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Dietary protein source significantly alters growth performance, plasma variables and hepatic gene expression in rainbow trout (*Oncorhynchus mykiss*) fed amino acid balanced diets

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ABSTRACT

The objective of the study was to evaluate the effect of dietary protein source on fish growth, nutrient utilization, plasma variables and hepatic gene expression in juvenile rainbow trout (*Oncorhynchus mykiss*). Fishmeal (FM) and soy protein isolate (SPI) were used as the main sources of protein in six isonitrogenous, isolipidic and isocaloric diets. The amino acid profiles of the diets were completely balanced to minimize differences between experimental treatments and formulated to contain increasing levels of branched-chain amino acids (BCAA) based upon dietary requirements for trout (NRC, 1993). Dietary protein source more consistently changed the measured variables while BCAA supplementation had an unexpected effect over whole body lipid content. Growth performance and protein retention efficiency were significantly reduced in fish fed SPI diets independently of BCAA supplementation. Total concentration of amino acids as well as circulating indispensable amino acids (IAA) were significantly elevated in the plasma of fish receiving SPI diets compared to fish fed FM diets. The change in IAA was large enough to increase ($p<0.05$) plasma IAA/DAA (DAA: dispensable amino acids) proportion even when the diets were formulated to have a ratio close to 1. Levels of circulating BCAA and alanine were also elevated in the fish fed SPI diets, possibly indicating a change in protein turnover. The use of SPI caused a reduction ($p<0.05$) in the hepatic expression levels of alanine amino transferase (*alt1*) and glutamine synthetase 2 (*gls02*), while an increase was observed for aspartate aminotransferase (*got2*), and asparagine synthetase (*asns*) compared with FM diets. Expression of the gene *tor* (target of rapamycin) declined over time for all treatments, while expression of a gene known to repress *tor* function, *redd-1*, was consistently higher in the liver of fish fed SPI diets. Glucose 6 phosphate dehydrogenase (*g6pd*) also showed a significantly higher expression in the liver of fish fed SPI diets but only at higher levels of BCAA supplementation.
In summary dietary protein source has a significant effect over growth performance, body composition and hepatic gene expression in rainbow trout. We also identified for the first time in fish changes in the expression of *redd-1*, which may represent another regulatory point in the TOR cascade.

1. Introduction

Feeds for intensive aquaculture still rely on fishmeal produced from marine resources as a major source of high quality protein (FAO, 2008; Tacon and Metian, 2008). Considering the decline in wild fish populations and the active growth of aquaculture worldwide, the sustainability of this industry heavily depends on the search for alternatives to replace fishmeal and new feed formulations to minimize negative impacts on fish growth performance and the environment (Naylor et al., 2009). Among the alternatives being evaluated, plant protein represents a viable choice and aspects such as inclusion levels, ingredient digestibility, presence of anti-nutritional factors, nutrient balance and ingredient processing have been evaluated (Aksnes et al., 2006; Barrows et al., 2008; Gatlin et al., 2007; Glencross et al., 2005; Gomes et al., 1995; Halver and Hardy, 2002; Iwashita et al., 2008; Kaushik et al., 1995). This research has advanced our understanding regarding how plant proteins can be used as fishmeal replacements, but has also revealed challenges in formulating a viable complete plant-based diet for carnivorous fish. For example, high levels of replacement of fishmeal by plant protein sources are still associated with lower efficiency of dietary nutrient use by carnivorous fish (Hansen et al., 2007; Hevroy et al., 2008; Mambrini et al., 1999). This effect may be related to most salmonid fish being able to preferentially use amino acids to accomplish different metabolic functions (Halver and Hardy, 2002) as well as to the fact that

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amino acids can act as metabolic signals (Li et al., 2009). As a result, the effect of the amino acid profile over protein metabolism and the factors influencing it are considered key research areas of research when evaluating the complex effects of replacing fishmeal as a protein source.

