Assessing Gut Transport of Methionine and Lysine in Fast-growing Rainbow Trout

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ABSTRACT

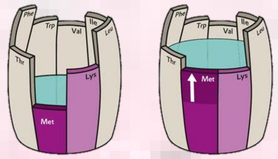
Supplies of fish meal are limited and the future growth of aquaculture depends on the discovery of alternative sources of protein to feed fish. Promising alternatives include plant-derived sources, however plant proteins often lack sufficient levels of methionine and lysine for fish dietary requirements. Gut transport of two limiting, essential, amino acids were compared between a line of rainbow trout selected for growth on a plant protein diet (CX) and a non-selected, commercial line (HC) in a factorial design. We hypothesized rainbow trout selected on a plant-derived protein diet would uptake lysine and methionine more efficiently in their gut compared to non-selected fish. Amino acid uptake and total amino acids were assessed in plasma over time using [C^{13}] and [N^{15}] isotopically labeled methionine and lysine and measured by LCMS. Amino acid transporter expression in the gut was assessed using qPCR. Results show amino acid uptake in rainbow trout selected for rapid growth on a plant-protein diet is greater than the uptake observed in non-selected fish. Expression of amino acid transporters in the proximal intestine show a significant up-regulation of transcription in several transporters over time consistent with levels of amino acid uptake.

INTRODUCTION

The aquaculture industry currently uses most of the fish meal produced worldwide as feed. Because rainbow trout are carnivorous and fish meal is limited, the discovery of feed alternatives is of the utmost importance. Considerable work has been done examining ingredients of potential feed, but evaluation of the fish consuming the products has been minimal.

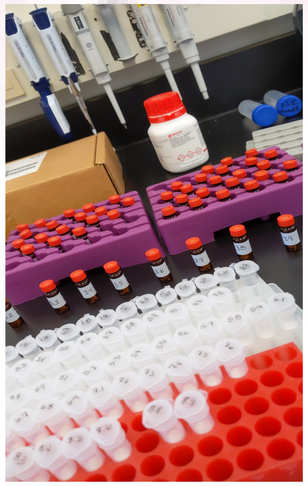
Recent studies have examined the impact of alternative diets on selected lines of rainbow trout[1]. This species shows a remarkable ability to shift metabolic patterns in response to changes in diet. Unfortunately, when reared on an all-plant based protein feed, growth was limited and mortality rates often increased. This is likely due to the fact that plant-derived proteins are often lack sufficient amounts of methionine and lysine which are considered essential for fish diet[2].

Leiblig's Law of the Minimum demonstrates how growth is determined by the least abundant nutrient. Each stave of Leiblig's Barrel represents a particular amino acid and the length is proportional to its abundance. The water held by the barrel represents potential growth and the excess amino acids are catabolized[3].



Nearly two decades of collaboration between the USDA-ARS and University of Idaho at the Hagerman Fish Culture Experiment Station, has produced a strain of rainbow trout selected for growth on a 100% plant-based feed[4]. This strain is referred to as the "CX" line and is now in its 9th generation. This line of trout grows at a more rapid rate on an all-plant protein diet than commercial strains, referred to as House Creek or "HC", on a fish meal diet. This is likely due to their increased ability to uptake limiting amino acids more rapidly than non-selected fish.

A mechanistic approach will further our understanding of rates of uptake, turnover, and metabolic fate of amino acids in the CX strain using the stable isotopes carbon [C^{13}] and nitrogen [N^{15}]. Analysis of amino acid transporters in the gut using qPCR will provide additional support showing that the CX line is better equipped to uptake these limiting amino acids. Analyzing how the CX line's metabolism differs will provide a greater understanding of how genetic and epigenetic factors can enhance the ability of fish to utilize plant proteins. This will allow for production of feed formulations and strains of fish that are optimized for sustainable aquaculture production.



CITATIONS

(1) Kamalam, B.S., Medale, F., Kaushik, S., Polakof, S., Skiba-Cassy, S. and Panserat S. 2012.
(2) Halver, J.E. and Hardy, R.W. 2002. Fish Nutrition 3rd ed.
(3) Baar, H. (2003, April 10). Von Liebig's Law of the Minimum and Plankton Ecology (1899–1991).
(4) Overturf, K., Welker, T., Towner, R., Schneider, R., Barrows, F., LaPatra, S. 2013. Variation in Rainbow trout, (*Oncorhynchus mykiss*), to biosynthesize EPA and DHA when reared on plant oil replacement feeds.

