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Comparative analysis of nutritional profiles of *Moina macrocopa* cultured in different media: An investigation into the effects of culture media on aquatic organism nutrition

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Abstract
Cladoceran *Moina macrocopa* is an important zooplankton for juvenile fish and crustacean, widely used as live feed in aquaculture. In this experiment, five different culture media denoted as mixed feed (Treatment-1), *Chlorella* (Treatment-2), *Spirulina* (Treatment-3), dried cattle (cow) manure (Treatment-4), and baker's yeast (Treatment-5) was prepared to culture *M. macrocopa*. According to the proximate composition data, *Spirulina* and *Chlorella* enriched zooplankton found significantly with higher protein content and lower lipid content among all the treatment groups. While, Out of all the treatment groups, palmitic acid (C16:0) stood out as the most abundant saturated fatty acid (SFA) available. The poly unsaturated fatty acids (PUFA) found to be the highest (35.44±1.43%) in zooplankton enriched in *Chlorella* followed by gamma-linolenic acid (GLA) (C18:3n-6) in *Spirulina* medium. Besides, a maximum of 17 amino acids were present in *Chlorella* and *Spirulina*-treated *M. macrocopa* 56.74±2.10% and 51.72±1.73%, respectively. Arginine, Histidine, and Lysine were dominant between these two treatment groups. Among the non-essential amino acid (NEAA), Aspartic acid and asparagine acid found significantly higher in Baker's yeast enriched *M. macrocopa*. Our results suggest that the *Chlorella and Spirulina* enriched treatment groups showed better findings on protein, lipid and fatty acid as well as essential amino acid content in *M. macrocopa*.

Keywords: *Moina macrocopa*, culture media, amino acid, fatty acid

1. Introduction
Zooplanktons are the common food for the aquaculture fish species. They are the primary source of nutrition such as protein, lipid, amino acids etc. Zooplankton is an essential food source for aquaculture fish species, providing crucial nutrients such as protein, lipids, and amino acids (Khan and Qayyum, 1971) [19]. Seasonal variations can affect the body nutrient composition of natural zooplankton, which may be influenced by the nutrient levels present in the water (Vijverberg and Frank, 1976, Donnelly *et al*., 1994) [44, 8]. However, these levels of nutrients appear to satisfy the requirements of necessary protein, lipid, and phosphorus of fish. Zooplankton's fatty acid composition is affected by the fatty acid composition of their diets (Watanabe *et al*., 1983; Proulex and de la Noile, 1985) [46, 35]. Additionally, the composition may vary as the seasonal succession of phytoplankton species takes place (Jeffries, 1970) [15]. The rich presence of unsaturated fatty acids in zooplankton indicates that it is a nutritious food for raising commercial fish larvae (Lokman; 1994) [21]. For the growth and development of fish, it is vital to have both eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) available in fatty acids (Kanazawa *et al*., 1979; Tucker, 1992) [18, 42]. Watanabe *et al*. (1983) and Lokman, (1994) [46, 21] stated the presence of these two components might be variable from species to species of zooplankton.
Several kinds of research on the growth performance of crayfish and other marine fishes as well as on freshwater fishes suggest that the rate of growth accelerates when nourished with zooplankton (Holm and Moller, 1984; Kamler *et al*., 1992) [13, 17] as compared to formulated

diets.

The flavor and texture of fish were also found to have improved with feeding zooplankton (Spenelli, 1978) [39]. It has been suggested that live zooplankton may contain enzymes such as amylase, proteases, exonuclease, and esterase, which have a significant impact on the digestion of fish larvae (Munilla-Moran *et al*., 1990) [26], and at the same time, the presence of free amino acids forms a powerful attractant and appetite stimulant for fish.

In this study, we have treated *Moina macrocopa* with five culture media in order to estimate their effects on the nutritional profiles. To overcome the essential nutrient contents diet deficiency for both crustacean and fin fish hatcheries and farms in the country, this part of the research work was scheduled and performed with a necessary step.

2. Materials and Methods

2.1 Collection, screening and isolation of *Moina macrocopa*

Adult cultivable *Moina macrocopa* were collected from various places of Dhanmondi and Gulshan lake of Dhaka Metropolitan City with the help of 250-500µm mesh size zooplankton net. Immediately, after collection, these were transported in live condition in a common Erlenmeyer Glass Jar to the experiment site at the BCSIR, Dr. Qudrat-i-Khuda Road, Dhanmondi, Dhaka, Bangladesh. These collection activities were performed early in the morning between 8:00-10:00 am. In the laboratory, the collected adult zooplanktons were screened and isolated from the respective species with the help of a microscope (Optica U2LCD) and a pipette. The isolated species *Moina macrocopa* was then identified according to Brook (1959) [4]. A pipette was used to place a few drops of the water samples on a glass slide mounted on a microscope. With the help of a pipette desired zooplankton (*M. macrocopa*) was collected and stored in a different Erlenmeyer glass beaker containing aerated water to mono-species culture in the pre-prepared research tank.

2.2 Experimental design and enrichment of *Moina macrocopa*

Five types of feed media, such as mixed feed, *Chlorella*, *Spirulina*, dried cattle (cow) manure, and baker's yeast were used individually for each pre-selected aquarium stocked with almost equal density. To culture different stages of each species and in an individual aquarium, the used feed mediums were named – Mixed ingredients as Treatment-1; *Chlorella* (cultured in Bristol's modified medium) as Treatment-2; *Spirulina* as Treatment-3; dried cattle (cow) manure as Treatment-4; and Baker's yeast as Treatment-5 with three replication for every treatment group.