The use of molecular techniques to study the effects of fishmeal replacement in carnivorous fish diets has provided valuable information regarding which pathways are involved and explaining some of the changes in fish metabolism when high levels of plant proteins are used in their diets. For example Vilhelmsson et al. (2004) working with rainbow trout, evaluated the effect of replacing fishmeal with a mixture of plant ingredients on protein retention efficiency and changes in liver protein profiles. They observed a reduction in protein efficiency ratio in rainbow trout fed the plant meal-based diets along with an increase in proteins associated with the generation of reductive (REDOX) power. This response was associated with significant elevations in the levels of the enzymes transaldolase and malate dehydrogenase as well as increased activity of *g6pd*. Along the same line, Olsvik et al. (2011) reported lower transcriptional levels for super oxide dismutase (*sod1*) and significant increases for glutathione peroxidase (*gshpx*) transcription in the liver of Atlantic salmon (*Salmo salar*) when transferred from a diet based on fishmeal to one based on plant protein. Also studying the effects of fishmeal replacement, Martin et al. (2003) evaluated fish growth performance and liver proteome changes of rainbow trout fed two diets with high inclusion levels of plant protein but differing in soybean meal content (SBM). Along with significantly lower protein efficiency utilization, they reported higher activities of hepatic transaminases (*glud*, *got2*, and *alt1*) in the fish fed the diet with increased SBM levels.

Molecular tools have also been used to study protein metabolism, revealing new and more specific aspects of its regulation in mammals. One pathway that has been the focus of many studies is the mammalian target of rapamycin pathway (mTOR) (Inoki and Guan, 2006). This pathway has been described as an integration point of several inputs that modulate protein metabolism and growth, processes that involves signaling from nutrients such as amino acids, growth factors (insulin/IGF; EGF) and energy status (Sarbassov et al., 2005).

Nutrient input affects several components of the TOR pathway and its regulation. Branched-chain amino acids, especially leucine, have been shown to play an important role in the activation of the TOR pathway, stimulating protein synthesis and inhibiting degradation (Garlick, 2005). Although recent molecular work in rainbow trout has shown that components of the TOR cascade are present in fish muscle and liver (Lansard et al., 2009, 2011; Seiliez et al., 2008) nothing is known about the effect of BCAA supplementation *in vivo* in fish. The use of a nutrigenomic approach should promote a better understanding of the effects of replacing fishmeal in fish protein metabolism, nutrient use and growth efficiency. Given that the liver plays a central role in nutrient metabolism it is therefore crucial to understand if decreases in efficiency of nutrient use are associated with changes in hepatic transcription in genes known to participate in protein metabolism. The present study used soy protein isolate (SPI) as a dietary protein source to completely replace fishmeal (FM) and study metabolic and hepatic transcriptional changes in rainbow trout.

2. Materials and methods

2.1. Experimental fish and diets

Five hundred and forty juvenile rainbow trout (RBT) of the House Creek strain (College of Southern Idaho, Twin Falls, Idaho), average weight 12 g, were randomly distributed in 18 fiberglass tanks (150 L), each supplied with 6 L/min, constant temperature (14.5 °C) spring water under a photoperiod of 14 h light 10 h dark at the Hagerman Fish Culture Experimental Station, University of Idaho. Six experimental diets were formulated using fishmeal or soy protein isolate as the main protein source, using NRC (1993) requirements as the reference for the formulation. Crystalline amino acids (L-isomers from Ajinomoto, North America, Inc.), were used to balance the amino acid profile of the diets and to increase the levels of branched chain amino acids as shown in Tables 1 and 2, based on NRC (1993) recommendations. Each diet was fed to triplicate tanks of fish. Fish were hand-fed to apparent satiation 3 times daily, 6 days a week for 12 weeks.

