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Research Article

The effects of L-lysine in the diet of silver catfish (*Rhamdia voulezi*) female broodstocks

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ABSTRACT. The objective of this study was to assess the L-lysine effect in silver catfish, *Rhamdia voulezi* female reproduction. Four hundred fish were used distributed in a random in 16 net cages (25 fish each × 4 replicates = 100 fish per treatment). The treatments consisted in four diets, isoproteic and isoenergetic (30% crude protein and 3,500 kcal kg^{-1} digestible energy) with different L-lysine levels: T1: 1.30; T2: 1.40; T3: 1.60 and T4: 1.95%. After 185 days of experiment 18 females per treatment were weighed, measured, and submitted to hormonal induction (0.5 and 5.0 mg kg^{-1} Carp Pituitary Extract). The females were then sacrificed and had their ovaries, liver and fat removed and the respective organosomatic indices were estimated. With regard to the reproductive parameters, the released oocytes, absolute fecundity and remaining ovaries were influenced ($P < 0.05$) by the diet, and the highest mean values were observed in the treatment with 1.95% L-lysine. Among the organosomatic indices, only the visceral fat was influenced ($P < 0.05$). The estradiol levels and the composition of essential amino acid in oocytes were not affected ($P > 0.05$). We can conclude that L-lysine affects the reproductive parameters and accumulation of visceral fat in *R. voulezi*.

Keywords: catfish, *Rhamdia voulezi*, amino acid, L-lysine, oocytes, reproduction, nutrition.

INTRODUCTION

Silver catfish, *Rhamdia voulezi* is a Siluriforme species, endemic and found in the region of the lower Iguaçu River, Paraná, Brazil (Baumgartner *et al*., 2006), has a rapid growth, even during the coldest months of the year, good feed conversion and tasty flesh without intramuscular bones that is well appreciated by the consumers (Diemer *et al*., 2012; Signor *et al*., 2013).

The scarcity of fingerlings has been one of the bottlenecks to meet the regional demand and allow the systematic distribution to the productive sector. Nevertheless, we have observed significant progress in the development of priority investigations in order to understand the relationship between nutrition and reproduction mainly because it is a species of native fish (Coldebella *et al*., 2011; Tessaro *et al*., 2012).

Parra *et al*. (2010) observed changes in the morphometry of the eggs and larvae survival as a result of diets with different protein sources for *Rhamdia quelen* kept in captivity. According to Reidel *et al*. (2010), diets with 3,250 kcal kg^{-1} digestible energy and 35% crude protein for catfish kept in net cages caused faster gonad development and an extension of the spawning period. Therefore, it is evident that an appropriate nutrition enables the enrichment of the ovarian and testicular tissues, and consequently benefits reproduction (Coldebella *et al*., 2011; Tessaro *et al*., 2012).

In this sense, it is believed that amino acids are essential for the diet of broodstock, because they promote increase in vitellogenin production, which is the main precursor of yolk sac formation (Izquierdo *et al*., 2001), mobilizing the endogenous reserves as sources of nutrients for the initial ontogenetic period until the functional development of the digestive tract is complete (Andrade *et al*., 2010).

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Among the amino acids required by fish, L-lysine is regarded as reference to diet formulation because it is strictly essential, does not present any endogenous synthesis pathway, has basic metabolism and is the only one intended for body protein deposition. Concomitantly, the laboratory analysis to determine its level in the ingredients, diets and tissues is quite precise (Khan & Abidi, 2011). Information about the requirements needed by the species is extremely scarce (1.5 to 1.7% of the diet or 4.5 to 5.1% crude protein for fingerling (1.5 to 10 g) of *R. quelen*, according to Montes-Girao & Fracalossi (2006).

Therefore, the objective of this study was to analyze the effect of L-lysine on the reproductive performance by evaluating the ovarian tissue (histomorphology), organosomatic indices (gonadosomatic, hepatosomatic and viscerosomatic index), the levels of estradiol and the composition of total amino acids of *Rhamdia voulezi* breeding females, during the first reproductive cycle, confined in net cages.

MATERIALS AND METHODS

Experimental design

This experimental design and diets followed Diemer *et al.* (2014). The study was conducted at the Center for the Development of Technologies for Net Cages of the Iguaçu River - Governor José Richa Reservoir, located in Boa Vista da Aparecida - Paraná/Brazil, in partnership with Study Group on the Management of Aquaculture (GEMAq) of the State University of West Paraná (Universidade Estadual do Oeste do Paraná-Unioeste), campus Toledo, Paraná, Brazil. We kept 400 juveniles of *Rhamdia voulezi* (initial length 15.1 ± 2.2 cm and weight 35.18 ± 0.25 g) in 16 net cages (0.40 m³ useful volume, 0.5 cm mesh size). The experiment was carried out using four treatments and four replications, distributed randomly. Each treatment corresponds to an L-lysine level, and the experimental unit consisted in 25 fish per net cage (25 × 4 replicates = 100 fish per treatment) during 185 days.

Experimental diets

We prepared four isoenergetic and isoproteic diets (3,500 kcal kg^{-1} of digestible energy; 30% of crude protein) with different total L-lysine levels: 1.30; 1.40; 1.60 and 1.95% of the diet (Table 1), following Diemer *et al*. (2014). The intermediate L-lysine levels were chosen according to the nutritional requirements of the species according to Montes-Girao & Fracalossi (2006). The ingredients were ground in a hammer mill, with 0.5 mm mesh size and extruded, and the requirement for each nutrient was calculated by the software SuperCrac® 5.7 Master. The experimental feed was sent to CBO Laboratory (Campinas, São Paulo, Brazil) for determination of amino acid and crude protein composition, measured by means of High Performance Liquid Chromatography (Table 2). The meal was offered twice a day (09:00 and 17:00 h), until the apparent satiation.

Reproduction study

During the reproductive period 18 females and 18 males per treatment were selected by external characteristics (females: bulging abdomen which was soft to touch, swollen and reddish genital papilla; males: color and fluidity of the milt extruded after gentle pressure on the abdomen) (Reidel *et al*., 2010; Diemer *et al*., 2014). The seminal parameters were described by Diemer *et al*. (2014). The selected fish were transferred to the laboratory (only to spawning) located on the riverbank, individually weighed and placed in 250 L tanks. The water temperature ranged from 23.0 to 25.0ºC.

