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Effects of carnosine supplementation to an all-plant protein diet for rainbow trout (*Oncorhynchus mykiss*)

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ABSTRACT

Fish meal may contain "unknown growth factors" that have yet to be identified for their physiological role. Carnosine is a histidine-β-alanine dipeptide found in muscle and nervous system tissue which has been demonstrated to have biological activity, but its physiological role is not well defined. A 9-week feeding study was conducted comparing diet FM, a 100% fish meal protein control diet, to fish fed three plant protein diets: diet SPI, 100% of the fish meal replaced with soy protein isolate; diet SPI + AA, diet SPI supplemented with methionine, lysine, threonine and glycine to diet FM levels; and diet CSN, diet SPI + AA supplemented with carnosine. Feeding diet SPI resulted in significant differences in feed conversion ratios (FCR), percent gain and protein retention efficiencies relative to fish fed diet FM. Feeding diets SPI + AA and CSN resulted in FCRs, percent gains and protein retention efficiencies that were not significantly different from fish fed diet FM. Fish fed diets SPI, SPI + AA and CSN resulted in reduced muscle ratio (MR) and feeding diets SPI + AA and CSN resulted in increased intraperitoneal fat ratio (IPFR) relative to fish fed diet FM. Supplementing carnosine to an all-plant protein diet resulted in elevated plasma carnosine and increased muscle free pool anserine. Feeding diets SPI, SPI + AA and CSN resulted in reduced muscle development and increased calpain induced proteolysis. In conclusion, carnosine supplementation did not significantly improve the 100% plant protein diets in regard to the measured growth characteristics above the amino acid supplemented treatments and other unidentified factors may be limiting in the diet causing the reductions in MR and elevated IPFR.

1. Introduction

It is predicted that global trout production; rainbow trout, *Oncorhynchus mykiss*; brook trout, *Salvelinus fontinalis* and brown trout *Salmo trutta*, will increase 51% and fish meal use for these feeds will have to decrease 35% by 2020 (Tacon and Metian, 2008). There have been numerous studies identifying the effects of replacing fish meal with plant meals, terrestrial animal meals or seafood processing waste on the growth performance of rainbow trout (Adelizi et al., 1998; Aksnes et al., 2006a, 2006b; Luo et al., 2006; Yanik et al., 2003). These studies have demonstrated that alternatives to fish meal are either lacking in various nutrients and contain amino acid profiles not optimized for piscivorous fish, or contain anti-nutritional factors that result in reduced growth when used to supply a large portion of dietary protein for aquafeed (Francis et al., 2001; Tacon, 1997). Mechanical processing of plant proteins can lead to the concentration of the protein fraction, drastically reducing the content of antinutritional compounds and improving nutrient digestibility and availability in plant protein ingredients (Drew et al., 2007). However, there is no single commodity crop that can provide the essential amino acid profile found in fish meal. This has led to the practice of supplementing the limiting amino acids directly to feeds and composing blends of either animal or plant proteins to best mimic the amino acid profile of fish meal (Cheng and Hardy, 2002; Cheng et al., 2004; Gaylord et al., 2004; Torstensen et al., 2008). Feeding these diets resulted in improved growth performance over complete fish meal replacement with single ingredients; however there are still deficiencies related to the sensitivity of metabolic pathways that are associated with protein source (Vilhelmsson et al., 2004).

Andrews and Page (1974) described the presence of water soluble growth factors in menhaden fish meal that alleviated the negative effects of replacing fish meal with soybean meal in diets fed to channel catfish (*Ictalurus punctatus*). These unknown growth factors or compounds have yet to be identified for their physiological role. The histidine dipeptides are water soluble nitrogenous compounds

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that consist of carnosine (β-alanine-L-histidine), anserine (β-alanine-L-1-methylhistidine), balenine or ophidine (β-alanine-L-3-methylhistidine) and homocarnosine (γ-aminobutyric-L-histidine). The distribution of these peptides in vertebrate tissue is well documented (Abe, 1983b; Christman, 1976; Crush, 1970). Carnosine is of particular interest as a possible lost nutrient or unknown growth factor because histidine dipeptides are lacking in plant proteins and are the predominant dipeptides in the muscle amino acid free pool of fish (Abe, 1983a). Carnosine, anserine and balenine are found exclusively in animal tissues (Schönherr, 2002).