2.2. Sampling procedure

The study was carried out in accordance to the guidelines of the University of Idaho's Animal Care and Use Committee. At the end of the 12 week experiment, fish in each tank were weighed and counted to calculate weight gain, feed intake, feed conversion ratio, specific growth rate, protein retention efficiency and energy retention efficiency. At the same time five fish per-tank were euthanized using an overdose of tricaine-methanesulfonate (250 mg L^{-1}, Argent Laboratories, Redmond, WA, USA), after which, each was individually weighed and samples of liver and blood were collected to measure changes in hepatic gene expression and plasma amino acid concentrations respectively. All samples were collected 36 h post-prandially to minimize confounding effects associated to periods of active nutrient absorption on liver metabolism. For gene expression analysis, liver samples (±100 mg) were immediately placed into tubes containing TRIzol® reagent (Invitrogen, Carlsbad, CA, USA), snap frozen in liquid nitrogen and stored at −80 °C until processing. Liver samples were homogenized by adding a 5 mm stainless steel bead to the RNA–TRIzol mix and then shaking twice for 2 min at 40 Hz using a Qiagen MM301 shaker (Valencia, CA, USA). Blood was collected from the caudal vein in sterile, heparinized syringes. Plasma was recovered after 5 min centrifugation at 3000×*g* and immediately frozen at −20 °C until processing.

Table 1
Ingredient formulation and nutrient composition of experimental diets.

	Diet 1	Diet 2	Diet 3	Diet 4	Diet 5	Diet 6
Ingredient formulation (as fed, g kg^{-1})						
Fish meal	44.0	40.3	35.1	0.0	0.0	0.0
Soy protein isolate	0.0	0.0	0.0	35.7	33.6	32.8
Wheat flour	20.0	20.0	20.0	20.0	20.0	20.0
Fish oil	14.8	15.5	16.2	18.2	18.3	18.4
Corn gluten	0.0	1.4	7.5	2.6	5.6	6.8
Wheat starch (g)	16.4	17.6	15.4	17.7	15.5	15.0
Vitamin C	0.3	0.3	0.3	0.3	0.3	0.3
Choline	0.5	0.5	0.5	0.5	0.5	0.5
TM salt[a]	0.1	0.1	0.1	0.1	0.1	0.1
Vitamin premix[b]	1.0	1.0	1.0	1.0	1.0	1.0
Di calcium phosphate	0.5	0.5	0.5	0.5	0.5	0.5
Amino acid mix[c]	2.4	2.9	3.4	3.3	4.6	4.6
Nutrient composition (as fed, g kg^{-1})						
Protein (%)	43.9	43.4	42.4	41.9	42.9	42.2
Fat (%)	21.2	20.1	20.3	21.5	21.1	20.9
Ash (%)	8.4	8.0	7.2	8.8	6.7	5.6
Gross energy (MJ kg^{-1})	24.5	25.3	25.3	25.4	25.6	25.8

(g): gelatinized.

[a] Supplies the following per kg dry diet: KI, 1.9 mg; $MnSO_4 \cdot H_2O$, 75.8 mg; $ZnSo_4 \cdot 7\text{-}H_2O$, 132.0 mg; Na_2SeO_3, 0.88 mg; $CoCl_3 \cdot 6\ H_2O$, 4.0 mg; $CuSO_4 \cdot H_2O$, 11.8 mg; $FeSO_4 \cdot H_2O$, 298.5 mg.

[b] Supplies the following per kg dry diet: thiamin mononitrate, 62 mg; riboflavin, 71 mg; niacin, 294 mg; calcium pantothenate, 153 mg; pyridoxine hydrochloride, 50 mg; folic acid, 22 mg; vitamin B_{12}, 0.08 mg; D-biotin, 0.8 mg; myoinositol, 176 mg; retinal acetate, 8818 IU; vitamin D_3, 588 mg; α-tocopherol acetate, 670 mg; menadione sodium bisulfite complex, 37 mg.

[c] Amino acid mixtures used in each diet (Arg-His-Iso-Leu-Lys-Met-Phe-Thr-Trp-Val-Glu, g/kg): D1:1.9–0.3–4.8–1.3–0–2.4–3.5–0.7–0.4–8.1–0; D2:3.4–1.1–1.5–0–0.2–3.4–5.8–2.2–0.5–3.9–6.1; D3: 1.4–0–8.9–5.0–0–1.9–2.0–0–0.6–13.1–0; D4: 0–1.3–1.3–0–5.9–6.9–1.7–4.3–1.5–6.4–17; D5: 0–0.8–3.9–0.7–4.5–6.2–0.6–2.9–1.5–9.9–1.8; D6: 0–0.7–8.6–6.5–5.0–6.1–0–1.5–1.6–14.5–0.