Metabolic Fuel Use of Rainbow Trout Feed with Varying Macronutrient Feeds in Low Oxygen Environment at Three Different Temperatures

Kevin Torben Stiller[1,2*], Carsten Dietz[2], Klaus Heinrich Vanselow[1], Damian Moran[3], Guid Riesen[4], Stefan Marx[5], Saskia Heckmann[5], Enrico Savino[6], Pompeo Moscetta[6], and Carsten Schulz[2]

[1] Forschungs- und Technologiezentrum Westküste der Universität Kiel, Hafentörn 1, 25761 Büsum, Germany

[2]Gesellschaft für Marine Aquakultur, Hafentörn 3, 25761 Büsum, Germany

[3]Department of Biology, Lund University, Sölvegatan 35, 223 62 Lund, Sweden

[4]Skretting Aquaculture Research Centre, 4001 Stavanger, Norway

[5]SubCtech GmbH, Wellseedamm 3, 241145 Kiel, Germany

[6]Systea SpA, Via Paduni 2/a, 03012 Anagni (FR), Italy

*Contact information: Hafentörn 3, 25761 Büsum, Germany
Phone: +494834604220; Fax: +494834604299; email: stiller@ftz-west.uni-kiel.de

Abstract

Metabolic fuel use (MFU) is becoming increasingly important for the evaluation of feedstuffs for intensive aquaculture. However standardized methods for measurement of MFU under culture-like conditions are not well developed. The feed industry is looking for the level of protein which can be replaced by cheaper fat and/or carbohydrates without affecting the protein retention rate negatively. From an economic perspective, fish should not waste expensive surplus protein for their energy metabolism (EM), but instead should invest it in muscle mass. The optimization of protein inclusion rates into aquaculture pellet diets is particularly important from a food production efficiency perspective, as fish power their metabolism using more protein than other farmed vertebrates. The calculation of respiratory quotients can be used to infer which metabolic fuels (carbohydrates, fat and protein) were used to power metabolism. There are two different respiratory quotients used in fish physiology. The classic respiratory quotient (RQ) is the ratio of CO_2 produced and O_2 consumed, and knowledge of this quotient gives a proxy of protein, carbohydrate and fat fuel use rates. The precise determination of aquatic animal RQ is extremely tough due to difficulties in accurately measuring the small quantity of excreted CO_2 against a comparatively large background of dissolved inorganic carbon. The ammonia quotient (AQ) is easier to measure for aquatic animals compared to RQ. The AQ is the ratio of produced total ammonia nitrogen (TAN) and consumed O_2. We investigated the EM of rainbow trout fed two isoenergetic diets (digestible energy = 19.5 kJ g^{-1}) in a recirculating aquaculture respirometer system (RARS) under different temperatures (12°C, 16°C and 20°C) and low evening oxygen conditions (4 PM to 8 AM).

A complete description of the RARS is given in Stiller et al. (2013). Briefly, the system consisted of 10 tanks (250 l volume each) included in a recirculating aquaculture system with mechanical (sedimentation) and biological (trickling filter) treatment units. The system utilizes a flow-through approach to measure respiration, where the water metabolite concentration of each tank is measured sequentially by a suite of water chemistry analysers. Respiration rates are calculated based on the differences of test and reference tank metabolite values and water flow rates. The water chemistry analysis unit measured the following parameters: O_2 (amperometric electrode); pH (intermediate junction electrode); temperature; TAN (loop flow orthophtalaldehyde fluorometric autoanalyer, Micromac 1000, Systea SpA, Italy) and dissolved CO_2 (flow throw headspace analyzer with non-dispersive infra-red detection, OceanPack pCO_2 RAS, SubCtech GmbH Germany). Analyzers were calibrated daily. The daily data acquisition was 12 measurements per day and tank, corresponding to a two hour measuring cycle in which everyone of the 10 tanks were measured. Daily water exchange was around 10% of total RAS volume. The diets were fed at 1% biomass (BM) once per day. The first feed had a protein content of 42.5% and the second 49.5%. To achieve a similar digestible energy at different protein levels in the 42.5% protein diet; 3.7% more fat, 2.2% more starch and 0.6% more crude fiber were used in that feed. The experiment was carried out at three sequential temperatures (12, 16 and 20°C, each for 16 days). Acclimation to the next test temperature was done in 1°C steps over 4 days, followed by 10 days of measurements at a constant temperature. For the first 5 measurement days, the water flow rate through the tanks were at maximum to maintain high dissolved oxygen levels (~ 79% (7.5 mg l^{-1}) at 12°C,~ 70% (6.1 mg l^{-1}) at 16°C and ~ 57% (4.6 mg l^{-1}) at 20°C). For the final 5 days of measurement the flow rates were down regulated between 4 PM to 8 AM to create a low oxygen environment (~ 50% (4.7 mg l^{-1}) at 12°C, ~ 50% (4.3 mg l^{-1}) at 16°C and ~ 40% (3.2 mg l^{-1}) at 20°C). On the final day of a particular temperature regime the fish were fasted without an oxygen challenge, and the following day the fish were weighed for growth data. Every temperature regime started with a fish biomass of ~3.70 kg per tank so that in every profile the same absolute amount of feed (~0.56 kg) was administered. The biomass was reduced after every temperature step starting with 50 fish per tank (73.4 ± 13.0 g) at 12°C, 42 fish per tank (90.2 ± 21.8 g) at 16°C and 35 fish per tank (105.5 ± 30 g) at 20°C. A minimum of 5 fish were sacrificed after every profile for whole body analysis. Digestibility of the diets was measured at the end of the experiment by re-acclimating the fish to 16°C and feces was collected by stripping. The photoperiod was controlled via artificial room lighting, and set to 12:12 light:dark, with sunrise starting at 06:00.

With a few exceptions, temperature, diet and oxygen availability had no significant effect on whole body composition, growth variables and digestibility results. The higher protein diet exhibited slightly higher digestibility (2.2%) than the other diet. The only significant differences in oxygen consumption were detected at 12°C, where fish fed the 49.5% protein diet consumed on average 1.1% more oxygen. The oxygen consumption rate decreased for all treatments during the one-day fasting period in a similar fashion. Fish fed the 49.5% protein diet exhibited higher rates of protein fuel use (11.5% at 12°C, 7.7% at 16°C and 7.9% at 20°C) compared to the lower protein diet. An oxygen challenge at night during the digestive process appears to have no significant influence

on the AQ value. The results demonstrate the utility of automated RARS to non-invasively evaluate the physiological performance of different fish diets in an aquaculture setting.