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The nutritional quality of drum-dried algae produced in open door mass culture

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Summary

The yield of three large scale cultures of *Scenedesmus acutus, Chlorella vulgaris,* and *Coelastrum proboscideum* was drum dried. The amino acid composition of the three species proved to compare well with the FAO (11) amino acid pattern except for methionine and isoleucine. Bio-assay evaluation of the three algal proteins gave the following values: protein efficiency ratio, 1.9–2.1; net protein ratio, 2,4–2.8; biological value, 75–78; digestibility coefficient 88–89; and calculated net protein utilization 67–69. Total nucleic acid content was about 4 %. Uric acid content in the plasma of rats fed Scenedesmus, Chlorella, and Coelastrum diets was significantly higher ($P < 0.05$) than in plasma of rats fed on a casein diet.

Zusammenfassung

Proben von Massenkulturen der Algen Scenedesmus acutus, Chlorella vulgaris und Coelastrum proboscideum wurden getrocknet und untersucht. Die Aminosäurezusammensetzung der Proteine der drei Stämme war mit der von der FAO (11) empfohlenen vergleichbar außer für Methionin und Isoleucin. Die biologische Auswertung der drei Algenproteine ergab folgende Werte: Protein efficiency ratio 1,9–2,1; net protein ratio 2,4–2,8; biologischer Wert 75–78; digestibility coefficient 88–89; berechnete net protein utilization 67–69. Der Gesamt-Nucleinsäuregehalt betrug etwa 4 %. Die Harnsäurekonzentration im Serum von mit Algen gefütterten Ratten war signifikant höher als bei Tieren mit einer Casein-Diät.

Key words: algae, Scenedesmus, Chlorella, Coelastrum, mass culture, nutritional quality

Introduction

The usefulness of microalgae as a source of protein for food or feed has been under investigation for many years.

The nutritional value of unicellular green algae such as Scenedesmus and Chlorella is greatly influenced by the method used for processing the raw material. The digestibility of algae is dependent on disrupting the

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algal cell wall by proper processing. The simplest method for disrupting algal cell walls is through exposing the cells for a few seconds to high temperatures (100–120 °C) (15, 18).

Jaleel and Soeder (13) studied the nutritional value of drum dried Scenedesmus in rat feeding experiments following the guidelines of the protein advisory group (PAG). The authors reported mean values of 2.7, 78.6 % for protein efficiency ratio (PER) and protein digestibility (DC), respectively. When correction was made for endogenous nitrogen, respective values for protein digestibility (DC), net protein utilization (NPU) and biological value (BV) were 96.4, 70.5 and 73.1 %.

Pabst (20) carried out nitrogen balance studies and fed diets containing 1.6 percent nitrogen in the dry matter equal to 10 % crude protein of casein + methionine, Scenedesmus, or Coelastrum. The respective mean values obtained for the three diets for the following nutritional indices were PER (2.50, 1.85, 1.86) NPU % (86, 61, 63) true digestibility % (95, 81, 80), and biological value (BV) % (92, 75, 75).

Recently, Gross et al. (12) reported a mean value of 2.63 for the PER of Scenedesmus cultivated in Peru.

Brune and Walz (5) found that, with reference to egg protein (100 %), Scenedesmus protein shows the best essential amino acid index (EAAI) (75.5 %) compared with alfalfa (64.2 %) or soybean protein (60.4 %). They mentioned that EAAI values are relevant for supplementing algae protein in diets for human and animal nutrition.

There has been some debate about health hazards posed by the nucleic acid content of microalgae (25) which is about 4 % (22). Kofranyi (14) mentioned that the routinely acceptable Scenedesmus ration of 15 g/head/day, would never pose any uric acid problems. The aim of the present work was to evaluate the nutritional value of three algae powders, *Scenedesmus acutus, Chlorella vulgaris,* and *Coelastrum proboscideum* cultured locally in rat feeding experiments.

