Nutritional properties of four marine microalgae for albino rats

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Abstract

The nutritive value of the marine microalgaeTetraselmis suecica, Isochrysis galbana, Dunaliella tertiolecta and Chlorella stigmatophora was studied in diets given to rats. Biological assays were carried out in order to determine the Protein Efficiency Ratio (PER) and the Food Conversion Efficiency (FCE). Each dried microalga was fed to weaning Wistar albino rats as the sole protein source at a protein level of 12%. Control rats were given diets containing 12 % casein. Food consumption was similar in all groups. PER values obtained were 1.14 with T. suecica diet, 1.13 with I. galbana diet, 2.07 with D. tertiolecta diet and 1.13 with C. stigmatophora diet (casein, 2.50). FCE values followed a similar pattern. The data showed that the marine microalga D. tertiolecta is a source of protein of good quality. Its PER is quite high, compared to vegetable and cereal proteins, and compares favourably with other microbial protein sources, such as yeasts or different freshwater microalgae. Haematological tests showed no significant differences among the groups in haemoglobin levels, red and white blood cell counts, differential count and mean corpuscular volume. Different blood parameters were also determined and a significant decrease in triglycerides levels appeared with all the microalgal diets, whereas a significant decrease in cholesterol appeared in D. tertiolecta and C. stigmatophora diets.

Introduction

The increasing demand for proteins has led to growing efforts to intensify the production of protein from conventional and unconventional sources. Microbial proteins (Single Cell Protein - SCP - or Biomass Protein - BMP -) have been considered as a protein source only fairly recently. In the context of SCP production, mass culture of microalgae is 4uile appealing, not only because of the high protein content of the cells, but also because of their ability of utilizing solar energy.

Microalgae provide an efficient means of converting solar energy into biomass. The utilization of microalgae has been encouraged by the high biomass yields with high protein levels observed both in the laboratory and outdoors. Besides their high protein content, micro algae contain water- and lipid-soluble vitamins and other valuable products (Becker, 1986; Borowitzka, 1988a, b; Richmond, 1986).

A variety of different microalgae have been investigated for their use as SCP (Becker & Venkataraman, 1982; 1984; Becker, 1986; 1988) or for the production of fine chemicals (Borowitzka, 1988a; Richmond, 1986). The species considered for these purposes have been freshwater ones, mainly of the genera *Chlorella, Dunaliella, Scenedesmus* and *Spirulina* (Becker, 1988). However, marine microalgal mass culture has been focused on its use in aquaculture systems (De Pauw & Persoone, 1988), although certain marine micro algae are a potential source of a variety of products (Fabregas & Herrero, 1985; 1986; 1990; Parkinson, 1987; Abalde *et al.,* 1991; Herrero *et al.,* 1992).

The marine unicellular algae *Tetraselmis suecica* (Prasinophyceae), *Isochrysis galbana* (Haptophyceae), *Dunaliella tertiolecta* and *Chlorella stigmatophora* (Chlorophyceae) have been suggested as potential sources of SCP and have already been evaluated for their protein quality (Fabregas & Herrero, 1985). Their composition in minerals and vitamins have also been found to be promising (Fabregas & Herrero, 1986; 1990). Therefore, they could be used as a potential food or feed or as a dietary supplement for human or animals. The aim of the present study was to carry out short-term feeding tests by weaning albino rats on unialgal diets of *T suecica, I. galbana, D. tertiolecta* or C. *stigmatophora* in order to establish their nutritive qualities of these algae.

Materials and methods

Four different marine photosynthetic micro algae were used. *Tetraselmis suecica* (Prasinophyceae), *Isochrysis galbana* (Haptophyceae), *Dunaliella tertiolecta* and *Chlorella stigmatophora* (Chlorophyceae). These microalgae were cultured, harvested and dried as previously reported (Fabregas & Herrero, 1985). The biochemical composition of the dried biomass of each microalga was determined and has been reported elsewhere (Fabregas & Herrero, 1985; 1986; 1990).

