Montana lech

Abstract

We investigated the transport of dietary amino acids into the blood using two different lines of rainbow trout, select (Select) and commercial (TL). Both groups were fed a commercial diet containing 40% protein and 15% lipid once a day at 1% body weight (BW) for two weeks. After two weeks, liver and distal intestine of fish were sampled 24 h postprandially and genes involved in amino acid, fatty acid, and glucose transporters were examined using qPCR. Both groups were also subsequently gavaged with 1%BW of a slurry of the commercial diet containing 1% of [¹⁵N] phenylalanine. Labeled phenylalanine was recovered in blood plasma using LC-MS. Twenty-four hour postprandial samples did not show differences in gene expression between lines of fish for all genes examined. However, examination of genes at 3, 6, and 9 h post-gavage did show differences in up-regulation of two amino acid transporters between groups. Recovery of isotopic phenylalanine in the blood peaked at 6 h in both fish groups but Select line fish had higher uptake and which corresponded with increased gene expression of amino acid transporter SLC1A5.

Hypotheses:

- Null Hypothesis- Both strains of fish will uptake amino acids equally
- Alternate Hypothesis- Select line of fish will have a higher uptake of amino acids

Introduction

Maximizing the acquisition and assimilation of dietary proteins is essential to improving the growth and feeding efficiency of cultured fishes. These processes rely on the efficient uptake of amino acids in the small intestine. A select line of rainbow trout has been bred for over 9 generations for growth on a plant-based diet. Over this time, the selected strain fish have shown large increases in growth over commercially available strains. Why this select line of rainbow trout grows at a faster rate than commercial lines of rainbow trout is still unclear, although it appears to be related to how amino acids are absorbed. This study focuses on whether the increased absorption or catabolism of specific amino acids, such as phenylalanine, contribute to the increased growth rate in select line rainbow trout.

Materials and Methods

Six tanks (140 L) with recirculated water at 15.0°C were stocked with TL and Select trout with an average initial weight of 202 \pm 4 grams. Fish were fed a commercial diet (4.5 mm Pond LE, Skretting, Toole, Utah) at a rate of 1%BW per day. Fish were fed once daily in the morning for two weeks, at which point five fish were sampled for analysis. In a separate trial, fish were fed the commercial diet for four weeks before gavaging at 1%BW with a feed slurry containing 1% ¹⁵N-labeled phenylalanine. Samples of blood, distal intestine, and liver from three fish were taken at 3, 6, and 9-hours post-gavage. Total RNA was isolated from distal intestine and liver, while blood was spun down to recover plasma. RNA was reverse-transcribed into cDNA for later analysis by qPCR. We measured expression of intestinal (GLUT4, SLC1A5, SLC1A14, SLC36-A1) and liver transporters (FABP2). The Livak method (Bustin 2006) was used to determine relative abundance in all samples. Additionally, the level of ¹⁵N-labeled phenylalanine in the plasma was measured using LC-MS.



Selection for Faster Growth Rate Affects Amino Acid Transport and Catabolism Between Commercial Lines of Rainbow Trout

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Citations

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• After two weeks, there were no observed differences in glucose transport, fatty acid binding protein, neutral amino acid transporters, or glutamate transporters between selected and commercial lines of rainbow trout

However, differences were observed at a finer scale when fish were gavaged with 1% of the diet and sampled at 3, 6, and 9 h.

By 6 h post gavage, expression of sodium-dependent, neutral amino acid transporter SLC1A5 was up-regulated in select line fish (Figure 6). Differential up-regulation of glutamate, neutral amino acid transporter

SLC1A14 was not observed until after 6 h in commercial line fish (Figure

Recovery of [N¹⁵] phenylalanine peaked at 6 h in select line fish and was significantly higher than observed in commercial line fish (Figure 8).



When fish were sampled after two weeks, there was no difference in the expression of fatty acid, glucose, or amino acid transporters. However, we did observe differences in the expression of amino acid transporters during the nine-hour period post-gavage. In terms of the specific amino acid transporter SLC1A14, the commercial line had higher expression at 9 h than the Select line. In other studies, SLC1A14 has been shown to be a QTL marker for increased growth in other monogastric animals, such as chickens (Mignon-Grasteau et al., 2015). Therefore, the results obtained by previous studies do not appear to align with the findings of this project. Conversely, Select fish up-regulated SLC1A5 much higher than the commercial line. Since SLC1A5 is a major transporter of the amino acid phenylalanine, the upregulation of this transporter could indicate that Select line fish are better able to absorb dietary phenylalanine than commercial line fish. This is supported by our LC-MS data, which show higher levels of phenylalanine in

These observed changes in expression of gut amino acid transporters provide evidence for the differences observed in performance between strains. The increased expression of SLC1A5, along with the higher levels of phenylalanine in the plasma, may result in improved utilization of dietary amino acids by ensuring that all essential amino acids are available in amounts necessary for muscle anabolism. Lack of certain essential amino acids necessitates the catabolism of other tissue to provide them. Reducing the rate of catabolism and muscle turnover in Select fish could be a possible mechanism as to why Select fish have an increased growth rate compared to

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