Aquaculture Reports 14 (2019) 100197

Contents lists available at ScienceDirect

Aquaculture Reports

journal homepage: www.elsevier.com/locate/aqrep

Dietary amino acid requirements of pebbly fish, *Alestes baremoze* (Joannis, 1835) based on whole body amino acid composition

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ARTICLE INFO

Keywords:
A/E ratios
Alestes baremoze
Amino acid composition
Dietary requirements

ABSTRACT

Alestes baremoze is a valuable food fish with a wide geographical distribution in East, North and West Africa. Currently, the nutritional requirements of *A. baremoze* have not yet been determined, which hinders attempts towards developing appropriate feed formulations for its culture. This study was thus conducted to estimate essential amino acid (EAA) requirements of *A. baremoze* using the A/E ratio method, as a guide in formulating its diet. Fish samples used in the study were categorised into four classes according to their fork lengths (1–12 cm; 13–24 cm; 25–36 cm and 37–48 cm), with each class consisting of 10 fish. Results from the amino acid composition analysis revealed significant difference ($P < 0.05$) in the concentration of tryptophan, lysine, methionine, threonine, phenylalanine, isoleucine and valine amongst the different class sizes of *A. baremoze*. The A/E ratios of *A. baremoze* muscle tissue were in the same range with those obtained from other fish species, except for tryptophan. When expressed as a percentage of dietary protein, the EAA requirements of *A. baremoze*, were however not significantly different ($P > 0.05$) within the four classes. The EAA requirement profiles for *A. baremoze* were found to be similar to those observed in other omnivorous fish species. Considering the importance of *A. baremoze* as a potential species for freshwater aquaculture, the present data provides guidance to the development of test diets with appropriate amino acid inclusions until dose response treatments are carried out.

1. Introduction

Alestes baremoze or pebbly fish is an omnivorous freshwater species usually found both in lacustrine and riverine conditions (Akinyi et al., 2010; Kasozi et al., 2017). In Uganda, the fish is found in Lake Albert and the Albert Nile (Mbabazi et al., 2012). It belongs to order Characiformes and family Alestidae (Akinyi et al., 2010). This species has greatly attracted scientists' and fish farmers' interest because of its high market price and good meat quality. Studies indicate that *A. baremoze* has been extensively harvested, putting it under threat (Mbabazi et al., 2012). As a result, small pelagic fishes such as *Brycinus nurse* and *Neobola bredoi* have currently replaced the once booming *A. baremoze* fishery. These now contribute to almost 80% of the fish catches on Lake Albert. Current strategies for increasing *A. baremoze* production point towards its culture (Kasozi et al., 2017). The sustainability of the culture programme will however greatly depend on the development of appropriate feeding technologies for this candidate species. Efforts towards its culture will be supported by generating knowledge informing its amino acid requirements which is of significant importance.

Amino acids (AA) are significant biomolecules that serve as protein building blocks and are intermediates in various metabolic pathways (Li et al., 2009; Mohanty et al., 2014). Due to their significance, it is important that specific amino acid requirements are determined so as to optimise amounts of dietary protein necessary for efficient animal production. Amino acid assays have been widely done to accurately determine the protein requirements for the culture of different fish species (Wilson and Cowey, 1985; Santiago and Lovell, 1988; Namulawa et al., 2012). Similarly, several studies on quantitative and qualitative properties of amino acid composition in several fish species have been done. In these, the EAA profile of fish carcass has been commonly used as an indicator of fish amino acids requirements (Saavedra et al., 2006). Fish amino acid requirements can also be determined through dose–response studies (Tibaldi and Lanari, 1991; Fournier et al., 2002) however these methods are costly and time-consuming, especially when determining the requirement for all essential amino acids (Akiyama et al., 1997). Consequently, other

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https://doi.org/10.1016/j.aqrep.2019.100197
Received 16 November 2018; Received in revised form 8 March 2019; Accepted 17 April 2019
Available online 03 June 2019

methods such as the daily increment method (Martinez-Palacios et al., 2007), and the whole body tissue A/E ratio method (Monentcham et al., 2010; Hossain et al., 2011; Namulawa et al., 2012) have been applied.

