CRUDE PROTEIN IN DIETS FOR NILE TILAPIA BROODFISH*

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ABSTRACT

The effect of different levels of crude protein (32A, 32B, 36, 38, 44 and 50% CP; 3,500 kcal digestible energy) on Nile tilapia broodstock was assessed. After 30 experimental weeks (Sept./14 to Mar./15), 91.0% of eggs from fish fed 44% CP hatched and produced 16.4% more viable larvae than the treatment with 32% CP. Egg production and absolute fecundity were similar between treatments (p>0.05). Sperm motility, average path, straight line and curvilinear velocities showed satisfactory values with 44% CP, unlike 36% CP. Lower profitability was observed with 32% CP; profit increased as protein level upped. Statistically significant responses were not found for reproductive performance of females. Results were satisfactory for commercial-scale production as crude protein increased. The initial hypothesis was demonstrated for most parameters assessed in males, larvae growth and economic viability. Therefore, it is recommend the use of diets with 44% CP for Nile tilapia brood fish.

Keywords: larvae; reproductive performance; semen; survival

PROTEINA BRUTA EM DIETAS PARA REPRODUTORES DE TILÁPIA DO NILO

RESUMO

O efeito de diferentes níveis de proteína bruta (32A, 32B, 36, 38, 44 e 50% PB; 3500 kcal energia digestível) em reprodutores de tilápia do Nilo foi avaliado. Após 30 semanas experimentais (set./14 a mar./15), 91,0% dos ovos de peixes alimentados com 44% PB, eclodiram e produziram 16,4% de larvas viáveis a mais do que o tratamento que recebeu 32% PB. A produção de ovos e a fecundidade absoluta foram semelhantes entre os tratamentos (p>0,05). A motilidade e a velocidade médias do esperma, em linha reta e curvilínea, apresentaram valores satisfatórios no tratamento ~~em~~ 44% CP, ao contrário de 36% PB. Observou-se baixa rentabilidade com 32% PB; todavia, o lucro aumentou com o nível de proteínas na dieta. Respostas estatisticamente significativas não foram encontradas para o desempenho reprodutivo de fêmeas. Os resultados foram satisfatórios para a produção em escala comercial com o aumento da proteína bruta. A hipótese inicial foi demonstrada para a maioria dos parâmetros avaliados em machos, crescimento de larvas e viabilidade econômica. Portanto, recomenda-se dieta com 44% PB para reprodutores de tilápia do Nilo.

Palavras-chave: larva; desempenho reprodutivo; sêmen; sobrevivência

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INTRODUCTION

Although in the last few years considerable progress has been made in the knowledge of the nutritional demands of Nile tilapia, there is still a need for further studies on nutrition that consider different categories of weight or age, as well as specific studies about broodfish (FURUYA *et al*., 2013), especially the supply of essential nutrients for the development of gonads, eggs and larvae (LUPATSCH *et al*., 2010), which is an immediate need since the aquaculture activity has shown rapid expansion recently.

In this context, it is important to emphasize the relevance of providing adequate diets to Nile tilapia broodfish, a species raised all over the world and characterized by presenting parceled asynchronic spawning, with low fecundity rate, whose reproductive process received special attention in the last few years (NG and ROMANO, 2013; LUPATSCH *et al*., 2010; COWARD and BROMAGE, 2000). Considering the limitations to gamete quality, and consequently the large scale production of larvae and fry (NAVARRO *et al*., 2014), effects of the addition of crude protein (CP) to follow the reproductive behavior of this species were described by GUNASEKERA *et al*. (1996); AL-HAFEDH *et al.* (1999); EL-SAYED and KAWANNA (2008) and OLIVEIRA *et al.* (2014). However, the results of those studies did not clarify what the adequate demand for protein of tilapia broodstock is, northe effect of protein on egg quality (LUPATSCH *et al.,* 2010).

A review published by NG and ROMANO (2013) on nutrition and feed management of Nile tilapia explained the need to add high quality nutrients, mainly during vitellogenesis, in order to avoid signs of delay in the process of gonad maturation, low values of hatching, fertilization and sperm motility rates. Those facts emphasize the importance of nutrition in reproductive performance. According to the authors, the levels of CP that showed the most satisfactory results were between 30 and 40%, which is such a wide range. Therefore, makes it difficult to prepare specific feed. In addition, comparatively to other nutrients, protein is the most expensive item in diet formulation for aquatic organisms (NRC, 2011), and that wide range may considerably interfere with the final cost of production (Âmbar Amaral Group, *Raguife Rações*® – Santa Fé do Sul, SP/BR; Gonçalves, G.S. 2016, per. comm.). Thus, the protein level in the feed for broodfish should be critically analyzed, considering that in practice, besides egg production and quality, the health condition of the fish must be assessed when facing the constant challenges of farming systems, as well as the economic impact of using different feed.

Due to the need to obtain an adequate diet for broodfish and knowing how important these results would be to the activity, the objective of this experiment was to assess the morphometric, reproductive and blood parameters of Nile tilapia broodfish fed diets with different levels of protein, in addition to economic aspects of larvae produced by the broodfish studied.

