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Reproductive performance of female Nile tilapia (*Oreochromis niloticus*) fed diets with different digestible energy levels

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ABSTRACT - This study aimed to evaluate the reproductive performance of female Nile tilapia (*Oreochromis niloticus*) fed diets containing different levels of digestible energy (DE). The fish were housed in 15 fiberglass tanks (500 L) in a recirculating system at an average temperature of 27.5 °C. The treatments consisted of five diets with increasing levels of DE (3,200; 3,400; 3,600; 3,800; and 4,000 kcal/kg). The levels of DE did not significantly influence the final weight or the hepatosomatic, gonadosomatic, and visceral fat indices. The absolute fecundity was influenced by the treatments, for which the highest values were observed from the 3,600 kcal/kg DE level and upward. The proximate composition of the fish also had a significant effect on the variables crude protein, ether extract, and ash; the fish fed diets with higher levels of DE exhibited the lowest body protein content, while the accumulation of ether extract exhibited the opposite response. A level of 3,600 kcal/kg of digestible energy should be used in diets with 380 g/kg crude protein and a starch/lipid ratio of 1.33 for female Nile tilapia.

Key Words: body composition, fecundity, fish, nutrition, reproduction

Introduction

Tilapia farming is a growing industry, given that tilapia is the second largest group of freshwater fish cultivated worldwide. Production of tilapias has a wide distribution, notably in Asia, Africa, and Americas. The global production of tilapia reached 4,507,002 t in 2012, representing 10.2% of total farmed fish production (FAO, 2014). In Brazil, Nile tilapia (*Oreochromis niloticus*) is the most cultivated species, comprising approximately 46% (253,000 t) of the freshwater fish production (Ministério da Pesca e Aquicultura, 2011).

Nile tilapia have early sexual maturation and are multiple spawners, meaning that they can reproduce throughout the year, with short vitellogenic periods (Izquierdo et al., 2001), a physiological process that demands a high metabolic rate. Nevertheless, the low fecundity combined with the asynchronous spawning are factors that influence its commercial expansion (Tsadik Getinet, 2008).

Energy is a property of the nutrients released during the metabolic process of oxidation. Lipids are the main source of metabolic energy to breeding, provide essential fatty acids, and are important components of membrane (Tocher, 2003) and cell signaling involved in steroidogenesis (Mercure and Van Der Kraak, 1996). Diet can influence the maturation process of the gametes and energy storage in the form of the yolk (Mañanos et al., 2008), composed primarily of lipids and protein, wherein the dietary oil sources may influence fecundity (Hajizadeh et al., 2008), and the protein may influence the tilapia spawning performance (El-Sayed and Kawanna, 2008).

Currently, the formulation of diets for animals has been performed to meet energy requirements. To this end, the energy:protein ratio is a point that deserves priority attention when determining the nutritional requirements of the concerned species.

The knowledge of the energy metabolism of fish is an indispensable tool for the preparation of suitable artificial diets during the pre-spawning period. The nutritional requirements for tilapia broodstock have not yet been defined in Brazil. Oliveira et al. (2014) defined the level of dietary crude protein needed for broodstock; however, it is

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still necessary to establish the most appropriate amount of energy. Thus, the present study was conducted to evaluate the performance of female Nile tilapia fed diets containing different levels of digestible energy (DE) by varying the starch/lipid ratio.

Material and Methods

The work was conducted in accordance with ethical standards and approved by the Ethics and Biosafety Committee of the Universidade Federal de Lavras, Minas Gerais, Brazil, certified by case number 008/12.

The experiment was conducted in Lavras, MG, Brazil, from September to November 2013. The experiment was conducted in a laboratory with a standard recirculating system and water temperature control (27±0.5 °C), using 15 water tanks with a total capacity of 500 L. The mean values of the temperature, pH, and dissolved oxygen parameters were 27.1 °C; 7.2, and 5.9 mg/L, respectively. The limnological parameters were maintained within the range recommended for tilapia breeding (Bhujel, 2000) and are considered suitable for tilapia embryonic development (Rana, 1990).

