



Full Length Research Paper

# Effect of diets of different protein levels for Nile tilapia broodstock on sperm quality characteristics and on egg biomass production and fecundity

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Accepted 29 January, 2018

This experiment aimed to assess the effects of diets for Nile tilapia broodstock with six months of age, fed different levels of crude protein (CP), 32, 38, 44 and 50%, on sperm quality characteristics and on egg biomass production and fecundity. In Dec./13 the fish began to be fed, however, the collections occurred the Jan. to May/14. Two earthen ponds (200 m<sup>2</sup> X 1.5 m) were used, and ten hapas (3.0 x 1.5 x 1.5m) were installed in each one, making a total of 20 experimental hapas, in two stages: (1) Reproduction (3M; 9F) and (2) Recovery, the females stayed in those 16 hapas, and the males were moved to the remaining four hapas. At the end of the experiment, 80.56% of females fed the highest level of CP spawned, in contrast to 69.44% of those fed the lowest level performing an efficiency of 11,12% in relation of spawn. In this work we emphasize the use of 44% diets for males. However, for females the improvement in reproductive performance was observed in spawning. It should be noted that the nutritional requirements of breeders for Nile tilapia, for both sexes, should be differentiated mainly during the gonadal maturation process.

**Keywords:** Nutrition, reproduction, semen, fry.

## INTRODUCTION

Nowadays, with prospects of population growth and the need to increase food production suggested by the FAO (2016), the animal production sector is one of the fastest growing in the world (Oliveira et al., 2014 and 2015). Aquaculture stands out for supplying a large range of products and sub-products, and tilapia is considered an important fish species produced worldwide (Ng & Romano, 2013; Oliveira et al., 2014 and 2015). Therefore, there has been greater demand for tilapia larvae and juveniles (Ng and Wang 2011), which is one of the biggest challenges of the production

chain (El Sayed and Kawanna, 2008; Lupatsch et al., 2010).

Aspects related to the nutrition of males still demand more studies, such as semen and sperm characteristics, which differ according to the nutritional state of the fish (Izquierdo et al., 2001) and have a fundamental role in the reproductive process (Billard and Cosson, 1992; Rurangwa et al., 1994). Semen and sperm parameters of tilapia are influenced by the addition of vitamin C (Mataveli et al., 2010), digestible energy (Bombardelli et al., 2010), oils

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(Navarro et al., 2014) and crude protein (Oliveira et al., 2014). Nevertheless, there are still no conclusive results about the ideal diet to improve the reproductive parameters in males of this species.

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 crude protein 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 (Ámbar Amaral Group, *Raguife Rações*<sup>®</sup> – Santa Fé do Sul, SP/BR; Gonçalves, G.S. 2016, per. comm.). Thus, the protein level in the feed for brood fish 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.

Thus, the objective of this study was to assess the reproductive aspects of Nile tilapia broodstock, GIFT strain, fed diets containing different levels of protein on sperm quality characteristics and on egg biomass production and fecundity.

## MATERIALS AND METHODS

The experiment was carried out at the premises of APTA/S.A.A. (São Paulo's Agency for Agribusiness Technology) in Pirassununga, São Paulo/Brazil, located at the east-central region of São Paulo state (latitude 21°59'46" South and longitude 47°25'33" West), with the approval of the Animal Ethics Committee, UNESP University, Campus/SP/BR (Process number 014944/14). In December/2013 the fish began to be fed (Table 1), however, the collections occurred from January to May/ 2014 (five months).

Four experimental diets containing different levels of crude protein (CP) (32; 38; 44 and 50% CP) and 3,500 kcal of energy (Table 1) were formulated (software Optimal FORMULA 2000) and processed at the experimental feed factory located in the Fishery Institute, APTA-UPD, in São José do Rio Preto, São Paulo/Brazil.

The raw materials were weighed, homogenized, ground (0.7 mm), and then immediately homogenized again, extruded in a FERRAZ<sup>®</sup> (E 62) machine, and dried in a forced ventilation oven at 55 °C for 24 h. Later, the feed was taken to the CBO-Analysis Laboratory in Campinas, SP/Brazil, for analysis of crude protein concentration, energy, mineral matter, ethereal extract and amino acid composition (Table 2), following the methodology recommended by El Sayed et al., (2003), according to AOAC (1990).

