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Diets containing crude glycerin damage the sperm characteristics and modify the testis histology of Nile tilapia broodstock

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ABSTRACT

The effects of crude glycerin in the diets of male Nile tilapia (*Oreochromis niloticus*) on growth, seminal and spermatic characteristics, blood parameters, histological aspects of the hepatic and testicular tissue, and on the chemical composition of different organs/tissues were evaluated. For 10 months, the animals were fed diets containing 32% digestible protein (DP), 3200 kcal digestible energy (DE)·kg ration^{-1} and five inclusion levels of crude glycerin (0, 4, 8, 12 and 16%). Growth, seminal and spermatic parameters were evaluated, and the plasmatic levels of calcium, triglycerides and glucose were measured. Gonadosomatic, viscerosomatic and hepatosomatic indexes were evaluated and combined with the histological parameters of the testicles and liver. Finally, the centesimal composition of the muscles, testis, liver and viscera were evaluated. The volume of semen released, fecundity, effective fecundity and plasmatic levels of glucose decreased proportionally ($p < 0.05$) to the increase of glycerin in the diet. The hepatosomatic index increased proportionally ($p < 0.05$) to the inclusion levels of crude glycerin in the diet. Fish fed on glycerin had the highest protein levels ($p < 0.05$) in the muscle and testicles. Histological assessment suggests that fish fed the lowest glycerin diet had the highest amounts of spermatozoids in the testicles. Other variables under analysis were not affected by diet ($p > 0.05$). The results show that, although crude glycerin does not impair growth, any inclusion level in the diet impairs the spermatogenesis process and damages the sperm characteristics of male Nile tilapia.

Statement of relevance: Do not use the crude glycerin to the Nile tilapia males.

1. Introduction

The breeding of Nile tilapia has become one of the main components of aquaculture worldwide. With a global production of approximately 3.95 million tons in 2011, it ranks second in fresh water fish (FAO, 2014).

The continuous supply of eggs, larvae and fries (both in quality and quantity) is highly relevant to supporting the high rates of tilapia culture growth (El-Sayed, 2006). In this context, studies examining the nutrition and feeding of broodstock (Hardy, 1999; Watanabe et al., 2002) are important because the diet affects the endocrine system, and consequently the reproduction performance of the fish (Watanabe and Vassalo-Agius, 2003), jeopardizing the fecundity (Bromage, 1995; Tyler and Sumpter, 1996) and the quality of gametes, embryos and larvae (Izquierdo et al., 2001). Scanty information exists on the nutrition of broodstock and fish matrixes (Bombardelli et al., 2009), and the majority focuses on females (El-Sayed et al., 2005; Ng and Wang, 2011; Oliveira et al., 2014) with little attention to the males. While it has been widely reported that diet can interfere with the quality of fresh semen and spermatozoa in the same way that it can affect the quality and the viability of spermatozoids after cryopreservation (Putstowka et al., 2000), information on the effects of diet on male reproduction in tilapia broodstock is exceedingly rare. The few extant results on the issue suggest that vitamin C supplementation in the diet improves the quality of spermatozoids (Mataveli et al., 2007) and that the levels of digestible energy in the diet affect spermatogenesis. In fact, diets with approximately 3450 kcal of ED·kg of diet^{-1} result in higher sperm concentrations and higher percentages of spermatozoids without any morphological changes (Bombardelli et al., 2010). Studies regarding feed resources that would trigger growth and reproduction should be undertaken by researchers in animal nutrition.

Byproducts or agro-industrial byproducts are highlighted due to their potential as alternative and low cost ingredients. Due to recent

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interest in the search for renewable energy sources, great importance has been given to the biodiesel industry (Ayoub and Abdullah, 2012), especially with regard to crude glycerin, which corresponds to 10% of biodiesel production (Swiatkiewicz and Koreleski, 2009). Glycerin is an ingredient with feed potential because it is mainly comprised of glycerol, which plays an important role in energy metabolism (Lin, 1977).

