

**FATTY ACIDS COMPOSITION AND NUTRITIONAL EFFECT  
IN RATS OF CUSHURO (*Nostoc sphaericum vaucher*)<sup>1</sup>**

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**Summary**

The green-blue algae cushuro (*Nostoc sphaericum vaucher*) grow spontaneously in lakes at high altitude and it is a food eaten for native dwellers from Peruvian sierra. Notwithstanding there is no studies whether its consumption is benefit or no. The objective of the study was to evaluate the effects of its ingestion, in rats eaten *at libitum*; and the composition of its fatty acid contents. Results: **a)** The chemical proximal analysis of cushuro was: 98 % water, 0.17 % protein, and the rest are fiber, chlorophyll and others. Chromatographic analysis of fatty acids is the following: Saturated: 14:0, 15:0, 16:0 y 18:0; monounsaturated: 16:1 (cis9), 18:1 (cis9) y 18:1 (cis11); polyunsaturated: 16:2 (cis9,cis12), 18:2 (cis9,cis12) y 18:3 (cis9,cis12,cis15). The plentiful fatty acids are 15:0, 16:0, 16:1 and 18:3. The fatty acids 15:0 and 18:1 (cis11) are uncommon in biology and not important in human diets. **b)** Weanling rats were feed with a normal standard diet (13.8 % protein and 38 % sugar) with addition 5 % of dry cushuro weight, during two months. They had a significantly weight loss ( $p = 0.000006$ ), a significantly excreta production ( $p = 0.000266$ ) and a moderate increase of water drinking ( $p = 0.032697$ ) than rats with a standard diet only. The ingestion of solid food was similar in two groups ( $p = 0.19999$ ). These data carry to conclude that the alga cushuro seem to contain antinutrients in the enough amount which may influence the weight loose in rats and influence negatively in the nutrient absorption of a normal diet.

**Keywords:** high altitude algae – uncommon fatty acids – antinutrients - weight loss

### Introduction

The cushuro (*Nostoc sphaericum vaucher*) is a native green-blue algae widely used in the high-altitude Peruvian sierra. It is widely distributed at different rural towns of Ancash, Amazonas, Cuzco, Junin, La Libertad, Lima, Puno and Tumbes (1). There are some technical works on proximal analysis and chemical composition with the following results: 1% of protein, 0.1 % fat and whether consumed it give between 250 to 300 Kcalories by 100 grams of dried matter (2, 3, 4). It is probably that method sensibility used in the chemical characterization did not detect some native xenobiotics which it is found at very low quantities (5).

A lot of substances partially characterized in the food have varied effects on the men and the animals which consume it in their diet (6). Some of them are: protease inhibitors (legumes and cereals) which prevent food absorption; cyanide release substances (almond and nut) which releases hydrogen cyanide; goitrogen compounds (cabbages and onions) which produce goiter; biogenic amines (banana, tomato and some beans) which excite the nervous system; diazo compounds (nuts) which shows carcinogenic effects; alkaloids (potatoes, beans, beet-roots, and spinach) with varied metabolic effects. All these substances were studied in experimental animals upon addition these compounds in their diets. The present work has as objective study their effects upon addition to the diets, in rats consuming *ad libitum*; also to characterize the chemistry of their fatty acids.

### Material and methods

It was used male and female Wistar rats as experimentation animals because the rat is widely studied as an animal nutritional, toxicological and carcinogenic point of view (7, 8). For the analysis, each group of rats (with algae en their diet) has a control group (without algae in their diet).

Weaning male rats of 25 days old were obtained from The Peruvian Institute of Nutrition from The Health Office. Each rat was maintained at his cage. They were feed with a standard diet (13.8 % protein) until day 30. Starting day 31 the rats were divided in two group: a) a group with standard diet containing alga cushuro (group A1); and b) a group with standard diet without alga cushuro (group A2). Both groups feed their diets "*ad libitum*" at each cage and they were maintained this way for two months to complete the study. The amount of food consumed, feces produced, water ingestion and body weight over a 24-hours period was registered. During the nights as environment temperature dropped, the animal room was maintained to 20 °C to avoid weight loss by cold exposure. Each rat was maintained at his respective cage where we collect their food, water and feces. See Table 1.

Table 1. Percentage of algae in their diets and initial weight before adding alga cushuro

Group	Standard diet (13.8 % protein + 38 % sugar)		p*
	A1	A2	
% dried cushuro added	5	0	
Initial weight (grams)	71.46	69.26	0.1692
Number of rats	14	14	

p\* The differences between with (A1) and without (A2) dried cushuro.

