ELSEVIER

Journal of Nutritional Biochemistry 15 (2004) 196–205

THE JOURNAL OF Nutritional Biochemistry

Intestinal steroids in rats are influenced by the structural parameters of pectin

Gerhard Dongowski[a,*], Angelika Lorenz[b]

[a]*Department of Food Chemistry and Preventive Nutrition, German Institute of Human Nutrition Potsdam-Rehbrücke, Bergholz-Rehbrücke, Germany*
[b]*Department of Gastrointestinal Microbiology, German Institute of Human Nutrition Potsdam-Rehbrücke, Bergholz-Rehbrücke, Germany*

Received 3 August 2002; received in revised form 13 May 2003; accepted 23 May 2003

Abstract

We investigated the effects of pectin with different degrees of methylation (34.5, 70.8, and 92.6%, respectively) on the composition and concentration of intestinal and fecal bile acids and neutral sterols in conventional and germfree rats. Diets containing 6.5% pectin (galacturonan) were given for 3 weeks. High concentrations of free and secondary bile acids appeared in cecum and colon of conventional rats. With increasing degree of methylation, more bile acids were transported into lower parts of intestinal tract and excreted whereas the proportion of secondary bile acids decreased. In contrast, the composition of bile acids in intestinal contents and feces was relatively unchanged in germfree rats. Exclusively cholesterol was found as a neutral sterol in germfree rats. Coprostanol appeared in cecum of conventional rats and additionally coprostanone in colon. Amounts of neutral sterols increased with increasing degree of methylation of pectin. Additionally, concentrations of bile acids in plasma decreased if the pectin-containing diets were given. Besides the degree of methylation, the molecular weight of pectin used in the diets influenced concentration and composition of intestinal and fecal steroids in rats.

Keywords: Bile acid; Neutral Sterol; Rat; Pectin; Degree of methylation

1. Introduction

The cell wall polysaccharide pectin present in fruits and vegetables consists mainly of long chains of α-1.4-linked galacturonic acid residues (homogalacturonan) interrupted by highly branched rhamnose-galacturonic acid sequences (rhamnogalacturonans I and II). As a dietary fiber (DF) it is not degraded by intestinal enzymes during the passage of stomach and small intestine. Therefore, pectin is present in the upper part of intestinal tract in macromolecular form [1]. Physicochemical and functional properties of pectin - and in consequence several physiological effects - are influenced by its structural parameters like the degree of methylation (DM) that means the degree of esterification of the carboxyl groups with methanol. In nutritional studies, pectin is applied mostly in isolated form consisting preferentially of homogalacturonan.

It is well known that DF may act in different way on the lipid metabolism [2]. Like other soluble DF, pectin is capable of reducing plasma LDL-cholesterol levels - a major risk factor for development of atherosclerosis – especially in hyperlipidemic humans and animals. This cholesterol-lowering effect of the soluble viscous DF seems to be connected with the transport of bile acids (BA) into lower parts of the intestinal tract and their increased excretion [3-7]. Thus, interactions between pectin and BA were found in in vitro and in vivo studies [8,9]. Despite different theories are discussed, the mechanisms of these interactions (binding, adsorption, etc.) are not yet clear [10-15].

The passage of pectin through the small intestine (of minipigs) was necessary for lowering serum cholesterol because intracecal infusion of pectin had no effect on total serum cholesterol levels [16]. Studies with ileostomy patients showed increased BA (35%) and cholesterol excretion (14%) after administration of 15 g pectin [17]. Because of the interactions between pectin and BA in small intestine, less BA are re-absorbed and therefore more BA are trans-

This work was supported by the German Federal Ministry for Research and Technology (BMFT) (FKZ 07 NBL 05/4).

* Corresponding author. Tel.: +49-33200-88268; fax: +49-33200-88444.

E-mail address: dongo@mail.dife.de (G. Dongowski).

0955-2863/04/$ – see front matter
doi:10.1016/S0955-2863(03)00080-9

ported into the (cecum and) colon where they are deconjugated and partly dehydroxylated by enzymes of the microflora [18]. The greater excretion of BA is connected with a decrease of the BA pool. This effect causes probably the increased hepatic synthesis of BA and liver depletion of cholesterol resulting in reduced serum cholesterol levels [3,19-21]. Further, the short-chain fatty acid (SCFA) propionate produced during fermentation of DF by the intestinal microflora may inhibit the hepatic cholesterol synthesis [22]. Additionally, formation of the SCFA causes a drop in colonic pH. This effect may lower the solubility of the BA and depress their passive re-absorption in the lower parts of the intestinal tract.

The effects of molecular and structural parameters of pectin on the interactions with BA have been hardly investigated. But it was shown that macromolecular and high-methoxyl pectins were most effective in lowering plasma cholesterol in rats [23]. Likewise, Yamaguchi et al. [24] found that a high molecular weight of pectin is necessary to repress serum cholesterol in rats. A diet with 3% macromolecular pectin was more effective in reducing plasma cholesterol and excretion of BA and neutral sterols (NS) in hamsters than pectin with a lower molecular weight [25]. Further it has been shown in vitro that the DM, the arrangement of free carboxyl groups in the galacturonan macromolecules, the molecular weight and a derivation of pectin as well as the pH influence the interactions with bile acids. However, structural parameters of the BA play also a role in the extent of interactions with pectin [26,27].

Altogether, the blood cholesterol lowering effect of pectin is mainly due to prevention of absorption of BA from the intestinal tract. Effects of the structure of pectin on the steroid transport into the lower parts of the intestinal tract and on the actions of the microflora (on pectin fermentation and steroid composition) has been scarcely reported in detail. Therefore, influences of pectin on physiological parameters in rats have been investigated in this study. Especially, the effects of structural parameters of pectin (like DM and molecular weight) on composition and concentration of BA and NS in intestinal contents and feces as well as on plasma lipids were examined in experiments with conventional ("normal") and germfree rats. In germfree rats, fermentation of pectin and actions of bacterial steroid degrading enzymes are prevented.

2. Methods and materials

2.1. Pectins

High- and low-methoxyl citrus pectins (H and L) without additives were obtained from Copenhagen Pectin A/S, Lille Skensved, Denmark, and purified by treatment with acidic aqueous ethanol. The DM of these macromolecular preparations H and L was 70.8 and 34.5%, respectively. For preparation of the very highly methoxylated pectin V (DM = 92.6%), preparation H was further methoxylated using methanol/conc. H_2SO_4. The intrinsic viscosities [η] of the pectins L, H, and V were 395, 692, and 421 ml/g galacturonan, respectively [1].

The galacturonan content of the pectin preparations was determined by the m-hydroxybiphenyl method [28]. Methyl ester groups were analyzed by the chromotropic acid method [29]. The intrinsic viscosity [η], which is empirically related to the molecular weight by the Mark-Houwink relation, was determined in 0.155 mol/L NaCl (high-methoxyl pectins) or in 0.05 mol/L NaCl/0.005 mol/L sodium oxalate (low-methoxyl pectins) at 25.0°C and pH 6.0 using an Ubbelohde viscosimeter.

