

THE SUPPLEMENTARY EFFECT OF ALGAE ON THE NUTRITIVE VALUE OF SOYBEAN MILK

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Vegetable foods are the chief dietary staples in Taiwan as well as in the other parts of Asia. They compose a large proportion of the Chinese diet, especially in the rural areas. The nutritive value of vegetable foods is generally lower than that of animals on account of its deficiency in some essential amino acids or some other factors.

During the past several years, it has been found that algae contains much nutrients not only rich in essential amino acid⁽¹⁾ but also a growth stimulating factor⁽²⁻⁸⁾. Earlier workers studied the nutritional adequacy of this material and considered it to be a potential food source for man⁽⁴⁻¹¹⁾. Even though interest in algae is wide-spread, feeding of algae to human as a dietary item has been a virtually unexplored field. Many questions remain to be answered.

The purpose of this investigation was to observe the effect of supplementary algae to soybean milk—which has been used as a popular human food in the place of cow's milk in China—on growth, food utilization and nitrogen balance of growing young rats. In addition, the possible change of body length

and hemoglobin content under the influence of algae supplementation was noted.

EXPERIMENTAL

Soybean milk powder, dried chlorella and C. G. F. (chlorella growth factor) were tested. All of them were supplied by Taiwan Chlorella Manufacture Co., Ltd. Their proximate analysis is shown in Table 1. Soybean milk powder was a kind of water extract of soybean dried into powder form by spray method. Chlorella was *Scenedesmus Obliquus*, and C. G. F. solution was a concentrated chlorella extract which was dissolved in 80% cane sugar sirup. 1.3 ml of C. G. F. solution was equivalent to 1 gm of chlorella.

Corn starch and cane sugar were also used as basal components of diets in the 2nd and the 3rd experiments. The composition of the basal rations and of the different test rations are given in Tables 2, 3 and 4.

Selected weanling rats of the Long Evans Strain were used as the experimental animals. In the 1st experiment, 32 female rats were divided into 4 groups and fed for 30 days. In the 2nd and the 3rd experiments, each 36

Table 1. The proximate analysis of chlorella, soybean milk powder and C. G. F.

	Chlorella % dry weight	Soybean milk % dry weight	C. G. F. in 80% sugar sirup
Moisture	5.87	7.52	16.16
Crude protein (N×6.25)	55.88	41.69	1.97
Crude fat	9.98	19.93	—
Carbohydrate	21.61	26.81	80.00
Ash	6.65	4.05	1.87

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male rats were divided into 6 groups. The animals were fed for 15 days in the 2nd experiment and 30 days in the 3rd experiment.

All animals were housed two together in a cage equipped with a bottle of distilled water. Test diet and water were given *ad libitum* and the body weight of animals was weighed twice a week. The constant amount of diet was charged every day, and food intake was measured twice a week.

Apparent nitrogen digestibility and biological value were determined according to the method of Mitchell⁽¹²⁾ principally. Two weeks after the start of taking test diets, the rats were housed in metabolic cages. The urine and feces were collected for 5 days in the third week. Nitrogen content of urine and feces were analyzed by the Kjeldahl method. After the animals were fed 30 days, hemoglobin content was determined as acid hematin by using 0.02 ml of blood collected from the tail. Additional experiment was the study of body length which was measured when the animal was laid on a table.

RESULT

The first experiment: Animals were fed with high protein diets of about 40% protein levels. Composition of the diet for each group

was shown in Table 2. There were some significant phenomena. The growth curves of the animals were shown in Fig. 1. The rate of growth increased as the content of algae increased. Six days after the rats started to take algae supplemented diets, the growth already increased significantly ($p < 0.05$). Both body weight gain and food intake increased as the algae was added (Table 2), such as body weight gains were 23, 51, 64 and 67 gm at 0, 2, 4 and 8% of algae levels respectively. Food intake was 150 gm at 0% algae and 244 gm at 8% of algae level. Feed efficiency (F.E.) was also improved with the addition of algae (Table 2).

Hemoglobin content and body length also increased significantly ($p < 0.01$) with algae addition as shown in Fig. 2. Both of them appeared to approach the maximum at 4% algae level.