MATERIAL AND METHODS

The experiment was carried out at the *Peixe Vivo Aquacultura*® fish farm, Santa Fé do Sul-SP/Brazil (20º12'40"S and 50º55'33"W), from September/2014 to March/2015, with the approval of the Animal Ethics Committee, UNESP University, Jaboticabal Campus/SP/BR (nº 014944/14). Fry of Nile tilapia, GIFT strain - Aqua América®1 were used in this study.

The mean values of water temperature remained at 28.0 ± 2.23 °C, dissolved oxygen 6.77 ± 2.03 mg L^{-1} and pH 6.8 ± 0.54, registered with digital potentiometers. In order to determine water transparency (m), the Secchi disc was used, and the values were 32.0 ± 2.67 cm during the seven months of experiment.

Three thousand eight hundred and forty Nile tilapia broodfish GIFT strain - Aqua América®1 were used: 2,880 females (197.63 ± 62.57 g) and 960 males (218.7 ± 65.05 g). The fish were weighed (g) and measured (cm) before being placed in "hapas" (10.0 x 3.0 x 0.80 m, 1.0 mm mesh size), at a proportion of three females to one male (3F:1M), a total of 160 fish per hapa, installed in a pond (2,000 m^2). In order to obtain better oxygenation in the hapas and avoid net clogging, a pump system was installed and pond water was sprinkled onto the hapas. A completely randomized design was used, composed by six treatments and four replications.

Six diets were used in the experiment; four experimental diets and two commercial diets

commonly used by large part of the fry producers. In the Table 1 are presented the ingredients of the experimental feed offered to Nile tilapia broodfish during the experiment. The four experimental diets contained different levels of crude protein (CP) (32A; 38; 44 and 50 %), 3,500 kcal of digestible energy (Table 2), and were formulated and processed at an experimental feed factory located in Fishery Institute, APTA - Centro de Pesquisa do Pescado Continental, São José do Rio Preto - SP/BR. The raw material was weighed, homogenized and ground to 0.7 mm particles, using a *Vieira*® hammer mill. Afterwards, it was homogenized again and taken to extrusion in a *FERRAZ*® E62 machine, and dried in a forced ventilation oven at 55°C for 24 h. The commercial diets (32B and 36% CP) and 3,500 kcal of digestible energy were purchased at a feed factory located in Santa Fé do Sul - SP/BR and offered to the fish (Table 2).

Table 1. Ingredients of the experimental feed offered to Nile tilapia broodfish during the experiment.

Ingredients (%)	Diet (% CP)			
	32	**38**	**44**	**50**
Feather meal	1.00	1.50	2.50	2.80
Poultry by-product meal	11.00	12.00	15.00	16.50
Soybean 60% SPC	20.00	24.00	29.00	32.50
Corn gluten meal 60%	2.00	3.00	4.50	5.52
Wheat meal	8.77	8.20	4.58	3.20
Wheat flour	0.00	1.00	4.00	5.00
Macrogard[1]	0.03	0.03	0.03	0.03
Active MOS[2]	0.50	0.50	0.50	0.50
Broken rice	34.75	27.06	18.45	11.50
Meat and bone meal 43% CP	2.06	4.00	0.68	0.00
Fish meal 55% CP	10.00	12.00	13.50	15.00
Spray dried blood meal	1.50	2.00	3.50	4.00
Salt	0.30	0.30	0.30	0.30
Dicalcium phosphate	1.50	0.89	0.82	0.57
Soybean oil	4.00	1.00	0.47	0.00
Vitamin C 35%	0.48	0.17	0.17	0.17
Choline chloride 70%	0.20	0.20	0.20	0.20
L-Lysine	0.10	0.20	0.20	0.19
L-Threonine	0.12	0.20	0.06	0.23
Taurine	0.10	0.10	0.10	0.10
DL-Methionine	0.24	0.40	0.19	0.44
Oxinyl Dry[3]	0.10	0.10	0.10	0.10
Mycotoxin adsorbent	0.20	0.20	0.20	0.20
Antifungal	0.30	0.20	0.20	0.20
Orego-Stim[4]	0.05	0.05	0.05	0.05
Premix[5]	0.70	0.70	0.70	0.70

[1]β- Glucan (Biorigin®); [2] Mannanoligosaccharide (Biorigin®); [3]Antioxidant; [4]Essential oils (Meriden Animal Health®); [5]Vitamin and Mineral Supplement (In Vivo®) – levels of guarantee per kg of the product: Vit. A= 12,000.00 UI kg^{-1}; Vit. D3 = 3,000.000 UI kg^{-1}; Vit. E = 150.0 mg; Vit. K3 = 15.00 mg; Vit. B1 = 20.00 mg; Vit. B2 = 20.00 mg; Vit. B6 = 17.50 mg; Vit. B12 = 40.00 mcg; Vit. C = 300.000 mg; Nicotinic Acid = 100.00 mg; Pantothenic Acid = 50.00 mg; Biotin = 1.00 mg; Folic Acid = 6.00 mg; Antioxidant = 25.00 mg; Copper Sulfate = 17.50 mg; Ion Sulfate = 100.00 mg; Manganese Sulfate = 50.00 mg; Zinc Sulfate = 120.00 mg; Calcium Iodide = 0.80 mg; Sodium Sulfate = 0.50 mg; Cobalt Sulfate = 0.40 mg; Inositol = 125.00 mg; Choline = 500.00.