Animals and diets: Biological assays were carried out to determine the Protein Efficiency Ratio (PER) and the Food Conversion Efficiency (FCE). In the test diets each alga was the sole source of protein at a protein level of 12 %. Casein was the protein source in the control diet, also at 12 % level. Taking into account the nutritional requirements of the rats (LASA, 1969) and the composition of each microalga (Fabregas and Herrero, 1985; 1986; 1990), the diets were formulated using a linear programme operated on Hewlett Packard HP9817 Computer. All diets were supplemented with methionine. The remaining nutrient requirements of the rat (fat, fibre, vitamins, minerals) must be given in sufficient amounts. All diets were adjusted to 4 % crude fibre and 8 % oil by appropriate additions of cellulose and olive oil. Since casein is a pure protein, all other components must be added to the control diet (oil, sugar, vitamin and mineral mixtures).

Microalgal biomass for the protein supply was sufficient for the vitamin and mineral requirements of rats, so neither vitamin nor mineral mixtures were added to the experimental diets; the oil and sugar present in the microalgal biomass must be completed by adding olive oil and sucrose in order to adjust the diet to the rat requirements.

Table 1. Composition of experimental diets. Protein, fibre and oil contents were 12%, 4% and 8%, respectively, in all diets. Mineral mixture contained (g kg^{-1} mixture): NaCl, 338; $CaHPO_4$, 334; $KHCO_3$, 219.6; KH_2PO_4, 72; $MgCO_3$, 38; $FeSO_4$, 9.3; $MnSO_4$, 1.18; $CuSO_4$, 0.16; $AIK(SO_4)_2$, 0.08; $CaCl_2$, 0.08; IK, 0.04; FNa, 0.008. Vitaminized starch mixture contained (mg g^{-1} starch): thiamine, 1.5, riboflavine, 0.2, niacin, 2.2, pyridoxine, 0.4; calcium panthothenate, 0.3; vitamin A, 6 I.U.; vitamin D, 20 I.U.

Component (g kg^{-1} diet)	Control diet	*T. suecia* diet	*I. galbana* diet	*D. tertiolecta* diet	*C. stigmatophora* diet
Casein + 0.5% Methionine	150	–	–	–	–
T. suecia + 0.3% Methionine	–	333	–	–	–
I. galbana + 0.3% Methionine	–	–	353	–	–
D. tertiolecta + 0.3% Methionine	–	–	–	253	–
C. stigmatophora + 0.3% Methionine	–	–	–	–	343
Sugar	40	27	31	38	28
Oil	80	67	41	54	76
Mineral mixture	50	–	–	–	–
Vitamin mixture	50	–	–	–	–
Starch	630	573	575	655	553

Standard AOAC (1980) procedures were used for protein, crude fibre and oil analysis of the feeds. The compositions of the control and algal diets are shown in Table 1.

Each diet was fed to weaning Wistar albino rats. Female Wistar weaning rats 42-48 g were randomly divided into five groups of ten. The rats were caged individually, and food and water were given *ad libitum.* The rats were maintained at 25ºC and subjected to alternating 12 h periods of light and darkness. The animals were given the test diets for 4 weeks, and individual body weights were recorded periodically and food consumption measured daily. PER was calculated for each diet as weight gain (g) per unit of protein consumed (g) by the animal. Casein PER is customarily adjusted to an assumed PER of 2.50, which requires a corresponding correction of the experimental values. FCE was calculated as feed consumed (g) per weight gain (g).

At the end of the experimental period, blood was collected by haematological tests, such as haemoglobin content, red and white blood cell counts, differential count and mean corpuscular volume, carried out in a TECHNICON H6000/ H601. The plasma obtained by centrifugation of total blood was analyzed for different parameters (electrotites, cholesterol, triglycerides, enzymes, proteins and others) using an automatic analyzer (HITACHI 737).

After killing the rats, the different organs were removed and relative organ weights (g organ/ 100 g body weight) of rats were recorded (Venkataraman *et al., 1980).*

Statistical analysis of the data were performed using the Mann-Whitney-Wilcoxon non-parametric test.

Table 2. Feed consumption, gain in weight, Protein Efficiency Ratio (PER) and Food Conversion Efficienct (FCE) of rats given diets containing casein and different marine microalgae at a protein level of 12% for 4 weeks ($n = 10$).

