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Fatty acid profiles and growth of African catfish (*Clarias gariepinus*, Burchell, 1822) larvae fed on freshwater rotifer (*Brachionus calyciflorus*) and *Artemia* as live starter feeds

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Abstract
The potential use of locally grown rotifers (*Brachionus calyciflorus*) as an alternative starter live feed to *Artemia* in the feeding of African catfish larvae was explored. Larvae cultured in experimental tanks under ambient hatchery conditions were fed on three experimental diets; freshly decapsulated *Artemia* cysts, rotifer, *Brachionus calyciflorus* and a combination of the two for three days following commencement of exogenous feeding. Change in Total Length (TL) measurements of larvae was used as a measure of growth and fatty acid profiles of six-day old larvae were determined using Gas chromatography-mass spectrometry (GC-MS) method. Overall, the growth of Rotifer-fed African catfish larvae was significantly better than *Artemia* – fed larvae (F= 47.605, P=0.000). Noteworthy, was the fact that catfish larvae fed on a mixture of rotifers and *Artemia* grew faster (10.04±0.45 mm, P=0.000) than those fed on either rotifers or *Artemia* (Rotifer-9.04±0.58 mm, *Artemia* -8.78±0.54 mm, P=0.147). Significantly higher composition of Arachidonic acid (AA) and Docosahexaenoic acid (DHA) were recorded for rotifer -fed larvae than for *Artemia*-fed larvae (AA: F=22.292 and P=0.016, DHA: F=28.740, P=0.011). These essential fatty acids play a significant role in the structural, physiological and functional development of larval fish and may explain the better growth recorded in this study. A combination of rotifers with *Artemia* was of an added advantage possibly due to the large-sized *Artemia* that makes catchability easy. The results demonstrated that partial or total replacement of *Artemia* with rotifers as a live starter feed for African catfish larvae is feasible because they compete favourably with *Artemia* in fish larvae growth performance.

Keywords: African catfish (*Clarias gariepinus*), Starter feed, Rotifer (*Brachionus calyciflorus)*, *Artemia*, Essential fatty acids.

1. Introduction
African catfish (*Clarias gariepinus*) (Burchell, 1822) is one of the commercially farmed fish in Uganda that has gained rapid popularity because of its fast growth and high yields. However, the absence of a readily available starter feed in commercial hatcheries remains a major obstacle in its production. Unlike other fresh water fish species whose newly hatched larvae accept formulated feeds with a defined composition to ensure maximal growth and survival [1], African catfish larvae rely on the yolk sac for its nutritional requirements [2] during early stages of growth. At the onset of exogenous feeding, African catfish larvae requires live feeds such as *Artemia* nauplii/ cyst, yeast, unicellular algae, rotifers, copepods, cladocerans as the most appropriate starter feeds because the larvae have difficulty in assimilating dry prepared diets due to their incomplete development of the digestive system [2]. These live feeds offer an appropriate size ingestible by a wide range of larval fish species and are rich carriers of digestive enzymes. They are therefore very paramount in producing maximum number of high quality fish seed from the available brood stock which is the main objective of any fish hatchery system [3]. Additionally, the high nutritional quality (presence of nutrients such as lipids) of these live starter feeds meets the demand for high growth and development rates that occurs at this stage of fish [4]. Lipids are particularly important in larval fish nutrition not only for supplying calorific energy but also for providing the essential polyunsaturated fatty acids (PUFA) that allow optimal physiological performance in the growth process including visual

development, optimal pigmentation and immunity, maintenance of cell membrane fluidity that are expressed in better growth and survival of fish larvae [5, 6, 1].

