Sperm characteristics and growth of Rhamdia quelen males fed on rations formulated with vegetable food and different digestible energy levels supplied by soy bean oil

Short Communication Características espermáticas e crescimento de machos Rhamdia quelen alimentados com rações formuladas com alimentos vegetais e diferentes níveis de energia digestível fornecidos pelo óleo de soja

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ABSTRACT
This research was carried out to evaluate the effects of rations formulated with vegetable foods and different levels of digestible energy supplied by soybean oil, on sperm characteristics and growth of Rhamdia quelen males. The fishes were fed on over 255 days with rations containing 350 g crude protein (CP) kg^{-1} and 2700, 2950, 3200, 3450 and 3700 kcal of digestible energy (DE) kg^{-1}. A randomized experimental design was used with five treatments and three replicates. A 8 m^2 tank containing six females and three males was an experimental unit. The fishes were weighted fortnightly to correct the feeding rate. All males receipted an intramuscular injection of 2.5 mg of crude pituitary extract (CPE) kg^{-1} to perform the sperm collection. The sperm characteristics were assessed on fresh sperm immediately after collection. All data were subjected to ANOVA and regression analysis. The growth, sperm volume, sperm concentration, or time of sperm motility were not affected ($p < 0.05$) by rations. In addition, ration containing 3318.75 kcal of DE kg^{-1} improved the integrity of sperm membrane, suggesting a theoretical higher sperm survival rate of 98.22%. In conclusion, ration formulated with vegetable foods and different levels of soy bean oil as energy source did not interfere on growth of Rhamdia quelen males. In addition, the diet energy supplied by mainly soy bean oil offered a nutritional condition that improved the sperm quality since males fed on ration with 3318.75 kcal of digestible energy kg^{-1} has been increased the membrane integrity of sperm cell.

Keywords: Fertility; Membrane Integrity; Motility; Silver Catfish; Spermatozoa.

RESUMO
Esta pesquisa foi realizada com o objetivo de avaliar os efeitos de rações formuladas com alimentos vegetais e diferentes níveis de energia digestível fornecida pelo óleo de soja,

nas características espermáticas e no crescimento de machos de Rhamdia quelen. Os peixes foram alimentados por mais de 255 dias com rações contendo 350 g de proteína bruta (PB) kg^{-1} e 2700, 2950, 3200, 3450 e 3700 kcal de energia digestível (ED) kg^{-1}. O delineamento experimental foi inteiramente casualizado, com cinco tratamentos e três repetições. Um tanque de 8 m^2 contendo seis fêmeas e três machos foi uma unidade experimental. Os peixes foram pesados quinzenalmente para correção da taxa de alimentação. Todos os machos receberam uma injeção intramuscular de 2,5 mg de extrato pituitário bruto (CPE) kg^{-1} para realizar a coleta de sêmen. As características seminais e espermáticas foram avaliadas em espermatozoides frescos imediatamente após a coleta. Todos os dados foram submetidos a ANOVA e análise de regressão. O crescimento, volume seminal, concentração espermática, ou tempo de motilidade dos espermatozoides não foram afetados ($p < 0,05$) pelas rações. Além disso, a ração contendo 3.318,75 kcal de DE kg^{-1} melhorou a integridade da membrana espermática, sugerindo uma taxa teórica de sobrevivência espermática superior de 98,22%. Em conclusão, a ração formulada com alimentos vegetais e diferentes níveis de óleo de soja como fonte de energia não interferiu no crescimento de machos de Rhamdia quelen. Além disso, a energia da dieta fornecida principalmente pelo óleo de soja ofereceu uma condição nutricional que melhorou a qualidade espermática, em que machos alimentados com ração com 3.318,75 kcal de energia digestível kg^{-1} houve o aumento da integridade de membrana da célula espermática.

Palavras-chave: Fertilidade, Integridade de Membrana, Motilidade, Bagre Prateado, Espermatozoide.

1 INTRODUCTION

The silver catfish Rhamdia quelen is a promising species for South American aquaculture, particularly south of Brazil, Argentina and Uruguay, due its easy adaptably to farming conditions, good growth index and especially its tolerance to low temperatures (Santos and Meurer, 2018). Several studies have focused on the reproductive performance of the Rhamdia genus, which has been helpful in the support of its productivity and market insertion. In light of them, to meets the demand by high quality gametes, embryos and larvae of the growing Rhamdia farming, in the last years the scientific community has focus on broodstock nutrition (Tessaro et al., 2012; Diemer et al., 2014; Hilbig et al., 2019).