Muscle is the largest tissue compartment in a fish, accounting for nearly 60% of its total mass. Muscle growth is achieved through myocyte addition, protein accretion and accrual of extracellular matrix proteins (Allen et al., 1979; Koumans et al., 1993; Mozdziak et al., 1997; Stickland, 1983; Wilschut et al., 2010). Increased muscle mass through myocyte addition due to satellite cell activation is of particular interest (Allen et al., 1979; Mozdziak et al., 1997; Valente et al., 1998). Carter et al. (1994) demonstrated that for every 1 g of protein consumed, 0.83 g of endogenous protein was synthesized; however, only about 30% of the ingested protein was actually retained as growth (Bowen, 1987). The effects of starvation, refeeding and dietary carbohydrate level on muscle growth and satellite cell activity have been evaluated in fish and poultry (Chapalamadugu et al., 2009; Fauconneau and Paboeuf, 2000; Halevy et al., 2000; Moore et al., 2005). However, little is known about the influence of protein source, crystalline amino acid or carnosine supplementation on muscle cell growth in rainbow trout

Vilhelmsson et al. (2004) showed that soy diets caused increased protein synthesis rates and Martin et al. (2003) showed that feeding compound plant protein diets resulted in increased protein degradation rates. Recent studies have also demonstrated the effects of nutrition (Chapalamadugu et al., 2009; Fernández-Navaro et al., 2008; Gómez-Requeni et al., 2003, 2004, 2005; Macqueen et al., 2010; Martin et al., 2002; Peragon et al., 1994; Rescan et al., 2007) and genetics (Gabillard et al., 2003; Tymchuk et al., 2009) on the metabolic pathways in muscle that regulate protein synthesis and protein degradation rates on a transcriptional level, and correlations have been made between differential expression of genes and enzyme activities in protein degradation pathways (Cleveland and Weber, 2010; Cleveland et al., 2009; Dobly et al., 2004; Overturf and Gaylord, 2009). There is a need to improve aquaculture production efficiency through the increased use of alternative ingredients that will result in feeding fish in a way that muscle yields are maximized. For our study, the regulation of various genes involved in muscle development and protein degradation in conjunction with the activity of proteolytic enzymes that have previously been determined to correlate with changes in muscle metabolic activity was investigated (Cleveland and Weber, 2010; Cleveland et al., 2009; Dobly et al., 2004; Johansen and Overturf, 2005, 2006; Overturf and Gaylord, 2009; Rescan et al., 2007; Salem et al., 2005a, 2005b, 2006a, 2006b;).

As fish meal levels in rainbow trout feeds are reduced, the dietary loss of water soluble nitrogen compounds may contribute to growth reductions. It is theorized that piscivorous fish obtain the bulk of their carnosine through dietary means and it is logical that supplementing this nutrient could improve growth performance of rainbow trout fed plant based diets that are devoid of carnosine. The objectives of this feed study were to determine the effects of dietary carnosine supplementation to an all plant-protein diet on growth performance and white skeletal muscle development in rainbow trout. A 9-week feeding study was conducted comparing plant protein diets devoid of carnosine to a fish meal control diet.

2. Materials and methods

2.1. Diet formulation, experimental design and animal husbandry

Four cooking extruded diets were formulated: 1) diet FM, containing 100% fish meal protein (47.9% CP, 22.73 MJ kg^{-1} GE), 2) diet SPI, replacing 100% of the fish meal with soy protein isolate (48.7% CP, 24.14 MJ kg^{-1} GE), 3) diet SPI + AA, diet SPI supplemented with 0.7% met, 0.31% lys, 0.51% thr and 1.05% gly (50.2% CP, 24.04 MJ kg^{-1} GE) and 4) diet CSN, diet SPI + AA supplemented with 0.39% carnosine (CSN) (50.4% CP, 24.08 MJ kg^{-1} GE) (Table 1). All diets were manufactured using a twin-screw cooking extruder (DNDL-44, Buhler AG, Uzwil, Switzerland) with an 18 s exposure to an average of 127 °C in the six extruder barrel sections. The die plate was water cooled to an average temperature of 60 °C and pressure at the die head averaged 260 psi. The 3.0 mm pellets were then dried in a pulse-bed air drier (Buhler AG, Uzwil, Switzerland) for 25 min at 102 °C with a 10 minute cooling period. Final moisture levels were less than 7%. All oil was included in the mix rather than top-coated.