Determination of fish amino acid requirements through the A/E ratio method is less expensive and quickly provides results (Monentcham et al., 2010). This method depends on the determination of requirements of a reference amino acid, usually lysine which has been suggested for fish species, whose EAA requirements have not yet been determined (Monentcham et al., 2010). For example, Namulawa et al. (2012) determined the amino acid requirement of the Nile perch, *Lates niloticus* using a conversion factor of 5.0 g lysine 100 g^{-1} protein. Similarly, Robinson et al. (1980) and Kaushik (1998) and) used the same factor with channel catfish, *Ictalurus punctatus* and turbot, *Scophthalmus maximus* respectively. In this study, the unknown EAA requirements for *A. baremoze* were also derived using similar methods. Apart from the information about the proximate composition and mineral contents of *A. baremoze*, its amino acid profile is unknown; hence its nutritional requirements have not yet been determined. Therefore, the objectives of this study were to apply methods used in previous studies to estimate the dietary requirements of *A. baremoze* of different size classes. Results from this study will be applicable in guiding the development of appropriate feeding strategies for this fish once domesticated.

2. Materials and methods

2.1. Sample collection and processing

Fish was collected from Abok fish landing site (02° 14.46‘N 31°19.15‘E) located on Lake Albert, Packwach district (Fig. 1). It was then categorised into four classes according to its fork lengths (1–12 cm; 13–24 cm; 25–36 cm and 37–48 cm), with each class consisting of 10 fish. Whole fish, excluding the viscera, liver and gonads, were individually chopped, minced on ice and frozen for further processing. All the prepared samples were then submitted to Chemiphar (U) Ltd for analysis, an independent analytical laboratory, internationally accredited according to ISO 17025:2005.

2.2. Crude protein analysis

Crude protein was determined using the Kjeldahl method, after acid digestion using copper (II) sulphate as a catalyst, according to AOAC 24.38-24.040 (ISO 937). After digestion, the ammonia from the sample was distilled into boric acid solution by steam distillation and titrated against hydrochloric acid. Crude protein was determined by multiplying the nitrogen value by the conversion factor of 6.25.

2.3. Amino acid profiles

Total amino acid profiles were determined according to the adapted method of the European Commission (Commission Directive 98/64/EC of 3 September 1998). The reproducibility of the results was within approximately 3%. Duplicate samples were hydrolysed with 6 N hydrochloric acid for 24 h at 110 °C. The hydrolysed samples were then analysed as outlined below:

a Cystine and methionine: Oxidative hydrolysis, amino acid analyzer with ninhydrin (ISO 13903:2005; EU 152/2009).
b Tryptophan: Alkaline hydrolysis, quantification by high-performance liquid chromatography (HPLC) techniques (ISO 13903: 2005; EU 152/2009).
c Other Amino acids: Acid hydrolysis, amino acid analyzer with ninhydrin (ISO 13903: 2005; EU 152/2009).

2.4. A/E ratio and estimation of EAA dietary requirements

The A/E ratios of the EAA composition of the whole fish body were calculated according to Arai (1981) and Hossain et al. (2011). Since there is no available data on the lysine requirement for *A. baremoze*, the EAA dietary requirement of *A. baremoze* was estimated based on the known 5.0% lysine requirement per 100 g protein using the following formulae:

A/E ratio = [(individual EAA / total EAA including cystine and tyrosine) × 1000].

EAA = [(Determined requirement value of lysine × A/E ratio of individual amino acid) / A/E ratio of lysine] using 5.0% lysine

