Amino acid composition of Arctic charr, *Salvelinus alpinus* (L.) and the prediction of dietary requirements for essential amino acids

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Abstract

Embryo somatic tissues, non-somatic yolk-sac materials, and whole, individual fingerlings (age 0+) of Arctic charr, *Salvelinus alpinus* (L.), as well as a commercial trout diet, were analysed for a wide spectrum of amino acids. Analytical material consisted of prefeeding swim-up fry that were separated into discrete yolk sac and somatic embryo tissue samples. Amino acid concentrations in fry somatic tissue and whole fingerlings were generally very similar to each other, but were lower than those measured in yolk materials. Higher correlations were observed between the majority of specific amino acid concentrations in the trout diet when compared with fingerling data ($r^2 = 0.91$) and fry somatic tissue data ($r^2 = 0.89$), than when correlated with fry yolk sac material ($r^2 = 0.76$). These results indicate that the essential amino acid profiles of fry somatic tissue and whole fingerlings are closer to that of a commercial feed than they are to the endogenous profiles found in the embryonic yolk sac material itself. The dietary ratios of individual essential amino acids were also compared with the total essential amino acid concentrations (A/E ratios) in whole fingerling tissues, and these ratios could be used to accurately estimate the apparent essential amino acid requirements of Arctic charr. The rationale for using carcass amino acid composition data to estimate the dietary essential amino acid requirements of Arctic charr is discussed.

KEY WORDS: amino acid, Arctic charr, dietary requirements *Salvelinus alpinus*

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Introduction

To supply the appropriate quantity and quality of dietary proteins and amino acids (AA) to allow for growth optimization in fish culture, a clear understanding of their requirements is essential. When compared with most mammals, fish, and particularly young immature fish, are thought to have a somewhat higher requirement for essential amino acids (EAA) (Conceiãço *et al*. 2003b). Arctic charr, *Salvelinus alpinus* (L.) is a relatively new aquaculture species in development, and there is little published information about its nutritional requirements. Several studies have been carried out to determine the protein requirements of Arctic charr (Jobling & Wandsvik 1983; Tabachek 1986; Gurure *et al*. 1995), but individual EAA requirements have not been precisely determined (Simmons *et al*. 1999; Johnston 2002). Consequently, Arctic charr diets are generally formulated on the basis of the EAA requirements of other salmonids, particularly rainbow trout, *Oncorhynchus mykiss* (Walbaum) and Atlantic salmon, *Salmo salar* (L.).

According to the National Research Council (NRC) (1993), many fish diets are still formulated on the basis of A/E ratios of different reference proteins, primarily because EAA requirements have not been determined for most species being used in aquaculture. A/E ratios which are commonly mimicked include those of casein: gelatin mixes, whole chicken egg, fish meal: soybean: ground nut meal composite, fish meal, zein and whole trout egg, among many others (Robinson 1992). Phillips & Brockway (1956) suggested that the nutritional value of dietary protein depends on the similarity of its AA profile to that of the animal being fed. For this reason, knowledge of either the fish's whole body or embryonic AA profiles is increasingly being used to determine the dietary EAA requirements for many fish species. Furthermore, high correlations have been observed between dietary EAA requirements and whole body A/E ratios for

several fish species (Cowey & Tacon 1983; Wilson & Poe 1985; Wilson 1994). Although Phillips & Brockway (1956) did not take into account AA turnover rates or maintenance of metabolic demands, they clearly demonstrated that the A/E ratios of egg or body protein often correspond to the A/E ratios required in the diet. However, there is some question about the use of embryo A/E ratios as an indicator of dietary EAA requirements. This method has been found to be less predictive than methods that compare indicators based on carcass and whole body ratios (Wilson 1994; Mambrini & Kaushik 1995). No information is currently available on either the embryo or whole body AA composition of Arctic charr, and such data may provide a relevant starting point for determining specific EAA requirements for this emerging aquaculture species.

The present study was designed to determine the AA composition of non-somatic yolk sac (YS) materials, fry somatic tissues (ST) and whole body (WB) fingerlings of Arctic charr, and to compare these with a commercial rainbow trout diet (TD) formula that is commonly employed in the production of this species. The relationship between the EAA concentrations in various fish tissues and those of the trout diet on which they were fed, was also determined and compared with other values reported for chinook salmon, *Oncorhynchus tshawytscha* (Walbaum) and chum salmon *Oncorhynchus keta* (Walbaum) (NRC 1993). Finally, the theoretical EAA requirements of Arctic charr were estimated using whole body EAA compositional data.