The algae cushuro was acquired from the local market (Huaraz) which arises from Aguascocha lagoon, located at 4,700 meter above sea level, in Catac, Ancash, Peru. The native food was washed with deionized water and dried at 50 °C into an oven until dried completely. This was keeping safe in a freezer to chemical composition and nutritional studies. The samples were pulverized and mixed with the standard pellets to rats obtained from The Purina Company. The crude water was previously boiled before the animals taken it. The amount of food taken, the produced excretes and the animal weight was registered by a scale previously calibrated. The liquid volume taken was registered by a volumetric cylinder.

The chromatographic analysis was performed by High Performance Liquid Chromatography using a HPLC: Hewelt Packard 1050 series VWD equipped with a C-18 column (Chomatopac C-R3A).

The obtained data were analyzed using a *t student*, for samples supposing unequal variables (9).

#### Results and Discussion

a) Rats were fed with a Standard diet (13.8 % protein + 38 % sugar) containing 5 % dried alga cushuro by two months. They had a significantly weight loss than rats that were fed by standard diet only. See table 2.

Table 2. Weight gain of rats under ingestion of cushuro in their diets

	Group A1	Group A2
% dried cushuro added	5	0
Mean (gr.)	98.61	114.68
Variance	107.83	94.81
Number of rats	14	14
p*(T<= t) two coils	0.000006	

p\* The differences between with (A1) and without (A2) dried cushuro.

The weight gain of rats not only depends of an adequate energy contribution (carbohydrates and fats) but it also the contribution of protein and micronutrient, and some active biologically compounds, as phytochemical and antinutrients which are present in the food. In first place, a deficient nutrition carries to a fat mobilization, then as the nutrient decreases, one o more metabolic functions, which depends on the presence of nutrients, starting to malfunction and then there is loss of cell mass. So the weight losing of the animals starts evident.

The significant weight lose under ingestion of cushuro was followed by an increase significant lose of excretas, which suggest a defective intestinal absorption of the foods. No differences in food consumption were noted between rats fed with and without cushuro in their diets; although there was a moderate difference upon ingestion of water. See Table 3.

Table 3. Mean of water and food intake and production of excretas of rats with diets with and without alga cushuro

	Group A1	Group A2	p*
% dried cushuro added	5	0	
Water intake (ml.)	105.99	100.63	0.0326
Food intake (gr.)	45.01	46.53	0.1994
Produced excretas (gr.)	44.3457	37.979	0.0002
Initial weight (gr.)	71.46	69.26	0.1692
Final weight (gr.)	98.61	114.68	0.000006

p\* The differences between with (A1) and without (A2) dried cushuro.

This data suggests that dried algae cushuro in the diet promotes a significantly weight loss in the rats. This weight lose would be related to a decreased of intestinal absorption due there was more production of excretas. The significance decreased of weight loss in rats taking algae cushuro does not seem to a decreased intake of food because the treated rats consumed the same amount of food that control rats but it would be to an exaggerate production of excretas, which it was related to a defective intestinal absorption.

b) The chemistry proximal analysis gave the following composition: 98 % water, 0.17 % protein and the rest were fiber, chlorophyll in small amounts. The fatty acid composition and the relative concentration are showed in Table 4.

Table 4. The fatty acids composition and the relative concentration of alga cushuro

Fatty Acids concentration	Common name	Systematic name	Relative
14:0	Miristic acid	Tretadecanoic acid	2.28
15:0		Pentadecanoic acid	23.42
16:0	Palmitic acid	Hexadecanoic acid	15.19
16:1(9)	Palmitoleic acid	9-hexadecenoic acid	9.55
16:2(9,12)	Palmitolinoleic acid	9,12-hexadecadienoic acid	3.38
18:0	Stearic acid	Octadecanoic acid	1.76
18:1(9)	Oleic acid	9-octadecenoic acid	2.67
18:1(11)	Vaccenic acid	11-octadecenoic acid	1.58
18:2(9,12)	Linoleic acid	9,12-octadecadienoic acid	3.04
18:3(9,12,15)	$\alpha$ -linolenic acid	9,12,15-octadecatrienoic acid	24.32

The fatty acids, little commons in prokaryotes (10), are 15:0, 18:1(*cis* 11); they are also found in the algae cushuro. These fatty acids are not present in marine algae (11). What call to attention is the relative abundance of the fatty acid 15:0, which probably is synthesized at the initial step using propionyl CoA instead of the normal acetyl Co A. The enzyme responsible for this step is the acetyl transacetylase which is highly unspecific and it can use acetyl or propionyl as a substrate (12). At the final step the metabolic pathway produces the pentadecanoic acid (15:0) instead of palmitic acid (16:0), which are also in abundance in cushuro. The fatty acid 18:1 (*cis* 11) is present at relative low concentration (13). The anabolic pathway may be starting with palmitic acid as a substrate to the enzyme *9-desaturase* to give 16:1 (*cis* 9) and then to the enzyme *elongase*, to finally give the 18:1 (*cis* 11), which is called vaccenic acid.

