ORIGINAL RESEARCH

The Effect of Blue-Green Algae *Aphanizomenon Flos-Aquae* on Nutrient Assimilation in Rats

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ABSTRACT

Blue-green algae *Aphanizomenon flos-aquae* (AFA), rich in protein, vitamins, and polyunsaturated fatty acids, are used as food supplements. We studied the effect of varying concentrations (0.05-0.4%) of AFA on growth, feed consumption, and nutrient digestion in rats. A diet supplemented with 0.05% algae (equivalent to human daily consumption) had a stimulatory effect on weight gain and feed consumption ($p<0.05$). Animals fed diets supplemented with AFA in different concentrations also demonstrated statistically significant changes in weight of viscera and increase in protein level in the liver and small intestinal mucosa. AFA in the diet did not affect pancreatic trypsin and intestinal sucrase and maltase activity, but was associated with increased pancreatic α-amylase ($p<0.05$; 0.2% AFA supplement), and decreased intestinal aminopeptidase N activity ($p<0.025$; 0.05% and 0.1% AFA supplement). Low concentrations of AFA in the diet are associated with increased growth.

Experimental studies have shown that blue-green algae are a source of protein, minerals, vitamins, and other biologically active substances that may be used in human diets as a food supplement.[1,2] Algae also are a potential source of nutrients for a rapidly growing world population.[2]

The most comprehensive studies have been done using *Spirulina* and include tests on subacute and chronic toxicity and the effects on reproduction, lactation, mutagenicity, and teratogenicity. Rodent studies using different algae concentrations resulted in no significant variation from controls.[1,2] *Spirulina* contains high levels of vitamin B12 and may also be used as a source of vitamin A.[3] However, the bioavailability to humans of vitamin B12 from some species of blue-green algae is questionable.[4] Blue-green algae have been a target for screening programs trying to identify novel substances of potential medical value. As a result, antiviral,[5] anti-inflammatory and antioxidant,[6] immunomodulating,[7] and enzyme-inhibiting (acetylcholinesterase, cAMP phosphodiesterase) activities[8] have been identified.

In the early 1980s, a new blue-green algae species *Aphanizomenon flos-aquae (AFA)* appeared on the market. This alga is rich in protein (63-69% dry weight), carotene, vitamin B12, and other biologically-active compounds.[9] AFA contains a high concentration of α-linolenic acid (18:3n3), which at a concentration of 10-15% in the rat diet represents an excellent source of n-3 polyunsaturated fatty acids.[10] Supplementation with AFA resulted in an elevation of n-3 fatty acids in rat plasma, and a decrease in cholesterol and triglyceride levels.[10,11] Toxicological studies on mice did not show any detrimental effects on animals fed diets supplemented with 65-500 mg AFA/day for 43 days.[12] Although many individuals consume this algae, data about its physiological effect, its nutritional value, and its safety are minimal. This study tests the efficiency of low doses of AFA in animal nutrition and evaluates its role in nutrient assimilation.

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MATERIALS AND METHODS

Animals and diets

Adult male Sprague-Dawley rats weighing 150-170 g were purchased from Charles River Laboratories (Wilmington, MA). Rats were divided randomly into five

groups of eight and placed into individual wire cages. Animals were maintained at 22°C with a light-dark cycle of 12 hours. Feed and water were supplied ad libitum. For four days the rats were fed the standard Purina chow #5012 (Purina Test Diets, Richmond, IN) for the purpose of adaptation to the new feed. The control group continued to receive the standard diet, but the four experimental groups were fed with the standard diet supplemented with 0.05%, 0.1%, 0.2%, and 0.4% AFA respectively. An algae concentration of 0.05% in a rat's diet corresponds to a human daily intake of 2.6 g, which is in the range of recommended doses for human consumption, 1.5-3 g (Table 1).

Experimental diets were stored at 4°C. Feed consumption was measured every third day and residual feed from the previous 3-day period was discarded. Growth was monitored weekly during the 28 days of the experiment. The protocol for this study was reviewed and approved by the institutional animal care and use committee.