Materials and methods

Sample collection and preparation

Samples of fry non-somatic YS material, fry ST and fingerling WB from a Labrador strain of Arctic charr were obtained from fish cultured at the Alma Aquaculture Research Station, University of Guelph, Ontario, Canada. Fingerlings were fed a commercial rainbow TD produced by Martin Mills Inc., Elmira, Ontario, Canada prior to sampling. Approximately 150 swim-up fry (~0.01 g per fish) were killed with an overdose of tricaine methanesulphonate (150 mg L^{-1} MS-222) and the YS were separated from the remaining fry ST using a pair of forceps under a dissecting microscope. This resulted in two separate tissue sample pools (YS and ST). Individual 0+ year old fingerling fish (20–35 g) were randomly sampled, and killed in MS-222. The intestines were removed and the gut contents were flushed out prior to obtaining the pooled WB sample. Samples of the employed commercial trout grower diet constituted the TD sample. Three subsamples from each of the pooled YS, pooled ST, pooled WB and the TD samples were then prepared for AA analyses. The YS, ST and WB samples were homogenized using a mortar and pestle, and distilled water was added to make a homogeneous slurry. The tissue samples were then freeze-dried and re-homogenized to a fine powder prior to AA analysis. TD samples were finely ground (mesh size = 0.5 mm) using a mortar and pestle using a similar procedure.

Analytical procedures

Prepared tissue and feed samples were hydrolysed in a vacuum chamber containing 6 M HCl at 110 °C for 24 h prior to AA analysis. AA concentrations were determined using high-performance liquid chromatography (HPLC) techniques (Beckman Model 120C AA analyser) after post-column derivatization with ninhydrin (ion exchange technique), following the procedures described by Mason (1980). As acid hydrolysis oxidizes and breaks down tryptophan and cystine, their specific concentrations were not determined.

Calculations

The concentration of each specific AA was expressed relative to the total AA content of the sample ($g_{(specific\,AA)}\ kg^{-1}_{(total\,AA)}$). Whole body AA concentrations were used to compute sample EAA ratios (A/E), as per the following formula (Arai 1981):

$$\text{A/E ratio} = \frac{\text{Individual EAA}_{\text{content}}}{\text{Total EAA}_{\text{content}}} \times 1000$$

The EAA requirements of Arctic charr were estimated using the EAA composition of the fingerling WB determined in this study.

Data analysis

The experiment was a completely randomized design with YS, ST, WB and TD as treatment units, each replicated three times. Mean AA concentrations were compared using analysis of variance (ANOVA). Statistical Application Software (version 8e, SAS Institute Inc.) was used for all statistical analyses; significance level was $P < 0.05$. The following statistical model was used to analyse the response data:

$$Y_{ijk} = U + T_i + R_j + E_{ijk}$$

where Y_{ijk} is the observed response, U is the overall mean value, T_i is the effect of the i th body tissue or diet ($i = 1, 2, 3,$

4), R_j is the effect of the j th replicate ($j = 1, 2, 3$) and E_{ijk} is the random experimental error.

Differences among treatments were assessed using multiple range tests (Duncan 1955) and were considered significant at a level of $\alpha = 0.05$. The experimental data was compared with information reported for other species. EAA compositions of the various samples were subjected to regression analysis to determine their relationships with EAA requirements for chinook salmon, *O. tshawytscha* (Walbaum) and chum salmon, *O. keta* (Walbaum) (NRC 1993). Arctic charr tissue A/E ratios were also regressed against the TD A/E ratios.

Results

The AA composition of Arctic charr tissues and the TD are presented in Table 1. AA concentrations were not significantly different among YS, ST and WB for proline only, while similarities between ST and WB were observed for threonine, glutamine, methionine, leucine and phenylalanine. AA composition of YS was generally different from that of ST and WB, being higher for threonine, sereine, alanine, valine, isoleucine, leucine, tyrosine and phenylalanine but lower for asparagine, glutamine, glycine, methionine and arginine. The AA composition of TD was also generally different from that of YS, ST and WB tissues. Correlations between the AA composition of TD and ST ($r^2 = 0.91$) or TD and WB ($r^2 = 0.89$) were higher than those between TD and YS ($r^2 = 0.76$).

The A/E ratios for EAA requirements of chinook salmon, chum salmon and the different tissues in the present study are shown in Table 2. Regression analysis demonstrated higher correlations between A/E ratios of chinook salmon EAA requirements with the A/E ratios of ST ($r^2 = 0.40$), or with that of the WB pattern ($r^2 = 0.39$), than compared with that of YS ($r^2 = 0.19$). Table 3 details the AA composition of the various charr tissues as well as those reported by other researchers for several different fish species. These data indicate that AA concentrations and ratios for Arctic charr determined in the present study are similar to those reported for several other fish species.

