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Full Length Research Paper

Whole body and egg amino acid composition of Nile perch, *Lates niloticus* (Linnaeus, 1758) and prediction of its dietary essential amino acid requirements

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Eggs and tissue of Nile perch juveniles were analysed in order to derive the essential amino acid dietary requirements for Nile perch larvae and juveniles, using the A/E ratio. Results revealed the presence of both essential and non-essential amino acids in Nile perch. A significant difference ($p < 0.05$) between the amino acids (AA) composition in the eggs and tissue and amongst the four class sizes of juveniles was observed. Estimates of the amino acid dietary requirements revealed that Nile perch has high arginine, leucine, threonine, valine and isoleucine dietary requirements.

Key words: Nile perch, amino acids, dietary requirements, larvae, juveniles.

INTRODUCTION

Nile perch (*Lates niloticus*) is a freshwater carnivorous fish and a couple of authors have reported its predatory effect on several indiginous fish species (Goldschmidt et al., 1993; Ogutu-Ohwayo, 1993). However, this fish is currently of great social economic importance in the East African region (Gumisiriza et al., 2009; Beuving, 2010). The Nile perch fishery is however under threat due to the intensive fishing pressure on the fishery that has resulted in a tremendus decline of Nile perch populations (Munyaho, 2004; Njiru et al., 2009). Current strategies for increasing Nile perch production point towards the culture of this species (Gregory, 2006); however, the sustainability of the Nile perch culture programe will greatly depend on the development of Nile perch aquaculture feeding technologies.

Proteins are the most expensive component of fish feed (Meyer and Fracalossi, 2005) and directly affect fish weight gain (Martinez-Palacios et al., 2007; Zuanon et al., 2009). Amino acid assays have been widely done to accurately determine the protein requirements for the aquaculture of different fish species (Kaushik, 1998; Conceição et al., 2003), to reduce wastage and pollution during culture. Several methods have been used to estimate the amino acid dietary requirements in fishes: the dose respose method (Tibaldi and Lanari, 1991; Yokoyama and Nakazoe, 1992; Fournier et al., 2002), the daily increment method (Martinez-Palacios et al., 2007), and the whole body tissue A/E ratio method (Mohanty and Kausik, 1991; Hossian et al., 2011). The A/E ratio is the concentration of each essential amino acid as a percentage of the concentration of total essential amino acids including tyrosine. This method has become popular due to the ease of its application and its direct relationship to the amino acid (AA) profile in fish because it gives important data for establishing AA baselines in the dietray requirements in fish (Cowey and Tacon, 1983; Wilson and Cowey, 1985; Gatlin, 1987.

Wilson and Poe (1985) have also observed a significant correlation ($r = 0.96$) between the estimated essential amino acids (EAA) and the AAs in the whole body tissue of Channel catfish *Ictalurus punctatus*. This correlation has been widely used to estimate the dietary

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Abbreviations: EAADR, Essential amino acids dietary requirements; **PITC,** phenylisothiocyanate; **PTC,** phenyltiocarbamyl.

EAA requirements in fishes, such as the silver pomfret *Pampus argenteus* (Hossain et al., 2011), the brook trout *Salvelinus alpinus* (Gurure et al., 2007) and the South American catfish *Rhamdia quelen* (Meyer and Fracalossi, 2005). This method is popular because it is less expensive and it quickly provides results, compared to the dose-response experiments (Akiyama et al., 1997), especially where no data on the quantitative AA requirements are known as in the case for Nile perch.

Studies have refined diets based on the A/E ratios of the whole body AA profiles, following the A/E ratio concept, where A/E = [(individual EAA / total EAA including cystine and tyrosine) × 1000], used to formulate test diets for Coho salmon *Oncorhynchus kisutch* (Arai, 1981). Studies of diet formulation further consider lysine as the AA reference by relating it to each AA in the formulated feeds (Miles and Chapman, 2007), because lysine is the least concentrated AA (Craig and Helfrich, 2002). Lacking known lysine concentrations for Nile perch, lysine concentrations were based on percent concentrations in Nile tilapia *Oreochromis niloticus*, 5.12% (Santiago and Lovel, 1988), common carp *Cyprinus carpio*, 5.7% (Nose, 1979), rainbow trout *Salmo gairdneri*, 4.3% (Walton et al., 1984), chinook salmon *Oncorhynchus tshawytscha*, 5.0% (Halver et al., 1958), channel catfish *I. punctatus*, 5.0% (Robinson et al., 1980), Mossambique tilapia *Oreochromis mossambicus*, 3.78% (Jauncey et al., 1983; Jackson and Capper, 1982) to 4.5% (Coloso et al., 1993), and the Asian sea bass *Lates calcarifer*, 4.9% (Glencross, 2004). These lysine values investigated through dose-reponse trials have been used to establish essential amino acid (EAA) requirements in other fish whose requirements are unknown, based on the fact that fish of the same feeding behaviour tend to have similar EAA dietary requirements (Meyer and Fracalossi, 2005). The unknown EAA requirements for Nile perch were therefore derived using similar methods: EAA = [(Determined requirement value of lysine × A/E ratio of individual amino acid) / A/E ratio of lysine] (Kaushik, 1998; Hossain et al., 2011), using 5.0% lysine concentration.