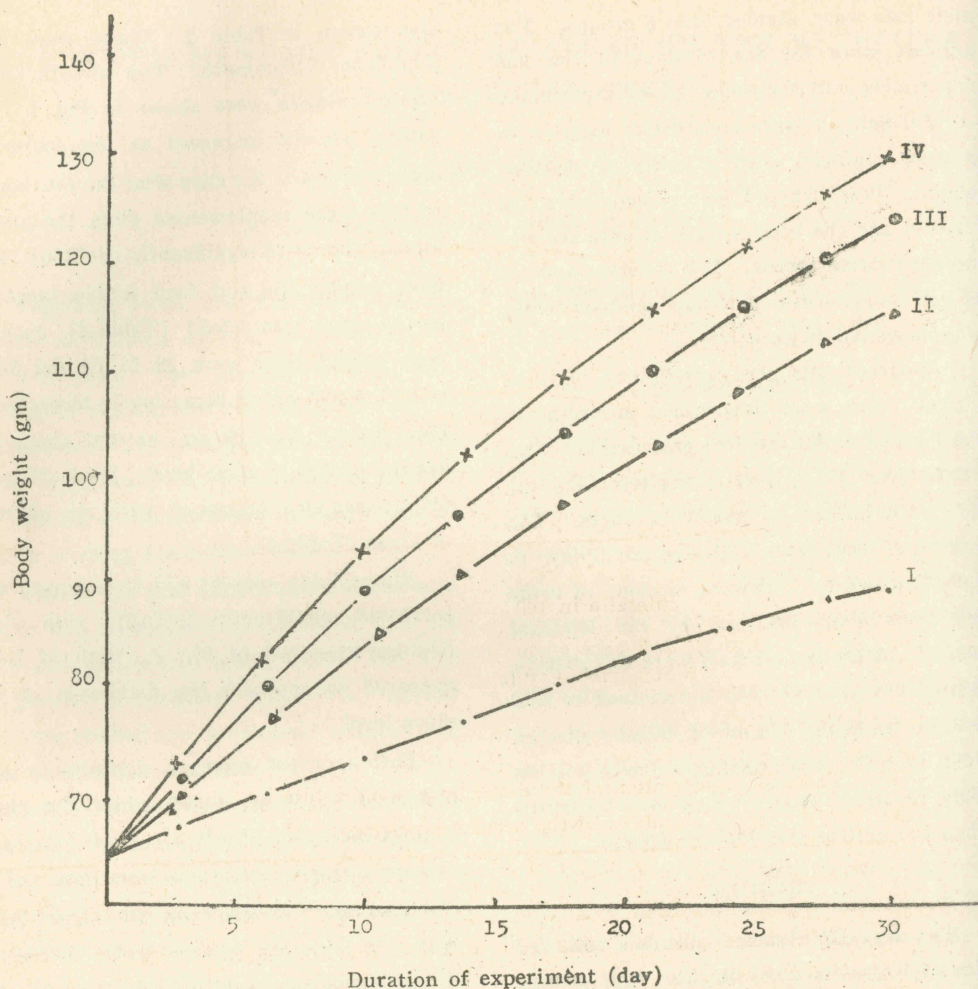
Both apparent nitrogen digestibility and biological value decreased while the algae content increased (Table 3). High fecal and urinary nitrogen excretions were observed as characteristic phenomenon of algae feed. Although nitrogen intakes were increased, the nitrogen retained in body was not increased in proportion with intake. The

Table 2. Composition of the diets, body weight gain and dietary intake of the first experiment

Groups		1	2	3	4
Ingredient of diet (%)	Soybean milk	100	98	96	92
	Chlorella	0	2	4	8
Components of diet (%)	Crude protein	41.69	41.97	42.26	42.83
	Crude fat	19.93	19.73	19.53	19.13
	Carbohydrate	26.81	26.71	26.60	26.39
Body weight gain in 30 days (gm)		23±4	51±7	64±8	67±6
Food intake in 30 days (gm)		150±3	253±21	238±22	244±13
F.E.		6.6±1.5	4.4±0.5	3.7±0.7	3.6±0.5

The data were the average of 8 female rats for each group

F.E.=Feed efficiency



I. ·—·: control group fed without chlorella in soybean milk powder
 II. △—△: test group fed with 2% chlorella in soybean milk powder
 III. ○—○: test group fed with 4% chlorella in soybean milk powder
 IV. ×—×: test group fed with 8% chlorella in soybean milk powder

Fig. 1. Animal growth response to algae supplement to soybean milk powder

apparent digestibility were 83, 76, 75 and 73%, while biological values were 63, 64, 58 and 54% for 0, 2, 4 and 8% algae levels respectively.

Second experiment: Corn starch was added to reduce the protein level of diets to 20% and at the same time, C. G. F. effect was also tested. The test diets for the second experiment was described in Table 3. The results obtained were similar to those of first experiment. Such as body weight gain was increased, F. E. or P. E. R. were improved

when algae were added in the diets. Even at the 8% level, the body weight gain, and P. E. R. were lower than those of control animals fed with complete diet. It seems that chlorella does not contain enough vitamins and minerals for the normal growth. Group 6 was tested for the C. G. F. effect. The amount of C. G. F. used in this experiment was equivalent to 2% chlorella in the diet. It was observed that chlorella could promote the growth (Table 3,

