

Effects of Dietary Addition of a Low-Pectin Apple Fibre Preparation on Rats

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The aim of this study was to scrutinise if the dietary addition of a low-pectin fibre preparation obtained from apple pomace, the by-product of apple concentrate processing, is able to favourably affect the gut metabolism, antioxidant status and blood bio-markers of the organism, as it takes place when apple fibre is present in the diet as an unprocessed ingredient. The nutritional experiment was performed on rats allocated to 2 groups of 10 animals each and fed for 2 weeks with either a control cellulose-containing diet or an experimental low-pectin apple fibre-containing diet. To induce metabolic disorders a diet rich in saturated fat and fructose was used in both diet-specific groups. The dietary apple fibre preparation (AFP) significantly reduced the activity of sucrase and maltase in the mucosa of the small intestine. In the caecal digesta, the dietary AFP significantly increased bacterial α -glucosidase and α -galactosidase activity, whereas bacterial β -glucuronidase activity was significantly reduced. Also, the content of short chain fatty acids in the caecal digesta was significantly increased after the AFP supplementation. In the blood serum, the dietary AFP significantly reduced the glucose concentration, and decreased the ratio of total cholesterol to HDL cholesterol. In conclusion, the tested dietary AFP is still able to favourably affect the gut metabolism and can also ameliorate blood glucose concentration, which seems to be related to the inhibition of mucosal disaccharidase activities. However, the analysed preparation has no influence on the antioxidant status of the organism and may trigger adverse effects on cholesterol metabolism.

INTRODUCTION

Dietary fibre is a well-known group of indigestible food components that can favourably affect the hindgut physiology, *i.a.* by increasing gut motility, digesta volume and faecal weight, being simultaneously an important nutrient for the gut microbiota [Anderson & Hanna, 1999; Sembries *et al.*, 2004]. Bacterial fermentation of dietary fibre in the hindgut leads to the production of short-chain fatty acids (SCFA), including acetate, propionate and butyrate. Numerous studies have demonstrated that SCFA promote colonic epithelial health through several mechanisms, such as the stimulation of water and NaCl absorption, the maintenance of barrier function and the regulation of cell proliferation [Wong *et al.*, 2006; Fung *et al.*, 2012]. Dietary fibre is also partly responsible for the regulation of lipid and glucose metabolism in the body; it can *i.a.* reduce blood cholesterol concentration and post-prandial glycaemia [Anderson & Hanna, 1999]. Moreover, dietary fibre is found in apples together with polyphenols which are able to improve the antioxidant status of the organism and can help protect it against chronic diseases related to oxidative stress [Maffei *et al.*, 2007; Bonarska-Kujawa *et al.*, 2012; Juśkiewicz *et al.*, 2012].

It has been estimated that the consumption of fresh and processed apples provides human body 10–30% of the overall daily ingested dietary fibre depending on individual eating habits [Aprikian *et al.*, 2003]. Apples are also one of the most popular fruits that are processed by the food industry in Europe, especially to obtain apple concentrate as a base for the apple juice production. Although the technology of apple concentrate production is highly efficient, still 12–20% of processed raw material becomes a by-product which is especially rich in dietary fibre and is usually dumped or composted [Sudha *et al.*, 2007]. In order to increase the efficiency of apple juice extraction and to clarify the obtained product pectolytic enzymes are added. Pectin breakdown reduces the viscosity of pectin-rich crude juice and thus increases juice flow and reduces the overall press time [Jayani *et al.*, 2005]. In an apple tissue, pectolytic enzymes cause its maceration, cell lysis, modification of cell wall structures and depolymerisation [Kabbert *et al.*, 1997], thus changing the overall fibre structure of the pomace. In fresh apples, the soluble fibre fraction, mainly pectins, represents around 50–30% of the overall fibre content [Suni *et al.*, 2000; Colin-Henrion *et al.*, 2009], whereas apple pomace consists mainly of the insoluble fibre (about 83%) [Dongowski *et al.*, 2002]. Numerous articles describe the beneficial effect of apple fibre consumption and its favourable effects on such diet-related diseases as obesity, diabetes, cardiovascular disease and cancer [Hyson, 2011]. Nevertheless, most of the studies are fo-

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TABLE 1. Basic chemical composition of apple fibre preparation (g/100 g).