Since the amino acid profiles of Nile perch are currently unknown, the objectives of this study were to apply methods used in previous studies (Hossain et al., 2002; Love, 1980; Ali et al., 2005; Yildiz et al., 2006; Razzaque et al., 2008; Chakraborty and Banerjee, 2009; Aberoumad and Pourshafi, 2010) to estimate the dietary requirements of Nile perch. In particular, we sought to derive diets for larval and juvenile stages of the Nile perch.

MATERIALS AND METHODS

Sample collection

Fish samples for this study were caught from Lake Victoria using a beach seine net (3 inch mesh size in the arms, 1.5 inch in the cod end) at Resort beach (32° 28′ 26″ E, 00° 3′ 51″ N), Uganda. The samples caught were between 1 to 95 cm long (Standard length, SL). The short fish between 1 and 30 cm were divided into four size classes as follows: 1 to 5, 6 to 10, 11 to 20 and 21 to 30 cm, respectively, prior to analysis. The long female fish between 90 and 93 cm were dissected to obtain yolked eggs for analysis.

Crude protein analysis

Oven dried samples of eggs and tissue of fish from each of the size classes (1 to 5, 6 to 10, 11 to 20 and 21 to 30 cm) were ground, sieved and analysed for crude protein using standard Association of Official Analytical Chemists, AOAC (2004) methods.

Amino acid profiling

Egg and tissue samples were analysed by the Pico-Tag method using a Waters Breeze high performance liquid chromatography (HPLC) with Empower software (Waters, Millipore Corp., Milford, MA). This was done by hydrolysing 400 mg of each of the samples with 6 N HCl for 24 h and then reacted with phenylisothiocyanate (PITC) to produce phenyltiocarbamyl (PTC) amino acids. These amino acids were then analysed by reverse phase HPLC. Sodium acetate buffer and acetonitrile-water (60:40) were used in the mobile phases with gradient separation (flow between 1 ml to 1.5 ml/min). Absorbance was detected at 254 nm using a UV detector in Pico-Tag Columns at 45°C. Amino acid standard H from Pierce (Prod no: 20088) and Internal Standard: L-α-AMINO-n-BUTYRIC ACID Sigma (A-1879) were used as standards.

For the determination of cystine and methionine, the samples were oxidized with performic acid over night at 0°C. Performic acid is an oxidizing reagent that converts cysteine quantitatively to cysteic acid and methionine to methionine sulfone. The reactions were stopped with hydrogen bromide and dried. This was followed by sample hydrolysis using 6 N HCl for 24 h and then reacting with phenylisothiocyanate (PITC) to produce phenyltiocarbamyl (PTC) amino acids. These amino acids were then analysed by reverse phase HPLC. Sodium acetate buffer and acetonitrile-water (60:40) were used in the mobile phases with gradient separation (flow between 1 ml to 1.5 ml/min). Absorbance was detected at 254 nm using a UV detector in Pico-Tag Columns at 45°C. Cysteic acid-Sigma (C-7630) and Methionine sulfone-Sigama (M-0876) were used as standards.

To identify tryptophan, 400 mg samples were hydrolysed under alkaline conditions with a saturated-barium hydroxide solution and heated to 110°C for 16 h, using DL-tryptophan-99+% (Cat No 16.269-8, Aldrich Sigma) as the standard. The hydrolysates were analysed by reverse phase liquid chromatography with UV detection at 285 nm, using a Waters Breeze HPLC with Empower software (Waters, Millipore Corp., Milford, MA). Duplicate samples were hydrolysed for two days. Spectrophotometeric absorbance was detected at 285 nm using an ultra violet (UV) detector (Waters 2487 Detector).

A/E ratio and estimating EAA dietary requirements

The A/E ratios of the EAA composition of whole body and yolked eggs were calculated using the method of Arai (1981) and Hossani et al. (2011): A/E ratio = [(Individual EAA content / Total EAA content) × 1000]. The EAA dietary requirement of Nile perch was estimated based on the known 5.0% lysine requirement per 100 g protein as in *L. calcarifer* (Glencross, 2004), using the formula suggested by Kaushik (1998). In this formula, EAA requirement = [(determined requirement value for lysine × A/E ratio of individual amino acid) / A/E ratio for lysine.

Table 1. Percentage amino acid composition (g/100g) of eggs and tissue of whole Nile perch of different size classes (cm), n = 10.

