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Interaction between dissolved oxygen concentration and diet composition on growth, digestibility and intestinal health of Nile tilapia (*Oreochromis niloticus*)

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ABSTRACT

The present study was undertaken to evaluate the individual and combined effects of oxygen concentration and diet composition on the growth, nutrient utilization and intestinal morphology of Nile tilapia (*Oreochromis niloticus*). Two recirculating aquaculture systems were used to create the difference in oxygen concentration: normoxia (6.9 mg·L^{-1}) and hypoxia (3.5 mg·L^{-1}). Two diets were formulated using a different soybean meal (SBM) content to create a contrast in the potential to affect the gut barrier function. Triplicate groups of 35 fish with initial mean body weight of 23 g were fed "Control" diet containing 20% fish meal and "Test" diet containing only plant protein source at normoxia and hypoxia for 8 weeks. Six fish per treatment were sampled for intestinal morphological analysis at the end of week 1, 4 and 8. The proximal, middle and distal intestine were processed for quantitative histology, in order to count goblet cells (GC) and eosinophilic granulocytes (EG); and to measure the thickness of lamina propria (LP) and sub-epithelial mucosa (SM). The study showed that growth was best in the "Control" diet under normoxia, while no interaction between oxygen and diet composition was found. Hypoxia reduced nutrient digestibility significantly ($p < 0.05$). For the "Test" diet, the decline in digestibility was larger than for "Control" diet over time. Both diet composition and oxygen level induced changes in intestinal morphology of Nile tilapia. We observed a thickening of the LP and SM caused by an increased infiltration of inflammatory cells, and an increased number of GC and EG among the enterocytes. The negative effect of increased soybean meal on intestinal morphology was enhanced at low oxygen level and aggravated in time. The SBM enteritis-like symptoms were more pronounced in the proximal than in the distal intestine of Nile tilapia.

Statement of relevance: Too many nutritional papers focus only on the growth response of fish. Here we describe effects of diet composition and oxygen on growth, digestibility and intestinal health. Modern aquaculture can benefit from this holistic approach.

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1. Introduction

Gut barrier function is vital for gut health and maintaining the general health of the fish (Jutfelt, 2011; Sundh, 2009). The gut acts as a physical and chemical barrier providing the first line of defence against invading organisms entering the body via consumed feed or through ingestion of water (Cain and Swan, 2010). Impairment of the gut barrier function enables an increased exchange of materials between gut lumen and body in two ways: paracellularly through the tight junctions or transcellularly through transcytosis which enhances the susceptibility to bacterial infection. The gut barrier function is influenced by several factors like dietary composition, environmental challenges, gut microbial population and immune functioning of the fish (Jutfelt, 2006).

In intensive rearing of salmonids, fluctuating concentrations of dissolved oxygen (DO) is known to create prolonged stress disrupting the tight junctions in the intestinal wall, therefore increasing the permeability (Olsen et al., 2002; Sundh et al., 2010). Paracellular permeability increased in both the proximal and distal intestine of Atlantic salmon (*Salmo salar*) subjected to 50% DO saturation (Sundh et al., 2010; Niklasson et al., 2011). Several reports were published on the reactions of fish to hypoxia in terms of growth, digestibility and oxygen consumption such as blue tilapia (*Oreochromis aureus*) (Papoutsoglou and Tziha, 1996), Nile tilapia (*Oreochromis niloticus*) (Ishibashi et al., 2002),

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European sea bass (*Dicentrarchus labrax*) (Thetmeyer et al., 1999) and juvenile turbot (*Scophthalmus maximus*) (Pichavant et al., 2000). The effects on the gut barrier function were not analysed. In general, data on impacts of oxygen concentration on gut barrier function in other fish species than salmonids are lacking.

Also dietary composition is associated with an impaired intestinal barrier (Jutfelt, 2011). In salmonids, for example, plant proteins and especially soybean meal cause histological, morphological and functional changes in the gastrointestinal tract. These changes may widen the lamina propria and increase the presence of inflammatory cells (a mixed cell population, including lymphocytes, macrophages and polymorphonuclear leucocytes) (Baeverfjord and Krogdahl, 1996); induce enteritis in the distal intestine (Urán et al., 2008b; Urán et al., 2009); and shorten villi and microvilli (van den Ingh et al., 1991). Soy saponins in combination with other plant protein ingredients are suspected to be the possible inducers of this intestinal inflammation (Knudsen et al., 2008). Some of these changes have also been found in species other than salmon, e.g., common carp (*Cyprinus carpio*) (Urán et al., 2008a), rainbow trout (*Oncorhynchus mykiss*) (Nordrum et al., 2000) and Gilthead sea bream (*Sparus aurata*) (Bonaldo et al., 2008), summer flounder (*Paralichithys dentatus*) (Bone, 2013). In Nile tilapia, a recent study suggested a mild enteritis after feeding a diet with 43% soybean meal (Mahmoud et al., 2014).

In the present work, it is hypothesized that one single stressor might have a minor impact on the intestinal morphology and digestion whereas the impact might increase when two stressor are combined. In the current study, the combined impact of oxygen stress and a dietary challenge on digestion and intestinal morphology was examined. Moreover, these impacts was assessed over time.