The sampled females, an ovarian sample was taken by biopsy from each fish per treatment: first sample (before the first hormonal dose) and second sample (at the moment of stripping). Each sample was removed using the intra-ovarian cannulation technique (inserting commercial urethral plastic catheter, external diameter = 20 mm), and separated by treatment. The biopsy sample was subdivided into three equal samples (subsamples): 1) placed in Serra solution (Brzuska, 1979) for 5 min to find the position of the germinal vesicle (GV) using a stereomicroscope (oc 10x; obj 17) (Bittencourt *et al*., 2012). Only the females which presented ≥60% GVs in a peripheral position were selected and separated by experimental, 2) preserved in Gilson's solution (Simpson, 1951) for 30 min, and then the diameters were measured under a stereomicroscope Zeiss-Stemi 2000 at 170x magnification (Romagosa *et al*., 1990; Bittencourt *et al*. 2012), 3) preserved in a buffered formaldehyde solution.

For induction of ovulation, these 18 females selected per treatment were injected with CPE at doses of 0.5 and 5.0 mg kg^{-1} body weight (CPE = Carp Pituitary Extract), with a 12 h interval between the doses. The males received one single dose of 2.5 mg CPE kg^{-1}.

After a period of 240 accumulated thermal units, the females were massaged so that the oocytes could be released. The eggs released from each female were collected in beakers (250 mL). Then, three 0.1 mL subsamples were collected, and the total number of released oocytes was estimated by extrusion: 1) 0.1 g quantify the number of released oocytes, 2) 100 oocytes fixed in Gilson´s solution (Simpson, 1951) to measure

Table 1. Ingredients and proximate composition of the experimental diets.

Ingredients (%)	L-lysine level (%)			
	1.30	1.40	1.60	1.95
Rice meal	35.00	34.90	34.80	34.70
60% Gluten	22.00	21.67	21.33	21.00
Corn grain	16.06	16.27	16.49	16.70
55% Fish meal	14.97	14.91	14.86	14.80
45% Soybean meal	9.15	9.07	8.98	8.90
Soybean oil	1.65	1.73	1.82	1.90
Mineral and vitamin mix[a]	0.50	0.50	0.50	0.50
Salt (NaCl)	0.30	0.30	0.30	0.30
Propionic acid	0.20	0.20	0.20	0.20
Antioxidant (BHT)	0.02	0.02	0.02	0.02
L-threonine	0.15	0.16	0.17	0.18
L-lysine ($HCL_{-99\%}$)	0.00	0.27	0.53	0.80
Total	100.00	100.00	100.00	100.00
Proximate composition				
Digestible energy (kcal kg^{-1})[b]	3500	3500	3500	3500
Crude protein (%)[c]	30.00	30.00	30.00	30.00
Total L-lysine (%)[c]	1.20	1.40	1.60	1.80
Crude lipid (%)[d]	4.73	4.73	4.73	4.73
Crude fiber (%)[d]	1.38	1.38	1.38	1.38
Total phosphorus (%)	0.80	0.80	0.80	0.80
Starch (%)[d]	38.62	38.62	38.62	38.62
Calcium (%)[d]	0.97	0.97	0.97	0.97
Total phosphorus (%)[d]	0.80	0.80	0.80	0.80

*Diet formulated by Diemer *et al*. (2014). [a]Basic composition: folic acid: 500 mg, pantotenic acid: 4000 mg; biotin: 40mg; Cu: 2000 mg; Fe: 12,500 mg; I: 200 mg; Mn: 7500 mg; niacin: 5000 mg; Se: 70 mg; vitamin A: 1,000,000 UI; vitamin B_1: 1900 mg; vitamin B_{12}: 3500 mg; vitamin B_2: 2000 mg; vitamin B_6: 2400 mg; vitamin C: 50,000 mg; vitamin D_3: 500,000 UI; vitamin E: 20,000 UI; vitamin K_3: 500 mg; Zn: 25,000 mg, [b]Digestible values to *Rhamdia quelen* according to Oliveira Filho & Fracalossi (2006), [c]HPLC laboratory analysis, [d]Calculated values.

the oocyte diameter at the moment of release (=spawning), and 3) the remainder were frozen (-20ºC, Freezer Continental, Brazil), identified and sent for analysis to determine amino acid composition of High Performance Liquid Chromatography. The amount of spawning females (percentage of females that released oocyte); absolute fecundity (total number of oocytes released by each female) and relative fecundity (number of oocytes released in relation to body weight) were calculated from oocytes samples (Tables 1-2).

After spawning, the fertilization was performed were homogenized separately gametes for each treatment. The eggs from each female was hydrated (5-7 min), and were transferred to 20 L conic fiberglass incubators. Eight hours after insemination, the progress of cleavage was observed under stereoscopic microscope (BEL-STM Pro, Italy).

Reproductive parameters and histology

The fertilization rate (FR% = 100 × the number of eggs which were observed to show cleavage - blastoporus closure/total number of oocytes) for each treatment was estimated by counting 100 eggs from each unit (in triplicate). The eggs showing irregular or opaque cleavage were discarded. The hatching rate (HR% = 100 × the number of hatched larvae/total number of larvae) was also estimated according to Romagosa *et al*. (1990).

After the gametes sampled all females were sacrificed with Eugenol® solution, as recommended by Diemer *et al*. (2012), dissected, had their organs removed (ovaries remnants, liver and visceral fat) and weighed (*g*) to calculate the indexes: gonadosomatic (IGS), hepatosomatic (HIS), and visceral fat. The indices were then calculated [Index = (weight of organ/total weight of the fish) × 100].

Fragments of the ovaries were fixed in buffered formol and processed according to the routine techniques for light microscopy (Romagosa, 2010). The tissues were dehydrated in a series of ethanol, infiltrated and embedded in glycol methacrylate, and then 5 µm sections were cut on a microtome (Sorvall

Table 2. Analysis of protein and amino acid composition of the experimental diets, formulated by Diemer *et al*. (2014).

Total amino acids (%)	L-lysine level (%)			
	1.30	1.40	1.60	1.95
Aspartic acid	2.44	2.35	2.28	2.46
Glutamic acid	5.28	5.13	4.95	5.26
Serine	1.52	1.49	1.42	1.49
Glycine	1.84	1.83	1.79	1.93
Histidine	0.65	0.59	0.62	0.63
Arginine	1.82	1.78	1.75	1.84
Threonine	1.14	1.10	1.04	1.15
Alanine	2.18	2.10	2.01	2.14
Proline	2.40	2.40	2.32	2.48
Tyrosine	1.12	1.13	1.13	1.17
Valine	1.40	1.32	1.34	1.42
Methionine	0.60	0.60	0.56	0.58
Cystine	0.35	0.46	0.59	0.48
Isoleucine	1.25	1.16	1.20	1.27
Leucine	3.44	3.34	3.27	3.46
Phenylalanine	1.47	1.42	1.39	1.46
L-lysine	1.30	1.40	1.60	1.95
Taurine	0.08	0.07	0.07	0.08
Crude protein (%)	30.47	29.79	29.61	31.40

Type JB-4, New York, USA), mounted onto glass slides and stained with hematoxylin-eosin. The material was processed using routine histological techniques. The analysis and photographic documentation were performed with a Nikon Eclipse-50 photomicroscope.