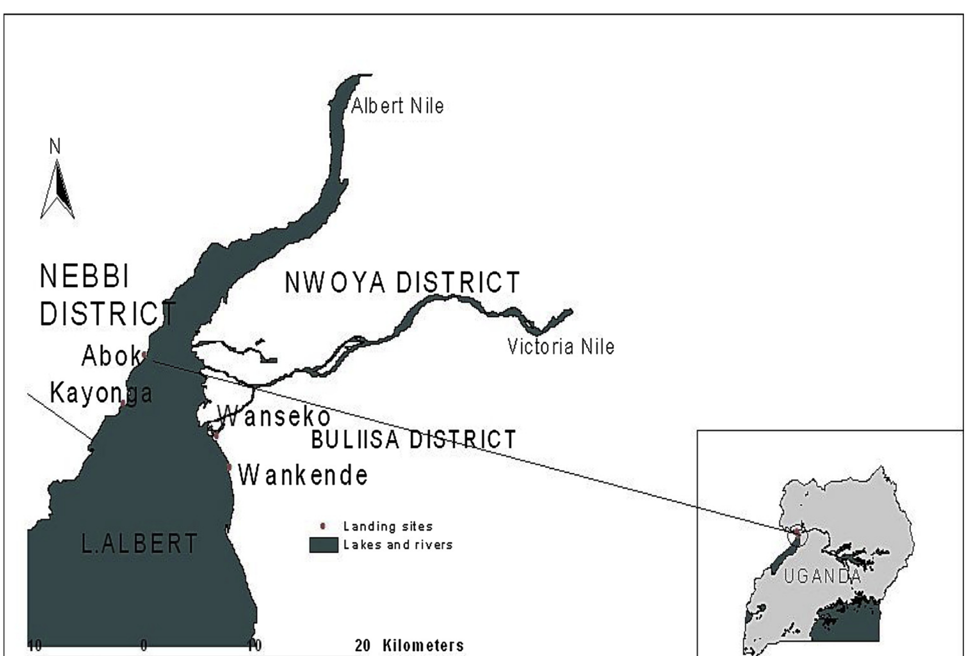

Fig. 1. Location of Abok fish landing site on Lake Albert, Uganda.

Table 1
Amino acid composition of whole body tissue from different size classes of *A. baremoze*.

Parameter	composition (% protein)					
Essential amino acids	1-12 cm	13-24 cm	25-36 cm	37-48 cm	*P*-value	Significance*
Tryptophan	0.16 ± 0.004	0.17 ± 0.007	0.20 ± 0.000	0.20 ± 0.008	0.009	*
Lysine	1.40 ± 0.049	1.59 ± 0.077	1.71 ± 0.084	1.69 ± 0.049	0.030	*
Methionine	0.45 ± 0.017	0.48 ± 0.018	0.50 ± 0.011	0.52 ± 0.009	0.033	*
Threonine	0.72 ± 0.000	0.79 ± 0.020	0.86 ± 0.013	0.83 ± 0.018	0.004	*
Arginine	1.00 ± 0.080	1.05 ± 0.049	1.18 ± 0.091	1.11 ± 0.056	0.230	ns
Phenylalanine	0.63 ± 0.022	0.74 ± 0.014	0.78 ± 0.011	0.74 ± 0.021	0.005	*
Histidine	0.41 ± 0.016	0.44 ± 0.028	0.49 ± 0.021	0.48 ± 0.019	0.072	ns
Isoleucine	0.68 ± 0.021	0.76 ± 0.043	0.84 ± 0.043	0.82 ± 0.031	0.037	*
Leucine	1.26 ± 0.035	1.39 ± 0.084	1.48 ± 0.070	1.45 ± 0.042	0.072	ns
Valine	0.74 ± 0.026	0.84 ± 0.034	0.93 ± 0.038	0.89 ± 0.035	0.020	*
Non –essential amino acids						
Tyrosine	0.48 ± 0.043	0.58 ± 0.011	0.60 ± 0.054	0.57 ± 0.031	0.131	ns
Cysteine	0.15 ± 0.016	0.15 ± 0.004	0.18 ± 0.008	0.18 ± 0.014	0.087	ns
Aspartic acid	1.57 ± 0.021	1.74 ± 0.084	1.86 ± 0.028	1.86 ± 0.084	0.025	*
Proline	0.65 ± 0.140	0.57 ± 0.007	0.79 ± 0.376	0.72 ± 0.175	0.766	ns
Glutamic acid	2.23 ± 0.035	2.44 ± 0.077	2.65 ± 0.000	2.53 ± 0.063	0.006	*
Serine	0.64 ± 0.026	0.66 ± 0.066	0.72 ± 0.014	0.73 ± 0.040	0.233	ns
Glycine	1.12 ± 0.346	0.93 ± 0.003	1.32 ± 0.641	1.14 ± 0.329	0.817	ns
Alanine	1.05 ± 0.113	1.06 ± 0.028	1.26 ± 0.197	1.17 ± 0.084	0.095	ns
Crude protein (%)	16.6 ± 0.070	17.2 ± 0.636	18.8 ± 0.777	20.7 ± 0.707	0.009	*

* significant difference ($P < 0.05$); ns = not significant.

concentration.

2.5. Statistical analysis

Amino acid analyses were performed in duplicate on each sample (two samples per fish stage). Statistical analyses were performed using GenStat 14th Edition (64bit): VSN International Ltd. Treatment means were separated by Fisher's protected *t*-test least significant difference (LSD) at 5% level of significance. Data for individual amino acids are expressed as means ± standard deviation.