Discussion

Based on the premise that the primary role of dietary AA is to synthesize body proteins, Phillips & Brockway (1956) suggested that the nutritional value of dietary protein depends on its similarity to the AA profile of the animal being considered. Although this concept does not account for the AA used for maintenance of metabolic demands, it is a reasonable starting point when attempting to define dietary EAA requirements. Our results, as well as previous research, shows an empirical relationship between EAA requirements of fish and the pattern of those same AA in eggs (embryo), or WB protein content (Kanazawa *et al.* 1989; Mambrini & Kaushik 1995). Beneficial outcomes of this information, such as increased growth rates, have been achieved by using WB A/E ratios of several fish species to formulate their diets. Rumsey & Ketola (1975) used A/E ratios of WB and fish eggs to establish EAA supplementation levels in casein and soybean-based diets for Atlantic salmon and rainbow trout, respectively. Arai (1981) observed improved growth in coho

Table 1 Amino acid (AA) composition [$g_{(specificAA)}$ $kg^{-1}_{(totalAA)}$] of Arctic charr yolk sac (YS), somatic tissue (ST), whole body and a rainbow trout grower diet[1]

AA	Fry YS	Fry ST	WB	Trout diet	*P*-value
Asparagine[2]	94 ± 1.1^{c}	101 ± 4.2^{b}	112 ± 2.5^{a}	99 ± 2.0^{b}	0.0004
Threonine	54 ± 0.6^{a}	50 ± 0.3^{b}	50 ± 0.3^{b}	45 ± 0.3^{c}	0.0001
Serine	65 ± 0.9^{a}	54 ± 0.7^{b}	52 ± 0.2^{c}	64 ± 2.1^{a}	0.0001
Glutamine[2]	124 ± 1.3^{c}	155 ± 1.3^{b}	157 ± 4.4^{b}	180 ± 4.7^{a}	0.0001
Proline	62 ± 3.8^{b}	63 ± 1.5^{b}	61 ± 2.6^{b}	71 ± 2.9^{a}	0.0150
Glycine	30 ± 1.0^{d}	62 ± 1.2^{b}	71 ± 3.6^{a}	57 ± 2.1^{c}	0.0001
Alanine	88 ± 0.9^{a}	65 ± 0.3^{c}	70 ± 0.5^{b}	69 ± 1.2^{b}	0.0001
Valine	58 ± 1.7^{a}	44 ± 0.2^{c}	41 ± 0.7^{d}	47 ± 2.1^{b}	0.0001
Methionine	21 ± 0.5^{b}	27 ± 0.1^{a}	28 ± 0.8^{a}	22 ± 1.7^{b}	0.0001
Isoleucine	41 ± 1.6^{a}	33 ± 0.2^{b}	31 ± 0.2^{c}	27 ± 0.3^{b}	0.0001
Leucine	92 ± 1.7^{a}	75 ± 0.4^{b}	70 ± 6.7^{b}	89 ± 2.0^{a}	0.0003
Tyrosine	49 ± 1.4^{a}	38 ± 3.2^{b}	31 ± 0.8^{c}	30 ± 1.0^{c}	0.0001
Phenylalanine	57 ± 0.8^{a}	48 ± 0.9^{c}	48 ± 0.9^{c}	56 ± 0.7^{b}	0.0001
Lysine	86 ± 1.2^{b}	91 ± 1.4^{a}	89 ± 2.6^{ab}	63 ± 2.6^{c}	0.0001
Histidine	23 ± 0.4^{c}	24 ± 0.8^{c}	25 ± 0.5^{b}	27 ± 0.2^{a}	0.0003
Arginine	56 ± 0.5^{c}	69 ± 0.8^{a}	63 ± 0.9^{b}	55 ± 1.2^{c}	0.0001

[1] Values (mean ± SE, $n = 3$) in the same row that do not share common superscript letters are significantly different ($P < 0.05$).
[2] Asparagine and Glutamine also include concentrations for cystine and tryptophan, respectively.