Crude glycerin has been successfully employed in swine (Shields et al., 2011; Berenchtein et al., 2010) and broiler chicken (Kroupa et al., 2011) feed. Glycerin may be highly useful in tilapia because it has a digestible energy (DE) similar to corn, corresponding to 3126.5 kcal DE·kg^{-1} (Meurer et al., 2012) or 2875.8 kcal DE·kg^{-1} (Gonçalves et al., 2015). Research suggests that diets containing up to 10% crude glycerin do not affect the growth of channel catfish (*Ictalurus punctatus*) (Li et al., 2010) and Nile tilapia fingerlings (Neu et al., 2012). A recent study showed that diets containing up to 12% crude glycerin did not alter the performance of Nile tilapia juveniles (Gonçalves et al., 2015). The results suggest that crude glycerin may be able to replace corn in these diets.

The current assay evaluates the effects of the inclusion of crude glycerin in the diet of Nile tilapia broodstock (*O. niloticus*) on growth, seminal and sperm characteristics, blood parameters, histological aspects of the hepatic and testicular tissues and on the chemical composition of different organs/tissues.

2. Materials and methods

2.1. Animals, installations and experimental design

The current study complied with the requirements of the Committee for Ethics in Animals of the Universidade Estadual do Oeste do Paraná (CEUA/Unioeste), according to protocol 03212-CEUA/Unioeste. Four hundred females (25.73 ± 0.11 g) and 200 males (25 ± 0.43 g) of the GIFT strain of *O. niloticus* were used during the first maturation age. The females and males were separately penned in forty 1-mm mesh hapas. The hapas were installed in a brick tank with an earthen floor. While females were stocked into twenty 3 m × 2 m hapas at 20 females per hapa, the males were stocked into twenty 2 m × 1 m hapas at 10 males per hapa. All broodstock were marked by electronic markers implanted under the skin. Marks featured ISO FDX-B 134.2 kHz and an external antimigration layer.

The animals were distributed in a totally randomized experimental design with five treatments and four replications. The treatments were comprised of iso-protein and iso-energy diets with 32% digestible protein (DP) and 3200 kcal of digestible energy (DE)·kg of diet^{-1} and five inclusion levels of crude glycerin at 0, 4, 8, 12 and 16% of diet dry weight.

2.2. Experimental diet, feed management and reproduction management

Ingredients were evaluated for their nutritional composition prior to diet formulation. The diets were formulated to contain 32% DP and 3200 kcal DE·kg diet^{-1} (Table 1). The digestibility coefficients of the ingredients (Boscolo et al., 2002; Meurer et al., 2003; Meurer et al., 2012) were used to calculate the digestible protein and digestible energy of the rations. The ingredients were ground in a hammer mill, passed through a 0.5-mm sieve and mixed. Next, heated water at 60 °C was added, and the ingredients were pelleted (3-mm diameter) to produce a sinking ration. Finally, the pelleted ration was dried at 55 °C in a stove with forced ventilation.

The fish were fed twice a day (10:00 h and 16:00 h) over 10 months (Siddiqui et al., 1998; El-Sayed et al., 2005) at a feed rate of 1% biomass per day (Bhujel, 2000), adjusted every 17 days. The fish were submitted to reproduction management during this period, with the males and females separated for a 12-day period (adapted Tacon et al., 1996; Bombardelli et al., 2009). After this period, the males were transferred to the hapas with females for mating for five days (adapted Macintosh and Little, 1995; Bombardelli et al., 2009). These procedures were used to maintain all males in reproductive activity and mating.

Table 1
Composition of feed and nutrient contents (%) of experimental diets at different inclusion levels of crude glycerin used in the feed of matrixes and broodstock of the Nile tilapia (*O. niloticus*).