Experimental materials and methods

After the dark green algae (Scenedesmus, Chlorella and Coelastrum) was spun in the centrifuge, it was passed into double drum drier for drying. The drying time for the algal slurry was not more than 10 seconds, at a temperature of about 120 °C. Drying the algae on the drum drier has the dual advantage of sterilizing the samples and breaking the cell walls, thus making the intracellular components more digestible. The algal material was produced in outdoor mass cultures in Egypt (10).

Rat feeding experiment

Male Sprague-Dawley rats aged 21 + 2 days were housed individually in cages modified to allow quantitative feces and urine collection. For the first three days the rats were fed a diet consisting of a mixture of equal parts of all experimental diets. After three days, the food was removed for 6 h, and the rats were weighed and distributed over five groups with six rats in each of approximately equal weights. One group received a protein free diet consisting of the following ingredients (in %): cotton seed oil, 5; salt mixture, (Muller), 6; vitamin mixture, (Muller), 2; cellulose, 4; and corn starch up to 100 %.

The second group received reference casein diet plus 0.5 % methionine. The diet contained 10 % of casein protein. Groups 3, 4, and 5 received algae diets containing

10 % of algal protein of species Scenedesmus, Chlorella, and Coelastrum, respectively.

Feed consumption was measured and nitrogen intake was calculated for each animal from the nitrogen content of the diets. The animals were weighed twice weekly. The feces and urine were collected and kept frozen until total nitrogen analysis. The bio-assay methods determined were: protein efficiency ratio (PER) described by the A.O.A.C., (2); net protein ratio (NPR) described by Bender and Doell, (6); and biological value (BV) and digestibility coefficient (DC) according to the method of Mitchell, (19). The net protein utilization (NPU) was calculated according to the equation $\frac{BV \times DC}{100}$ of Chick et al. (8).

At the end of the bio-assay experiments, blood was collected from the optic vein of each individual rat in the presence of an anticoagulant. The plasma was separated and kept frozen for uric acid analysis.

Chemical analyses

Total nitrogen: The nitrogen content of the diets, feces, and urine was determined by the micro-Kjeldahl method (1) using a steam distillation apparatus for micro-Kjeldahl analysis (17).

Amino acids

Finely ground algal samples (mg) were hydrolyzed with 6 N HCl in sealed ampoules at 120 °C for 18 h. The hydrolysates were evaporated to remove the excess hydrochloric acid and the residue was redissolved in citrate buffer pH 2.2. The amino acids were separated on a Beckman automatic amino acid analyser according to the method of Spackman, Moore and Stein. The results were expressed in mg/g nitrogen. The amino acid reference pattern of the FAO (11) was used for comparison.

Nucleic acid

The extraction of RNA and DNA from the cells was done according to the method of Wanka (26). The ribonucleic acid was determined according to the method of Ashwell (3) and the deoxyribonucleic acid by the diphenyl amino method (7).

Plasma uric acid

Plasma uric acid was determined by using a uric acid kit according to the method of Biomerieus, Charbonnieres – Les Bains/France.

Results and discussion

The mean values of crude protein content of the three green algae investigated in the present study were about 50 % (Table 1). The essential amino acid patterns of the three algae are presented in Table 1. Under the present experimental conditions, cysteine could not be determined, so that the data for the sulfur-containing amino acids is represented only by methionine. The Table shows that the protein score of the three algae products ranged between 37 and 46 % with methionine being first limiting AA, and Chlorella being the lowest and Coelastrum highest in the methionine content.

Isoleucine appears to be second limiting, with protein score ranging between 80–90 % compared to FAO amino acid pattern (11).

Table 1. Amino acid composition of Scenedesmus, Chlorella and Coelastrum with reference to the FAO reference pattern.