2.2. Steroids

The reference steroids were obtained from the following sources: Steraloids (Wilton, NH): α-, β- and ω-muricholic acids (MCA), 7-ketodeoxycholic acid (KDCA), 12-ketolithocholic acid (KLCA); Sigma (St. Louis, MO): taurodeoxycholic acid (TDCA), cholic acid (CA), lithocholic acid (LCA), hyodeoxycholic acid (HDCA), 5α-cholestane and 5α-cholestan-3-one (cholestanone); Fluka (Neu-Ulm, Germany): chenodeoxycholic acid (CDCA), deoxycholic acid (DCA), ursodeoxycholic acid (UDCA), cholesterol, 5β-cholestan-3-one (coprostanone); Calbiochem (La Jolla, CA): taurocholic acid (TCA), taurochenodeoxycholic acid (TCDCA), tauroursodeoxycholic acid (TUDCA), 7α,12β-dihydroxy-5β-cholanic acid; Serva (Heidelberg, Germany): 5β-cholestan-3β-ol (coprostanol).

2.3. Animals and diets

Male conventional rats (strain Shoe:Wist) weighing 202 ± 11 g were obtained from Tierzucht Schönwalde GmbH, Germany. The rats were housed individually in temperature and humidity controlled cages (22 ± 2°C and 55 ± 5%) on a 12-h light-dark cycle (light, 06 00–18 00 h).

Adult germfree male rats of the inbred strain AVN/Ipcv-Wistar (Rehbrücke) weighing approximately 270 g were obtained from the Germfree Animal Unit of the German Institute of Human Nutrition, Potsdam-Rehbrücke. The animals were maintained in positive-pressure isolators (Metall und Plastik, Radolfzell, Germany) and housed individually in polycarbonate cages on irradiated wood chips (Altromin, Lage, Germany) at 22 ± 2°C and 55 ± 5% humidity on a 12-h light-dark cycle (light, 06 00–18 00 h).

Animals were fed the control diet C (conventional rats) or the irradiated control C# (germfree rats) for 7 days after arrival. Then the rats were randomly divided into 4 groups (germfree rats) or 5 groups (conventional rats) and assigned to the experimental pectin-free (control) or pectin-containing diets. The animals had free access to diets and water. The pelleted diets for the experimental groups contained 6.5% pectin (galacturonan). For the experiments with germfree rats, the diets were γ-irradiated (20 kGy) [1].

2.4. Sample procedure

Blood was collected at the 11th and 21st day of the experiment after overnight food deprivation (12 h). Plasma was separated by centrifugation at 1500 g for 20 min at 4°C. Complete feces were collected within two periods (period A: 8th–10th day; period B: 18th-20th day). At the end of the experiments and 2 h after last feeding, rats were sacrificed and the contents of ileum, cecum and colon were immediately prepared for analysis (stored at −20°C and then freeze-dried).

2.5. Plasma lipid measurements

Plasma lipids were determined enzymatically using a COBAS MIRA/S analyzer from Hoffmann-La Roche using Cobas enzyme kits (Basel, Switzerland) and the 3-hydroxysteroid dehydrogenase/NADH kit (Sigma, Deisenhofen, Germany).

2.6. Extraction and determination of bile acids in intestinal contents and feces

After addition of 7α,12β-dihydroxy-5β-cholanic acid as internal standard, freeze-dried intestinal contents or feces materials were extracted for 30 min at 37°C with 50% tert. butanol (end-concentration) [30] and then centrifuged for 15 min at 4°C and 5000 g. The supernatant was diluted with water and the BA were purified by solid phase extraction on Bakerbond spe C_{18} columns in the BAKER spe-12G system (J.T. Baker, Gross Gerau, Germany). The BA were estimated by HPLC using pre-column derivatization and fluorescence detection [31]. Free and glycine conjugated BA were directly derivatized with 4-bromomethyl-7-methoxycoumarin (BMC) in presence of 18-Crown-6 as a catalyst. Taurin conjugates which cannot react with BMC were enzymatically hydrolyzed with choloylglycine hydrolase (Sigma, Deisenhofen, Germany) before their derivatization and analysis as free BA. The BMC labeled derivatives were separated on a non-polar stationary phase (Nucleosil 100 Å; C_{18}; 5 μm; 250 × 4.6 mm) at 40°C in HPLC equipment from Gynkotek (Germering, Germany) with online-degasser DG 1310, gradient pump M 480, injection automate GINA 160, column oven (Peltier), fluorescence detector RF 1002 (excitation λ 320 nm; emission λ 385 nm) (Shimadzu Europe, Duisburg, Germany) and GynkoSoft software. Linear gradients consisting of acetonitril (30-100%), methanol (40-0%) and water (30-0%) were applied. Extractions were conducted in duplicates and each HPLC analyses were two times repeated.

2.7. Extraction and determination of neutral sterols in intestinal contents and feces

After addition of 5α-cholestane as internal standard, freeze-dried intestinal contents or feces materials were first hydrolyzed for 2 h at 75°C with 1 mol/L NaOH in 90% ethanol. After addition of water, the non-polar NS were separated by extraction with hexane (three times). The purified hexane extract was dried in vacuum and dissolved in ethanol. The NS were determined using HPTLC. The solutions (500-3000 nL) were applied 8 mm from the bottom of 20 × 10 cm HPTLC silica gel 60 F_{254} plates (Merck, Darmstadt, Germany) by spraying (6 mm streaks) with the automatic TLC sampler III (Camag, Muttenz, Switzerland). The chromatograms were developed with ether-heptane (55: 45, v/v) in an automatic developing chamber (Camag); run distance 80 mm. The plates were then dipped for 3 s in a copper sulfate-phosphoric acid reagent using a chromatogram immersion device III (Camag) and heated for 5 min at 180°C. After cooling, the spots were measured at 405 nm using a TLC scanner II with CATS software (Camag). Extractions were conducted in duplicates and each HPTLC analyses were two times repeated.

2.8. Statistical analysis

Results are expressed as mean values and standard deviations (SD). Statistical significance was determined using one-way analysis of variance followed by Student's t test. $P < 0.05$ was taken to indicate a statistically significant difference.

2.9. Ethical considerations

The experimental protocol was performed according to international and national guidelines. The animal experiment was approved by the Ethics Committee of the Ministry of Nutrition, Agriculture and Forestry of the State Brandenburg, Germany.

3. Results

3.1. General effects of pectins with different structural parameters in rats

Because of small differences in composition (including the contents of methoxyl groups) of the pectin preparations it was decided to use the pectins in the diets on the basis of their galacturonan content. All diets used are characterized in Table 1.

The diets for the germfree animals were sterilized by γ-irradiation (20 kGy). Irradiation under the used conditions resulted in a partially depolymerization of the pectins whereas the DM was not changed. Intrinsic viscosities [η] of the irradiated pectins L#, H#, and V# isolated from the diets were 284, 446, and 267 ml/g galacturonan, respectively [1]. In the experiments with conventional rats, the diet with the irradiated pectin H# was additionally used. Thus it was possible to study the effects of the molecular weight of pectin on the intestinal steroids.