2. Materials and methods

2.1. General design

The experiment was conducted at the Fisheries Faculty of Nong Lam University, Ho Chi Minh city, Vietnam. Juvenile all sex reversed male Nile tilapia (*Oreochromis niloticus*) were obtained from a local hatchery. A 2×2 factorial design was used to evaluate the effects of diet and dissolved oxygen (DO) concentration in water on growth, digestibility and intestinal morphology of tilapia. Two diets were formulated and tested in triplicate tanks under normoxia and hypoxia conditions. To study for temporal effects on digestibility and intestinal morphology, faeces and intestinal tissues were collected and analysed at different time points (after 1, 4 and 8 weeks). The experiment lasted for 56 days.

2.2. Housing

Two identical recirculating aquaculture systems were used to create differences in DO concentration. Each system, was composed by a large water tank (1 m^3) connected to six cylindrical tanks (150 L) holding the fish. Each cylindrical tank was equipped with a small bio-filter. Water was pumped from both the water storage tanks via the small bio-filters to the fish tanks (total $N = 12$). Each tank was stocked with 35 tilapia with an initial mean body weight of 23 ± 0.3 g.

In the normoxia treatment, the DO concentration was aimed to be closed to 100% saturation. Saturation was achieved by 1) aerating water in the water storage tank with air stones before supplying the fish-tanks; and 2) adding air stones inside each fish-tank. In the hypoxia treatment, the aeration was reduced to reach approximately 50% saturation in the storage tank by adjusting the output of air stones, and the fish-tanks were not aerated. DO concentration was measured daily using an oxygen meter (MW600 model, Milwaukee Instruments Inc., Rocky Mountain, NC, USA). The measured DO concentration inside the fish-tanks at the normoxia and hypoxia treatments were 80% (6.9 ± 0.2 mg L^{-1}) and 40% (3.5 ± 0.4 mg L^{-1}), respectively. During the trial, the water temperature was at 27 °C and pH at 6.7. Total ammonia nitrogen (TAN) was remained below 2 mg L^{-1} in both systems. TAN was measured with a Sera kit (SERA GmbH, Heinsberg, Germany) and pH with a pH meter (digital mini-pH meter, BASF, Kuantan, Malaysia). The rate of system water renewal was calculated based on TAN and DO concentration. One third of volume of the storage tank was renewed if TAN was above 2 mg L^{-1} and/or DO concentration below 6 and 3.5 mg L^{-1} at the normoxia and hypoxia, respectively. Minimally, every three days, water was added to compensate water losses due to faces collection and evaporation.

2.3. Diets

Two diets were formulated using a different soybean meal content to create a contrast in the potential effect on the gut barrier function. The "Control" diet contained 20% fish meal and the "Test" diet contained only plant proteins. Both diets were formulated to be isoproteic and isolipidic. In both diets, the amino acids and other nutrients were exceeding the minimum requirements of Nile tilapia (NRC, 2011). Extruded pellets (2 mm diameter) were produced and the marker Cr_2O_3 was added to the diets at 1.0 g kg^{-1} to measure digestibility coefficients. Formulation and diet analyses are shown in Table 1.

2.4. Experimental procedures

Fish were fed initially with the same amount of feed corresponding to 3% of body weight per day; twice daily at 9:00 h and 16:00 h. In case of fish refusing feed in any tank, the feeding of all tanks was reduced to prevent uneaten feed. The trial lasted for 8 weeks, feed intake, growth, feed conversion ratio and survival were assessed at the end. Feed intake was adjusted every 2 weeks.

Faeces were collected twice a day, prior to feeding, using sedimentation columns as described by Cho et al. (1982). Faecal collection bottles were placed in a thermostatic box with ice to avoid the bacterial degradation of nutrients in faeces. To follow the changes in digestibility over time, the faeces collected during week 1, 4 and 8 were kept separately and stored at −20 °C until analysis.

Table 1
Formulation and nutrient content of experimental diets.

	Diets	
	Control	Test
Ingredient (%)		
Fish meal	20	–
Soybean meal	21.3	54.5
Rice bran	18	10
Distillers dried grains with soluble	20	10.7
Cassava	13	13
Fish oil	0.5	0.5
Soybean oil	2.2	4.3
DL-Methionine	1.0	1.0
Dicalcium phosphate	1.0	3.0
Vitamin and mineral premix[a]	2.0	2.0
Chromic oxide	1.0	1.0
Analysed nutrient content on DM basis (g kg^{-1})		
Dry matter (DM; g kg^{-1} diet)	924	930
Crude protein	302	314
Crude fat	81	86
Crude fiber	44	45
Total carbohydrates[b]	517	518
Ash	100	82
Phosphorus	11	10
Chromic oxide	16	15

[a] Vitamin and minerals premix (per kg of feed): contain vitamin A 40,000 IU; D_3 9600 IU; E 300 mg; C 700 mg; K_3 60 mg; B_1 54 mg; B_2 64 mg; B_6 64 mg; niacine 96 mg; pantothenic acid 132 mg; choline 60% 800 mg; Fe 259.2–336.8 mg; Cu 43.2–52.8 mg; Zn 1060–1540 mg; Mn 216–264 mg; Co 0.44–0.52 mg; I 17.28–21.12 mg; Se 2.16–2.64 mg; folic acid 20 mg; biotin 1 mg; inositol 192 mg; carrier.

[b] Calculated as, total carbohydrates = 1000 − (crude protein + crude fat + ash).

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