The production of nutritionally adequate live starter feeds therefore, is a bench mark for successful fish seed production of African catfish. However, their appropriate culture in adequate quantities of for propagation of African catfish remain a challenge resulting into high larvae mortality at early life stages [7] and thus low numbers of fish larvae obtained in hatcheries. In Uganda, the present practice among fish farmers (hatchery operators) is the use of decapsulated cysts of different *Artemia* strains following commencement of exogenous feeding and this has resulted into a low survival rate in hatchery-based catfish seed production as low as 15% attributed to mainly poor larval nutrition. It is documented that the nutritional quality of *Artemia* may vary considerably according to the geographical strain, processing batch and development stage [8] as observed by [9] while culturing Sole larvae (*Solea solea* L.) on two different strains of *Artemia* nauplii. The farmers are therefore not able to identify the best already packaged strain to use and yet not all strains of *Artemia* guarantee equal culture success in aquaculture hatcheries [10]. These factors together with the high cost and occasional scarcity of *Artemia* also make it us suitable for commercial aquaculture [11]. There is a need therefore to explore alternative starter feeds (especially live feeds) to this *Artemia* to counteract these challenges.

Rotifers especially *Brachionus calyciflorus* rotifers have been viewed as potential substitutes for *Artemia* as a live starter feed in African catfish larvae rearing because of their good morphological, behavioural and nutritional characteristics [12, 13, 14, 15]. A partially bigger mouth in African catfish larvae [16] than most cyprinid larvae permits newly born larval *Clarias gariepinus* to consume rotifers with sizes greater than 200 µm. This study therefore compared the growth performance and fatty acid profiles of African catfish larvae fed on *B. calyciflorus* and *Artemia* larvae in an attempt to present *B. calyciflorus* as alternative starter feed to *Artemia*, partially or in totality for the culture of African catfish larvae.

2. Materials and methods

2.1 Study area

This research project utilised the facilities of the wet laboratory of the Department of Biological Sciences-Makerere University for plankton culture, Ssenya Commercial Fish Farm located in Central Uganda; Masaka district for growth performance experiments of African catfish larvae raised in an indoor hatchery and Chemistry Department-Makerere University for fatty acid profiling.

2.1 Culture of algae *(Chlorella* sp.)

Chlorella sp. was cultured to act as a source of food for *B. calyciflorus*. 25 litres rectangular glass tanks were used for this culture experiment with continuous supply of aeration supplied through perforated air stones, to keep the *Chlorella* cells in constant circulation prevent settling and facilitate maximum exposure to light. 10 g of each of Diammonium Phosphate and Urea were supplied to the *Chlorella* culture as a source of nutrients. The cultures were also supplied with 24 hour constant lighting using a single 40W (daylight) fluorescent tube (equivalent 1000 Lux). Water quality parameters; ammonia and pH were monitored daily for the entire period of the experiment and always regulated to fit in the suitable parameters for *Chlorella* growth [17] by refreshing the cultures with chlorine free water. Counts of cells/ml were taken using a magnification of x200 on an inverted microscope (WILOVERT®) and a *Sedgwick*-Rafter Cell counting chamber to ensure that enough food for the rotifers is available before initiating their culture.

2.2 Acquisition of *B. calyciflorus*

Seed rotifers were collected by selective netting with 200µ, 100µ and 50µ zooplankton nets from "green" pond (eutrophic) water at the botanical gardens in Makerere University. To achieve a culture of only rotifers, 'Basudine' an organophosphoric acid ester was applied at a rate of 1.2mgl^{-1}, following [18]. This chemical at the set concentration, knocks off copepods, cladocerans, and mosquito larvae but does not harm rotifers, thereby allowing a clean rotifer population to flourish [19].

2.3 Population growth and enumeration of *B. calyciflorus*

This was carried out in 10 and 20 litres plastic jerry cans. Batch and semi continuous culture techniques [20] were used to culture sufficient numbers of *B. calyciflorus*. *B. calyciflorus* rotifers were transferred into 8 litres of the treatment food (*Chlorella* sp.) suspensions. *B. calyciflorus* were counted at x20 magnification and transferred into fresh algal suspensions (initial algal densities, x 10^4 cells/ml). Once a day, a 1ml sample was taken from which the number of individuals/ml were counted and recorded. All experiments were carried out at room temperature 25^{o}C with a 12:12h light: dark cycle. To minimize sedimentation, the experimental tanks were gently bubbled with air from a compressor. At day seven of this culture, an adequate number of rotifers for the fish larvae was attained. These *B. calyciflorus* rotifers were then transported from culture units at Department of Biological Sciences, Makerere University to Ssenya commercial fish farm in 20 litres plastic jerry cans.

