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Bioavailability of nutrients in rats fed on edible seaweeds, Nori (*Porphyra tenera*) and Wakame (*Undaria pinnatifida*), as a source of dietary fibre

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Abstract

A comparative study of the influence of two edible seaweeds, Nori (*Porphyra tenera*) and Wakame (*Undaria pinnatifida*), on dietary nutritive utilization was performed. Male adult Wistar rats were fed, for 2 weeks, diets containing Nori, Wakame or cellulose as source of dietary fibre. All diets contained similar amounts of dietary fibre (5%), protein (14%) and ash (5%). Intake, body weight gain, food efficiency (weight gain/food intake), apparent digestibility and retention coefficients for protein, fat, and minerals (calcium, iron, magnesium, zinc, sodium, and potassium) were evaluated. The addition of Wakame or Nori did not affect the gain in body weight of rats or food efficiency. Fresh and dry stool weights were higher in rats fed seaweeds than in the control group. Seaweed-fed animals showed significantly lower apparent digestibilities of protein and fat but absorbed nitrogen was more effectively used by animals. Apparent digestibility and retention coefficients for calcium, magnesium, zinc, iron, sodium and potassium were lower for seaweed-fed rats, and showed lower values for Wakame than Nori. The seaweeds could be a good source of dietary fibre in diet but they may modify digestibility of dietary protein and minerals.

Keywords: Seaweeds; Dietary fibre; Protein; Minerals

1. Introduction

Marine algae are frequently consumed in Asia and occasionally in the rest of the world (Nisizawa, Noda, Kikuchi, & Watamaba, 1987). From a nutritional point of view, edible seaweeds are rich in non starch polysaccaharides [Dietary Fibre, (DF)], (Lahaye, 1991; Wong & Cheung, 2000), proteins, (Jurkovic, Kolb, & Colic, 1995), minerals (Jimenez-Escrig & Goñi-Cambrodón, 1999) and vitamins (Watanabe, Nakamo, Tamura, & Yamanaka, 1991). They have a low lipid content and they provide a very low amount of energy. However, seaweed composition could interfere with bioavailability of dietary components (Lahaye, 1991; Pak & Araya, 1996; Suzuki, Nakai, Yoshie, Shiron, & Hirano, 1993). As seaweed polysaccharides cannot be entirely digested by human intestinal enzymes, they are considered to be a source of DF (Lahaye, 1991; Mabeau & Fleurence, 1993).

Seaweed DF differs in composition, chemical structure, physico-chemical properties and biological effects from those of land plants. Therefore, seaweeds could constitute sources of a wide variety of DF.

Wakame and Nori are brown (*Phaeophyceae*) and red (*Rhodophyceae*) algae, respectively. Their compositions are different. Alginates, fucans and cellulose constitute the main cell-wall polysaccharides of *Pheophyceae*, whereas sulphated galactans, xylans, mannans and cellulose compose the cell wall of *Rhodophyceae* (Nishide, Anzai, Uchida, & Nisizawa, 1990). This diversity of polysaccharides corresponds to different physico-chemical, rheological, and chemical properties as well as biological properties (Lahaye, 1991; Lahaye, & Kaeffer, 1997). Chemical properties and biological behaviour of seaweeds have been poorly investigated.

The aim of this work was to study the influence of two seaweeds on the growth of animals and on the

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bioavailability of dietary components in order to evaluate their potential use as ingredients in the food industry.

2. Materials and methods

2.1. Materials

Nori (*Porphyra tenera*) and Wakame (*Undaria pinnatifida*) were purchased in local health stores. They were dried for 16 h at 60 °C and milled by using a cyclotec mill (Tecator, 1093 Sweden) to pass through a screen with an aperture of 0.5 mm. The composition of seaweeds has been previously reported (Rupérez & Saura-Calixto, 2001). Both seaweeds contain 34% of total DF on dry matter.

2.2. Animals and diets

Thirty adult male Wistar rats from the same litter were obtained from the breeding centre at the Faculty of Pharmacy (Universidad Complutense, Madrid, Spain). The study was approved by the Department of Nutrition of the Universidad Complutense of Madrid. Rats were handled according the Guide for the Care and Use of Laboratory Animals National Research Council (1985).