Amino acid	FAO pattern (mg/g N) (11)	Amino acid composition (mg/g N)			Protein score* (%)		
		Scenedesmus	Chlorella	Coelastrum	Scenedesmus	Chlorella	Coelastrum
Lysine	344	400	400	419	116	116	122
S-contg.**	219	87	81	100	40	37	46
Aromatic	375	362	344	350	143	138	142
Leucine	438	600	594	600	137	136	137
Isoleucine	250	212	200	225	85	80	90
Valine	313	337	437	375	108	140	120
Threonine	250	419	331	437	168	133	175
Tryptophan	62.5	106	94	87	170	150	140
Crude protein %		52.3	51.1	50.8			

* Relative to the FAO reference pattern (11).
** Only methionine in experimental samples.

Bio-assay evalution

Lubitz (16) obtained PER values of 2.19 and 3.3 for Chlorella and casein diets, respectively, compared to 2.03 and 2.72 for the same diets in the present study. The alga Scenedesmus was tested by Jaleel and Soeder (13), and Becker and Venkataraman (4), resulting in PERs of 2.7 and 2.2, respectively. The same species gave PER of 2.12 in the present investigation. The alga Coelastrum in a study by Pabst (20) gave PER of 1.86 compared to 1.9 in present study. These comparisons indicate the similarity of our results with results of other investigators for the same species grown under different conditions.

The NPRs exceeded the PERs for casein, Scenedesmus, Chlorella, and Coelastrum diets by 14, 32, 30 an 26 %, respectively. The relatively high increase of NPR values of algae protein over its PER values upgrades the quality of algae protein compared to casein diet. This finding could be mathematically observed by comparing NPR with the PER value relative to that of casein (Table 2). For instance, the PER and NPR values of Scenedesmus diet relative to the respective value of casein were 78 and 90 %.

Kraut et al. (15) reported that the biological value of protein from roller-dried Scenedesmus averaged about 90 % of that of casein. In the present study BV values of algae diets relative to that of casein were around 86 %. Jaleel and Soeder (13) obtained lower values of BV (73 %), DC (78 %) and higher NPU (70.5 %) for diets containing Scenedesmus, compared to values obtained in this study (Table 2). For Scenedesmus or Coelastrum containing diets (10 % protein level), Pabst (20) reported a BV value of 75 % which is around the figure obtained for the two algae in the present study.

Pabst (20) obtained NPU values of 61 and 63 % for Scenedesmus and Coelastrum, respectively. Becker and Venkataraman (4) reported NPU to be 65.8 % for Scenedesmus. This value is lower than the calculated value in the present study (Table 3), which was around 68 %. However, the correlation between PER and calculated NPU values was significant ($P > 0.01$) with a correlation coefficient of + 0.825.

Table 4 indicates that the total nucleic acid content (on a dry weight basis) of Scenedesmus, Chlorella and Coelastrum ranged between 3.6–4.7,

Table 2. Average protein efficiency ratio (PER) and net protein ratio (NPR) values* of diets containing casein, Scenedesmus, Chlorella and Coelastrum as sole source of protein (10 % level).

Diet	PER	%	NPR	%
Casein	2.7 ± 0.04	100	3.1 ± 0.07	100
Scenedesmus	2.1 ± 0.08	77.9	2.8 ± 0.07	90.3
Chlorella	2.0 ± 0.015	74.6	2.6 ± 0.05	85.2
Coelastrum	1.9 ± 0.046	70.2	2.4 ± 0.07	77.7

* Average of 6 rats ± standard deviation.

Table 3. Average biological value (BV), digestibility coefficient (DC), and net protein utilization (NPU) values* of diets containing casein, Scenedesmus, Chlorella and Coelastrum as sole source of protein (10 % level).

