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Effect of temperature and varying level of carbohydrate and lipid on growth, feed efficiency and nutrient digestibility of brook trout, *Salvelinus fontinalis* (Mitchill, 1814)

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ABSTRACT

Temperature is the most important environmental factor determining the growth rate of fish. Increasing summer temperatures in salmonid growing regions worldwide are presenting new challenges for effective nutrient delivery, particularly as feeds increasingly replace fish oil with cheaper and available energy sources. The effect of temperature and non-protein energy sources on growth performance, nutrient utilisation and digestibility of brook trout, *Salvelinus fontinalis* was evaluated at temperatures reflective of optimum, 15 °C, and elevated summer conditions, 19 °C. The experiment was a 2 × 4 factorial design with two temperatures (15 °C and 19 °C) and four iso-nitrogenous (protein: 440 g/kg) and iso-energetic (22 MJ/kg) diets. Diets were formulated with increasing carbohydrate level (180–260 g/kg) balanced by decreasing lipid level (170–130 g/kg). A 12-week study was conducted using triplicate group of brook trout with initial weight 48.0 ± 3.46 g at each temperature. Fish were fed 2% body weight ration daily. Growth rate was higher and feed utilisation was more efficient at 15 °C than 19 °C (P = 0.00). Reduced fish oil inclusion and increased gelatinised carbohydrate had no effect on growth and feed utilisation at both temperatures. The apparent digestibility coefficients of nutrient and energy were significantly higher at 15 °C than 19 °C (P = 0.00). Dry matter, gross energy and energy from carbohydrate were more digestible as dietary carbohydrate levels increased, at both temperatures. Increasing dietary carbohydrate generated an increase in the intestinal activity of α-amylase, which was higher at 15 °C. Across the levels of dietary carbohydrate tested; there were no pathological changes to liver or intestine histology. Fish oil can be substituted with gelatinised carbohydrate under challenging high water temperatures in brook trout feeds, with no detriment to health, nutrient digestibility or growth performance.

1. Introduction

Many aquaculture species are currently exposed to increasing water temperature and the general trend is predicted to continue (Lorentzen, 2008; Lough and Hobday, 2011; Pittock, 2003). Similarly, salmonid culture in Australia is approaching

Abbreviations: GC, goblet cells; GCHO, gelatinised carbohydrate; LP, lamina propria; MF, mucosal fold; PAS, periodic acid-schiff; SPSS, statistical package for the social sciences; SNV, supranuclear vacuolisation; SM, submucosa.

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to the upper thermal tolerance limit for this species (Barnes et al., 2011; De Silva, 2012; Hobday and Lough, 2011; Pankhurst and King, 2010). All cultured aquatic species are poikilothermic and increasing water temperatures cause an exponential increase in metabolic rate and energy demand (Barnes et al., 2011; Jobling, 1997; Katersky et al., 2006). Since fish consume to meet their energy requirement, feeding fish at elevated temperatures means fish will consume enough food to meet their metabolic demand until appetite is inhibited (Jobling, 1997; Kaushik and Médale, 1994).

Protein and lipid are used as energy sources in carnivorous fish, which are obtained from fish meal and fish oil, respectively. Expansion of the aquaculture industry in areas where temperatures approach the upper thermal tolerance level for a given species means an increasing demand of wild harvested fish to provide protein and oil (Hobday et al., 2008). In spite of the increasing demand, the production of fish meal and fish oil remains static; and their price has increased annually (FAO, 2012). In order to produce cost effective aquafeeds at elevated temperatures, alternative energy sources from either plant protein and oil or carbohydrate need to be added and balanced. Carbohydrate is more efficient during summer and it is found that winter feeds should contain less carbohydrate with high protein and lipid level as compared to summer, to maximise feed utilisation (Hemre and Sandnes, 2008; Hemre et al., 1995b).