Energy in the diet is essential to maintain the vital processes of growth and reproduction (NRC, 2011). Specifically, lipids represent an important source of metabolic energy to feed fish (Melo et al., 2016). Lipids are a source of important fatty acids for male and female reproduction (Bombardelli et al., 2010; Hilbig et al., 2019), which affect the gonadal development and reproductive performance of fishes (Sargent et al., 2002).

Although fish oil is considered the main source of energy and essential fatty acids for fish nutrition, it has been gradually replaced by vegetable oil, such as soybean oil (Lazzari et al., 2016). Vegetable oils are useful because they provide a good fatty acid profile, which is involved in several reproductive processes in fish (Wassef et al., 2012); thus, soybean oil has been widely used in freshwater fish rations (Ayisi et al., 2019). Soybean oil is an abundant source of linoleic acid (18:2 n-6), which can be easily elongated to the highly unsaturated fatty acids (HUFAs) C_{20} and C_{22} by Rhamdia quelen (Juliana et al., 2016). Some of these HUFAs are metabolic precursors of series 2 prostaglandins, which are important in spermatogenesis (Sargent et al., 1995).

This preliminary research was carried out to evaluate the effects of rations formulated with vegetable foods and different levels of digestible energy supplied by soybean oil, on sperm characteristics and growth of silver catfish (Rhamdia quelen) males.

2 MATERIALS AND METHODS

The experiments were carried out in Toledo, PR, Brazil (-24.7806403 latitude; -53.7235581 longitude; and 476 m elevation) and were approved by the Institutional Ethical Committee on Animal Use (protocol 03112/2012 – CEUA). Over 255 days, a total of 90 females (44.67 ± 0.60 g) and 45 males of Rhamdia quelen (44.22 ± 1.17 g) were housed in 15 excavated tanks coated with masonry with a total area of 8 m^2. The tanks had supply of water only to compensate the loss by evaporation and seepage.

Fish were distributed in a randomized experimental design, consisting of five treatments and three replicates. The treatments were animals fed with rations containing 350 g CP kg^{-1} and 2700; 2950; 3200; 3450 and 3700 kcal of DE kg^{-1} (Table 1). Each 8 m^2 tank, containing six females and three males, was considered an experimental unit.

Table 1. Ingredient and calculated composition of experimental diets for Rhamdia quelen breeders

Ingredients (g kg^{-1} as fed)	Diets (kcal of digestible energy kg^{-1})				
	2700	2950	3200	3450	3700
Soybean meal	629.5	630.9	632.2	633.6	638.7
Corn	226.4	226.4	226.3	226.3	197.0
Inert	97.7	68.8	40.0	11.3	0
Dicalcium phosphate	23.0	23.0	23.0	23.1	23.3
Limestone	5.3	5.3	5.4	5.5	5.4
Mineral and vitamin mix[1]	10.0	10.0	10.0	10.0	10.0
DL – methionine	2.9	2.9	2.9	2.9	2.9
Salt (NaCI)	5.0	5.0	5.0	5.0	5.0
Antioxidant (BHT[2])	0.2	0.2	0.2	0.2	0.2
Soybean oil	0	27.5	55.0	82.2	117.5

Calculated composition (g kg^{-1} dry matter)[3]					
Crude protein	350.0	350.0	350.0	350.0	350.0
Digestible protein	304.5	305.1	305.8	306.4	306.8
Crude fiber	64.1	54.9	45.7	36.5	32.6
Fat	11.9	38.9	65.8	92.8	127.1
Digestible energy (kcal kg^{-1})	2700	2950	3200	3450	3700
Starch	159.9	160.0	160.0	160.0	160.0
Calcium	10.0	10.0	10.0	10.0	10.0
Total phosphorus	9.0	9.0	9.0	9.0	9.0
Met + cystine Total	12.5	12.5	12.6	12.6	12.5
Total methionine	7.5	7.5	7.5	7.5	7.5

[1]Basic composition: Folic acid: 200 mg, pantothenic acid:4000 mg; biotin: 40 mg; Cu: 2,000 mg; Fe: 12,500 mg; I:200 mg; Mn: 7,500 mg; niacin: 5,000 mg; Se: 70 mg; vitamin A: 1,000,000 UI; vitamin B1: 1,900 mg; vitamin B12: 3,500 mg; vitamin B2: 2,000 mg; vitamin B6: 2,400 mg; vitamin C: 50,000 mg; vitamin D3: 50,0000 UI; vitamin E:20,000 UI; vitamin K3: 500 mg; Zn: 25,000 mg. [2]Butylated hydroxytoluene. [3]The nutrient content were calculated by software Super Crac Premium®.