Component	Mean	SD
Dry matter	94.76	0.14
Ash	3.2	0.02
Fat	3.01	0.07
Protein	8.56	0.23
Total dietary fibre	75.3	0.58
Soluble dietary fibre	9.21	0.11

Mean value and standard deviation (SD, *n* 3).

cused on the dietary supplementation with apple fibre whose structure was not modified by the addition of pectolytic enzymes [Aprikian *et al.*, 2001, 2003; Maffei *et al.*, 2007; Dé-cordé *et al.*, 2008; Kim *et al.*, 2013].

The aim of this study was to scrutinise if the dietary addition of a low-pectin fibre preparation obtained from apple pomace, the by-product of apple concentrate processing, is able to favourably affect the gut metabolism, antioxidant status and blood bio-markers of the organism, as it takes place when apple fibre is present in the diet as an unprocessed ingredient. The nutritional experiment was performed on rats and a modified diet rich in saturated fat and fructose was used to induce metabolic changes related to the Westernisation of human eating habits.

MATERIAL AND METHODS

Apple fibre preparation (AFP)

Apple pomace as a waste product obtained from dessert apple cultivars during clarified juice production with an enzyme-assisted pressing was provided from the ALPEX Company (Łęczeszyce, Poland). The fresh pomace from the Bücher-type press was dried in a convection oven at a temperature of 70°C until the moisture content was lower than 5%, and then the dried material was sieved (mesh size: 5 mm and 3 mm) to discard the seeds. Afterwards, the sieved material was ground to powder in a disc mill. The obtained apple fibre preparation was then assayed in duplicate for the content of dry matter, crude protein, fat, ash, total dietary fibre and insoluble dietary fibre using standard AOAC methods [AOAC, 2005]. The chemical composition of the apple fibre preparation is shown in Table 1. The preparation was especially rich in dietary fibre (75.3 g/100 g), but contained also some amounts of protein and fat (8.56 g and 3.01 g per 100 g, respectively)

Animals and diets

The animal protocol used in the present study was approved by the local Institutional Animal Care and Use Committee. The experiment was conducted on twenty male Wistar rats aged 49 days that were randomly allocated to two groups of ten animals each. The average initial body weight was comparable between both groups and equalled 189.1 ± 1.6 g in total. All animals with comparable body weight were housed individually over 2 weeks in metabolic cages (Tecniplast Spa, Buguggiate, Italy) with free access to water and semi-

TABLE 2. Composition of diets (g/100 g)

Component	Diet	
	C	A
Casein ¹	11.35	11.35
DL-methionine	0.15	0.15
Lard	7	7
Cholesterol ²	1	1
Cellulose ³	5	-
Apple fibre preparation	-	6.7
Mineral mix ⁵	3.5	3.5
Vitamin mix ⁶	1	1
Fructose ⁴	69	69
Maize starch ⁷	2	0.3
<i>Calculated content of selected dietary ingredients</i>		
Dietary protein	10.1	10.6
Dietary fibre	5.0	5.0

