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Marine microalgae as a potential source of minerals in fish diets

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Abstract

The incorporation of powdered marine microalgae in fish diets can substitute, at least in part, for the addition of minerals to the diet. In diets for freshwater fishes, the incorporation of 33% of powdered marine microalgae can supply some of the mineral element requirements. The incorporation of microalgal powder in diets for marine fishes appears more effective, since lower percentages are needed to cover the mineral requirements. Most mineral needs of turbot can be covered with low percentages of marine microalgal powder in the diet: 3.8% of *Tetraselmis suecica*, 5.7% of *Isochrysis galbana*, 3.57% of *Dunaliella tertiolecta* and 3.9% of *Chlorella stigmatophora*. Mn and Co must, however, be added. Thus, incorporation of small amounts of marine microalgae in diets can replace a mineral mixture.

Introduction

Marine microalgae growing naturally in seawater constitute an essential and huge link in the global food chain and global oxygen production (Berend et al., 1980).

The ability of marine microalgae to accumulate trace elements is well documented (Jensen et al., 1974; 1976; Sakaguchi et al., 1981) but very little work on the mineral content of marine microalgae has been done with laboratory cultures, and none has focussed on their utilization as feed. Most of the research on inorganic elements has been confined to osmoregulation (Ginzburg, 1981; Rebhun and Ben-Amotz, 1984), toxicity (Cain et al., 1980; Wong, 1980) and related physiological functions (Azam et al., 1974; Adshead-Simonsen et al., 1981).

Mineral nutrition is one of the most neglected areas in the field of aquaculture (Lall, 1979). Minerals which have demonstrable biological functions, either in elemental form or when incorporated into specific compounds, include calcium, phosphorus, magnesium, sodium, potassium, sulfur, chlorine, iron , copper, cobalt, iodine, manganese, zinc, molybdenum, selenium and fluorine (Nose, 1972). Little, if any, published information exists on the needs of fish, shellfish and molluscs for these minerals. Hence, the mineral mixtures designed for warm-blooded animals have frequently been used in studies on nutrient requirements of fish (Lall, 1979). In recent years, active research in the area of mineral nutrition has been undertaken, specially with freshwater fishes (Tacon and De Silva, 1983), although the mineral requirements of marine fishes remain less known.

Some species of microalgae can be useful sources of minerals for animals in aquaculture systems (Stanley and Jones, 1976) and activated-sludge single-cell protein has been successfully incorporated into pelleted feeds for trout (Tacon, 1979).

Tetraselmis suecica, Isochrysis galbana, Dunaliella tertiolecta and *Chlorella stigmatophora* are marine microalgae at present widely used in marine aquaculture. These marine micro algae have been recently suggested as a new source of single cell protein (SCP) (Fabregas and Herrero, 1985). We report here the mineral composition, in terms of major and trace elements, of these four microalgae with regard to their use in aquaculture.

Materials and methods

Four different marine photosynthetic microalgae were used. *Tetraselmis suecica* (Prasinophyceae) was isolated from Ria de Arosa waters (N.W. Spain) (Fabregas et aI., 1984). *Isochrysis galbana* (Haptophyceae), *Dunaliella tertiolecta* and *Chlorella stigmatophora* (Chlorophyceae) were obtained from The Culture Centre for Algae and Protozoa, Cambridge, England. They were cultured in seawater filtered through a *0.45-µm* Millipore filter, autoclaved at 120ªC for 20 min and enriched with $NaN0_3$, 2 *mM;* NaH_2PO_4, 100 *µM;* $ZnCI_2$ 1 *µM;* $MnCI_2$ 1 *µM;* Na_2MoO_4, 1 *µM;* $CoCI_3$ 0.1 *µM;* $CUSO_4$, 0.1 *µM;* ferric citrate, 20 *µM ;* thiamine, 35 µg/I; biotin, 5 µg/I; B_{12} 3 µg/ I; EDTA, *26.4 µM;* Tris-HCI 5 *mM;* pH, 7.6; salinity, 35%$_o$ (Fabregas et aI., 1984).

Cultures were carried out in 10-1 flasks with 9 I of culture medium. All the cultures were maintained in a controlled environmental incubator at 15 ± 11C and illuminated with 11 fluorescent lamps (Osram daylight L55/10). A 12: 12 light-dark period was maintained. Cultures were continuously pumped with air at a rate of 151/min (Fabregas et aI., 1985).