2.4 Growth performance (Total length) of African catfish larvae

Commercial fish hatchery unit located at Ssenya commercial fish farm was utilized for this feeding experiment. This fish farm's hatchery was utilized for testing performance of African catfish larvae fed on Chinese *Artemia* strain (most commonly utilized *Artemia* strain in Uganda), Rotifer *B. calyciflorus* and mixture of Chinese *Artemia* strain and Rotifer *B. calyciflorus* as live starter feeds. All water supplied to the hatchery was sieved through a 50 µm mesh to eliminate zooplankton contamination from the water supply ponds. Experimental plastic basins (30 L) were used as culture tanks in triplicate of the three feed experiments and modified to fit in the flow – through system of the hatchery unit.

The African catfish larvae were obtained following induced breeding of adult African catfish and subsequent hatching of eggs following routine procedures used at the farm. To each of the experimental tanks (30 litres capacity), five hundred (500) larvae of a uniform initial mean Total Length (TL) of 7.54 mm were randomly distributed and maintained under ambient hatchery conditions. These larvae appeared healthy and active and had no signs of disease. Water temperature, dissolved oxygen levels, pH and ammonia levels were monitored and maintained regularly after every one hour to appropriate catfish hatchery conditions (Temperature: 24-28 ^{o}C, Dissolved Oxygen: 5-8 mg/l, pH: 6-8, and Ammonia: less than 0.1mgl-^{1}) during the experiment. African catfish larvae were fed for three days on the starter feeds (*Artemia, B. calyciflorus* and mixture of the two diets) following commencement of exogenous feeding (day three) as is the practice of fish farmers

in Uganda. This experiment was conducted using the China-*Artemia* strain since most catfish hatcheries (more than 90% of famers) in Uganda use this strain. Feeding rate of 400 rotifers per larvae per day [21] was applied. Decapsulation of *Artemia* cysts followed standard decapsulation procedures [22] and a similar feeding rate of the fish larvae as for rotifers adopted. Fish larvae were fed five times a day at an interval of two hours and feed provided slightly above required estimates to allow feeding to satiation.

Starting on day four to the sixth day, 30 larvae were randomly picked from each experimental basin one hour after the first feeding (9.00 am) for measurement of total length (TL) as an indicator of growth since it was easier to measure, unlike wet weight measurement which was not feasible since the larvae were too small and fragile. Each of the 30 larvae were placed on filter paper to allow absorption of excess water and create a situation of inactivity before taking length measurements using a Vernier caliper to the nearest 0.05 mm. Daily mean total length and Specific Growth Rate (SGR) were determined following the formulae provided by [4], to indicate the differences in the impact of test starter diets on growth of the larvae.

2.5 Fatty acid profiles (nutrition status indicators) of African catfish larvae

On the sixth day of the feeding experiment, a sub sample of 30 larvae was randomly collected from each of the experimental tanks of each treatment, dried with filter paper and wrapped in aluminium foil taking special care to eliminate any contamination. Each sub sample was then labelled based on the treatment, stored under ice and immediately transported to the department of Chemistry, Makerere University for fatty acid profiling.