Parameter	Egg	Amino acid composition (g/100 g) in fish of varying class sizes (cm)				Statistical F	Difference among class sizes
		1-5 cm	6-10 cm	11-20 cm	21-30 cm		
Essential amino acids							
Tryptophan	0.28±0.01	0.50±0.03	0.53±0.00	0.53±0.01	0.45±0.01	43.78	*
Methionine	0.86±0.01	1.35±0.01	0.73±0.73	1.49±0.04	1.43±0.01	1.17	**
Cystine	0.36±0.01	0.55±0.03	0.28±0.28	0.58±0.01	0.61±0.00	1.35	**
Histidine	0.92±0.01	1.41±0.01	1.45±0.00	1.35±0.01	1.31±0.01	514.07	*
Valine	1.93±0.04	2.76±0.00	2.88±0.00	2.63±0.04	2.61±0.03	160.06	*
Isoleucine	1.73±0.01	2.44±0.03	2.50±0.04	2.29±0.01	2.32±0.03	130.97	*
Leuine	2.91±0.01	4.58±0.03	4.73±0.06	4.37±0.00	4.32±0.01	630.85	*
Tyrosine	1.27±0.00	2.05±0.01	2.07±0.05	1.98±0.06	1.93±0.05	64.45	*
Arginine	2.76±0.03	6.37±0.04	6.12±0.14	5.61±0.11	5.27±0.12	210.55	*
Threonine	1.59±0.04	2.65±0.02	2.67±0.02	2.46±0.03	2.56±0.01	530.39	*
Phenylalanine	1.47±0.02	2.63±0.02	2.68±0.02	2.49±0.02	2.42±0.03	440.27	*
Lysine	2.37±0.00	3.18±0.24	2.80±0.36	2.37±0.00	2.20±0.09	4.11	**
Non-essential amino acids							
Aspartic acid	2.65±0.01	5.71±0.01	5.47±0.10	5.22±0.11	5.04±0.00	336.06	*
Proline	1.75±0.01	3.30±0.00	3.27±0.37	2.95±0.42	2.70±0.07	269.97	*
Glutamic acid	4.72±0.10	8.85±0.14	9.27±0.00	8.56±0.05	8.29±0.00	537.82	*
Serine	1.60±0.01	2.61±0.04	2.65±0.01	2.49±0.00	2.48±0.03	429.63	*
Glycine	1.64±0.01	5.12±0.04	5.03±0.02	4.52±0.01	3.89±0.71	1440.02	*
Alanine	2.48±0.02	4.31±0.02	4.46±0.10	3.99±0.09	3.75±0.09	110.7	*
Protein crude composition (%)	33.78	64.11	67.81	65.03	59.68		

*Significant difference ($p < 0.05$), **no significant difference ($p > 0.05$).

Statistical analysis

The SPSS statistical software version 16.0 for Windows was used for analysis. Analysis was performed to test the following null hypotheses: 1; there is no significant difference in the concentration of the different AA in Nile perch at different developmental stages, 2; there is no significant difference in the percentage essential amino acid dietary requirements (EAADR) for Nile perch at different developmental stages. Data were expressed as mean percentage AA (g/100 g ± STD). Comparison amongst the mean AA percentages of the different juvenile size classes (1 to 5, 6 to 10, 11 to 20 and 21 to 30 cm) and eggs at $\alpha = 0.05$, followed by one-way ANOVA.

RESULTS

Results revealed the presence of lysine, arginine, histidine, threonine, valine, leucine, isoleucine, methionine, cystine, phenylalaine, tyrosine, aspartic acid, glutamic acid, serine, proline, glycine, alanine and tryptophan in tissue and eggs of Nile perch. All AA found in eggs were at much lower concentrations than those observed in body tissue (Table 1). There was no significant difference ($p > 0.05$) in the concentration of tryptophan, methionine, cystine, tyrosine and lysine amongst the different fish class sizes. However, a significant difference ($p < 0.05$) was observed in the concentration of arginine, histidine, threonine, valine, leucine, isoleucine, phenylalaine, aspartic acid, glutamic acid, serine, proline, glycine, and alanine amongst the different class sizes investigated (Table 1).

Analysis further indicated a significant difference ($p < 0.05$) in the A/E ratio for all the essential amino acids save for tyrosine and leucine (Table 2). Observations also indicated an increase in the dietary demand for all estimated amino acids with increasing length (age), save for lysine, which was kept constant at 5% in all the size classes considered in this investigation as observed in Table 3. Investigations further revealed that Nile perch had higher arginine, leucine, valine, threonine, and lysine dietary requirements compared to tryptophan, cystine and histidine (Figure 1).

DISCUSSION

The amino acid profiles of Nile perch eggs and tissue performed in this study indicate presence of both essential and non-essential amino acids in Nile perch.

Table 2. A/E ratios of eggs and Nile perch tissue of different size classes.