	Initial weight (g) ($\bar{x} \pm$ SD)	Gain in weight (g) ($\bar{x} \pm$ SD)	Feed consumed (g) ($\bar{x} \pm$ SD)	$PER_{corr.}$	FCE
Casein diet	44.9 ± 1.28	94.72 ± 3.79	274.3 ± 11.99	2.50	2.89
T. suecia diet	47.4 ± 3.79	46.18 ± 5.28	293.6 ± 23.99	1.14	6.35
I. galbana diet	48.35 ± 2.16	41.24 ± 4.19	264.9 ± 12.99	1.13	6.42
D. tertiolecta diet	42.1 ± 1.57	82.82 ± 4.10	289.9 ± 11.66	2.07	3.50
C. stigmatophora diet	45.27 ± 1.9	40.51 ± 5.88	261.6 ± 20.41	1.13	6.45

Results

The food consumption and the weight gain of the rats during the 4 weeks feeding period are shown in Table 2. There were no significant differences in the food intake among the five groups of rats, whereas the group fed on *I. galbana* diet showed a significantly higher intake of water than the remaining groups. The weight of the rats did not follow a comparable trend to that of diet consumption (Table 2). Rats fed on casein (control group) showed a weight increase significantly higher than those fed on the different microalgal diets. Among the latter, rats fed on *D. tertiolecta* had higher weight increase, close to that of the control group.

PER values were similar to weight increase values (Table 2). For a casein PER of 2.50, PER values of the diets with the different marine microalgae were 1.14 for *T. suecica,* 1.13 for *f. galbana.* 2.07 for *D. tertiolecta* and 1.13 for C. *stigmatophora.* The PER value of *D. tertiolecta* was almost double that of the other microalgae. A similar pattern occurred with the FCE which gave values of 2.89 in the control diet and 3.50 in the *D. tertiolecta* diet, and values above 6 for the remaining diets. The differences between the *D. tertiolecta* and control diets were less than those between the *D. tertiolecta* and the remaining diets.

Table 3. Relative organ weights (g of organ weight/100 g body weight) of rats fed diets containing 12% of algal protein or 12% casein for 4 weeks ($n = 10$). Those marked with asterisk (*) differ significantly from the control ($P < 0.01$).

	Casein ($\bar{x} \pm$ SD)	*T. suecica* ($\bar{x} \pm$ SD)	*I. galbana* ($\bar{x} \pm$ SD)	*D. tertiolecta* ($\bar{x} \pm$ SD)	*C. stigmatophora* ($\bar{x} \pm$ SD)
Liver	5.189 ± 0.419	4.897 ± 0.197	5.514 ± 0.311	5.338 ± 0.248	4.413 ± 0.206*
Kidneys	1.084 ± 0.062	1.200 ± 0.063*	1.147 ± 0.094	1.051 ± 0.061	1.196 ± 0.140
Heart	0.477 ± 0.036	0.438 ± 0.031*	0.414 ± 0.033*	0.407 ± 0.035*	0.459 ± 0.062
Lungs	0.720 ± 0.044	0.749 ± 0.069	0.775 ± 0.060	0.589 ± 0.043*	0.764 ± 0.144
Brain	1.458 ± 0.115	1.792 ± 0.147*	1.405 ± 0.110	1.358 ± 0.099	1.640 ± 0.536
Spleen	0.310 ± 0.030	0.205 ± 0.021*	0.219 ± 0.029*	0.227 ± 0.030*	0.249 ± 0.023*
Adrenal gland	0.026 ± 0.006	0.032 ± 0.006	0.032 ± 0.003	0.026 ± 0.006	0.029 ± 0.013
Thymus	0.367 ± 0.087	0.312 ± 0.043	0.315 ± 0.072	0.317 ± 0.070	0.384 ± 0.046