The feed was then taken to CBO - Analysis Laboratory, in Campinas, SP/Brazil, for analysis of crude protein concentration, gross energy, mineral matter, ethereal extract and amino acid

composition (Table 2), following the methodology recommended by EL-SAYED *et al.* (2003), according to the AOAC methodology (1980).

The adjustment periodof the broodfish to the feed lasted 30 days before the beginning of harvesting (Sep./2014). One percent of the total live weight was offered to the fish during the whole experimental period, similarly to what had been suggested by SIDDIQUI *et al.* (1997; 1998) and LUPATSCH *et al.* (2010).

Table 2. Centesimal composition of diets offered to Nile tilapia broodfish during the experiment.

Composition	Treatment (% CP)					
	T1/32A	**T2/32B**	**T3/36**	**T4/38**	**T5/44**	**T6/50**
Crude Protein (%)	31.80	32.21	36.27	38.34	44.61	50.78
Ethereal Extract (%)	7.66	7.78	9.08	9.68	9.18	9.41
Crude Fiber (%)	2.89	2.40	2.72	2.57	2.46	2.49
Calcium (%)	2.43	2.69	3.09	3.13	3.64	3.10
Total Phosphorus (%)	1.76	1.50	1.79	1.63	1.96	1.63
Arginine (%)	2.07	2.10	2.58	2.44	2.77	3.00
Lysine (%)*	1.45	2.00	2.27	2.51	2.85	3.20
Met. + Cyst.[1] (%)*	1.10	1.08	1.13	1.26	1.26	1.59
Threonine (%)*	1.17	1.44	1.72	1.82	2.09	2.36
Tryptophan (%)*	0.29	0.34	0.38	0.40	0.48	0.52
Methionine (%)*	0.82	0.86	1.05	1.13	1.03	1.41
Digestible Energy (kcal)*	3,565.81	3,550.61	3,589.76	3,454.79	3,523.41	3,546.36
Digestible Protein (%)*	26.97	27.03	31.29	32.00	37.08	40.93
Starch (%)*	29.86	30.00	27.24	25.27	20.50	16.00

**Calculated value; [1]Methionine (Met.) + Cysteine (Cyst.).*

During the reproductive period, females and males remained together in the hapas, and reproduction occurred spontaneously, as males and females paired up. They were in their first gonadal maturation. The eggs were collected weekly: all the females were checked individually and when eggs were detected in their mouth, they were removed by immersing the female in a bucket with pond water, opening the operculum and forcing the water through it, so that all the eggs could be removed. The eggs were then taken to the laboratory, where the volume (mL) produced per hapa was recorded, samples were taken and eggs were counted and had their diameter measured (µm). The remaining eggs were kept individually in hatcheries (2000 mL), one per replication, at a total of 24 hatcheries with water recirculation and control of the physical and chemical variables, until the eggs hatched into larvae, with total absorption of the yolk sac. The larvae were counted, using a strainer with a previously known number of larvae, for verification of hatching rates. All the procedures involved from egg harvesting until the moment the larvae were counted, as mentioned before, were based on the same protocols, both in the experimental reproduction ponds and at the commercial fish farm.

Samples of 100 eggs per treatment, collected throughout the whole reproductive cycle were taken for the assessment of the mean diameter of eggs. At first, the samples were photographed under stereomicroscope attached to a digital camera (Bel – 150 X). The diameter was calculated by the arithmetic mean between the largest horizontal and vertical axis by using free software, and then the means were compared between treatments.

Seventy mature males were selected for semen sampling: 10 in the first sampling (initial sampling – August/2014), 30 males after 90 days (intermediate sampling – November/2015) and 30 males after 210 days (final sampling – March/2015). In the initial and final sampling, the semen was collected directly from the testes. In order to do so, the fish were anesthetized with Eugenol solution (60.0 mg L^{-1}) (RANZANI-PAIVA

et al., 2013), euthanized by spinal cord dissection, and had their testes removed. In the intermediate sampling, semen was collected with insulin syringes without a needle after ventral massage in a cephalocaudal direction. The semen collected was immediately assessed with regard to computerized sperm parameters.

The sperm motility parameters were assessed via CASA method (Computer Assisted Sperm Analysis), employing the procedure adopted by WILSON-LEEDY and INGERMANN (2007) for analysis using open source software ImageJ/Plugin CASA. The videos were captured (Basler® camera A640-120gc) at 100 fps (640x480 pixels) and processed according to what had been described by WILSON-LEEDY and INGERMANN (2006) and SANCHES *et al.* (2010; 2013), but the configurations used were adapted to Nile tilapia. The sperm parameters obtained from three replications per male were: motility rate (MOT), curvilinear velocity (VCL), average path velocity (VAP) and straight line velocity (VSL).

