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http://www.aquaculturehub.org

ISSN 0792 - 156X

PUBLISHER:
Israeli Journal of Aquaculture - BAMIGDEH -
Kibbutz Ein Hamifratz, Mobile Post 25210, ISRAEL
Phone: + 972 52 3965809
http://siamb.org.il

MOISTURE, PROTEIN, AND AMINO ACID CONTENTS OF THREE FRESHWATER ZOOPLANKTON USED AS FEED FOR AQUACULTURED LARVAE AND POSTLARVAE

Solomon I. Ovie* and Stella O. Ovie

National Institute for Freshwater Fisheries Research, P.M.B. 6006, New Bussa, Niger State, Nigeria

(Received 20.7.05, Accepted 4.9.05)

Key words: amino acid profile, *Brachionus calyciflorus*, *Diaphanosoma excisum*, *Moina micrura*, moisture content, protein level

Abstract

The moisture content, crude protein level, and amino acid profile of three freshwater zooplankton (*Moina micrura, Diaphanosoma excisum, Brachionus calyciflorus*) commonly used for rearing fish larvae were analyzed using standard methods. The moisture contents and crude protein levels were similar, as follows: *M. micrura* 89% and 52.4%, *D. excisum* 89.3% and 57.3%, and *B. calyciflorus* 91.6% and 50.3%, respectively. The samples contained 17 amino acids: nine essential and eight non-essential amino acids. The dominant essential amino acids (per 16 g N) in *M. micrura* were lysine (10.73 g), arginine (8.17 g), leucine (8.0 g), and histidine (5.09 g); in *D. excisum*, lysine (9.95 g), leucine (8.0 g), valine (6.23 g), and arginine (4.78 g), and in *B. calyciflorus*, leucine (8.95 g), lysine (8.64 g), arginine (6.37 g), phenylalanine (5.20 g), and valine (4.83 g). In all three species, glutamine and aspartic acid dominated the non-essential amino acid profile, and methionine was the essential amino acid in the smallest concentration. Differences among amino acid profiles were insignificant ($p>0.05$).

Introduction

Rearing larvae of fin and non-fin fish heavily and almost exclusively depends on live zooplankton. For the carnivorous larvae of commercially cultured African catfish, this is most important to avoid mass mortality. For several decades, imported cysts of marine *Artemia* was the only live feed for commercially cultured fish larvae. Since the 1980s, the high cost of *Artemia* has been a major bottleneck in the commercial production of fish larvae and fingerlings in fish hatcheries. This problem is especially prevalent in developing countries such as Nigeria that need to import the commodity (Ovie et al., 1993; Adeyemo et al.,

* Corresponding author. E-mail: soloovie@yahoo.com

1994; Ovie and Egborge, 2002) and many hatcheries have been forced to close. Scientific research efforts were initiated to identify and culture suitable cost-effective local zooplankton as a replacement for imported *Artemia*. Pioneering efforts of the National Institute for Freshwater Fisheries Research in New Bussa is noteworthy in this regard, as it has completely replaced imported *Artemia* with locally-cultured indigenous freshwater zooplankton for larvae rearing.

The nutritive value of live fish food organisms is vital to their success as larvae feed. Because of their long and dominant use in aquaculture, the amino acid profiles of *Artemia* and some other marine zooplankton have been well established (Watanabe et al., 1978, 1983). The reverse is true of freshwater zooplankton whose amino acid profiles have been poorly researched and documented although these organisms are growing in importance as larvae feeds in freshwater fish hatcheries.

This study reports on the amino acid profiles of three species of freshwater zooplankton commonly used for indoor hatchery rearing of larvae of the catfish *Clarias, Heterobranchus,* and their hybrid. It is hoped that results of this study will provide valuable information on the nutritive value of these freshwater zooplankton which are known to be cosmopolitan in the tropics (Ovie, 1993; Lavens and Sorgeloos, 1996).

Materials and Methods

Laboratory-cultured *Moina micrura*, *Diaphanosoma excisum* (Cladocera), and *Brachionus calyciflorus* (Rotifera) were reared on the micro-algae *Scenedesmus*, harvested with a 50 µm zooplankton net, and air-dried on Whatman no.1 filter paper. The caked sample was peeled off the filter paper and stored in dry marked screw-cap specimen bottles pending analysis. Samples were analyzed for moisture content, crude protein, and amino acid profiles according to AOAC (1984).

Moisture content was determined by taking a known weight of each sample, drying it to a constant weight at 100-150°C, and calculating the percent difference between the initial and final weights. Crude protein was determined by the micro-Kjedahl method, following the three major steps of digestion, distillation, and filtration. To determine the amino acid profile, samples were defatted, hydrolyzed, and evaporated in a rotary evaporator before being loaded into a Technicon Sequential Multi-Sample Auto-Analyser (SMAA) model TSM-1, equipped with a pen recorder.

Statistical differences in amino acid profiles were determined by Analysis of Variance (ANOVA) followed by student's Neuman Keuls to determine differences among means. The Statistical Package for Social Scientists (SPSS) was used for the analysis.

Results

The moisture contents, crude protein concentrations, and amino acid profiles of the three organisms were fairly similar (Table 1). The 17 amino acids comprised nine essential acids and eight non-essential acids. Lysine was the most abundant of the essential amino acids in all three zooplankton. In addition, arginine, leucine, and histidine were dominant essential amino acids in *M. micrura,* arginine, leucine, and valine dominated in *D. excisum*, and arginine, leucine, and phenylalanine in *B. calyciflorus*. Among non-essential amino acids, glutamine and aspartic acid dominated in all three species. The amino acid profiles of the three zooplankton did not significantly (p>0.05) differ from each other.