Amino acid	Egg/Larvae	A/E ratio			
		1-5 (cm)	6-10 (cm)	11-20 (cm)	21-30 (cm)
Tryptophan	15.17±0.26	16.24±0.88	17.43±0.06	18.70±0.13	16.38±0.75
Methionine	46.58±0.83	44.40±0.68	47.80±0.05	53.03±1.23	52.10±0.99
Cystine	19.37±0.43	17.90±0.98	18.40±0.02	20.75±0.44	22.10±0.23
Histidine	50.10±0.62	46.27±0.56	47.65±0.61	48.03±0.48	47.70±0.04
Arginine	149.75±1.72	209.05±0.02	201.10±4.89	199.13±2.91	192.08±2.62
Threonine	86.12±0.13	86.99±0.23	87.59±0.82	87.25±1.49	93.45±0.66
Tyrosine	68.88±0.15	67.34±0.82	67.88±1.85	70.20±1.68	70.53±0.97
Valine	104.69±2.11	90.70±0.58	94.75±0.02	93.43±2.09	95.19±2.11
Isoleucine	93.75±0.29	80.12±1.40	82.00±1.44	81.33±0.85	84.43±0.28
Leucine	157.66±0.41	150.31±1.99	155.44±1.20	155.39±0.10	157.42±1.41
Phenyl	79.52±0.98	86.34±1.24	88.08±0.82	88.40±0.23	88.39±0.29
Lysine	128.45±0.06	104.35±7.16	91.88±1.18	84.35±0.60	80.18±2.33

Table 3. Estimated amino acid dietary requirements (using the ideal protein concept) for Nile perch of different size classes, n = 10.

Amino acid	Egg/Larvae	1-5 (cm)	6-10 (cm)	11-20 (cm)	21-30 (cm)	F	Difference among class sizes
Tryptophan	0.59±0.10	0.79±0.01	0.96±0.12	1.11±0.02	1.02±0.77	10.45	*
Methionine	1.81±0.03	2.14±0.18	2.65±0.34	3.14±0.10	3.25±0.16	15.09	*
Cystine	0.75±0.03	0.87±0.11	1.02±0.13	1.23±0.02	1.38±0.05	14.91	*
Histidine	1.95±0.03	2.23±0.18	2.64±0.33	2.85±0.01	2.98±0.08	6.11	*
Arginine	5.83±0.06	10.06±0.69	11.16±1.69	11.80±0.26	11.98±0.21	9.32	*
Threonine	3.35±0.01	4.19±0.23	4.85±0.67	5.17±0.05	5.83±0.21	7.97	*
Tyrosine	2.68±0.01	3.24±0.26	3.77±0.58	4.16±0.13	4.40±0.07	5.66	*
Valine	4.08±0.08	4.37±0.33	5.24±0.67	5.54±0.08	5.95±0.30	4.70	*
Isoleucine	3.65±0.01	3.86±0.33	4.55±0.66	4.82±0.03	5.27±0.14	3.99	**
Leucine	6.14±0.01	7.24±0.59	8.61±1.21	9.21±0.01	9.83±0.37	5.76	*
Phenyl	3.10±0.04	4.16±0.34	4.88±0.67	5.24±0.05	5.52±0.14	8.00	*
Lysine	5.00±0.00	5.00±0.00	5.00±0.00	5.00±0.00	5.00±0.00	0.00	**

*Significant difference ($p < 0.05$), **no significant difference ($p > 0.05$).

Similar profiles have been reported in several fish species as observed in Tables 4 and 5, and this proves the premise that amino acids are an important body composition and requirement in fish. Amino acids are reportedly important as regulators of key metabolic pathways are important for body maintenance, growth, feed intake, nutrient utilization, immunity, behavior, larval metamorphosis, reproduction and resistance to environmental stress and pathogenic organisms in fish (Li et al., 2008). It is therefore important that these are appropriately provided in Nile perch feeds to ensure proper growth of cultured Nile perch.

Results from this study reveal a much lower concentration of all amino acids in the eggs compared to that observed in the body tissue. This finding is contrary to reports made by Hossain et al. (2011), who observed that the fraction of arginine, isoleucine, and valine in eggs is significantly higher than that observed in the body of *Pampus argenteus* and by Ng and Hung (1994) who indicated that isoleucine, leucine, threonine and valine content in the eggs is also higher than that observed in the body in *Acipenser transmontanus*. Both of these reports have suggested that the high amino acid levels observed in the eggs are caused by the relatively high protein content in the eggs (27.4 ± 0.7%, *A. transmontanus*; 48.55% *P. argenteus*) of these fish compared to that available in the tissue (16.9 ± 0.7, *A. transmontanus*; 47.43% *P. argenteus*), yet the reverse is observed in Nile perch in this study. The low amino acid content observed in Nile perch eggs is probably due to