Estradiol analysis

The same females from each treatment were then anesthetized with Eugenol ® solution (60 mg L^{-1}), as recommended by Diemer *et al*. (2012) for the withdrawal of blood aliquots by caudal puncture (with syringes). After centrifugation at 3000 rpm for 10 min, the plasma was kept in frozen (-20ºC, Freezer Continental, Brazil) for later analysis using Interkit immunoassay commercial kits Elisa, according to Barcellos *et al*. (2002).

Water quality

The quality of the water of the area covered by the net cages was monitored by analyzing the variables: temperature (22.1 ± 1.9°C), pH (7.44 ± 0.42), electrical conductivity (268 ± 51 µS cm^{-1}) and dissolved oxygen (7.43 ± 1.49 mg L^{-1}) measured weekly *in situ* by means of portable potentiometers by Hanna Instruments® and water transparency (3.1 ± 0.5 m) measured by visual disappearance of the Secchi disk.

Statistical analysis

Data were submitted to analysis of variance (ANOVA) followed by regression, and then the Tukey test was applied at 5% significance. The statistical analysis was carried out, including normality and homoscedasticity tests, by the free Software R-302.

RESULTS

The females selected for hormonal induction exhibited over 60% ($P \geq 0.05$) of relocated germinal vesicle (nucleus). We observed that the oocytes from treatment with 1.40% of L-lysine showed lower percentage of relocated nucleus (64.72%) when compared with the other diets (72.64; 71.18; 74.17% diets 1.30, 1.60 and 1.95% of L-lysine, respectively), and were not different from one another ($P \geq 0.05$). Ovaries at final maturation, showing relocated nucleus may be seen in Figure 1a (Fig. 1c). Released *R voulezi* oocytes could be seen (Fig. 1c).

For *R. voulezi,* the percentage distribution of the oocyte diameters before the 1st hormonal injection (1st sample) exhibited similar patterns between the four treatments, with polimodal tendency, with modes of 570; 670; 750 and 860 µm (Fig. 2a). Nevertheless, at the moment of spawning (2nd sample), the configurations were similar for the diets with levels of 1.30, 1.40 and 1.95% L-lysine keeping the modes at 590, 670 750 and 860 µm, respectively (Figs. 2b, 2c, 2e). Still in Figure 2c we can verify that the females which had received diets containing 1.40% L-lysine displayed three modes with smaller diameters (520, 590 and 790 µm) when compared with the other three diets offered. However, it is noteworthy that at the moment of release, the quality of the oocytes (color, aspect and uniformity) was inadequate, with the presence of numerous white (opaque), irregular and bloody cells, indicating residual oocytes.

The reproductive parameters: released oocytes, fertilization and hatching rates, absolute fecundity and remaining ovaries were influenced ($P < 0.05$) by the diet (Table 3). The average percentage of spawning females of *R. voulezi* ranged from 50.0 to 78.0% and was not influenced ($P > 0.05$) by the addition of different levels of L-lysine in the feed (Table 3). We did not observe effect ($P > 0.05$) of L-lysine on the concentrations of estradiol, the concentrations found ranged from 0.54 to 0.70 ng mL^{-1} (Fig. 3).

Ramdhia voulezi ovaries presented analogous anatomical and morphological archetypes during the differentiation of ovarian follicles for the four treatments (diets) (Figs. 1a, 1b and 4a, 4b). In the present study, the ovarian walls are covered by an albu-