Table 1
Composition of control diets (C, C#) and experimental diets (L, L#, H, H#, V, V#)

Component	Diets C and C#	Diets L and L#	Diets H and H#	Diets V and V#
	(g/kg diet)			
Casein[1]	200	200	200	200
Wheat starch[2]	630	56.6	530.8	542.6
Pectin[3]	0	83.4	99.2	87.4
Microcrystalline cellulose[4]	50	50	50	50
Sun flower oil[5]	50	50	50	50
Vitamin mixture[6]	20	20	20	20
Mineral mixture[7]	50	50	50	50

[1] Dauermilchwerk Peiting GmbH, Landshut, Germany.
[2] Heller and Strauss, Berlin, Germany.
[3] Low-methoxyl (L) and high-methoxyl pectin (H) from Copenhagen Pectin A/S, Lille Skensved, Denmark. Very highly methoxylated pectin V was prepared by from pectin H. Pectins L#, H#, and V# are γ-irradiated (20 kGy). Pectin concentration: 65 g galacturonan/kg diet.
[4] Rettenmeier GmbH, Ellwangen, Germany.
[5] Thomy GmbH, Karlsruhe, Germany.
[6] Composition of the vitamin mixture (in mg/kg diet): retinol palmitate, 7; cholecalciferol, 0.02; α-tocopherol acetate, 240; menadione, 15; thiamine, 30; riboflavin, 30; pyridoxine, 22.5; cyanocobalamin, 0.05; niacin, 75; pantothenic acid, 75; folic acid, 15; biotin, 0.03; choline, 1500; *p*-aminobenzoic acid, 150; myo-inositol, 150 (Altromin GmbH, Lage, Germany).
[7] Composition of the mineral mixture (in mg/kg diet): Ca, 9300; P, 7300; K, 7100; Na, 4400; Cl, 3600; S, 1700; Mg, 800; Fe, 200; Mn, 100; Zn, 30; Cu, 8; F, 4; I, 0.4; Se, 0.2; Co, 0.1 (Altromin GmbH).

The diets were well accepted by the rats. It was shown that the weight gain, the food intake and feed efficiencies of all conventional rat groups were not different during the experimental period. In germfree rats fed pectin-enriched diets, food consumption tended to be higher. Further, the walls of ileum, cecum, and colon were heavier and most of the intestinal contents were greater in rats when pectin-containing diets were fed [1]. Pectin passes the small intestine as a macromolecule and was fermented in cecum and colon of conventional rats under formation of SCFA. Low-methoxyl pectin was fermented faster than high-methoxyl pectins. Total anaerobic and Bacteroides counts were greater in groups fed pectin. Pectin-fed rats had greater intestinal weights [1].

LDL-cholesterol was lowered significantly in conventional rats fed the pectin-containing diets after 21 days as well as in germfree rats fed diets containing pectins H# and V# both after 11 days and 21 days. HDL-cholesterol was not changed (data not shown). Plasma concentrations of BA were decreased both after 11 and 21 days in all groups of conventional rats fed the not irradiated pectins as well as in all germfree groups fed pectin-containing diets compared to day 0 (Table 2).

3.2. Effects of pectin on bile acids

The profiles of the individual BA (in %) as well as the total BA (in μmol) in intestinal contents of conventional rats are summarized in Tables 3 and 4. BA composition in ileal, cecal, and colonic contents (in nmol) of germfree rats is shown in Table 5.

Table 2
Bile acids in plasma of conventional rats fed control diet C or diets containing low-methoxyl pectin L, high-methoxyl pectin H, and very highly methoxylated pectin V or irradiated diet containing pectin H# as well as of germfree rats fed irradiated control C# or irradiated diets containing low-methoxyl pectin L#, high-methoxyl pectin H# or very highly methoxylated pectin V#

Conventional rats			Germfree rats		
Group	Day	Bile acids (μmol/L)	Group	Day	Bile acids (μmol/L)
C	0	19.7 ± 2.4	C#	0	9.7 ± 2.1
	11	21.4 ± 2.6		11	8.5 ± 0.4
	21	19.5 ± 1.1		21	9.4 ± 1.3
L	0	21.2 ± 1.4	L#	0	10.3 ± 1.3
	11	18.6 ± 0.7*		11	8.4 ± 0.7*
	21	18.4 ± 1.1*		21	7.7 ± 1.2*
H	0	21.5 ± 0.7	H#	0	9.4 ± 0.7
	11	18.4 ± 0.8*		11	7.9 ± 0.8*
	21	18.6 ± 1.2*		21	6.9 ± 0.5*
V	0	20.9 ± 1.4	V#	0	9.7 ± 1.2
	11	17.6 ± 1.1*		11	8.2 ± 0.6*
	21	17.5 ± 0.3*		21	6.1 ± 0.6*
H#	0	20.0 ± 2.1			
	11	18.9 ± 1.7			
	21	18.8 ± 0.7			

Values are means ± SD, $n = 8$. * $P < 0.05$ compared to day 0.

3.2.1. Comparison of pectin effects on total bile acid content

The total amount of BA in ileal contents was 8.7 μmol in control group and more than 15 μmol in rats fed the pectin with the highest DM. Use of irradiated pectin H# (lower molecular weight than pectin H) resulted in lower concentrations of BA in ileal contents. Relatively high concentrations of BA were found in cecal contents. Approximately 28 μmol BA were measured in the control group, whereas more than 66 μmol total BA were present in cecal contents of rats fed the diet with pectin V. In colonic contents, 11.2 μmol total BA were found in the control group, but up to 20.4 μmol in animals fed pectin-containing diets. Like in the other intestinal contents, the concentration of BA increased significantly with the DM of the pectin used (Table 4).

3.2.2. Comparison of pectin effects on constitution of bile acids in intestinal contents and in feces of conventional rats

In ileal contents, TCA was the predominant bile acid (43.7–49.2%) followed by βMCA (33.2–34.5%). Furthermore, α/ωMCA (3.1–7.0%) which could not be separated by the applied HPLC technique, TDCA (4.2–7.9%), TCDCA (3.9–6.1%), and HDCA (2.1–5.1%) were found in distinct concentrations in ileal contents. With increasing

Table 3
Composition of primary bile acids (in percent) in intestinal contents of conventional rats fed control diets C or diets containing low-methoxyl pectin L, high-methoxyl pectin H, and very highly methoxylated pectin V or irradiated diet containing high-methoxyl pectin H#

Intestinal content	Group	TCA	CA	KDCA	TCDCA	CDCA	α/ωMCA	βMCA	UDCA
		(%)							
Ileum	C	45.26 ± 6.18	0	0.58 ± 0.30	6.12 ± 1.32	0	5.94 ± 1.48	33.23 ± 6.30	0.90 ± 0.33
	L	43.63 ± 10.33	0.07 ± 0.04	1.29 ± 0.26*	3.92 ± 0.88*	0.79 ± 0.24	7.00 ± 1.67	33.43 ± 10.22	0.07 ± 0.07*
	H	46.11 ± 8.59	0	0.33 ± 0.09	6.06 ± 2.05	0	3.33 ± 0.84*	34.13 ± 5.52	0
	V	45.38 ± 11.25	0	0.70 ± 0.11	4.54 ± 0.38*	0	4.98 ± 0.53	34.44 ± 8.89	0.35 ± 0.17*
	H#	49.18 ± 7.03	0	0.51 ± 0.33	5.49 ± 1.44	0	3.06 ± 0.88*	34.53 ± 7.66	0.18 ± 0.11*
Cecum	C	9.83 ± 1.66	9.18 ± 2.23	0.60 ± 0.11	0.12 ± 0.08	3.26 ± 1.25	10.56 ± 2.27	16.44 ± 4.34	0.21 ± 0.14
	L	8.54 ± 1.62	12.41 ± 3.13	0.13 ± 0.05*	0	4.37 ± 0.86	12.83 ± 1.83	18.36 ± 3.22	0.83 ± 0.17*
	H	12.37 ± 2.22	11.53 ± 1.49	0.56 ± 0.41	0.25 ± 0.09	3.63 ± 1.03	12.48 ± 3.30	19.34 ± 2.16	0
	V	14.60 ± 1.18*	8.82 ± 1.06	0	1.33 ± 0.22*	1.81 ± 0.33*	15.36 ± 1.19*	21.33 ± 1.34	0.72 ± 0.40*
	H#	9.88 ± 1.09	10.36 ± 2.11	0.33 ± 0.21	1.20 ± 0.39*	3.11 ± 0.43	14.26 ± 2.05	17.74 ± 3.22*	0.83 ± 0.25*
Colon	C	0.35 ± 0.22	3.21 ± 0.42	0.44 ± 0.08	0	1.74 ± 0.44	14.63 ± 2.22	10.44 ± 0.89	0
	L	0	5.22 ± 1.03*	0.51 ± 0.09	0	2.22 ± 0.40	14.41 ± 1.26	10.82 ± 1.34	0
	H	1.22 ± 0.26*	5.41 ± 0.46*	1.21 ± 0.26*	0	1.37 ± 0.60	18.14 ± 1.88	12.55 ± 1.44	0.40 ± 0.33*
	V	1.26 ± 1.00	6.83 ± 1.22*	0.83 ± 0.31*	0	2.49 ± 0.39	20.32 ± 2.61*	12.44 ± 0.88*	0.23 ± 0.17
	H#	0	3.44 ± 0.46	0.25 ± 0.07*	0	2.33 ± 0.66	18.34 ± 0.92*	11.26 ± 0.96	1.03 ± 0.11*