Diets were prepared from a Fibre Free AIN-93M Purified Rodent Diet (DYETS, Inc. Bethlehem, Pennsylvania) that conforms to or exceeds the nutrient requirements for the maintenance of adult rats, set forth by the National Research Council (1985).

Rats were randomly divided into three groups of 10 with an average body weight of 195 g. A control group was given diet containing cellulose (50 g/kg of diet) as DF, and the other two groups were given Nori or Wakame (147.1 g/kg of diet) at the expense of cellulose. All diets contained 14% of protein and 5% of DF. The composition of diets is shown in Table 1.

Table 1
Composition of experimental diets (g/kg)

	Control	Test
Casein	140	140
Corn starch	465.5	368.39
Corn starch, dextrinized	155.2	155.2
Sucrose	100	100
Cellulose	50	–
Salt Mix AIN-93M [34]	35	35
Vitamin Mix AIN-VX [34]	10	10
L-Cystine	1.8	1.8
Choline Bitartrate	2.5	2.5
Tert-butylhydroquinone (TBHQ)	0.01	0.01
Soybean oil	40	40
Nori (*Porphyra tenera*) or Wakame (*Undaria pinnatifida*)	–	147.1

Rats were housed in individual metabolic cages for 2 weeks in a room maintained at 22±1 °C, with 12 h light–dark cycles. Rats were allowed free access to the powdered diet and mili-Q water. Food intake and body weight were measured every 48 h. During the last experimental week, faeces and urine were collected every 48 h. Urine was collected in hydrochloric acid (5 ml/l), and faeces were weighed, frozen at −20 °C, freeze-dried, and milled to a particle size < 0.5 mm.

2.3. Analytical methods

2.3.1. Total DF (TDF)

TDF content in diets was determined by the AOAC enzymatic-gravimetric method (Lee & Prosky, 1995).

2.3.2. Protein

Diets, urines and faeces were analyzed for total nitrogen by the micro-Kjeldahl method with a Leco FP-2000 Dumas nitrogen/protein determinator (Rupérez & Saura-Calixto, 2001). Samples were weighed into ceramic boats and loaded into the FP-2000, where they were combusted in the pure oxygen environment of the furnace. After passing through a thermo-electric cooler to drop out water, an aliquot from the combustion gasses was taken. Gasses were bubbled, all nitogen-containing materials reduced to nitrogen and detected by a thermal-conductivity cell. An air blank was carried out and the instrument calibrated with EDTA. Protein was calculated as nitrogen ×6.25 (Rupérez & Saura-Calixto, 2001).

2.3.3. Lipids

Lipids were extracted from diets and faeces in a Soxhlet extractor (Soxtec System HT 1043 Tecator, Sweden) with petroleum ether at 40–60 °C (AOAC, 1995). Lipid content was determined gravimetrically after drying at 60 °C to constant weight.

2.3.4. Ash and minerals

Aliquots of diets, faeces, and urines were digested in 50% HNO_3 (suprapure) at 550 °C to total calcination. Ash was gravimetrically quantified and was dissolved in 50 ml of 50% HNO_3 with 5 g/l $LaCl_3$. Calcium, magnesium, iron and zinc were determined in the samples by flame atomic absorption spectrophotometry, and sodium and potassium were determined by emission, with a Perkin-Elmer Model 5100 PC atomic absorption spectrophotometer with an air-acetylene flame (impact besd and corrosion resistant nebulizer; Vanhoof & De Schrijver, 1996).

2.3.5. Calculations of nutritional indices

The following parameters were considered in order to evaluate protein, fat and mineral digestibilities of the diets:

Table 2
Effect of Nori (*Porphyra tenera*) and Wakame (*Undaria pinnatifida*) on body weight, food intake and food efficiency in rats[a]

	Control	Nori	Wakame
Body weight gain (g)[b]	57.30±10.14	49.75±8.18	66.25±10.69
Food intake (g dry matter)[b]	234.61±13.72	209.58±1.04	235.04±21.51
Food efficiency[b,c]	0.24±0.04	0.24±0.04	0.28±0.10