Each treatment feed was applied manually in an amount of 1g/L of water (Treatment-1); 0.5×10^6 *Chlorella* cells/ml (Nandini and Sarma, 2000) [27] (Treatment-2); *Spirulina* powder 0.05 gm/L of water (Treatment-3); dried cattle (cow) manure 1.5g/L of water (Rottman, 1992) [37] (Treatment-4) and baker's yeast 24×10^6 cells/ml of water (Treatment-5) respectively two times (morning and afternoon) daily in each culture medium respectively. The density of *Chlorella* and baker's yeast was estimated by using a hemocytometer.

In the preparation of each feed medium, *Spirulina* and dried cattle manure were dried and crushed into small sizes and dried once again in a laboratory oven (Binder FED 53-UL) and cooled overnight in a desecrator before preserving in an air-tight container for use in research activities. The experiment was initiated through the stocking of 50 individual/L of *M. macrocopa*, in each replicated aquarium for 5 different feed media. Once initiated, each aquarium was aerated for 24 hours with an aerator and fed regularly with the respective feed medium. At the same time, 50% water from each aquarium was exchanged every 5 days and continued up to the end of the experiment.

2.3 Estimation of Proximate Composition

Freshly collected *M. macrocopa* dried to constant weight in an air oven drying method (AOAC, 1984) [2]. The moisture content of the sample was determined as a loss of weight. The percentage of moisture was calculated by the AOAC standard equation. Organic matter was burned off at as low a temperature as possible and the remaining inorganic material was the ash content. The percentage of ash content was calculated by the AOAC standard equation (AOAC, 1984) [2]. The crude protein content was determined by the Micro-Kjeldhal method based on the conversion of nitrogen of protein into ammonium sulfate. The total N content was then converted into crude protein by multiplying with the factor 6.25 and moisture factor. A mixture of chloroform: and methanol (2:1) was used for lipid content determination by Floch's (1957) [9] method. The mixture was allowed to face overnight and the solution was transferred to a weighed conical flask and heated to dryness. A total of 5g dry, homogenized zooplankton sample was taken in a conical flask. Then, add chloroform: methanol (2:1) mixture until the sample is submerged and kept overnight. The samples were then filtered through filter paper and collected into a pre-weighed conical flask. The conical flask was kept in the water bath until the solution was fully evaporated and only oil remains in the flask. Finally, weighed the conical flask and calculated the lipid amount using the formula**.**

2.4 Estimation of Amino acid profile

An amino acid analyzer (SYKAM S4300) was used to detect the amino acid profile of selected zooplankton. A 0.20g sample was homogenized with 25 ml 7N HCl by mortar pestle and filtered**.** The samples were hydrolyzed for 22-24 hours approximately**.** Then 250 ml solution of neutralized HCl was prepared using 7.5N and NaOH solution in a volumetric flask with a sample dilution buffer (pH 3.4). Then the solution was taken in a microfilter (0.45 mm) to remove any foreign particles if present. A vial filled with a total of 100 µL solution. Then, added 900 µL sample dilution buffer (pH 3.4) to the vial was to make the volume 1 mL (10 times dilution). Simultaneous analysis was performed on the standard amino acids. Based on the retention time and the peak area of the standard amino acids, the identification of each amino acid in the unknown sample was made.

2.5 Estimation of fatty acid profile

The hydrolytic method was used for fat and fatty acids extraction from zooplankton. Petroleum ether was used to extract fat, which was subsequently methylated to form fatty acid methyl esters (FAMEs), and their quantitative measurement was carried out using gas chromatography (GC). The process of methylation using HCl and methanol solution was applied to the sample, resulting in the formation of fatty acid methyl ester (FAME) that can be easily detected by GC. Subsequently, the mixture of hexane and anhydrous diethyl ether was used to isolate the fatty acid methyl ester.

To clean the organic phase, a base wash was performed using aqueous NaOH, and the resulting organic layer was separated. Next, a small amount of the sample, specifically 2-3 µl, was injected into a gas chromatograph equipped with a capillary column and flame ionization detector for analysis. To perform the calculation, a chromatogram was utilized while concurrently analyzing standard fatty acids. The identification of each fatty acid in the unidentified pattern was determined by analyzing its retention time and peak area, and comparing it to those of standard fatty acids.

2.6 Statistical analysis

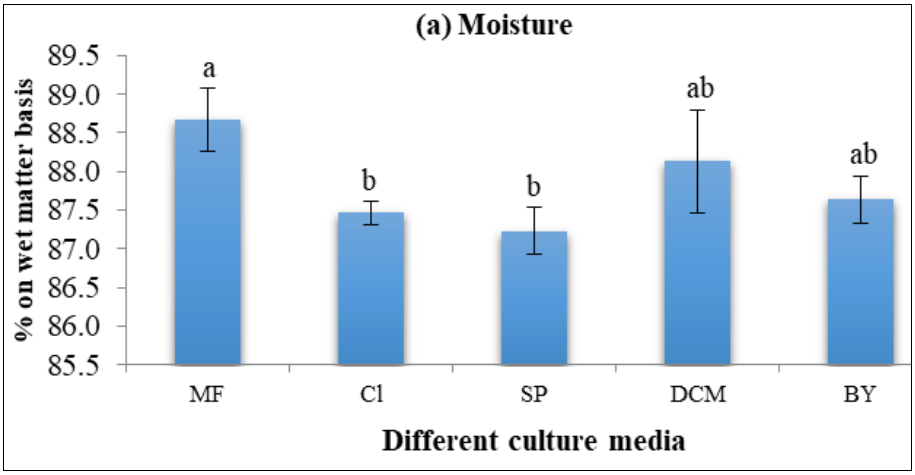
The data were examined to identify the descriptive metrics, such as the mean and standard deviation, minimum and maximum value. To test the equality of population density in five culture media one-way analysis of variance (ANOVA) test was used. Multiple correlations and regression analyses for different food media and population densities were used to show the dependency of these parameters on the mass culture of selected live feed. Microsoft Office Excel 2010 was used for data analysis. The Statistical Package for the Social Sciences (SPSS) v. 20.0 software package (SPSS, SAS Institute Inc. Gary, USA) was used to analyze the data on proximate composition, amino acid, fatty acid profile, and growth performance indicators were compared by t-test and ANOVA followed by Tukey's HSD post hoc for multiple comparisons.