2.5.1 Fatty Acid Analysis and Estimation

Fish larvae samples were dried in a hot air oven at a constant temperature of 60 °C. The dried samples were then used for estimation of lipid [23]. Fatty acids were saponified and methylated using 2% NaOH in methanol, 14% BF /methanol and heptane. The fatty acid methyl esters sufficient (FAME) were determined on a Hewlett Packard HP 5890 gas chromatograph equipped with a flame ionization detector. The sample were injected at 190 °C onto a J and W Scientific DB23 fused silica capillary column (30 m x 0.25 mm i.d., 0.25-µm film thicknesses) with hydrogen as the carrier gas. The column was operated isothermally at an oven temperature of 180 °C and a detector temperature of 210 °C. Fatty acids of the three settings were identified by comparing with authentic standards [24].

2.5 Data analysis

The data obtained were tested for normality and homogeneity of variances and later compared in the feeding trials (treatments) using one-way Analysis of Variance (ANOVA) after the collected data conformed to all the ANOVA assumptions to test significant differences (p<0.05) in growth and fatty acid composition.

3. Results

3.1 Comparison of growth of catfish larvae fed on decapsulated *Artemia* (Chinese strain) and Rotifer *B. calyciflorus*

Figure 1 shows the impact of three different diets on the catfish larvae growth. One way ANOVA revealed significant differences (F= 47.605, P=0.000) in African catfish larvae growth measured as Total Length (TL) among treatments. Rotifer-*Artemia*-fed African Catfish larvae had the highest overall TL (10.04±0.45) mm at the end of the experiment followed by Rotifer-fed larvae (9.04±0.58) mm and finally *Artemia* (8.78±0.54) mm. Significant variation in Specific Growth Rate (SGR) of catfish larvae was similarly observed across the three diets (F=46.162, P = 0.000) as indicated in Figure 2.

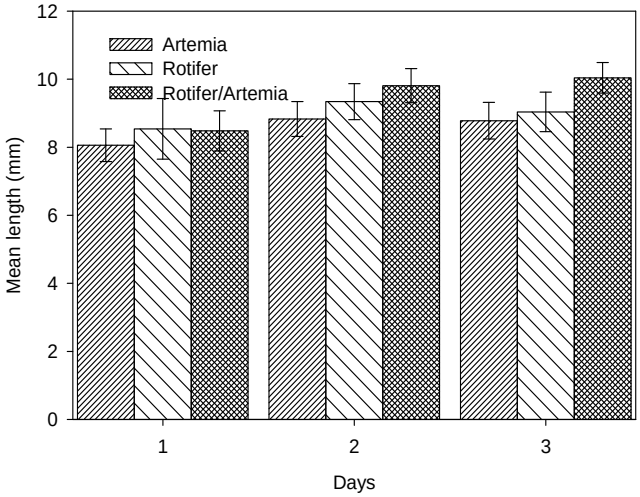

Fig 1: Mean length (mm) ± SD of African catfish larvae fed on three experimental diets (*Artemia*, rotifer, and a mixture of *Artemia* and rotifer) from day 1 to day 3 following commencement of exogenous feeding

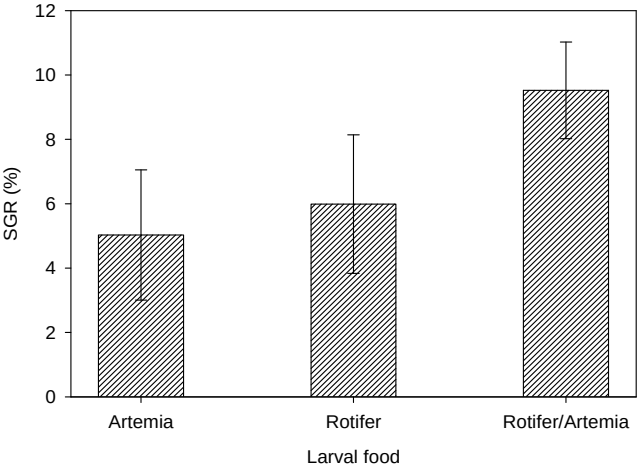

Fig 2: Specific Growth Rate (SGR % ± SD) of African catfish larvae fed on three experimental diets (*Artemia*, rotifer, and a mixture of *Artemia* and rotifer)