Fish diets should provide a surplus amount of non-protein energy sources (carbohydrate or lipid) which can be metabolised to meet general energy requirements, leaving an organism to direct the maximum level of available dietary protein into growth (Johnston et al., 2003). It is generally believed that carnivorous fish have limited capacity to utilise carbohydrates (Stone, 2003; Wilson, 1994). The effect of different carbohydrate levels on growth, protein efficiency and nutrient digestibility has been studied in several carnivorous fish and showed that an increases in dietary carbohydrate up to a certain level did not affect the growth performances (Dias et al., 2004; Guerreiro et al., 2014; Moreira et al., 2008), feed efficiency (Dias et al., 2004; Guerreiro et al., 2014), starch digestibility (Couto et al., 2012), protein efficiency and protein digestibility (Moreira et al., 2008). There is little information available on nutrition in brook trout, *Salvelinus fontinalis* (Amin et al., 2014; Jobling et al., 2010), carbohydrate utilisation and effects of energy source on liver and gut have not been established. However, in brook trout increasing carbohydrate improved protein efficiency (Amin et al., 2014). An iso-lipidic and iso-energetic diet, achieved by replacing dietary protein content with carbohydrate showed no difference in the energy efficiency and positive effect on protein efficiency in rainbow trout (Krogdahl et al., 2004). Considering these, there may be a potential of using gelatinised carbohydrate to replace lipid in brook trout.

Water temperature influences the dietary carbohydrate utilisation in rainbow trout (Brauge et al., 1995). Dietary imbalance in relation to temperature might be a factor of poor carbohydrate utilisation (Ringrose, 1971). It has been shown in gilthead sea bream and European sea bass that feeding excessive levels of carbohydrate at low temperatures exhibited low carbohydrate assimilation due to low enzymatic activity, while coldwater species (Atlantic salmon, *Salmo salar*) can adapt their enzymatic digestion and utilise carbohydrate at low temperatures (Papoutsoglou and Lyndon, 2005). Furthermore, it is confirmed in Senegalese sole, *Solea senegalensis* that glycolysis is not affected by the starch levels of diets and elevated levels of lipid inhibited lipid biosynthesis from carbohydrate (lipogenesis) (Dias et al., 2004) which is increased at lower temperature (Guerreiro et al., 2012; Guerreiro et al., 2014).

Like other salmonids, brook trout are susceptible to warmer temperatures, which reduce growth (Robinson et al., 2010). Brook trout inhabit water ranging from 0 °C to 20 °C (Power, 1980), but the optimum temperature for growth is 15.6 °C (Raleigh, 1982). However, in Australia, summer water temperatures often reach 19–20 °C where brook trout are farmed (Lough and Hobday, 2011; Miller et al., 2006; Ng et al., 2010). This increased summer temperature can increase energy demand, thermal stress and reduce growth and feed formulations need to be adjusted with season (Hemre and Sandnes, 2008). To the best of our knowledge, there are no studies available on the effect of temperatures on the nutrient utilisation in brook trout fed a carbohydrate rich diet (Amin et al., 2014). Therefore, the overall objectives of this study were to determine the effect of high carbohydrate in their feed on nutrient utilisation, health, protein and carbohydrate digestion, energy utilisation at ambient temperature (15 °C) and high summer temperatures experienced in Australian salmonid production (19 °C).

2. Materials and methods

2.1. Experimental diet

Four iso-nitrogenous (440 g/kg) and iso-energetic (22 MJ/kg) diets with different lipid and carbohydrate levels were prepared from dry ingredients (Table 1). The main protein source (fish meal) and fish oil were supplied by Skretting (Cambridge, Tasmania, Australia). Carbohydrate was supplied as pre-gelatinised maize starch (BO11C). All the dietary ingredients were mixed thoroughly by a Brice mixer (Model: VFM–20C, Brice Australia Pty Ltd, Burwood, VIC) and approximately 12% water was added, then pelleted through 3 mm-die in a California Laboratory Pellet Mill (California Laboratory Pellet Mill Co., San Francisco, USA). The diets were dried in an oven (Model: 68732-1, Forma Scientific, Division of Mallinckrodt. Inc. Marietta, Ohio, USA) to below 10% moisture content.

2.2. Experimental system and design

The experiment had a 2 × 4 factorial design with two temperatures, four diets and was conducted in triplicate with a total of 24 tanks each with a 300 L capacity. The trial was conducted in two independent freshwater recirculation systems