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Short communications

Purple potato flake reduces serum lipid profile in rats fed a cholesterol-rich diet

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ABSTRACT

The effect of purple potato flake on cholesterol metabolism was investigated in rats fed a high-cholesterol diet. The hypocholesterolemic action of dietary purple potato flake might be related with caecal fermentation and steroid excretion due to the phosphorus and polyphenols including anthocyanin.

1. Introduction

Potato is one of the best origins of carbohydrates in human diet. Usually as starch form it is used to make industrialized product like as noodles, instant soups, and potato chips. It has been reported that some potato starches like as dietary fiber has a hypocholesterolemic action in rodents (Demigné, & Rémésy, 1982; Younes, Levrat, Demigné, & Rémésy, 1995; Robert, Nancy, Rayssiguier, Mazur, & Rémésy, 2008). This is thought as resistant starch mostly as raw and retrograded. However, other investigators have reported that no change in serum cholesterol in rats fed a raw potato starch (Chezem, Furumoto, & Story, 1997; Wang, Yu, Liu, & Chen, 2008). Recently this difference has been considered with phosphorus contents in potato starch (Kanazawa et al., 2008) because covalently bound phosphorus residues contribute to limit enzymatic α-amylase hydrolysis. Meanwhile, new developed potato varieties have been paid more and more attention on health beneficial effects due to the augmented

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Abbreviations: ALP, alkaline phosphatase; ALT, alanine amino trasnferase; AST, aspartate amino trasnferase; CONT, a control diet; GAPDH, glyceraldehydes-3-phosphate dehydrogenase; HDL, high-density lipoprotein; HMG-CoA, 5-hydroxy-3-methylglutaryl-CoA; LDL, low-density lipoprotein; PPF, a purple potato flake diet; RS, resistant starch; SEM, standard error of mean; SREBP-2, sterol regulatory binding protein-2

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nutrients such as mineral and polyphenol (Andre, Ghislain, et al., 2007; Andre, Oufir, et al., 2007; Mori et al., 2010). For example, Noda et al. (2006) reported that colored potato cultivars (Inca Purple, Kitamursaki, Shadow-Queen, Inca Red, and Northern Ruby), which contain anthocyanins, have a relatively higher phosphorus content (891–1065 ppm). Furthermore, Yoon et al. (2008) reported that the ethanol-extracted pigment as a good source of anthocyanin from purple potato dose-dependently lowers serum LDL-cholesterol level in rats. To date, however, there has been little information on cholesterol metabolism of colored potato in animals.

In this study it was investigated the effect of purple potato flake compared to cornstarch on serum and liver lipids levels, fecal sterol excretion, caecal fermentation, and hepatic mRNA expression in exogenous cholesterol-loaded rats.

2. Materials and methods

2.1. Plant material and preparation

Purple potato (*Solanum tuberosum* cv. Shadow-Queen) flake which contains anthocyanin (87 mg/100 g power; Han, Matsumoto, Shimada, Sekikawa, & Fukushima, 2007) and phosphorus (910 ppm; Noda et al., 2006), was prepared according to our previous report (drum-dried method) (Han et al., 2008).

2.2. Analysis of polyphenol content

For analysis of polyphenol content in purple potato flake, high-performance liquid chromatography (HPLC)–mass spectroscopy (MS) analysis was carried out. Ground purple potato flake (100 mg) was mixed with 10 mL of 10% phosphoric acid containing 0.1% methanol, incubated at 37 °C for 3 h, and then vigorously vortexed. Next, the mixture was centrifuged at 1000*g* for 5 min and filtered with a PTFE hydrophilic syringe filter (0.45 μm). Finally the filtrate was applied to LC–MS system consisting of an Agilent Technologies 1200 series (Palo Alto, CA) coupled to a 4000 Qtrap LC/MS/MS (Applied Biosystem instrument, Framingham, MA). For the negative ion MS $[M-H]^-$ performed at a capillary temperature of 550 °C and voltage of 5.5 kV, a nebulizing pressure of N_2 50 psi was used as sheath gas. HPLC was carried out on an analytical column C18 (Capcell PAK-C18, 250 × 4.6 mm, 5.0 micron; Shiseido, Tokyo, Japan). The mobile phase A was a mixture of methanol, acetic acid, and water (5:2.5:92.5, v/v/v), and B was a mixture of methanol, acetic acid, and water (95:2.5:2.5, v/v/v). The injection volume was 10 μL, and the flow rate was 0.8 mL/min. The polyphenol content was determined by the peak areas of the extracted ion chromatogram (gallic acid *m/z* 169, $[M-H]^-$; chlorogenic acid *m/z* 353, $[M-H]^-$; caffeic acid *m/z* 179, $[M-H]^-$; *p*-coumaric acid *m/z* 163, $[M-H]^-$; myricetin *m/z* 317, $[M-H]^-$; quercetin *m/z* 301, $[M-H]^-$; and kaempferol *m/z* 285, $[M-H]^-$) using a standard curve derived from commercial polyphenols.