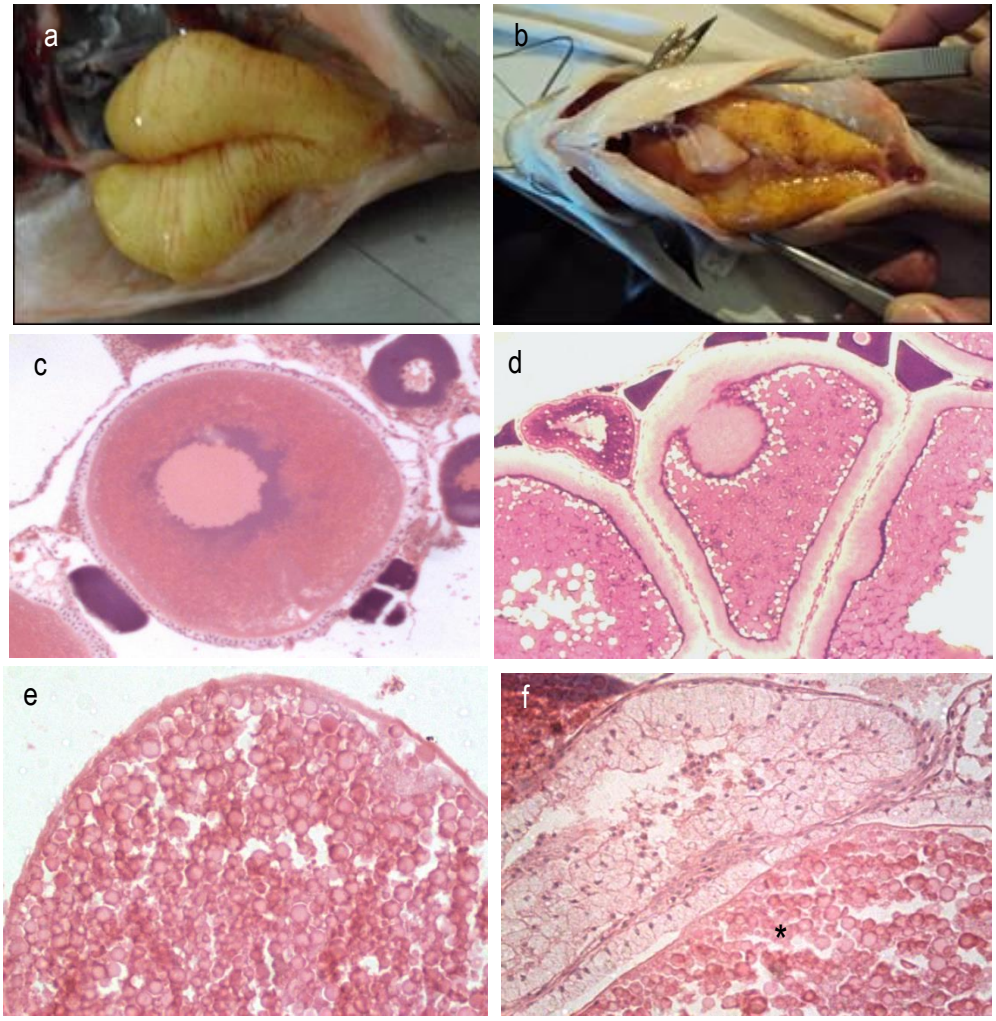

Figure 1. Anatomical organization (1a-1b) and light micrographs (1c-1f) of *Rhamdia voulezi* female reproductive system: (1a, 10x). Ovaries in stage final maturation (1c, 20x). Oocyte before the first hormone - germination vesicle slightly shifted toward the periphery. (1e, 20x). Time of release of the oocyte (1b, 10x). Ovaries oocyte after spawning; (1d, 10x) micropyle (arrow); (1f, 20x) mature oocyte (*) and post-ovulatory follicle (arrow). No differences in the anatomical and morphological archetypes during the differentiation of ovarian follicles for the four treatments (diets).

gineous tunic that emits septa towards the center, forming the ovuligerous lamellae, where the germinal epithelium houses all the oocyte development (Figs. 1a-1b). The analysis of the germinal epithelium associated to the stage of development of the germ cells present during the reproductive cycle of *R. voulezi* adult females allowed us to propose four reproductive phases throughout the year: 1 Developing, 2 Spawning capable, 3 Regressing, and 4 Regenerating, according to the terminology proposed by Reidel *et al*. (2010) and Brown-Peterson *et al*. (2011) and adapted to this species.

The ovaries reach their maximum size, with dark-yellow color (Fig. 4b) and vitellogenic oocytes are predominant (final maturation or mature), with relocated germinal vesicle or nucleus (Fig. 1c) towards the micropyle, which is formed by invagination of the zona radiata in its extremity (Fig. 1d). Fewer reserve previtellogenic oocytes are observed. At this phase, some remaining structures called postovulatory follicles (Fig. 1f) can be observed after ovulation (Fig. 1e).

In some of the females used in the present study we could notice that after oocyte release (expulsion), the ovaries remained in the area of the abdominal cavity (Figs. 4a-4b), exhibiting two distinct regions: *i*) left - macroscopically, full of yellow and white oocytes, with quite apparent irrigation (Fig. 4a); microscopically, at first, the atretic follicles mark the fragmentation and absorption of zona radiata and breakup of yolk granules

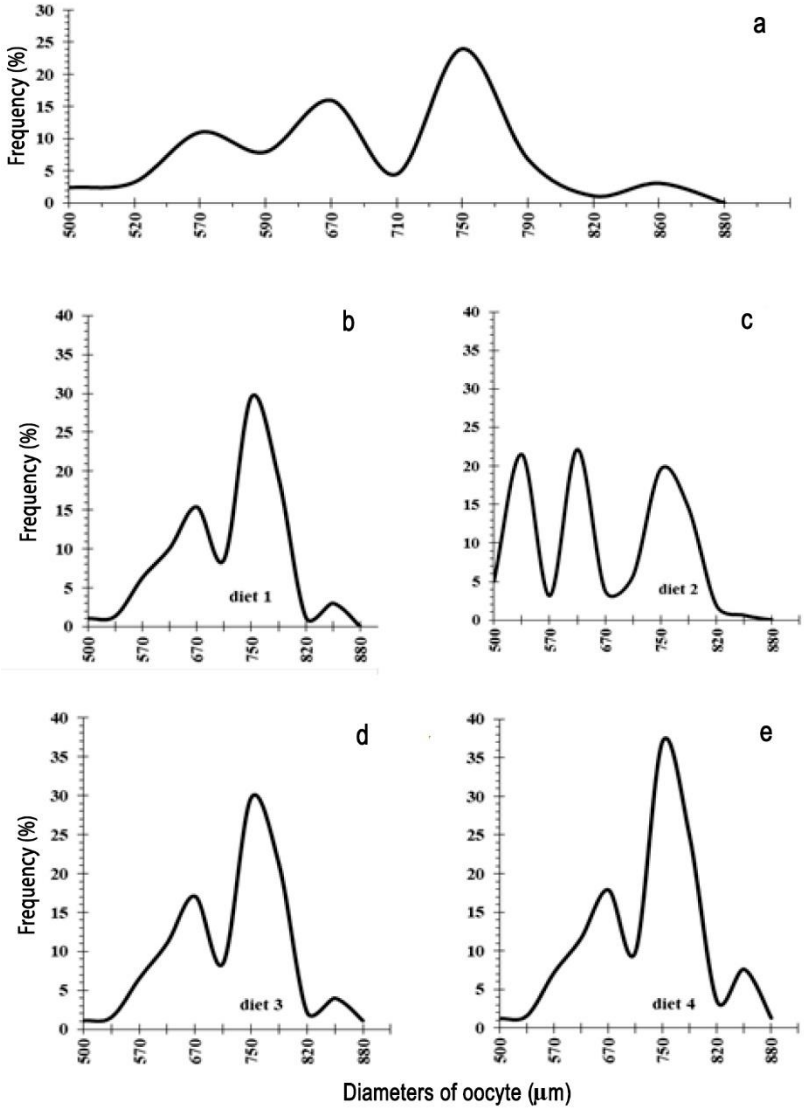

Figure 2. The diameters of oocyte frequency distributions of *Rhamdia voulezi*: (a) before the 1st application of hormones - the 1st sample of the four treatments grouped, a) during oocyte release- the 2nd sample, 2b) 1.30, 2c) 1.40, 2d) 1.60, 2e:1.95% L-lysine. Frequencies are presented as mean values (n = 6 by treatment).

(Fig. 4c). The follicular envelope is then totally broken and the follicular cells invade the ooplasma by phagocytosis (Fig. 4d). Thus, it could be noted that the *R. voulezi* females were at regressing stage (partial and total), signalizing the end of the reproductive cycle; and *ii*) right - remnants of this region of the ovary are congested and thick (Fig. 4b). Still at this stage it is possible to see the presence of oocytes at different stages of development during the reproductive period, noting that the species presents group asynchronous development (Fig. 4e), when more than one group of oocytes follow the same stage of development until the end of the cycle, characterizing them as multiple or parceled spawning (Reidel *et al*., 2010).