The absolute weights of the various organs followed the same trend as the body weights, being higher in the control and *D. tertiolecta* group. However, the differences are lower if the relative organ weights (g organ weight/ 100 g body weight) are considered (Table 3). There were differences among the groups in the weights of some of these organs. No significant differences occurred in relative weights of adrenal glands and thymus, whereas the relative weight of the spleen was significantly higher in the control group. Other significant differences respect to the control were: relative liver weight was lower in rats fed on C. *stigmatophora;* relative kidney weight was higher in rats fed on *T. suecica;* relative heart weight was lower in rats fed on *T.*

suecica, D. tertiolecta and *I. galbana,* and the same occurred with the relative weight of lungs in *D. tertiolecta;* relative brain weight was higher in rats fed on *T. suecica.*

There were no haematological abnormalities in any of the groups (Table 4). No significant differences were found among the groups in haemoglobin levels, red blood cell counts, white blood cells counts, relative proportions of the various white cells and mean corpuscular volume.

In the blood parameters analyzed at the end of the experimental period significant differences only occurred in the following cases (Table 5): urea content was significantly higher in rats fed on *T. suecica* and *I. galbana;* uric acid and creatinine were significantly higher in rats fed on *T. suecica;* phosphorus was lower in rats fed on C. *stigmatophora;* CPK was lower in *D. tertiolecta* group; triglycerides were lower in all the groups fed on microalgae than in the rats fed on control diet and hypocholesterolemic effects were showed by *D. tertiolecta* and C. *stigmatophora.*

Table 4. Heamatological data for rats fed diets containing 12% of algal protein or 12% casein for 4 weeks ($n = 10$).

Parameter	Casein	*T. suecica*	*I. galbana*	*D. tertiolecta*	*C. stigmatophora*
Haemoglobin (g/100 ml whole blood)	10.85 ± 0.85	11.77 ± 0.85	11.64 ± 0.88	10.82 ± 0.88	11.63 ± 0.55
Red blood cells (10^6/mm^3)	3.61 ± 0.34	3.42 ± 0.31	3.35 ± 0.80	3.44 ± 0.57	3.38 ± 0.52
While blood cells (WBC)					
Total (10^3/mm^3)	2.40 ± 0.40	2.47 ± 0.35	2.79 ± 0.33	2.12 ± 0.55	2.46 ± 0.22
Lymphcytes (% of total WBC)	77.00	79.71	77.80	77.95	78.92
Neutrophils (% of total WBC)	17.81	16.77	18.56	18.01	17.95
Eosinophils (% of total WBC)	0.70	0.26	0.50	0.25	0.37
Monocytes (% of total WBC)	1.68	1.88	1.28	1.64	1.64
Basophils (% of total WBC)	0.40	0.30	0.54	0.78	0.50
Mean corpuscular volume (μ^3)	62.0 ± 4.2	65.0 ± 5.3	63.3 ± 5.0	64.2 ± 5.3	60.0 ± 2.4

Table 5. Blood parameters for rats fed diets containing 12% of algal protein or 12% casein for 4 weeks. Those marked with asterisks differ significantly from the control ($P < 0.01$).

Variable	(unit)	Casein	*T. suecica*	*I. galbana*	*D. teriolecta*	*C. stigmatophora*
Glucose	(g l^{-1})	1.40	1.31	1.44	1.35	1.32
Urea	(g l^{-1})	0.18	0.30*	0.62*	0.19	0.17
Uric acid	(mg l^{-1})	10.8	26.2*	8.2	11.3	9.7
Creatinine	(mg l^{-1})	3.3	4.2*	3.0	3.0	3.2
Na	(mEq l^{-1})	147	148	145	148	147
K	(mEq l^{-1})	4.54	4.74	3.50	5.09	3.81
Cl	(mEq l^{-1})	117	115	116	115	115
Ca	(mg l^{-1})	88.5	91.3	82.0	85.1	93.5
P	(mg l^{-1})	80.8	71.1	67.3	83.1	45.5*
Total protein	(g l^{-1})	44.3	42.3	42.7	44.3	43.4
Albumins	(g l^{-1})	25.1	25.3	25.6	27.0	24.3
Globulins	(g l^{-1})	19.2	17.0	17.1	17.3	19.1
Albumins/globulins		1.30	1.48	1.49	1.56	1.27
Total bilirrubine	(mg l^{-1})	1.0	0.5	1.5	1.5	2.0
Alkaline phosphatase	(U.I. l^{-1})	610	590	649	651	598
Alalnine aminotransferase	(U.I. l^{-1})	23.42	23.44	21.37	22.66	24.20
Glutamic-oxalacetic transaminase	(U.I. l^{-1})	17.03	16.58	18.29	17.71	16.50
γ-glutamyl-transpeptidase	(U.I. l^{-1})	0.44	0.30	0.33	0.30	0.40
Lactate dehydrogenase	(U.I. l^{-1})	326	367	308	377	366
Creatine phosphokinase	(U.I. l^{-1})	312	310	321	175*	318
Triglycerides	(g l^{-1})	0.69	0.49*	0.16*	0.37*	0.33*
Cholesterol	(g l^{-1})	0.86	0.90	0.80	0.69*	0.71*

