Aquacultural Engineering 67 (2015) 59–66

Contents lists available at ScienceDirect

Aquacultural Engineering

journal homepage: www.elsevier.com/locate/aqua-online

Interaction of dietary energy levels and culture density on growth performance and metabolic and oxidative status of rainbow trout (*Oncorhynchus mykiss*)

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ARTICLE INFO

Article history:
Received 22 November 2014
Received in revised form 17 June 2015
Accepted 17 June 2015
Available online 22 June 2015

Keywords:
Culture density
Dietary energy
Growth
Fish
Intermediary metabolism
Oncorhynchus mykiss
Rainbow trout
Oxidative stress
Welfare

ABSTRACT

Rainbow trout (100 g initial weight) were subjected to the combined effect of two culture densities (15 and 40 kg m^{-3}, D15 and D40, respectively) and two dietary energy levels (22 and 27 MJ kg^{-1} E22 and E27, respectively) during a 75-days experimental period. At the end of the experiment, the growth rate as well as the metabolic and oxidative status of liver and muscle of fish were studied.

The results showed that combination of culture density and dietaryenergy level negatively affected growth, cholesterol and LDL plasma levels and oxidative stress in muscle. Higher culture density negatively affected the values of total protein, triglycerides, and HDL in plasma, values of hepatic and muscular metabolic activities pyruvate kinase (PK), citrate synthase (CS), and hydroxiacil-CoA dehydrogenase (HOAD); glutamate pyruvate transaminase (GPT) and glutamate oxaloacetate transaminase (GOT) activities in plasma, liver, and muscle; glucose 6P dehydrogenase (G6PDH) activity in muscle; and oxidative stress in liver.

High energy intake, adversely affected the hepatic activity of G6PDH, HOAD, GPT and oxidative stress in muscle.

Consequently our results indicate that a combination of high culture density and a high level of dietary energy (27 MJ kg^{-1} in diet) exert a negative impact on the physiology and consequently on the welfare of the farmed fish.

1. Introduction

Rainbow trout is one of the main fish species in aquaculture; Spain holds the 19th place in world ranking of aquaculture producers, and rainbow trout accounts for 99% of the country's freshwater aquaculture production (MARM, 2011).There have been major technological advances in the rainbow trout industry resulting in the production of fish with significant amounts of high-quality lean muscle (Cohen et al., 2005).

However, intensive fish-farming practices often cause stress and poor health in the fish. The improvement of rearing conditions would benefit fish welfare as well as aquaculture profitability (Ashley, 2007). Aspects such as water quality, culture density, feeding, nutritional conditions, and management procedures all directly influence fish stress levels (Conte, 2004; Ashley, 2007).

The effect of culture density on fish welfare is species dependent; high densities are appropriate in those species that live naturally in large tanks, while for territorial species low densities are preferred (Ashley, 2007).

In most cultured fish species, high density stunts growth, reduces food intake, and lowers food-conversion efficiency (Boujard et al., 2002). These results are attributed to social interactions by competition for food and/or space (Narejo et al., 2005), leading to the establishment of hierarchies (Trenzado et al., 2007). Excessive high densities can raise the incidence of physical injuries, stress, and susceptibility to disease; can alter swimming behaviour; and can intensify aggression (Anras and Lagardere, 2004). Experimental evidence indicates that water quality is the key factor in relation between culture density and fish welfare (Ellis et al., 2002).

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http://dx.doi.org/10.1016/j.aquaeng.2015.06.001
0144-8609/

There is considerable evidence for decreased welfare associated with high culture densities in rainbow trout *Oncorhynchus mykiss* (Ellis et al., 2002); however, excessive aggressive behaviour and poor feeding response may also occur when trout are held at very low densities (Ellis, 2002; Ellis et al., 2002).

Diet composition can also affect fish health and welfare. High-energy diets are advantageous for reducing the protein level in the feed (Sargent et al., 2002), decreasing nitrogen and phosphorus loads from high-protein diets, and improving feed-conversion ratios (Chaiyapechara et al., 2003). However, high levels of dietary energy lead to greater fat deposition in the entire body of fish (Chaiyapechara et al., 2003), increasing fish susceptibility to lipid oxidation (Rueda-Jasso et al., 2004). Also, previous studies in rainbow trout displayed a synergistic effect between dietary-energy levels and stocking density (Trenzado et al., 2007).

Under stressful conditions, the metabolic rate of fish can be accelerated in order to cover higher energy demands (Lupatsch et al., 2010) involving an activation of oxidative processes which in turn augment the production of reactive oxygen species (ROS, Guerriero et al., 2002). In this sense, the metabolic and oxidative status of fish may be altered if culture density is inappropriate. It has been verified that the fish intermediary metabolism is affected by factors such as physical activity (Lushchak et al., 2001), confinement (Trenzado et al., 2003), dietary composition, and energy content (Suárez et al., 2002). Also intermediary metabolism and oxidative status is affected by food deprivation in several fish species (Furné et al., 2009a,b).

Although influence of dietary energy level and culture density on growth and metabolic activities of cultured fish has been reported in the literature, the effect of both parameters at once hasn't studied. This study examines the combined effect of the dietary level of energy and culture density on growth, metabolic status, and oxidative status of rainbow trout. The final aim is to assess whether both stressful fish-farming practices at once or separately influence fish physiology.