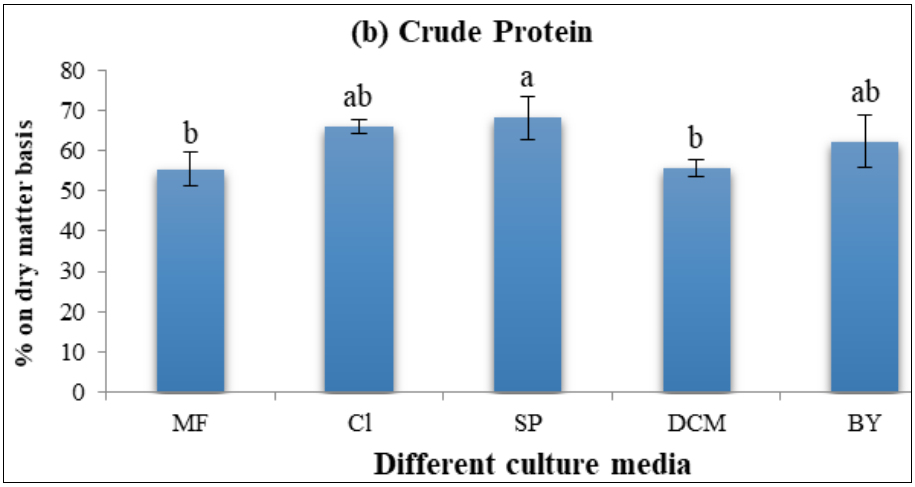
3. Results

Various biochemical compositions *i.e*., proximate (moisture, crude protein, lipid and ash content), fatty acid and amino acid content of experimental zooplankton cultured in five different media were analyzed.

Laboratory-analyzed moisture content in *M. macrocopa* reared in five different culture media is presented in Fig. 1a. It was found to be in the range of 86.9% to 89.1%. The mean highest content (88.67±0.4%) of moisture was found fed with mixed feed in, which was significantly (P<0.05) higher than those measured in the other four treatments. While significantly (P<0.05) lower mean moisture content was found in *Chlorella* (87.47±0.15%) and *Spirulina* (87.23±0.31%) treated *M. macrocopa*. The mean moisture content was 88.13±0.67%, and 87.63±0.31% in dried cattle manure and Baker's yeast-treated *M. macrocopa* respectively.

Fig. 1(b) depicted the crude protein content in *M. macrocopa* reared in five different culture media. It was found to be in the range of 52.8% to 71.8% on a dry matter basis. The mean crude protein content (68.1±5.48%) was found significantly higher (P<0.05) in *Spirulina* feed, while the mean lowest 55.4±4.16% was found in mixed feed treated *M. macrocopa*. Otherwise, the mean crude protein content in *Chlorella*, dried cattle manure, and Baker's yeast-treated *M. macrocopa* was 65.87±1.65%, 55.70±1.97%, and 62.27±6.54% respectively.