The adjustment period of the broodstock to the feed lasted 30 days before the beginning of harvesting (December/2013). One percent (1%) of the total live weight was offered during the whole experimental period, similarly to what had been suggested by Siddiqui et al., (1997) and Lupatsch et al. (2010). The diets (females and males) were weighed and provided twice daily to the fish until they were apparently satiated<sup>1</sup>(Soybean Protein Concentrate- 60% CP), <sup>2</sup>Protenose (Corn Gluten - 60% CP), <sup>3</sup>β- Glucan (Biorigin<sup>®</sup>), <sup>4</sup>Mannan oligosaccharide (Biorigin<sup>®</sup>), <sup>5</sup>Antioxidant, <sup>6</sup>Essential oils (Meriden Animal Health<sup>®</sup>), <sup>7</sup>Vitamin and Mineral Supplement (In Vivo<sup>®</sup>) – levels of guarantee per kg of the product: Vit. A=12,000.00 IU/kg; Vit. D3= 3,000.000 IU/kg; 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; Iron 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.

One hundred and ninety-two Nile tilapia broodstock GIFT strain were used: 144 females (280.30 ± 69.60 g) and 48 males (372.5 ± 110.68 g) with six months of age (1<sup>st</sup> gonad maturation). At first they were electronically identified with microchips (AnimallTAG<sup>®</sup>). Monthly all animals (100%) were weighed (± 0.01 g) and a mean total weight and the total length (±0.1 cm).

Two earthen ponds (200 m<sup>2</sup> X 1.5 m deep each) were used, and ten (10) hapas (3.0 x 1.5 x 1.5m) were installed in each one, making a total of 20 experimental hapas. Sixteen hapas were used for **Stage 1- Reproduction** (3 males and 9 females). Later, at **Stage 2- Recovery**, the brood fish were separated: the females stayed in those 16 hapas, and the males were moved to the remaining four (4) hapas. For better illustration, the experimental design is represented in Figure 1.

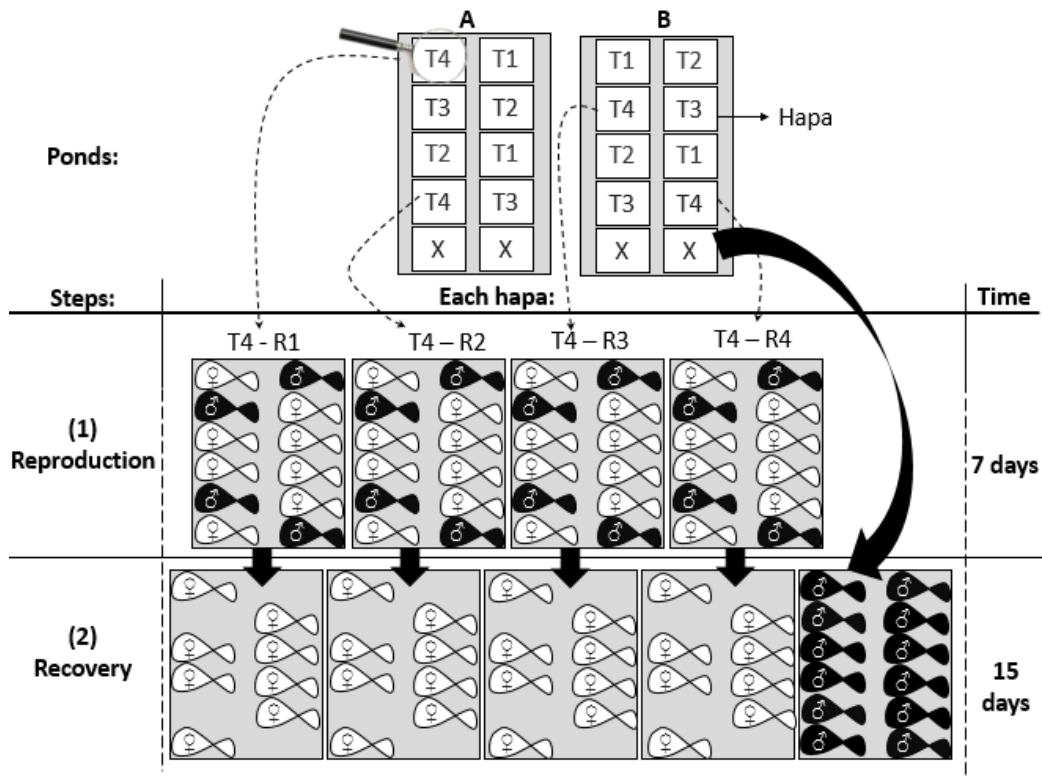
**Table 1:** Ingredients of the experimental feed offered to the Nile tilapia brood fish.