1. Casein preparation contained 89.7% protein, 0.3% fat, 2.0% ash and 8.0% water (Lacpol Company, Murowana Goslina, Poland).
2. Sigma-Aldrich (Poznań, Poland).
3. α -Cellulose (Sigma).
4. ADM Corn Processing (USA).
5. AIN-93G-MX (per kg mix): 357 g calcium carbonate anhydrous (40.04% Ca), 196 g potassium phosphate monobasic (22.76% P and 28.73% K), 70.78 g potassium citrate, tripotassium monohydrate (36.16% K), 74 g NaCl (39.34% Na and 60.66% Cl), 46.6 g potassium sulphate (44.87% K and 18.39% S), 24 g magnesium oxide (60.32% Mg), 6.06 g ferric citrate (16.5% Fe), 1.65 g zinc carbonate (52.14% Zn), 1.45 g sodium meta-silicate 9H₂O (9.88% Si), 0.63 g manganous carbonate (47.79% Mn), 0.3 g cupric carbonate (57.47% Cu), 0.275 g chromium potassium sulphate 12H₂O (10.42% Cr), 81.5 mg boric acid (17.5% B), 63.5 mg sodium fluoride (45.24% F), 31.8 mg nickel carbonate (45% Ni), 17.4 mg lithium chloride (16.38% Li), 10.25 mg sodium selenate anhydrous (41.79% Se), 10 mg potassium iodate (59.3% I), 7.95 mg ammonium paramolybdate 4H₂O (54.34% Mo), 6.6 mg ammonium vanadate (43.55% V) and 221.026 g powdered sucrose.
6. AIN-93G-VM (g/kg mix): 3.0 nicotinic acid, 1.6 calcium pantothenate, 0.7 pyridoxine-HCl, 0.6 thiamine-HCl, 0.6 riboflavin, 0.2 folic acid, 0.02 biotin, 2.5 vitamin B12 (cyanocobalamin, 0.1% in manitol), 15.0 vitamin E (all-rac- α -tocopheryl acetate, 500 mg/g), 0.8 vitamin A (all-trans-retinyl palmitate, 275 mg/g), 0.25 vitamin D3 (cholecalciferol, 10 mg/g), 0.075 vitamin K1 (phylloquinone), 974.655 powdered sucrose.
7. AVEBE (Veendam, Holland).

purified casein diets. The diet intake was monitored in daily intervals. The environment was controlled with a 12 h light and 12 h dark cycle, a temperature of 21 ± 1°C and twenty air changes/h. The control diet contained cellulose as a fibre source (group C), whereas in the experimental diet, the AFP was added at the expense of cellulose and a part of maize starch (6.7% of the diet, group A). Both diets were similar in terms of the content of dietary ingredients and their detailed composition is shown in Table 2.

Analytical procedures

During the first 10 days of experimental feeding, the nitrogen excretion pattern was determined. The initial 5-day preliminary period was aimed at adapting the gut micro-

biota to the diets, followed by a 5-day experimental period, in which faeces and urine were collected once daily from all rats. Nitrogen in faeces and urine was determined according to the Kjeldahl's method. Apparent nitrogen digestibility and nitrogen retention were used as the criteria of nutritional quality of the diets.

At the termination of the experiment, the rats were anaesthetised with the intraperitoneal injection of sodium pentobarbital (60 mg/kg body weight) according to the recommendations for euthanasia of experimental animals. After laparotomy, blood samples were taken from the caudal vein, and then small intestine, caecum, colon, liver, heart and kidneys were removed and weighed.

The small intestine was divided into four equal parts, and the second part from the stomach side was rinsed with ice-cold physiological saline and cut open. The mucosal samples were collected by scraping with glass slides on an iced glass plate, weighed and subsequently stored at -20°C . The mucosal sucrase and maltase activity was assayed by the method of Dahlqvist [1964] with modifications described elsewhere [Jurgoński *et al.*, 2013] and expressed as micromoles of disaccharide hydrolysed per min per g of protein.

Samples of the caecal digesta were used for the immediate determination of ammonia, pH value and dry matter, whereas the rest of the digesta was transferred into tubes and stored at -70°C . Ammonia was extracted from the caecal digesta, trapped in a solution of boric acid in Conway's dishes and determined by direct titration with sulphuric acid. The pH value was measured using a microelectrode and a pH/ion meter (model 301; Hanna Instruments, Vila do Conde, Portugal). The dry matter was determined at 105°C .

SCFA content in the caecal digesta was determined using a gas chromatograph (Shimadzu GC-2010, Kyoto, Japan). The 0.2 g sample was mixed with 0.2 mL formic acid, diluted with deionized water and centrifuged at $7211 \times g$ for 10 min. The supernatant was then injected (1 μL) onto a capillary column (SGE BP21, 30 m \times 0.53 mm; SGE Europe Ltd, Milton Keynes, UK) using an on-column injector. The initial oven temperature was 85°C and was raised to 180°C by 8°C per min and held there for 3 min. The temperatures of the flame ionization detector and the injection port were 180°C and 85°C , respectively.

