

• • • • • **NUTRITIONAL VALUE OF ALGAE GROWN UNDER  
STERILE CONDITIONS**

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**INTRODUCTION**

Algae obtained as a byproduct from a photosynthetic gas exchanger have been considered as a food source for humans during extraterrestrial habitation by Haldane (2) and Powell *et al.* (4). Spoehi (5) and Burlew (1) have considered the use of algae as a possible food source for man in overpopulated countries. In all of these experiments, the algae used were produced under unsterile conditions. Studies carried out in the Aerospace Medical Research Laboratories indicate that the bacteria population can be one-third of that of algae population in a mature autotrophic culture.<sup>1</sup> In addition, there is no assurance that the population of bacteria will be similar in algal cultures found in different environments. With such high unstable levels of bacteria present a true evaluation of the nutritional value of algae can only be made on algae grown under sterile conditions. In addition, there is good evidence to believe that some of the toxicity associated with algae as a food is caused by the bacterial contamination.

Sterile cultures of heterotrophically grown algae can be maintained with less effort than sterile cultures of autotrophically grown algae. There is no indication, however, that algae grown under these two conditions should have the same nutritional value. This paper reports the data obtained from digestion trials carried out using sterile heterotrophically and autotrophically grown algae as the sole protein source in the diets of rats.

**EXPERIMENTAL PROCEDURE**

Three digestion trials using 12 weanling male rats per trial were carried out to compare the digestibility of energy and protein in sterile heterotrophic, sterile autotrophic, and bacterially contaminated autotrophic algae.

The sorokin high temperature strain of *Chlorella Pyrenoidosa* was used for the production of both the heterotrophically and autotrophically grown sterile algae. The composition of the culture medium used to produce the heterotrophically grown sterile algae is found in Table 1. The medium was made up in 5-gallon carboys and sterilized by autoclaving for 1 hour at 121° C. These carboys were then inoculated aseptically and turned in electric motor driven racks to maintain the algal cells in suspension.

<sup>1</sup> Unpublished data

TABLE 1  
CULTURE MEDIUM

	Grams/Liter
KNO <sub>3</sub>	1.21
MgSO <sub>4</sub> ·7H <sub>2</sub> O	2.46
KH <sub>2</sub> PO <sub>4</sub>	1.23
Sodium Citrate	0.195
Ferric Ammonium Citrate 16.5% - 18.5% Fe <del>44</del>	0.0853
Ca (NO <sub>3</sub> ) <sub>2</sub> ·4H <sub>2</sub> O	0.020
EDTA	0.004
Dextrose	5.0
Arnons A5 Trace Element Solution	1 ml/liter

The autotrophically grown sterile algae were produced in the same culture medium as described in Table 1 except that the glucose was omitted. This medium also was made up in 5-gallon carboys and sterilized. These carboys were equipped with glass tubing inserted through a rubber stopper to permit the delivery of air to the algal suspension. Room air enriched with approximately 8% carbon dioxide was passed through a bacterial filter and bubbled through several of these carboys connected in series. When the algal suspension reached the desired density, it was drawn off and new sterilized medium was added aseptically to these carboys. The algae were then removed from the suspension by centrifuging, washed with distilled water, and dried by lyophilization. Sterility checks were made on the algae when they were harvested. Algae produced in the above methods were pooled in a large container and thoroughly mixed before being added to the diet. The control diet contained a high temperature strain of Chlorella Pyrenoidosa produced in mass culture under non-sterile conditions by an industrial firm.

The rats were housed in individual cages and weighed each day. Water and a diet composed of 42% algae, 48.3% corn starch, 5% corn oil, 0.5% methionine, 2.2% vitamin mix, and 2% mineral mix was fed ad libitum. All of the protein in these diets was supplied by the algae. The rats were placed on the diets for a three-day preliminary period which was followed by a three-day digestion trial. Data on food consumption and fecal weights were recorded each day of the experiment.

Individual fecal samples were dried in a vacuum oven at 50° C. The caloric content of each sample was determined with an Oxygen Bomb Calorimeter. Protein nitrogen was determined by the micro-Kjeldahl method.

## RESULTS AND DISCUSSION

The analysis of the diets showed that the gross energy and crude protein content of the diet supplemented with contaminated algae (CA diet) was higher than the diets containing sterile algae. The diet which contained sterile heterotrophically grown algae (SH diet) had slightly higher crude protein content than the diet which contained the sterile autotrophically grown algae (SA diet). These data are recorded in Table II.

The average daily rat weight gains for the three diets were 5.7g for the SH diet, 5.4g for the SA diet and 5.7g for the CA diet. These are normal weight gains for young weanling rats; and show no significant difference due to diet. Feed efficiency data for the three algal diets are recorded in Table III. The rats which received the SH diet had a higher feed efficiency than those which received the SA diet or the CA diet. These differences were statistically significant at the 1 per cent level. The SA diet had a higher feed efficiency than the CA diet but these differences were not statistically significant.

The energy digestion coefficients for the three diets are recorded in Table IV. These data show that SH diet was higher in digestible energy than the SA diet which was, in turn, higher in digestible energy than the CA diet. These differences were statistically significant at the 1 per cent level. The protein digestibility coefficients are recorded in Table V. These data indicate that the SH diet had a greater protein digestibility than either the SA diet or the CA diet which was statistically significant at the 1 per cent level. The SA diet had a slightly higher digestibility of protein than the CA diet which approaches being statistically significant at the 5 per cent level.