A total number of 60 breeding individuals of Nile tilapia, UFLA (Universidade Federal de Lavras) strain, that had not previously reproduced, were used, 45 females and 15 males with mean initial weights of 71.71 g and 82.6 g, respectively. This strain underwent genetic improvement programs over the last 25 years (Dias, 2014).

Microchips were implanted in females to identify them during the collection of eggs. The selected males released semen after light cephafalocaudal-directed abdomen massage (Sanches et al., 2009). Males and females were distributed in 15 500-L tanks in a completely randomized design with five treatments and three replicates; the male: female sex ratio used was 1:3 as recommended by Little and Hulata (2000) under aquarium conditions.

The adaptation period of females to males and to the environment was 20 days. During this period the animals received the experimental diet with the lowest energy content: 3,200 kcal of DE (Table 1). After adaptation period, the respective experimental diets were offered for 60 days.

The treatments consisted of five diets (Table 1) that were formulated to contain increasing levels of DE (3,200; 3,400; 3,600; 3,800; and 4,000 kcal of DE/kg of diet) (Table 2) according to the table of ingredient chemical composition, described by Furuya (2010). The digestible energy levels were obtained by increasing the levels of soybean oil in the feed and varying the starch/lipid ratio, with a crude protein content of 380 g/kg, according to Oliveira et al. (2014). During the experiment, fish were fed twice a day (at 08.00 and 14.00 h) until apparent satiation to avoid leftovers and the tanks were cleaned daily to remove excreta.

Every six days, the oral cavity of the females was assessed individually for the collection of eggs. When present, the eggs were gently collected by counter-flow of the oropharynx with the aid of a wash bottle and beaker.

Table 1 - Formulation of the experimental diets

Ingredient (g/kg)	Digestible energy (kcal/kg)				
	3,200	3,400	3,600	3,800	4,000
Soybean meal	680.00	680.00	680.00	680.00	675.00
Fish meal (salmon)	100.00	100.00	100.00	100.00	100.00
Corn meal	103.00	85.00	65.00	50.00	38.00
Wheat bran	35.00	35.00	35.00	30.00	28.00
Alginate	2.00	2.00	2.00	2.00	2.00
Cellulose	4.00	5.00	6.00	7.00	7.00
DL-methionine	3.00	3.00	3.00	3.00	2.00
Soybean oil	20.00	45.00	81.50	105.00	140.00
Dicalcium phosphate	10.00	9.80	9.90	1.50	1.00
Vitamin C[1]	0.60	0.60	0.60	0.60	0.60
Common salt	1.00	1.00	1.00	1.00	1.00
Vitamin/mineral supplement[2]	5.00	5.00	5.00	5.00	5.00
Kaolin	36.20	28.40	12.00	15.00	7.00
Butyl-hydroxy-toluene (antioxidant)	0.20	0.20	0.20	0.20	0.20

[1] Ascorbyl monophosphate with 35% active ingredient.
[2] Guaranteed levels, calculated in this diet, of vitamin and mineral supplements (Mogiana Alimentos S/A - GUABI): vitamin A, 16,000 IU; vitamin D, 4,500 IU; vitamin E, 250 mg; vitamin K, 30 mg; vitamin B1, 32 mg; vitamin B2, 32 mg; vitamin B12, 32 mcg; vitamin B6, 32 mg; vitamin C, 42,000 mg; pantothenic acid, 80 mg; niacin, 170 mg; biotin, 10 mg; folic acid, 10 mg; kaolin, 2,000 mg; cobalt, 0.5 mg; copper, 20 mg; iron, 150 mg; iodine, 1 mg; manganese, 50 mg; selenium, 1 mg; zinc, 150 mg; and antioxidant additive, 150 mg.