The videos were obtained immediately after sperm activation with distilled water, and were then edited and assessed at three distinct moments: 10, 30 and 60 seconds after sperm activation. In the initial sampling, the sperm parameters were not assessed 60 seconds after activation.

Samples of eight larvae per treatment were collected on the seventh day of life after hatching and total absorption of the yolk sac for registration of total length (mm), using the same procedures employed to measure egg diameter .

In the economic analysis, only the costs with production were considered; the investments, and consequently the depreciation of durable goods were not taken into account. In the calculations, we assumed a similar production situation to the one proposed in this study, where 3,840 broodfish were used per treatment (2,880 females and 960 males), distributed in 24 hapas in a 2,000 m^2 pond, and the feed offered corresponded to 1% of the live weight on alternate days, during an experimental period of 210 days. The methodology proposed by the Institute of Agricultural Economics in São Paulo (MATSUNAGA *et al.*, 1976; MARTIN *et al.*, 1994) was adapted to estimate the monthly production costs. In the calculation of actual operating costs (AOC), the expenses with broodstock feed, feed used during sexual reversion, and the employee responsible for feeding and taking care of larvae and broodfish were considered. In this calculation, the expenses with packaging, commercialization, marketing and sales tax were not considered.

The total operating costs (TOC) were composed by AOC plus social charges from recruitment (social security contributions, vacations and other expenses), which were considered as 43% of the salary (AYROZA *et al.*, 2011) (Table 3). In the calculations, the values obtained in the present study for the number of larvae produced in each treatment (post-hatch larvae) were used as a basis, and a 17.50% mortality rate was considered during the reversion phase. Later, the production cost of one thousand larvae after reversion (Cost of one thousand larvae = n larvae / total operating costs x 1000) was calculated. The gross income (GI) was estimated as the number of larvae obtained for each group multiplied by the sale price (post-hatch larvae, 1,000 units: R$ 50.00; larvae after reversion, 1,000 units: R$ 115.00; GI = n larvae x sale price / 1,000). The operating profit (OP) was calculated as the difference between GI and TOC (OP = GI – TOC), and the gross margin (GM) was calculated as the profit margin obtained in relation to TOC: GM = (OP / TOC) x 100).

The software Statistica 7.0 (Statsoft 2005) was used for the statistical analysis of the morphometric and reproductive assessed, and the results were submitted to one-way analysis of variance (ANOVA), and when differences were observed (p<0.05), the Tukey's test of comparison of means was applied to the same level of significance. The values of sperm parameters obtained during final sampling were also compared with the values obtained during the first sampling. The Dunnett's method of comparison of means was conducted at 5% significance, and the starting point was considered as control for 10 and 30 seconds after sperm activation. The assumptions of homogeneity of residues and homoscedasticity of variance were evaluated by the Shapiro-Wilk and Levene tests, respectively, at the same level of significance.

Table 3. Items and values of actual operating costs (AOC) and total operating costs (TOC) of Nile tilapia larvae production obtained from broodstock fed different levels of crude protein.

Treatments (% CP)	Items AOC					AOC (R$)	TOC (R$)
	Broodstock feed		Reversion feed		Employee (R$)*		
	Cost kg (R$)	Total cost (R$)	Cost kg (R$)	Total cost (R$)			
T1/32A	1.58	1,925.50	5.25	72,338.68	1,7420.45	91,684.63	116,595.88
T2/32B	2.43	2,961.37	5.25	70,459.00	1,7420.45	90,840.83	115,752.08
T3/36	2.24	2,729.83	5.25	77,994.46	1,7420.45	98,144.74	123,055.99
T4/38	2.50	3,046.68	5.25	78,420.92	1,7420.45	98,888.05	123,799.30
T5/44	2.58	3,144.17	5.25	84,236.53	1,7420.45	104,801.16	129,712.41
T6/50	2.74	3,339.16	5.25	88,221.59	1,7420.45	108,981.20	133,892.45

**Expenses with one employee working 59 hours per week, considering a salary of R$ 1,500.00.*

RESULTS

Females

The means of final weight (FW), weight gain (WG) and final length (FL) of the females did not show significant differences (p>0.05) between the six diets offered (Table 4).

The volume and total number of eggs produced per treatment did not show significant differences (p>0.05) at the end of 30 weeks of harvesting (Table 4). Absolute fecundity did not exhibit significant differences (p>0.05) either, and the same happened to egg diameter, percentage of spawnings per female, and larvae survival rate (Table 4).

Although we have noticed that the highest levels of CP presented higher values of total number of produced larvae, they were not different between treatments (p>0.05). However, the increase in protein level in the diets increase significantly (p>0.05) the total length of the larvae (Table 4).

Table 4. Morphometric and reproductive parameters (mean ± standard values) of Nile tilapia female broodfish fed different levels of crude protein.