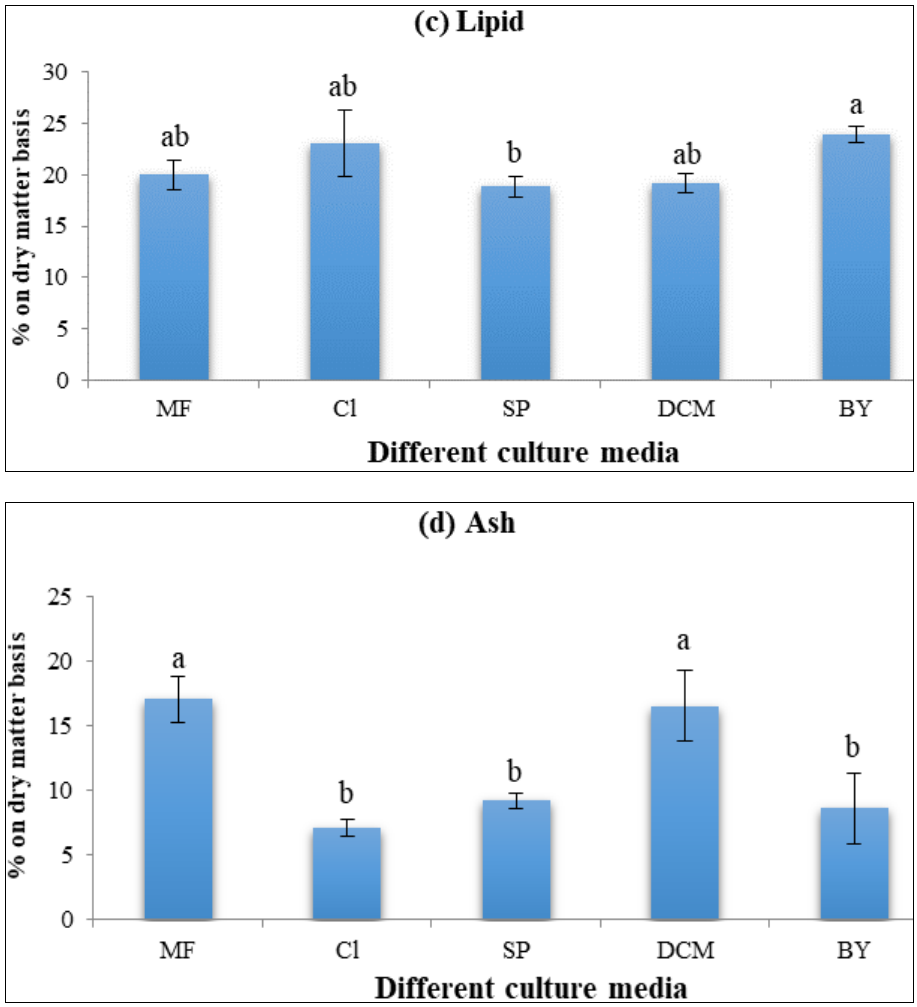


Fig 1(a-d): Proximate composition of *M. macrocopa* a) Moisture b) Crude protein c) Lipid and d)Ash content. (MF=Mixed feed, Cl=*Chlorella*, SP=*Spirulina*, DCM=Dried cattle manure, BY=Baker's yeast). Bars (mean ± SD) with different letters denote significant differences (ANOVA, HSD; $p<0.05$)

In this study, fat content was found to be in the range of 17.8% to 26.6% on a dry matter basis (Fig. 1c). Significantly ($p<0.05$) higher mean lipid content (23.93±0.78%) was found with baker's yeast fed sample, while significantly ($p<0.05$) lower (18.83±1.05%) mean lipid content was quantified in *Spirulina* treated *M. macrocopa*. Besides, the mean lipid contents in mixed feed, *Chlorella*, and dried cattle manure treated samples were 19.97±1.48%, 23.03±3.26%, and 19.17±0.98 respectively (Fig. 1c).

The ash content of *M. macrocopa* was found within the range of 6.4% to 18.8% on a dry matter basis (Fig. 1d). The mean lowest (7.1±0.66%) and highest (17.07±1.79%) ash contents were noted in *Chlorella* and mixed feed-treated *M. macrocopa* respectively. Besides, with other feed groups, *Spirulina* had a mean ash content of 9.2±0.56%, while, 16.53±2.73% in cattle manure, and 8.63±2.75% in Baker's yeast-treated samples (Fig. 1d). Ash content in mixed feed and dried cattle manure treated *M. macrocopa* was significantly different from *Chlorella, Spirulina* and baker's yeast treated *M. macrocopa*.

The total body fatty acid profiles of *Moina macrocopa* are summarized in Table-1, in which fatty acids with single bond have been categorized as Saturated Fatty Acid (SFA), one double bond has been categorized as Mono Unsaturated Fatty Acid (MUFA) and those with more than one double bond are categorized as Poly Unsaturated Fatty Acid (PUFA).

The SFA was significantly ($p<0.05$) highest in mixed feed (40.77±2.56%) and least in *Chlorella* (28.00±1.17%) treated *M. macrocopa*. Whereas it was in *Spirulina*, dried cattle manure and baker's yeast-fed samples were 33.46±1.08%, 38.26±0.8%, and 35.47±1.07% respectively. Individual composition and the availability of respective ingredients of SFA (Table 1) revealed that among all the saturated fatty acids available and in the five feed-treated media used for raising *M. macrocopa*, Palmitic acid (C16:0) was the most prevalent. However, in the mixed feed medium, Myristic acid (C14:0), Margaric acid (C17:0), and Stearic acid (C18:0) were found to be significantly higher ($p<0.05$) than the others, while Pentadecylic acid (C15:0), Arachidic acid (C20:0), were found significantly higher ($p<0.05$) in cattle manure medium.

In a similar culture condition in *M. macrocopa*, the highest MUFA (44.22±2.05%) was found in baker's yeast-treated media, while the lowest (34.81±1.1%) was found in dried cattle manure treatment media. Again in the obtained range, individual findings showed 36.32±1.86%, 36.36±1.94% and 35.45±1.09% in mixed feed, *Chlorella* and *Spirulina* treated media for *M. macrocopa* respectively (Table 1). An individual component in MUFA, the Oleic acid (C18:1n-9) was found dominant ingredient within all the respective media. The Vaccenic acid (C18:1n-7) was found significantly ($P<0.05$) higher in *Spirulina* treated sample.

The PUFA was highest (35.44±1.43%) in *Chlorella* and least (20.24±1.13%) in baker's yeast fed *M. macrocopa*. In the rest of the culture media, it was 22.47±3.48%, 31.02±1.71%, and 26.92±0.32% for the mixed feed, *Spirulina* and dry cattle

manure fed samples respectively (Table 1). Among the PUFA's components, Alphalinolenic acid (ALA) (C18:3n-3) was significantly (p<0.05) dominant in *Chlorella*, followed by gamma-linolenic acid (GLA) (C18:3n-6) in *Spirulina* fed samples of *M. macrocopa*.