Table 2 Essential amino acid (EAA) requirements (g kg^{-1}) and A/E ratios for chinook salmon, chum salmon, Arctic charr (fry yolk sac, fry whole body, fingerling whole body) and rainbow trout diet

	Chinook salmon[1]		Chum salmon[2]		Arctic charr[3]				Rainbow trout
					Fingerling whole body		Fry yolk sac	Fry whole body	Diet
EAA	g req. kg^{-1} diet	A/E ratio*	g req. kg^{-1} diet	A/E ratio*	g req. kg^{-1} diet	A/E ratio*	A/E ratio*	A/E ratio*	A/E ratio*
Arginine	24	180	26	177	24	141	114	150	128
Histidine	7	54	7	47	9	56	48	51	63
Isoleucine	9	66	10	71	12	70	85	72	62
Leucine	16	117	15	112	26	156	188	163	207
Lysine	20	150	19	142	34	201	177	197	148
Methionine	16	120	12	89	11	64	44	59	51
Phenylalanine	21	153	25	186	18	108	117	103	129
Threonine	9	66	12	89	19	112	110	109	105
Valine	13	96	12	89	16	92	119	95	108

[1] EAA requirements of chinook salmon expressed as per cent of a 380 g CP kg^{-1} diet (NRC 1993).
[2] EAA requirements of chum salmon expressed as per cent of a 400 g CP kg^{-1} diet (NRC 1993).
[3] EAA requirements for Arctic charr expressed as per cent of a 380 g CP kg^{-1} diet, estimated from fingerling whole body (WB) A/E ratios.
* A/E ratio = [essential amino acid concentration (g)/total essential amino acid concentration (g)] × 1000.

Table 3 Amino acid composition of body tissues of Arctic charr compared with published values for other fishes (g kg^{-1})

Essential amino acid	Goldfish[1]	Golden shiner[1]	Fathead[1] minnow	Catfish[2]	Atlantic salmon[3]	Rainbow trout[4]	Coho salmon[4]	Cherry salmon[5]	Charr somatic tissue[6]	Charr whole body[6]
Arginine	68	64	64	67	66	64	60	62	69	63
Histidine	26	26	25	22	30	29	30	24	24	25
Isoleucine	40	41	42	43	44	43	37	39	33	31
Leucine	75	76	78	74	77	76	75	75	75	70
Lysine	86	88	89	85	93	85	86	88	91	89
Methionine	31	32	30	29	18	29	35	31	27	28
Phenylalanine	41	45	46	41	44	44	41	46	48	48
Threonine	47	50	52	44	50	48	51	46	50	50
Tryptophan	9	9	11	8	9	9	14	8	–	–
Valine	45	44	44	52	51	51	48	49	44	41

[1] Data from Gatlin (1987).
[2] Data from Wilson & Poe (1985).
[3] Data from Wilson & Cowey (1985).
[4] Data from Arai (1981).
[5] Data from Ogata *et al.* (1983).
[6] Data from present study.

salmon, *Oncorhynchus kisutch* (Walbaum), when fed diets containing supplemental EAA at levels comparable with its WB A/E ratios. Similarly, Ogata *et al.* (1983) reported improved fry growth for cherry salmon, *Oncorhynchus masou* (Brevoort), and amago salmon, *Oncorhynchus rhodurus* (Jordan & McGregor), when fed purified diets supplemented with EAA levels simulating the A/E ratios of cherry salmon embryos.

The observed similarities between the AA composition of Arctic charr ST and WB fingerlings supports previous findings (Wilson & Poe 1985; Ng & Hung 1994) and suggests that there are very few differences in AA composition among different sizes of fish within the same species. This implies that the AA composition of a species (as a function of its genetic make-up) does not likely change dramatically with age or size. This is however, not always the case. Differences in the AA content of different sized Siberian sturgeon (*Acipenser baeri*) (Kaushik *et al.* 1991) and dolphin fish (*Coryphaena hippurus*) (Ostrowski & Divakaran 1989) have been reported.

Given the variability in methods used to formulate fish diets, it is important to note that research suggests that

carcass and WB A/E ratios are better indicators of EAA dietary requirements than are egg A/E ratios (Wilson 1994; Mambrini & Kaushik 1995). In general, the AA composition is more variable in eggs as compared with WB data (Wilson 1994). With that said, the AA composition of eggs has also provided useful data and guidance for the formulation of diets for Atlantic salmon and rainbow trout (Ketola 1982).

Within the family salmonidae, current and previous findings (Table 3) indicate that AA compositions across a range of species are generally quite similar. Arctic charr patterns observed in the present study compared favourably with chinook salmon data (Wilson & Poe 1985). However, Wilson & Poe (1985) also reported a higher correlation ($r^2 = 0.96$) between WB A/E ratios and the A/E ratio requirement in chinook salmon as compared with channel catfish ($r^2 = 0.68$).