Ingredients (%)	Diets (% 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
SPC <sup>1</sup>	20.00	24.00	29.00	32.50
Protenose <sup>2</sup>	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 <sup>3</sup>	0.03	0.03	0.03	0.03
Active MOS <sup>4</sup>	0.50	0.50	0.50	0.50
Broken rice	34.75	27.06	18.45	11.50
Meat and bone meal	2.06	4.00	0.68	0.00
Fish meal	10.00	12.00	13.50	15.00
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
Oxynyl Dry <sup>5</sup>	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 <sup>6</sup>	0.05	0.05	0.05	0.05
Premix <sup>7</sup>	0.70	0.70	0.70	0.70
Total	100.00	100.00	100.00	100.00

**Table 2:** Centesimal composition of the experimental feed offered to the Nile tilapia brood fish.

Composition	Diets (% CP)			
	32	38	44	50
Humidity %	5.65	7.15	6.11	5.26
Crude Protein %	32.21	38.34	44.61	50.78
Ethereal extract %	7.78	4.00	5.31	4.90
Crude Fiber %	2.40	2.57	2.46	2.49
Calcium %	2.69	3.13	3.64	3.10
Phosphorus %	1.50	1.63	1.96	1.63
Arginine %	2.10	2.44	2.77	3.00
Lysine %*	2.00	2.51	2.85	3.20
Met.+ Cyst. %*	1.08	1.26	1.26	1.59
Threonine %*	1.44	1.82	2.09	2.36
Tryptophan %*	0.34	0.40	0.48	0.52
Methionine %*	0.86	1.13	1.03	1.41
Digestible Energy <sup>1*</sup>	3,550.61	3,454.79	3,523.41	3,546.36
Digestible Protein %*	27.03	32.00	37.08	40.93
Starch %*	30.00	25.27	20.50	16.00

\*Calculated value, <sup>1</sup>kcal; Methionine (Met.)+ Cysteine (Cyst.)

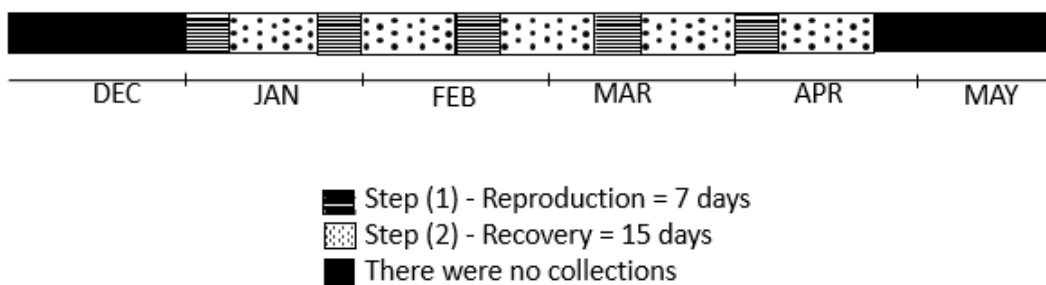


**Figure 1:** Detailed experimental design and reproductive management. (A) and (B) excavated ponds (200m<sup>2</sup> each); (T) Treatments (% of crude protein: T1-32, T2-38, T3-44, T4-50); (R) Replications; **Stage 1- Reproduction** – males and females kept together in the hapas for 7 days, with four replications per treatment; **Stage 2- Recovery** - males were separated from the females for 15 days. Four hapas were used only at stage (2) to receive the males during the recovery period.

Before the beginning of **Stage 1- Reproduction**, the fish from each treatment were fed the respective diets for a period of 30 days, and later throughout the whole experimental period.

