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Effect of dietary lipid content and stocking density on digestive enzymes profile and intestinal histology of rainbow trout (*Oncorhynchus mykiss*)

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ABSTRACT

The effect of both dietary lipid level and stocking density on digestive function was tested in young rainbow trout under culture conditions. Two dietary lipid levels (14 and 33%) and three culture densities (15, 30 and 40 kg/m^3) were assayed in triplicate lots: L14/D15, L14/D30, L14/D40, L33/D15, L33/D30 and L33/D40. Intestinal activities of amylase, lipase, trypsin, chymotrypsin, total protease (at different pHs) as well as some biometric parameters such hepatosomatic index (HSI) and digestivesomatic index (DSI) as indicative of perivisceral fat accumulation, were determined after a 12-weeks experimental period. A histological study of intestine was carried on to evaluate changes in tissue architecture. Fish fed on higher lipid diets grew more and accumulated a higher amount of fat in perivisceral tissue, although higher stocking density reduced this trend. According to digestive capacity, higher lipid intake induced an activation of lipase activity that was not apparent when fish were held under the highest rearing density. By other hand, dietary lipids promoted a significant inhibition of protease activity in fish under high density conditions. These results seem to indicate a clear interaction between both variables. In this study, it was manifested a general effect of dietary lipids level and culture density on digestive profile. These interactions should be taken into account when foreseeing the digestive utilization of a commercial feed at different culture conditions to improve fish production.

1. Introduction

Nowadays fish welfare is one of the main aspects to consider in aquaculture both for the increasing importance of this industry and for the social awareness of animal health. The concept of animal welfare is not objectively easy to define (Ashley, 2007; Lymbery, 2002). According to North et al. (2006) "welfare represents the physical and mental state of an animal in relation to its environment. This state is reflected in their health, quality of life and freedom from suffering". Therefore it could be assumed that physical health is the most universally accepted measure for welfare.

Then, how to assess fish health? In order to answer this question there are many studies that evaluate different aspects related to fish intake, growth performance, hormonal and metabolic changes. It is not difficult to conclude that these parameters are affected by sustained compromised conditions and factors such stocking density, an inappropriate diet, feeding techniques, handling, transportation and water quality (Ashley, 2007).

Rearing density is a main factor to be considered in the aquaculture industry since involves intensive fish culture systems. This condition tends to increase as the fish growth or to decrease after grading process (Ashley, 2007; Ellis et al., 2002). How inadequate density can affect fish welfare remains controversial, and its effects have been related to water quality and social interactions in some species (Ellis et al., 2002). Some of the variables used to measure the influence of high stocking density are related to parameters related to chronic stress response. Several studies reported an affect of density on cortisol and thyroid hormones (Di Marco et al., 2008; Fatima et al., 2018), metabolic activity (Suárez et al., 2015) feed intake, growth rate, immune system and reproduction (Lupatsch et al., 2010; Merino et al., 2007) as well as survival, fin erosion and abnormal behaviour (Martins et al., 2012; Schram et al., 2006). These alterations can finally lead to a decreased fillet quality

Abbreviations: BTEE, *N*-benzoyl-L-tyrosine ethyl ester; CNP, 4-chloro-2-nitrophenol; CNPG3, 2-chloro-4-nitrophenol-α-D-maltotrioside; BAPNA, Nbenzoyl-DL-arginine-4-nitroanilide; PAS, Periodic Acid Schiff; TA, taurocholic acid

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(Suárez et al., 2014).

Stocking density influence on digestive enzyme activities has been previously studied (Bolasina et al., 2006) and despite recent results in *Anguilla marmorata* stated an affected growth, a relevant effect on digestive activity was not reported (Tan et al., 2018). Impaired digestive capacity can be a cause of decreased growth of fish under stressing conditions. In this sense, the use of suitable diets that can enhance digestive capacity under certain rearing conditions would be a useful tool to improve animal culture conditions, which ultimately would ensure a product of higher quality.

Protein is essential to ensure a suitable fish performance, but a dietary overload can lead to an oxidation of excess amino acids with associated costs of nitrogenous waste production (Wu and Gatlin III, 2014) that can impair environmental water quality. Dietary lipids have a key role in fish nutrition, but the presence of commercial diets with high lipid concentration has generated controversy among fish farmers. On one hand they are cheaper, more environmental friendly and according to previous studies their supplementation improves efficient utilization of protein (Kaushik and Medale, 1994). But, on the other hand, several studies have revealed that high dietary lipid levels can increased lipid deposition affecting to body composition, hepatosomatic and visceral somatic index (Hansen et al., 2008; Wang et al., 2005). What is more, the location and composition of fat deposits could affect the flavour, nutritional qualities and shelf life of fish fillets (Mohanta et al., 2008; Suárez et al., 2014). In this sense, optimization of protein/lipids levels in diets should be essential to assure the quality of the production and animal welfare.

The ability to process feed has to be taking into account when replacing nutrients in fish diets. This depends on the enzymatic profile of the digestive tract and the adaptive traits of these enzymes to nutritional changes (Murashita et al., 2015). In this sense, different studies stated the influence of dietary content on digestive profile. Ducasse-Cabanot et al. (2007) reported in rainbow trout fed on diets without fish oil, a decrease on lipase activity of digestive extracts and in others enzymes, such peptidases and alkaline phosphatase, associated to brush-border membrane of enterocytes. Also, Rungruangsak-Torrissen et al. (2009) showed an activation of trypsin and chymotrypsin specific activity in pyloric caeca of rainbow trout fed on diets with high protein content. Studies on the digestive capacity of the fish should be considered in dietary formulation because they provide complementary information on nutritional requirements that differ according to species (Iqbal et al., 2016; Zeng et al., 2016).