Table 2 - Proximate composition of the experimental diets

Proximate composition	Digestible energy (kcal/kg)				
	3,200	3,400	3,600	3,800	4,000
Dry matter	934.6	937.0	935.6	937.5	936.3
Ash	100.3	91.3	78.4	75.5	65.9
Ether extract	51.6	84.5	123.0	142.5	169.7
Crude fiber[1]	48.8	49.2	49.5	49.5	48.8
Crude protein	382.1	380.5	385.0	384.9	381.0
Lysine[1]	2.63	2.63	2.63	2.62	2.60
Methionine[1]	0.84	0.84	0.84	0.83	0.72
Arginine[1]	2.72	2.72	2.71	2.70	2.68
Threonine[1]	1.44	1.43	1.43	1.42	1.41
Tryptophan[1]	0.46	0.46	0.46	0.45	0.45
Linoleic acid (18:2n-6)[1]	1.9	3.2	5.2	6.4	8.2
Linolenic acid (18:3n-3)[1]	0.2	0.4	0.7	0.8	1.1
Linoleic/linolenic[1]	8.25	7.93	7.79	7.73	7.70
EPA (20:5n-3)[1]	1.13	1.13	1.13	1.13	1.13
DHA (22:6n-3)[1]	0.94	0.94	0.94	0.94	0.94
EPA/DHA[1]	1.21	1.21	1.21	1.21	1.21
Arachidonic acid (20:4n-6)[1]	0.07	0.07	0.07	0.07	0.07
Digestible energy (kcal/kg)[1]	3,200.0	3,400.0	3,600.0	3,800.0	4,000.0
DE/CP (kcal DE/g CP)	8.37	8.94	9.35	9.87	10.50
Starch/lipid	3.77	2.23	1.33	1.00	0.73

DE - digestible energy; CP - crude protein; EPA - eicosapentaenoic acid; DHA - docosahexaenoic acid.
[1] Calculated values.

During this procedure, the weight of the females was measured using a digital precision scale (0.01 g). Then, the spawning females were identified by the microchip reader and returned to their respective tanks until the next collection.

The collected eggs were counted and weighed on a digital precision scale (0.001 g) to estimate the relative fecundity (number of eggs per gram of female weight), absolute fecundity (total number of eggs per spawning), weight of spawning, and spawning index (spawning weight per gram of female weight). The number of spawning per tank, average number of spawning per female, and percentage of spawning females during the experiment were also determined at the end of the experiment.

After each spawning, a sample of 15 eggs was collected and fixed in 4% buffered formalin (Eiras et al., 2000) for later morphometric analysis. The remaining eggs were artificially incubated in experimental incubators made of PVC, with a 200-mL capacity in each unit and constant aeration. The temperature in the incubation system was adjusted to 28±0.5 °C.

The absorption of the yolk sac occurred at 5-8 days after hatching. After that, a sample of 15 post-larvae was collected from each incubator and fixed in 4% buffered formalin for subsequent determination of the total length. The morphometric analyses of post-larvae and eggs were performed using a Motic® SMZ 168 stereomicroscope, with a Moticam® camera coupled to Motic Image Plus 2.0 image analysis software, in which the mean diameter of the eggs (longest length + shortest length / 2) and total length of the post-larvae were determined.

At the end of the experiment, all females were euthanized using a 2-phenoxyethanol solution (0.06%) after 24 h of fasting. Then, the fish were dissected and the weights of the liver, gonads, and visceral fat were measured to calculate the following indices:

Gonadosomatic index (GSI) = (weight of the gonads × 100) / fish weight;

Hepatosomatic index (HSI) = (weight of liver × 100) / fish weight;

Visceral fat index (VFI) = (weight of visceral fat × 100) / fish weight.

The collected carcasses were stored at –80 °C for later analysis of the proximate composition (Detmann et al., 2012) of the whole fish.

