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Effects of seaweed-restructured pork diets enriched or not with cholesterol on rat cholesterolaemia and liver damage

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ABSTRACT

Seaweed enriched-restructured pork (RP) is a potential functional food. However, indications of adverse effects associated with herbal medications, which include among others liver failure, toxic hepatitis, and death have been reported. Cholesterol feeding produces hepatomegalia and fat liver infiltration. The effect of seaweed-RP diet, cholesterol-enriched or not, on plasma cholesterol, liver damage markers, structure, and cytochrome CYP4A-1 were evaluated after 5 wk. Eight rat groups were fed a mix of 85% AIN-93M rodent-diet plus 15% RP. The Cholesterol-control (CC), Cholesterol-Wakame (CW), Cholesterol-Nori (CN) and Cholesterol-Sea Spaghetti (CS) groups respectively consumed similar diets to control (C), Wakame (W), Nori (N), and Sea Spaghetti (S) but as part of hypercholesterolaemic diets. CN and CS significantly blocked the hypercholesterolaemic effect observed in CC group. After 5-wk, N and S diets increased the CYP4A-1 expression. However, seaweed-RPs were unable to reduce the histological liver alterations observed in CC group. Larger and more abundant hepatocellular alterations were found in CS and CN rats suggesting that the hypocholesterolaemic effects of these seaweed-RPs seem to be a two-edged sword as they increased liver damage. Future studies are needed to understand the involved mechanisms.

1. Introduction

Functional foods represent a new category of products whose healthy properties have already made them very popular (Arvanitoyannis and van Houwelingen-Koukaliaroglou, 2005; Ashwell, 2002). Restructured pork (RP) is one of the most consumed meat derivates and appears to be a suitable vector for including functional ingredients improving the “image” of the meat product by modifying its composition (i.e. saturated fat, cholesterol, and sodium) (Cofrades et al., 2008; Jiménez Colmenero et al., 2010). Seaweeds, as Wakame (*Undaria pinnatifida*), Nori (*Porphyra umbilicalis*) and Sea Spaghetti (*Himanthalia elongata*) have gained importance as foodstuffs in Western countries (Bocanegra et al., 2009; Mohamed et al., 2012). Seaweeds have been included as components of potentially functional meats or meat derivates because their consumption induces positive effects on cholesterolaemia, antioxidant status and adipose tissue enzyme expression (González-Torres et al., 2012; Olivero-David et al., 2011; Mišurcová et al., 2011; Moreira et al., 2010).

Our group found that cholesterol-enriched casein diets induced severe hypercholesterolemia and liver damage (Sánchez-Muniz et al., 1992, 1996). In addition, cholesterol feeding caused rat splenomegaly (Bastida et al., 2006) and hepatomegaly (Sánchez-Muniz et al., 2003; Viejo et al., 2003), which was reduced when the casein and olive oil in the diet was substituted by olive oil-fried sardines as the only source of protein and fat. Bocanegra et al. (2003, 2006) found that cholesterol feeding induced hypercholesterolaemia, liver fat infiltration and peroxidation in rats, which were partially checked by including 7% Nori in the diet, while Konbu was unable to do that. Similarly, differences in the hypolipaemic and antioxidant properties of the seaweed-enriched RP-diets were attributed to differences in the seaweed composition (e.g. total and soluble dietary fibre, mineral, vitamin, and phytochemical content) of the Nori, Wakame, and Sea Spaghetti included in those RPs (González-Torres et al., 2012; Moreira et al., 2010; Olivero-David et al., 2011). Moreover, differences in the results of the studies by Bocanegra et al. (2006) and Moreira et al. (2010) also suggest that

Abbreviations: ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; C, control-group; CC, Cholesterol-control; CN, Cholesterol-Nori; CS, Cholesterol-Sea Spaghetti; CW, Cholesterol-Wakame; CYP4A-1, Cytochrome 4A-1; N, Nori; PUFA, polyunsaturated fatty acids; RP, restructured pork; S, Sea Spaghetti; TBARS, thiobarbituric acid reactive substances; W, Wakame.

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some meat compounds (e.g. iron, the meat matrix itself) can interact with seaweed compounds, which could explain those results.