[a] For details of diets see Table 1. Values are means±standard deviation; ($n=10$).
[b] Not significantly different.
[c] Food efficiency: (weight gain/food intake)

Table 3
Effect of Nori (*Porphyra tenera*) and Wakame (*Undaria pinnatifida*) on protein and fat utilization in rats[a]

	Control	Nori	Wakame
Stool			
Fresh weight (g/week)	6.15±1.78a	16.69±4.49b	17.83±9.15b
Water content (%)[b]	36.32±13.40	56.14±3.11	49.73±18.51
Ash (% dry matter)[b]	30.73±6.02	25.09±0.26	32.38±5.01
Protein intake (g/week)[b]	19.3±0.80	19.7±1.27	21.6±2.35
Faecal protein (g/week)	1.42±0.32a	2.73±0.64b	2.94±0.65b
Urinary protein (g/week)	6.49±0.50a	5.59±0.51b	4.11±0.50c
Protein-apparent digestibility	92.6±1.82a	86.1±3.38b	86.2±3.63b
Protein efficiency ratio	1.29±0.34a	1.37±0.30a,b	2.02±0.55b
Fat intake (g/week)[a]	5.45±0.23	5.48±0.35	6.08±0.66
Fat excretion (g/week)	0.05±0.01a	0.10±0.02b	0.11±0.02b
Fat-apparent digestibility	99.00±0.24a	98.10±0.46b	98.14±0.49b

[a] Values are means±standard deviation ($n=10$). Different letter in a row indicate significant difference ($P<0.005$).
[b] Values not statistically different.

2.3.5.1. Food efficiency. Relationship between weight gained by the animal and the food consumed (weight gain/food intake).

2.3.5.2. Protein efficiency ratio. Weight gain per weight of eaten protein (weight gain/protein intake).

2.3.5.3. Apparent digestibility. Proportion of intake absorbed [(intake–faecal excretion) / intake]×100.

2.3.5.4. Apparent balance. Proportion of intake that is retained [(intake–excretion)/intake]×100.

2.4. Statistical analysis

Results are expressed as mean values and standard deviations (S.D.). One-way analysis of variance (ANOVA) was used to determine the significance of mean differences between groups, by using the StatGraphics computer program (SAS/STAT version 6, SAS Institute, Cary, NC). Significance level was $P<0.05$.

3. Results and discussion

The addition of seaweeds as a source of DF did not significantly affect body weight gain or food intake (Table 2).

Seaweed-fed animals showed an increase in fresh and dry stool weight as compared with the control group (Table 3). This result seems to be due to the high water holding capacity of indigestible components of diet, mainly from seaweeds (Rupérez & Saura-Calixto, 2001; Suzuki, Ohsugi, Yoshie, Shirai, & Hirano, 1996) and to higher excretion of dry matter, which may be composed of material of both endogenous and exogenous origin (Cumming & MacFarlane, 1991).

The intake of protein was slightly higher in seaweed-fed animals than in the control group, and faecal nitrogen excretion was significantly higher when the diet contained seaweed, so that protein digestibility was apparently lower. Faecal protein could derive from an increase of bacterial nitrogen (Hasen, Bach Knudsen, & Eggum, 1992; Stephen & Cummings, 1980), or from dietary protein resistant to digestion in the small intestine.

DF could block the access of digestive enzymes (Onning & Asp, 1995), and also decrease the activity of proteolytic enzymes (Pacheco-Delahaye, 1999), producing a decrease of apparent protein digestibility.

The dietary fat intakes of all the animals were comparable. However, the addition of seaweeds to the diet caused a significantly higher percentage of excreted fat when compared with the control group (Table 3), so that apparent fat digestibility was lower in the case of seaweeds. The results of protein and fat digestibility are

consistent with those reported by other authors (Suzuki et al., 1993).

DF is an important component of seaweeds. Total DF content is 34% in both Wakame and Nori, while insoluble DF as dry matter constitutes 16.3 and 19.2% of Wakame and Nori, respectively (Rupérez & Saura-Calixto, 2001). DF is frequently included in diets as a source of potential agents for prevention of diseases, although it is recommended that the effects of these fibre sources on metabolism of essential minerals be measured very carefully.

