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The effect of digestible protein to digestible energy ratio and choline supplementation on growth, hematological parameters, liver steatosis and size-sorting stress response in Nile tilapia under field condition

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ABSTRACT

This study evaluated growth performance, hematological parameters, histological liver analysis, and production costs of Nile tilapia fed increasing levels of digestible protein, digestible energy, and choline. Twelve thousand Nile tilapia (148 ± 6.7 g) were randomly distributed into 80 1 m^3 net cages, in a 5 × 2 × 2 factorial design with five digestible protein (DP) levels (24, 26, 28, 30, and 32% DP), two digestible energy (DE) levels (13.4 and 14.65 MJ DE kg^{-1} diet), and two choline levels (0.0 and 1000 mg kg^{-1} diet), with four replicates per treatment. Fish fed the higher energy level showed a sparing effect of protein; the higher protein level determined the highest fillet yield. Fish fed diets with 24% DP showed the highest liver lipid, and independently of treatment all analyzed fish showed hepatocyte degeneration. The best benefit cost ratio for whole fish production was achieved with 28% DP/13.4 MJ DE kg^{-1}, and for fillet production with 30% DP/13.4 MJ DE kg^{-1}. The results of the hematological assay showed alterations in red blood cells, mean corpuscular volume, albumin, Albumin:Globulin ratio, and glucose after size-sorting stress. Overall, these results indicate a lower resistance to stress, mainly for fish fed with no choline and oil supplementation. In this study we determined that the best performance was achieved with DP:DE ratios of 21.45 g MJ^{-1} (28.74% DP/13.4 MJ DE kg^{-1}) and 18.60 g MJ^{-1} (27.25% DP/14.65 MJ DE kg^{-1}). The highest fillet yield was obtained with 30% DP, regardless of the dietary energetic level. Sustained homeostasis was observed in this setting, and even though size-sorting stress altered some hematological parameters, they were still within the range recognized as healthy. Choline was not effective in protecting the liver against hepatic steatosis, but was able to buffer some of the negative effects of stress under these rearing conditions.

Statement of Relevance: This research has been approved by the Ethics Committee of our Institution. Our team has been working with nutrition and fish health since 2000. According to the NRC (2011), only a few studies have been conducted to estimate the dietary nutrient requirements of farmed fish under intensive culture conditions. Moreover, a number of factors may affect the dietary and nutritional requirements of fish differentially in the laboratory and under intensive culture. For example, climatic conditions may fluctuate widely in the field, directly affecting physiological responses, hence nutritional requirements. Similarly, fish densities are much higher under intensive farming. Establishing the appropriate nutritional requirements in these settings must also consider the trade-off between growth performance and production costs. Based on this, we investigated the effect of different levels of digestible protein and digestible energy on growth performance and hematological responses of the Nile tilapia in a commercial fish farm in Brazil, where fish farmers are known to use diets with excessive levels of crude protein throughout fish culture and hepatic steatosis is often reported. Therefore, we also investigated the potential hepatic protective effect of choline against such conditions. Finally, we analyzed the health status, as measured by hematological parameters, of fish subjected to handling-induced stress procedures that are usual on fish farms.

1. Introduction

Dietary protein is one of the main factors that influence fish production and nitrogen waste into the water which, in excess, may impair fish

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growth and water quality under intensive fish farming (Mires et al., 1990, Tibbetts et al., 2000). Previous research conducted under laboratory conditions has estimated a dietary requirement of 25% (El-Sayed and Gaber, 2005) and 27.5% crude protein (Balarin and Haller, 1982) for tilapia. According to the NRC (2011), however, digestible protein to digestible energy ratio is a more rational way of expressing protein requirements. This is because dietary energy levels are also tightly linked to nutrient consumption. If energy is available in excess, it may impair nutrient consumption, whereas when in deficit protein can be catabolized as energy source (NRC, 2011). For the Nile tilapia, previous research conducted in the laboratory has determined optimal digestible protein:digestible energy ratios ranging from 21.4 to 32.7 g MJ^{-1} depending on fish size (Trung et al., 2011). However, the digestible protein:digestible energy ratio has not been evaluated under field and stress conditions.

According to the NRC (2011), only a few studies have been conducted to estimate the dietary nutrient requirements of farmed fish under intensive culture conditions. Studies developed in these conditions are needed especially considering the constant environmental stress that fish are subjected to in intensive commercial settings. Moreover, a number of factors may affect the dietary and nutritional requirements of fish differentially in the laboratory and under intensive culture. For example, climatic conditions may fluctuate widely in the field, directly affecting physiological responses, hence nutritional requirements. Similarly, fish density is much higher, and fish management, such as sorting, is also more severe under intensive farming. Therefore, the nutritional demands of these fish are likely much higher, and may impair performance and immune responses, and increase susceptibility to pathogens when not met. On establishing the appropriate nutritional requirements in these settings the trade-off between growth performance and production costs must also be considered.

Several metabolic syndromes, such as hepatic steatosis, have been associated with nutritional disorders. Since choline plays an important role on the maintenance of cell structure, lipid transportation and phosphatidilcholine precursor, which is responsible for hepatic steatosis prevention, its dietary inclusion is necessary since fish cannot synthesize this compound in adequate amount (Ketola, 1976; Halver, 2002; McDowell, 1989; NRC, 2011).