For the proximate analyses, the samples were pre-dried in a freeze dryer (LIOBRAS, L 202, São Carlos, SP, Brazil) at –50 °C for 72 h. The material was homogenized and the dry matter content was determined after drying for 12 h at 105 °C until a constant weight was reached. The ash was determined by the incineration of the organic components for 5 h at 550 °C using a furnace (LF 0213, JUNG, São Paulo, Brazil). The Kjeldahl method was used for the evaluation of crude protein content (N × 6.25). The concentration of lipids was determined by extraction with ethyl ether in a Soxhlet extraction system.

The data were subjected to analyses of the residuals for normality, correlation, and homogeneity of variance using the Shapiro-Wilk, Durbin-Watson, and Levene tests, respectively. Subsequently, the data were subjected to analysis of variance (ANOVA) followed by the Scott-Knott test ($\alpha = 0.05$ significance). To analyze the percentage of spawning females, the methodology of generalized linear models with binomial distribution was used. All statistical analyses were performed using the R Development Core Team software (2013).

Results

The present study demonstrates that there was no significant effect ($P>0.05$) of the dietary levels of DE on the performance variables of final weight, GSI, HSI, and VFI (Table 3).

The different treatments significantly affected ($P<0.05$) the absolute fecundity of the females, while the other spawning performance variables (weight of spawning, spawning index, relative fecundity, average number of spawning per female, and number of spawning per tank) were not significantly influenced by the diets as well as percentage of spawning females during the experiment period ($P>0.05$) (Table 4). The mean diameter of eggs and total length of post-larvae were not significantly different either.

Females that received diets with DE levels of 3,600, 3,800, and 4,000 kcal displayed a higher absolute fecundity. The lowest values for this variable, 374 and 348, were observed in females fed dietary DE levels of 3,200 and 3,400 kcal, respectively.

The experimental diets significantly affected ($P<0.05$) the variables crude protein, ether extract, and body ashes (Table 5).

Fish fed diets with the lowest energy level showed higher body protein deposition (64.59%) and increased ash content, while fish fed diets with 3,600 kcal/kg DE showed less protein deposition (60.42%). The same treatment also resulted in higher levels of body ether extract.

Table 3 - Means ± standard error of performance parameters of female Nile tilapia (*Oreochromis niloticus*) fed diets with different digestible energy levels

Variable	Digestible energy (kcal/kg)				
	3,200	3,400	3,600	3,800	4,000
Final weight	80.95±2.02	64.50±1.00	75.44±2.56	77.61±4.30	72.65±1.28
Gonadosomatic index	3.51±0.39	2.96±0.50	2.73±0.53	1.86±0.34	2.27±0.57
Hepatosomatic index	1.37±0.06	1.47±0.09	1.39±0.11	1.35±0.18	1.26±0.46
Visceral fat index	0.09±0.005	0.09±0.004	0.09±0.004	0.07±0.005	0.09±0.005

Table 4 - Reproductive performance of female Nile tilapia (*Oreochromis niloticus*) fed diets with different digestible energy levels (mean±SEM)

Variable	Digestible energy (kcal/kg)				
	3,200	3,400	3,600	3,800	4,000
Absolute fecundity	374.02±14.10b	348.50±10.00b	467.82±9.15a	457.33±15.42a	449.32±13.48a
Relative fecundity	5.15±0.42	5.10±0.15	6.32±0.06	5.60±0.30	6.57±0.23
Weight of spawning	5.00±0.63	3.83±0.37	5.70±0.56	4.64±0.57	5.58±1.12
Number of spawning per tank	4.67±1.20	3.00±1.00	3.67±0.88	4.67±0.88	5.33±0.33
Average spawning per female	1.56±0.40	1.00±0.33	1.22±0.29	1.56±0.29	1.78±0.11
Spawning females (%)	100.00	77.77	77.77	88.88	100.00
Spawning index	6.81±0.48	5.48±0.35	7.37±0.21	6.47±0.65	9.43±1.23
Total length of larvae	8.26±0.04	8.06±0.03	8.37±0.10	8.21±0.10	8.43±0.03
Mean diameter of eggs	2.17±0.03	2.05±0.04	2.16±0.04	2.19±0.19	2.21±0.27

Means followed by different letters in the same row are significant (P<0.05) by Scott-Knott test.
SEM - standard error of the mean.

