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Basic Biochemical Constituents in the Laboratory Cultures of Six Species of Micro Algae

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Abstract

Among live feeds, micro algae constitute the primary food of almost all aquatic organisms. Motility, cell size and cell constituents are the main criteria in selecting suitable live feed to various culture organisms at different stages of their growth. Levels of assimilates such as soluble protein, free aminoacids, total soluble sugars and lipids were estimated from six cultures of microalgal feed to compare the nutritive efficiency of these live feeds. The phytoflagellate *Isochrysis* and the diatom *Chaetoceros* were found to contain all the basic assimilates in satisfactory levels, although the species of *Chlorella, Tetraselmis, Dunaliella* and *Nannochloropsis* contained more levels of protein (45 mg/g dry wt). The results of investigation were compared and discussed in the light of manipulating the dietary requirement of cultivable molluscs and crustaceans at their different growth stages.

Introduction

Micro algal species used in an algal diet for bivalve culture may be characterized by its size, chemical composition, digestibility and non-toxicity (Webb and Chu, 1982). The lipid and fattyacid composition of certain species of *Chlorella, Pseudoisochrysis* and *Pyramimonas* were reported by Chu and Dupy (1980). Monosaccharide composition of eight micro algal feeds for bivalves was reported by Chu *et al.*, (1982) and protein and aminoacid composition in nine species were reported by Parsons *et al.*, (1961). In the present study a comparison of the basic biochemical constituents in some of motile and non-motile phytoplankton feeds cultured in our laboratory was made in order to understand and compare the levels of these constituents in them as well as in their mixture in different proportions to supply a balanced diet to bivalve molluscs as well as in the formulation of compounded feed.

Materials and Methods

Cultures (not bacteria free) of *Isochrysis galbana* (Haptophyceae, size 7-8 μ), *Tetraselmis gracilis* (12-14 μ), *Dunaliella salina* (8-10 μ), *Nannochloropsis salina* (2-3 μ) and *Chlorella marina* (3-4 μ) all belong to Chlorophyceae and *Chaetoceros calcitrans* (Bacillariophyceae, 2-5 μ) were maintained in the laboratory in Walnes medium (Walne, 1970) in sterilized seawater (28-32 ppt) at room temperature(27-29°C) with a light intensity of 1500 lux from fluorescent lamps and generally a photoperiod of 14/10 hrs light/dark. Another set of these cultures was maintained in Walnes medium with varied concentrations of kinetin (5-15 μg/l) in reduced photoperiod (10/14 hrs of L/D) to study the effect of kinetin and altered photoperiod. Ten liters of these cultures at their exponential phase (10th day after inoculation) were centrifuged in polypropylene tubes at 6000 rpm for 10 min. The cells were washed in 0.5% ammonium formate isotonic with sterile seawater and dried at 80 +_1° C overnight. From the dry cells protein (Lowry *et al.*, 1951), lipids (Barnes and Blackstock, 1973), soluble sugars (Dubois *et al.*, 1956) and free aminoacids (Troll and Canan, 1953) were determined spectrophotometrically with suitable standards.

Results and Discussion

Among the six species of micro algae studied, *Isochrysis* contained maximum levels of protein (59.62 mg/g dry wt.). *Chaetoceros* contained soluble sugars and lipids to a maximum of 33.62 mg/g dry wt and 16.17 mg/g dry wt respectively and *Chlorella* registered higher levels of aminoacids (4.03 mg/g dry wt) than the other species (Table 1). In an attempt to understand the levels of micronutrients and growth hormones and the rate of photoperiod on the accumulation of basic biochemical constituents in these micro algal cultures, 10 μg/l of kinetin was observed to be effective in increasing the levels of protein ranging from 4% in *Isochrysis* to 20% in *Dunaliella* (Table 2).

Table 1. Levels of basic biochemical constituents in six species of microalgae (mg/g dry wt ± Standard Error)

Species	Protein	Soluble sugars	Lipids	Amino acids
Isochrysis	59.62±6.2	26.52±5.8	14.43±2.4	2.57±1.7
Tetraselmis	51.54±4.8	17.15±2.9	10.54±2.6	2.73±1.3
Dunaliella	42.16±7.8	12.28±2.5	07.20±1.9	2.04±0.9
Chaetoceros	56.37±5.7	33.62±5.2	16.71±3.3	3.05±0.8
Chlorella	54.11±7.4	30.65±4.3	10.23±2.8	4.03±2.1
Nanochloropsis	49.67±6.3	15.20±3.4	11.28±2.4	3.11±1.3

Table 2. Effect of Kinetin (10 μg/l) and reduced photoperiod (10/14 of L/D on the levels of protein (mg/g dry wt ± S.E)

Species	Control conditions (Walne's medium and L/D=14/10hr)	Experimental conditions (10 μg/l kinetin in walne's medium and L/D=10/14 hr)	Increase over the Control (%)
Isochrysis	59.62±6.1	62.30±5.4	104
Tetraselmis	51.54±4.8	59.35±4.8	116
Dunaliella	42.16±7.8	48.34±3.7	120
Chaetoceros	56.37±5.7	60.49±4.1	107
Chlorella	54.11±7.4	58.22±5.9	108
Nanochloropsis	49.67±6.3	52.16±3.6	105

Protein, aminoacids, fatty acids and carbohydrate composition of microalgae are qualitatively similar but are markedly different quantitatively (Chu and Dupy,1980; Web and Chu, 1982). Composition of the micro algae reported here (Table 1) pertained to growing cells in their exponential phase of growth which were sampled on the 10th day after inoculation. The reported values in mg/g dry wt of algal cells can be of use while incorporating these algae in compounded feed.

According to Parsons and his co-workers (1961), a protein:carbohydrate:lipid ratio of approximately 4:3:1 is suitable for zooplankton and bivalve larvae. Our results in Table 1 showed all the unialgal species contained proteins-sugars-lipids-aminoacids in decreasing order. Although the composition of basic assimilates is sound, the cell size, motility and cellwall composition and thickness are the important factors determining suitability. Non-motile forms like *Chlorella, Nannochloropsis* and *Chaetoceros* may settle down if the water is not agitated sufficiently. Size of cells also determine the filtering efficiency and mouth parts orientation of the species or the life history stage of the organisms being reared (Babinchak and Ukeles, 1979). Feeding experiments have proved that diet consisting of more than one species of alga is superior over diet consisting single species of micro alga (Walne, 1970; Epifanio, 1979). Hence our results presented in Table 1 can be of use in providing bivalve larvae and juveniles a balanced algal diet.

Our finding that 10 µg/l of kinetin in the culture medium even at low photoperiod caused an increase of 4-20% in the protein levels of algal cells (Table 2) is parallel to the illustrations of Spoehr and Milner (1949) that light intensity and temperature do alter the nutrient composition in *Chlorella* and of Burkiewicz (1987) that gibberellins and cytokinins stimulate cell division and increase the dry weight of these species of micro algae. Attempts in this line of micro-agriculture is of some advantage in manipulating the nutritive value of phytoplankton feeds in culture so as to provide the candidate species under culture a balanced, blended and natural diet.

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