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Food Safety on Utilization of Solar-dried Thai *Spirulina*

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ABSTRACT

Solar-dried Thai Spirulina strain TH-S-02 was analysed for possible mycotoxin, pesticides, heavy metals including microbial contaminations. The safety on utilization of algae was evaluated by animal trials in rats for 12 wks with feeding diets containing 0, 10, 20 and 30% algae. No significant differences in hematology, blood chemistry and urine sediments in animals of the same sex were observed. However, cholesterol levels in the control females were significantly lower than the algal diet groups.

At termination, gross autopsy findings and histopathological studies on important organs showed no evidence of abnormalities. Besides, the highest total intake of nucleic acid obtained from this investigation was still below 2.6 g as recommended by PAG guidelines for an acceptable daily intake of nucleic acid from unconventional source.

Key words : safety, animal trials, *Spirulina*, contamination

INTRODUCTION

The use of blue-green algae *Spirulina* as human food has been known for centuries being a part of the diet by natives of Lake Chad in Africa and Aztecs in Mexico. The last three decades have drawn the interest of scientists in USA, Japan, France, Czechoslovakia and Germany, etc. in developing a system to exploit the algae productivity for being used as health food (Clement *et al*., 1967). *Spirulina* has certain basic advantages over other well studied algae like *Chlorella* or *Scenedesmus* as its amenability to a low level of technology. A high protein content and rich sources of many vitamins and minerals are the other important attributes of *Spirulina* as well as its excellent digestibility.

During the past decades, a very detailed study about toxicological evaluations of *Spirulina* extending over several years was published by the United National Industrial Development Organization. The tests performed included subacute and chronic toxicity, reproduction and lactation, mutagenicity and teratogenicity using Wistar rats of both sexes. It was concluded that none of the parameters tested showed any acute variations from those of the controls (Venkataraman *et al*., 1980; Chung *et al*., 1978).

The National Inland Fisheries Institute with the support of FAO and USAID in cooperation with Israel during 1984-1989 was recently successful in selection and isolation 7 strains of

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Thai *Spirulina*. Investigation on nutritive values of these *Spirulina* spp. were already performed earlier by the authors and the strain TH-S-02 was discovered to possess the highest nutritive value with moderately low content of nucleic acid and was selected to be used in this safety test.

A major limitation in the use of single cell protein (SCP) as food source is the high nucleic acid content, which is approximately 4-6% for algae. As constituents of nucleic acid, purine compounds in human diet mostly metabolized to yield uric acid whose high concentration may lead to gout or renal stones (Zollner *et al*., 1972). Therefore, data concerning the highest total intake of nucleic acid from this local *Spirulina* spp. by short- term feeding trials in rats will be necessary as part of the detailed tests stipulated by the Protein Advisory Group (PAG, 1972) to provide basic information regarding the safe use as natural food or incorporation into conventional dishes.

MATERIALS AND METHODS

Spirulina sample

Thai *Spirulina* strain TH-S-02 was cultivated by National Inland Fisheries Institute, Department of Fisheries, Ministry of Agriculture and Cooperatives, Thailand. Approximately 40 Kgs. algae were used in this study.

Analysis of toxic substances and microbial contaminations

Mycotoxin, pesticides, heavy metals as well as microbial contaminated were analysed by Department of Medical Science, Ministry of Public Health, Thailand.

Biological test on experimental animals

40 weanling rats of Sprague-Dawley strain of each sex were divided into 4 groups. The algae was incorporated in the diets at 0, 10, 20 and 30%. Animals were kept individually in stainless steel cages with food and water given ed libitum. Temperature was maintained at 22-25ºC, light/ dark cycle of 12/12 hrs. Daily food intake and weekly body weight changes were recorded. Physical appearance and behavior were observed daily during 12 wks studies.