Parameters	Treatment (% CP)[1]					
	T1/32A	T2/32B	T3/36	T4/38	T5/44	T6/50
Morphometric						
IWF (g)	197.63 ± 62.57	197.63 ±6 2.58	197.63 ± 62.59	197.63 ± 62.60	197.63 ± 62.61	197.63 ± 62.62
FWF (g)	299.44 ± 71.73	300.55 ± 79.86	317.22 ± 68.83	297.77 ± 112.30	250.55 ± 33.67	322.22 ± 67.36
IWM (g)	218.7 ± 65.05	218.7 ± 65.05	218.7 ± 65.05	218.7 ± 65.05	218.7 ± 65.05	218.7 ± 65.05
FWM (g)	280.0 ± 42.27	310.0 ± 64.71	289.44 ± 51.01	323.3 ± 55.62	368.55 ± 73.16	341.66 ± 38.16
Reproductive*						
TVE (mL)	15.83	14.66	16.75	14.34	15.25	16.38
TNE	246.86	228.62	261.21	223.56	237.82	255.36
ED (mm)	2.19 ± 0.18	2.09 ± 0.05	2.18 ± 0.17	2.15 ± 0.12	2.25 ± 0.16	2.16 ± 0.17
AF	1.171 ± 520.08	1.019 ± 486.07	1.081 ± 509.75	948 ± 425.74	884 ± 342.18	1.137 ± 804.00
S (%)	20.00	21.00	23.00	22.00	24.00	22.00
TL	185.57	180.75	200.08	201.17	216.09	226.31
SL (%)	75.00	79.00	77.00	90.00	91.00	89.00
TLL (mm)	6.78 ± 0.24^{a}	6.80 ± 0.18^{a}	6.79 ± 0.28^{ab}	7.99 ± 0.20^{bc}	8.01 ± 0.18^{bc}	8.19 ± 0.17^{c}

*[1]Diets (% CP); 32A - commercial feed; 32B - experimental feed; IWF - Initial Weight of Females; FWF - Final Weight of Females; IWM - Initial Weight of Males; FWM - Final Weight of Males; TVE - Total Volume of Eggs; TNE - Total Number of Eggs; ED - Egg Diameter; AF - Absolute Fecundity; S - Spawnings; TL - Total Larvae; SL - Survival of Larvae; TLL - Total Length of Larvae.*Values obtained during the whole experimental period. Different letters in the same row show significant difference (post hoc multiple comparisons test, groups means, p<0.05).*

Males

The values of sperm motility rate and velocities evaluated (VCL, VAP and VSL) did not show effect (p>0.05) for the different levels of crude protein in the feed 10, 30 and 60 seconds after activation during the 90-day feeding period.

The values of sperm parameters assessed 10 seconds after activation in the final sampling showed influence (p<0.05) of the levels of crude protein only for the values of VAP (average path velocity) and VSL (straight line velocity), and the lowest means were observed at the 38% CP level (Table 5).

Thirty seconds after activation, VCL, VAP and VSL were influenced (p<0.05) by the different levels of crude protein in the feed. The lowest values were obtained in the fish fed diet with 36% CP, and the highest values were observed for 44 % CP (Table 6).

Sixty seconds after activation, only VCL was not influenced (p>0.05) by the levels of CP in the feed; for MOT, VAP and VSL, the highest values were observed with 44% CP, and the lowest ones with 36% CP (Table 7).

Table 5. Computerized sperm parameters (mean ± standard values) assessed 10 seconds after activation in Nile tilapia fed different levels of crude protein for 210 days.

Treatment (% CP)	MOT (%)	VCL (μm s^{-1})	VAP (μm s^{-1})	VSL (μm s^{-1})
T1/32A	80.00 ± 7.14	106.82 ± 9.13	56.98 ± 7.59^{a}	51.36 ± 6.07^{a}
T2/32B	70.76 ± 10.33	104.64 ± 13.90	63.56 ± 7.27^{a}	59.15 ± 6.30^{a}
T3/36	80.38 ± 7.14	112.68 ± 7.56	65.26 ± 2.96^{a}	60.25 ± 2.50^{a}
T4/38	71.79 ± 16.18	89.71 ± 15.62*	46.13 ± 8.01^{b}	43.54 ± 7.27^{b}
T5/44	73.14 ± 10.98	108.40 ± 8.69	58.64 ± 5.26^{a}	53.80 ± 6.84^{a}
T6/50	74.46 ± 7.98	110.37 ± 13.57	59.65 ± 4.62^{a}	54.60 ± 6.03^{a}

*MOT – Sperm motility rate. VCL - Curvilinear velocity. VAP - Average path velocity. VSL - Straight line velocity. 32A - commercial feed. 32B - experimental feed. Different letters in the same column show significant difference (p<0.05) between the levels of protein according to the Tukey's test of comparison of means. *Significant difference (p<0.05) when compared with the initial sampling according to the Dunnett's test of comparison of means.*

Table 6. Computerized sperm parameters (mean ± standard values??) assessed 30 seconds after activation in Nile tilapia fed different levels of crude protein for 210 days.