Discussion

Although data on the chemical composition of the algae give valuable information about their nutritional value, they cannot be considered as a substitute for biological appraisals of protein quality in the animal. PER has been shown to be the most useful test to estimate the nutritive quality of protein s, based on short-term feeding trials with weaning rats, and has been used with most of the microalgae studied as food. Food consumption was similar in all groups; therefore, results of this study cannot be ascribed to decreased food intake during the experimental period. However, food consumption has been reported to be less in some diets with *Spirulina* and *Scenedesmus* than in control diets with casein (Becker & Venkataraman, 1982; Venkataraman, 1983). However, the *I. galbana* diet provoked an increase in water intake, probably due to the fact that this microalga has the highest ash and chloride content (Fabregas & Herrero, 1985; 1986).

PER and FCE data (Table 2) show important differences between *D. tertiolecta* and the remaining microalgae assayed. However, data on the chemical composition of these microalgae and on the MEAA index did not show this difference (Fabregas & Herrero, 1985); this fact encourages the importance of biological tests of protein quality in animals. Table 6 shows the PER values obtained with freshwater micro algae, other microbial proteins and conventional foods, and illustrates the good quality of *D. tertiolecta* protein (Table 2). Its PER value is higher than that found for drum-dried, sun-dried and freeze-dried *Scenedesmus* (1.99, 1.14, 1.12) and lower tan drum-dried *Scenedesmus* supplemented with methionine (2.20). It is also higher than *Chlorella* (0.84, 1.89, 1.31) and *Spirulina* (1.78, 1.89), cereals such as corn (1.23) or wheat (1.21), and close to fish flour (2.33), soybean (2.35) or casein (2.50). The yeasts, probably the microorganisms most used as a protein source (SCP), show a PER value considerably lower than that of *D. tertiolecta* (1.05); this is also lower than those of other freshwater and marine microalgae. PER values between 1.70 and 1.90 are considered as excellent for this type of SCP (Becker, 1980).

As a rule, algal proteins are poorly utilized when intact cells are given to monogastric animals or humans and special treatments are required to disrupt the algal cell wall, thus making the protein accessible to proteolytic enzymes. The effect of various methods for processing and drying the algae on their digestibility has been studied extensively and different processing methods give differences in PER values (Table 6). In the present experiments the marine microalgae were dried in an oven, without using any method to break the cell wall. PER values obtained with *T. suecica, I. galbana* and

C. *stigmatophora* were lower than that reached for *D. tertiolecta* diets, probably because *D. tertiolecta* lacks a cell wall (Oliveira *el al.,* 1980). PER values obtained with all these marine microalgae were higher than that of un processed *Chlorella.* Marine microalgae also compare favourably with other protein sources, such as the cereals wheat or corn. PER values of *T. suecica, I. galbana* and C. *stigmatophora* were very similar to those of wheat and corn, and the PER value of *D. tertiolecta* was higher than both (Tables 2, 6).

Table 6. Comparative PER values of different protein sources (DD, drum-dried; SD, sun-dried; FD, freeze-dried).