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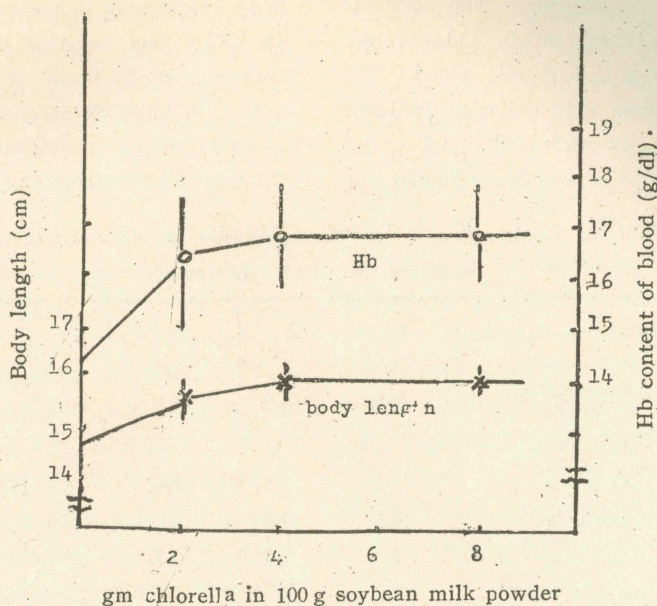


Fig. 2. Influence of chlorella on body length and hemoglobin content of blood

Table 3. Composition of the diets, body weight gain and dietary intake of the second experiment

Groups		1	2	3	4	5	6
Ingredients of diet (%)	Soybean milk	51.3	51.3	51.3	51.3	51.3	51.3
	Chlorella	—	—	2	4	8	—
	C. G. F. in 80% sugar sirup	—	—	—	—	—	2.6
	Corn starch	38.7	33.7	36.7	34.7	30.7	38.7
	80% sugar sirup	10	10	10	10	10	7.4
	Vit. mixture	—	1	—	—	—	—
	Salt mixture	—	4	—	—	—	—
Body weight (gm)	Beginning	71±2	70±3	70±3	70±3	70±3	69±3
	Final	103±4	121±6	113±6	115±6	117±8	109±6
	Gain	32±2	51±3	43±3	45±3	47±5	40±3
	% of increase		59	33	40	49	27
Food intake (gm)		140±2	156±7	157±6	159±8	163±4	159±5
F. E.		4.3±0.9	3.0±0.3	3.6±0.2	3.5±0.3	3.4±0.5	4.0±0.6
P. E. R.		1.2±0.1	1.7±0.2	1.4±0.1	1.4±0.1	1.5±0.2	1.3±0.3

Each diet contain 1 20% crude protein, 10% crude fat and about 60% carbohydrate.

The data were the average value for each male rat in 15 days.

C. G. F.=chlorella growth factor

In the third experiment: The complete diet was used as basal ration, and different amount of algae or C. G. F. was added. The composition of diets were described in Table 4. As to C. G. F. an equivalent of 1% or 4% chlorella was added to the completed diet, the

body weight gain increased up to 14% (Table 4). This fact suggests that some growth factors exist in algae, though the effect of C. G. F. or chlorella on complete diet was not so great as on incomplete diet (Table 3). Another phenomenon was that the further

Table 4. Composition of the diets, body weight gain and dietary intake of the third experiment

Groups		1	2	3	4	5	6
Ingredients of diet (%)	Soybean milk powder	51.3	51.3	51.3	51.3	51.2	51.3
	Chlorella	—	—	—	—	3	4
	C. G. F.	—	1.3	2.6	5.2	—	—
	Corn starch	38.5	36.5	39.5	30.5	36.5	34.5
	80% sugar sirup	5.2	3.9	2.6	—	5.2	5.2
	Vit. mixture	1	1	1	1	1	1
	Salt mixture	4	4	4	4	4	4
Body weight (g)	Beginning	63	62	63	64	62	64
	Final	148	159	157	154	153	162
	Gain	85±9	97±4	94±7	88±4	91±8	98±7
	% of increase		14	10	4	7	15
Food intake		350±42	360±44	342±61	330±43	333±30	365±21
F. E.		4.1±0.4	3.6±0.7	3.6±0.7	3.7±0.5	3.7±0.8	3.7±0.5
P. E. R.		1.2±0.1	1.4±0.1	1.4±0.1	1.4±0.2	1.4±0.2	1.4±0.1

Each diet contained 20% crude protein, 10% crude fat and about 60% carbohydrate. The data were the average of each male rat in 30 days.