3. Results

3.1. Amino acid composition

Whole-body amino acid composition for different class sizes of *A. baremoze* was determined (Table 1). The profiles indicated the presence of both essential and nonessential amino acids. No major differences were observed in the concentrations of amino acid in the different size classes investigated in this study although a statistical difference ($P < 0.05$) was observed in the concentrations of tryptophan, lysine, methionine, threonine, phenylalanine, isoleucine and valine. Among the nonessential AA, aspartic acid and glutamic acid were significantly ($P < 0.05$) different.

3.2. A/E ratios and EAA dietary requirements

A comparison of A/E ratios for *A. baremoze* muscle tissue and that for other fish species was undertaken (Table 2). This showed similarity between the *A. baremoze's* A/E ratio and that of other omnivorous fish species, however A / E values of tryptophan were slightly higher than those observed in other omnivorous fish species. The EAA requirements obtained in our study showed considerable similarity at different sizes (Table 3) and also with other omnivorous species such as Nile tilapia and common carp (Table 4).

4. Discussion

4.1. AA concentrations of different size classes

AA are classified as nutritionally essential or nonessential for fish (Li et al., 2009). EAA are those that either cannot be synthesized or are inadequately synthesized de novo by animals relative to needs (Akiyama et al., 1997). Conditionally, EAA must be provided from the diet under conditions where rates of utilisation are greater than rates of synthesis. On the other hand, all nonessential AA can be synthesized adequately by aquatic animals (Mohanty et al., 2014). Although AA concentrations of different size classes occurred in the same range, slightly high values were recorded in adult fish from 25 to 48 cm fork length. In addition, significantly high protein content was recorded in the fish samples within the same range, an indication that broodstock fish contains higher levels of protein per unit than the immature fish. These differences could also be related to the quality food eaten by fish in this class size. It has been observed that *A. baremoze* has a flexible diet, shifting from zooplankton to zoobenthos, detritus, and macrophytes as plankton densities decline (Campbell et al., 2005; Kasozi et al., 2017).

The protein composition of fish is affected by season, sexual changes, and environment but the amount and quality of food that the fish eats is critical. Although this study didn't conduct the AA composition of eggs, differences in the pattern of AA in eggs and in the carcass have been reported with the concentrations of nutrients in the eggs always higher than in the carcass (Arai, 1981; Hossain et al., 2011).

Among the AA, the concentration of glutamic acid was significantly highest while that of tryptophan was the lowest. Similar trends have been reported by Namulawa et al. (2012) for *Lates niloticus*, Meyer and Fracalossi (2005) for *Rhamdia quelen* and by Monentcham et al. (2010) for *Heterotis niloticus*. Previous studies have also indicated that glutamic acid is one of the most abundant free AA in fish plasma and muscle where it's required for synthesis of purine and pyrimidine nucleotides in all cells (Li et al., 2009).

4.2. A/E ratio and EAA requirement profiles

The A/E ratio has been the most widely used method for estimating amino acids requirements of fish (Hossain et al., 2011). Several authors have found significant correlations between the whole body A / E ratios of different species (Wilson and Poe, 1985; Monentcham et al., 2010; Hossain et al., 2011). The occurrence of a similar A / E ratio between different species suggests that amino acid requirements of such species expressed as percentage of dietary protein are very similar (Wilson and Poe, 1985; Hossain et al., 2011). Many fish diets are formulated on the

Table 2
Comparison of A/E ratios of *A. baremoze* tissue of different size classes with other omnivorous fishes.
Source: [a]Wilson and Poe (1985); [b]Meyer and Fracalossi (2005); [c]Monentcham et al. (2010).

Parameter	Estimated A/E ratio *A. baremoze*				Omnivorous		
Amino acid	1-12 cm	13-24 cm	25-36 cm	37-48 cm	Channel catfish[a]	Nile tilapia[b]	African bonytongue[c]
Tryptophan	20.31	19.01	20.08	21.27	15.00	18.30	10.20
Lysine	172.22	176.22	175.65	177.39	168.00	160.20	154.70
Methionine	55.49	53.03	51.26	54.85	–	–	–
Threonine	89.32	88.33	88.08	87.59	87.00	93.20	90.10
Arginine	123.83	116.18	120.70	116.85	132.00	142.00	120.70
Phenylalanine	78.15	82.05	79.92	77.90	–	–	–
Histidine	50.62	49.14	49.97	50.48	43.00	44.00	78.90
Isoleucine	83.83	84.44	85.93	86.48	85.00	93.60	90.10
Leucine	154.94	154.54	152.03	152.65	146.00	160.00	136.60
Valine	91.48	93.22	95.33	93.48	102.00	91.30	100.00
Tyrosine[*1]	59.51	64.82	60.97	59.80	–	–	–
Cysteine[*2]	18.61	17.48	18.72	19.80	–	–	–
Met + Cys	74.10	70.51	69.98	74.65	75.00	72.10	76.40
Phe + Tyr	137.66	146.87	140.89	137.7	147.00	129.20	142.30