Algae are rich in a wide variety of minerals, but they are also rich in other components, such as DF and resistant protein, that may pass through the intestine without being absorbed and can retain dietary mineral components (Holland, Unwin, & Buss, 1991).

In this work, the addition of algae to diets did not significantly modify dietary ash content (3.66% dry matter in control, 3.80 and 4.00% dry matter for Nori and Wakame diets, respectively).

Some reports demonstrate that the type of DF does not impair retention of minerals (Nickel, Nielsen, Smart, Mitchell, & Belury, 1997), although many dietary and endogenous factors may influence mineral bioavailability when DF products are consumed, but the fibre level does not seem to affect mineral absorption as much as fibre composition (Coudray, Bellanger, Castiglia-Delavaud, Rémésy, Vermorel, & Rayesiguier, 1997). Many dietary components may react with minerals and form highly stable complexes which may decrease apparent absorption of divalent cations (Kashumura, Kimura, & Itokawa, 1996). On the other hand, some dietary components may increase the solubility of mineral complexes and decrease faecal excretion of minerals such as zinc or calcium.

In this experiment, the intake of magnesium, zinc and iron was less in seaweed-fed animals, although the latter presented greater total excretion (urine and faeces). Thus, apparent digestibility and retention coefficients were lower in seaweed-fed rats, the values being lower for Wakame than for Nori. Calcium values followed the same trend, but the differences were not significant.

Some authors accept that effects of dietary components on magnesium absorption have no nutritional impact, although it has been demonstrated that excessive intake of calcium and phosphorus in rats reduce intestinal absorption of magnesium (Vanhoof & De Schrijver, 1996; Table 4).

Zinc is known to interact with many dietary components which affect its utilization, such as the amount of phytate and type and amount of DF; however, DF per se has little or no effect on zinc availability (Rossander, Sandberg, & Sandström, 1992). Zinc availability can be significantly determined by the level and source of dietary protein (Sandström & Lönnerdal, 1989).

In this case, zinc availability was low, as is usual in non-animal products. Apparent digestibility and retention coefficients were lower when Wakame was included in the diet, but the addition of Nori produced no difference in apparent digestibility. The difference in the behaviour of the two seaweeds could be due to differences in amino acid composition; some amino acids, such as histidine or cysteine, form a soluble complex with magnesium that facilitates their absorption by preventing zinc precipitation (Sandström, 1991; Wapnir, Khani, Bayne, & Lifshitz, 1983). Nori and Wakame both contain a high proportion of protein (34 and 14%, respectively; Jurkovic et al., 1995). The proteins contain hystidine and cysteine, although these are limiting

Table 4
Effect of Nori (*Porphyra tenera*) and Wakame (*Undaria pinnatifida*) on intake and excretion of calcium, magnesium, zinc, iron, sodium and potasium in rats[a]

	Control	Nori	Wakame
Calcium			
Intake (mg)	855±356a	695±45b	791±86a
Faecal excretion (mg)[b]	448±99	441±15	520±115
Urine excretion (mg)	13±0.2a	10±0.1b	9±0.1c
Apparent digestibility (%)	47.4±12.9	36.4±15.5a,b	33.3±17.6b
Apparent retention(%)	45.9±13.0	34.9±15.5a,b	32.1±17.6b
Magnesium			
Intake (mg)	270±11a	175±11b	219±24c
Faecal excretion (mg)	46±10a	62±14.5a	113±25b
Urine excretion (mg)	19±0.001a	17±0.001b	25±0.001c
Apparent digestibility (%)	82.9±4.2a	64.6±8.6b	47.5±13.8b
Apparent retention (%)	75.9±4.4a	54.7±8.9b	35.8±14.7b
Zinc			
Intake (mg)	11±0.4a	10±0.7a	7±0.8b
Faecal excretion (mg)[b]	4±0.9a	3±0.8a	5±1.0b
Urine excretion (mg)	0.4±0.004a	0.3±0.002b	0.4±0.005c
Apparent digestibility (%)	61.7±9.4a	66.9±8.1a	37.0±16.6b
Apparent retention (%)	60.0±9.4a	65.2±8.1a	34.3±16.8b
Iron			
Intake (mg)	24±1	11±0.7b	14±2c
Faecal excretion (mg)[b]	4±0.9	4±0.8	5±1.0
Urine excretion (mg)	0.5±0.001a	0.4±0.001b	0.4±0.001b
Apparent digestibility (%)	83.8±4.0a	61.0±9.5 b	62.5±9.9b
Apparent retention (%)	82.8±4.0a	59.3±9.5 b	61.2±10.0b
Sodium			
Intake (mg)	378±6a	569±37b	647±70.31c
Faecal excretion (mg)	12±3a	36±9b	70±16c
Urine excretion (mg)	108±0.4a	190±0.4b	258±0.4c
Apparent digestibility (%)	96.7±0.8a	93.6±1.6a	89.0±2.9b
Apparent retention (%)	68.2±1.8a	60.0±3.1a	48.8±6.8b
Potasium			
Intake (mg)	650±7a	748±48a	834±91b
Faecal excretion (mg)	19±4a	64±15b	112±25c
Urine excretion (mg)[b]	346±0.4	395±0.8	569±0.8
Apparent digestibility (%)	97.0±0.7a	91.4±2.1a	86.3±3.6b
Apparent retention (%)	43.6±2.7a	38.3±4.7b	17.5±10.5c