Table 5 - Body composition (% of dry matter) of broodstock fed diets with different digestible energy levels (mean±SEM)

Variable	Digestible energy (kcal/kg)				
	3,200	3,400	3,600	3,800	4,000
Dry matter	17.52±0.89	18.94±0.04	19.87±0.24	19.21±1.62	19.48±0.56
Crude protein	64.59±0.7a	62.35±0.19c	60.42±0.06d	62.99±0.13b	63.19±0.12b
Ether extract	13.82±0.25c	17.71±0.44b	20.26±0.55a	16.72±0.52b	17.06±0.28b
Ash	20.57±0.07a	18.33±0.04b	18.05±0.58b	18.97±0.89b	18.17±0.16b

Means followed by different letters in the same row are significant (P<0.05) by Scott-Knott test.

Discussion

The present study demonstrates that dietary DE levels influence the body composition and fecundity of the female tilapia. Tilapia use large amounts of energy in their reproductive process, including for the aggressive behavior of males, mating, brood care, territorial defense, and mouth-brooding of eggs (El-Sayed and Kawanna, 2008).

Tilapia species can suppress the growth to maintain their reproductive capacity (Coward and Bromage, 1999). If their energy reserves are not sufficient to support these functions, tissue protein is mobilized and catalyzed to be used as an energy source. In the present study, no significant effects were observed regarding female final wejght, because females under reproductive activity route much of their energy reserves to reproduction, not for growth.

The HSI has been used as indicator of the reproductive period being correlated with GSI, since the liver has an important role in reproduction, regarding expressing estradiol receptors to determine the vitellogenin synthesis, which is deposited subsequently in pre-vitellogenic oocytes. No significant effects were observed regarding female body indices (GSI, HSI, and VFI). The effects observed for GSI and HSI corroborate the results reported by Bombardelli et al. (2009), who worked with different energy levels in the diets of female Nile tilapia and also found that there were no significant effects of diet on these parameters. Coldebella et al. (2013) evaluated different levels of soil oil for female catfish (*Rhamdia quelen*) and also observed no differences between treatments for the same variables; however, females fed diets with 140 and 200 g/kg lipids displayed greater accumulations of visceral fat. In contrast to these results, Oliveira et al. (2014) observed an inverse relationship between hepatosomatic and gonadosomatic indices with a 380 g/kg crude protein level in the diet of adult female Nile Tilapia, with an initial average weight of 764 g.

Females fed higher DE levels exhibited higher body ether extract, while protein deposition had the opposite response. The increased energy levels in the diet were obtained by including higher levels of soybean oil and this probably affected female body composition due to the decrease of the starch:lipid ratio.

Proteins and lipids are major components of the diet, are present in the egg, and are used as nutrient sources during embryogenesis. Proteins are present in fish eggs as lipoproteins, hormones, and enzymes, determining the egg quality (Brooks et al., 1997). Lipids, incorporated into the oocyte during vitellogenesis, are from the diet or the maternal body reserves. In this study, we achieved the required energy levels with increase in soybean oil and decrease in carbohydrates in the diets; therefore, the increase in linoleic and linolenic fatty acids in treatments must be considered, since tilapias are able to convert these precursors into highly unsaturated fatty acids: eicosapentaenoic, docosahexaenoic, and arachidonic acids. In the present work, the linoleic:linolenic acid ratio ranged from 8.25 to 7.7. Eicosapentaenoic and arachidonic acids are precursors of eicosanoids, like prostaglandins (Bell et al., 1986; Sargent et al., 1995; Tocher, 2003), which have a large variety of physiological actions in fish, including oocyte final maturation and ovulation (Sorbera et al., 2001; Lister and Van der Kraak, 2008; Planas and Swanson, 2008).