3.2 Fatty acid profiles of catfish larvae fed on different diets for three days

The analysis of fatty acids in the African catfish larvae samples indicated variation in fatty acid composition across feed treatments (Table 2). The composition of saturated fatty acids was higher in rotifer- fed African catfish larvae (38.92±0.65) % as when compared to *Artemia*-fed (35.16±1.41) % and Rotifer/*Artemia*-fed larvae (34.66±0.67) %. Noteworthy was the higher concentrations of 16:0 and 18:0 of the saturated fatty acids presented across the diets and with a higher concentration in the rotifer-fed catfish larvae. Rotifer-fed catfish larvae demonstrated a comparably low composition of total monounsaturated fatty acids (MUFAs) (16.18±0.45)%.

Artemia + Rotifer-fed larvae had a higher composition of total MUFAs (26.96±1.10) % followed by *Artemia* fed larvae (21.3±0.30) %. Of the MUFAs present, 16:1n5, 18:1n7 and 18:1n9 were generally high across diets but comparably much higher in *Artemia*-fed larvae than in Rotifer-fed larvae. The composition of polyunsaturated fatty acids was higher in Rotifer-fed larvae (43.10±1.51) % followed by *Artemia* + Rotifer-fed larvae (38.38±1.38) and finally *Artemia* -fed larvae (37.85±2.15) %. The high composition of DHA; 22:6n3 (17.68±0.43) % in Rotifer-fed larvae as when compared to *Artemia*-fed larvae (14.58±0.86) % was notable. Rotifer- fed larvae also recorded higher proportions of DHA/EPA and AA/EPA than was the case for *Artemia*-fed larvae.

Table 1: Main fatty acid composition (% of total fatty acids) of catfish larvae fed on three different experimental diets

Fatty acid	Artemia	Rotifer	Artemia + Rotifers	
14:0	0.59±0.00	0.54±0.01	0.86±0.03	
15:0	0.80±0.16	1.02±0.03	0.78±0.01	
iso15:0	0.37±0.03	0.47±0.04	0.47±0.01	
iso 17:0	0.0	0.38±0.00	0.40±0.00	
16:0	18.39±0.73	20.18±0.33	18.80±0.27	
17:0	0.92±0.04	0.99±0.07	1.03±0.01	
18:0	14.11±0.45	15.35±0.19	12.33±0.33	
∑SFAs	**35.16±1.41**	**38.92±0.65**	**34.66±0.67**	**F=20.979, P=0.017**
14:1n5	0.0	0.0	0.36±0.03	
16:1n4	0.88±0.05	1.17±0.03	0.86±0.01	
16:1n5	3.35±0.08	1.54±0.03	5.04±0.45	
16:1n7	0.48±0.06	0.49±0.04	0.74±0.07	
17:1n9	0.0	0.0	0.85±0.09	
18:1n7	5.10±0.05	3.30±0.06	6.11±0.22	
18:1n9	11.49±0.05	9.67±0.28	13.00±0.23	
∑MUFAs	**21.3±0.30**	**16.18±0.45**	**26.96±1.10**	**F=140.182, P=0.001**
18:2n6(LA)	6.50±0.37	6.49±0.16	8.25±0.23	
18:3n3(LNA)	1.45±0.19	1.89±0.26	2.77±0.06	
20:2n6	1.13±0.05	1.41±0.06	0.98±0.01	
20:3n6	1.98±0.15	2.47±0.02	1.84±0.01	
20:4n6(ARA)	6.20±0.37	7.32±0.25	5.50±0.18	
20:5n3(EPA)	3.71±0.01	3.16±0.15	4.43±0.16	
22:5n6	0.93±0.14	1.37±0.10	0.84±0.07	
22:5n3	1.36±0.02	1.30±0.09	1.18±0.01	
22:6n3(DHA)	14.58±0.86	17.68±0.43	12.59±0.67	
∑PUFAs	**37.85±2.15**	**43.10±1.51**	**38.38±1.38**	**F=13.492, P=0.032**
DHA/EPA	3.93	5.59	2.84	
AA/EPA	1.67	2.32	1.24	
DHA/AA	2.35	2.42	2.29	

