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The effects of different protein levels in the diet on reproductive indexes of *Rhamdia quelen* females

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ABSTRACT

The nutrition of broodstock is one of the least studied areas because biological mechanisms, such as gonadal maturation, are extremely complex processes and are affected by several nutrients, especially in continuous spawning fish with short vitellogenic periods as *Rhamdia quelen*. For the present experiment, 66, 14-month-old female *R. quelen* were randomly distributed in six cages and fed for 90 days with three isocaloric diets containing either 28%, 34%, or 40% crude protein. Data were collected at the beginning of the experiment (day zero) and after 45 and 90 days, for evaluation of several parameters as final weight, length, condition factor, hepatosomatic, gonadosomatic and visceral fat indexes, amino acids in the ovaries, plasma enzymes aspartate aminotransferase (AST) and alanine aminotransferase (ALT), total plasma protein, testosterone, 17β-estradiol, free amino acids (AAL), and triglycerides (TG). From these females, the reproductive cycle, egg characteristics and larval size were evaluated. The results showed that the different protein levels in diets had no effect on the main physiological and reproductive parameters. In conclusion, the 28% crude protein dietary level was sufficient for maintenance of the broodstock and the reproductive indexes of *R. quelen* females.

1. Introduction

The silver catfish *Rhamdia quelen* has wide distribution in South America (Gomes et al., 2000; Baldisserotto and Radünz Neto, 2005). In fish production systems at a density of 2 to 4 fish per square meter, this species can reach 600–800 g of body weight in 8 months (Barcellos et al., 2001). Silver catfish attain sexual maturity at 1 year of age, and, when in good nutritional status, can achieve high rates of hatching in response to hormonal induction (Gomes et al., 2000). This species presents an asynchronous spawning with reproductive cycle occurring from August to March (Barcellos et al., 2001). The increasing demand for fingerlings of this species requires the maximization of their production through efficient reproductive management and development of suitable diets that meet the nutritional requirements of broodstock.

The nutrition of broodstock is one of the least studied areas because the biological mechanisms, such as gonadal maturation, are extremely complex processes (Izquierdo et al., 2001; Chong et al., 2004; Khan et al., 2005). The gonadal development and fecundity are affected by several nutrients, especially in continuous spawning fish with short vitellogenic periods (Izquierdo et al., 2001) as *R. quelen* (Barcellos et al., 2001). The effect of nutrition is significant in fish maturation and fecundity. Proteins and lipids are the major components of the yolk, acting as sources of nutrients used during the biosynthesis of early embryogenesis (Khan et al., 2005), and allow greater survival of the embryo and larvae (Silva, 2004).

According to Brooks et al. (1997), proteins are present in the eggs of fish, such as lipoproteins, hormones, and enzymes, determining egg quality and consequently the production of fry and fingerlings on a large scale. According to these authors, despite the considerable efforts that have been directed towards unraveling the importance of dietary components in determining egg quality, the evidence that diet can directly affect egg quality is very limited.

In general, the nutritional status of the female can influence gonadal development and limit the amount and the quality of the eggs (Johnston et al., 2007; De Silva et al., 2008). Gunasekera et al. (1996) reported that the level of dietary protein influences the viability of offspring, with very low levels (10–20%) resulting in low fertilization rates of eggs and a large percentage of deformed larvae.

In the Brazilian species, few studies have evaluated the effects of protein levels in the diet on the reproductive performance of fish, especially of *R. quelen*. Thus, the aim of this study was to evaluate the effect of dietary protein level on animal performance and reproductive and hematologic parameters of female catfishes.

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2. Materials and methods

All procedures involving animals were conducted in accordance with standards approved by the Ethics Committee for Animal Welfare (CEBEA), Universidade Federal de Santa Maria, and protocol number 23081.006974/2009-96.

The work was conducted at the Aquaculture Experimental Station of the Polo de Modernização Tecnológica do Médio Alto Uruguai–Universidade Regional Integrada do Alto Uruguai e das Missões–Campus de Frederico Westphalen, Rio Grande do Sul, Brazil, (27°38′S/53°43′W, 465 ma.s.l.) between July 15 and October 18, 2008, including the feeding period of breeding and birth of the larvae, and Laboratory of Fish Culture, Department of Animal Science, Universidade Federal de Santa Maria, Rio Grande do Sul, Brasil (29°43′S/53°42′W, 95 ma.s.l.), in the postlarval evaluations for 14 days. The determination of the spawning period was in accordance to the highest gonadosomatic index obtained by Barcellos et al. (2001) for *R. quelen*.

2.1. Experimental diets

We evaluated three isocaloric (4000 kcal gross energy kg^{-1}) diets containing 28%, 34%, and 40% crude protein (Table 1). The foods were pelleted for females in a meat grinder with water (50% of the dry weight of ingredients), dried with forced air circulation between 48 and 50 °C for 48 h, ground in a manual grinder, sieved between 6 and 8 mm, and then stored in a freezer (−18 °C).

The diet used before the experimental trials to evaluate the postlarvae performance, in all treatments, was prepared with 37% sugar cane yeast, 20% cooked egg yolk, 30% poultry liver (33% dry), 2% soy lecithin, 8% rice bran, and 3% vitamin and mineral mixture, ground and sieved into particle sizes between 100 and 400 μm.