Clinical and histopathological investigations

At termination, urine collection for urinalysis, determination of allantoin by method of Borchers (1977) as well as observation on crystals of urinary sediments were performed. Animals were anesthetized by ether inhalation and blood collected by heart puncture. Hematological study with Sysmex F-800, blood chemistry and allantoin determination were determined using Hitachi photometer model 4020, Boehringer Mannheim GmbH, Germany. Important organs such as lung, heart, brain, liver, kidney, spleen, adrenal gland and gonads, etc. were examined and weighed and histopathological of each organ was studied. Significant difference between control and treatment were performed using Duncan's Multiple Range Test.

RESULTS AND DISCUSSION

Composition of the experimental diets are shown in Table 1. Standard diet was incorporated with *Spirulina* at dietary levels of 0, 10, 20 and 30%. All experimental diets prepared consisting of 23-24% protein, 4-5% fat, 3-4% fiber and 6-8% ash. Table 2 shows data on analysis of toxic substances and microbial contaminations. Among the different metals found in algae, lead, cadmium, mercury and arsenic were still at low level. However, high residue of aluminium in algae resulting from aluminium containing materials used in the solar-drying process was not found to be harmful. No biogenic toxins were presented in the samples

investigated including aflatoxin. Besides, microbial contamination was within the safety ranges since solar-drying might provide sufficiently high temperature exposure to destroy the pathogens.

Data on food consumption and body weight changed are shown in Table 3. The consumption of algal diets were a little lower than those of the controls, the body weight gained of the rats followed a comparable trend to that of diet consumed. Furthermore, none of the tested animals showed any sign of discomfort.

Table 4 shows data on blood chemistry studies. There were no significant differences in serum uric acid contents and cholesterol levels among the tested groups except with the control, males and females, respectively. However, data collected in Germany and Thailand in clinical studies from volunteers did not reveal any alarming increase of uric acid concentration due to the consumption of algae-containing diet (Scenedesmus). The obtained value was acceptable only for healthy adults but could not be applied for children since they were more sensitive to an increase in their uric acid metabolism (Feldheim, 1972). Effect of nitrite on mutagenicity of Thai *Spirulina* spp. was reported by Butryee *et al*., 1995, that nitrosated *Spirulina* spp. extracts contained not only the nitroso but also non-nitroso mutagenic compounds. Avoid consuming this algae with nitrite rich food especially in peptic ulcer patients were recommended. Hematological responses of rats fed with algal diets are shown in Table 5. No differences were found in the hemoglobin content or the number of the red cells among the diets. The lymphocyte were observed to be slightly high in all groups including the controls though in differential leukocyte count, the relative proportions of the various component cells were similar.

Data on allantoin determination in urine and observation of crystals in urine sediments are shown in Table 6. Detailed urine analysis did not reveal any noticeable abnormalities. Microscopic examination on urine sediment indicated some calcium oxalate and triple phosphate crystals in all

Table 1 Composition of experimental diets.

Ingredients	Experimental diets			
	Dietary level of *Spirulina* (%)			
Spirulina	0	10	20	30
Fish meal	20	10	5	2
Maize meal	24	24	24	24
Soybean extract	12	12	7	-
Wheat bran	15	15	15	15
Rice flour	20	20	20	20
Soybean oil, Sugar, Vitamin mixture, Mineral mixture	9	9	9	9
Total	100	100	100	100

tested groups including controls. Allantoin determination, a urinary indicator of purine catabolism in most mammals was also found in the normal ranges.

No major differences were detected in the terminal weight of lung, heart, brain, liver, spleen, kidney, adrenal gland or gonads. The body weight, however, remained a little higher in the controls of both sexes as summarized in Table 7. No histological changes in the vital organs could be observed. Assuming the highest daily intake of 30 g food with 9 g. algae in the highest of 30% algal diet group would obtain nucleic acid uptake of only 1.21 g/Kg BW. which was still within the given limit of maximum tolerable daily intake (MTD) of 4.33 g/Kg BW from the ADI of 2.6 g/head as recommended by PAG guidelines for acceptable daily intake of nucleic acid from the unconventional source. Hence, intake of 10 *Spirulina* tablets / day as recommended for health food uses would obtain approximately 1.2 g / head of nucleic acid in 20 g of pure *Spirulina* which is still within the safe use

Table 2 Analysis of toxic substances and microbial contamination in solar-dried *Spirulina* TH-S-02 and standard diets.