Table 1: Fatty acid composition (%) and availability of its individual components in *M. macrocopa* reared in different culture media

Fatty acids	Mixed feed	*Chlorella*	*Spirulina*	C. manure	Baker's yeast
C14: 0	4.48±0.45^{a}	1.84±0.47^{d}	2.31±0.45^{cd}	3.28±0.44^{bc}	3.97±0.37^{ab}
C15: 0	3.63±1.09^{a}	1.93±0.33^{b}	1.79±0.25^{b}	4.12±0.47^{a}	0.99±0.25^{b}
C16: 0	20.76±2.14^{a}	18.28±1.92^{a}	18.32±1.34^{a}	18.33±1.82^{a}	21.10±1.98^{a}
C17: 0	4.28±0.62^{a}	1.55±0.51^{b}	3.39±0.78^{a}	3.34±0.62^{a}	-
C18: 0	5.55±1.15^{a}	1.32±0.28^{c}	3.42±0.42^{b}	3.80±0.39^{ab}	3.99±0.63^{ab}
C20: 0	-	0.91±0.27^{ab}	1.48±1.14^{ab}	2.25±0.72^{a}	1.96±0.36^{a}
C22: 0	2.07±0.85^{a}	2.18±0.27^{a}	2.75±0.23^{a}	3.15±0.46^{a}	3.46±0.81^{a}
∑ SFA	40.77±2.56^{a}	28.00±1.17^{d}	33.46±1.08^{c}	38.26±0.8^{ab}	35.47±1.07^{bc}
C16: 1n-7	7.20±0.70^{b}	7.15±0.98^{b}	8.08±0.38^{b}	7.24±0.45^{b}	13.84±1.23^{a}
C18: 1n-9	22.40±2.73^{ab}	20.63±2.52^{abc}	16.10±0.97^{c}	17.88±1.23^{bc}	24.89±2.14^{a}
C18: 1n-7	4.54±0.70^{b}	6.38±1.22^{ab}	8.22±0.56^{a}	5.70±1.00^{b}	-
C20: 1n-9	2.18±0.26^{ab}	2.21±0.59^{ab}	1.60±0.33^{b}	2.67±0.43^{ab}	3.37±0.86^{a}
C22: 1n-9	-	-	1.45±0.37^{a}	1.33±0.27^{a}	2.12±0.57^{a}
∑ MUFA	36.32±1.86^{ab}	36.36±1.94^{ab}	35.45±1.09^{b}	34.81±1.1^{b}	44.22±2.05^{ab}
C18: 2n-6	5.42±1.12^{b}	8.37±1.15^{ab}	9.00±1.52^{ab}	5.53±0.78^{ab}	9.13±1.96^{a}
C18: 3n-3	2.86±0.87^{b}	18.35±1.75^{a}	-	4.29±0.53^{b}	3.64±0.54^{b}
C18: 3n-6	-	-	12.91±1.67	-	-
C20: 3n-6	2.71±1.03^{b}	1.44±0.26^{b}	1.69±0.77	4.59±0.71^{a}	1.62±0.37^{b}
C20: 4n-6	1.37±0.53^{ab}	1.34±0.34^{ab}	1.47±0.27^{ab}	1.97±0.20^{a}	0.99±0.17^{b}
C20: 5n-3	7.07±1.01^{a}	1.88±0.49^{b}	2.05±0.80^{b}	6.46±1.18^{a}	1.20±0.43^{b}
C22: 5n-3	3.04±0.78^{a}	2.64±0.37^{a}	2.43±1.06^{a}	4.08±0.56^{a}	2.63±0.57^{a}
C22: 6n-3	-	1.42±0.32^{a}	1.47±0.35^{a}	-	1.02±0.63^{a}
∑ PUFA	22.47±3.48^{cd}	35.44±1.43^{a}	31.02±1.71^{ab}	26.92±0.32^{bc}	20.24±1.13^{d}

Values (mean ± SD) with different subscripts letters in the row are significantly different (t-test, p<0.05).

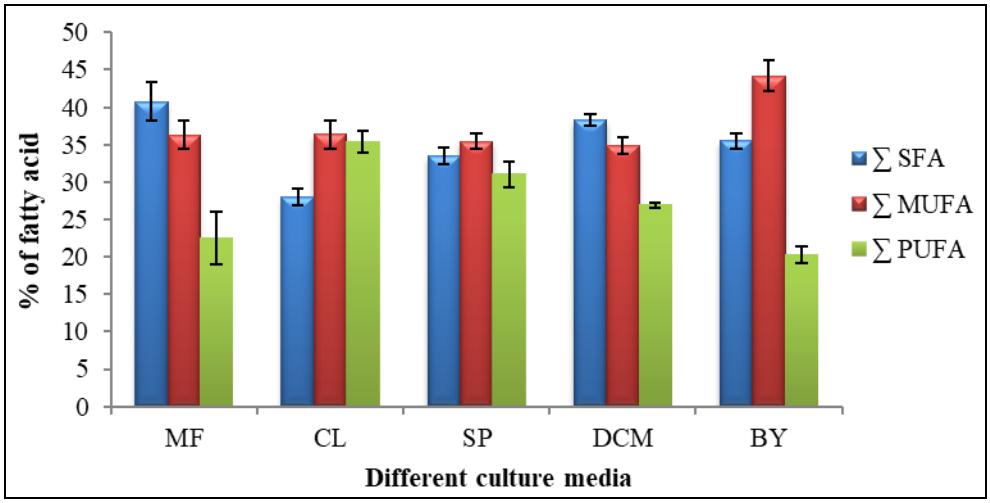


Fig 2: Total body fatty acid composition of *M. macrocopa* with mixed feed (MF); *Chlorella* (CL); *Spirulina* (SP); dried cattle manure (DCM) and baker's yeast (BY) cultured media

The Docosahexaenoic acid (DHA) (C22:6n-3) was found significantly (p<0.05) highest in *Spirulina* (1.47±0.35%) followed by *Chlorella* (1.42±0.32%) fed samples, but it was below the traceable amount in mixed feed and dried cattle manure culture media. Eicosapentaenoic; EPA (C20:5n-3) were quantified significantly (P<0.05) higher in mixed feed medium, while Dihomolinolenic (C20:3n-6), Arachidonic acid (C20:4n-6) and Docosapentaenoic acid (C22:5n-3) were found significantly (P<0.05) higher in cattle manure medium.