TCA, taurocholic acid; CA, cholic acid; KDCA, 7-ketodeoxycholic acid; TCDCA, taurochenodeoxycholic acid; CDCA, chenodeoxycholic acid; MCA, muricholic acid; UDCA, ursodeoxycholic acid.
Values are means ± SD, $n = 8$. * $P < 0.05$ compared to control group C.

DM of the pectin in the diet, the amounts of TCA, βMCA and of total BA increased in ileum.

More than 50% of the BA belonged to the cholic acid family having a hydroxyl or keto group at the 12th C atom of the steroid nucleus. The part of secondary BA (Table 4) was found to be between 7.0 and 10.0%. In ileal contents, most of the BA were conjugated with taurine (53.2–60.0%).

Enzymatic action of the intestinal microflora resulted in distinct variations of the BA composition in cecum: The proportion of tauroconjugated BA decreased to 14.7–21.6% and that of secondary BA increased to 36.0–49.8% (Table 4). In detail, a broader spectrum of the individual BA was present in cecal contents compared to ileum. The dominant bile acids were here HDCA (24.3–31.0%) and βMCA (16.4–21.3%). The proportions of TCA, CA, α/ωMCA, and DCA were each approximately 10%. The concentrations of most of the individual BA and of the total BA increased with the DM of pectin used in the diets. The pectin with the lower molecular weight (H#) was less effective in transport of BA into the cecum (Tables 3 and 4).

Table 4
Composition of secondary bile acids (in percent) as well as total bile acids (in μmol) in intestinal contents of conventional rats fed control diet C or diets containing low-methoxyl pectin L, high-methoxyl pectin H, and very highly methoxylated pectin V or irradiated diet containing high-methoxyl pectin H

Intestinal content	Group	TDCA	DCA	KLCA	LCA	HDCA	Total bile acids
		(%)					(μmol)
Ileum	C	4.21 ± 2.21	0	0	0	3.75 ± 1.04	8.73 ± 0.63
	L	5.63 ± 1.63	0	0	0	4.17 ± 1.16	10.66 ± 1.12*
	H	7.85 ± 0.71*	0.05 ± 0.03	0	0	2.11 ± 0.36	13.74 ± 0.85*
	V	4.53 ± 0.62	0	0	0	5.08 ± 0.92	15.47 ± 1.59*
	H#	4.92 ± 2.28	0	0	0	2.12 ± 1.44	11.44 ± 0.92*
Cecum	C	5.82 ± 0.84	11.77 ± 4.41	0	1.21 ± 0.88	31.00 ± 7.77	27.77 ± 1.79
	L	6.22 ± 1.59	9.19 ± 1.16	0.52 ± 0.39	0.81 ± 0.36	25.79 ± 2.39	53.17 ± 1.59*
	H	4.93 ± 1.67	9.44 ± 1.82	0.09 ± 0.05	0.25 ± 0.42	25.13 ± 4.04	61.76 ± 2.58*
	V	5.66 ± 1.39	6.03 ± 0.51*	0	0	24.33 ± 2.06	66.47 ± 1.62*
	H#	4.07 ± 0.41*	11.40 ± 1.83	0	0	26.82 ± 1.76	53.96 ± 1.67*
Colon	C	0.38 ± 0.10	20.26 ± 2.55	12.41 ± 1.15	0.65 ± 0.23	35.48 ± 2.44	11.17 ± 0.25
	L	0.63 ± 0.12*	18.94 ± 1.66	12.54 ± 2.26	1.38 ± 0.21*	33.30 ± 2.17	14.71 ± 0.33*
	H	2.15 ± 0.27*	17.55 ± 1.24	10.56 ± 0.90	1.53 ± 0.51*	27.90 ± 1.54*	16.62 ± 0.30*
	V	1.81 ± 0.30*	15.83 ± 2.20	8.16 ± 0.55*	0.22 ± 0.18	29.58 ± 3.13*	20.38 ± 0.61*
	H#	0	15.68 ± 0.94*	12.87 ± 2.05	0.90 ± 0.21	33.90 ± 4.29	14.82 ± 0.54*

TDCA, taurodeoxycholic acid; DCA, deoxycholic acid; KLCA, 12-ketolithocholic acid; LCA, lithocholic acid; HDCA, hyodeoxycholic acid.
Values are means ± SD, $n = 8$. * $P < 0.05$ compared to control group C.

Table 5
Bile acids in intestinal contents of germfree rats fed irradiated control diet C# or irradiated diets containing low-methoxyl pectin L#, high-methoxyl pectin H# or very highly methoixylated pectin V#

Intestinal content	Group	Primary bile acids					Secondary bile acids			Total bile acids
		TCA (nmol)	TCDCA	α/ωMCA	βMCA	TUDCA	TDCA	DCA	HDCA	
Ileum	C#	1057 ± 92	0	14 ± 11	117 ± 60	0	0	0	46 ± 53	1234 ± 80
	L#	2587 ± 133*	44 ± 32	67 ± 42	522 ± 111*	0	0	0	122 ± 44*	3342 ± 121*
	H#	3328 ± 458*	34 ± 26	61 ± 27	520 ± 158*	0	0	0	205 ± 21*	4148 ± 281*
	V#	2696 ± 417*	37 ± 26	5 ± 11	465 ± 55*	0	0	0	160 ± 3*	3363 ± 215*
Cecum	C#	6241 ± 542	35 ± 24	85 ± 12	816 ± 41	0	0	0	462 ± 41	7639 ± 433
	L#	7623 ± 481*	124 ± 8*	68 ± 16	1046 ± 153*	60 ± 88	0	23 ± 25	393 ± 33	9337 ± 381*
	H#	16175 ± 517*	266 ± 97*	47 ± 22	2094 ± 130*	80 ± 75	118 ± 20*	47 ± 32	496 ± 21	19323 ± 477*
	V#	18147 ± 606*	0	85 ± 16	2135 ± 142*	0	302 ± 32*	0	608 ± 42*	21277 ± 558*
Colon	C#	968 ± 183	0	5 ± 12	117 ± 13	0	0	0	42 ± 3	1132 ± 172
	L#	1933 ± 255*	0	0	263 ± 20*	0	0	0	139 ± 20*	2335 ± 240*
	H#	3097 ± 285*	0	10 ± 2	450 ± 29*	0	14 ± 20	22 ± 4	112 ± 61*	3705 ± 291*
	V#	3824 ± 411*	0	37 ± 17*	453 ± 164*	0	0	0	82 ± 12*	4396 ± 369*

TCA, taurocholic acid; TCDCA, taurochenodeoxycholic acid; MCA, muricholic acid; TDCA, taurodeoxycholic acid; DCA, deoxycholic acid; HDCA, hyodeoxycholic acid; TUDCA, tauroursodeoxycholic acid.
Values are means ± SD, $n = 8$. * $P < 0.05$ compared to control group C#.