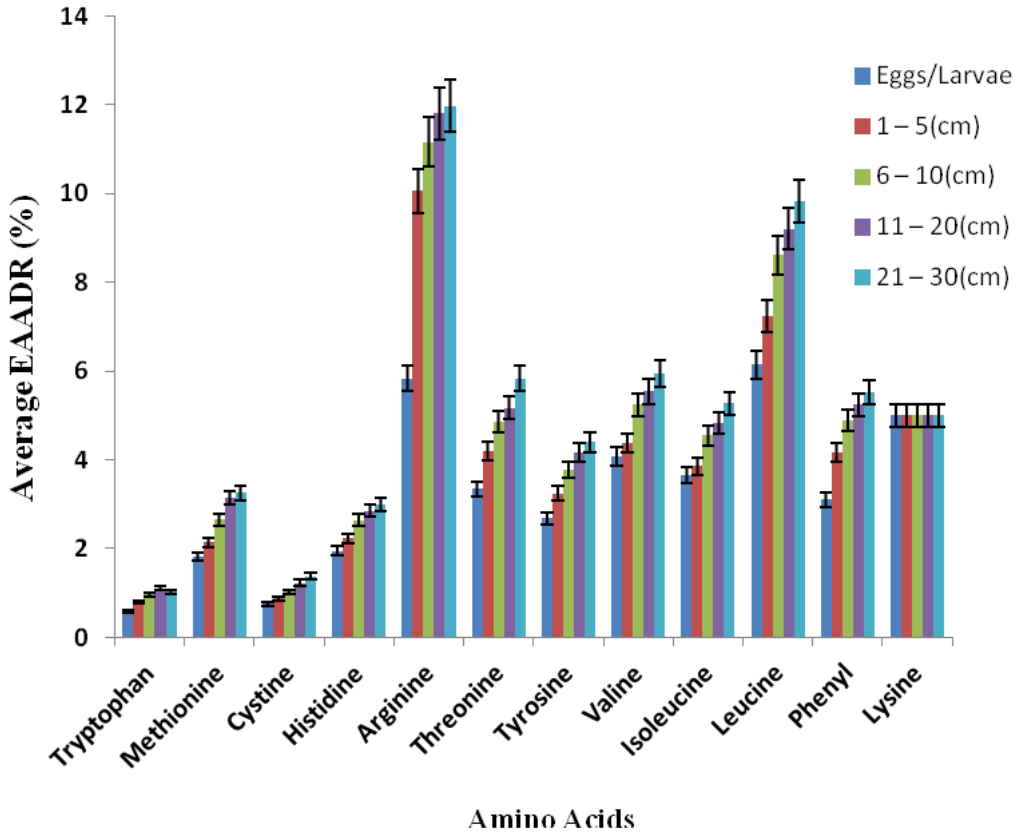

Figure 1. Percentage average EAADR for Nile perch at different developmental stages.

its low protein content (33.78%), compared to that observed in the tissue of this fish (64.11 to 67.81%) in this study.

No major differences were observed in the concentrations of amino acid in the different size classes investigated in this study, though a statistical difference ($p < 0.05$) was observed in the concentration of arginine, histidine, threonine, valine, leucine, isoleucine, phenylalaine, aspartic acid, glutamic acid, serine, proline, glycine, and alanine amongst the different class sizes. This statistical difference is however negligible, given that the concentrations of amino acids in the different size classes occur in the same ranges (Table 1). Studies performed by Meyer and Fracaloss (2005) on *Rhamdia quelen* and by Kaushik (1998) on *Dicentrarchus labrax*, *Sparus aurata* and *Psetta maxima*, indicate similarities among amino acid composition for all fish groups investigated, irrespective of their weight, length or dietary history. This observation suggests that the amino acid concentration in Nile perch is similar at all developmental stages investigated in this study.

The amino acid concentrations observed in Nile perch in this study differ from those observed in several other fishes (Tables 4 and 5), probably due to differences in the analytical procedures used in the different investigations. To establish the EAADR for Nile perch, the A/E ratios were calculated. In these calculations, all the amino acid concentrations, except arginine, tyrosine, and phenyalanine expressed as A/E ratios in Nile perch are similar to those observed in some omnivorous and carnivorous fishes observed in Table 6. This observation is in agreement with results obtained by Meyer and Fracaloss (2005) who compared the amino acid concentration of *R. quelen* to that of other fish species, by Nurullah et al. (2003) who studied the nutritional quality of small fish species in Bangladesh and by Mohanty and Kaushik (1991) who investigated the whole body amino acid composition of Indian major carps. These observations indicate that fishes contain similar amounts of amino acids, since muscle is the major tissue in teleost and is composed of amino acids that do not vary significantly between species, irrespective of their feeding behaviour (Cowey and Luquet, 1983).

The A/E ratios obtained for arginine (149.75 ± 1.72 to 209.05 ± 0.02), tyrosine (67.34 ± 0.82 to 70.53 ± 0.97), and phenyalanine (79.52 ± 0.98 to 88.39 ± 0.29) are higher than those observed in other fish species presented in Table 7. Studies performed by Kaushik (1998) on *D. labrax*, *S. aurata* and *P. maxima* indicated similar variations in the A/E ratios of some amino acids in these fish species. These potential differences may derive from differences in the proportions of structural

Table 4. Amino acid composition of eggs of various fish species (all values are expresses as percentage of protein).