Sample collections

At the end of the experiment, animals fasted overnight and were euthanized by carbon dioxide inhalation. Blood was collected by heart puncture and centrifuged for 15 min at 3,000 rpm to obtain serum. Viscera (liver, kidney, small and large intestine, cecum, and spleen) was extirpated, blotted, weighed, frozen in liquid nitrogen, and kept at -80°C for further biochemical testing. Before freezing, the small and large intestine were washed with ice-cold phosphate-saline buffer (PBS). The cecum was weighed with its content.

Liver and intestinal mucosa scraped from the middle part of the small intestine were homogenized (10 cycles) in cold deionized water (1:10) using a Potter-Elvehjem type homogenizer. Homogenates were centrifuged at 4°C for 10 min at 1,000 g to eliminate nuclei and cell debris. The thawed pancreas was homogenized in Polytron (Brinkman Instruments, Wesbury, NY) for 20 sec at a maximum speed in ice-cold PBS and centrifuged at 1000 g for 15 min at 4°C. Supernatants of liver, pancreas, and intestinal mucosa were used to study enzyme activities and protein level.

Biochemical analysis

Pancreatic a-amylase (EC 3.2.1.1) was tested with the Pierre et al.[13] method using Cobas Bio (Roshe Analytical Instruments, Inc). The trypsin (EC 3.4.21.4) activity was measured using the synthetic substrate p-tosyl-L-arginine methyl ester hydrochloride after activation with enteropeptidase.[14] Pancreatic enzyme activities were expressed in U/mg of protein. Sucrase (EC 3.2.1.48-10) and maltase (EC 3.2.1.20) activities were tested by the method of Dahlquist,[15] and aminopeptidase N (EC 3.4.11.2) by that of Fujita et al.[16] Intestinal enzyme activities were expressed in µmol of hydrolyzed substrate/min/g protein. The protein amount in intestinal mucosa was measured by the Lowry[17] method, while protein in liver and pancreas was measured by the Bradford[18] method. Serum protein[17] and glucose (Sigma Diagnostic Kit) levels were also measured.

Table 1. Relationship of algae amount in rat and human diets

Algae amount in rat's diet	Algae consumption by rat/day*	Equivalent algae consumption by 150 lb person
0.05%	7.5 mg	2.62 g
0.10%	15.0 mg	5.24 g
0.20%	30.0 mg	10.48 g
0.40%	60.0 mg	20.96 g

*Calculations were made for the average 200 g rat consuming 15 g/day of Purina chow

Statistics

Data are represented as mean±SE. Differences between control and experimental groups were established using unpaired Student's t-test. Differences of $p<0.05$ were considered significant.

RESULTS

Growth and feed consumption

The initial average weight of rats in all groups was similar and progressively increased during the experiment. A statistically significant difference in the weight of animals in different groups was not found at any time point; however, total weight gain was significantly greater (16%, $p<0.05$) in the group receiving 0.05% algae supplementation than in controls (Table 2). Total amount of food consumed during the experiment was higher only in rats fed 0.05% algae supplement ($p<0.05$; Table 3). However, the

Table 2. Effect of algae AFA on rats weight and weight gain (g)

Group	Initial weight	Final weight	Weight gain
Control	211.12±2.55	405.14±13.56	192.57±12.36
0.05% AFA	209.25±4.27	431.88±10.52	*222.62± 9.48
0.10% AFA	211.50±1.96	412.12±11.72	200.69±11.23
0.20% AFA	204.00±4.78	407.62± 9.47	203.62±11.26
0.40% AFA	208.00±2.38	422.88± 6.67	218.88± 5.17

Values are mean±SE. n=7 in control group. In experimental groups n=8. *$p<0.05$ versus control.

feed efficiency ratio, which characterizes consumption per unit of weight gain, did not show any significant difference between animal groups (Table 3). Therefore, weight gain on the diet containing 0.05% algae supplement seems to be related to the amount of food consumed.

Dietary supplementation with algae also affected the weight of viscera. We found (Table 4) a statistically significant ($p<0.05$) increase in the relative weight of the liver in rats receiving 0.05% of algae and in the weight of the pancreas in rats receiving 0.1% ($p<0.025$), 0.2% ($p<0.025$), and 0.4% ($p<0.05$) of algae. However, the relative weight of small intestine in rats fed 0.2% of algae, large intestine in rats fed 0.4% of algae, and spleen in rats fed 0.05% and 0.4% of algae was significantly lower than in controls (p correspondingly < 0.025; 0.005; 0.025; 0.025).