During the experiment (150 days), eggs were collected five times between December and May (Figure 2), and each collection was carried out for seven days (**Stage 1- Reproduction**). After that period of collection, the fish (males and females) were separated so as to enable better recovery from the reproduction stage (**Stage 2- Recovery**). Males and females remained separated for 15 days, when the

females stayed in their reproduction hapas, and the males were withdrawn and placed in auxiliary hapas. The males were kept under the same experimental conditions they had been during the reproductive period together with the females. After 15 days apart and receiving the same feeding, the males were brought back to the females hapas (previously identified by microchips) for another period of 7 days of reproduction. That procedure was repeated five times, until the females stopped producing eggs due to low water temperature (near winter).



**Figure 2:** Reproductive management adopted throughout the experimental period. **Stage 1- Reproduction:** males and females together for egg collection; **Stage 2- Recovery:** males and females were kept separate.

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 four treatments and four replications.

At the end of each reproductive stage (after seven days of reproduction), the eggs were collected from each hapa. The females were individually verified by using a hand net and a microchip scanner, so that the presence of eggs in the oropharyngeal cavity could be detected, according to the methodology described by Lupatsch et al. (2010). The eggs were removed with a wash bottle and water from the pond, and taken to the laboratory to be weighed (g) and have the volume produced by each brood fish measured (mL). Samples (1g) were taken and the eggs were counted and had their diameter measured ( $\mu\text{m}$ ). The remaining eggs were kept, separated according to the treatment, in hatcheries (400 mL) with water recirculation and control of the physical and chemical variables until they hatched into larvae and the yolk sac was totally absorbed. During the incubation and larvae hatching at mean temperature was  $28,0 \pm 1,0^\circ\text{C}$ .

Samples of 50 oocytes (6 females X 4 treatments = 24 samples) were taken for mean diameter assessment. At first, the samples were photographed under stereomicroscope attached to a digital camera (Bel – 150 X). The diameter was calculated by arithmetic mean between the largest horizontal and vertical axis by using free software (Table 3).

Five fish per diet were selected for semen sampling at two moments: zero (Mz - Jan/14) and final (Mf - May/14). Semen was collected directly from the testes, so as to avoid the contamination with urine and blood; in order to do so, the fish were anesthetized with Eugenol solution ( $60.0 \text{ mgL}^{-1}$ ) (Ranzani-Paiva et al., 2013) and euthanized by spinal cord dissection.

Sperm survival was estimated by counting 600 fixed cells per fish and stained with eosin-nigrosine as recommended by Kavamoto and Fogli da Silveira (1986) and Sanches et al., (2009). The percentage of normal spermatozoa was assessed after the sperm cells had been fixed in buffered formol-saline and stained with rose Bengal (Streit Jr. et al., 2004) and 600 spermatozoa were then classified following the recommendations made by Caneppele et al., (2013).

The sperm motility parameters were assessed with the free software CASA (*Computer Assisted Sperm Analysis*), employing the procedure adopted by Wilson-Leedy and At the final moment, the values of motility rate were influenced ( $p < 0.05$ ) by the levels of crude protein in the feed, and the highest values ( $93.9 \pm 6.1\%$ ) were observed in the diet with 44% CP, and the lowest ones with 50% CP ( $75.4 \pm 11.0\%$ ) (Figure 1). Similar behavior was verified for sperm velocity: the lowest values ( $p < 0.05$ ) were observed for the diet with 50% CP. At this level of protein, we observed  $128.8 \pm 12.0$ ;

Ingermann (2006; 2007) for analysis using open source software Image J/Plugin CASA. The videos were captured at 101fps (640x480 pixels) and processed according to Sanches et al., (2010; 2013), but the configurations used were adapted for 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 software Statistica 7.0 (Statsoft 2005) was used for the statistical analysis of morphometric and reproductive data, and the results were submitted to analysis of variance (one-way ANOVA). The principal components analysis was applied to the sperm parameters, considering males as a blocking factor and the different levels of crude protein as treatment. For all the parameters, when differences were observed ( $p < 0.05$ ), the Tukey test was applied at the same level of significance. Besides, the sperm parameters were compared with the initial moment according to the Dunnett's test.