Protein source	Processing	PER	Reference
Casein		2.50	
Scenedesmus acutus	DD	1.99	Becker *et al.*, 1976
Scenedesmus acutus	SD	1.14	Becker *et al.*, 1976
Scenedesmus acutus	FD	1.12	Becker *et al.*, 1976
Scenedesmus acutus + 0.3% Methionine	DD	2.20	Becker *et al.*, 1976
Chlorella vulgaris	Raw	0.84	Cheeke *et al.*, 1977
Chlorella vulgaris	DD	1.89	Thanamunkul *et al.*, 1977
Chlorella vulgaris	Autoclaved	1.31	Cheeke *et al.*, 1977
Spirulina	SD	1.78	Becker & Venkataraman, 1984
Spirulina platensis + 0.3% Methionine	SD	1.89	Becker & Venkataraman, 1984
Wheat		1.21	Venkataraman, 1983
Soybean		2.35	Venkataraman, 1983
Corn		1.23	Becker, 1984
Yeasts		1.05	Venkataraman, 1983
Fish-flour		2.33	Cheeke *et al.*, 1977

One of the parameters generally considered in the evaluation of the toxicological effects of microalgal diets is the weight of different organs (Becker, 1980; Payer *et al,* 1980; Venkataraman *et al.,* 1980; Becker & Venkataraman, 1982). The relative organ weights of rats fed on the different microalgal diets were normal, although there were some significant differences with respect to the control (Table 3). Differences in relative organ weights also were found in rats fed on diets with freshwater micro algae, especially the organ weights of liver, kidneys, lungs and spleen, although no pathological effects could be observed.

It has been assumed that, since these organs are more metabolic ally active, they will be the most affected by changes in the diet (Payer *et a/., 1980).* Blood counts indicate no abnormalities in any group (Table 4). Assays with *Scenedesmus* showed that the density of white blood cells was lower in blood from rats given the algal diets than in that from rats given the control diet of casein, although the differential white blood cell counts and the relative proportions of the various white cells were similar (Venkataraman *el al.,* 1980). In other experiments with this microalga the haemoglobin content in blood from rats given the algal diet was 3 % lower than in that from rats given the casein diet (Payer *et al.,* 1980). None of these differences were observed in the rats given the marine micro algal diets.

It has been reported that there is an inverse relation between the blood urea content and the biological value of the diet which is sufficiently accurate to provide a useful method for the prediction of protein quality from measurement of urea levels (Eggum, 1970). This is in accordance with the results obtained here for *T. suecica, I. galbana* and *D. tertiolecta* diets, since rats fed on *D. tertiolecta* showed an urea content similar to casein and a good P ER, whereas rats red on *T. suecica* and I. *galbana* showed a significantly higher urea content than rats red on casein and a significantly lower PER (Table 5). However, C. *stigmatophora* did not fit with this rule, because rats fed on it presented a low PER and a normal urea content.

From the different plasma enzymes determined, only CPK values were significantly lower in rats fed on *D. tertiolecta* diets than in rats fed on the control diet, but this enzyme shows very disperse and divergent values and can be strongly altered by physical effort (Wallach, 1979). The remaining enzymes, and the plasma proteins, were similar in all groups. All the groups fed on micro algae showed significantly lower triglycerides levels, and a significant decrease in cholesterol was shown by rats fed on *D. tertiolecta and* C. *stigmatophora.* There are numerous reports on the hypocholesterolemic properties of a variety of materials derived from biological sources and foods (Vahouny, 1985) but the hypocholesterolemic properties of microorganisms are less well known. In trials involving the feeding of weaning rats with freshwater microalgae, it was observed that the serum cholesterol levels were lower in animals given the microalgal diets than in controls fed on a casein-based standard diet. This hypocholesterolemic effect increased as the percentage of micro algae in the diet increased (Anusuya- Devi *el al.,* 1979; Rolle & Pabst, 1980; Venkataraman *et al.,* 1980; Becker & Venkataraman, 1982; Anusuya-Devi & Venkataraman, 1983). This cholesterol lowering property was also previously observed in studies with *Dunaliella tertiolecta* (Fabregas *el al.,* 1988). High colesterol levels have been correlated with the incidence of coronary diseases and other artherosclerotic lesions, so that the hypocholesterolemic effect of these algal-protein diets can constitute an added advantage.

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