The mean amino acid composition of *M. macrocopa* fed with five different feeds is shown in Table 2. Analyzed results show that a maximum of 17 amino acids were present in *Chlorella* and *Spirulina*-treated *M. macrocopa*. Among them, nine were essential amino acids (EAA), and rest eight were non-essential amino acids (NEAA). While a minimum of 15 amino acids were estimated in mixed feed-treated *M. macrocopa*. Among them, eight were EAA, and seven were NEAA. On the other hand, 16 amino acids were detected in dried cattle manure treated *with M. macrocopa*. Among them equal eight no. of EAAs and NEAAs were in cattle manure and nine EAAs and seven NEAAs were in baker's yeast-treated *M. macrocopa*. The values of each essential amino acid in *M. macrocopa* were found to vary in different culture medium and even different within other related amino acids.

Illustrated (Fig. 3a) essential amino acid concentrations (%) in *M. macrocopa* suggest its highest availability in *Chlorella* (56.74±2.10%) followed by *Spirulina* (51.72±1.73%) treated media. In other cases, the proportion was 39.94±0.9%, 40.89±1.57%, and 47.18±1.67% in mixed feed, cattle manure, and baker's yeast treatments respectively.

Obtained ingredients in the form of Arginine, Leucine, and Lysine were the major derivatives of the EAAs in mixed feed, cattle manure, and baker's yeast treatment samples, while it was in *Chlorella* and *Spirulina* treated media, the Arginine, Histidine, and Lysine were dominant.

Methionine acid was absent in mixed feed and cattle manure treated *M. macrocopa* but, was present in the rest of the three treatments. Similarly, the Tyrosine acid was absent in mixed feed and baker's yeast treated media, whereas, present in *Chlorella*, *Spirulina*, and cattle manure media. The Arginine and Histidine acid were significantly ($p<0.05$) higher in *Chlorella* (12.18±1.43% and 9.15±0.79%) followed by *Spirulina* (10.03±0.80% and 8.03±0.74%) treatment. Among the NEAAs Glutamine, acid was found in a higher percentage in all treatments except baker's yeast. In baker's yeast treated *M. macrocopa*, Aspartic acid and asparagine acid were found significant ($p<0.05$) in the highest percentage. Isoleucine, lysine, phenylalanine, and threonine acid did not differ significantly ($p<0.05$) in different treatments. Fig. 3b depicts Non-essential amino acids concentration (%) in *M. macrocopa*.

Table 2: Amino acid concentrations (%) *M. macrocopa* reared in five different media

Amino acids	Mixed feed	*Chlorella*	*Spirulina*	C. manure	Baker's yeast
	Essential Amino acid (Mean ± SD)				
Arginine	6.42±1.18^{c}	12.18±1.43^{a}	10.03±0.80^{ab}	7.65±0.38^{bc}	7.93±1.16^{bc}
Histidine	3.14±0.40^{b}	9.15±0.79^{a}	8.03±0.74^{a}	3.29±1.06^{b}	3.66±0.71^{b}
Isoleucine	3.61±0.59^{a}	5.25±0.71^{a}	5.36±0.81^{a}	3.93±0.47^{a}	4.00±0.76^{a}
Leucine	7.19±0.45^{b}	8.38±0.79^{ab}	6.59±0.87^{b}	6.95±0.42^{b}	9.40±1.03^{a}
Lysine	7.78±0.88^{a}	10.09±1.13^{a}	8.55±1.06^{a}	8.97±0.37^{a}	8.42±0.81^{a}
Methionine	-	2.11±0.25^{a}	2.04±0.47^{a}	-	1.90±0.17^{a}
Phenylalanine	4.10±0.21^{a}	3.37±1.83^{a}	4.24±2.19^{a}	2.85±0.51^{a}	4.64±0.71^{a}
Threonine	3.25±0.80^{a}	4.55±1.01^{a}	3.50±1.19^{a}	3.24±0.35^{a}	4.37±1.95^{a}
Valine	4.46±0.81^{a}	1.66±0.89^{a}	3.39±1.03^{ab}	4.01±0.82^{ab}	2.86±1.13^{ab}
	Non-essential Amino acid (Mean ± SD)				
Alanine	9.65±0.88^{a}	5.77±0.81^{c}	7.27±0.78^{bc}	8.96±0.99^{ab}	7.47±0.76^{abc}
Asparagine	6.60±1.16^{a}	1.38±1.11^{c}	3.27±0.41^{bc}	4.16±0.56^{b}	7.65±1.01^{a}
Aspartic	5.74±1.11^{bc}	4.56±0.66^{c}	8.84±0.79^{ab}	6.47±2.44^{bc}	11.90±1.61^{a}
Glutamine	13.23±1.62^{a}	12.92±1.22^{a}	14.34±0.9^{a}	14.44±0.94^{a}	6.23±1.90^{b}
Glycine	9.46±0.75^{a}	6.94±0.40^{a}	6.25±0.50^{a}	7.58±2.60^{a}	7.18±1.68^{a}
Proline	5.06±1.04^{a}	3.24±0.74^{a}	4.25±0.41^{a}	3.95±0.35^{a}	4.32±0.65^{a}
Serine	10.30±1.24^{a}	6.20±0.83^{bc}	3.07±0.71^{c}	8.03±1.43^{ab}	6.17±1.69^{bc}
Tyrosine	-	2.25±1.33^{ab}	0.98±0.50^{bc}	4.20±0.82^{a}	-

Values (mean ± SD) with different superscript letters in the row are significantly different (*t*-test, $p<0.05$).