## RESULTS

The study showed that the growth of the Nile tilapia brood fish was not affected by the different diets ( $p > 0.05$ ). The mean values of weight gain (Wg) and final weight (Wf) were similar in the four diets (Table 3), as well as for the mean values of final length (Lf).

At the end of 25 weeks of experiment, Nile tilapia females during their first reproductive cycle fed with different levels of CP exhibited differences for the values of egg production at the first harvesting ( $p < 0.05$ ). The total number of eggs produced did not vary in consequence of the diets offered ( $p > 0.05$ ) (Table 3).

The level of CP influenced ( $p < 0.05$ ) egg diameter (Table 3). The treatment with 38% CP had the lowest values when compared with the other treatments, whereas the diet with 44% CP showed the highest values. There were no differences regarding egg diameter and weight between treatments ( $p > 0.05$ ).

Neither the values of absolute fecundity nor the percentage of females that spawned showed significant differences. The number of spawned per female did not suffer any change when receiving diets with different levels of CP (Table 3).

$59.8 \pm 6.4$  and  $53.8 \pm 6.3 \text{ m s}^{-1}$  for VCL, VAP and VSL respectively. However, for 44 % CP the highest values were found:  $153.9 \pm 16.3$ ;  $79.3 \pm 8.5$  and  $67.8 \pm 9.1 \text{ m s}^{-1}$  for VCL, VAP and VSL, respectively. Similar behavior was also observed for sperm survival and number of normal spermatozoa (Figure 2), where the diet with 50% CP presented the lowest values ( $p < 0.05$ ) for both variables.

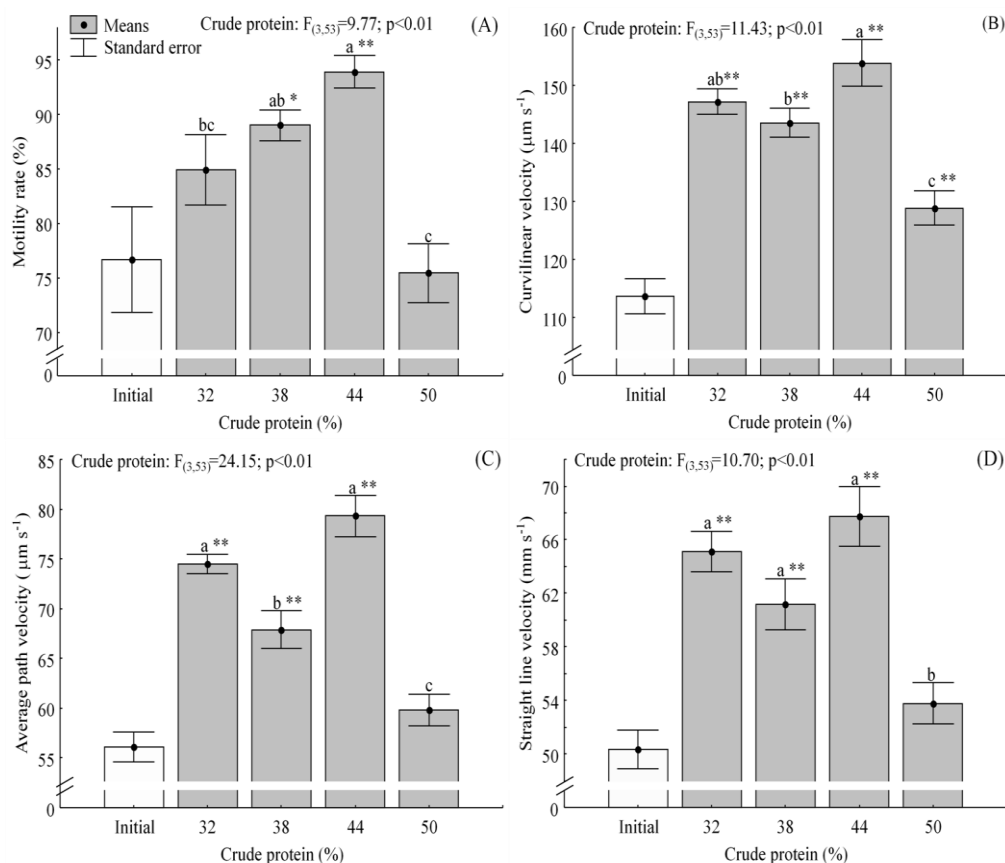
**Table 3:** Morphometric and reproductive parameters of Nile tilapia females fed different levels of crude protein.