The proportion of BA of the cholic acid family was between 32.2 and 38.9% in cecal and colonic contents. This points to a lower absorption of BA of the chenodeoxycholic acid family, having no hydroxyl or keto group at the 12th C atom of the steroid nucleus, from the ileum.

The enzymatic modification of BA continued by action of the microflora. In detail, the amount of tauroconjugated BA diminished further to ≤ 3.1%. Additionally, the proportion of secondary BA increased to 69.2% in control and to 66.8–55.6% in the pectin groups. With increasing DM of the pectin in the diets, the proportion of secondary BA in colon decreased continuously. Compared to cecal contents, higher proportions of HDCA, α/ωMCA, DCA, and KLCA were found, whereas especially the parts of CA and βMCA decreased in colonic contents (Tables 3 and 4).

Further microbial conversions of the acidic steroids were observed in feces. Tauroconjugated BA were not found in these preparations. The proportion of CA, CDCA, and βMCA decreased and that of α/ωMCA and HDC increased in all groups. The BA composition of feces in the collecting period A is shown in Figure 1. With rising DM of pectin, especially an increase of the MCA and DCA as well as a decrease of the secondary BA were observed. In the collecting period B the total amount of excreted BA was 22.1

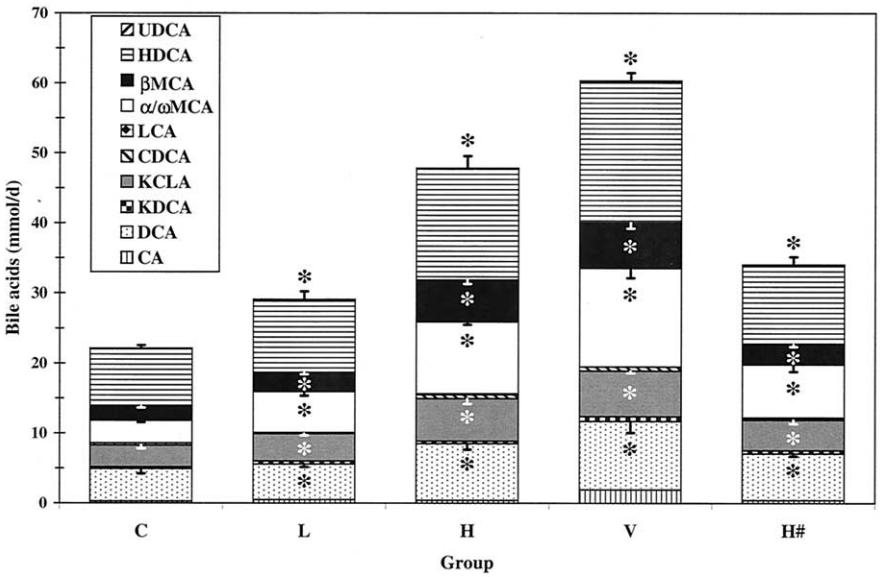

Fig. 1. Concentrations of bile acids in feces (collecting period A) of conventional rats fed control diet C or diets containing low-methoxyl pectin L, high-methoxyl pectin H, and very highly methoxylated pectin V or irradiated diet containing high-methoxyl pectin H#. Values are means ± SD, n = 8. * $P < 0.05$ compared to control group C.

Table 6
pH values in feces of conventional rats fed control diet C or diets containing low-methoxyl pectin L, high-methoxyl pectin H, and very highly methoxylated pectin V or irradiated diet containing pectin H# as well as of germfree rats fed irradiated control C# or irradiated diets containing low-methoxyl pectin L#, high-methoxyl pectin H# or very highly methoxylated pectin V#

Group	Day 0	Day 7	Day 14	Day 21
	Conventional rats			
C	7.39 ± 0.23	7.30 ± 0.17	7.25 ± 0.16	7.26 ± 0.06
L	7.41 ± 0.19	6.61 ± 0.20*	6.57 ± 0.14*	6.36 ± 0.13*
H	7.38 ± 0.26	6.67 ± 0.15*	6.64 ± 0.21*	6.43 ± 0.22*
V	7.41 ± 0.16	6.67 ± 0.12*	6.58 ± 0.18*	6.36 ± 0.20*
H#	7.39 ± 0.20	6.69 ± 0.24*	6.52 ± 0.17*	6.40 ± 0.22*
	Germfree rats			
C#	6.46 ± 0.11	6.60 ± 0.29	6.54 ± 0.12	6.51 ± 0.16
L#	6.40 ± 0.25	6.62 ± 0.24	6.72 ± 0.09	6.66 ± 0.10
H#	6.62 ± 0.19	6.50 ± 0.25	6.69 ± 0.12	6.68 ± 0.16
V#	6.52 ± 0.13	6.30 ± 0.24	6.63 ± 0.10	6.48 ± 0.21

Values are means ± SD, $n = 8$. * $P < 0.05$ compared to control groups C or C#.

μmol/day in the control group. With rising DM of the pectin in the diet, the excreted amount of acidic steroids increased to more than 60 μmol/day. Pectin H# was less effective for excretion of BA (34 μmol/day). Obviously, the viscosity of pectin plays a role in absorption of BA from intestine. Altogether, the concentrations of most of the individual BA in feces was higher in all pectin-fed groups and increased additionally in most cases with the DM of pectin used in the diets of the conventional rats.

Before starting the experiment, the pH values in feces of the conventional rats were approximately 7.40. In all pectin groups, a significant decrease in fecal pH was found during the experiment. After 3 weeks, the pH values in feces were between 6.36 and 6.43 in the pectin groups but 7.26 in the control (Table 6). This decrease in intestinal and fecal pH – caused by the formation of SCFA during the fermentation of pectin – is directly connected with reduced proportion of secondary BA.

3.2.3. Comparison of pectin effects on bile acids in intestinal contents and in feces of germfree rats

In general, lower concentrations of acidic steroids were found in germfree rats in comparison with conventional rats (Table 5). Feeding pectin resulted in higher amounts of BA in intestinal contents and in feces. In cecal and colonic contents of the germfree rats, the total concentration of BA increased with rising DM of the pectins used. But in ileal contents and in feces, no systematic correlation was found between the excreted BA and the DM. In feces, the total BA concentrations were in the control group 3.1 and 4.8 μmol/day, respectively, in both collecting periods but in the pectin groups between 9.1 and 13.7 μmol/day. A similar qualitative composition of BA was found in all intestinal contents and feces samples from germfree rats. The predominant BA were TCA (82.7 ± 3.6%), βMCA (11.3 ± 3.8%) and HDCA (4.1 ± 1.4%). Furthermore, α/ωMCA, TCDC, TUDC, and TDC were present in concentrations of <2%. Secondary BA were practically not present. Only in single samples, a low amount of DCA was found in intestinal contents.