Regarding these aspects, the aim of this study is to evaluate the possible effects of dietary lipid content on the main digestive enzymes activity (total proteases, trypsin, chymotrypsin, lipase and amylase) in rainbow trout (*Oncorhynchus mykiss*) under different density conditions. All focused to get a better understanding of how digestive profile could be modulated by dietary composition in fish under culture conditions similar to fish farming.

2. Material and methods

2.1. Fish and Experimental design

The study was carried out in rainbow trout (*Onchorynchus mykiss*) one-year-old (100 ± 10 g initial mean weight ± standard error of mean, SEM) from a fish farm (Piscifactoría Caviar de Riofrío S.L., Riofrío, Granada, Spain, 37° 9′ 0″ N, 4° 12′ 0″ W). Two dietary-lipid levels (14% and 33%) and three culture densities (15 kg/m^3, 30 kg/m^3 and 40 kg/m^3) were studied. According to this, six experimental groups were assayed: L14/D15, L14/D30, L14/D40, L33/D15, L33/D30 and L33/D40. Animals were distributed into 18 tanks, being the six treatments assayed in triplicates. At the end of 12 weeks-long period, preceded by an acclimation of 15 days, fish were randomly extracted from each tank for biometric, histological and digestive parameters determination as described below.

Table 1
Proximate composition of experimental diets.

Proximate composition (g/kg)	L14	L33
Dry matter	978	954
Crude protein	455.3	453.7
Crude lipid	136.9	329.9
Ash	75.5	61.0
Nitrogen-free extract (NFE)[a]	332.3	155.3
Gross energy (MJ/kg diet)[b]	22.2	26.8
P/E ratio (g protein/MJ)	20.5	16.9

[a] Calculated as 1000-crude protein (g/kg) - crude lipid (g/kg) - ash (g/kg).
[b] Calculated on the basis of 24.3, 39.7 and 17.2 kJ/g of protein, lipid and NFE, respectively.

2.2. Fish maintenance and diets

The farm operated in a flow-through system supplied with high-quality well water. Fish were reared in outdoor tanks (1 m diameter, 0.60 m depth, 400 L effective volume) continuously supplied with 1 L/min of water; 14 ± 0.4 °C; dissolved oxygen never dropped below of 7.0 ± 1.4 ppm; pH 7 ± 0.5, total ammonia nitrogen 0.2 ± 0.1 mg/L).

Two commercial isoprotein extruded pellets diets (closed-formulae) used were based on fish meal and oil, soy protein and oil, wheat meal and gluten, pea meal, vitamins and minerals, according to the manufacturer (Table 1). Both diets differed in fat content (14% and 33%) and in the amount of nitrogen-free extract, total energy content, and therefore in P/E ratio. The diet with higher lipid content has a greater vegetable-oil fraction than in lower lipid content one (15%).

Fish were fed twice a day (seven days a week) with a total daily ration of 1% body weight. Every 3-week period, the fish were weighed to adjust culture density and ration size.

2.3. Sampling and samples treatment

At the end of the experimental period, after 24 h food deprivation, fish were quickly sampled at random from each tank (eight per tank and twenty-four per treatment) and killed according to the regulations of the Directive 2010/63/EU (overdose of metacaine, approved killing protocol). Animals were weighed in a portable scale (Sartorius) and immediately afterwards, liver and digestive tract were carefully removed and weighed. Digestive tracts were frozen in liquid nitrogen and stored at −80 °C until enzymatic activities analysis. After defrosting, peridigestive fat was careful removed and each complete digestive tract was homogenized in ice cold buffer (100 mM Tris–HCl, 0.1 mM EDTA, 0.1% triton X-100, pH 7.8) at a ratio 1:4 (w/v) with an electric homogenizer (Heidolph Instruments). Homogenates were centrifuged at 30000 ×*g* for 30 min at 4 °C (Sigma model 3 K30 centrifuge). After centrifugation, the supernatant was removed and frozen at −80 °C for further analysis.

2.4. Analytical procedures

The chemical composition of the diets was determined following AOAC (2004) standard procedures: water content by desiccation in an oven at 105 °C until constant weight; ash by incineration in a muffle furnace at 450 °C for 16 h; crude protein by the Kjeldhal method (crude protein = N × 6.25) and total lipid extraction by Soxhlet's method.

The α-amylase (EC 3.2.1.1) activity was assayed with a commercial kit (Chemelex, S.A., 08420, Barcelona, Spain) by measuring the generation of 4-chloro-2-nitrophenol (CNP) from the hydrolysis of 2-chloro-4-nitrophenol-α-D-maltotrioside (CNPG3) at 405 nm and 37 °C. Lipase (EC 3.1.1.3) activity was measured according to Faulk et al. (2007), being the enzymatic reaction mixture 0.35 mM 4-nitrophenyloctanoate as substrate in 0.5 mM Tris–HCl, 6 mM sodium taurocholic acid (TA), 1 M NaCl pH 7.4 buffer. Nitrophenol generation