Microbial glycolytic activity in the caecal digesta (α - and β -glucosidase, α - and β -galactosidase and β -glucuronidase) were measured spectrophotometrically using the rate of *p*- or *o*-nitrophenol release from the respective nitrophenyl-glycosides and expressed as μmol product formed per h per g of the digesta [Juśkiewicz *et al.*, 2012].

Serum concentration of triacylglycerols (TAG), total cholesterol and its HDL fraction were estimated with reagents from Alpha Diagnostics Ltd. (Warsaw, Poland). The activity of superoxide dismutase (SOD) in erythrocyte lysate was determined using reagents from Randox Laboratories Ltd. (Crumlin, Antrim, UK). The serum antioxidant capacity of water- and lipid-soluble substances was determined by a photochemiluminescence detection method using a Photochem (Analytik Jena AG, Jena, Germany). In the photochemiluminescence assay, the generation of free radicals was partially eliminated by the reaction with antiox-

idants present in the serum samples, and the remaining radicals were quantified by luminescence generation. The results were expressed as mmol of ascorbate or Trolox equivalents per mL of serum.

The extent of lipid peroxidation in selected tissues (kidneys, liver and heart) was determined as the content of thio-barbituric acid-reactive substances (TBARS) according to Uchiyama & Mihara [1978] and expressed in nmol of malondialdehyde per gram of tissue.

Statistical analysis

Results were analysed statistically using unpaired two-tailed Student's *t*-test at a significance level of $P \leq 0.05$. Results are expressed as mean value and standard error of the mean (SEM, $n=10$). Calculations were done using STATISTICA 8.0 (StatSoft Corporation, Kraków, Poland).

RESULTS

The dietary AFP used in the study had no significant effect on the body weight of rats (Table 3). The rats fed with the A diet showed a significant increase of N intake and N excretion with faeces, therefore their N apparent digestibility index significantly decreased. Conversely, the N retention index that takes into account losses of N both with faeces and urine significantly increased in this group. The dietary supplementation with AFP had effect on the dry matter of digesta, which was significantly decreased both in the ileum and caecum. In the mucosa of the small intestine, the sucrase and maltase activity was also found to be significantly lower in group A. Moreover, the 2-week administration of dietary AFP to rats tended to decrease the pH of caecal digesta ($P=0.065$) and significantly decreased the pH of colonic digesta. The AFP supplementation lowered also the ammonia concentration in the caecal digesta.

Bacterial enzyme activity and SCFA content in the caecal digesta of rats are shown in Table 4. The dietary supplementation with AFP significantly increased the α -glucosidase activity and to more than two times the α -galactosidase activity. Furthermore, the β -glucuronidase activity was significantly lower in group A than group C. The total SCFA content was significantly elevated in group A, which resulted from the increased content of acetic, propionic, butyric and valeric acids. The content of branched-chain fatty acids (*iso*-butyric and *iso*-valeric acids) did not differ significantly between both groups. Also, the proportions of acetate, propionate and butyrate did not change after AFP supplementation.

Group A was characterised by a significantly lower serum glucose level (Table 5). Blood triglyceride concentration was similar between both groups, however, a trend towards increased serum total cholesterol concentration ($P=0.07$) and a trend towards decreased HDL cholesterol concentration ($p=0.09$) was noted in the blood serum of rats of group A. As a result, a significantly decreased ratio of total cholesterol to HDL cholesterol was observed in this group. Moreover, the AFP supplementation had neither significant effect on antioxidant parameters measured in blood (SOD activity, ACW and ACL) nor on the tissue TBARS content of selected internal organs.

TABLE 3. Body weight (BW), nitrogen excretion pattern and indicators of gastrointestinal tract function of rats.