Fish species	Arg	Cys	His	Ile	Leu	Lys	Met	Phe	Thr	Trp	Tyr	Val	Reference
Siver pomfret (*Pampus argenteus*)	6.70±0.12	0.98±0.03	2.61±0.05	3.78±0.18	7.63±0.05	8.71±0.05	2.68±0.03	4.64±0.14	5.56±0.17	-	3.18±0.08	5.43±0.23	Hossain et al. (2011)
Yellow perch (*Perca flavescens*)	5.81	2.62	2.50	5.37	7.91	6.78	2.42	5.50	5.16	-	3.70	5.67	Hossain et al. (2011)
Largemouth bass (*Micropterus salmoides*)	9.06	1.13	2.33	3.32	8.14	7.39	2.67	4.33	5.00	1.07	3.67	6.17	Hossain et al. (2011)
Rainbow trout (*Salmo gairdneri*)	5.70	1.03	2.47	4.71	9.45	7.30	2.89	5.48	4.82	0.98	4.23	6.23	Suyama and Ogino (1958)
University of Washington Rainbow trout (*Salmo gairdneri*)	7.60	-	2.80	4.70	7.60	8.30	2.10	5.20	6.00	-	-	8.90	Satia et al. (1974)
Atlantic salmon (*Salmo salar*)	6.40	1.70	2.70	6.40	10.30	8.80	2.70	5.30	5.90	0.98	1.10	6.20	Cowey et al. (1972)
Chinook salmon (*Oncorhynchus tschawyischa*)	7.70	-	2.60	6.80	9.40	8.80	3.00	4.80	5.80	0.90	-	7.00	Seagran et al. (1954)
Coho salmon (*O. kisutch*)	7.00	-	2.80	7.50	10.00	8.80	2.70	4.90	5.90	0.90	-	7.10	Seagran et al. (1954)
Sockeye salmon (*O. nerka*)	7.20	-	2.70	7.50	10.20	8.50	2.80	4.80	5.80	0.90	-	7.30	Seagran et al. (1954)
Pink salmon (*O. gorbuscha*)	7.23	-	2.85	6.94	9.44	8.86	3.04	4.87	5.14	0.10	-	8.12	Seagran et al. (1954)
Atlantic silversides (*Menidia menidia*)	6.50	0.50	4.70	5.00	7.70	7.90	3.90	3.60	5.20	-	4.60	7.20	Schauer et al. (1979)
Walleye (*Stizostedion ritreum*)	5.70	-	2.90	6.40	8.30	7.80	3.10	4.80	5.20	1.20	4.10	7.10	Ketola (1982)
Catfish (*Ictalurus punctatus*)	5.40	-	2.20	5.10	9.70	7.70	3.40	3.90	6.00	-	3.90	5.90	Ketola (1982)

proteins between species and differences in the metabolic and physiological needs for specific amino acids for particular fish species (Wilson and Poe, 1983). It is therefore possible that Nile perch contains more arginne, tyrosine and phenyalanine, which are especially important for particular structures and metabolic activities that occur in this fish and do not probably occur in some other fish species.

In this study, the EAADR for the different developmental stages of Nile perch were estimated using the known average lysine requirement value (5%) of most carnivorous fishes [5% for *Salmo salar*, (NRC, 1973), 5% for *Sparus aurata* (Kaushik, 1998), 5% for *P. maxima* (Kaushik, 1998), 4.9% for *Lates calcarifer* (Glencross, 2004)]. This was done because the lysine requirement for Nile perch is yet to be established. These estimated requirements will be important as guide for the appropriate AA percentages to be included in the artificial diets formulated for Nile perch. Estimation of amino acid dietary requirements is vital and fundamental for determining the appropriate formulation of aquaculture feeds needed to sustain the domestication of fish species (Conceição et al., 2003).

In an attempt to investigate the appropriate EAADR for Nile perch larvae, Nile perch eggs were used in this study. A similar procedure has been used with the eggs of *Pampus argenteus* (Hossain et al., 2011) and *Acipenser transmontanus* (Ng and Hung, 1994) since the egg amino acid compositions provide an index for the requirements of EAA for the larvae (Shcherbina et al., 1988). The EAADR profile developed in this study will be used in the development of appropriate diets for Nile perch larvae.

The EAADR profile obtained in this study indicates that dietary amino acid demands increase with age. Similar observations have been cited in *S. aurata* and *Solea senegalensis* (Aragão et al., 2004c). This is attributed to the marked ontogenetic changes in the essential amino acid requirements from larval to juvenile stages. This observation correlates with changes in Nile perch feeding habits from plankton as larvae to insects as juveniles and finally to fish as late juveniles. For these reasons, different combinations and concentrations of AA should be considered for formulation of feeds at different growth stages in the Nile perch following the EAADR profile developed herein.