The different levels of L-lysine evaluated did not influence ($P > 0.05$) the GSI. The same happened with regard to HSI ($P > 0.05$). However, the visceral fat was different ($P < 0.05$) between the treatments, with the lowest values observed in the treatment with 1.30 and 1.95% L-lysine, with mean values of 1.08 and 1.07%, respectively. The GSI ranged between 11.86 and 14.08%, and HIS between 1.84 and 2.43%.

The concentrations of total amino acids in the oocytes were not affected by the different levels of L-lysine in the diet (Table 4). Therefore, the profile of amino acids found in the oocytes of the females remained constant, even with the variation of L-lysine.

Table 3. Reproductive performance of female breeding *Rhamdia voulezi*, fed with diets containing different L-lysine levels. *Different small letters in the line indicates significative difference, ANOVA followed by Tukey test. Absolute fecundity (oocytes female^{-1}); Relative fecundity (oocytes.g of female).

Variables	L-lysine level (%)				*P*
	1.30	1.40	1.60	1.95	
Spawning females (%)	66.83 ± 24.05	78.00 ± 26.94	50.00 ± 27.60	66.86 ± 36.51	0.58
Oocytes released (g fish^{-1})	6.41 ± 3.70^{b}	5.77 ± 3.11^{b}	5.35 ± 2.50^{b}	11.00 ± 5.43^{a}	0.002*
Rate of fertilization (%)	49.92 ± 19.65^{ab}	26.32 ± 16.55^{b}	52.77 ± 20.84^{a}	43.20 ± 34.98^{ab}	0.022*
Rate of hatching (%)	69.72 ± 29,91^{ab}	35.21 ± 2.59^{b}	80.45 ± 16.97^{a}	49.88 ± 43.64^{ab}	0.034*
Absolute fecundity	7,643 ± 4,638^{b}	7,300 ± 3,568^{b}	7,266 ± 2,621^{b}	13,210 ± 6,520^{a}	0.008*
Relative fecundity	97.65 ± 44.47	82.79 ± 31.04	85.60 ± 28.57	111.02 ± 39.17	0.229
Remnants ovaries (g)	4.90 ± 3.36^{b}	4.90 ± 2.22^{b}	3.60 ± 2.80^{b}	7.60 ± 5.22^{a}	0.004*

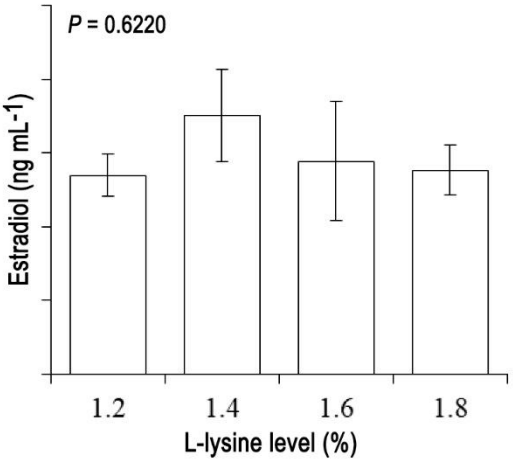

Figure 3. Effect of L-lysine in the concentration of estradiol of female breeding *Rhamdia voulezi*, fed with diets containing different L-lysine levels. Bars denote standard deviation. Means within columns sharing superscripts are not significantly different ($P > 0.05$).

DISCUSSION

The characteristics observed on the ovaries and oocytes macro and microscopic evaluations of the fish fed with diets containing different levels of L-lysine ratifying that although those females were kept in limited environments and confined, the sequence of reproductive events occurred normally, including the process of ovarian maturation (Reidel *et al*., 2010; Coldebella *et al*., 2011). Signor *et al*. (2013) reported that *R. voulezi* presented great potential to be reared in net cages and the broodstocks were viable for 180 days.

Another parameter assessed was the size or diameter of the oocytes, which is recommended as an indicator (monitor) of ovarian development (Leonardo *et al*., 2004; Bittencourt *et al*., 2012), and also supplies an estimate of parental investment of the progeny (Romagosa *et al*., 2013). The type of distribution found in this study for *R. voulezi* had been expected, since the species is characterized by asynchronous development, presenting four modes, released in parcels in the wild, during the reproductive period (Reidel *et al*., 2010). Those findings corroborate the ones described by Signor *et al*. (2013), who showed that *R. voulezi* may reproduce several times during the same reproductive season.

It is known that not all the females reach ovarian maturation at the same time (Phelps *et al*., 2011). It is important to point out that the *R. voulezi* females used in this study were at 1st maturation (first reproductive cycle, 100% of the fish were 11 months old), which justified the wide variation in the reproductive parameters. According to Signor *et al*. (2013), the capacity of egg production of *R. voulezi* is directly related to the class of body weight, in other words, larger broodfish produce larger number of oocytes. In addition, the absence of effect on the average of spawning females observed in this study was also described by Tessaro *et al*. (2012) for *R. quelen* females, but the values were slightly higher, ranging between 76.72 and 89.63% with diets supplemented by different energy levels.

According to Bombardelli *et al*. (2006), the relative fecundity for *R. quelen* when reared in captivity varied from 116 to 156 oocytes per gram of female. In this study, L-lysine did not affect relative fecundity, and the results ranged between 82.79 and 111.02 (Table 3), lower than the ones reported for *R. quelen*, probably due to the system of confinement adopted. According to Romagosa *et al*. (1990), the number of released oocytes per female (297.308 and 377.643 oocytes) increased with age (3 and 4 years, 1st and 2nd ovarian maturation, respectively) for *Piaractus mesopotamicus* kept in ponds.

We can see that L-lysine did not present effect on estradiol. However, the concentrations measured were low, may be due to the first maturation, or the fact that the blood was collected after reproduction. According to Iseki *et al*. (2008), studying the seasonal variations

