makes up the semen (Vassallo-Agius, 2001) acting in the

protection of sperm in osmosis and thermal shock processes (Simpson et al., 1986), in addition, there is a low proportion of docosahexaenoic acid, responsible for the fluidity of the sperm cell membrane (Roqueta-Rivera et al., 2010; Engel et al., 2020), hence the efficiency in the reproductive process.

Regarding the hormonal levels, the diets containing different sources of lipids did not influence the twestosterone levels in silver catfish (Fig. 1), possibly related to similar reproductive development time in all fish indicating their final maturation process (Barcellos et al., 2001), a fact observed that corroborates with the histological evaluation of the gonads that presented an advanced state of maturation (Fig 2).

A last important comment is that we did not determine the fatty acid profiles in the different lipid sources tested. In fact, we used the consolidated literature together with our data to form the empirical basis of our discussion correlating the fatty acid profile of each lipid source with male R. quelen reproductive traits (steroid profiles, gonadal development, and sperm quality). We recognize that this lack of fatty acid measurements may be a limitation of our work, however, this does not lessen the importance of this work, since data about the effects of lipid sources on male jundiá reproductive traits are very scarce.

5 CONCLUSION

In a practical perspective, we concluded that soybean and sunflower oils can be used as replacement to fish oil in diets for male specimens of *R. quelen* while the canola and olive oils should be avoid, because decrease the sperm quality.

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DECLARATION OF COMPETING INTEREST

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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