Previously, in the formulation of diets, ingredients were evaluated by the centesimal composition. The value of CP and DE was calculated according to Oliveira Filho and Fracalossi (2006). For the formulation of experimental diets, the ingredients were ground in a hammer mill, sieved through a 0.5 mm mesh, and pelleted at 3 mm diameter (Tessaro et al., 2012).

Fishes were fed twice daily (2% of biomass) at 10:00 h and 15:00 h. Every two weeks, fish were weighed (Marte® AS2000C; ± 0.01 g) to correct the feeding rates. At the end of the experiment, body weight was determined individually. From this data, the final weight and body weight gain were determined (Tessaro et al., 2012).

Water temperature was measured daily with a mercury thermometer (± 0.1 °C; winter and early spring: 18.8 ± 2.8 °C; spring and summer: 24.1 ± 2.5 °C; mean throughout the experimental period: 20.99 ± 3.91 °C). Every two weeks, we measured the pH (7.3 ± 1.0; Tecnal® Tec 5) and dissolved oxygen of the water (5.7 ± 1.7 mg L^{-1}; YSI 550A), both at 10:00 h and 15:00 h (Tessaro et al., 2012).

During the breeding season (january), fish were evaluated for semen and sperm parameters. All males receipted a single intramuscular injection with 2.5 mg CPE kg^{-1} to synchronism the spermiation. The sperm collection was performed 10 hours (water at 24 °C) after the hormonal application by gentle abdominal massage (Tessaro et al., 2012). Semen was collected with graduated test tubes (± 0.1 mL) and the volume of semen released (mL or ml kg^{-1}) was measured. Immediately after collection, semen was kept cool (± 12 °C) for the time required to carry out all sperm analysis (Tessaro et al., 2012). In fresh sperm were assessed the time of sperm motility, sperm survival rate and the sperm concentration (Tessaro et al., 2012).

All results were underwent to analysis of variance and regression analysis both at 5% significance level. Normality and homoscedasticity presuppositions were checked. Indexes in percentage were transformed into square root arc sen. The software Statistica 7.0® was used for proceed the statistical analyses.

3 RESULTS

The final weight and weight gain of Rhamdia quelen breeders was not influenced ($p > 0.05$) by diets containing different levels of DE. In general, males had a mean final weight between 94.22 and 120.17 g, which represented a weight gain between 50.00 and 75.72 g (Table 2).

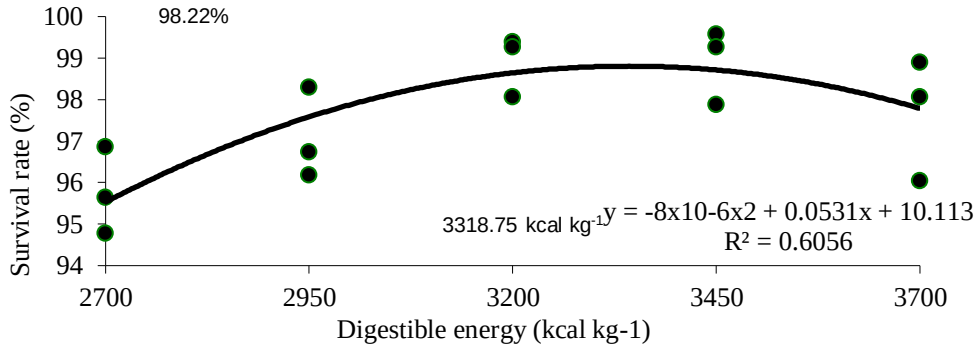
Table 2. Growth, semen and sperm parameters of breeder of Rhamdia quelen fed diets containing different levels of digestible energy and formulated with plant origin ingredients