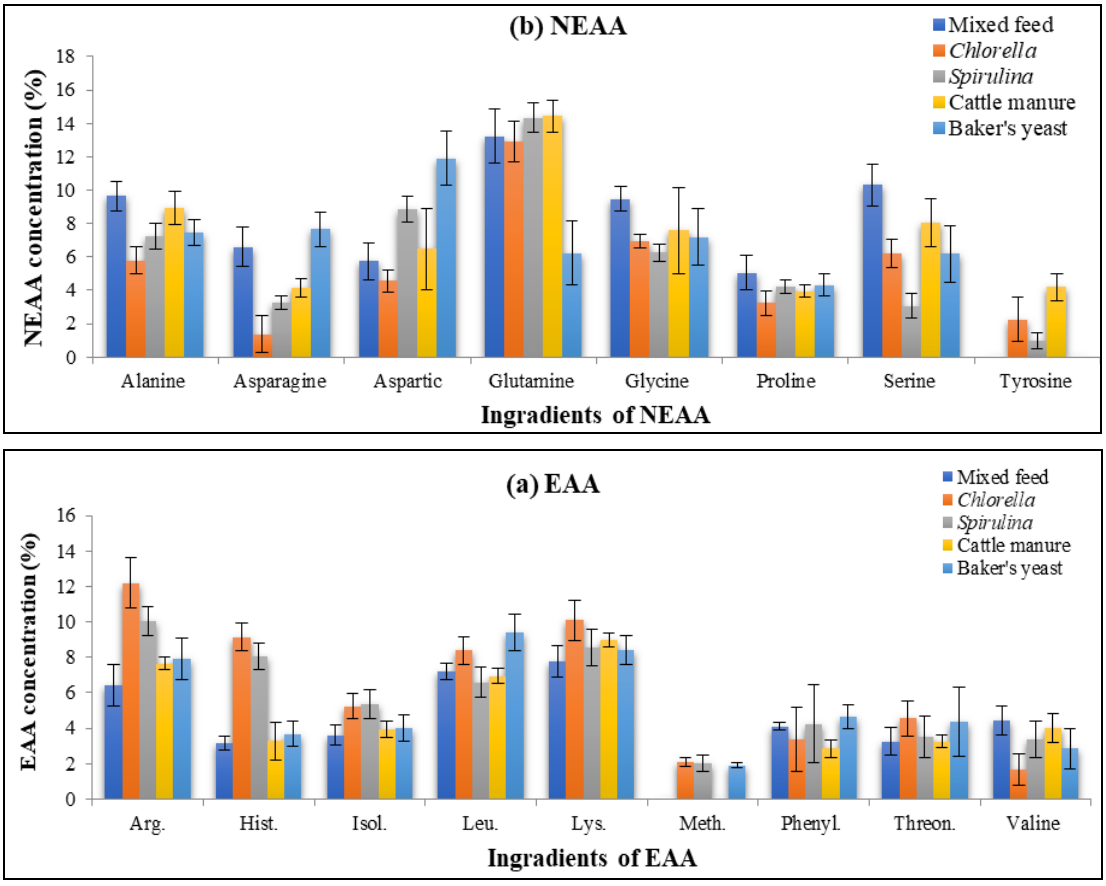


Fig 3(a-b): Percentage composition of essential amino acids EAA (a) and non- essential amino acids NEAA (b) in *M. macrocopa*

4. Discussion

Zooplankton absorbs nutrients major portion through food (Habib *et al*., 1997) [12]. *M. macrocopa* is a freshwater Cladoceran zooplankton that inhabits freshwater. It possesses a rich nutritional profile and serves as an excellent protein source for the growth and development of fish larvae (Pennak, 1978; Villegas, 1990) [39, 45]. The type of food consumed is a determining factor for the variations in the chemical composition of *M. macrocopa*. (Manklinniam *et al*., 2018) [23]. This segment deals with the nutritional composition analysis of *M. macrocopa* cultured in five different media during the study period. Various proximate compositions of *M. macrocopa* found significantly varied with different culture media.

In general, the various culture groups had comparable levels of moisture, which were similar to the moisture levels found in living organisms. Specifically, the moisture content in these groups was approximately 90% of their wet weight (Pace and Orcutt, 1981; Ovie *et al*., 1993) [32, 31]. The moisture contents of *M. macrocopa* in this study were very similar to Watanabe *et al*., (1982) [48] investigate where it was found to be in the range of 87.9-89.0% which is similar to the present study (87.23±0.31 to 88.67±0.4). Creswell (1993) [6] in a study on zooplankton biochemistry found the ranges of moisture content 87.2-89% in *Moina* sp. In a previous study on another similar cladoceran; *Moina micrura*, Ovie *et al*., (2006) [30] has been demonstrated that moisture content was about 89.0%, which is almost similar to present findings.

The mean crude protein levels (55.4±4.16% to 68.1±5.48%) compared favorably with concentrations reported by the other researchers. Watanabe *et al*., (1982) [48] quantified 59.95-62.6% of crude protein in *Moina* sp., while Ovie *et al*., (2006) [30] reported 52.4% crude protein in *M. micrura*, both findings are almost similar to present studies. Also, Manklinniam *et al*., (2018) [23] observed that the average crude protein content was 50% in *M. macrocopa*. Gogoi *et al.*, (2016) [11] stated that the nutritional quality of *Moina* sp. varies considerably on the availability of food and the protein contents remain at an average of 50% on a dry weight basis, which slightly differs from than present findings but quietly identical to Creswell (1993) analyzed 59.12% crude protein fed with poultry manure. Oleksii *et al*., (2018) [28] worked on the differences in the nutritional value of *M. macrocopa* by using *Saccharomyces cerevisiae* and *Rhodotorula glutinis* and obtained 51.04±4.56% and 55.51±2.41% crude protein, respectively. These findings are almost similar with mixed feed and cattle manure treated *M. macrocopa* but lower in of *Chlorella*, *Spirulina*, and Baker's yeast treated *M. macrocopa* of this study.

