NUTRIENT CONTENT OF VARIOUS ALGAE AND AMINO ACID ADEQUACY FOR GROWTH OF RATS AND CHICKS*

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INTRODUCTION

Numerous reports have been concerned with the nutritional potential of algae and plankton. Schwimmer and Schwimmer (8) have recently reviewed these reports. Much research in recent years has been directed to systems for algae production; however, the use of this material as a food source has received relatively little attention.

The possibility of employing algae in space systems has stimulated an interest in algae production and in its nutritional value. Our interest in algae has been directed more towards its possible use as a food in crises of various sorts. These situations would be such as to enable the processing and supplementations which may be necessary to make a palatable and adequate food from algae.

As a consequence of this interest, studies have been designed to analyze the nutrient content of algae chemically and studies have been initiated with experimental animals and human subjects to determine the biological availability of the nutrients present in algae. The present report is concerned with analyses of various algae for certain water-soluble vitamins, amino acids, differential nitrogen analyses, as well as routine proximate analyses. Data are also presented on the ability of algae protein to support growth in rats and chicks and also on the amino acid deficiencies of algae protein.

EXPERIMENTAL

Analytical Procedures

Listed below are the procedures employed in the nutrient analyses of algae.

*A portion of the material presented in this paper is to be published, Leveille et al. (5).

Crude protein - (Nitrogen x 6.25) macro Kjeldahl, AOAC (1).

Total lipid - Acid hydrolysis with extraction of lipids from the hydrolyzed mixture, AOAC (1).

Chromogen free lipids - Eluted from a magnesia:supercell (1:1) column with petroleum ether:acetone (9:1).

Caloric content - Bomb calorimetry (2).

Amino acids - Determined by the Moore Stein procedure (4).

Tryptophan was determined microbiologically, AOAC (1).

Amide N - By the procedure outlined by West and Todd (11).

Ammonia nitrogen - By distillation from an algae sample in a basic solution, trapping of the ammonia in boric acid and titrating with standard sulfuric acid.

Humin N - Micro Kjeldahl analysis of the residue remaining after acid hydrolysis of the algae.

Vitamins - Thiamine and ascorbic acid were determined by the procedures of Ziporin et al. (12) and Schaffert et al. (8), respectively. Riboflavin, pantothenic acid, pyridoxine and niacin were determined microbiologically. Niacin and pantothenic acid were assayed with Lactobacillus arabinosus 17-5; a modification of the AOAC (1) procedure was employed for niacin and a modification of the method of Novelli et al. (6) for pantothenic acid. Riboflavin was assayed with Lactobacillus casei #7469 by a modification of the AOAC procedures (1). Pyridoxine was assayed with the yeast Saccharomyces carlsbergensis #9080.

Animal Studies

Male Hy-line chicks were fed a commercial diet for one week and were then assigned to various treatment groups on the basis of weight. The chicks were housed in cages with raised wire floors. Body weight and food consumption were determined weekly. The composition of the diet employed for chick studies was as follows in gm/100 gm of diet: Starch 16.20; salt mix** 5.31; vitamin mixture** 0.40; choline Cl 0.20; corn oil 5.00; non-nutritive fiber 3.00; glucose to 100. Addition of algae and amino acids to the basal diet were made at the expense of glucose.

In the rat studies, male, weanling rats of the Holtzman strain weighing 40-50 gm were distributed to the various treatment groups after receiving a stock diet for three or four days. The animals were housed individually in cages having raised wire floors. The experimental diets were fed for approximately three weeks; the exact length of the periods is indicated in the tables of results. The composition of the basal diet employed for the rat studies was as follows in gm/100 gm of diet: Starch 13.0; salt mixture (USP XIV) 4.00; vitamin mixture** 0.40, Choline Cl 0.3; non-nutritive fiber 3.0; corn oil 5.0; glucose to 100. As in the chick diets, additions to the basal diet were made at the expense of glucose.

**For composition see Leveille et al. (5).

Three algae were employed in these studies, 1) a mixture of Scenedesmus obliquus and Chlorella ellipsoida,† 2) Chlorella pyrenoidosa‡ and 3) Spongiococcum excentricum.§ The analytical data for the algae samples employed in the feeding studies are presented in Table 1.