Table 5. Comparative amino acid profiles of selected fish species (All values are expresses as percentage of protein).

Amino acid	*Clarias garipienus*[a]	*Tilapia zilli*[a]	*Dicentrarchus labrax*[b]	*Sparus aurata*[b]	*Psetta maxima*[b]	*Citharinus citharinus*[c]	*Clarias anguillaris*[c]	*Hemisynodnotis membranaceus*[c]	*Male Silver pomfret*[d]	*Female silver pomfret*[d]	*Juvenile silver pomfret*[d]	*Adult silver pomfret*[d]	*Lates calcarifer*[e]
Cystine	1.16±0.03	1.15±0.07	1.00±0.02	1.00±0.10	1.10±0.10	-	-	-	1.03±0.09	1.04±0.10	-	-	-
Taurine	0.53±0.03	1.51±0.09	-	-	-	-	-	-	-	-	-	-	-
Aspartic acid	11.35±0.61	11.17±0.69	9.48±0.42	9.00±0.83	10.3±1.08	10.00	9.89	12.84	9.00±0.31	9.09±0.35	10.21	7.60	-
Methionine sulphone	3.17±0.17	3.17±0.19	2.58±0.01	2.71±0.19	3.40±0.06	3.04	2.31	-	2.62±0.15	2.66±0.17	3.42	2.33	3.00
Threonine	4.81±0.26	4.80±0.29	4.29±0.31	4.24±0.31	4.63±0.49	4.08	5.01	4.89	5.49±0.25	5.46±0.23	4.26	4.02	4.50
Serine	4.48±0.24	4.47±0.27	4.65±0.23	4.48±0.41	5.21±0.52	2.57	5.18	4.56	4.54±0.18	4.39±0.26	3.46	3.72	-
Glutamic acid	17.81±0.96	18.16±1.11	15.55±1.72	14.10±0.45	16.45±1.76	15.6	15.68	15.76	13.98±0.50	13.78±0.57	13.97	11.46	-
Glycine	5.07±0.27	5.20±0.32	7.14±0.54	7.36±0.47	9.68±0.91	2.52	9.98	6.25	7.04±0.23	7.52±0.24	5.99	4.01	-
Analine	6.45±0.35	6.77±0.41	6.44±0.36	6.30±0.36	7.29±0.63	4.06	6.05	6.61	5.35±0.21	5.44±0.22	6.43	4.93	-
Valine	5.34±0.29	5.18±0.32	4.55±0.36	4.44±0.23	4.68±0.42	4.18	4.36	5.18	5.35±0.22	5.38±0.22	6.86	5.15	4.40
Isoleucine	5.22±0.28	5.04±0.31	4.14±0.38	4.09±0.22	4.31±0.50	2.49	3.01	4.65	3.80±0.27	3.87±0.32	3.66	4.42	3.60
Leucine	9.53±0.51	9.49±0.58	7.21±0.56	6.95±0.43	7.53±0.60	-	7.68	8.1	7.66±0.19	7.64±0.20	6.21	7.66	7.10
Tyrosine	1.15±0.06	1.47±0.09	3.90±0.21	3.94±0.22	4.11±0.55	3.27	2.96	2.98	3.25±0.15	3.28±0.17	4.42	3.55	-
Phenylaianine	4.19±0.23	4.06±0.25	4.46±0.22	4.41±0.34	4.46±0.49	2.54	4.68	4.87	4.53±0.23	4.54±0.21	4.88	4.26	4.20
g-aminobutyric acid	0.51±0.03	0.66±0.04	-	-	-	-	-	-	-	-	-	-	-
Ornithine	0.65±0.04	0.27±0.02	-	-	-	-	-	-	-	-	-	-	-
Lysine	10.64±0.57	10.37±0.63	7.61±0.68	7.27±0.63	8.13±0.78	8.39	6.33	10.62	8.63±0.21	8.67±0.23	11.88	8.27	6.20
Arginine	6.82±0.37	11.66±0.72	8.36±0.21	8.49±0.49	7.73±0.88	3.89	6.74	5.88	6.61±0.20	6.70±0.23	6.123	5.56	6.90
Hyroxyproline	0.30±0.00	0.30±0.02	-	-	-	-	-	-	-	-	-	-	-
Proline	3.81±0.21	3.95±0.24	4.90±0.05	5.06±0.26	5.52±0.86	2.76	5.39	3.87	6.50±0.23	6.47±0.27	4.09	2.55	-
Histidine	-	-	2.43±0.19	2.82±0.44	2.48±0.45	3.07	3.49	3.24	2.66±0.23	2.59±0.21	2.35	2.00	1.50

Source: [a]Osibano et al. (2009), [b]Kaushik (1998), [c]Effiong and Mohammed (2008), [d]Hossain et al. (2011) and [e]Glencross (2004).