Fecundity is represented by the total number of eggs produced per fish, which can be expressed as the number of eggs per spawning or the relationship between the number of eggs and body weight (Izquierdo et al., 2001). The size of a fish influences fecundity, with larger females producing more eggs per spawning than smaller females, although the number of eggs per spawning is highly variable (Lupatsch et al., 2010). Fecundity can also vary between different strains of tilapia and this variation can also occur within the same species, depending on the farming conditions (Gómez-Márquez et al., 2003). The present results indicated an increase in absolute fecundity with increased DE levels in the diet, which demonstrates that the females fed the highest DE level were able to produce more eggs in each spawning. A similar result was obtained by Hajizadeh et al. (2008), who used a mixture of palm oil and cod oil, changing the ratio of n-3 and n-6 fatty acids, and achieved an increase in absolute fecundity.

The number of clutches per female and per tank were not influenced by the experimental diets or by the relative fecundity. The number of females that spawned during the experimental period was satisfactory, whose lowest percentage (77%) was obtained in the treatments with 3,400 and 3,600 kcal DE, representing seven out of nine females belonging to treatment.

Ng and Wang (2011) compared the inclusion of palm oil with fish oil and linseed oil for Nile tilapia broodstock and found no significant differences in the absolute and relative fecundity. The results for the relative fecundity in this study are close to those observed by other authors (Gunasekera et al., 1996; Hajizadeh et al., 2008; Bombardelli et al., 2009). El-Sayed and Kawanna (2008) obtained improved fecundity by combining the levels of 16.7 MJ (3,988 kcal/kg) of gross energy and 400 g/kg protein in the diet of Nile tilapia; however, fecundity decreased with increased energy content to 18.8 MJ (4,490 kcal/kg), regardless of the protein levels used.

The yolk of the fish larvae is an endogenous nutrient reserve that provides nutrition to the larvae (Mazorra et al., 2003) until they are able to capture and digest the food available in the surrounding environment. The range of the mean egg diameter obtained in this study was from 2.05 to 2.21 mm, which is in accordance with Coward and Bromage (2000), who reported that eggs produced in mouth brooders normally exceed 2 mm in diameter. Similar results were reported by Hajizadeh et al. (2008), who used different sources of oil in the diet of tilapia. Ng and Wang (2011) verified that the utilization of crude palm oil in the diet of tilapia broodstock influenced the egg production, hatchability, and larval normality; however, they found no significant difference between treatments on the mean diameter of the eggs of female tilapia.

The nutrition of broodstock may influence the quality of the offspring because the nutrients of the diets of females are incorporated into the eggs during vitellogenesis and will therefore be reflected in the quality of the post-larvae. However, according to Sousa et al. (2013), little is known about the effect of maternal diet regarding the performance of the progeny after the end of the vitelline reserves. The same authors assessed the development of post-larvae in the sexual reversion phase to observe the effect of different levels of DE and protein in the maternal diet up to 30 days of development and did not find significant effects on post-larval survival and growth. In the present study, no significant difference to the total length of post-larvae was observed and a mean value of 8.26 mm was observed for the total length of the post-larvae. These results are close to those found by Ng and Wang (2011), but they differ from those found by El-Sayed and Kawanna (2008), who alternated the levels of energy and protein in tilapia diets and observed a length of approximately 11.6 mm for the post-larvae.

Conclusions

We recommend the level of 3,600 kcal/kg of digestible energy in diets with 380 g/kg protein and a starch/lipid ratio of 1.33 as the most appropriate diet for female Nile tilapia in this reproductive period.

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