In this study we investigate the effect of different levels of digestible protein and digestible energy on growth performance and hematological responses of the Nile tilapia in a commercial fish farm in Brazil, where fish farmers are known to use diets with excessive levels of crude protein throughout fish culture and hepatic steatosis is often reported. Therefore, we also investigated the potentially protective effect of choline (Ogino et al., 1970; Osol et al., 1982; Wilson and Poe, 1988) against hepatic steatosis, a recurrent problem in fish raised under intensive conditions. Finally, we analyze the health status, as measured by hematological parameters, of fish subjected to size-sorting stress procedures that are typical of commercial practice.

2. Materials and methods

The study is made of two phases. In phase I, fish received diets with different levels of digestible protein, digestible energy and with or without supplemented choline. Growth performance, fillet yield, visceral fat, liver lipid concentration and economical analyses were evaluated. In phase II, we compared fish physiological condition considering their health status, before and after sorting-induced stress.

2.1. Study area (phases I and II)

This study was developed to evaluate the growth performance, physiological response, and production cost of Nile tilapia in net cages fed diets containing levels of digestible protein, digestible energy, and choline for 119 days in the autumn and winter (9 March to 5 July). The research was carried out in the Fernandes Fish Farm, Canoas II reservoir, São Paulo State, Brazil (22°56′41.47″ S and 50°10′39.06″ W). This reservoir is located between São Paulo and Parana State. The water average temperature is 23.3 ± 7 °C, with maximum and minimum temperatures of 30 and 16 °C, respectively.

2.2. Fish and experimental procedure (phase I)

Fish were stocked into ten 6 m^3 (2.0 × 2.0 × 1.5 m) net cages at a density of 2300 male Nile tilapia (Supreme Tilapia strain) per net cage during 25 days, in order to adapt to experimental conditions. Then, twelve thousand male Nile tilapia juveniles with an initial body weight of 148 ± 6.7 g (mean ± SD) were randomly stocked into 80 1 m^3 net cages (1.0 × 1.0 × 1.0 m) at a density of 150 fish per net cage (Ridha, 2006). The experimental design was a 5 × 2 × 2 factorial design with five digestible protein (DP) levels (24, 26, 28, 30, and 32% DP), two digestible energy levels (DE) (13.4 and 14.65 MJ DE kg^{-1} diet), and two choline levels (Cho) (0.0 and 1000 mg kg^{-1} diet) totalizing 20 treatments. All treatments were randomly distributed among net cages with four replicates per treatment. Fish were hand-fed two or three times daily, depending on the water temperature. Briefly, the amount of feed was previously weighed, and then distributed to each net cage using a boat. The feeding management took about 40 min, in which the mortality was recorded.

At the beginning of the experiment the water temperature was 24 °C (9 March); it gradually decreased to 21 °C (4 May), reached 17 °C on 4 June, and maintained until the end of the experiment (5 July, 2011). Therefore, the average water temperature was 20.5 °C, which is considered below ideal temperature for optimal growth and health (Lim and Webster, 2006). The amount of diet offered during the feeding trial considered the water temperature, as recommended for Nile tilapia (Lim and Webster, 2006). At above 21 °C fish were fed three times daily, and at lower temperatures twice daily. The average amount of feed fed to the fish was the same regardless of treatment (133.6 kg per net cage).

2.3. Water quality (phase I)

Water temperature, pH, dissolved oxygen (DO), dissolved oxygen saturation (DOS), total dissolved solids (TDS), and total ammonia were measured every morning through a multi probe system (YSI Environmental, Yellow Spring, OH, USA). The average water temperature was 20.5 °C ± 3.5 and other water quality parameters were: pH 7.25 ± 0.2; DO 7.23 ± 0.2 mg L^{-1}; DOS 76.0% ± 5.0; TDS 0.63 ± 0.6 mg L^{-1}; total ammonia 0.06 ± 0.01 mg L^{-1}; Secchi depth 3.67 ± 0.5 m; and cyanobacteria concentration <0.1 ppb. These values were within the comfort range for the species (Boyd, 1996).

2.4. Growth performance parameters (phase I)

At the end of the experimental period (119 days) final biomass (FB), specific growth rate (SGR), feed conversion ratio (FCR), visceral fat (VF), and fillet yield (FY) were determined.

Where:

$$\text{FB (g)} = \text{weight of all fish in the net cage;}$$
$$\text{SGR}\left(\%\text{day}^{-1}\right) = (\text{Ln final weight–Ln initial weight/total no.of the experimental days}) \times 100$$
$$\text{FCR} = \text{dry feed intake (g)/wet weight gain (g);}$$
$$\text{VF (\%)} = [\text{visceral fat weight (g)} \times 100]/\text{fish weight (g); and}$$
$$\text{FY (\%)} = [\text{weight of the fillet (g)} \times 100]/\text{fish weight (g).}$$

2.5. Diets

Two diets were formulated to contain 24 and 32% of DP with 13.4 and 14.65 MJ DE kg^{-1}. Others diets were obtained by the dilution