RESULTS

Analytical Studies

Data obtained for the proximate analysis of the same species of algae employed in the feeding studies are presented in Table 2. The data illustrate the high nutritive potential of algae. These algae are high in protein and fat and therefore in caloric content. The algal content of various vitamins is also quite high as demonstrated by the data presented in Table 3.

The high protein content of algae shown in Table 2 is for crude protein, N x 6.25, and does not necessarily represent the true protein content. In order to obtain a more valid value for protein content the samples were analyzed for various forms of non-protein nitrogen as shown in Table 4. It is evident from these data that in the samples analyzed, only about 80% of the total nitrogen represents amino nitrogen. The values presented for amino nitrogen were obtained by difference; however, a few samples were assayed for amino nitrogen and the determined values and those obtained by difference agreed excellently.

The values obtained for amino acid analyses of the various algae samples are presented in Table 5. The one obvious deficiency common to all of these samples is the low level of sulfur amino acids. These samples were essentially devoid of cystine and contained extremely low levels of methionine. This is particularly true of the algae S. excentricum. Considerable variation was noted between samples for certain of the other amino acids; lysine, histidine and threonine appeared low in certain instances.

Feeding Studies

Protein efficiency ratios (gm gain/gm protein consumed) were used as a criterion of the protein quality of algae protein. The results of such studies in chicks and rats are presented in Tables 6 and 7. These data illustrate the inferiority of algae protein to soybean or casein controls used for the chick and rat studies, respectively. The mixture of S. obliquus and C. ellipsoida was superior to either of the other two algae fed. The data presented in Table 7 further demonstrate the methionine deficiency in rats

† Obtained from Micro Algae Research Institute of Japan, Tokyo, Japan.

‡ Generously supplied by Dr. R. H. Lowry, Boeing Airplane Co., Aerospace Division, Seattle 24, Washington.

§ Purchased from Grain Processing Corp., Muscatine, Iowa. This material was specially prepared for this laboratory.

fed the algae S. excentricum. Rats were fed a diet in which the protein was supplied by the algae S. excentricum for three weeks; they were then fed the same diet to which had been added 0.5% DL-methionine for 11 days. As can be seen from the data in Table 7, the rats gained 23 gm in 11 days and had a protein efficiency ratio of 1.22 when fed the methionine-containing diet as compared to a gain of 9 gm and a protein efficiency ratio of only 0.34 in the preceding three-week period when fed the unsupplemented diet.

The data presented in Table 8 illustrate the influence of amino acid supplementation to algae diets on growth and feed efficiency of chicks. Chicks were fed diets in which the mixed algae S. obliquus and C. ellipsoida served as the sole source of protein. This basal diet was supplemented with methionine, lysine, tryptophan, arginine, glycine, threonine and histidine or the foregoing mixture from which one amino acid was omitted. Only the omission of methionine or glycine depressed growth significantly indicating a deficiency of these amino acids for the growing chick. The omission of histidine from the mixture resulted in a slight growth stimulation, an indication of a possible amino acid imbalance.

In order to determine more conclusively the existence of amino acid deficiencies, a second study was initiated with chicks in which amino acids were added to the same diet as used in the previously described study. The design and the results of this second study are presented in Table 9. The addition of methionine or glycine to the diet had little influence on growth while the simultaneous addition of these two amino acids resulted in a significant growth stimulation. The addition of threonine to the methionine-glycine supplemented diet was without effect. The addition of lysine to the diet containing added methionine, glycine and threonine depressed growth and the addition of histidine depressed growth further. The addition of histidine alone resulted in a growth depression as compared to chicks fed the unsupplemented control diet. The addition of leucine alone or in combination with the other five amino acids did not influence growth.

A similar study was initiated with weanling rats using the algae C. pyrenoidosa the results of which are presented in Table 10. The basal diet improved growth and feed efficiency when supplemented with methionine, tryptophan, lysine, threonine and histidine. Omitting single amino acids from the mixture depressed growth only in the case of methionine and histidine, indicating a deficiency of these two amino acids for the growing rat. Methionine supplementation alone also improved growth, but not feed efficiency.