In that 100% of the fish meal was replaced with isolated soy protein in the three isolated soy protein-based diets (SPI, SPI + AA, and CSN), a

Table 1
Diet formulations, proximate composition and amino acid composition of experimental diets on an as fed basis (g kg^{-1}).

	Diet designations			
	FM	SPI	SPI + AA	CSN
Ingredient				
Mexican sardine meal	601.5	0	0	0
Soy protein isolate [a]	0	545	537.2	537.2
Wheat flour	245.8	193.2	175.3	171.4
Menhaden fish oil	136.7	199	199	199
Stay-C 35 [b]	3	3	3	3
Choline Cl, 60%	4	4	4	4
Vitamin premix 30 [c]	8	8	8	8
Trace mineral premix 3 [d]	1	1	1	1
Dicalcium phosphate 21%	0	46.8	46.8	46.8
DL-methionine [e]	0	0	7	7
Lysine HCL [e]	0	0	3.1	3.1
Threonine [e]	0	0	5.1	5.1
Glycine [e]	0	0	10.5	10.5
Carnosine [e]	0	0	0	3.9
Analyzed composition as fed basis (g kg^{-1})				
Protein (N x 6.25)	445.9	451.9	474.6	494.2
Gross energy (MJ kg^{-1})	22.73	24.14	24.04	24.08
Crude lipid	190.3	192.7	190.6	195.7
Amino acid concentration as fed basis (g kg^{-1} diet)				
Asparagine/aspartic acid	38.8	50.4	51.8	54.3
Glutamine/glutamic acid	68.9	91.5	93.6	96.1
Serine	18.9	23.7	22.7	24.6
Threonine	17.8	16.4	21.4	24.6
Lysine	27.7	24.3	27.1	27.1
Arginine	26.3	32.7	33.3	32.9
Histidine	8.2	11.1	11.3	13.8
Carnosine [f]	1.7	*	*	6.5
Taurine	0.2	0.0	0.0	0.0
Phenylalanine	16.5	22.6	23.2	23.9
Tyrosine	11.7	15.1	15.2	15.6
Valine	19.5	19.3	22.0	20.9
Leucine	30.2	34.7	36.0	36.4
Isoleucine	16.5	18.9	21.2	20.3
Alanine	27.2	18.7	19.1	19.6
Methionine	11.1	6.1	12.3	12.7
Glycine	33.3	19.1	29.9	30.7
Proline	22.9	24.2	26.3	27.3
Cystine	3.9	5.4	5.4	5.5
Tryptophan	4.5	6.2	6.3	6.7
Sum	408.2	440.4	478.1	506.5

[a] ARDEX AF, Archer Daniel Midland Co., Decatur, IL, USA.
[b] Rovimix Stay-C 35, L-ascorbyl 2-polyphosphate, 35% ascorbic acid activity, DSM Nutritional Products, Switzerland.
[c] Contributed per kilogram of diet: vitamin A (as retinol palmitate), 10,000 IU; vitamin D_3, 720 IU; vitamin E (as DL-α-tocopherol acetate), 530 IU; niacin, 330 mg; calcium pantothenate, 160 mg; riboflavin, 80 mg; thiamin mononitrate, 50 mg; pyridoxine hydrochloride, 45 mg; menadione sodium bisulfate, 25 mg; folacin, 13 mg; biotin, 1 mg; vitamin B_{12}, 30 μg.
[d] Contributed in mg kg^{-1} of diet: zinc, 37; manganese, 10; iodine, 5; iron 3; copper, 1.
[e] Sigma-Aldrich Co., St. Louis, Missouri, USA.
[f] Asterisk indicates carnosine detected below 5 nmol ml^{-1} limit of quantification.