Treatments (% CP)	MOT (%)	VCL (μm s^{-1})	VAP (μm s^{-1})	VSL (μm s^{-1})
T1/32A	68.78 ± 16.67	74.90 ± 7.73^{a}	37.98 ± 4.93^{a}	35.64 ± 4.60^{a}
T2/32B	60.61 ± 12.96	70.89 ± 8.46^{a}	40.00 ± 4.61^{a}	38.07 ± 4.71^{a}
T3/36	56.43 ± 9.98	64.61 ± 8.87^{b}*	33.31 ± 3.79^{b}	31.32 ± 3.36^{b}
T4/38	65.52 ± 20.30	67.72 ± 10.99^{a}*	35.19 ± 5.10^{a}	33.66 ± 4.95^{a}
T5/44	67.39 ± 7.02	81.92 ± 6.03^{c}	43.66 ± 4.08^{c}	40.66 ± 4.16^{c}
T6/50	60.80 ± 8.59	74.85 ± 6.69^{a}	37.73 ± 2.72^{a}	35.64 ± 2.98^{a}

*MOT – Sperm motility rate. VCL – Curvilinear velocity. VAP – Average path velocity. VSL – Straight line velocity. 32A – commercial feed. 32B – experimental feed. Different letters in the same column show significant difference (p<0.05) between the levels of protein according to the Tukey's test of comparison of means. * Significant difference (p<0.05) when compared with the initial sampling according to the Dunnett's test of comparison of means.*

In short, at the three moments of assessment (10, 30 and 60 seconds after activation), the highest values were observed in the treatment containing 44% CP, and the lowest ones were found with 36 and 38% CP (Figure 1).

According the economic analysis, positive values were observed for operating profit (Table 8), considering six reproduction ponds. The treatment with 50% CP exhibited the highest values of operating profit, as well as gross margin (%) of the activity.

Table 7. Computerized sperm parameters (mean ± standard values) assessed 60 seconds after activation in Nile tilapia fed different levels of crude protein for 210 days.

Treatment (% CP)	MOT (%)	VCL (μm s^{-1})	VAP (μm s^{-1})	VSL (μm s^{-1})
T1/32A	40.96 ± 15.38^{a}	50.53 ± 7.28	21.87 ± 2.44^{a}	20.47 ± 2.32^{a}
T2/32B	28.12 ± 14.32^{a}	49.66 ± 5.98	23.66 ± 2.93^{a}	21.61 ± 3.04^{a}
T3/36	14.38 ± 6.70^{b}	45.54 ± 10.74	20.32 ± 4.66^{b}	18.24 ± 3.80^{b}
T4/38	42.35 ± 17.29^{a}	49.06 ± 7.34	22.83 ± 3.65^{a}	21.63 ± 3.34^{a}
T5/44	49.71 ± 11.48^{c}	58.94 ± 8.04	29.95 ± 4.98^{c}	28.45 ± 4.94^{c}
T6/50	36.32 ± 6.03^{a}	52.90 ± 4.16	25.07 ± 1.17^{a}	23.09 ± 1.75^{a}

MOT - Sperm motility rate. VCL – Curvilinear velocity. VAP – Average path velocity. VSL – Straight-line velocity. 32A – commercial feed. 32B – experimental feed. Different letters in the same column show significant difference (p<0.05) according to the Tukey's test of comparison of means.