TABLE 1

PROTEIN, TOTAL LIPID, CHLOROPHYLL-FREE LIPID, ASH CONTENT OF VARIOUS ALGAE

Algae	Crude protein (N x 6.25)	Lipid	Chlorophyll-free* lipid	Ash
	%	%	%	%
Scenedesmus obliquus				
+ Chlorella ellipsoida	55.56	4.49	0.72	3.62
Chlorella pyrenoidosa	59.96	11.93	7.60	4.91
Spongiococcum excentricum	31.04	11.32	7.18	5.29

* Total lipid extract freed of chlorophyll and other chromogens.

TABLE 2

PROTEIN, FAT, ASH AND CALORIC ANALYSES OF VARIOUS ALGAE*

Algae**	Protein (N x 6.25)	Total fat	Chromogen-free fat	Ash	Calories/gm
	%	%	%	%	
1	61.9	9.88	6.08	5.11	5.266
2	60.4	7.28	6.26	5.07	5.230
3	53.3	11.70	7.79	4.32	5.406
4	57.7	5.15	4.18	3.75	5.543
5	31.6	11.97	5.18	5.57	4.552

* Values expressed on a dry-weight basis.

**Code: 1 and 2 Chlorella pyrenoidosa; 3 mixture of Scenedesmus obliquus and Chlorella ellipsoida freeze-dried; 4 mixture of S. obliquus and C. ellipsoida oven-dried at 60° C.; 5 Spongiococcum excentricum.

TABLE 3

THE VITAMIN CONTENT OF VARIOUS ALGAE

Algae*	Vitamin (mcgm/gm of dry algae)					
	Thiamine	Riboflavin	Pantothenic acid	Pyridoxine	Niacin	Ascorbic acid
1	3.50	37.04	34.36	0.527	198.59	139.6
2	1.36	29.79	34.33	3.168	252.33	135.8
3	0.20	0.16	1.61	0.235	4.68	18.0
4	0.04	0.27	1.51	0.148	5.72	74.4
5	0.18	47.78	11.23	2.489	78.16	283.5

* Code: 1 and 2 *Chlorella pyrenoidosa*; 3 mixture of *Scenedesmus obliquus* and *Chlorella ellipsoida* freeze-dried; 4 mixture of *S. obliquus* and *C. ellipsoida* oven-dried at 60° C.; 5 *Spongiococcum excentricum*.

TABLE 4

NITROGEN ANALYSES OF VARIOUS ALGAE

Algae*	Total N	Amide + NH_3 N	Humin N	Amino N by diff	Amino N
	%	%	%	%	% of total N
1	9.44	0.84	0.82	7.78	82.42
2	9.26	0.75	0.53	7.98	86.18
3	7.70	0.56	1.33	5.81	75.45
4	8.88	0.61	1.11	7.16	80.63
5	4.90	0.61	0.79	3.50	71.43

* Code: 1 and 2 *Chlorella pyrenoidosa*; 3 mixture of *Scenedesmus obliquus* and *Chlorella ellipsoida* freeze-dried; 4 mixture of *S. obliquus* and *C. ellipsoida* oven-dried at 60° C.; 5 *Spongiococcum excentricum*.

TABLE 5

THE AMINO ACID COMPOSITION OF VARIOUS ALGAE

Amino acid	Algae* 1	2	3	4	5
	grams amino acid/16 grams N				
Lysine	5.09	7.51	5.87	3.63	2.37
Histidine	0.94	1.30	1.51	1.18	0.75
Arginine	2.34	5.11	5.70	4.27	3.58
Aspartic acid	4.14	5.65	7.40	6.37	3.10
Threonine	1.58	2.59	3.95	2.26	1.95
Serine	1.02	2.29	3.15	1.08	2.29
Glutamic acid	3.35	9.49	9.46	7.71	4.79
Proline	2.76	4.71	4.87	3.79	4.58
Glycine	1.94	5.70	4.99	4.10	2.28
Alanine	2.52	5.63	6.03	3.17	3.10
Cystine	-	0.34	-	-	-
Valine	1.81	7.32	5.10	4.65	3.17
Methionine	0.59	1.94	2.96	1.48	0.38
Isoleucine	1.39	4.35	3.71	3.16	1.49
Leucine	3.22	9.60	8.22	6.86	3.74
Tyrosine	1.05	2.84	3.01	1.68	1.16
Phenylalanine	1.92	5.19	6.88	4.49	2.23
Tryptophan	1.53	1.76	1.71	1.80	1.24

* Code: 1 and 2 Chlorella pyrenoidosa; 3 mixture of Scenedesmus obliquus and Chlorella ellipsoida freeze-dried; 4 mixture of S. obliquus and C. ellipsoida oven-dried at 60° C.; 5 Spongiococcum excentricum.