Whole body A/E ratios appear to be suitable indicators of EAA requirements for a number of fish species. In the present study, and in Wilson & Poe (1985), the highest correlations were observed between WB A/E ratios and dietary A/E ratios (in the commercial feed). Wilson (1991) reported reasonable agreement between the EAA requirements for catfish, as estimated using an ideal protein procedure (100% true digestibility and 100% biological value), and those determined directly by dose–response experiments. The estimates based on the ideal protein procedure were calculated using catfish WB A/E ratios in conjunction with lysine requirements estimated from a dose–response experiment. Using the relative amounts of EAA in the carcass of a fish alone to predict dietary requirement, necessarily assumes that all EAA ingested are used for growth. This approach is questionable when applied to developing larvae, as their WB EAA levels may include a significant proportion of free EAA, which are only partly used for protein synthesis (Rønnestad & Fyhn 1993).

Millward (1994) compared the AA patterns required for tissue protein, growth and routine metabolic demands, observing that the AA pattern required for the growth for equivalent amounts of protein is similar to the tissue protein pattern itself. However, maintenance patterns were only similar for the sulphur AA, arginine, histidine and tryptophan, and were slightly lower for threonine, and lower still for lysine, leucine, valine and isoleucine. These results suggest that tissue AA profiles could be used to reasonably predict dietary requirements for the sulphur AA, histidine, tryptophan and arginine, but are less useful for the determination of lysine and the branched chain AA. Our estimates of EAA requirements for Arctic charr are similar to those values reported in chinook and chum salmon for arginine, methionine and histidine (NRC 1993). This also explains the slightly higher values in charr for valine, threonine, lysine, leucine and isoleucine.

It is important to note that published research sometimes reports different EAA requirements even for a single species. For example, lysine requirements for rainbow trout have been reported to range from 13 to 29 g kg^{-1} diet (NRC 1993). According to Kim *et al.* (1992), these differences may be a result of laboratory variations, including fish size, temperature, and experimental diets composition, and/or differences in metabolic losses which may be due in part, to variations in physiological conditions encountered in various experiments or because of genetic differences in the test stock of fish being used. These factors affect the metabolic status of the fish, thereby directly or indirectly influencing the efficiency of protein accretion and growth, and consequently, affecting estimates of nutrient requirements. Thus, EAA requirements can be expected to differ according to the energy needs required to satisfy routine metabolic demands of fish cultured under different conditions. Consider also that AA requirements for catabolism can be substantial for larval fish. After the onset of first feeding, AA may account for 60% or higher of metabolic energy dissipation (Rønnestad *et al.* 1999). Although AA profiles for larger fish remain relatively constant between, and within species (Wilson 1994), considerable variance is observed in AA profiles for various larval fishes. This may be related to differences in allometric growth as different tissues develop at different times and rates throughout ontogeny (Oikawa & Itazawa 1984; Osse & van den Boogaart 1995; Conceiãço *et al.* 2003b). It is essential that these conditions be properly defined so that dietary requirement values can be interpreted correctly.

The present study estimated the dietary EAA requirements of young Arctic charr. These values did not take into account metabolic losses, or the digestibilities or efficiencies of utilization of any individual EAA. When expressed as a percentage of diet, the values for methionine, arginine, isoleucine, threonine and histidine, are generally similar to EAA requirements reported for chinook and chum salmon (NRC 1993). In contrast, the current estimates for lysine and leucine requirements are much higher than the reported values for chinook and chum salmon (NRC 1993). Similarly, Ostrowski & Divakaran (1989) observed higher A/E ratios for lysine and leucine, as well as phenylalanine and arginine, in the dolphin fish, *Coryphaena hippurus* (L.). These A/E ratios were derived from daily EAA requirements for turnover in muscle, gills and the gastrointestinal

tract, based on metabolic demand estimates (Fauconneau & Arnal 1983). Interestingly, Ostrowski & Divakaran (1989) noted that methionine, an EAA involved in complex metabolic pathways, did not exhibit a high metabolic rate A/E ratio. Similar observations by Fuller *et al*. (1989) show maintenance patterns for the sulphur AA to be similar to WB patterns.

Finally, work by Wilson (1991) demonstrates that there is a strong case for promoting this 'ideal protein' approach for determining the EAA requirements in fish. It will be beneficial to compare estimates of EAA requirements in the current study with values determined from future empirical dose–response studies. Conceiãço *et al*. (2003b) encouraged further studies to elucidate those factors which could affect the relative requirements and bioavailabilty of individual AA, in order to further improve requirement estimates. New methods, such as those proposed by Conceiãço *et al*. (2003a) will be helpful to this end. Considerable savings in research resources may be achieved if approaches like the ideal protein concept are applied to the determination of EAA requirements of non-ruminant animals.

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