The common saturated fatty acids of the cushuro (14:0, 16:0, 18:0) are produced by releasing the fatty acids from their fatty acyl Co A respectively to give myristic, palmitic and stearic acids (14). The common monounsaturated fatty acids 16:1(*cis*9) and 18:1 (*cis* 9) are produced starting from the corresponding saturated substrates (16:0 and 18: 0) by the 9-desaturase action which is localized in bacteria and plants (10).

The polyunsaturated fatty acids 16:2(*cis*9,*cis*12) y 18:2(*cis*9,*cis*12) are produced from their corresponding monounsaturated fatty acids 16:1(*cis*9), 18:1(*cis*9) by the 12-desaturase action; while the polyunsaturated fatty acid 18:3(*cis*9,*cis*12,*cis*15) is produced by using the polyunsaturated fatty acid 18:2(*cis*9,*cis*12) as a substrate to the enzyme 15-desaturase. The bacteria cannot synthesize fatty acids with more than two double bonds but this green-blue alga can as some plants and green algae.

All these fatty acids contained in the cushuro, except the fatty acids 15:0 and 18:1 (cis 11), can be metabolized efficiently by rats (15) and they seem not interfere the food absorption at the intestinal level. In spite of high content of  $\alpha$ -linolenic acid (ALA) in rat diets and data that the intake of 3 % ALA-diacylglycerol oil significantly suppressed body weight in mice (16), we suggest, but does not prove, that both unusual fatty acids (pentadecanoic and 11-octadecenoic acids) could interfere with the intestine absorption of foods (due to significant feces production) playing a role as antinutrients.

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### **References**

1. Aldave A. Algas. Trujillo, Perú: Editorial La Libertad. 1989
2. Collazos. et al. Composición de Alimentos de mayor consumo en el Perú. Lima, Perú: Instituto Nacional de Salud Press. 1996.
3. Freyre A. Análisis Químico Bromatológico de Algas (*Nostoc Sphaericum vauch.*) de Aguas Continentales. Lima, Perú: Universidad Nacional Federico Villarreal, Tesis. 1980.
4. Torres A. Estudio Técnico y Biológico para la obtención de mermelada a base de *Nostoc Sphaericum vaucher*. Lima, Perú: Universidad Nacional Federico Villarreal, Tesis. 1986.
5. Guengerich FP. Effects of nutritive factors on metabolic processes involving bioactivation and detoxification of chemicals. *Ann. Rev. Nutr.* 1984; 4:207-231.
6. Lindner E. Toxicología de los Alimentos. Zaragoza: Editoreal Acribia. 1978.
7. Gill T.J. The rat in biomedical research. *Physiologist* 1985; 28: 9-17.
8. Council of Scientific Affairs. Animal in research. *JAMA*, 1989; 261: 3602-3606.
9. Boeckx R. Laboratory statistics. In: Hicks JM and Boeckx RL, eds. *Pediatric Clinical Chemistry*. Philadelphia, PA: W.B. Saunders Company, 1984: 18-48.
10. Vance DE and Vance JE. 1985. *Biochemistry of Lipids and Membranes*. Menlo Park, CA: Benjamin/Cummings.
11. Shahidi H. Nutraceutical lipids. In: Huang YS, Lin SJ and Huang PC, eds. *Essentials Fatty Acids and Eicosanoids*. Champaign, Illinois: AOCS Press 2003: 300-304.
12. Wakil S, Stoops JK, and Joshi VC. Fatty acid synthesis and its regulation. *Annual Review of Biochemistry* 1983; 52:537-579.
13. Kim KH et al. Role of reversible phosphorylation of acetyl-CoA carboxylase in long chain fatty acid synthesis. *FASEB journal*, 1983; 3:2250-2256.

14. Wakil S. Fatty acid synthase, a proficient multifunctional enzyme. *Biochemistry*, 1989; 28:4523-4530.
15. Nunez J. Rol Biológico de los Alimentos en la salud Humana: Bioquímica y Biología Molecular. Huaraz, Perú. In press, 2006.
16. Hase T and Itakura H. Antiobesity effect of long-term consumption of dietary diacylglycerol in experimental animals models. In: Katsuragi Y, Yasukawa T, Matsuo N, Flickinger BD, Tokimitsu I, and Matlock MG, eds. *Diacylglycerol Oil*. Champaign, Illinois: AOCS Press 2004: 86-95.