(% Crude Protein, CP)	Diets <sup>1</sup>			
	32	38	44	50
<b>Morphometric Parameters</b>				
Initial weight female (Wi, g)	284.30 ± 69.60	284.30 ± 69.60	284.30 ± 69.60	284.30 ± 69.60
Final weight female (Wf, g)	535.55 ± 110.08	501.80 ± 67.18	521.83 ± 73.43	532.19 ± 108.80
Weight gain female (WG, g)	251.25 ± 117.68	217.50 ± 71.81	237.53 ± 78.49	247.89 ± 116.31
Initial weight male (Wi, g)	372.5 ± 110.68	372.5 ± 110.68	372.5 ± 110.68	372.5 ± 110.68
Final weight male (Wf, g)	583.58 ± 134.42	658.68 ± 99.87	568.28 ± 91.74	650.96 ± 80.81
Weight gain male (WG, g)	211,08 ± 134,42	286,18 ± 99,87	195,78 ± 91,74	278,46 ± 80,81
<b>Reproductive Parameters</b>				
Total volume of eggs (mL) <sup>2</sup>	606.00	615.00	678.00	723.00
Total number of eggs <sup>2</sup>	91,021.00	94,435.00	86,802.00	109,964.00
Total weight of eggs (g) <sup>2</sup>	459.83	478.59	443.62	562.22
Egg diameter (mm)	2.03 ± 0.23 <sup>ab</sup>	2.02 ± 0.19 <sup>c</sup>	2.06 ± 0.21 <sup>a</sup>	2.03 ± 0.21 <sup>b</sup>
Absolute fecundity	2,275 ± 878	2,208 ± 1009	2,411 ± 1097	2,199 ± 1038
Females that spawned (%)	69.44	77.78	75.00	80.56
Females that did not spawn (%)	30.56	22.22	25.00	19.44
Spawnings per female/treat.	1.05	1.25	1.00	1.38

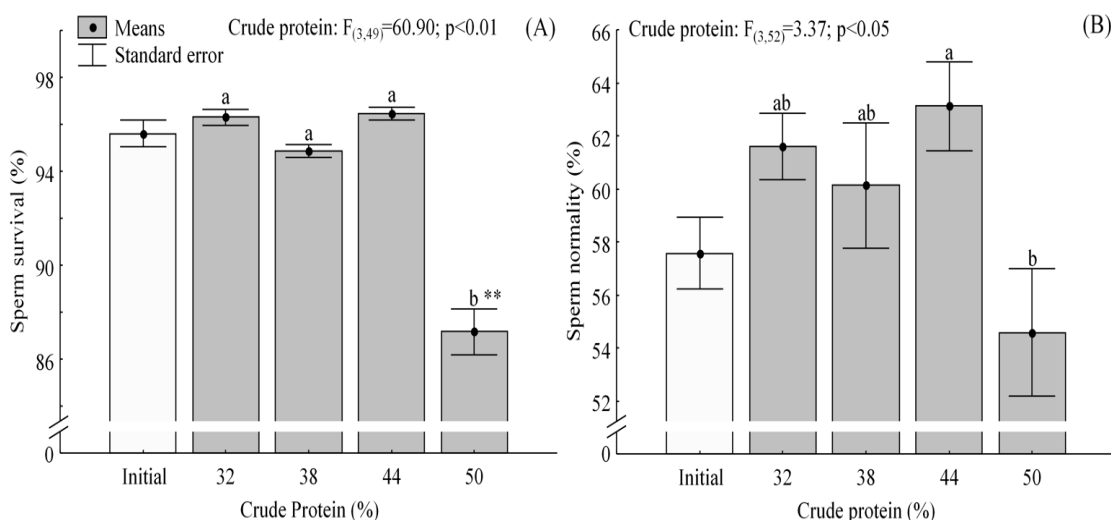
<sup>1</sup>Diets (% CP); <sup>2</sup> Values obtained during the whole experimental period. Egg diameter: 6 females X 4 treatments = 24 samples/treatment. \*Different letters in the same row show significant difference (post hoc test of multiple comparisons, means of the groups, p<0.05). Monthly all animals were weighed and measured (100%).