No differences in fecal pH values were found in all germfree groups during the experimental period. The measured pH values were between 6.40 and 6.72 (Table 6).

3.3. Effects of pectin on neutral sterols

In conventional rats, only cholesterol was found as a NS. Coprostanol and coprostanone, the microbial degradation products of cholesterol, appeared first in cecal or colonic contents (Figure 2). The concentration of cholesterol was lowest in control group and increased with rising DM of pectin used in the diet. The irradiated pectin H# was less effective in transport of neutral NS into lower parts of the gastrointestinal tract. In detail, amounts of cholesterol found in the ileal contents were 1.88 and 2.50 μmol, in cecal contents 4.51 and 8.30 μmol and in colonic contents 1.42 and 3.14 μmol, respectively, in the control group and in the group fed pectin V. Furthermore, concentrations of coprostanol and coprostanone were more than doubled using diets with the very highly methoxylated pectin.

Like in cecal contents, coprostanol was the predominant NS in feces of conventional rats. In presence of pectin in the diet, the concentration of coprostanol was increased to values between 14.7 ± 2.7 and 16.5 ± 1.6 μmol/day (control group: 9.2 ± 0.9 μmol/day) whereas the total NS amount was increased to values between 23.1 ± 2.9 and 29.8 ± 2.3 μmol/day (control group: 13.5 ± 1.0 μmol/day).

In all contents of ileum, cecum, and colon and additionally in feces of germfree rats, exclusively cholesterol was present as a NS. With exception of ileum contents, the concentration of cholesterol increased in trend with the DM of pectin used in the diets. Compared to control, the cholesterol levels in rats fed with the highest methoxylated pectin were approximately doubled.

3.4. Effect of pectin on total steroids

The concentrations of total steroids (sum of bile acids and neutral sterols) in intestinal contents and feces (collecting period B) of both the conventional and the germfree rats are shown in Figure 3. With a few exceptions, the concentration of total steroids increased continuously with increasing DM of the pectin used in the diets. Additionally, the sum of BA and NS was lower in conventional rats given the irradiated pectin H# compared with pectin H (not shown).

In general, the proportion of BA (of the total steroids) was higher in intestinal contents and feces samples of conventional rats compared with that of germfree rats and decreased from ileal contents to feces. Thus, proportion of BA was approximately 65% in colonic contents of conven-

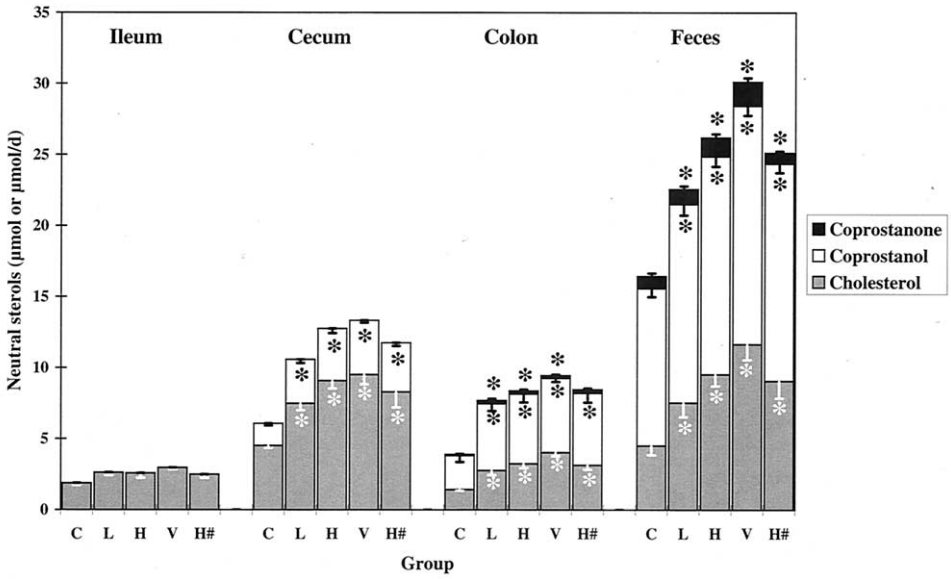

Fig. 2. Composition of neutral sterols in intestinal contents (in μmol) and in feces (collecting period A) (in μmol/day) of conventional rats fed control diet C or diets containing low-methoxyl pectin L, high-methoxyl pectin H, and very highly methoxylated pectin V or irradiated diet containing high-methoxyl pectin H#. Values are means ± SD, n = 8. * $P < 0.05$ compared to control group C.

tional rats but only approximately 55% in germfree rats. It seems that BA are evidently absorbed to a relatively higher degree in the lower parts of the gastrointestinal tract than the NS.

4. Discussion

Several physiological effects of pectin in the gastrointestinal tract are influenced by its functional or physicochemical properties which are caused – on the other hand - by its structural parameters (molecular weight, DM, arrangement of free and methoxylated carboxyl groups, etc.). It has been shown that pectin can interact with BA [8-15], but only few studies report an effect of pectin structure on serum cholesterol level and BA excretion [23-25]. We found that molecular parameters of the pectin may influence the qualitative and quantitative composition of acidic and neutral sterols in intestinal contents and feces of conventional and germfree

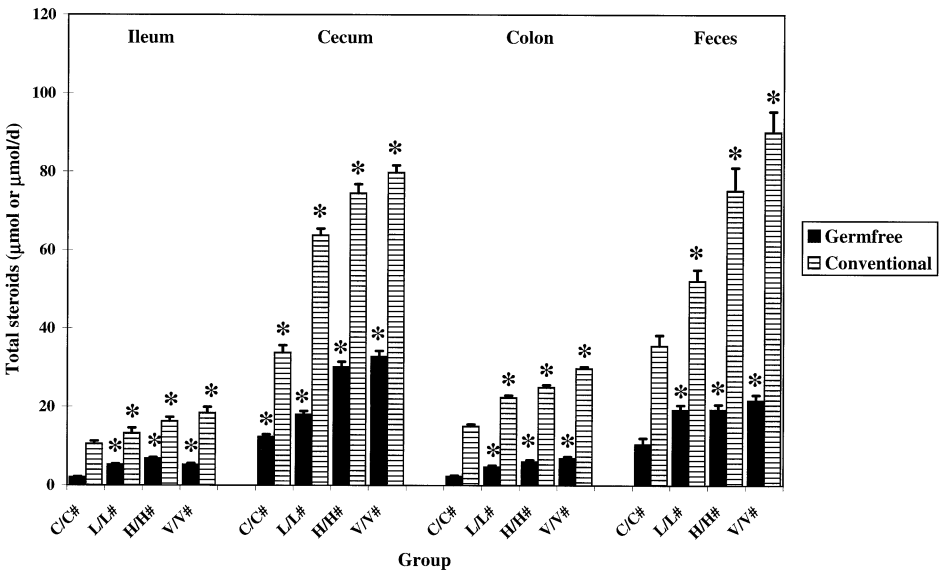

Fig. 3. Total steroids in intestinal contents (in μmol) and in feces (collecting period B) (in μmol/day) of conventional fed control diet C or diets containing low-methoxyl pectin L, high-methoxyl pectin H, and very highly methoxylated pectin V as well as germfree rats fed irradiated control diet C# or irradiated diets containing low-methoxyl pectin L#, high-methoxyl pectin H#, or very highly methoxylated pectin V#. Values are means ± SD, n = 8 (conventional rats) or 6-8 (germfree rats). * $P < 0.05$ compared to control groups C or C#.

rats. The pectins used differed in the DM (34.5, 70.8, and 92.6%, respectively) and additionally in their intrinsic viscosity [η]. Pectin can disturb or influence the micelle formation as well as the lipid digestion and absorption in small intestine. In these processes, BA and NS are involved. In presence of viscous DF like pectin, less steroids are reabsorbed and transported to liver via the enterohepatic circulation. As a result of this effect, they may be transported into the lower parts of the intestinal tract. Here they are continuously transformed by enzymes of the microflora (i.e., by deconjugation, dehydroxylation and other reactions).