Biochemical studies

To test the effect of algae on nutrient absorption, we studied the activity of intestinal enzymes responsible for carbohydrate and protein digestion. The addition of 0.2% algae to the diet increased more than two times ($p<0.05$) pancreatic α-amylase activity (Table 5); however, algae supplementation did not affect intestinal disaccharidase (sucrase and maltase) specific activity. Trypsin activity in experimental animals was not statistically significant when compared to controls. However, the activity of aminopeptidase N, responsible for intermediate and final stages of protein hydrolysis, was lower ($p<0.025$) in rats receiving diets with 0.05 and 0.1% of algae than in controls. Higher doses of algae (0.2% and 0.4%) did not affect aminopeptidase N activity.

The amount of protein in different animal tissues is represented in Table 6. The amount of protein in small intestinal mucosa of rats receiving diets supplemented with 0.05% and 0.4% of algae was significantly higher ($p<0.025$) than in controls. Similar results were obtained by testing the amount of protein in liver. Tissue protein levels for the most part were higher; however, a statistically significant difference ($p<0.05$) with controls was observed only in rats receiving supplementation of 0.1% of algae. The amount of protein in the pancreas in all animal groups was similar, except for the group fed 0.1% algae supplement, in which pancreatic protein was lower ($p<0.025$) than in the controls. The levels of protein and glucose in the blood of controls and experimental rats were similar (Table 7).

Table 3. Effect of algae AFA on total feed consumption (g) and feed efficiency ratio

Group	Feed consumption	Feed efficiency ratio
Control	795.86±28.33	4.20±0.20
0.05% AFA	*867.25±23.17	3.91±0.08
0.10% AFA	817.88±27.61	4.02±0.21
0.20% AFA	808.38±21.70	4.01±0.12
0.40% AFA	834.88±14.40	3.89±0.06

Values are mean±SE. n=7 in control group. In experimental groups n=8. *p<0.05 versus control.

DISCUSSION

Experimental data on rats fed diets supplemented with low doses of AFA comparable to that ingested by people demonstrated an increase in weight gain and feed consumption in comparison with controls. The most beneficial effect on animal growth and food consumption was observed at the level of 0.05% diet supplementation with algae. Food efficiency ratio, in contrast to weight gain and food consumption, was similar in all animal groups. Dietary supplementation with algae also increased the relative weight of the liver and pancreas and decreased the relative weight of the small

Table 4. Effect of AFA on the relative weight of viscera (g/100 g body weight)

Group	Liver	Kidney	Pancreas	SI	LI	Cecum	Spleen
Control	3.00 ±0.13	0.79 ±0.03	0.06 ±0.01	2.05 ±0.04	0.40 ±0.02	1.32 ±0.10	0.19 ±0.01
0.05% AFA	a3.31 ±0.08	0.77 ±0.03	0.09 ±0.02	1.95 ±0.04	0.38 ±0.02	1.35 ±0.07	^{b}0.16 ±0.01
0.10% AFA	3.08 ±0.08	0.71 ±0.03	b0.09 ±0.01	2.13 ±0.08	0.38 ±0.01	1.43 ±0.09	0.18 ±0.01
0.20% AFA	2.98 ±0.08	0.79 ±0.03	b0.09 ±0.01	^{b}1.92 ±0.04	0.34 ±0.02	1.36 ±0.05	0.16 ±0.02
0.40% AFA	3.05 ±0.07	^{b}0.71 ±0.02	a0.08 ±0.01	2.13 ±0.03	c0.31 ±0.01	1.33 ±0.04	^{b}0.15 ±0.01

SI - small intestine, LI - large intestine. Values are mean ±SE. n=7 in control group. In experimental groups n=8. ap<0.05, bp<0.025, cp<0.005 versus control.