Nile perch requirements for arginine (10.01 to 11.98%), leucine (7.20 to 9.81%), threonine (4.17 to 5.83%), valine (4.35 to 5.93%), and isoleusine (3.34 to 5.26%) are high when compared to those reported in other fishes (Table 7). Limited information is available on the specific function of some of these amino acids in fish; however, arginine and leucine have been reported to be particularly important for immunity, reproduction, extra-endocrine signalling, neurological function, blood flow, osmoregulation, growth and development (Bordieri et al., 2005; Hyndman et al., 2006; Li and Gatlin, 2007). Some fish such as the West African lungfish *Protopterus annectens* have also been reported to produce extra arginine (Li et al., 2008) and have arginine vascotocin receptors (Konno et al., 2009) that are important in osmoregulation, and may be related to air breathing and aestivation in the lungfish. It is possible that Nile perch requires higher concentrations of these AA compared to

Table 6. A/E ratio (essential amino acid/total essential amino acid × 1,000) of selected omnivorous and carnivorous fish species.

Amino acid	A/E ratios					
	Omnivorous			Carnivorous		
	Jundia	Channel catfish	Nile tilapia	Pintado	Rainbow trout	Atlantic salmo
Arginine	115.5	132	140	133	125.5	123.3
Histidine	40.8	43	44	58.7	57.4	56.9
Isoleucine	78.8	85	93.6	82.5	83.8	83.4
Leucine	156.1	146	160	154	146.6	145.9
Lysine	180.2	168	160.2	166.2	176.3	163.2
Met+cys	96.4	75	72.1	69.3	52.8	70.8
Phe+tyro	148.3	147	129.2	139.9	149.3	149.2
Threonine	93.2	87	89.3	91.7	94	91.5
Tryptophan	8.4	15	18.3	18.1	17.7	17.9
Valine	82.4	102	91.3	86.6	96.7	97.9

Adapted from Meyer and Fracalossi (2005).

Table 7. Estimated EAA dietary requirements of selected fish species.

Fish species	Arg	His	Lys	(Met+	Cys)	Trp	(Phe+	Tyr)	Leu	Ile	Val	Thr	Reference
Atlantic salmon (*Salmo salar*)	6.00	1.80	5.00	-		0.50	-	5.10	3.90	2.20	3.20	2.20	NRC (1973)
Silver pomfret (*Pampus argenteus*)	3.46	1.37	4.50	1.87		-		3.79	3.98	2.00	2.79	2.85	Hossain et al. (2011)
Nile tilapia (*Oreochromis niloticus*)	4.20	1.72	5.12	2.68	0.53	1.00	3.75	1.79	3.39	3.11	2.80	3.75	Santiago and Lovell (1988)
Channel catfish (*Ictalurus punctatus*)	4.30	1.50	5.10	2.30	-	0.50	5.00	0.30	3.50	2.60	3.00	2.00	NRC (1993)
Common carp (*Cyprinus carpio*)	4.30	2.10	5.70	2.10	-	3.60	3.40	3.60	3.30	2.50	0.80	3.90	NRC (1993)
Jundia (*Rhamdia quelen*)	3.72	1.31	5.80	2.13	0.98	0.27	2.69	2.10	5.03	2.54	2.65	3.00	Meyer and Fracalossi (2005)
European sea bass (*Dicentrachus labrax*)	4.60	1.60	4.80	2.30		0.60	2.60		4.30	2.60	2.90	2.70	Kaushik (1998)
Gilthead sea bream (*Sparus aurata*)	5.40	1.70	5.00	2.40		0.60	2.90		4.50	2.60	3.00	2.80	Kaushik (1998)
Turbort (*Psetta maxima*)	4.80	1.50	5.00	2.70		0.60	5.30		4.60	2.60	2.90	2.9	Kaushik (1998)
Milk fish (*Chanos chanos*)	5.60	2.00	4.00	4.80		0.60	5.20		5.10	4.00	3.00	4.9	Borlongan and Coloso (1993)
Asian Sea bass (*Lates calcarifer*)	3.6	-	4.5	2.4		0.5	-		-	-	-	-	Coloso et al. (1993)
Red drum (*Sciaenops ocellatus*)	3.7	1.7	5.7	3		0.8	4.5		4.7	2.9	3.1	2.8	Moon and Gatlin (1991)

concentrations reported in other fishes as a metabolic adaptation to warmer water temperatures or low dissolved oxygen (DO) concentrations. I recommend that diets formulated for Nile perch should contain essential amino acids in the percentages provided in the profile

developed in this study, with corresponding high percentages of arginine and leucine.Future studies on this aspect of Nile perch feed formulation should also investigate the EAADR of Nile perch using the dose response experiments. And from these results, a correlation between the EAA profiles established in this study and the EAADR profiles from those experiments should be done. Results from such correlations have been used by several authors (Mohanty and Kaushik, 1991; Meyer and Fracalossi, 2005; Hossain et al., 2011) to affirm the strong relationship between the fish composition and its dietary requirements (Wilson and Poe, 1985) and to precisely determine the EAA requirements.

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