Ingredients (%)	Crude glycerin (%)				
	0	4	8	12	16
Soy meal[a]	62.58	63.53	64.48	65.43	66.37
Corn[a]	24.05	19.19	14.33	9.47	4.61
Fish flour[b]	5.00	5.00	5.00	5.00	5.00
Soy oil[a]	4.41	4.29	4.17	4.05	3.93
Salt	0.50	0.50	0.50	0.50	0.50
Bi-calcium phosphate	2.44	2.48	2.51	2.54	2.58
Crude glycerin[c]	0.00	4.00	8.00	12.00	16.00
Mineral and vitamin suppl[d]	1.00	1.00	1.00	1.00	1.00
Antioxidant[e]	0.01	0.01	0.01	0.01	0.01
Nutrients[f]					
Calcium (%)	1.04	1.05	1.06	1.07	1.08
Methionine + cystine (%)	1.01	1.00	1.00	0.99	0.99
Crude energy (kcal·kg^{-1})	4250.26	4232.34	4214.41	4196.48	4178.56
Digestible energy (kcal·kg^{-1})	3200.00	3200.00	3200.00	3200.00	3200.00
Crude fiber (%)	2.60	2.37	2.14	1.91	1.69
Total phosphorus (%)	1.00	1.00	1.00	1.00	1.00
Fat (%)	6.41	6.17	5.94	5.71	5.47
Crude protein (%)	35.70	35.72	35.74	35.76	35.78
Digestible protein (%)	32.00	32.00	32.00	32.00	32.00

[a] Digestibility rates of nutrients according to Boscolo et al. (2002).
[b] Digestibility rates of nutrients according to Meurer et al. (2003).
[c] Digestibility rates of nutrients according to Meurer et al. (2012).
[d] Mineral and vitamin supplementation, basal composition: folic acid: 200 mg; pantothenic acid: 4.000 mg; Biotin: 40 mg; Copper: 2.000 mg; Iron: 12.500 mg; Iodine: 200 mg; Manganese: 7.500 mg; Niacin: 5.000 mg; Selenium: 70 mg; Vitamin A: 1.000.000 UI; Vitamin B1: 1.900 mg; Vitamin B12: 3.500 mg; Vitamin B2: 2.000 mg; Vitamin B6: 2.400 mg; Vitamin C: 50.000 mg; Vitamin D3: 500.000 UI; Vitamin E: 20.000 UI; Vitamin K3: 500 mg; Zinc: 25.000 mg.
[e] Propionic acid.
[f] The nutrient contents were calculated using Super Crac Premium® software.

At the end of the mating period, the fish were weighed using a digital scale (Marte® AS2000; 0.01 g) and measured individually by a precision ichthyometer (0.1 cm). Then, the males and females were separated once more in their hapas for another period of isolated rearing. The same procedure was repeated during the entire experimental period.

2.3. Monitoring the physical and chemical parameters of water

The minimum (25.39 ± 1.85 °C) and maximum (28.22 ± 2.06 °C) temperatures in the earthen tanks were measured daily (at 10 h) using a max-min mercury thermometer (±1 °C). Dissolved oxygen (6.49 ± 1.48 mg·L^{-1}; Oximeter YSI® 550A) and water pH (8.01 ± 0.48; digital pH meter Tecnal® Tec 5) were both measured at 6 h fortnightly.

2.4. Seminal and spermatic parameters

In December, after the isolated rearing period and prior to mating, five males of each experimental unit were randomly selected and anaesthetized using a solution of 100 mg benzocaine hydroxide·L^{-1}. The fish were weighed and measured, and their semen was collected for assessment of seminal and spermatozoid counts.

The semen was collected by abdominal massage applied in a cephalic-caudal direction. The first drop of semen was discarded to avoid possible contamination with urine, mucus and feces (Khara et al., 2012). The total volume of semen provided by each male was measured in 1.0 mL syringes, with a precision of 0.01 mL (adapted to Sanches et al., 2013). Seminal pH was immediately measured after collection using the colorimeter method with litmus paper (Merck®) (Tessaro et al., 2012). After collection, the semen was kept under refrigeration (±12 °C) until seminal and spermatic analyses (Kanuga et al., 2012).