2.2. Fish, facilities, and experimental design

For the experiment, 66, 14-month-old female *R. quelen* weighing between 394.2 and 690.3 g from a single spawn were used. The females were reared in ponds and adapted during 90 days in cages. After the adaptation period, the females were randomly distributed in six, 1-m^3 cages (two cages per diet) at the rate of eight per cage and, for time point zero, 18 females were slaughtered for collection of blood and ovaries, prior fish distribution in cages. At each time point (45 and 90 days), six females were sampled per diet. The cages were installed in a 2000-m^2 earthen pond with an average depth of 3.5 ± 0.4 m at the site where cages were set.

In each cage, a submerged tubular feeder was installed. In addition to the females, 24 male *R. quelen* of the same age were kept in a 1000-m^2 pond, in two cages with individual capacity of 1 m^3. From these males, 12 fish were selected for semen collection and oocyte fertilization.

For spawning, the couples were induced with carp pituitary extract and transferred individually to Zoug-type incubators with a capacity of 56 l of water, as part of a recirculating water system with temperature regulation (set at 23 °C) and aeration (Radünz Neto et al., 1987).

A pen net was installed in each incubator, allowing the couple to remain at the top of the incubator. Immediately below the net, a funnel attached to a transparent hose was installed to permit visualization of the first eggs to determine the timing of spawning. After fertilization, only the couples were removed from the incubators, and the eggs were retained.

To evaluate the performance of postlarvae, they were placed in two water recirculation systems with individual capacity of 8 l. The larvae were divided into three treatments and three replicates according to maternal diet.

Table 1
Percentage composition and chemistry of diets with different protein levels used for female *Rhamdia quelen* expressed in dry matter.

Ingredients (%)	Protein level (%)		
	D28[a]	D34[a]	D40[a]
Swine meat meal	24.1	32.5	41.1
Soybean meal	24.1	32.5	41.1
Rice meal	22.28	14.48	3.9
Corn	19.2	9.9	3.0
Soybean oil	7.3	7.9	8.2
Limestone	0.6	0.2	–
DL-Methionine (99%)	0.42	0.52	0.7
Salt (NaCl)	0.5	0.5	0.5
Vit/Min supplement[b]	1.5	1.5	1.5
Agglutinative[c]	1.5	1.5	1.5
Proximate composition (%)			
Nutrients			
Dry matter[d]	92.84	92.34	93.23
Crude protein[d]	28.5	34.14	40.60
Gross energy (Kcal kg^{-1})[g]	4.000	4.000	4.000
Lipid[d]	14.22	16.21	15.32
Nitrogen-free extract[g]	18.30	18.70	18.00
Ash[d]	10.96	11.72	12.33
Crude fiber[d]	4.14	3.91	3.70
Calcium[d]	1.83	2.03	2.60
Phosphorus[d]	1.62	1.57	1.65
Lisine[f]	1.47	1.85	2.21
Methionine + cystine[f]	1.22	1.46	1.70
Tryptophan[e]	0.27	0.33	0.38
Threonine[f]	0.61	1.20	1.40
Arginine[f]	2.03	2.51	2.96
Valine[f]	1.28	1.54	1.79
Isoleucine[f]	1.00	1.22	1.43
Leucine[f]	2.01	2.39	2.72
Histidine[f]	0.64	0.77	0.89
Phenylalanine + tyrosine[f]	1.95	2.38	2.76

Diets adjusted according to Parra et al. (2008).
[a] D28 = diet 28% crude protein; D34 = diet 34% crude protein; D40 = diet 40% crude protein.
[b] Ingredient kg^{-1} diet: (MigPlus®): Folic acid = 250 mg; Pantothenic acid = 5000 mg; B.H.T. = 0.60 g; Biotin = 125 mg; Cobalt = 25 mg; Copper = 2000 mg; Iron = 820 mg; Iodine = 100 mg; Manganese = 3750 mg; Nicotinamide = 5000 mg; Selenium = 75 mg; Vitamin A = 1,000,000 UI; Thiamine = 1250 mg; Vitamin B12 = 3750 mcg; Riboflavin = 2500 mg; Pyridoxine = 2,485 mg; C = 28,000 mg; D3 = 500,000 UI; E = 20,000 UI; K3 = 500 mg; Zinc = 17,500 mg.
[c] Ca^{2+} and Mg^{2+} Lignosulphonate (Melbond®).
[d] According analysis by NUTRON® Alimentos Ltda, São Paulo, Brazil.
[e] Calculated from the ingredient analysis NUTRON® Alimentos Ltda, São Paulo, Brazil.
[f] According analysis by LAMIC® (Laboratório de Micotoxinas) Universidade Federal de Santa Maria, Rio Grande do Sul, Brazil.
[g] Calculated from Rostagno et al. (2005).

2.3. Broodstock and postlarvae feeding management

The females were fed for 90 days according to Fernández Palacios et al. (1995), daily at 9:00 and 16:00 h. The feed was weighed and furnished according to the biomass of each cage at the beginning and after 45 days, ranging from 2% to 3% by the end of the experiment. Males were fed daily at 3% biomass, with a commercial extruded food (SUPRA® Alisul Alimentos Ltda), containing 32% crude protein.

The postlarvae were fed every 2 h, from 8 to 18 h, up to 14 days during the transition between the absorption of the yolk sac for food tracking juvenile as proposed by Sink et al. (2010). The food residue and fish waste were removed daily before the first feeding.

2.4. Liver, gonads and blood sampling

Biometric data were collected at the beginning of the experiment (day zero) and at 45 and 90 days, for evaluation of final average weight (LW, g), total length (TL, cm), and condition factor [K = (weight × 100)/(length total^3)] (Steffens 1987).