Item	Diets (kcal of digestible energy kg^{-1})					P*
	2700	2950	3200	3450	3700	
FW	94.22±18.07	120.17±22.15	106.00±34.77	111.89±3.85	103.60±16.84	0.670
WG	50.00±18.41	75.72±20.70	61.56±34.47	67.67±3.77	59.82±17.78	0.672
VOL	3.54±2.48	4.73±1.17	2.10±1.50	3.63±0.36	4.20±1.37	0.357
VKG	45.80±38.21	41.54±11.82	21.73±17.36	33.46±2.57	40.93±5.84	0.614
SMD	28.96±5.95	24.14±3.61	25.83±1.08	23.71±1.10	24.59±0.67	0.329
SC	1.54±0.24	1.77±0.03	1.78±0.56	1.61±0.29	2.02±0.25	0.466

FW: mean final weight (g); WG: mean weight gain (g); VOL: volume of released semen (mL); VKG: volume of released semen in relation to body weight (mL kg^{-1}); SMD: sperm motility duration (s); SC: sperm concentration (spermatozoa x 10^{10} mL^{-1}); *P: P-value.

The experimental diets supplied to Rhamdia quelen breeders interfered in the production of viable spermatozoa. The highest percentage of live sperm or undamaged sperm membrane was achieved ($p < 0,05$) with diets containing 3318.75 kcal of DE kg diet^{-1} (Figure 1).

Figure 1 - Sperm survival rate in semen of Rhamdia quelen fed diets containing different levels of digestible energy and formulated with plant origin ingredients.



Finally, the volume of semen released (2.10 to 4.73 mL), the volume of semen released kg^{-1} (21.73 to 45.80 mL kg^{-1}), time of sperm motility (23.71 to 28.96 s) and sperm concentration (1.54 to 2.02 x 10^{10} spermatozoa mL^{-1}) were not affected ($p > 0.05$) by diets (Table 2).

4 DISCUSSION

The knowledge about ED requirement and the indirect effects of soy bean oil amount in the ration supplied to Rhamdia quelen breeders on sperm characteristics is determinant to improve the male fertility. The higher integrity of sperm membrane in fresh sperm from males fed ration with 3318.75 kcal of DE kg $diet^{-1}$ is a relevant event that deserves attention because survival rate is a sperm parameter commonly evaluated in andrologic routines and refers to sperm quality (Garcia et al., 2015). Since this parameter is strongly related to sperm motility in mammals (Lee and Park, 2015) and fish (Garcia et al., 2012), this index may be an appropriate indicator of cell viability (Cuevas-Uribe et al., 2011) considering its strong relationship with the successful fertilization of oocytes (Prunskaite-Hyyrylainen et al., 2014).

In a first assay on Rhamdia quelen breeders fed ration with different levels of DE, Tessaro et al. (2012) reported that 2700 to 3700 kcal DE kg^{-1} did not influenced the sperm characteristics. On the other hand, our results showed that ration with a vegetable source of energy, especially from soy bean oil, can improve the sperm quality as reported by Bombardelli et al. (2010) to males of Nile tilapia. These effects may be related to the fatty acid profile of the ration (Asturiano et al., 2001) rather than the energy level (Bombardelli et al., 2015). It is possible since arachidonic fatty acid (ARA; 20:4 n-6), which is a product of the elongation of linoleic fatty acid (18:2 n-6), is related to the production of eicosanoids and their metabolism. In light of them, ARA is related to the production of series 2 prostaglandins, which affect several functions of male reproduction and play an important role in spermatogenesis regulation (Norambuena et al., 2013a; Norambuena et al., 2013b; Asil et al., 2017).

In addition, similar to our data, poor results of sperm quality from males of piauçu (Leporinus macrocephalus) fed on ration with lower DE has been associated to changes in the rhythm of spermatogenesis that reduced the presence of Sertoli cells and the proliferation of spermatogonia (Navarro et al., 2006). Moreover, low sperm quality reported at higher levels of energy cannot be associated to limiting nutrient intake, since growth was not influenced by diets. The loss of sperm viability at higher levels of DE

may be associated to metabolic overload due accumulation of fat in hepatocytes (Bombardelli et al., 2010; Tessaro et al., 2012). The high lipogenic activity of soybean oil may favour the lipid accumulation in hepatocytes and after reduce the rates of lipid oxidation in these cells, promoting the loss of available metabolic energy for breeders (Caballero et al., 2004).

5 CONCLUSION

In conclusion, ration formulated with vegetable foods and different levels of soy bean oil as energy source did not interfere on growth of Rhamdia quelen males. In addition, the diet energy supplied by mainly soy bean oil offered a nutritional condition that improved the sperm quality since males fed on ration with 3318.75 kcal of digestible energy kg^{-1} has been increased the membrane integrity of sperm cell.

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