Despite benefits already due to seaweeds consumption reported, indications of adverse effects associated with herbal medications, which include among others liver failure, toxic hepatitis, and death have been suggested (Ernst, 2003). Cytochrome 4A-1 (CYP4A-1) plays a role in detoxification (Tabrez and Ahmad, 2012) and could be a marker of liver protection, but no studies have been performed on animal models fed seaweed-enriched RP.

As hypercholesterolaemia and antioxidant status are recognized to play a central role in liver damage (Bocanegra et al., 2009; Sánchez-Muniz et al., 1992, 1996), the hypothesis of the present paper is that seaweed-enriched RP can partially check liver inflammation and structural tissue modification and damage. Moreover, the protective effects are different and depend on the seaweed included in the RP.

Thus, the objective of this paper was to evaluate the effect of a seaweed-RP diet, cholesterol-enriched or not, on (a) serum cholesterol, (b) liver damage markers (alkaline phosphatase, ALP; aspartate aminotransferase, AST; and alanine aminotransferase, ALT); (c) CYP4A-1 expression; and (e) liver histological changes.

2. Materials and methods

2.1. Materials

Wakame (*U. pinnatifida*), Nori (*P. umbilicalis*) and Sea Spaghetti (*H. elongata*) were obtained from a local supplier (Algamar C.B., Redondela, Pontevedra, Spain). These seaweeds were ground in a mill (ZM 200, Retsch GmbH and Co., KG, Haan, Germany), passed through a screen with an aperture of 0.25 mm and stored in plastic flasks at 4 ± 2 °C until used. Meat raw materials (post-rigor pork and pork backfat), seaweeds and additives (sodium chloride, sodium tripolyphosphate and sodium nitrite) were used as reported by Cofrades et al. (2008). Table 1 gives information on protein, fat, soluble and insoluble fibre, and ash of the different RP employed for diet preparation.

2.2. Diet preparation and experimental design

Eight experimental semi-synthetic diets (Table 1) were prepared: (a) the control RP diet (C) contained 85% rodent feed (AIN-93M, purified rodent diet; Dyets #180729, DYETS, Inc., Bethlehem, PA, USA) and 15% freeze-dried control RP to which 4% cellulose had been added; (b) the Wakame RP diet (W) consisted of a mixture of AIN-93M #180729 feed (85%) and freeze-dried Wakame RP (15%); (c) the Nori RP diet (N) consisted of a mixture of AIN-93M #180729 feed (85%) and freeze-dried Nori RP (15%); (d) the Sea Spaghetti RP diet (S) consisted of a mixture of AIN-93M #180729 feed (85%) and freeze-dried Sea Spaghetti RP (15%); (e) the Cholesterol-control RP diet (CC) was identical to the C diet but with 2.43% cholesterol (95–98% purity) and 0.49% cholic acid (>98% purity) substituting an equal amount of starch (AIN-93M #180730 diet); (f) the Cholesterol-Wakame RP diet (CW) was the W diet enriched with cholesterol and cholic acid, and (g) the Cholesterol-Nori RP diet (CN) consisted of the N diet enriched with cholesterol and cholic acid; (h) the Cholesterol-Sea Spaghetti RP diet (CS) consisted of the S diet enriched with cholesterol and cholic acid.

Details of experimental diet composition are summarized in Table 1.

The experiments were approved by the Spanish Science and Technology Advisory Committee and by an ethics committee of the Universidad Complutense de Madrid (Spain). All experiments were performed in compliance with Directive 86/609/EEC of November 24, 1986.

2.3. Animal maintenance and sampling

Eighty growing male Wistar rats with a body weight of approximately 90 g at the outset were obtained from Harlan Laboratories Models, SL, Barcelona (Spain). The animals were housed individually in metabolic cells in a temperature-controlled room (22.3 ± 1.8 °C) with a 12 h light–dark cycle. Rats were fed commercial rat pellets (Panlab, Barcelona, Spain) for 1 wk to adapt to environmental conditions. The rats were later distributed into eight groups of 10 animals each, according to average body weight. Diets contained approximately 20.7% protein, 8.7% fat, and 4.2% total dietary fibre. Water and food were provided *ad libitum* over the 5-wk experimental period. At the end of the experiment, rats were anaesthetized, in fasting conditions, with an intraperitoneal injection of sodium pentobarbital (45 mg/kg body weight) and euthanized by extracting blood from the descending aorta with a syringe, taking one animal at a time from each of the eight groups. The liver was removed and weighed. The hepatosomatic index was calculated as the percent contribution of liver weight to the animal weight. Liver was dissected and its major lobe snap-frozen in liquid nitrogen and stored at −80 °C or stored in formalin prior to analysis.