Note: ∑SFAs: Sum of Saturated Fatty Acids; ∑MUFAs: Sum of Monounsaturated Fatty Acids; ∑PUFAs: Sum of Polyunsaturated Fatty Acids; DHA: Docosahexaenoic Acid; EPA: Eicosapentaenoic Acid; AA: Arachidonic Acid; LA: Linoleic Acid; LNA: Linolenic acid

Of the essential fatty acids, variation in composition was similarly observed across diets (Figure 3). Fish larvae fed on Rotifer indicated significantly high percentage composition of Docosahexaenoic acid (DHA, 22:6n3; F=28.740, P=0.011) and Arachidonic Acid (AA, 20:4n6; F=22.292 P=0. 016). On the other hand, *Artemia*-fed larvae, had slightly higher levels of Linoleic Acid (LA, 6.5%) and Eicosapentaenoic Acid (EPA, 3.71%), than Rotifer - fed larvae. Consequently, larvae fed on a combination of *Artemia* and Rotifer had significantly higher percentages of three of the essential fatty acids: Linoleic acid (LA, 18:2n6; F= 29.405, P=0.011), Linolenic acid (LNA, 18:3n3; F=25.926, P=0. 013) and Eicosapentaenoic acid (EPA, 20:5n3; F=51.908, P=0.005), and higher composition of all the three essential fatty acids when compared to *Artemia* only-fed larvae.

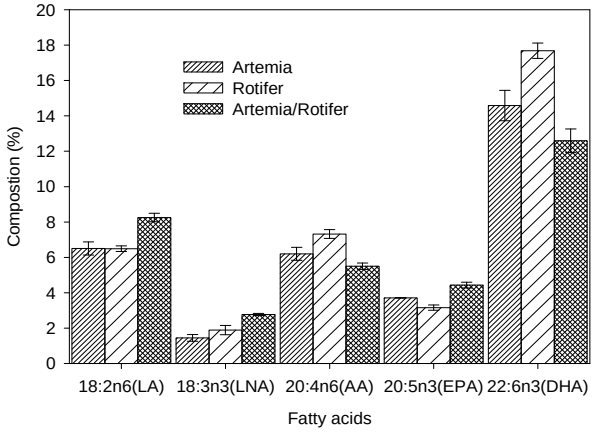

Fig 3: Essential fatty acid composition of African catfish larvae fed on different starter fe

4. Discussion

4.1 Comparison of growth of catfish larvae fed on different starter live food (*B. calyciflorus*, *Artemia* (Chinese strain) and *B. calyciflorus*-*Artemia* mixture)

The observed differences in growth performance of *B. calyciflorus*- fed catfish larvae compared to *Artemia* could be attributed to several reasons: The fact that *B. calyciflorus* rotifers offer a much smaller live feed than the size of decysted *Artemia*, could have favoured the initial stages of African catfish larvae. Jeje [25] reports that the larvae of African catfish are small at hatching less or equal to 4mg and 7mm in weight and length respectively. The small size of this larvae possibly thrives better on small zooplanktons especially rotifers whose ideal size ranges from 50-200 microns [26] compared to decysted *Artemia* cysts whose size range from 200 to 300 microns, depending upon the strain [27,28]. This size suitability coupled with their relative mobility makes it easier for them to be found and captured as food with lower energetic cost [29].

Additionally, differences in the nutritional composition of rotifers and *Artemia* could explain the growth trends revealed in this study. Rotifer-fed larvae were richer in essential fatty acids and similarly larvae fed on the combination rotifers and *Artemia*, which accounts for improved growth rate. This observation is in accordance with other previous researchers [12, 30] who indicated that rotifers confer better nutritional benefits to fish larvae since they are able to transfer fatty acids and other nutrients through the algae-rotifers-larvae food chain.