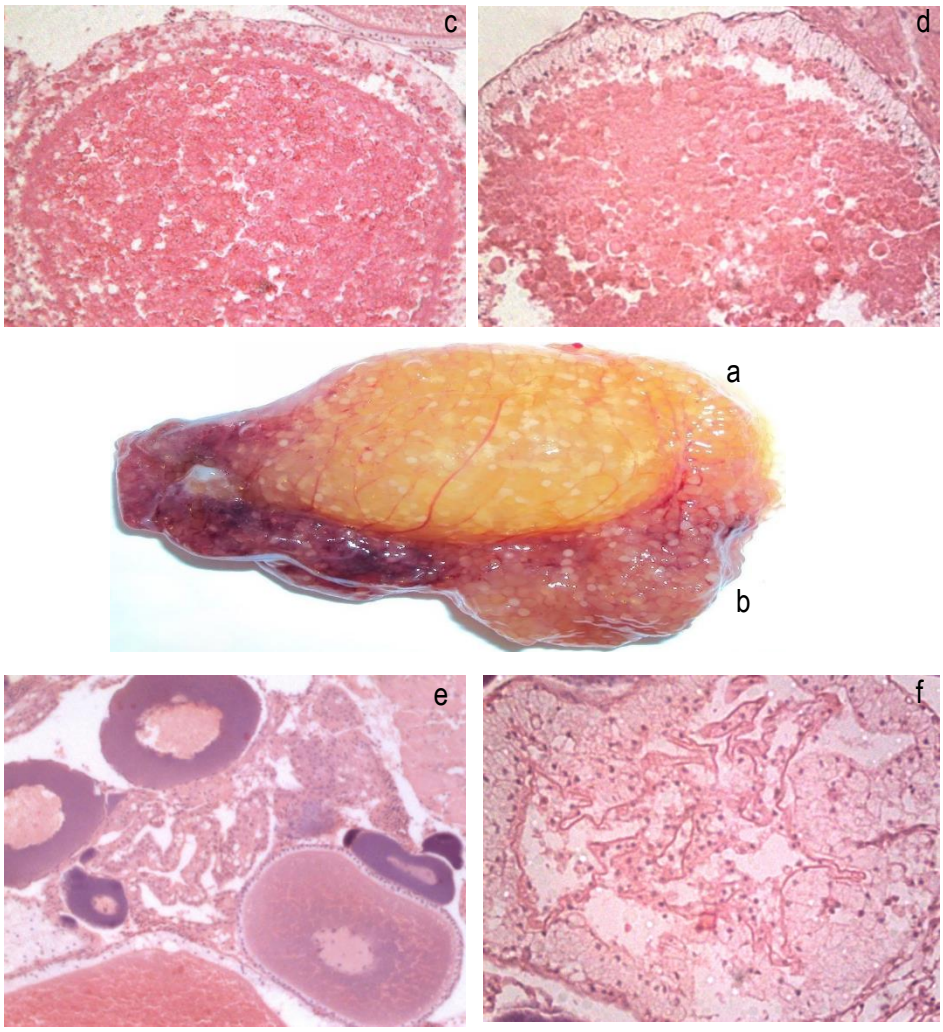

Figure 4. Ovaries partial spawn (4a, 10x) and remnants (4b, 10x) of *Rhamdia voulezi* (in the middle) (4c, 20x; 4d, 20x) it can be observed a large number of atresic follicles and (4e, 10x) it is show the post-ovulatory follicle (arrow) and oocytes in another phases; (4c, 40x) detail of post-ovulatory follicles. No differences in the anatomical and morphological archetypes during the differentiation of ovarian follicles for the four treatments (diets).

in the plasma levels of sexual steroids during the reproductive cycle of *P. mesopotamicus* females, the levels of estradiol varied according to gametogenesis, exhibiting the lowest values at the initial stages of development. At the phase of final maturation, the *Dicentrarchus labraxos* females presented progressively decreasing plasma levels of estradiol, without effect during spawning (Prat *et al*., 1999).

In relation of anatomical and morphological archetypes found in this study it is important to highlight that the fish presented a regular distribution of the organs described for the generous *Rhamdia*. Certainly, the ovarian structure variations reported by Reidel *et al*. (2010) and Ghiraldelli *et al*. (2007) for *R. quelen*, such as paired, elongated and bag-like ovaries located in the area of the abdominal cavity were equivalent to the ones found in *R. voulezi.*

Ramdhia voulezi developing ovaries, in general, occur from September to February, occupy much of the abdominal cavity (Fig. 1a), have light-yellow color, showing they are at the stage of advanced vitellogenesis, together with the previtellogenic oocytes. After that, the females become spawning capable - spawning (November to February), presenting hyperemic urogenital orifice, soft and large abdomen. After ovulation and spawning, postovulatory follicles were observed. Those follicles are formed by the involution of the follicular envelope of the granulosa cells (Romagosa *et al*., 2005). According to the authors, those postovulatory follicles do not have endocrine function and are quickly absorbed, which involves programmed cell death or apoptosis of the follicular cells. At this moment, the postovulatory follicles remain in the lamella after oocyte release and are composed of

Table 4. Composition of essential amino acids in oocytes on wet basis of females breeding *Rhamdia voulezi*, fed with diets containing different L-lysine levels. Values did not differ ($P > 0.05$) statistically.

Total amino acids (%)	L-lysine total level (%)				*P*
	1.30	1.40	1.60	1.95	
Aspartic acid	1.71 ± 0.34	1.60 ± 0.78	1.67 ± 0.45	1.46 ± 0.06	0.922
Glutamic acid	2.40 ± 0.41	2.23 ± 1.10	2.32 ± 0.63	2.05 ± 0.08	0.922
Serine	1.28 ± 0.27	1.23 ± 0.53	1.26 ± 0.31	1.11 ± 0.04	0.921
Glycine	0.72 ± 0.12	0.70 ± 0.34	0.73 ± 0.20	0.64 ± 0.03	0.944
Histidine	0.49 ± 0.04	0.46 ± 0.23	0.48 ± 0.12	0.43 ± 0.01	0.925
Arginine	1.25 ± 0.25	1.19 ± 0.60	1.16 ± 0.37	1.08 ± 0.03	0.925
Threonine	0.90 ± 0.11	0.80 ± 0.37	0.83 ± 0.21	0.72 ± 0.02	0.791
Alanine	1.54 ± 0.29	1.49 ± 0.69	1.56 ± 0.43	1.43 ± 0.06	0.981
Proline	0.95 ± 0.20	0.96 ± 0.44	1.01 ± 0.28	0.90 ± 0.05	0.970
Tirosine	0.63 ± 0.11	0.60 ± 0.31	0.63 ± 0.17	0.55 ± 0.05	0.953
Valine	1.19 ± 0.18	1.03 ± 0.52	1.03 ± 0.31	0.94 ± 0.06	0.795
Methionine	0.45 ± 0.05	0.40 ± 0.22	0.38 ± 0.14	0.38 ± 0.03	0.910
Cistine	0.33 ± 0.07	0.34 ± 0.15	0.39 ± 0.14	0.30 ± 0.03	0.792
Isoleucine	1.16 ± 0.16	1.02 ± 0.51	1.11 ± 0.30	0.97 ± 0.06	0.875
Leucine	1.91 ± 0.30	1.81 ± 0.88	1.91 ± 0.52	1.70 ± 0.09	0.954
Phenylalanine	0.68 ± 0.10	0.62 ± 0.33	0.65 ± 0.18	0.56 ± 0.03	0.899
L-lysine	1.33 ± 0.26	1.27 ± 0.66	1.33 ± 0.34	1.18 ± 0.06	0.959
Taurine	0.03 ± 0.01	0.02 ± 0.01	0.02 ± 0.01	0.02 ± 0.01	0.480

follicular layer, theca and basal membrane in the process of degeneration and absorption.