Observing the initial sample, we noticed that only the percentage of normal spermatozoa was not influenced (p<0.05) by the experimental diets (Figure 4). The other parameters were influenced (p<0.05), such as higher values for motility rate and velocity in diets with 44%

CP (Figure 3). Decrease in the values (p<0.05) was verified only for the sperm survival of fish fed 50% CP (Figure 4). Males considered as blocking factor influenced the parameters assessed (p<0.05).



**Figure 3:** Computerized sperm parameters of Nile tilapia fed different levels of crude protein in the diet. (A) Sperm motility rate - MOT. (B) Curvilinear velocity - VCL. (C) Average path velocity - VAP. (D) Straight line velocity - VSL. Different letters in the columns of each graph represent significant difference ( $p<0.05$ ) between treatments according to the Tukey test. Asterisks represent significant difference (\*\* $p<0.01$ ; \* $P<0.05$ ) between the initial moment (Initial) and the respective treatment according to the Dunnett's test of comparison of means.



**Figure 4:** Sperm survival rate (A) and Rate of normal spermatozoa (B) of Nile tilapia fed different levels of crude protein in the diet. Different letters in the columns of each graph represent significant difference ( $p<0.05$ ) between the different levels of protein according to the Tukey test. Asterisks represent significant difference (\*\* $p<0.01$ ) between the initial moment and the respective treatment according to the Dunnett's test of comparison of means.

## DISCUSSION

In addition to nutrition, reproduction is a fundamental biological process of the organisms, considering that survival and perpetuation of species depend on it. Thus, the possibility of controlling the reproductive cycle of confined organisms is one of the most important factors to ensure the success of fish farming (Romagosa et al., 2013).

Lupatsch et al., (2010) demonstrated that during the reproductive period, most nutrients consumed by the broodfish are directed to gonad development, egg and larvae performance, building nests, progeny protection, territory defense, among others. It is worth bearing in mind that Nile tilapia incubate their eggs in the mouth, and therefore do not eat during that period (El Sayed and Kawanna, 2008; Ng and Wang 2011). The low values of weight gain (WG) of the Nile tilapia brood fish during the 25 weeks of experiment corroborate the information mentioned above, as well as the data reported by Lupatsch et al., (2010) and Ng and Wang (2011), who described a moderate value of WG in brood fish when comparing levels of addition of oil to the feed during the reproductive period.

Analyzing the reproductive performance of tilapia brood fish in the present study, the number and volume of eggs produced during the experimental period did not show significant differences ( $p>0.05$ ), similarly to the results found by Gunasekera et al., (1996) and Siddiqui et al., (1998). However, El Sayed and Kawanna (2008) obtained better performance as the CP concentration increased in the diet of the brood fish (30-40%).

There are relevant studies on increase in protein concentration and energy in the diet of tilapia brood fish; however, they are a bit unclear and somewhat inconsistent, due to some practical aspects involved in the reproductive management, such as size of fish, stocking density, history of spawning and duration of studies; quality in feed formulation and use of correct raw material can also be mentioned (El Sayed and Kawanna, 2008). Therefore, until an ideal diet for tilapia broodfish has been determined, most reproduction fish farms will have to use feed formulated for other purposes, such as fattening or termination diet, for example. Nevertheless, it is important to understand the difference between nutrition of tilapia brood fish when compared with other stages of the life cycle, because the quality of progeny may be affected (Ng and Romano, 2013).

The difficulty in determining the ideal level of CP in feed for tilapia broodstock has a direct impact on the feed industry, as well as on the producers. These values are extremely large, and a difference of 10% in CP may mean increase or decrease in the final cost of production per tonne of feed. Obviously, the investment does not become profitable, and the companies cannot scientifically justify those values, so keeping a line of

rations for broodstock becomes impractical (Âmbar Amaral Group, Raguife Rações®- Santa Fé do Sul-SP/BR).