Parameters	C		A	
	Mean	SEM	Mean	SEM
Final BW (g)	223.4	6.6	232.4	3.4
Nitrogen balance:				
N intake (mg/5 d)	1150	7.6	1289*	7.1
N in faeces (mg/5 d)	128	0.8	182*	3.1
N in urine (mg/5 d)	561	15.4	534	19.5
N apparent digestibility ¹ (%)	88.9	0.1	85.9*	0.1
N retention ² (%)	40.1	1.3	44.5*	1.5
Small intestine				
pH of ileal digesta	7.2	0.13	7.04	0.12
Dry matter (%)	22.2	0.68	19.2*	0.92
Mucosal disaccharidase activity ($\mu\text{mol}/\text{min}/\text{g}$):				
Sucrase	40.3	1.67	33.4*	1.56
Maltase	50.8	1.97	45.1*	1.28
Caecum				
Tissue (g/100 g BW)	0.32	0.01	0.32	0.86
Digesta (g/100 g BW)	1.19	0.1	1.21	0.83
pH of digesta	7.06	0.04	6.96	0.28
Dry matter (%)	22.14	0.89	19.32*	0.67
NH ₃ (mg/g)	0.43	0.01	0.36	0
Colon				
Tissue (g/100 g BW)	0.64	0.02	0.65	0.03
Digesta (g/100 g BW)	0.64	0.06	0.45	0.07
pH of digesta	6.98	0.03	6.71*	0.04

C, control saturated fat-rich high-fructose diet; A, saturated fat-rich high-fructose diet supplemented with 6.7% apple fibre preparation. ¹Apparent digestibility: $[\text{N intake} - \text{N faecal} / \text{N intake}] \times 100$; ²Retention: $[\text{N intake} - \text{N faecal} - \text{N urinary} / \text{N intake}] \times 100$. * Significantly different from group C ($P < 0.05$).

DISCUSSION

Bhushan *et al.* [2008] have reported that apple pomace contains 35–60 g/100 g of total dietary fibre depending on the type of apple and the method of processing employed. The AFP used in the present experiment was rich in insoluble dietary fibre (66.1 g/100 g) and poor in soluble dietary fibre (9.21 g/100 g), which in apples consists mainly of pectins (Table 1). Depectinization of apple pomace results from the application of pectolytic enzymes during the production of apple concentrate [Kashyap *et al.*, 2001].

In the present nutritional experiment, the dietary fibre content was the same in both groups of rats. However, the AFP contained also some amounts of protein (8.56 g/100 g), which resulted in slightly increased overall content of this nutrient in the diet of group A (group C, 10.1% vs. group A, 10.6%). That difference seemed to be sufficient to affect significantly nitrogen excretion pattern of rats from group A; they consumed and excreted with faeces significantly more protein

than animals from the control group. Another important factor that could increase nitrogen content in the faeces of rats from group A was intensified bacterial fermentation. Although the AFP consisted mainly of insoluble fibre, there were still some amounts of the soluble fibre fraction (9.21 g/100 g) which is a known energetic substrate for the hindgut microbiota and could stimulate their anabolic processes connected with increased protein production [Howard *et al.*, 2000]. The aforementioned factors were decisive in the reduction of apparent digestibility observed after the AFP supplementation. Moreover, group A was also characterised by the increased nitrogen retention. Indeed, some studies point that more fermentable oligosaccharides are able to reduce N excretion in urine [Younes *et al.*, 1995; Howard *et al.*, 2000]. The increased nitrogen retention in the present study may explain why rats from group A had similar final BW to the control animals despite the decreased apparent nitrogen digestibility. A lack of the effect of apple dietary fibre on BW of rats is also suggested by other authors, who have not found any negative effects neither on diet intake nor on BW gain of rats when 5% pectin-rich apple preparations were added to the diet [Aprikian *et al.*, 2003].