Table 5. Effect of algae on rat pancreatic and intestinal enzyme activities

Enzymes	Diets				
	Control	0.05% AFA	0.1% AFA	0.2% AFA	0.4% AFA
α-Amylase	457.48 ±70.57	518.99 ±80.16	633.51 ±91.02	a1013.95 ±236.06	500.00 ±87.00
Trypsin	110.76 ±17.19	135.05 ±12.40	201.72 ±11.68	167.82 ±31.00	154.52 ±19.00
Sucrase	204.41 ±30.72	191.09 ±47.28	145.09 ±17.36	177.04 ±16.58	153.20 ±33.87
Maltase	839.39 ±104.66	976.59 ±221.38	787.58 ±80.41	965.63 ±122.17	708.58 ±150.33
ApN	188.17 ±33.84	b92.33 ±25.24	b93.04 ±18.25	182.79 ±24.47	142.46 ±22.64

Values are mean ±SE. n=7 in control group. In experimental groups n=8. ApN aminopeptidase N. Amylase activity in U/mg protein x 103. Trypsin activity in U/mg protein x 105. Intestinal enzyme activities are expressed in μmol of hydrolyzed substrate/min/g protein. ap<0.05, bp<0.025 versus control.

Table 6. Effect of algae AFA on viscera protein level (mg/g wet tissue)

Groups	Intestinal mucosa	Liver	Pancreas
Control	32.45±8.83	171.08±14.44	29.48±5.40
0.05% AFA	b64.11±11.18	203.97±11.95	25.06±5.44
0.10% AFA	48.96±5.56	a207.36±10.90	b18.38±1.41
0.20% AFA	49.28±5.11	193.75±8.94	24.61±3.33
0.40% AFA	b55.02±5.74	186.78±8.15	28.38±4.43

Values are mean ±SE. n=7 in control group. In experimental groups n=8. ap<0.05, bp<0.025 versus control.

Table 7. Effect of algae on protein and glucose levels in rat blood serum

Index	Diets				
	Control	0.05% AFA	0.1% AFA	0.2% AFA	0.4% AFA
Protein	9.33 ±0.62	9.45 ±0.23	10.03 ±0.57	9.60 ±0.30	9.78 ±0.57
Glucose	91.72 ±7.30	119.48 ±22.68	95.95 ±10.77	95.78 ±6.38	96.84 ±6.01

Values are mean±SE. n=7 in control group. In experimental groups n=8. Glucose concentration in mg/100 ml; protein concentration in mg/ml.

intestine, large intestine, and spleen in some animal groups. However, in spite of liver weight changes, the liver weight/body weight index was similar in all animal groups (0.030±0.001 - 0.033±0.001).

Protein content in some viscera demonstrated an increase in intestinal mucosa (diets with 0.05% and 0.4% algae), liver, and pancreas (diet with 0.1% algae). The highest increase (double) was found in intestinal mucosa in rats fed the diet with 0.05% algae supplement. One can speculate that the increased protein concentration in intestinal mucosa may be related to increased protein biosynthesis of different enzymes and transporters participating in nutrient digestion, absorption, and metabolism. This observation correlates with a statistically significant increase in weight gain and food consumption in rats fed a diet supplemented with 0.05% algae.

Analysis of digestive enzymes demonstrated an increase in α-amylase activity in the pancreas of experimental rats fed a diet with 0.2% algae. Intestinal dissacharidases did not change activity in the presence of algae. However, intestinal aminopeptidase N was decreased in animals fed diets with 0.05% and 0.1% algae supplement. These data do not show any consistent effects of algae on enzymes responsible for the initial (α-amylase, trypsin) and final stages (sucrase, maltase, aminopeptidase N) of carbohydrate and protein digestion.

Thus, our observations suggest a beneficial stimulatory effect of low concentrations of AFA (0.05-0.4%) on rat growth. The effect of such low blue-green algae doses on growth is not known. Higher doses (10-30%) of another blue-green algae, *Spirulina*, used as a protein source in rat diets did not demonstrate any significant difference between control and experimental groups.[1] Even higher doses of *Spirulina* (up to 73%) did not show any significant difference between experimental and control animals except for an increase in relative weight of some viscera.[19] AFA used in higher concentrations (10% and 15%) did not demonstrate any adverse effect on rat or mice survival and activity.[11,12] Because AFA appears to be well tolerated and is a good source of polyunsaturated fatty acids,[10,11] its use may increase the nutritional value of the diet.

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