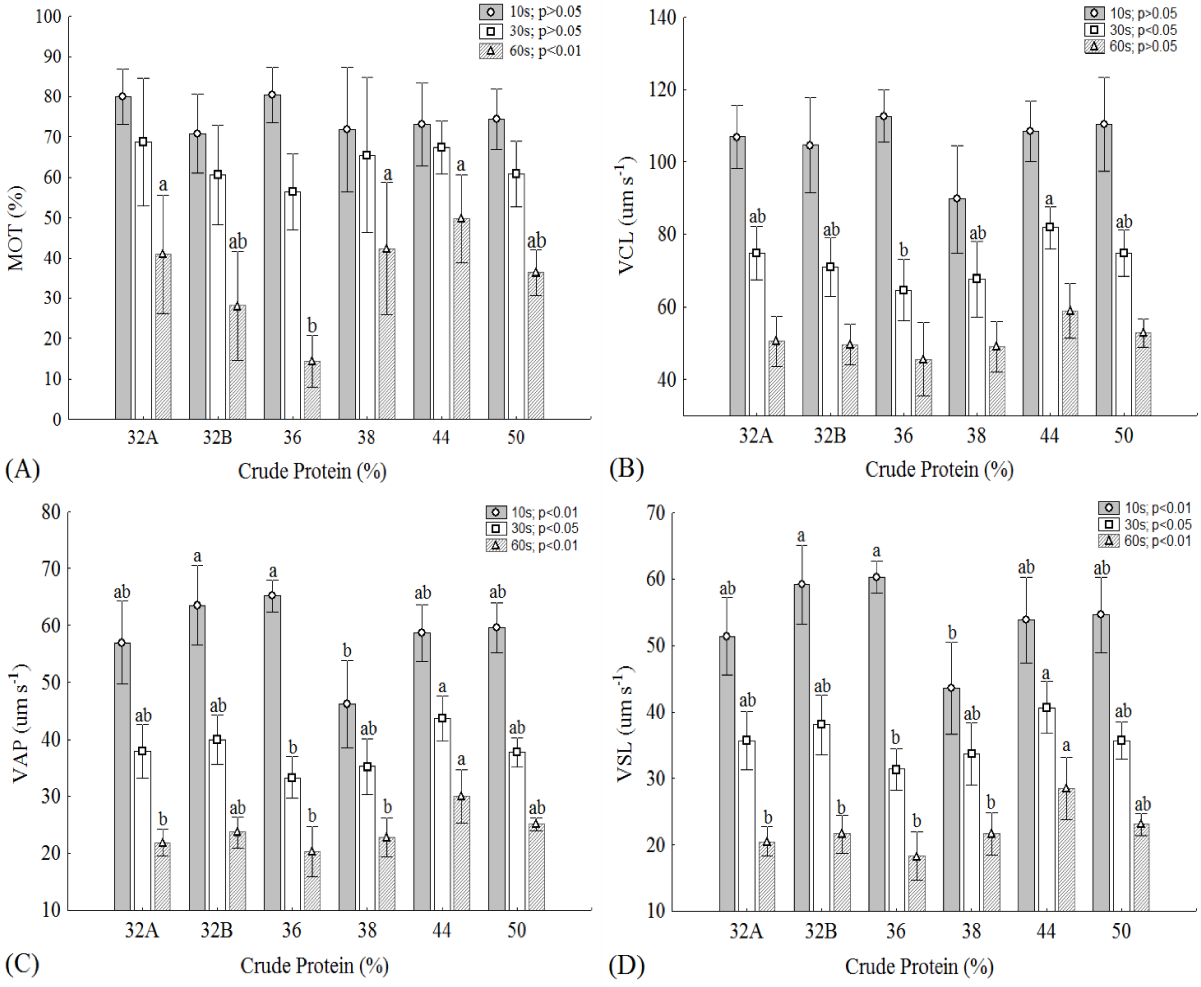

Figure 1. Sperm parameters (mean ± standard values) of Nile tilapia males submitted to different levels of crude protein in the diet for 210 days (final sampling), obtained 10, 30 and 60 seconds after sperm activation. (A) – Sperm motility rate. (B) - Curvilinear velocity (VCL). (C) – Average path velocity (VAP). (D) Straight line velocity (VSL). 32A – Commercial feed. 32B – Experimental feed. Different letters in the columns show significant difference (p<0.05) between the levels of CP according to the Tukey's test of comparison of means for the different levels of crude protein.

Table 8. Economic indicators of the production of Nile tilapia larvae obtained from broodfish fed different levels of crude protein.

Treatment	Total of larvae[1]	Cost/1,000 larvae (R$)	Gross income (R$)	Operating profit (R$)	Gross margin (%)
T1/32A	918,586	107.97	105,637.43	6,462.00	6.52
T2/32B	894,717	109.90	102,892.51	4,560.88	4.64
T3/36	990,406	106.66	113,896.68	8,261.14	7.82
T4/38	995,821	106.83	114,519.44	8,140.59	7.65
T5/44	1,069,670	104.98	123,012.08	10,720.12	9.55
T6/50	1,120,274	103.97	128,831.52	12,359.52	10.61

[1]Production increased by six times (representing six reproduction ponds) as a starting point for the profitability of the activity; subtracting from the total number of larvae per reproduction pond a 17.5% mortality rate.

DISCUSSION

Proteins are very important sources of nutrients for fish, and are responsible for the maintenance of physiological mechanisms related to reproduction, such as gonad maturation with gamete formation, vitellogenesis, egg fertilization, and initial ontogenetic development (WASHBURN *et al*., 1990). In Nile tilapia, proteins are used as source of energy for the reproductive process, including the aggressive behavior of the males, mating, territory defense and oral incubation (EL-SAYED and KAWANNA, 2008).

However, at the beginning of the period of oral incubation, some species of Cichlids, among which Nile tilapia, stop searching for food, and eventually suppress their growth, using physical resources in order to keep reproductive success (COWARD and BROMAGE, 1999; 2000). In the present study, that kind of behavior was observed, so at the end of 30 weeks of experiment the values of weight gain (WG) and final length (FL), both in males and females, were minimal and without significant differences between the treatments, corroborating what had been mentioned by COWARD and BROMAGE (2000) and LUPATSCH *et al.* (2010), who had found low values of WG in broodfish fed different levels of CP.

Broodfish nutrition is an important non-genetic factor with potential to determine the viability and quality of eggs and newly hatched larvae (DA SILVA *et al.*, 2008). It is interesting to consider the reproductive success of broodfish, since a well-nourished female that is adapted to confinement conditions has full possibilities of performing its maximum capacity of egg production. Taking that information into account, we observed that volume, number and size of the eggs produced by the broodfish assessed during this experiment did not show significant differences between the diets. However, the numbers obtained were sizeable and similar to the ones found by NG and WANG (2011).