TABLE 6

INFLUENCE OF VARIOUS ALGAE PROTEIN ON GROWTH AND PROTEIN EFFICIENCY OF THE GROWING CHICK

Protein source*	3-week gain	Protein efficiency ratio
	gm	gm gain/gm protein consumed
Soybean + DL-methionine**	237 ± 26 †	3.04
Scenedesmus obliquus + *Chlorella ellipsoida*	87 ± 22	1.55
Chlorella pyrenoidosa	9 ± 13	0.31
Spongiococcum excentricum	13 ± 8	0.43

* All proteins supplied at a level of 18% of the diet (% N x 6.25).

**0.54% added DL-methionine.

† Mean ± standard deviation.

TABLE 7

THE INFLUENCE OF VARIOUS ALGAE PROTEINS ON GAIN AND PROTEIN EFFICIENCY RATIO IN GROWING RATS

Protein source	Dietary protein level (N x 6.25)	3-week gain	Protein efficiency ratio
	%	gm	gm gain/gm protein consumed
Casein	14.88	112 ± 10*	2.50 ± 0.09
Scenedesmus obliquus + Chlorella ellipsoida	15.31	60 ± 7	1.38 ± 0.14
Chlorella pyrenoidosa	15.31	38 ± 12	0.94 ± 0.19
Spongiococcum excentricum	14.81	9 ± 6	0.34 ± 0.24
Spongiococcum excentricum + DL-methionine	15.12	23 ± 4**	1.22 ± 0.18

* Mean ± standard deviation.

**The animals receiving the S. excentricum diet were maintained on the same diet to which had been added 0.5% DL-methionine for 11 days (21st through 32nd day).

TABLE 8

INFLUENCE OF AMINO ACID SUPPLEMENTATION OF ALGAE PROTEIN (SCENEDESMUS OBLIQUUS + CHLORELLA ELLIPSOIDA) ON GROWTH AND FEED EFFICIENCY IN THE GROWING CHICK*

Amino acid mixture**	3-week gain	Feed efficiency
	gm	gm gain/gm feed consumed
None	83 ± 11†	0.32
Complete	124 ± 21	0.47
Minus threonine	117 ± 15	0.45
Minus glycine	96 ± 9	0.38
Minus arginine	122 ± 19	0.47
Minus tryptophan	124 ± 14	0.47
Minus methionine	78 ± 9	0.36
Minus lysine	114 ± 14	0.42
Minus histidine	132 ± 15	0.47

* All diets supplied 23.45% crude protein (% N x 6.25) from algae.

**Amino acids were supplemented at the following levels as a percentage of the diet: DL-methionine, 0.80; DL-tryptophan, 0.20; L-arginine HCl, 0.73; glycine, 0.75; DL-threonine, 0.60; L-histidine HCl.H_2O, 0.20; L-lysine HCl (95% purity), 0.59.

†Mean ± standard deviation.

TABLE 9

EFFECT OF AMINO ACID SUPPLEMENTATION OF ALGAE PROTEIN (<u>SCENEDESMUS OBLIQUUS</u> + <u>CHLORELLA ELLIPSOIDA</u>) ON GROWTH AND FEED EFFICIENCY OF THE GROWING CHICK

Amino acid supplement*						3-week gain	Feed efficiency
DL-methionine 0.80%	Glycine 1.00%	DL-threonine 1.20%	L-lysine HCl (95% purity) 1.10%	L-histidine HCl.H_2O 0.30%	L-leucine 0.70%		
						gm	gm gain/gm feed consumed
-	-	-	-	-	-	87 ± 22**	0.28
+	-	-	-	-	-	93 ± 19	0.34
-	+	-	-	-	-	89 ± 25	0.28
+	+	-	-	-	-	127 ± 28	0.39
+	+	+	-	-	-	124 ± 28	0.40
+	+	+	+	-	-	102 ± 30	0.34
+	+	+	+	+	-	89 ± 41	0.34
+	+	+	+	+	+	81 ± 41	0.32
-	-	-	-	+	-	71 ± 22	0.26
-	-	-	-	-	+	86 ± 25	0.30

* All diets supplied 18.00% protein (% N x 6.25) from algae.