Whereas the composition of BA was practically unchanged in intestinal contents of germfree rats, intense changes occurred in composition of BA during their passage the ileum, cecum, and colon of conventional animals. With increasing DM of pectin, more BA were transported into lower parts of the intestinal tract and finally excreted. Further, proportions of secondary BA were lower in conventional rats fed pectin-containing diets. Some secondary bile acids (i.e., DCA) formed may play a promotive role in colon carcinogenesis [32]. Fermentation of DF acts indirectly on the composition of steroids. Formation of SCFA (especially acetate, propionate, and butyrate) results in a decrease of pH in cecum and colon. A lower pH partially inhibits the activity of the bacterial enzyme 7α-dehydroxylase responsible for formation of secondary from primary BA.

The type of DF used in the diet may influence the BA metabolism. Thus, Ide and Horii [33] found that pectin, but not cellulose, increased the BA concentration in small intestine and cecum as well as the BA excretion in rats. Ebihara et al. [34] showed that methoxyl content, molecular weight and viscosity of pectins are important factors for their cholesterol-lowering effects in rats. After feeding male Wistar rats a diet with 7% pectin for 4 weeks, fecal BA excretion as well as hepatic activities of cholesterol 7α-hydroxylase and activities of 3-hydroxy-3-methylglutaryl-CoA reductase were higher whereas liver and serum cholesterol levels were reduced [21]. Feeding rats diets with 7.5% fermentable DF (including pectin) resulted in higher BA excretion, in a decrease in serum cholesterol, in higher cecal concentration of SCFA (propionate) as well as in higher microsomal hydroxymethylglutaryl CoA reductase and 7α-hydroxylase activities [35]. Matheson and Story [5] found generally a higher pool size for individual BA (especially CA, HDCA, αMCA, and βMCA) in pectin-fed rats compared with a cellulose-fed group. Further, pectin consumption resulted in a lower hydrophobicity of the BA pool and a lower ratio of circulating 12α-hydroxylated to non-12α-hydroxylated BA. The reduced hydrophobicity may lower the feedback inhibition of BA synthesis leading to the larger BA pool size.

Like in this study, a broad spectrum of BA in intestinal contents and feces was shown in other reports: Madsen et al. [36] found in feces of germfree Wistar rats 40.5% CA and 56.0% βMCA as main BA, whereas in conventional animals 34.0% DCA, 18.7% ωMCA, 15.6% DCA, 8.6% KLCA, and 3.9% CA were present. The predominant BA in the third quarter of the small intestine of conventional rats were CA (69.8%), βMCA (18.8%), and HDC (5.4%). Besides small concentrations of ωMCA, CDCA, and UDCA ($< 4\%$), the main BA in feces of germfree Fischer rats were βMCA (34.1%) and CA (25.3%) after feeding diets containing 5% pectin. On the other hand, 26.8% HDCA, 23.8% keto bile acids, 13.7% DCA, 6.3% ωMCA, and 4.1% αMCA were found in feces of conventional rats [37]. These data point to intense enzymatic actions of bacteria in cecum and colon on the BA. Further, the BA composition changed with the age of rats [38,39].

Whereas exclusively cholesterol appeared as a NS in germfree rats, additionally its microbial degradation products (especially coprostanol and coprostanone) were found in the lower parts of the intestinal tract in conventional rats. It was shown in our study that the amount of NS in intestinal contents is influenced by the DM as well as by the molecular weight of pectin. Besides, a correlation seems to exist between the fermentation of pectin and the extent of microbial conversion of cholesterol.

It was found that levels of some plasma lipids (e.g., LDL-cholesterol and BA) were lower in rats fed pectin-containing diets. A decrease in serum cholesterol and other serum lipids and an increase of excreted steroids in rats given pectin-supplemented diets were also reported in several studies [40-43]. In contrast, application of low-viscosity soluble DF containing up to 15 g of gum arabic and pectin (ratio 4:1) per 720 ml apple juice per day for 8 weeks did not change serum lipids in hypercholesterolemic subjects [44].

In conclusion, the amount of excreted of BA and NS as well as their composition during passage the intestinal tract are influenced by molecular and structural parameters of pectin used in the diet. The most distinct effects were found if macromolecular and very highly methoxylated pectins were applied. The qualitative composition of steroids in feces is a result of enzymatic transformation of the BA and NS by the microflora, which is additionally affected by the fermentation of the DF pectin.

Acknowledgments

The authors thank Dr. Wolfgang Lüder for analysis of the plasma lipids and Horst Maischack for valuable technical assistance.

References

[1] Dongowski G, Lorenz A, Proll J. The degree of methylation influences the degradation of pectin in the intestinal tract of rats and in vitro. J Nutr 2002;132:1935–44.

[2] Kritchevsky D, Story JA. Influence of DF on cholesterol metabolism in experimental animals. In: Spiller GA, editor. CRC Handbook of