In the present study, the degree of bacterial fermentation in the caecal digesta was followed *i.a. via* the determination of microbial enzymatic activity. Both α -glucosidase and α -galactosidase activity was significantly increased in rats fed the AFP-supplemented diet. The increased bacterial activity in the caecal digesta might have led to the increased ammonia utilization as a nitrogen source, and the significantly lowered ammonia concentration observed in the caecal digesta of group A supports that supposition. Topping [1996] in his study has shown that the main source of nitrogen necessary for proliferation of intestinal bacteria is ammonia derived from urea which is produced by ureolytic bacteria. Furthermore, the decreased ammonia concentration is considered a positive change because this compound can destroy cells, alter nucleic acid synthesis, induce cancerous cell growth and increase virus infections in the lower bowel upon administration of the usual Western diet [Visek, 1978]. In contrast to the above-mentioned enzymes, the activity of β -glucuronidase in the caecal digesta of group A was significantly decreased. Robertson *et al.* [1982] have suggested that the most important factor in the modulation of β -glucuronidase activity in the rat's large bowel is the bile flow. Indeed, dietary fibre is the main factor that intensifies peristalsis and thus the bile flow through the large intestine [Anderson & Hanna, 1999]. Some studies have also reported that polyphenols present in an apple pomace and cereals can inhibit the enzymatic activity of undesirable intestinal microbiota, such as tryptophanase, β -glucuronidase and β -glucosidase, which may play positive roles in the limitation of colon cancer [Ohkami *et al.*, 1995; Lahouar *et al.*, 2012].

The major metabolites of bacterial fermentation in the distal section of the digestive tract are SCFA. In the present study, the AFP significantly increased the concentration of total and particular SCFA in the caecal digesta of rats, especially the concentration of propionate and butyrate whose presence in the large intestine is an important factor in sustaining health of the local epithelium and suppression of cancerous

TABLE 4. Bacterial enzyme activity and SCFA content in the caecal digesta of rats.

Parameter	C		A	
	Mean	SEM	Mean	SEM
Enzyme activity ($\mu\text{mol/h per g digesta}$):				
α -Glucosidase	6.61	0.52	9.32*	1.09
β -Glucosidase	1.85	0.36	2.08	0.59
α -Galactosidase	2.76	0.34	6.87*	0.69
β -Galactosidase	36.9	1.96	39.6	1.87
β -Glucuronidase	5.78	0.45	3.95*	0.32
SCFA ($\mu\text{mol/g digesta}$):				
Acetic acid	44.9	3.11	56.3*	2.71
Propionic acid	9.84	0.8	13.6*	0.62
<i>Iso</i> -butyric acid	0.64	0.08	0.51	0.07
Butyric acid	5.24	0.41	7.36*	0.6
<i>Iso</i> -valeric acid	0.69	0.07	0.58	0.02
Valeric acid	0.62	0.06	0.86*	0.02
Total SCFA	62.3	3.89	79.3*	3.46
SCFA profile (% total content):				
Acetic acid	72	0.8	71	0.97
Propionic acid	16	0.4	17	0.7
Butyric acid	8	0.6	9	0.6

C, control saturated fat-rich high-fructose diet; A, saturated fat-rich high-fructose diet supplemented with 6.7% apple fibre preparation; SCFA, short-chain fatty acids. * Significantly different from group C ($P \leq 0.05$).

cell growth [O'Keefe *et al.*, 2009]. It has been reported that in colorectal tumour cells butyric acid can induce apoptosis and differentiation, as well can act as an active inhibitor of proliferation, cell cycle, angiogenesis and histone deacetylase [Fung *et al.*, 2012]. Moreover, acetate and propionate which are also significantly increased in the present study can differentially influence fat metabolism; acetate seems to promote lipogenesis in the liver, whereas propionate has been reported to inhibit this process [Nishina & Freedland, 1990; Demigné *et al.*, 1995], however, there are strong evidences suggesting that the inhibitory effect of propionate is rather unlikely [Illman *et al.*, 1988]. In the present study, the intensification of SCFA synthesis in group A resulted in just slightly lowered pH value of the caecal digesta and significantly lowered pH of the colon digesta. It is noteworthy that the acidification of the digesta promotes positive microbiota proliferation and decreases the growth of pathogenic bacteria species [Topping & Clifton, 2001].