The better growth performance of rotifer-fed African catfish larvae also corresponds with the high levels of DHA in the rotifer diet. The larval fatty acid profiles are always reflection of the diet profiles [31]. DHA, an essential fatty acid that accumulates in the brain of fish during early development and functions to increase neural functions [32], is easily incorporated in rotifers, unlike *Artemia* which catabolizes this fatty acid [33]. Docosahexaenoic acid (DHA) also has important structural and functional roles in all membranes, but especially neural membranes [34, 35, 1]. It is also noted that n-3 polyunsaturated (PUFA), principally DHA, has a role in maintaining the structure and functional integrity of fish cells with a specific and important role in neural (brain and eyes) cell membranes [36]. Fish larvae are thought to be visual feeders, adapted to attacking moving prey in nature [37]. Therefore higher DHA content in rotifer-fed larvae compared to *Artemia*- fed larvae in this study could explain better growth due to improved visual performance of the larva leading to more larval feeding responses. These results are in accordance with some studies that revealed that DHA is very paramount for various physiological functions, including survival, growth, and pigmentation success [38].

The superior growth performance of catfish larvae fed on a combination of rotifer *B. calyciflorus* and *Artemia* agrees with previous studies that proved that live feed mixture containing different live feeds provides a wide spectrum of live feeds for choice over the experimental period [39, 12]. In this case, the availability of different prey sizes as the mouth gape of fish larvae undergoes ontogenic development provide all the possibilities of preferred prey size for the growing larvae.

4.2 Fatty acid profiles of catfish larvae fed on different diets for three days

Lipids occur naturally in the *Artemia* embryos (cysts) and zooplankton like rotifers, and because of their significance in fish nutrition, fatty acid composition was used to evaluate the quality of the feed for larval fish in this study. The fatty acids of *B. calyciflorus* rotifers and *Artemia* were not determined since fish larvae fatty acid composition reflects the composition in their diets [40, 41].

The predominance of PUFAs and SFAs as when compared to MUFAs across the test diets in this study, is in accordance with previous findings [42]. Preferential catabolism of MUFAs along with preferential retention of DHA, EPA and AA, and specific SFAs, usually 16:0 or 18:0 by embryos of a variety of species has been recorded [42]. This reflects the essential structural role of DHA in membranes, the importance of AA and EPA in eicosanoid production and specific roles of SFAs in the sn-1 position of structural phospholipids [43, 44]. Amongst the SFAs, Palmitic acid (16:0) was predominant. This is in agreement with other previous researchers [45, 46] who observed that Palmitic acid (C16:0) is a key metabolite in fish.

In this study, total replacement of *Artemia* with *B. calyciflorus* rotifers conferred quantitatively higher composition of three (LNA, AA, DHA) of the five recorded essential fatty acids. Interestingly, DHA composition was remarkably high compared to AA and LNA compositions. This suggests higher DHA composition in rotifers, attributed to the ability of the rotifer *B. calyciflorus* to convert linoleic acid (18:3n3) to AA and finally DHA [47]. Similarly, rotifer *B. calyciflorus* converts linolenic acid (18:3n3) to EPA (20:5n3) and consequently to DHA (22:6n3) [48]. This further explains the reason for higher DHA levels in the *B. calyciflorus*- fed fish larvae compared to *Artemia* fed- larvae thus demonstrating the nutritional superiority of *B. calyciflorus* rotifers.

This higher composition of DHA in the *B. calyciflorus*-fed larvae in this study corresponded with high specific growth rate of the larvae which agrees with studies [38, 49, 50], that revealed the significance of DHA in controlling various physiological functions such as growth and survival. The remarkably high level of polyunsaturated fatty acids in rotifer-fed larvae in this study is a reflection of the fatty acid levels obtained from their food (rotifers). This observation is in accordance with other researchers [51] who demonstrated that rotifers catabolize fats easily and can store highly unsaturated fatty acids (HUFA). These fatty acids are later passed onto the fish along the food chain.