Discussion

The moisture contents of the three organisms in this study (89-91.6%) were very similar to those of other similar living food organisms such as *Acartia clausi* (86.9-87.8%), *Tigriopus japonica* (86.0-87.3%), *Daphnia* (88.1-89.3%), *Brachionus plicatilis* (86.4-91.8%), and *Moina* (87.9-89.0%; Watanabe, 1978, 1983). In general, the moisture contents were similar to moisture levels of living organisms, i.e., about 90% of the wet weight (Pace and Orcutt, 1981; Ovie et al., 1993).

The crude protein levels (50.3-57.3%) compared favorably with concentrations reported for other zooplankton such as *Tigriopus* (55.9-62.95%), *Daphnia* (62.6%), *Moina* sp. (59.95-62.6%), and *B. plicatilis*

Table 1. Moisture content, crude protein, and amino acid profile of *Moina micrura, Diaphanosoma excisum,* and *Brachionus calyciflorus.*

	M. micrura	*D. excisum*	*B. calyciflorus*
Moisture content (%)	89	89.3	91.6
Crude protein (%)	52.4	57.3	50.3
Essential amino acids (g/16 g N)			
Lysine	10.73	9.95	8.64
Arginine	8.17	4.78	6.37
Leucine	8.00	8.00	8.95
Histidine	5.09	2.60	1.83
Valine	4.44	6.23	4.83
Isoleusine	4.18	2.72	4.32
Phenylalanine	3.75	3.75	5.20
Threonine	2.93	3.84	3.92
Methionine	1.12	2.45	0.93
Non-essential amino acids (g/16 g N)			
Glutamine	15.39	13.61	12.22
Aspartic acid	9.84	10.23	10.53
Glycine	3.90	7.80	3.37
Serine	3.42	2.65	3.45
Proline	3.18	6.44	6.03
Tyrosine	3.00	3.21	2.82
Cystine	2.89	1.26	1.55
Alanine	2.48	4.46	4.00

(52.15-60.57%; Watanabe, 1983). The concentrations were also similar to common tropical freshwater fish species such as tilapia (51.69%) but slightly lower than concentrations in two species of freshwater sardines, *Pellonula afzeluisi* (71.33%) and *Sierrathrissa leonensis* (68.47%). Freshwater sardines represent a common and major source of protein in fish feed preparations (Eyo, 1991)

The amino acid profiles of the three zooplankton are compared with those of similar live fish food organisms from other geographical regions in Table 2, showing a close relationship. The amino acid concentrations of *Moina* in this study were higher than those obtained for *Moina* by Watanabe et al. (1983), except for alanine, proline, serine, threonine, and tyrosine, and higher than those obtained for *Artemia*, except for alanine, glycine, proline, serine, and tyrosine. The amino acid profile of *Diaphanosoma* was higher than that of *Artemia*, except for arginine, serine, and tyrosine. *Brachionus calyciflorus* had higher amino acid levels than *B. plicatilis*, except for

Table 2. Comparison of amino acid compositions of *Moina micrura, Diaphanosoma excisum,* and *Brachionus calyciflorus* with live food organisms from other studies.

	Moina		*Diaphanosoma*	*Brachionus*		*Artemia*
	1	*2*	*1*	*1*	*2*	*2*
Alanine	2.84	4.9	4.46	4.00	3.9	4.1
Arginine	8.17	5.1	4.78	6.37	5.2	5.0
Aspartic acid	9.84	8.3	10.23	10.53	9.8	7.5
Cystine	2.89	0.6	1.26	1.55	0.7	0.4
Glutamine	15.39	9.8	13.61	12.22	10.1	8.8
Glycine	3.90	3.7	7.80	3.37	3.6	4.7
Histidine	5.09	1.6	2.60	1.83	1.7	1.3
Isoleucine	4.18	2.5	2.72	4.32	4.4	2.6
Leucine	8.00	6.0	8.00	8.95	6.9	6.1
Lysine	10.73	5.8	9.95	8.64	6.6	6.1
Methionine	1.12	1.0	2.45	0.93	1.0	0.9
Phenylalanine	3.75	3.6	3.75	5.20	4.5	3.2
Proline	3.18	4.2	6.44	6.03	5.0	4.7
Serine	3.42	4.0	2.65	3.45	3.7	4.6
Threonine	2.93	3.8	3.84	3.92	4.0	1.7
Tyrosine	3.00	3.3	3.21	2.82	3.0	3.7
Valine	4.44	3.2	6.23	4.83	4.4	3.2

1 = this study
2 = Watanabe et al., 1983

glycine, isoleucine, methionine, serine, threonine, and tyrosine, and *Artemia,* except for alanine, glycine, serine, and tyrosine.

The economic implications of this study for aquaculture are largely two-fold. Firstly, as a basic dietary requirement for larvae and post-larvae feed, *M. micrura, D. excisum*, and *B. calyciflorus* contain adequate crude protein and amino acid concentrations that are vital to the good growth and survival of fish species. Therefore, they can serve as good and cheap replacements for the expensive imported marine *Artemia*. Lavens and Sorgeloos (1996) found that *Artemia* cysts are less nutritionally valuable than natural zooplankton populations. Jeje (1992) recorded improved performance of *Clarias anguillaris* larvae fed natural zooplankton as compared to hatched *Artemia* nauplii. Secondly, large quantities of harvested and dried zooplankton are cheap sources of protein for incorporation into artificial fish feeds that could allow for reduction of the use of freshwater sardines and tilapias, freeing them for human consumption.

Acknowledgement

We are grateful to the Federal Ministry of Agriculture and Rural Development, Nigeria, for providing funds for this study under the Program of Research and Development for Aquaculture Production.

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