Toxic substances	Algal diet	Max. level in SCP (IUPAC)[1]	Standard diet
Heavy metals (mg/kg)			
Pb	Not found	5.0	0.58
Cu	3.78		6.16
Cd	Not found	1.0	0.32
As	0.02	2.0	
Hg	0.012	0.1	0.02
Al	183.17		112.50
Microbial contamination			
colony forming unit/g	12×10^3		10×10^3
molds (colonies/g)	190		110
MPN coliforms/g	9.1		<3
MPN *E. coli*/g	<3		-
Pathogenic bacteria			
S. aureus	Not found		Not found
C. perfringens/0.1g	Not found		Not found
Salmonellae / 25g	Not found		Not found
Mycotoxin			
Aflatoxin	Not found		Not found
Pesticide residue (mg/kg)			
Aldrin	Not found		<0.01

Analysed by Department of Medical Science, Ministry of Public Health, Thailand.

1/ International Union of Pure and Applied chemistry

Table 3 Mean values of food intake and body weight gain of rats fed with *Spirulina* at dietary level of 0–30 %.

Sex / Diet		At the end of wk											
(% Spirulina)		1	2	3	4	5	6	7	8	9	10	11	12
Food intake (g/rat)													
Males	0	113	165	208^{a}	234^{a}	218^{a}	225^{a}	199	204	212	213	231^{a}	193^{a}
	10	120	170	199^{ab}	215^{a}	206^{a}	211^{ab}	197	193	200	204	208^{b}	163^{b}
	20	117	165	189^{ab}	199^{b}	196^{a}	199^{b}	197	190	204	199	210^{b}	171^{b}
	30	115	164	187^{b}	189^{b}	182^{b}	199^{b}	194	195	195	199	199^{b}	162^{b}
Females	0	107	144	167	174^{a}	166^{a}	172^{a}	148	155	161	161	169	141^{a}
	10	112	147	161	163^{ab}	154^{ab}	161^{ab}	155	152	159	156	163	121^{b}
	20	110	149	167	158^{ab}	149^{ab}	147^{b}	149	148	160	157	163	125^{b}
	30	110	141	152	147^{b}	141^{b}	144^{b}	149	147	157	161	158	116^{b}
Weight gain (g/rat)													
Males	0	42^{b}	54	51^{a}	47	44	34	27	21^{a}	15	15	10	9^{a}
	10	46^{a}	52	48^{ab}	49	43	29	23	14^{b}	17	14	11	7^{ab}
	20	47^{a}	52	44^{b}	48	41	29	25	17^{ab}	15	13	10	11^{a}
	30	45^{ab}	52	42^{b}	39	40	32	23	17^{ab}	15	14	14	5^{b}
Females	0	37^{b}	37	29	20	21^{a}	15	13	6	9	6	5	2
	10	42^{a}	37	24	22	19^{a}	15	13	4	8	3	7	1
	20	40^{ab}	37	24	21	20^{a}	11	13	6	10	4	8	3
	30	40^{ab}	32	22	22	14^{b}	11	12	5	7	7	5	2

Figures on the same column with the same letters were not significant differences at 95 % level.

Table 4 Data on clinical blood chemistry in expeimental rats.