The following phase, *Regenerating* is the moment when the ovaries get ready to start a new reproductive cycle. At this phase, the walls of the ovaries become thick and the ovigerous lamellae reorganize, the process of folliculogenesis is active and there is great mitotic proliferation of the germ cells, and nests of oogonia.

The accumulation of visceral fat can influence the reproductive parameters of females of diverse fish species and is an important parameter to evaluate. This condition was verified in the present study and showed differences among the levels of L-lysine supplementation in the diet. Other studies reported less accumulation of fat in fish fed diets with different levels of L-lysine in *Oncorhynchus mykiss* (Encarnação *et al*., 2004); *Cyprinus carpio* (Zhou *et al*., 2008); *P. mesopotamicus* (Abimorad *et al*., 2010); *Oreochromis niloticus* (Furuya *et al*., 2013). The reduction in visceral fat may have occurred due to the fact that L-lysine acts as a precursor of carnitine, which is involved in the transport of long-chain fatty acids to the mitochondria (Zhou *et al*., 2010).

Amino acid supplementation is important to balance diets for fish and in case of broodstock females these molecules can influence the composition of oocytes. This fact reflects also in the vitellus composition. In the present study, although the diets were formulated with different levels of L-lysine, we did not observed influence on the essential amino acids composition of oocytes. Since there was no difference in the amino acid composition of the oocytes, it is assumed that the diets were sufficient for the development of the oocytes. Similarly, according to Gunasekera *et al*. (1997), the profile of amino acids found in the oocytes of *O. niloticus* females remains constant, even if the level of protein in the diet is below the nutritional demand of the species. Besides, Khan *et al*. (2005) stated that the influence of diets with different levels of protein on the composition of fish oocytes has not been totally clarified yet. On the other hand, Kabir *et al*. (2013) suggest that the levels of protein in the muscle, liver and oocytes might be related to weight gain, although the protein content in the oocyte is influenced by the diet, and the nutritional quality of the protein affects the reproductive development and quality of the *Pangasianodon hypophthalmus* eggs.

The alterations detected must be related to the different requirements of amino acids by the organs, mainly by the ovaries. According to Kabir *et al*. (2013), the relationship between the content of amino acids in the liver and the oocytes is due to the fact that during oocyte maturation, essential nutrients are transferred from the liver to the oocytes by the blood, and in general, greater protein deposition is observed in the muscles, followed by oocytes and liver. Assem *et al*. (2005) reported that seven amino acids (proline, alanine, valine, methionine, isoleucine, leucine and histi-

dine) exhibited significant increase in mature ovaries followed by reduction in spawning for *Trachinotus ovatus*. Thus, new studies are necessaries to help us elucidate the processes involved with nutrition and reproduction in order to define the metabolic importance of amino acids, both for oocyte development, embryonic stages and larval nutritional intake and survival.

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REFERENCES

Abimorad, E.G., G.C. Favero, G.H. Squassoni & D.J. Carneiro. 2010. Dietary digestible L-lysine requirement and essential amino acid to L-lysine ratio for pacu *Piaractus mesopotamicus.* Aquacult. Nutr., 16: 370-377.

Andrade, V.X.L., R.M. Honji & E. Romagosa. 2010. Processo de maturação das gônadas de pintado, *Pseudoplatystoma corruscans* alimentado com dois níveis protéicos e suplementados com óleo de milho. Arq. Bras. Med. Vet. Zootecn., 62: 332-342.

Assem, S.S., S.S. El-Serafy, M.M. El-Garabawy, M.El-G. Elabsawy & K.S. Kaldus. 2005. Some biochemical aspects of reproduction in female *Trachinotus ovatus* (Carangidae). Egypt. J. Aquat. Res., 31: 315-317.

Barcellos, L.J.G., J.F. Wassermann, A.P. Scott, V.M. Woehl, R.M. Quevedo, I. Itzzés, M.H. Krieger & F. Lulhier. 2002. Plasma steroid concentrations in relation to the reproductive cycle of cultured male *Rhamdia quelen*. J. Fish Biol., 61: 751-763.

Baumgartner, D., G. Baumgartner, C.S. Pavanelli, P.R.L. Silva, V.A. Frana, L.C. Oliveira & M.R. Michelon. 2006. Fish, Salto Osório Resevoir, rio Iguaçu basin, Paraná, State, Brazil. Check List, 2(1): 1-4.

Bittencourt, F., B.E. Souza, T.A. Lui, M.I. Borella, W.R. Boscolo, A. Feiden & E. Romagosa. 2012. Protein diets promote the maturation of oocytes and spawning of *Piaractus mesopotamicus* kept in cages. J. Appl. Ichthyol., 28: 886-893.

Bombardelli, R.A., E.F. Mörschbächer, R. Campagnolo, E.A. Sanches & M.A. Syperreck. 2006. Dose inseminante para fertilização artificial de ovócitos de jundiá-cinza, *Rhamdia quelen* (Quoy & Gaimard, 1824). Rev. Bras. Zootecn., 35: 1251-1257.

Brown-Peterson, N.J., D.M. Wyanski, F. Saborido-Rey, B.J. Macewicz & S.K. Lowerre-Barbieri. 2012. A standardized terminology for describing reproductive development in fishes. Marin. Coast. Fish. Dynam. Manage. Ecosys. Sci., 3: 52-70.

Brzuska, E. 1979. The in vivo method of estimating the stages of oocyte maturation in carp (*Cyprinus carpio* L). Acta Hydrobiol., 21: 423-433.

Coldebella, I.J., J. Radünz-Neto, C.A. Mallmann, C.A. Veiverberg, G.T. Bergamin, F.A. Pedron, D. Ferreira & L.J.G. Barcellos. 2011. The effects of different protein levels in the diet on reproductive indexes of *Rhamdia quelen* females. Aquaculture, 312: 137-144.

Diemer, O., D.H. Neu, F. Bittencourt, A. Signor, W.R. Boscolo & A. Feiden. 2012. Eugenol como anestésico para jundiá (*Rhamdia voulezi*) em diferentes pesos. Semina, 33: 1495-1500.

Diemer, O., F. Bittencourt, L.J.G. Barcellos, W.R. Boscolo, A. Feidin & E. Romagosa. 2014. L-lysine in the diet of *Rhamdia voulezi* male broodstocks confined in net cages. Aquaculture, 434: 93-99.