2.3. Animals and diet

Seven-week-old male F344/DuCrj rats (Charles River Japan; Yokohama, Japan) were used and handled with the Guide for the Care and Use of Laboratory Animals (NRC, http://newton.nap.edu/html/labrats/). And all procedures were approved by the Animal Experiment Committee of Obihiro University of Agriculture and Veterinary Medicine (the animal protocol approval No. 2450 and the validity dates until March 31th, 2013). Rats were individually placed in a conventional room under a temperature of 23 ± 1 °C and relative humidity of 60 ± 5% controlled with a 12-h light/12-h dark cycle. Rats were fed a commercial chow for 1 week, and then randomly divided into 2 groups of 5 rats weighing approximately 199–214 g. And the animals were provided with free access to either a control diet (CONT) or a purple potato flake diet (PPF) for 4 weeks. The CONT contained (in g/kg): 200 casein, 100 sucrose, 549 α-cornstarch, 50 cellulose, 50 soybean oil, 35 AIN-93G mineral mixture, 10 AIN-93G vitamin mixture, 3 L-cystine, 2.5 choline bitartrate, 5 cholesterol, 1.25 sodium cholate, and 0.014 *t*-butyl hydroquinone. In the PPF, a part of α-cornstarch was replaced by purple potato flake, resulting in final flake concentration of 30%. Body weight and food consumption were recorded weekly and daily, respectively. All feces were collected during the last 3 days of the experimental period. At the end of the experimental period of 4 weeks, the rats were anesthetized with Nembutal (pentobarbital sodium, 40 mg/kg body weight; Abbott Laboratories, Abbott Park, IL) after fasting overnight and killed. The blood samples were withdrawn, and tissue samples (liver and cecum) were quickly removed and weighed before freezing for storage at −80 °C.

2.4. Biochemical analysis

Blood samples were collected from jugular veins of fasting rats every week (on the 0th, 14th, 21st, and 28th days of experiment) to analyze serum total cholesterol, high-density lipoprotein (HDL)-cholesterol concentrations, and alanine amino trasnferase (ALT), aspartate amino trasnferase (AST) and alkaline phosphatase (ALP) activities by using commercially available reagent kits (Abbott Laboratories, Abbott Park, IL). Serum non-HDL-cholesterol (VLDL + IDL + LDL-cholesterol) concentration was calculated as follows: [non-HDL-cholesterol] = [total cholesterol] − [HDL-cholesterol]. Total lipid in liver and feces were extracted by a mixture of chloroform/methanol (2:1, v/v) (Folch, Lees, & Sloane-Stanley, 1957). Neutral sterols in the total lipid content were analyzed by the method of Matsubara, Sawabe, and Iizuka (1990). Bile acids in feces were measured by the method of Grundy, Ahrens, and Miettinen (1965). Short-chain fatty acids (SCFA) in caecum were measured by the method of Hara, Saito, Nakashima, and Kiriyama (1994). And all producers for total RNA isolation in liver, RT-PCR, and Southern blot analysis were followed as described previously (Han et al., 2005). In brief, the abundances of mRNA encoding a low-density lipoprotein (LDL) receptor, 5-hydroxy-3-methylglutaryl (HMG)-CoA reductase, cholesterol 7α-hydroxylase, sterol regulatory binding protein-2 (SREBP-2), and glyceraldehydes-3-phosphate dehydrogenase (GAPDH, used as an invariant control) were analyzed by semi-quantitative RT-PCR and subsequent Southern hybridization of the PCR products with each inner oligonucleotide probe. The used SREBP-2 primers of oligonucleotides were 5′-CGC AAC CAG CTT TCA AGT CCT-3′ as upstream primer and 5′-CCG GTA CCG CTT CTC AAT GAT-3′ as downstream primer. And the blot was hybridized with SREBP-2