In this study, egg diameter exhibited difference between treatments ( $p>0.05$ ), in contrast to the values described by Gunasekera et al. (1996), who did not find significant differences when levels of CP between 20 – 35% were used. However, Oliveira et al. (2014) determined by means of LRP (Linear Response Plateau) analysis that tilapia females fed diets with 38% CP obtained larger eggs (2.7 mm) when compared with the other treatments. Coward and Bromage (2000), in a review about reproductive physiology of tilapia, suggested that both food offer and feed quality may influence egg diameter. Nonetheless, they emphasized that only a few studies involving this matter are available, warning that there are other interrelated factors (size and age of brood fish) involved in the determination of these values. In other words, there is a gap to be filled in order to define which main factors can influence the production of larger or smaller eggs. Fecundity is another important parameter to determine the reproductive performance of fish. It is known that it may be affected by nutritional deficiencies of the diet (Izquierdo et al., 2001; Khan et al., 2005). The values of absolute fecundity and the number of females that spawned did not suffer influence of different levels of CP in the present study.

Santiago et al., (1985) demonstrated that the variation in levels of protein (20 – 50%) did not cause significant change, which corroborates the studies conducted by Gunasekera et al., (1996), who noticed improvement in absolute fecundity in females fed high levels of CP, 20 – 35%, when compared with the treatment with 10% CP. However, Oliveira et al., (2014) recommended by means of LRP analysis a maximum point of CP addition (38%), and the values of absolute fecundity were higher (3,757) when compared with the treatments with 32, 34 and 36 % CP, which exhibited values of 2,860, 3,285 and 3,418 eggs, respectively, except when 40% CP were used (3,753). Nevertheless, there was no significant difference.

The females exhibited low fecundity rate and asynchronous spawning, which is still one of the most significant restrictions for larvae production (Tsadik and Bart, 2007); however, they tend to spawn even with limited food or nutrient supply, using resources from their own body, channeling energy and protein into the gonads in order to ensure egg quality (Lupatsch et al., 2010). Nevertheless, after a long period the mean values of absolute fecundity and the intervals of spawning tend to decrease (Siddiqui et al., 1998). Using that information in order to interpret the results of the present study, we suppose that something similar might have occurred to the females, justifying the symmetry between treatments ( $p>0.05$ ). It is important to emphasize that the reproductive management proposed by the study was after each week of collection, males and females remained separated and



rested for 15 days in order to recover, justifying the similar values of final WG means between treatments.

For the males, the lowest values of sperm motility rate and velocities were found in the treatment containing 50% CP, indicating that high levels of protein may cause damage in reproductive performance. However, the variation in the levels of crude protein in the feed between 32 and 44% seems not to be a limiting factor for Nile tilapia males. Similar results were found by Oliveira et al., (2015), who did not observe effect of the levels 32, 34, 36, 38 and 40% CP on motility rate and duration of sperm motility of fresh semen for the species. It was also verified that diets containing 32, 38 and 44% CP improved the reproductive performance of males, when compared with the initial stage of the fish, especially the diet with 44% CP. The improvement in the condition of brood fish fed well-balanced diets is corroborated by Izquierdo et al. (2001) and Ruragawa et al. (2004), who discussed the importance of good quality diets for males. In addition, those authors suggested that sperm motility in fish may be influenced by other nutritional factors, such as vitamins and fatty acids. In spite of that, the effect of the levels of CP on the diet of brood fish has virtually not been addressed and the results are conflicting.

## CONCLUSION

In this work we emphasize the use of 44% diets for males. However, for females the improvement in reproductive performance was observed in spawning. It should be noted here that the nutritional requirements of Nile tilapia breeders, for both sexes, should be differentiated, and special attention should be given to diets during the gonadal maturation process.

## CONFLICT OF INTEREST

The author (s) have not declared any conflict of interests.

## ACKNOWLEDGMENTS

The authors would like to thank the *Fundação de Amparo à Pesquisa do Estado de São Paulo* - FAPESP (nº 2013/24474-1) and the *Coordenação de Aperfeiçoamento de Pessoal de Nível Superior* – CAPES. They also wish to thank APTA/UPD – Pirassununga, Fishery Institute – SP/BR and São José do Rio Preto, SP/BR and their coworkers.

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