Diet	BV	%	DC	%	NPU**	%
Casein	88.4 ± 1.15	100	94.0 ± 0.46	100	83.1 ± 1.4	100
Scenedesmus	76.2 ± 1.5	86.2	88.6 ± 0.84	94.3	67.5 ± 1.97	81.2
Chlorella	77.9 ± 2.3	88.1	89.3 ± 0.64	95.0	69.6 ± 2.42	83.8
Coelastrum	75.3 ± 2.1	85.2	89.2 ± 0.8	94.9	67.2 ± 2.42	80.4

* Average of 6 rats ± standard deviation.

** Calculated ($\frac{BV \times DC}{100}$).

3.3–4.6 and 4.1–4.2 % respectively. Average values of total nucleic acid content of the three algae, were 4.15, 4.06 and 4.15 %. Also, results indicate that RNA represents about 88.93, 87, and 88.93 % of total nucleic acids for Scenedasmus, Chlorella and Coelastrum, respectively, and the rest is DNA.

Table 4. Analysis of nucleic acid content (% dry basis) of green algae *Scenedesmus acutus* 276–3 a, *Chlorella vulgaris* and *Coelastrum proboscideum.*

Algae	Sample No.	RNA	DNA	Total nucleic acids
Scenedesmus	1	4.01	0.68	4.69
	2	3.32	0.44	3.76
	3	3.30	0.34	3.64
	4	3.71	0.52	4.23
	5	3.62	0.45	4.07
	6	3.94	0.57	4.51
Average ± SD		3.65 ± 0.27	0.50 ± 0.11	4.15 ± 0.41
Chlorella	1	3.12	0.14	3.26
	2	4.30	0.31	4.61
	3	3.91	0.40	4.31
Average ± SD		3.78 ± 0.49	0.28 ± 0.11	4.06 ± 0.71
Coelastrum	1	3.71	0.53	4.24
	2	3.58	0.51	4.09
	3	3.53	0.58	4.11
Average ± SD		3.61 ± 0.07	0.54 ± 0.03	4.15 ± 0.08

Table 5. Plasma uric acid concentration of rats at the end of feeding (17 days) of algae and casein diets.

Diets	Plasma uric acid (mg/100 ml) Mean ± SD*
Casein	2.75 ± 0.13 a
Scenedesmus	3.15 ± 0.10 b
Chlorella	3.50 ± 0.15 b
Coelastrum	3.83 ± 0.27 b

* Average ± standard deviation. The difference between mean values labelled with a and b is statistically significant ($P < 0.05$).

The concentration of nucleic acids in algae is of great importance from the nutritional stand-point. Senez (21), reported that generally algae and fungi have a lower nucleic acid content, while yeast and bacteria have a high content. The obtained results are in agreement with those obtained by Soeder and Pabst (23) who reported that the total nucleic acid concentration of microalgae biomass (Scenedesmus) comprises 4.0 ± 0.5 % of dry matter. Also, Jaleel and Soeder (13) found that total nucleic acid in Scenedesmus is of the order of 3.7 ± 1.0 % on dry weihgt basis.

El-Fouly et al. (9) found that the nucleic acid content of Chlorella was 6 % in samples harvested from normal and nitrogen deficient cultures. They also reported that 95 % of total nucleic acids were RNA and 5 % DNA, while Subbulaksmi et al. (24) found that the total nucleic acid content of Scenedesmus ranged from 5.3 to 6 % with RNA contributing the major part as compared with DNA.

Table 5 shows the plasma unic acid content (mg/100 ml) of rats at the end of the feeding experiment (17 days) on casein, Scenedesmus, Chlorella and Coelastrum diets (10 protein level).

The plasma uric acid values of rats maintained on diets based on algae proteins were significantly higher ($P < 0.05$) than those of rats maintained on the reference casein diet. The highest mean value was obtained amongst rats receiving the Coelastrum algae diet. This type of algae was the highest with respect to its methionine content and protein score but gave inferior bio-assay values in rat feeding experiments. It is thus most likely that the high nucleic acid content of this algae resulted in high plasma uric acid concentration and interfered with the normal metbolism of the protein and, consequently, the growth of the animals.

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