[a] Values are means±standard deviation ($n=10$). Different letters in a row indicate significant difference ($P<0.005$).

[b] Not significantly different.

amino acids in both seaweeds. Hystidine and cysteine concentrations are higher in Nori than in Wakame (Jimenez-Escrig & Goñi-Cambrodón, 1999), and apparent protein digestibility and retention coefficients have been found to be higher in Nori-fed rats than in Wakame-fed rats.

Results indicated that seaweeds decreased the efficiency of iron absorption, as shown in the decrease in the iron absorption and iron retention percentage. These values decreased because of both low iron availability and lower intake of iron in seaweed-fed animals (Gillooly et al., 1983; South, House, & Miller, 1997; Tuntawiroon et al., 1991). Polyphenolic compounds and peptides from partially digested protein in the samples could contribute to low iron digestibility and retention coefficients (House & Van Campen, 1994).

Cysteine is the only free amino acid to have an enhancing effect on iron absorption in man (Martinez-Torres & Layrisse, 1970). Its potential to chelate iron could account for the enhancing effect of cysteine-containing peptides on iron absorption, but this amino acid is limiting in the seaweeds studied.

Wakame and Nori contain high proportions of sodium and potassium (Jimenez-Escrig & Goñi-Cambrodón, 1999), and the intake of these minerals was higher in seaweed-fed animals (Table 4). Faecal and urinary excretion of sodium and potassium were also higher for seaweed-fed groups. Apparent sodium digestibility was lower for seaweeds, although the difference was significant only in the case of Wakame. Apparent potassium digestibility was similar, while rats fed with Wakame presented lower retention coefficients than other groups.

It is therefore important to consider the contribution of the colon to the overall absorption of minerals in the presence of fermentable substances (Michel & MacFarlane, 1996). In fact, the caecal concentrations of potassium, calcium and phosphorus in fermentable carbohydrates increase, but the concentrations of magnesium and sodium do not (Demigné, Levrat, & Rémésy, 1989). However, the effects of carbohydrates on mineral availability are to some extent controversial in that the digestive microbiota can express enzymatic activity and microbial fermentations can increase the solubility of divalent cations in the large intestine, which may improve their absorption in situ across the caecal wall. Thus, there is a possible shift of absorptive sites from the small intestine towards the large intestine, with potential enhancement of availability for absorption, especially of calcium and magnesium (Younes, Deigné, & Rémésy, 1996).

The experimental diets reported here contained equal amounts of mainly fermentable substrates, resistant starch and DF. Therefore, the only difference between them was the type of DF. On the other hand, cellulose is an insoluble DF which presents low fermentability (Barry et al., 1995), while seaweeds contribute soluble and insoluble compounds that may be more fermentable than cellulose. The availability of some minerals may have been enhanced by the fact that seaweed DF is more fermentable than cellulose DF.

In conclusion, seaweeds could help increase consumption of DF and minerals. More specific studies are necessary to determine the possible health implications for consumers.

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