The different microalgal species were harvested by centrifugation at the end of the logarithmic phases of growth. The slurries obtained were dehydrated in an oven at 60°C for 24- 30 h. The dehydrated microalgae were analyzed for phosphorus, calcium, iron, magnesium, cobalt, zinc, copper, manganese, sodium, chloride and potassium contents in ash. Ash was obtained by calcining the microalgae in a muffle oven at 600° C until all the organic matter was destroyed (AOAC, 1980). The standard procedures described in Furman and Welcher (1966) were used for calcium, phosphorus and chloride. The remaining ions were analyzed in an atomic absorption spectrophotometer. We used a preparative elemental method, valid for determining different elements at the same time (Hughes et aI., 1980). Warm HCI *6N* was added to the ashes and the resultant solutions were filtered and diluted with bi-distilled water in order to determine the metallic ions by atomic absorption spectrophotometry, choosing suitable conditions in each case.

Results and discussion

The mineral composition of the four marine microalgae used is presented in Table 1. In comparison with freshwater algae, marine algae generally have a higher content of inorganic ions, related to their greater osmotic potential (Raven, 1980). This is especially marked in the Cl and Na contents. *D. tertiolecta* and C. *stigmatophora,* both Chlorophyceae, show similarities and differences between themselves, but also in comparison with the other two species which belong to other taxonomic groups. Thus, mineral distribution does not appear to be correlated with taxonomy. These results are in general accordance with those of Riley and Roth (1971). On the other hand, the mineral composition of all these microalgae includes all the macro and microelements used in aquaculture, so that a small incorporation of these species into diets can replace a mineral mixture. There is considerable variation in the concentration of certain elements, such as Cu or Zn, among the different species, whereas the concentration of other elements appears very similar. This characteristic could allow us to choose different mixtures of marine microalgae in order to cover specific mineral requirements in aquaculture, taking into account that some microalgae are easier to culture than others and, in addition, that the trace metal content of microalgae could be increased by increasing the concentration of metals in the medium in which the organisms are grown (Riley and Roth, 1971). Therefore, data are correct as such, but represent only the composition obtained under particular growth conditions.

TABLE 1

Content of mineral elements in different marine microalgae, on dry weight basis

	T. suecica	*I. galbana*	*D. tertiolecta*	*C. stigmatophora*
P (%)	0.65	1.02	0.73	0.64
Ca (%)	2.08	1.62	2.09	1.51
Na (%)	1.04	0.72	0.92	0.98
K (%)	1.20	0.56	0.74	1.12
Cl (%)	3.72	5.08	2.42	2.45
Fe (%)	0.10	0.36	0.20	0.18
Mg (%)	0.78	1.15	0.63	0.78
Zn (%)	0.15	0.06	0.03	0.03
Mn (ppm)	47.49	40.47	56.51	40.46
Co (ppm)	5.53	10.51	5.80	7.81
Cu (ppm)	652.00	204.00	65.00	108.00

Use of unicellular algae as food for warm water fish was considered in a number of studies conducted on small samples in aquaria or tanks. Most of the experiments showed favourable results, the freshwater fish utilizing the microalgae quite effectively (Stanley and Jones, 1976).

On the basis of different feeding trials it was concluded that activated sludge single-cell protein (ASCP) can be successfully incorporated into pelleted feeds for trout, at dietary levels of up to 33% (Tacon, 1979). The mineral composition of this ASCP is very similar to those of the marine microalgae described here. The ASCP has a greater content of Fe and Mn, whereas any of the four marine microalgae studied contain higher concentrations of Mg, K, Na and Zn. The remaining metals are present in similar concentrations.

TABLE 2

Dietary requirements for mineral elements of different fishes, and percentage of marine microalgae in diet that cover each requirement