Table 5. Apparent digestibility and biological value of the first and third experiment

Groups		mg N. intake/ rat/day	mg N. in fecal	mg N. absorbed	Digesti- bility %	mg N. in urine	mg N. retained in body	Biologi- cal value
1st Exp.	Control 1	390±19	66±13	324±22	83±8	120±14	204±18	63±4
	2% 2	548±39	129±26	419±62	76±8	152±27	267±50	64±5
	Algae 4% 3	568±22	136±40	431±60	75±11	175±12	255±49	58±8
	8% 4	592±28	159±36	432±44	73±8	200±21	232±30	54±3
3rd Exp.	Control 1	416±47	70±7	346±48	83±8	102±26	244±19	71±3
	2	428±42	75±13	353±50	83±5	101±10	252±41	71±1
	C. G. F. 3	412±65	78±11	334±54	81±6	110±16	224±33	67±6
		400±24	78±10	328±27	82±4	103±15	225±18	69±4
	Chlorella 5	395±18	90±10	305±26	77±2	108±7	197±24	65±3
		420±22	100±12	320±17	76±6	120±7	200±9	63±2

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improvement of animal growth did not occur when the amount of C. G. F. increased in the diet. It seemed that a little amount of C. G. F. was enough to stimulate the animal growth.

DISCUSSION

The result of this experiment indicated that the supplement of chlorella to soybean milk would cause significant increase of body weight gain and improve F.E. either at complete or incomplete condition. These facts showed that algae could improve the nutritional value of soybean milk.

Furthermore the supplementary effect on the unbalanced or incomplete diet was greater than that on the complete one (see Tables 2, 3, and 4). These results suggested not only growth stimulating factor existed in chlorella, but also that it supplied some nutrients, such as minerals and vitamins to the unbalanced or incomplete diet. Takechi discovered a growth stimulating factor in chlorella⁽³⁾, named it as C. G. F. (chlorella growth factor) and extracted it with boiling water. It is considered that C. G. F. may be some nucleic acid substances, but many questions still remain in this field.

Nevertheless, the supplement of algae could improve the nutritional value of soybean milk. The nitrogen utilization of the diet decreased as algae was added. It was found that a large amount of nitrogen was excreted when animal was fed with algae diet. This result agreed with a previous report which pointed out high fecal excretion of nitrogen being characteristic of all algae foods⁽⁸⁾. The reason for high fecal nitrogen excretion was discussed as follows:

- 1). Resistance of the algae cell wall to the normal digestive process.
- 2). Resistance of nitrogen from the cell in a form which can not be digested or absorbed from the digestive tract⁽⁸⁾.

Other workers reported the disturbance of digestion when fed with algae diet⁽⁷⁾ and the

occurrence of gastro-intestinal symptoms. When 100 gm or more algae were fed⁽¹¹⁾, the algae toxicity evidenced by the gastro-intestinal distress may be due to bacterial contamination or the presence of anti-bacterial substances present in those algae species⁽¹³⁻¹⁴⁾. The urinary nitrogen also increased as the chlorella content increased. It may be thought that some nitrogen substances of chlorella which had been absorbed but found useless for body was excreted in urine. Chlorella is rich in amino acid, however it contains only a small quantity of s-amino acid. It is easy to cause the unbalanced amino acid pattern in animal body and increase nitrogen excretion.

SUMMARY

The effect of supplementary relation between chlorella and soybean milk powder was investigated in regard to growth and nitrogen utilization of growing rats. Various amounts of chlorella and its extract (C. G. F.) were added into the diet.

The supplement of chlorella to soybean milk resulted in increased body weight gain and improved F.E., especially with the incomplete diet, the effect was very significant.

Both body length and hemoglobin content of blood also increased and appeared to approach the maximum at 4% chlorella level.

The nitrogen utilization of diet decreased when algae was added. High fecal and urinary nitrogen excretions were observed in the rats that took the algae diet. Those were the major factors in the reduction of the apparent digestibility and biological value.

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綠藻對豆漿粉之補助效果

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植物性食品為亞洲各國之主要食物。其營養價值低於動物性食品。因為植物性食品往往會缺乏某些必須氨基酸或其他因素。在過去幾年間發現了一種綠藻不但含有豐富的氨基酸礦物質，而且含有一種促進生長的因素 (CGF)，因此許多人士在研究綠藻要它當為人類的食物之一。綠藻雖已普遍的被認識了，但要當為食物時尚有許多問題還未解。

本實驗是用臺灣綠藻公司所供給的綠藻，綠藻精液 (CGF) 及豆漿粉配成各種飼料，來飼養幼鼠，

觀察幼鼠的生長情形，飼料效率 (FE) 及蛋白質的利用等。

實驗結果證實了，添加綠藻及綠藻精於豆漿粉中能使幼鼠體重增加得多，促進發育及改善 FE，又能增加其身長及血紅素量。可知綠藻能提高豆漿的營養價值，但只吃添加綠藻的動物，其發育還是比吃添加維生素及礦物質的對照動物差一些。此現象在暗示綠藻不能充分的供給動物正常發育所需要的維生素及礦物質。