Fecundity is defined as the number of eggs released by a female, which depends ultimately on the available volume in the celomic cavity to hold the mature ovaries, and the size of those eggs (VAZZOLER, 1996). According to COWARD and BROMAGE (2000), food quality and availability influence both the number and the size of tilapia eggs. Nevertheless, more studies on that subject are necessary. RANA (1990) claims there is evidence that fecundity is more closely related to the age of broodfish than to their size or nutrition, so the number of eggs increases as the female becomes more mature. Furthermore, TREWAVAS (1983) states it is clear that larger eggs are produced by larger females.

The results of absolute fecundity obtained in this study did not show statistical differences between treatments, so we may assume that diets with low concentration of CP (32%) were as effective as diets with high level of protein (50%). These results are similar to the ones found by NG and WANG (2011). However, OLIVEIRA *et al*. (2014) found that diets containing 38% CP were more effective than the ones with 32% CP.

It is worth emphasizing that there are interrelated factors which may be involved in determining the number of eggs in tilapia (COWARD and BROMAGE, 2000), such as the

size of broodfish, since larger females produce more eggs than smaller females. Therefore, there is great variety in the number of eggs per spawning (LUPATSCH *et al*., 2010). Other factors include food offer, time of study, or even the number of fish per treatment, so further studies on this subject are needed.

GUNASEKERA *et al.* (1996) reinforced the idea of the fundamental role of nutrients in the success of fish farming, stating that the contribution of proteins in reproductive performance, quality and production of viable larvae is evident, in as much as they influence the development and production of eggs, considering the nutritional history of the female during spawning.

The results obtained in this study presented growing number of viable larvae as the concentration of CP increased in the feed: the treatment with 50% CP produced 21.9% more larvae (226,318) than the treatment with 32% CP (32A; 185,573); the treatment with 44% CP exhibited survival rate 16.4% higher than the treatment with 32% CP (32A), nevertheless these values do not show statistical differences.

On the other hand, significant differences were found for the total length of tilapia larvae as the percentage of CP increased in the diets. Such results were similar to the ones described by EL-SAYED and KAWANNA (2008), when the growth of tilapia larvae rose from 8.1 ± 0.17 to 12.5 ± 0.39 g with the addition of protein and energy in the diets offered to broodfish.

Protein intake is necessary to meet the demands of the fish, since amino acids are continuously used to build new proteins during growth and reproduction, and to substitute the existing proteins for maintenance (FURUYA, 2010).

Among proteins there are some fundamental hormones to the reproduction process, such as gonadotropins, which act on testicular somatic cells, the main regulators of Leydig cells, involved in steroidogenesis, and the Sertoli cells, responsible for the nutritional, structural and regulatory support of germ cells (SCHULZ *et al*., 2010). In order to observe possible changes in Nile tilapia males during the experiment, given that proteins play an important role in their reproductive performance, the males were evaluated at the beginning, middle and end of the studies.

The parameters motility (MOT), average path velocity (VAP), straight line velocity (VSL) and curvilinear velocity (VCL) did not show significant differences ($p>0.05$) between the treatments from the starting point until the 90th day of experiment, 10, 30 and 60 seconds after gamete activation (10s, 30s and 60s). These results were similar to the ones observed by OLIVEIRA *et al.* (2015), who did not verify effect of the levels 32, 34, 36, 38 and 40% CP in the diets for motility rate and sperm motility duration. However, after 210 days of experiment, the variables VAP and VSL were significantly lower ($p<0.05$) in the treatments containing 38% CP 10 seconds after activation, whereas after 30 seconds the best values ($p<0.05$) of VAP, VSL and VCL were found in the treatment with 44% CP, and the worst means were observed with 36% CP. Motility did not show significant difference for either of them. Nevertheless, 60 seconds after activation, MOT, VAP and VSL were significantly higher ($p<0.05$) in the treatments with 44% CP, and lower in the ones with 36% CP, and for both of them, VCL did not show any difference.

In general, observing the results, diets with 44% CP are presumably advantageous for the reproductive performance of Nile tilapia males.

The results of the economic analysis conducted at the end of the experiment demonstrated that using high crude protein levels might be economically viable. However, the profitability may not be considered advantageous in the treatment with 50% crude protein, if we take into account the higher input of nutrients in the ponds, increasing the nitrogen compounds and affecting water quality, the negative results in reproductive performance of the males used in the study, in addition to technical issues related to processing and extruding the feed. Bearing this in mind, we could observe that the treatments containing 44% CP presented interesting results, offered satisfactory reproductive performance and generated profit, making the farming system less overloaded.

CONCLUSION

It can be concluded that the increase in crude protein level in the diets did not bring significant improvement in the reproductive performance of the females. However, after assessing the

performance of the males, it is recommended the use of diets containing 44% CP for Nile tilapia broodfish.

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