Species	Element	Dietary requirement	% of marine microalgae in diet			
			T. suecica	*I. galbana*	*D. tertiolecta*	*C. stigmatophora*
Anguilla japonica	P[1]	2.9 g/kg	44	28	41	45
	Ca[1]	2.7 g/kg	13	16	13	18
	Fe[2]	0.17 g/kg	16	5	8	9
	Mg[2]	0.4 g/kg	6	3.4	6	5
Cyprinus carpio	P[3]	6—7 g/kg	92	51	85	93
	Mg[4]	0.4—0.5 g/kg	5.8	3.5	6	5.1
	Zn[5]	15—30 mg/kg	12	3.7	7.5	7.6
	Cu[6]	3 mg/kg	0.4	1.5	4.6	2.7
	Mn[6]	12—13 mg/kg	25	30	21	30
Salmo gairdneri	P[7]	7—8 g/kg	100	60.8	100	100
	Mg[7,8]	0.5—0.7 g/kg	7.3	4.3	7.9	6.4
	Zn[9]	5—30 mg/kg	1.2	3.7	7.5	7.6
	Cu[6]	3 mg/kg	0.46	1.5	4.6	2.7
	Mn[6]	12—13 mg/kg	25.5	30	21.4	30
Scophthalmus maximus[a]	P	0.25 g/kg	3.8	2.45	3.57	3.9
	Ca	0.45 g/kg	2.16	2.77	2.15	2.98
	Fe	0.025 g/kg	2.4	0.7	1.23	1.35
	Mg	0.039 g/kg	0.5	0.33	0.61	0.5
	K	0.32 g/kg	2.66	5.7	4.3	2.8
	Na	0.072 g/kg	0.75	1	0.78	0.70
	Cl	0.050 g/kg	0.13	0.09	0.20	0.20
	Cu	1.2 mg/kg	0.21	0.62	1.95	1.17
	Zn	8 mg/kg	0.45	1.38	2.76	3.18
	Mn	5 mg/kg	9.4	11.05	7.94	11.09
	Co	1.9 mg/kg	30	18	32	24

[a]The mineral requirements are not known; data are based on the different mineral mixtures of the diets for turbots (Adron et al., 1976; 1978; Mahajan and Agrawal, 1980).
[1] Arai et al., 1974; [2] Nose and Arai, 1979; [3] Ogino and Takeda, 1976; [4] Ogino and Chiou, 1976; [5] Ogino and Yang, 1979a; [6] Ogino and Yang, 1980; [7] Ogino and Takeda, 1978; [8] Knox et al., 1981; [9] Ogino and Yang, 1979b.

Considering the mineral requirements of some cultured freshwater fish species (Table 2), the incorporation of one or more of these microalgae into diets for these fishes at a level of 33% (as for the ASCP) can supply all their mineral requirements except that of phosphorus, for which large quantities of microalgae would be needed.

As can be seen in Table 2, these marine microalgae could be useful for covering the Cu and Mg needs of freshwater fishes and, probably, for other microelements which have not been studied. To meet the requirements of the remaining minerals, incorporation of the algae at dietary levels higher than 5% is needed, and such additions could influence the final formulation of the diet, since constituents of the marine micro algae other than minerals are included.

If we consider the mineral composition of commercial fish feeds instead of the mineral requirements, higher percentages of micro algae would be needed in the diets, since the concentrations of certain elements, including Zn, Mn, Cu and Co, are considerably higher in commercial fish feeds than those normally recommended (Tacon and De Silva, 1983).

In contrast to freshwater fishes, the mineral requirements of cultured marine fishes are not known. However, considering the different mineral mixtures of the basal diets for turbot *(Scophthalmus maximus)* (Adron et al., 1976, 1978; Mahajan and Agrawal, 1980), it can be shown that incorporation into the diets of small amounts of the marine microalgae studied can substitute in part for these mineral mixtures. Mineral needs of turbot can be met by low percentages of marine microalgae in the diets: 3.8% of *T. suecica,* 5.7% of 1. *galbana,* 3.5% of *D. tertiolecta* and 3.9% of C. *stigmatophora* (Table 2), except for Mn and Co which must be added. Nevertheless, we are working with the concentration of these elements in the mineral mixtures used and not with the exact requirements for these elements. If the Mn and Co requirements were known, it is very likely that they would be lower than the amounts supplied in mineral mixtures, and that it would be possible to supply them with low quantities of microalgae, since, as has been mentioned above, the mineral contents of the mixtures used generally surpass the dietary requirements.

There are great differences in the amounts of microalgae to be added to diets for freshwater and marine fishes. The requirements for mineral elements are lower in marine than in freshwater species because marine fishes have the ability to absorb certain minerals from the surrounding water as well as from the food ingested (Tacon and De Silva, 1983).

Another interesting aspect in the use of marine microalgae as a source of minerals is that incorporation of 5% in the diet also results in a protein supply between 2 and 2.7%, since the protein content of these species is between 39 and 54% of the dry weight (Fabregas and Herrero, 1985).

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