2.4. Food consumption and body weights

Food intake was checked daily and body weight variations were measured on alternate days.

2.5. Plasma cholesterol

Plasma cholesterol was determined by the enzymatic colorimetric method of SPINREACT (Sant Esteve de Bas, Girona, Spain).

Table 1
Composition of the Control, Wakame (*Undaria pinnatifida*), Nori (*Porphyra umbilicalis*) and Sea Spaghetti (*Himanthalia elongata*) enriched restructured pork without or with supplementary cholesterol diets.[a]

	Without supplementary cholesterol				With supplementary cholesterol			
	Control	Wakame	Nori	Sea Spaghetti	Control	Wakame	Nori	Sea Spaghetti
Diet AIN-93M								
Maize starch	382.75	382.75	382.75	382.75	353.59	353.59	353.59	353.59
Cholesterol					24.31	24.31	24.31	24.31
Cholic acid					4.85	4.85	4.85	4.85
Pork (freeze-dried)[b] (g/kg diet)								
Control restructured pork[b]	150				150			
Wakame-, Nori- or Sea Spaghetti-restructured pork[b]		150	150	150		150	150	150
Soluble fibre (g/100 g wet matter)[c]		1.1	1.33	1.35		1.1	1.33	1.35
Insoluble fibre (g/100 g wet matter)[c]	2.81	1.2	0.66	1.46	2.81	1.2	0.66	1.46
Polyphenols (as gallic acid equivalent/100 g wet matter)[c]	Tr	820	2170	2570	Tr	820	2170	2570

AIM-93M mineral mix (g/kg): calcium carbonate, 357.00; potassium phosphate monobasic, 250.00; potassium citrate·H_2O, 28.00; sodium chloride, 74.00; potassium sulfate, 46.60; magnesium oxide, 24.00; ferric citrate U.S.P., 6.06; zinc carbonate, 1.65; manganous carbonate, 0.63; cupric carbonate, 0.30; potassium iodate, 0.01; sodium selenate, 0.01025; ammonium paramolybdate $4H_2O$, 0.00795; sodium metasilicate·$9H_2O$, 1.45; chromium potassium sulfate·$12H_2O$, 0.275; lithium chloride, 0.0174; boric acid, 0.0815; sodium fluoride, 0.0635; nickel carbonate, 0.0318; ammonium vanadate, 0.0066; finely powdered sucrose, 209.806.

AIN-93VX vitamin mixture (g/kg): niacin, 3.00; calcium pantothenate, 1.60; pyridoxine HCl, 0.70; thiamine HCl, 0.60; riboflavin, 0.60; folic acid, 0.20; biotin, 0.02; vitamin E acetate (500 IU/g), 15.00; vitamin B12 (0.1%), 2.50; vitamin A palmitate (500 000 IU/g), 0.80; vitamin D3 (400 000 IU/g), 0.25; vitamin K1–dextrose mix (10 mg/g), 7.50; sucrose, 967.23.

[a] Other ingredients (g/kg diet): casein, 127.5; soyabean oil, 34; dyetrose (carbohydrate composition: monosaccharides, 10; disaccharides, 40; trisaccharides, 50; tetrasaccharides and higher, 900), 131,75; sucrose, 85; microcrystalline cellulose, 42.5; AIN-93M mineral mix, 29.75; AIN-93VX vitamin mixture, 12.16; choline bitartrate, 3.06; L-cystine, 1.53; t-butylhydroquinone, 0.0068.

[b] Protein (g), fat (g), minerals (g) (ash) and fibre (g) present in the 150 g of Control, Wakame, Nori and Sea Spaghetti freeze-dried restructured-pork; Control restructured pork: 76.99, 51.52, 15.61 and 4 (microcrystalline cellulose), respectively; Nori-restructured pork: 73.94, 53.10, 17.40 and 3.0, respectively; Wakame-restructured pork: 73.94, 53.10, 17.40 and 3.45; Sea Spaghetti restructured pork: 69.56, 51.25, 15.05 and 14.12.

[c] Data in restructured pork (source López-Lopez et al., 2009).