*1 Non-essential amino acid, capable of sparing dietary phenylalanine (Wilson, 1989; Meyer and Fracalossi, 2005).
*2 Non-essential amino acid, capable of sparing dietary methionine (Moon and Gatlin, 1991; Li et al., 2009).

basis of A / E ratios of different reference proteins, primarily because EAA requirements have not been determined for most species used in aquaculture (NRC, 1993). However, its application depends on the determination of requirements of a reference amino acid, usually lysine (Akiyama et al., 1997; Monentcham et al., 2010). Basing on the variability of methods used to formulate fish diets, it is essential to note that the whole body A / E ratios are better indicators of EAA dietary requirements than the egg A / E ratios (Mambrini and Kaushik, 1995; Hossain et al., 2011). The estimated A/E ratio of tryptophan obtained in our study was slightly higher than those reported for other omnivorous fish species. Tryptophan is largely required for synthesis of other compounds besides muscle protein (NRC, 1993).

Fish do not have a specific protein requirement but rather a definite requirement for EAA that comprise proteins. One of the important factors in determining the efficiency of protein utilisation for fish is the pattern of EAA in the diet. It is therefore, the essential amino acids in dietary protein that a fish requires and not necessarily the protein. The existing data on EAA requirements of different species of fishes shows similarities as well as differences (Tacon and Cowey, 1985; Cowey, 1994; Kaushik, 1998). These differences can be related to a number of factors such as changes in basal diet composition, size and age of fish, genetic differences, feeding rate and culture conditions (Cowey, 1994; Fournier et al., 2002; Hossain et al., 2011). There were no significant differences in the estimated EAA requirements of *A. baremoze* at different sizes. Similar trends were observed in African bonytongue, *Heterotis niloticus*, riverine fish from the Central and West Africa basin (Monentcham et al., 2010). This observation might correlate with the feeding habits of *A. baremoze* which is predominantly insectivorous (Kasozi et al., 2017). The estimated amino acid requirements obtained in this study indicates that dietary amino acid demands don't vary much with size and also with other omnivorous species such as Nile tilapia and the common carp. These results suggest that the EAA requirements, when expressed as a percentage of dietary protein, were not affected by the fish body size.

A higher requirement for leucine, compared to other omnivorous fish species was also observed. Leucine has been reported to be particularly important for immunity, reproduction, extra-endocrine signalling, neurological function, blood flow, osmoregulation, growth and development (Namulawa et al., 2012). It is possible that *A. baremoze* requires higher concentrations of leucine compared to other omnivorous fishes as a metabolic adaptation to riverine conditions since it is a fast moving fish. Leucine is a limiting essential AA in fish, and it has been demonstrated that its supplementation improves growth in different species (Li et al., 2009). For (phenylalanine + tyrosine), a lower requirement as compared to Nile tilapia and Channel catfish fish species was observed. Phenylalanine can be converted to tyrosine by tetrahydrobiopterin- dependent phenylalanine hydroxylase in liver (Li et al., 2009). Research on supplementing phenylalanine and tyrosine to aquafeeds and their potential influences on aquatic animals is currently limited. Adequate amounts of both sulfur amino acids (methionine and

Table 3
Estimated amino acid dietary requirements for *A. baremoze* of different size classes.