The amino acid analysis of both the sterile heterotrophic and sterile autotrophic algae were carried out by U.S. Army Medical Research and Nutrition Laboratory in Denver, Colorado. These data are recorded in Table VI.

TABLE II  
ANALYSIS OF EXPERIMENT DIETS

	% Crude Protein <sup>1</sup>	K cal/gr
Sterile Heterotrophically grown algae	20.00	4.67
Sterile Autotrophically grown algae	20.52	4.67
Contaminated Autotrophically grown algae	25.00	4.85

<sup>1</sup>Total Nitrogen x 6.25

TABLE III  
RATIO OF WEIGHT GAIN TO FEED INTAKE

Animal	Contaminated Autotrophic (CA Diet)	<u>ALGAE DIET</u>	
		Sterile Heterotrophic (SH Diet)	Sterile Autotrophic (SA Diet)
1	0.49	0.61	0.54
2	0.47	0.63	0.59
3	0.51	0.75	0.59
4	0.52	0.43	0.62
5	0.49	0.47	0.46
6	0.49	0.60	0.52
7	0.48	0.69	0.49
8	0.42	0.64	0.59
9	0.47	0.53	0.52
10	0.46	0.63	0.42
11	0.52	0.67	0.67
12	0.61	0.70	0.46
AVERAGE	0.49	0.62	0.54

TABLE IV

DIGESTIBLE ENERGY

Animal	<u>ALGAE DIET</u>		
	Contaminated Autotrophic (CA Diet)	Sterile Heterotrophic (SH Diet)	Sterile Autotrophic (SA Diet)
ENERGY DIGESTION COEFFICIENTS IN %			
1	82.70	87.93	84.66
2	80.71	88.92	83.18
3	79.60	87.73	83.75
4	76.01	86.96	84.44
5	82.04	88.41	83.85
6	82.69	86.54	84.22
7	81.44	88.15	85.25
8	81.13	88.73	83.91
9	81.55	86.71	83.62
10	80.58	88.24	84.28
11	80.77	87.39	83.11
12	82.21	88.27	84.48
AVERAGE	80.95	87.83	84.06

TABLE V  
DIGESTIBLE PROTEIN.

Animal	<u>ALGAE DIET</u>		
	Contaminated Autotrophic (CA Diet)	Sterile Heterotrophic (SH Diet)	Sterile Autotrophic (SA Diet)
PROTEIN DIGESTION COEFFICIENTS IN %			
1	74.47	79.34	73.17
2	70.32	80.07	69.95
3	68.65	77.83	70.83
4	67.71	75.70	68.95
5	74.16	77.35	73.11
6	76.87	77.47	73.29
7	70.76	79.58	76.30
8	75.76	80.10	73.41
9	65.67	77.69	72.36
10	72.73	79.38	73.73
11	68.93	78.12	73.69
12	75.47	79.45	72.10
AVERAGE	71.75	78.50	72.57

TABLE VI

AMINO ACID	STERILE HETEROTROPHIC ALGAE	STERILE AUTOTROPHIC ALGAE
Lysine	8.304	6.720
Histidine	1.690	1.408
Arginine	5.876	5.152
Asparagine	5.643	5.614
Threonine	7.843	8.017
Serine	3.835	3.445
Glutamic acid	11.481	10.728
Proline	3.680	3.541
Glycine	5.227	5.299
Alanine	9.587	9.078
Valine	4.724	4.042
Methionine	2.048	1.823
Isoleucine	2.835	2.524
Leucine	7.492	7.263
Tyrosine	3.061	2.910
Phenylalanine	4.373	4.428
Nitrogen as %	6.636	7.347

#### SUMMARY

Data are presented on the digestibility of protein and energy in diets containing sterile heterotrophic, sterile autotrophic, and contaminated autotrophic algae. These data show that the digestibility of protein and energy of sterile algae were higher than those from contaminated algae. The digestibility of both protein and energy of sterile heterotrophically grown algae were higher than those of the sterile autotrophically grown algae. These differences would disqualify the use and application of data obtained from heterotrophically grown algae to autotrophically grown algae.

The differences noted in the digestibility of protein and energy between sterile and contaminated algae warrant further study.



LITERATURE CITED

1. Burlew, John S., ed. Current Status of Large-Scale Culture of Algae. Algal Culture: From Laboratory to Pilot Plant. Carnegie Institution of Washington, Washington, D. C., publication 600: 1953.
2. Haldane, J.B.S., Biological Problems of Space Flight. J. Brit. Interplanet. Soc., 10:154, 1951.
3. Lubitz, Joseph A. The Protein Quality, Digestibility and Composition of Chlorella 71105, ASD Technical Report 61-535, W-P AFB, Ohio, 1961.
4. Powell, Richard C., Nevels, Elizabeth, M., and McDowell, Marion E. Algae Feeding in Humans. J. Nutrition 76:7, 1961.
5. Spoehi, H. A. and Milner, H. W. Chlorella as a Source of Food. Proc. Am. Phil. Soc., 95:62, 1951.