Sex / Diet		Choles-terol	Trigly-ceride	Glu cose	Bun	Uric acid	Phos-phorus	Total protein	Akaline phosphatase	SGOT	SGPT
(% *Spirulina*)		(mg/dl)	(mg/dl)	(mg/dl)	(mg/dl)	(mg/dl)	(mg/dl)	(g/dl)	(U/1)	(U/1)	(U/1)
Males	0	83.80	55.70	101.30^{a}	16.85	1.60^{a}	4.80	6.32^{a}	26.89	130.70	31.60
	10	82.22	51.67	96.78^{ab}	15.41	0.89^{b}	5.00	6.01^{b}	41.89	121.11	36.22
	20	93.70	48.10	92.70^{ab}	16.16	1.00^{b}	4.60	5.95^{b}	43.50	122.00	33.40
	30	84.30	56.20	84.00^{b}	15.80	1.00^{b}	4.90	6.15^{ab}	33.10	149.30	39.30
Females	0	82.70^{c}	42.00	100.10^{a}	17.47^{a}	1.10	3.90	6.24	18.30	123.00	29.20
	10	92.70^{b}	38.60	105.30^{a}	14.51^{ab}	1.20	4.10	6.23	22.80	145.40	29.70
	20	117.60^{a}	36.00	97.40^{ab}	15.49^{ab}	1.00	3.90	6.02	24.80	114.50	28.30
	30	113.00^{ab}	34.90	80.60^{b}	13.29^{b}	1.40	4.10	6.06	20.67	109.80	31.90

Table 5 Hematological response of rats fed with *Spirulina* diets for 12 wks.

Sex / Diet (% Spirulina)		WBC (10^3/µl)	RBC (10^6/µl)	HGB (g/dl)	HCT (%)	MCV (fl)	MCH (pg)	MCHC (g/dl)	PLT (10^3/µl)
Males	0	7.77	8.03	14.34	43.80	54.47	17.86	32.79	865
	10	7.22	8.24	14.52	44.98	54.57	20.72	32.44	969
	20	7.63	7.82	14.73	43.11	55.05	18.81	34.18	934
	30	6.94	7.83	14.39	42.38	53.87	18.32	33.99	872
Females	0	4.55	7.24	13.83	40.89	56.29	19.07	33.90	812
	10	4.69	7.23	13.26	41.18	56.93	18.45	32.47	800
	20	4.10	7.32	14.33	41.69	56.94	19.59	34.37	851
	30	4.29	7.08	13.85	40.50	57.13	19.55	34.20	849

Table 6 Allantoin determination in urine and crystals of urine sediment.

Sex/Diet (% Spirulina)		Allantoin (mg/dl)	Calcium Oxalate (observations,%)	Triple phosphate (observations,%)
Males	0	405	22	100
	10	367	33	33
	20	425	30	50
	30	410	40	90
Females	0	344^{ab}	67	89
	10	412^{a}	70	60
	20	350^{ab}	78	33
	30	308^{b}	10	70

Table 7 Final body weight and organ weight of rats fed with *Spirulina* diets for 12 wks (g.).

Sex / Diet (% Spirulina)		Body weight	Lung	Heart	Brain	Liver	Spleen	Kidney	Adrenal gland	Testis/ ovary
Males	0	420	1.85	1.45	2.12	16.03	0.85	1.78	0.04	1.93
	10	402	1.80	1.37	2.10	15.77	0.79	1.71	0.04	1.69
	20	405	1.77	1.38	2.09	15.52	0.77	1.70	0.04	1.84
	30	385	1.88	1.36	2.08	14.82	0.81	1.64	0.04	1.76
Females	0	254	1.38	0.94	1.96	8.76	0.62	1.11	0.04	0.14
	10	250	1.35	0.99	1.93	7.81	0.57	0.99	0.04	0.13
	20	248	1.33	1.04	1.94	8.69	0.56	1.04	0.04	0.15
	30	235	1.27	0.94	1.93	8.37	0.53	1.00	0.04	0.13

level of this algae.

CONCLUSION

Higher nucleic acid uptake is reported to increase the serum uric acid level leading to gout and stone formation in the kidney. However, nucleic acid is not a toxic component and it causes only physiological effects at higher levels like any other essential dietary ingredients, e.g. salt, Fe, vitamin A, fat, etc. taken in larger amounts. Besides, utilization of algae as a novel protein source to supplement human food will depend not only upon the cost of algae itself but the acceptance of the consumer are also a great factor. (Bhumiratana and Payer, 1973) The findings of this report were similar to the results of other studies with *Spirulina* performed so far which have proved its nutritional values and toxicological safety as human food in this regard.

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