**Mean ± standard deviation.

TABLE 10

INFLUENCE OF AMINO ACID SUPPLEMENTATION OF ALGAE PROTEIN (*CHLORELLA PYRENOIDOSA*) ON BODY WEIGHT GAIN AND FEED EFFICIENCY OF ALBINO RATS

Amino acid supplement*					19-day gain	Feed efficiency
DL-methionine 0.40%	DL-tryptophan 0.40%	L-lysine HCl (95% purity) 1.33%	DL-threonine 1.00%	L-histidine HCl.H_2O 0.53%		
					gm	gm gain/gm feed consumed
-	-	-	-	-	51 ± 8**	0.23 ± 0.02**
+	+	+	+	+	70 ± 18	0.33 ± 0.04
+	+	+	+	-	52 ± 10	0.26 ± 0.04
+	+	+	-	+	76 ± 14	0.28 ± 0.03
+	+	-	+	+	70 ± 14	0.29 ± 0.03
+	-	+	+	+	71 ± 16	0.32 ± 0.03
-	+	+	+	+	50 ± 8	0.22 ± 0.02
+	-	-	-	-	65 ± 11	0.23 ± 0.03

* All diets supplied 18.00% crude protein (% N x 6.25) from algae.

**Mean ± stndard deviation.

DISCUSSION

The analytical data obtained on the various algae samples indicate that algae would be an excellent source of nutrients. However, the data also reveal that the nutrient content is lower than appears at first glance. The protein content, as shown by the analyses for non-protein nitrogen (Table 4) is considerably lower than total nitrogen analyses would indicate, since approximately 20% of the total nitrogen is non-protein, an observation which agrees well with the data of Fowden (3). Similarly, a considerable portion of the total lipids are chromogens (Table 2); chlorophyll, which is largely undigested, would constitute a high percentage of these chromogens and therefore would reduce the caloric value of algae. The vitamins and ash, however, would presumably be available.

The data obtained in the animal studies demonstrate the methionine deficiency existing in all of the algae fed. This observation is in accord with the amino acid analyses showing this amino acid to be low in these samples (Table 5). The algae C. pyrenoidosa was shown to be deficient in histidine as well as methionine for growth in the rat. The deficiency in glycine noted for growth of chicks fed the mixture of algae (S. obliquus and C. ellipsoida), is also in accord with the amino acid analyses of this material. However, this amino acid would not influence the nutritional value of algae for man or the rat since these species do not require this amino acid. Similarly the histidine requirement of the adult human is negligible (7).

The growth depressing effect of histidine supplementation in chicks fed the mixed algae (S. obliquus and C. ellipsoida) is indicative of an amino acid imbalance (Harper and Kumta, 4). However, more data are required to definitely establish the existence of an amino acid imbalance and to determine the relationship of the amino acids involved in such an imbalance.

The data presented demonstrate that algae fed as the sole source of protein to young rats or chicks supports a poor rate of growth even when supplemented with amino acids. The reason for this is not evident from the work reported; however, other studies indicate lack of availability of nutrients to be involved. Since the proteins and other nutrients of algae are enclosed in a cellulose membrane, it is plausible to expect poor digestibility. At present, studies are being devised to develop means of improving the digestibility of algae.

SUMMARY

Analytical data are presented demonstrating a high nutritive potential for algae. Data are presented on the content of protein, amino acids, certain vitamins, lipid, ash and calories in various algae. The protein analyses demonstrate that only 80% of the nitrogen of algae is amino nitrogen.

Studies on the protein quality and amino acid deficiencies of three algae samples for growing rats and chicks are reported. Chicks or rats fed diets in which the protein was supplied by 1) a mixture of Scenedesmus obliquus and Chlorella ellipsoida, or 2) Chlorella pyrenoidosa, or 3) Spongiococcum excentricum grew less well and had lower protein efficiency ratios than their respective controls receiving soybean oil meal or casein protein.

Amino acid supplementation studies showed all the algae fed to be deficient in methionine. In addition, the mixed algae was deficient in glycine for the chick and the algae C. pyrenoidosa was deficient in histidine for the growing rat.

Data are presented which indicate that the amino acids of the mixture of algae (S. obliquus and C. ellipsoida) may be imbalanced for the growing chick.

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