Encarnação, P., C. Langes, M. Rodehutscord, D. Hoehler, W. Bureau & D.P. Bureau. 2004. Diet digestible energy contents affects L-lysine utilization, but not dietary L-lysine requirements of rainbow trout, *Oncorhynchus mykiss* for maximum growth. Aquaculture, 235: 569-586.

Furuya, W.M., M. Michelato, T.S. Graciano, L.V.O. Vidal, T.O. Xavier, V.R.B. Furuya & L.B. Moura. 2013. Exigência de lisina digestível para a tilápia-do-Nilo de 87 a 226 g alimentada com dietas balanceadas para a relação arginina: lisina. Semina, 34: 1945-1954.

Ghiraldelli, L., C. Machado, D.M. Fracalossi & E. Zaniboni-Filho. 2007. Desenvolvimento gonadal de jundiá, *Rhamdia quelen* (Teleostei, Siluriformes), em viveiros de terra, na região sul do Brasil. Acta Scient. Biol. Sci., 29: 349-356.

Gunasekera, R.M., K.F. Shim & T.J. Lam. 1997. Influence of dietary protein content on the distribution of amino acids in oocytes, serum and muscle of tilapia, *Oreochromis niloticus.* Aquaculture, 152: 205-222.

Iseki, K.K., J.A. Negrão & A.M.L. Castrucci. 2008. Variações sazonais nos níveis plasmáticos dos esteroides sexuais (17 beta-estradiol e testosterona) durante o ciclo reprodutivo de fêmeas de pacu, *Piaractus mesopotamicus*. Ensaios e Ciência: Ciênc. Biol. Agrar. Saúd., 12: 151-162.

Izquierdo, M.S., H. Fernandez-Palacios & A.G.J. Tacon. 2001. Effect of broodstock nutrition on reproductive performance of fish. Aquaculture, 197: 25-42.

Kabir, M.A., A. Ghaedi, A.D. Talpur & R. Hashim. 2013. Effect of dietary protein levels on reproductive development and distribution of amino acids in the body tissues of female *Pangasianodon hypophthalmus* (Sauvage, 1878) broodstock in captivity. Aquacult. Res., 46: 1-12.

Khan, M.A., A.K. Jafri & N.K. Chadha. 2005. Effects of varying dietary protein levels on growth, reproductive performance, body and egg composition of rohu, *Labeo rohita* (Hamilton). Aquacult. Nutr., 11: 11-17.

Khan, M.A. & G.F. Abidi. 2011. Effect of varying dietary L-lysine levels on growth, feed conversion, nutrient retention, L-lysine retention efficiency and haemato-logical indices of *Heteropneustes fossilis* fry. Aquacult. Nutr., 17: 657-667.

Leonardo, A.F.G., E. Romagosa & M.I. Borella. 2004. Induced spawning of hatchery-raised Brazilian catfish, cachara *Pseudoplatystoma fasciatum* (Linnaeus, 1766). Aquaculture, 240: 451-461.

Montes-Girao, P.J. & D.M. Fracalossi. 2006. Dietary L-lysine requirement as basis to estimate the essential dietary amino acid profile for jundiá, *Rhamdia quelen.* J. Aquacult. World Soc., 37: 388-396.

Parra, J.E.G., J. Radünz-Neto, C.A. R. Veiverberg, Lazzari, G.T. Bergamin, V. Corrêia, C.C. Ferreira & F.W. Ferreira. 2010. Desempenho reprodutivo de fêmeas de jundiá alimentadas com diferentes fontes protéicas. Arch. Zootecn., 59: 255-265.

Phelps, R.P., R. Hastey, J. Broach, A. Pendetar, L. Linley, N. Papanikos & R.A. Dunham. 2011. Broodstock selection criteria for induced spawning of channel catfish for the production of channel X blue catfish hybrid fry and the influence of temperature. N. Am. J. Aquacult., 73: 180-186.

Prat, F., S. Zanuy, N. Bromage & M. Carrillo. 1999. Effects of constant short and long photoperiod regimes on the spawning icrocephal and sex steroid levels of female and male sea bass. J. Fish Biol., 54: 125-137.

Reidel, A., W.R. Boscolo, A. Feiden & E. Romagosa. 2010. The effect of diets with different levels of protein and energy on the process of final maturation of the gametes of *Rhamdia quelen* stocked in cages. Aquaculture, 298: 354-359.

Romagosa, E. 2010. Reproductive status in females of the Brazilian catfish, *Pseudoplatystoma fasciatum* reared in cages. J. Appl. Ichthyol., 26: 806-811.

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Romagosa, E., P. Paiva & H.M. Godinho. 1990. Pattern of oocyte diameter frequency distribution in females of the pacu, *Piaractus mesopotamicus* (Holmberg, 1887) induced to spawn. Aquaculture, 86: 105-110.

Romagosa, E., F. Bittencourt & W.R. Boscolo. 2013. Nutrição e alimentação de reprodutores In: D.M. Fracalossi & J.P.E. Cyrino (eds.). Nutrição e alimentação de espécies de interesse para a aquicultura brasileira. Editora Copiart Ltda., Florianópolis, pp. 167-179.

Romagosa, E., S.R. Batlouni, M.I. Borella & A.F.G. Leonardo. 2005. Involução dos icroceph pós-ovulatórios em *Pseudoplatystoma fasciatum* (Pisces, Teleostei). Bol. Inst. Pesca, 31(2): 129-135.

Signor, A., A. Feiden, W.R. Boscolo, A.A. Signor, G.S. Gonçalves, C. Sary & S. Klein. 2013. Eventos reprodutivos do jundiá *Rhamdia voulezi* cultivado em tanques-rede. Rev. Bras. Repr. Anim., 37: 272-277.

Simpson, A.C. 1951 The fecundity of the plaice. Fish Invest., 2: 27 pp.

Tessaro, L., C.P.R. Toledo, G. Neumann, R.A. Krause, F. Meurer, M.R.M. Natali & R.A. Bombardelli. 2012. Animal performance and reproductive aspects of female *Rhamdia quelen* fed on different levels of digestible energy. Aquacult. Res., 45: 1425-1433.

Zhou, X.Q., C.R. Zhao, J. Jiang, L. Feng & Y. Liu. 2008. Dietary L-lysine requirement of juvenile Jian carp (*Cyprinus carpio*). Aquacult. Nutr., 14: 381-386.

Zhou, F., J. Shao, R. Xu, J. Ma & Z. Xu. 2010. Quantitative L-L-lysine requirement of juvenile black sea bream (*Sparus icrocephalus*). Aquacult. Nutr., 16: 194-204.