2. Materials and methods

2.1. Fish and experimental design

Rainbow trout (*O. mykiss*) one year old (100 g approx. initial weight, 18 cm approx. initial total length) (Table 2), from a fish farm (Piscifactoría Caviar de Riofrío S.L., Riofrío, Granada, Spain, 37°9′0″N, 4°12′0″W) were used. The farm was operated in a flow-through system supplied with high-quality well water.

The fish were distributed into 12 outdoor circular tanks (1 m diameter, 0.60 m depth, 400 l effective volume) continuously supplied with 30 l min^{-1} of water. Quality characteristics of inflow water were within values: temperature 14.1–14.4 °C; dissolved oxygen 7.0–8.9 mg l^{-1}; pH 6.8–7.5; Total ammonia nitrogen 0.1–0.3 (mg l^{-1}).

Two variables were considered together: dietary energy level (DEL), where 22 MJ kg^{-1} (E22) and 27 MJ kg^{-1} of energy (E27) where tested; and culture density (CD), where 15 kg m^{-3} (6 kg fish per tanks, loading rate of 0.5 kg l^{-1} min^{-1}, D15) and 40 kg m^{-3} (16 kg fish per tank, loading rate of 1.3 kg l^{-1} min^{-1}, D40) were assayed. According to this, four treatments were assayed in triplicates: E27/D15, E27/D40, E22/D15, and E22/D40. The experimental period was 75 days, preceded by a 15-day acclimation period.

Two commercial (closed-formulae) isoprotein extruded-pellet diets used and 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). Diets differed in the amount of lipid, nitrogen-free extract and total energy content, and therefore, in *P*/*E* ratio; being the higher energy diet (E27) higher lipid, too.

Table 1
Proximate composition (g kg^{-1} on dry weight) of the experimental diets.

Proximate composition	E22	E27
Crude protein	455.3	453.7
Crude lipid	136.9	329.9
Ash	75.5	61.0
Nitrogen-free extract (NFE)[1]	332.3	155.3
Energy (MJ kg^{-1})[2]	22.2	26.8
Protein/energy ratio (g MJ^{-1})	20.5	16.9

[1] Calculated as 1000—(crude protein (g kg^{-1}) + crude lipid (g kg^{-1}) + ash (g kg^{-1})).
[2] Calculated on the basis of 24.3. 39.7 and 17.2 KJ g^{-1} of protein, lipid and NFE, respectively.

These diets have also lower carbohydrate contents (lower values of nitrogen-free extract). The higher lipid content of the diet E27 has a greater vegetable-oil fraction than in diet E22, which has affected the different fatty acid composition (see Suárez et al., 2014).

The fish were fed twice a day with a total daily ration of 1% body weight.

The first day and every 25-day period, the fish of each tank were individually weighed wet. Based on the time course of the weight data, the ration size and culture density was periodically adjusted. Growth performance and feed efficiency were determined by evaluating specific growth rate (SGR) and feed-conversion ratio (FCR).

2.2. Collection and sample treatment

After 24 h of food deprivation the fish were quickly killed according to the regulations of the Directive 2010/63/EU (overdose of metacaine, approved killing protocol). Immediately afterwards, samples were extracted to determine the liver and digestive composition ($n = 5$ from each tank) and plasma metabolites, as well as liver and muscle enzymatic activities ($n = 5$ from each tank) at the end of the experimental period.

Blood samples were rapidly taken from the caudal vein using heparinized syringes and kept under cold conditions, until centrifuged ($1000 \times g$, 10 min). Plasma was separated and stored at −80 °C until analysed for glucose, total protein, triglycerides, cholesterol, HDL, LDL, GOT, and GPT.

Tissues (liver and muscle) were homogenized (1:9) in 100 mM of ice-cold Tris–HCl buffer containing 0.1 mM EDTA and 0.1% (v:v) Triton X-100, at pH 7.8 After centrifugation (27,000 × *g* for 30 min at 4 °C), the resulting supernatant was aliquoted and stored at −80 °C for later determination of the intermediary metabolism activity (PK, CS, HOAD, G6PDH, GOT, GPT) and antioxidant status (SOD, GR, CAT, GPX activities in liver; GR and GPX activities in muscle and TBARS in both).

2.3. Composition and plasma metabolite assays

The proximate composition of liver, digestive tract, and diets was determined following AOAC (2000) standard procedures: water content by oven drying at 105 °C to constant weight; ash by incineration in a muffle furnace at 450 °C for 16 h; crude protein by the Kjeldhal method (crude protein = $N \times 6.25$) and total lipid extraction by Soxhlet's method.

Plasma glucose, total lipids, triglycerides, and cholesterol levels were assayed by using standard colorimetric tests (Labkit 30232, 30345, 30360, 30180, CHEMELEX S.A. Barcelona, Spain). Protein concentration in plasma was analysed using the Bradford (1976) method, with bovine-serum albumin as a standard.

2.4. Metabolic enzymes in liver, muscle, and plasma

All intermediary metabolism enzyme assays were conducted at 25 °C using a Power Wavex microplate scanning