Dietary Fiber in Human Nutrition. Boca Raton: CRC Press, 1986. p. 129–42.
[3] Kay RM, Truswell AS. Effect of citrus pectin on blood lipids and fecal steroid excretion in man. Am J Clin Nutr 1977;30:171–5.
[4] Arjmandi BH, Craig J, Nathani S, Reeves RD. Soluble dietary fiber and cholesterol influence in vivo hepatic and intestinal cholesterol biosynthesis in rats. J Nutr 1992;122:1559–65.
[5] Matheson HB, Story JA. Dietary psyllium hydrocolloid and pectin increase bile acid pool size and change bile acid composition in rats. J Nutr 1994;124:1161–5.
[6] Kishimoto Y, Wakabayashi S, Takeda H. Hypocholesterolemic effect of dietary fiber: relation to intestinal fermentation and bile acid excretion. J Nutr Sci Vitaminol Tokyo 1995;41:151–61.
[7] Vergara-Jimenez M, Furr H, Fernandez ML. Pectin and psyllium decrease the susceptibility of LDL to oxidation in guinea pigs. J Nutr Biochem 1999;10:118–24.
[8] Story JA. Modification of steroid excretion in response to dietary fiber. In: Vahouny GV, Kritchevsky D, editors. Dietary Fiber. Basic and Clinical Aspects. New York: Plenum Press, 1986. p. 253–64.
[9] Story JA, Lord SL. Bile salts: In vitro studies with fibre components. Scand J Gastroenterol 1987;22(Suppl 129):174–80.
[10] Pfeffer PE, Doner LW, Hoagland PD, McDonald GG. Molecular interactions with dietary fiber constituents. Investigation of the possible association of pectin and bile acids. J Agric Food Chem 1981; 29:455–61.
[11] Falk JD, Nagyvary JJ. Exploratory studies of lipid-pectin interactions. J Nutr 1982;112:182–8.
[12] Koseki M, Kitabatake N, Doi E, Yasuno T, Ogino S, Kazama M, Doguchi M. Binding of taurocholate by pectin in the presence of calcium ions. J Food Sci 1987;52:1744–5.
[13] Furda I. Interaction of dietary fiber with lipids-mechanistic theories and their limitations. In: Furda I, Brine CJ, editors. New Development in Dietary Fiber. New York: Plenum Press, 1990. p. 203–27.
[14] Pandolf T, Clydesdale FM. Dietary fiber binding of bile acid through mineral supplementation. J Food Sci 1992;57:1242–5.
[15] Klavons JA, Bennett RD. Preparation of alkyl esters of pectin and pectic acid. J Food Sci 1995;60:513–5.
[16] Ahrens F, Hagemeister H, Pfeuffer M, Barth CA. Effects of oral and intracecal pectin administration on blood lipids in minipigs. J Nutr 1986;116:70–6.
[17] Bosaeus I, Carlsson N-G, Sandberg A-S, Andersson H. Effect of wheat bran and pectin on bile acid and cholesterol excretion in ileostomy patients. Hum Nutr Clin Nutr 1986;40C:429–40.
[18] Baron SF, Hylemon PB. Biotransformation of bile acids, cholesterol, and steroid hormones. In: Mackie RI, White BA, editors. Gastrointestinal Microbiology. Vol. 1. Gastrointestinal Ecosystems and Fermentations. New York: Chapman and Hall, 1996. p. 470–510.
[19] Miettinen TA, Tarpila S. Effect of pectin on serum cholesterol, fecal bile acids and biliary lipids in normolipidemic and hyperlipidemic individuals. Clin Chim Acta 1977;79:471–7.
[20] Stasse-Wolthuis M, Albers HFF, van Jeveren JGC, de Jong JW, Hautvast JGAJ, Hermus RJJ, Katan MB, Brydon WG, Eastwood MA. Influence of dietary fiber from vegetables and fruits, bran or citrus pectin on serum lipids, fecal lipids, and colonic function. Am J Clin Nutr 1980;33:1745–56.
[21] Garcia-Diez F, Garcia-Mediavilla V, Bayon JE, Gonzales-Gallego J. Pectin feeding influences fecal bile acid excretion, hepatic bile acid and cholesterol synthesis and serum cholesterol in rats. J Nutr 1996; 126:1766–71.
[22] Scott RW, Anderson JW, Bridges SR. Propionate inhibits hepatocyte lipid synthesis. Proc Soc Exp Biol Med 1990;195:26–9.
[23] Judd PA, Truswell AS. The hypocholesterolaemic effects of pectin in rats. Br J Nutr 1985;53:409–25.
[24] Yamaguchi F, Uchida S, Watabe S, Kojima H, Shimizu N, Hatanaka C. Relationship between molecular weights of pectin and hypocholesterolemic effects in rats. Biosci Biotech Biochem 1995;59:2130–1.
[25] Terpstra AHM, Lapre JA, de Vries HT, Beynen AC. Dietary pectin with high viscosity lowers plasma and liver cholesterol concentration and plasma cholesteryl ester transfer protein activity in hamsters. J Nutr 1998;128:1944–9.
[26] Dongowski G. Influence of pectin structure on the interaction with bile acids under in vitro conditions. Z Lebensm Unters Forsch 1995; 201:390–8.
[27] Dongowski G. Effect of pH on the in vitro interactions between bile acids and pectin. Z Lebensm Unters Forsch 1997;205:185–92.
[28] Blumenkrantz N, Asboe-Hansen G. New method for quantitative determination of uronic acids. Anal Biochem 1973;54:484–9.
[29] Bäuerle G, Otterbach G, Gierschner K, Baumann G. Bestimmungen des Polyuronidgehaltes und des Veresterungsgrades des Pektinanteils in Handelspräparaten, Apfelsäften und Apfelmaceraten. 1. Mitt: Bestimmung des Polyuronidgehaltes und des Veresterungsgrades von Handelspektinpräparaten. Dtsch Lebensm Rundsch 1977;73:281–6.
[30] van der Meer R, de Vries H, Glatz JFC. t-Butanol extraction of feces: a rapid procedure for enzymatic determination of fecal bile acids. In: Beynen AC, Geelen MJH, Katan MB, Schouten JA, editors. Cholesterol Metabolism in Health and Disease: Studies in the Netherlands. Wageningen: Ponsen & Looijen, 1985. p. 113–5.
[31] Wang G, Stacy NH, Earl J. Determination of individual bile acids in serum by high performance liquid chromatography. Biomed Chromatogr 1990;4:136–40.
[32] Velázquez OC, Zhou D, Seto RW, Jabbar A, Choi J, Lederer HM, Rombeau JL. In vivo crypt surface hyperproliferation is decreased by butyrate and increased by deoxycholate in normal rat colon: associated in vivo effects on c-Fos and c-Jun expression. J Parenteral Enteral Nutr 1996;20:243–50.
[33] Ide T, Horii M. Predominant conjugation with glycine of biliary and lumen bile acids in rats fed on pectin. 1989;61:545–57.
[34] Ebihara K, Kiriyama S, Manabe M. Cholesterol-lowering activity of various natural pectins and synthetic pectin-derivatives with different physico-chemical properties. Nutr Rep Intern 1979;20:519–26.
[35] Moundras C, Behr SR, Demigné C, Mazur A, Rémésy C. Fermentable polysaccharides that enhance fecal bile acid excretion lower plasma cholesterol and apolipoprotein E-rich HDL in rats. J Nutr 1994;124:2179–88.
[36] Madsen D, Beaver M, Chang L, Bruckner-Kardoss E, Wostmann B. Analysis of bile acids in conventional and germfree rats. J Lipid Res 1976;17:107–11.
[37] Saquet E, Leprince C, Riottot M. Dietary fiber and cholesterol and bile acid metabolism in axenic (germfree) and holoxenic (conventional) rats. II. Effect of pectin. Reprod Nutr Develop 1982;22:575–81.
[38] Kambe A, Uchida K, Takase H, Noruma Y, Adachi Y. Bile acid metabolism in analbuminemic rats. Lipids 1998;33:93–9.
[39] Uchida K, Takase H, Nomura Y, Satoh K, Igimi H, Takeuchi N. Bile acid metabolism in young-old parabiotic rats. Lipids 1997;32:383–90.
[40] González M, Rivas C, Caride B, Lamas A, Tabaoda C. Effects of orange and apple pectin on cholesterol concentration in serum, liver and faeces. J Physiol Biochem 1998;54:99–104.
[41] Nishina PM, Schneeman BO, Freedland RA. Effects of dietary fibers on nonfasting plasma lipoprotein and apolipoprotein levels in rats. J Nutr 1991;121:431–37.
[42] Sudheesh S, Vijayalakshmi NR. Lipid-lowering action of pectin from Cucumis sativus. Food Chem 1999;67:281–6.
[43] Reddy BS, Watanabe K, Sheinfil A. Effect of dietary wheat bran, alfalfa, pectin and carrageenan on plasma cholesterol and fecal bile acids and neutral sterol excretion in rats. J Nutr 1980;110:1247–54.
[44] Davidson MH, Dugan LD, Stocki J, Dicklin MR, Maki KC, Coletta F, Cotter R, McLeod M, Hoersten K. A low-viscosity soluble-fiber fruit juice supplement fails to lower cholesterol in hypercholesterolemic men and women. J Nutr 1998;128:1927–32.