Creswell (1993) [6] showed that the amount of fat in *M. macrocopa* is within the range of 11.81% to 27.22% on a dry weight basis. In an earlier experiment, on another similar cladoceran *M. micrura*, Tay *et al.*, (1991) [41] found only 8.7% lipid content using agro-industrial wastes as feed, which is very lower than the present study. However, Macedo and Pinto-Coelho (2001) [22] stated fat content in *Moina micrura* ranges from 11.1% to 22.1%. Alam *et al.*, (1993) [1] reported 9.94% of crude fat in *M. micrura*, while fed on chicken manure, but comparatively lower than the present findings with five types of media. Alam *et al.*, (1993) [1] obtained 6.8% ash content of *M. micrura* fed on chicken manure. In another investigation, Kibria *et al*., (1997) [20] stated 6.82% ash in another cladoceran *M. australiensis*, while reared on sewage and both the findings are slightly lower than the mean value of the present investigation.

Fatty acids are chemically diverse, often incorporated into organisms unchanged but different organisms have distinct profiles (Dalsgaard *et al.*, 2003) [7]. Additionally, fatty acids have the potential to serve as dietary tracers in the food chain and as indicators of the general quality of food (Iverson *et al*., 2004). Most living organisms require certain types of dietary fatty acids to support their somatic development and overall fitness (Masclaux *et al.*, 2012) [24]. Besides, being energy storage molecules, some fatty acids also have very important physiological functions (Minna Hiltunen, 2016) [25].

MUFA and PUFA are very important for aquatic organisms usually bio-accumulated through feeding (Persson & Vrede, 2006; Gladyshev *et al.*, 2010; Ravet *et al.*, 2010; Burns *et al*., 2011) [34, 10, 36, 5]. In an experiment on the fatty acid composition of microalgae Otleo & Pire (2001) [29] reported high PUFA content in *Chlorella* (36-43%) represented by a higher percentage of Linoleic acid (C18:2n-6), and Alpha-linolenic acid; ALA (C18:3n-3). They also noticed high PUFA content in *Spirulina* (30-42%) with a higher percentage of Linoleic acid (C18:2n-6) and Gamma Linolenic acid; GLA (C18:3n-6). In the present study, the PUFA content in *M. macrocopa* reared in *Chlorella* and *Spirulina* exhibited more or less similarity with the culture media. Where the PUFA content was 35.44±1.43% in *Chlorella* with a higher percentage of C18:2n-6 and C18:3n-3. In the case of *Spirulina-treated M. macrocopa*, PUFA was 31.02±1.71% with a higher percentage of C18:2n-6, and C18:3n-6. On the other hand, Lower PUFA (18.83±0.30%) was noticed in baker's yeast by P. Vijayagopal *et al.*, (2012) [43]. In the present experiment, 20.24±1.13% PUFA has observed in the baker's yeast-treated culture of *M. macrocopa.* Thus, the suggested fatty acid content is influenced by the fatty acid-containing foods. Similarly, Brett *et al*., (2006) [3]; Taipale *et al*., (2009) [40], and Gladyshev *et al*., (2012) [48] stated that the type of food consumed plays a crucial role in determining the accumulation of PUFA in aquatic invertebrates. But this is not consistent with the finding of Schlechtriem *et al*., (2006) [38]; Gladyshev *et al*., (2010) [10]; Masclaux *et al*., (2012) [24] who have concluded that PUFA accumulation in aquatic invertebrates depends on ecological factors.

The percentage EAA concentrations of *M. macrocopa* in this study were higher than those obtained for *M. micrura* (32.6%) by Watanabe *et al.*, (1983) [46]. Similarly, a higher percentage was in found in *Chlorella* (56.74±2.1%) and *Spirulina* (51.72±1.73%) treatments in this study compared to those obtained for *M. micrura* (48.45%) by Ovie and Ovie (2006) [30], but lesser in mixed feed as (39.94 ± 0.90%), cattle manure (40.89 ± 1.57) and baker's yeast (47.18 ± 1.67). Ovie and Ovie (2006) [30] estimated the amino acid profile of *Moina micrura* and reported a total of 17 amino acids (nine EAAs and eight NEAAs). The same number of EAAs and NEAAs were found in *Chlorella* and *Spirulina* treatments but slightly lesser in mixed feed, cattle manure, and baker's yeast-treated *M. macrocopa* in the present study. Arginine, Histidine, Leucine, and Lysine are the dominant EAAs in *M. micrura* (Ovie and Ovie, 2006) [30]. In almost every treatment of the present study Lysine, Arginine, Leucine, and Histidine were found as the dominant EEA. Watanabe *et al.*, (1986) stated Arginine, Leucine, and Lysine are the foremost EAAs in *M. micrura* which also revealed the present study. In addition, Histidine is also dominant as EAA in *Chlorella-treated M. macrocopa* in the present study.

5. Conclusion

The findings of this study suggest that the different culture media beneficially effected on the proximate composition, amino acid and fatty acid compositions of *M. macrocopa*. Especially, *Chlorella and Spirulina* enriched zooplankton showed better findings on protein, lipid and fatty acid as well as essential amino acid content. Therefore, *Chlorella* and *Spirulina* enriched *M. macrocopa* is recommended as a potential juvenile fish feed and better culture practice of live feed production for aquaculture farmers or industry.

6. Ethical Approval

This research work was approved by the Appropriate Committee under the project of Bangladesh Council of Science and Industrial Research, project ref. no. 39.02.0000.011.14.134.2021/900.

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8. Conflicts of Interest

The authors declare that they have no financial interest or personal relationship that could have appeared to influence the research work outlined in the paper.

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