HYPOTHESES

Objective 1.0: Assess methionine and lysine uptake in tissues and serum of selected (CX) vs. non-selected (HC) lines of rainbow trout using stable isotopes.

H_o: *CX line fish will show significantly enhanced gut transport (uptake) of essential, limiting amino acids methionine and lysine compared to non-selected fish when fed an all plant-based diet.*

H_a: *Diet and/or selection will have no effect on gut transport of essential, limiting amino acids in rainbow trout.*

Objective 2.0: Assess gene expression of amino acid transporters in the gut of selected vs. non-selected (HC) lines of rainbow trout.

H_o: *CX line fish will show significantly increased transcription of amino acid transporters of essential, limiting amino acids methionine and lysine in the gut compared to non-selected fish when fed an all plant-based diet.*

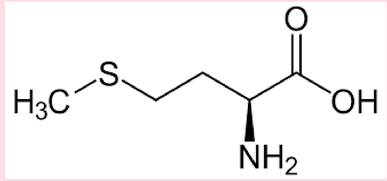
H_a: *Diet and/or selection will have no effect on amino acid transporter transcription in the gut of rainbow trout.*

MATERIALS AND METHODS

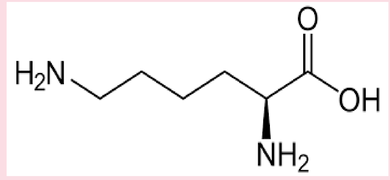
A random sample of 12 fish from each of 4 treatments (CX fish on the plant meal (PM) diet, CX fish on the fish meal (FM) diet, non-selected fish (HC) on the PM diet and HC fish on the FM diet) were separated from the general populations and held off feed for 24 hours. Each fish was then gavaged with a slurry of 1% body weight of feed containing 1% of [^{15}N] L-lysine or [^{15}N] L-methionine. At 0, 6, 9, and 12 h post-gavage, 3 fish were sampled for blood, liver, and intestine. Tissue samples were snap frozen in liquid nitrogen and stored until further analysis.

Total amino acid profiles in all tissue samples were determined using a Biochrom 30+ amino acid analyzer. Stable isotopes in samples were quantified on an Agilent 1100 liquid chromatograph. Gene expression in the gut was assessed using quantitative PCR and primers previously designed for 2 major amino acid transporters.