Parameter	estimated requirement (% of dietary protein)					
Amino acid	1-12 cm	13-24 cm	25-36 cm	37-48 cm	*P*-value	Level of significance
Tryptophan	0.59 ± 0.017	0.54 ± 0.022	0.57 ± 0.002	0.60 ± 0.025	0.113	ns
Lysine	5.00 ± 0.000	5.00 ± 0.000	5.00 ± 0.000	5.00 ± 0.000	1.000	ns
Methionine	1.61 ± 0.063	1.50 ± 0.058	1.46 ± 0.033	1.55 ± 0.029	0.125	ns
Threonine	2.59 ± 0.002	2.51 ± 0.065	2.51 ± 0.040	2.47 ± 0.055	0.207	ns
Arginine	3.59 ± 0.288	3.30 ± 0.156	3.44 ± 0.269	3.29 ± 0.168	0.563	ns
Phenylalanine	2.27 ± 0.081	2.33 ± 0.045	2.27 ± 0.033	2.20 ± 0.063	0.300	ns
Histidine	1.47 ± 0.060	1.39 ± 0.089	1.42 ± 0.207	1.42 ± 0.057	0.756	ns
Isoleucine	2.43 ± 0.076	2.40 ± 0.136	2.45 ± 0.126	2.44 ± 0.094	0.967	ns
Leucine	4.50 ± 0.126	4.38 ± 0.268	4.33 ± 0.064	4.30 ± 0.126	0.754	ns
Valine	2.66 ± 0.096	2.65 ± 0.109	2.71 ± 0.112	2.64 ± 0.105	0.879	ns
Tyrosine[*1]	1.73 ± 0.157	1.84 ± 0.036	1.74 ± 0.159	1.69 ± 0.092	0.665	ns
Cysteine[*2]	0.59 ± 0.058	0.54 ± 0.013	0.57 ± 0.025	0.60 ± 0.044	0.527	ns

ns = not significant ($P > 0.05$).
*1 Non-essential amino acid, capable of sparing dietary phenylalanine (Wilson, 1989; Meyer and Fracalossi, 2005).
*2 Non-essential amino acid, capable of sparing dietary methionine (Moon and Gatlin, 1991; Li et al., 2009).

Table 4
Amino acid requirements for channel catfish (*Ictalurus punctatus*), Nile tilapia (*Oreochromis niloticus*) and common carp (*Cyprinus carpio*), and estimated amino acid requirements for pebbly fish (*Alestes baremoze*).
Source: [a]Santiago and Lovell, 1988; [b]National Research Council (NRC), 1993; [c]Monentcham et al. (2010).

Parameter	*A. baremoze* requirements				Omnivorous requirements			
Amino acid (% dietary protein)	1-12 cm	13-24 cm	25-36 cm	37-48 cm	Nile tilapia[a]	Channel catfish[b]	Common carp[b]	African bonytongue[c]
Tryptophan	0.59	0.54	0.57	0.60	1.00	0.50	0.80	3.00
Lysine	5.00	5.00	5.00	5.00	5.12	5.10	5.70	5.10
Threonine	2.59	2.51	2.51	2.47	3.75	2.00	3.90	3.00
Arginine	3.59	3.30	3.44	3.29	4.20	4.30	4.30	4.00
Histidine	1.47	1.39	1.42	1.42	1.72	1.50	2.10	2.60
Isoleucine	2.43	2.40	2.45	2.44	3.11	2.60	2.50	3.00
Leucine	4.50	4.38	4.33	4.30	3.39	3.50	3.30	4.50
Valine	2.66	2.65	2.71	2.64	2.80	3.00	3.60	3.30
Met + Cys	2.20	2.04	2.03	2.15	3.20	2.30	3.10	2.50
Phe + Tyr	4.00	4.17	4.01	3.89	5.54	5.00	3.40	4.70

cysteine) are also necessary for protein synthesis and various physiological functions in the body (Moon and Gatlin, 1991; Lall and Anderson, 2005; Li et al., 2009). Although cysteine can spare 40–60% of methionine in the diets for various fishes, there is limited information about the nutritional importance of cysteine for fish (Li et al., 2009). Cysteine can be synthesized from methionine, however the conversion of methionine to cysteine in the body is irreversible (Lall and Anderson, 2005; Brosnan and Brosnan, 2006). Physiological methionine requirement can only be met by methionine supplied in the diet whereas cysteine requirement can be met by either of these two sulfur amino acids (Moon and Gatlin, 1991; Lall and Anderson, 2005).

5. Conclusion

This study is the first prediction of dietary requirements for EAA of *A. baremoze*, a promising species for sub-Saharan Africa fish farming. The results of the present study indicate a slightly higher requirement for leucine than other omnivorous fish species. The EAA requirements obtained has also showed considerable similarity at different sizes of *A. baremoze* as well as with other omnivorous species such as Nile tilapia and the common carp. Meanwhile, until data from dose-response experiments are available for *A. baremoze*, the estimated values proposed in this study can be used when formulating experimental and practical diets for *A. baremoze* at different stages of growth.

Conflict of interest

We certify that there is no conflict of interest.

Acknowledgments

This study was conducted with grant from the Competitive Grant Scheme (CGS Project ID/no: CGS/4/32/14) of the National Agricultural Research Organisation (NARO) under the World Bank fundedAgricultural Technology and Agribusiness Advisory services (ATAAS) project.

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