The high composition of AA in *B. calyciflorus* rotifer-fed catfish larvae in this study may further explain improved growth performance of catfish larvae as compared to when *Artemia* was used. Arachidonic acid (20:4n6; AA) is believed to be the chief source of eicosanoids in fish [1]. Eicosanoids produce highly bioactive molecules following regulated dioxygenase enzyme-catalysed oxidation of HUFA 20:4n6 (AA) and 20:5n3 (EPA). In fish, these molecules are involved in a great variety of physiological functions including blood clotting, immune and inflammatory responses, cardiovascular tone, renal and neural functions [52].

The low levels of essential fatty acids in *Artemia* fed fish larvae in this study also agrees with other previous studies that reported a deficiency of some essential fatty acids in *Artemia* [53, 54, 55] that are necessary in larval development, larval health, proper growth, prevention of anaemia and survival. The concentration of DHA in the polar lipid fraction of *Artemia* is very low [53] further explaining the low levels of DHA in *Artemia*-fed larvae in this study. Larvae fed on a diet combination had the highest composition of 2 essential fatty acids (LA and LNA) and thus explaining better growth performance of larvae fed on diet combination.

A similar observation was made [56, 57] and explained that LA

and LNA are true EFAs in freshwater fish species; these is because fish, like other vertebrates, cannot synthesize de novo polyunsaturated fatty acids and consequently require a dietary supply of these essential fatty acids (EFAs). Since fish do not possess the Δ12 and Δ15 desaturase enzymes necessary to produce 18:2n−6 (linoleic acid, LA) and 18:3n−3 (linolenic acid, LNA), respectively from 18:1n−9 (oleic acid, OA), and because freshwater teleosts have an innate capacity to desaturation and elongate LA to 20:4n−6 (arachidonic acid, AA) and LNA to 20:5n−3 (Eicosapentaenoic acid, EPA) and ultimately 22:6n−3 (Docosahexaenoic acid, DHA) [57].

Although freshwater fishes have this ability to modify dietary LA and LNA; the rate at which they do so may be too low to satisfy the high DHA requirement especially during early larval growth [24]. Besides young fish deposit zooplankton fatty acids in total lipids with little change [58], therefore DHA, EPA and ARA can be designated as "Essential Fatty Acids" for fish larvae too. In addition, all vertebrate species require both n-6 and n-3 PUFA [1], but the biologically active forms of EFA are generally the C20 and C22 metabolites of 18:2n-6 and18:3n-3, specifically 20:4n-6 (arachidonic acid -ARA), 20:5n-3 (eicosapentaenoic acid -EPA) and 22:6n-3 (docosahexaenoic acid -DHA), which in aquaculture are often termed highly unsaturated fatty acids (HUFA). It is also noted that EPA (20:5ω3) and DHA (22:6ω3) acids are essential fatty acids for larviculture of both marine and freshwater fish and crustaceans [59, 60].

5. Conclusion

It can be inferred from the results obtained from the study that *B. calyciflorus* rotifers confer a better specific growth rate to the African catfish larvae as compared to the case of utilizing decysted *Artemia* cysts. Notably however, is the best specific growth rate conferred to the African catfish larvae by a diet combination of *B. calyciflorus* and *Artemia*. Secondly, the better growth performance of *B. calyciflorus*-fed catfish larvae can be explained well based on the nutritional superiority of *B. calyciflorus* coupled with its suitable size of prey and mobility qualities as compared to *Artemia*. This factor favours B. *calyciflorus* as suitable substitutes for *Artemia* in the feeding of African catfish larvae. However, a combined diet of decysted *Artemia* and *B. calyciflorus* as investigated in this study provides even a better substitute to *Artemia* alone.

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