Essential Limiting Amino Acids



Methionine



Lysine

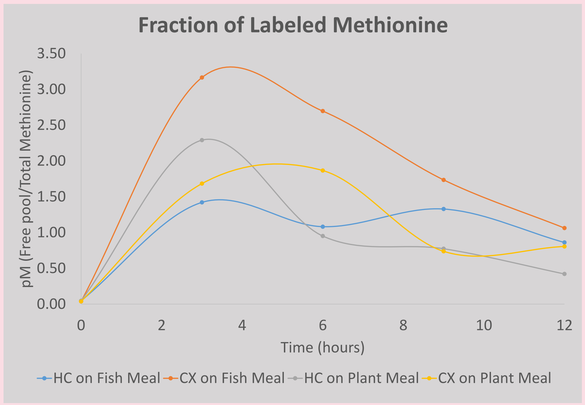


Figure 1

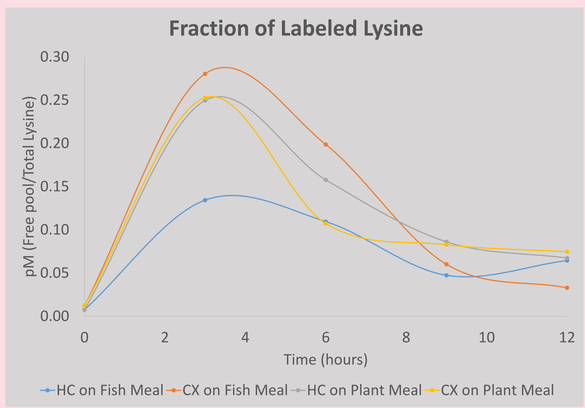


Figure 2

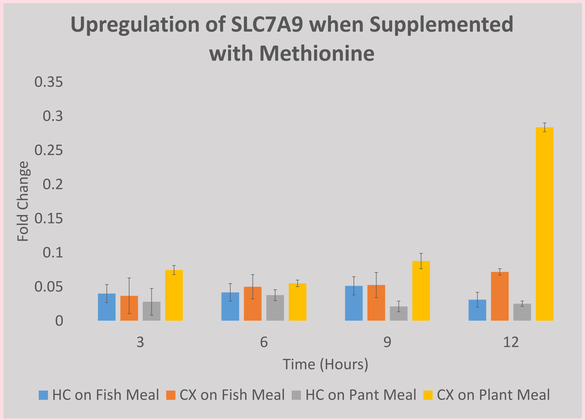


Figure 3

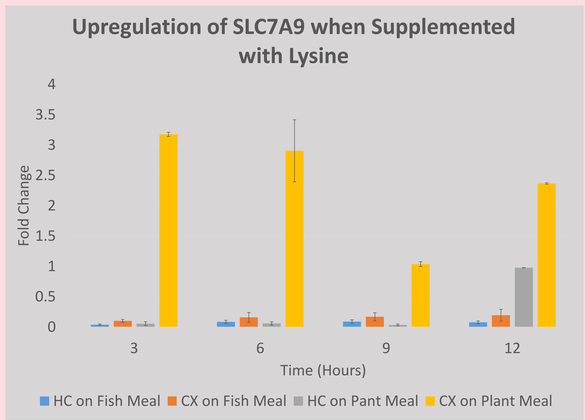
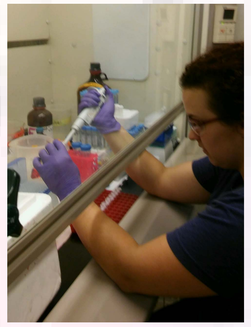


Figure 4

RESULTS

- **Objective 1.0:**
  - CX line of rainbow trout on fish meal showed the largest fractional uptake of labeled methionine and lysine over total amino acid uptake. (Figure 1,2)
  - CX and HC lines of rainbow trout on plant meal showed similar fractional uptake of labeled methionine and lysine over total amino acid uptake. (Figure 1,2)
  - HC line of rainbow trout on fish meal showed limited uptake of labeled methionine and lysine over total amino acid uptake. (Figure 1,2)
- **Objective 2.0:**
  - At three and six hours post-gavage with labeled methionine, there is no change in expression of SLC7A9 between different lines of rainbow trout. (Figure 3)
  - At nine hours post-gavage with labeled methionine, the HC line of rainbow trout on plant meal showed lower expression of SLC7A9 compared to other treatments. (Figure 3)
  - At twelve hours post-gavage with methionine, the CX line of rainbow trout show significantly increased upregulation of SLC7A9 compared to other treatments. (Figure 3)
  - Upregulation of SLC7A9 was greatly increased in the CX line of rainbow trout on the plant meal diet with labeled lysine. (Figure 4)
  - At twelve hours post-gavage with lysine, the HC line on plant meal showed increased expression of SLC7A9.



DISCUSSION/CONCLUSIONS

The CX line rainbow trout on fish meal had an increased fractional uptake of the limiting amino acids over total amino acids. This is significant because the fish meal diet already contains a sufficient amounts of methionine and lysine, yet these selected fish were still able to uptake increased amounts of the free limiting amino acid. Both selected and non-selected strains on the plant meal diet showed increased uptake of fractional labeled methionine and lysine. Because these amino acids are limited in the plant diet, more of the free labeled amino acids were absorbed into the bloodstream. The HC strain on the fish meal diet showed the lowest uptake of the labeled, limiting free amino acids. Because the fish meal already contains high levels of methionine and lysine, they do not need to supplement this diet using the free labeled amino acids.

Figures 3 and 4 show expression of SLC7A9, an amino acid transporter found in the apical membrane of intestinal epithelial cells. Expression of SLC7A9 is higher in the CX line on the plant meal diet. This corresponds with the increase shown in Figures 1 and 2. This increase is significant and allows this select line to absorb more of the limiting amino acids which may help explain the increased growth rate observed in this line of fish. The CX line on the fish meal diet showed no significant difference from the non-selected strain on either diet. This suggests that rates of uptake are similar among strains regarding these amino acids since they are not limiting in fish meal.

The provided data suggests that the CX line of rainbow trout have the ability to uptake limiting amino acids, such as lysine and methionine, at a greater rate on a plant-based diet than the non-selected line on a fish meal diet. Because of the metabolic plasticity of rainbow trout, it is possible to select lines of fish that are well suited for alternative feedstuffs. This will allow for not only an increase in the use of more sustainable plant-based fish feed, but also increased growth rates compared to non-selected fish on a fish meal diet.

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