In this study, dietary fructose and lard were used to induce metabolic changes related to the Westernisation of human eating habits. Numerous studies have reported that high fructose and lard contents in the diet are associated with various unfavourable metabolic disturbances in rodents, such as hyperlipidemia and hyperinsulinemia [Buettner *et al.*, 2006; Poudyal *et al.*, 2010]. It is noteworthy, however, that lard was the sole dietary source of fat in the present study, thus rats were deprived of essential fatty acids for 2 weeks. On the other hand, Aprikian *et al.* [2001] proved in an experiment on rats that a moderate dietary supplementation of lyophilized apple containing low amount of pectin (15% of the diet) exerted slight plasma cholesterol-lowering effects. The same authors in another study on rats have shown that the administration of diets

TABLE 5. Blood biochemical parameters and antioxidant status of rats.

Parameter	C		A	
	Mean	SEM	Mean	SEM
Glucose (mg/dL)	237	6.5	194*	7.9
TAG (mg/dL)	137	4.9	140	20.1
Total cholesterol (mg/dL)	122	8.1	141	5.8
HDL cholesterol (mg/dL)	49.3	1.5	45.7	1.3
HDL cholesterol (% total cholesterol)	40.4	1.8	32.4*	2.4
SOD (U/mL)	298	14.1	312	14.5
ACW (nmol/mL)	50.7	4.9	49.1	4.3
ACL (nmol/mL)	52.3	1.6	54.3	2.0
TBARS (nmol/g tissue):				
Kidney	129	7.1	121	6.4
Liver	77.1	1.9	76.9	0.9
Heart	81.7	3.9	77.9	0.4

C, control saturated fat-rich high-fructose diet; A, saturated fat-rich high-fructose diet supplemented with 6.7% apple fibre preparation; TAG, triacylglycerol; HDL, high-density lipoprotein; SOD, superoxide dismutase; ACW, serum antioxidant capacity of water-soluble substances; ACL, serum antioxidant capacity of lipid-soluble substances; TBARS, thiobarbituric acid-reactive substances. * Significantly different from group C ($P \leq 0.05$).

containing 5% apple pectin and 10% high polyphenol freeze-dried apples effectively lowered circulating cholesterol and triglyceride concentrations [Aprikian *et al.*, 2003]. Moreover, Krzysik *et al.* [2011] in the study on rats demonstrated that 5% content of pectins in the diet significantly reduced plasma total cholesterol level. In the present study, the dietary addition of apple fibre preparation unfavourably decreased the proportion of HDL cholesterol in the blood. It may be concluded that the negative effect of the AFP supplementation was probably a consequence of the reduced pectin content.

In the reported study, a favourable decrease of blood glucose concentration was observed in rats fed with the AFP-supplemented diet. Takahashi *et al.* [2005] have suggested that the ingestion of cellulose increases digesta viscosity and thus may delay glucose diffusion from the lumen of the upper gastrointestinal tract. Another potential mechanism by which dietary fibre can reduce postprandial glycaemia is the inhibition of mucosal disaccharidase activity as it was observed in the present study. Both the mucosal sucrase and maltase activities were significantly lowered in the small intestine of rats from group A. It is noteworthy that such inhibitory effect is especially desirable if the organism is at the risk of diabetes and the reduced absorption of glucose and other monosaccharides may improve the regulation of blood glucose level [Juśkiewicz *et al.*, 2008]. Also, the blood glucose-lowering effect of apple consumption is suggested in human studies [De Oliveira *et al.*, 2003].

Ghibu *et al.* [2013] have shown that a high-fructose diet (60% of the diet) can be used as a model to induce oxidative stress in rats. Also, Zagrodzki *et al.* [2007] have shown that the diet high in fructose significantly changes the antioxidant defence status of rats, which may be counteracted by the ad-

dition of antioxidants. Furthermore, Maffei *et al.* [2007] have suggested that the consumption of whole apple provides a useful dietary source of active scavengers to protect cells and tissues from oxidative stress, whereas Juśkiewicz *et al.* [2012] have demonstrated that the polyphenol-rich fibre complexes from apple pomace exert positive effects on the antioxidant status of rats. In the present experiment, the parameters of antioxidant status did not change after AFP administration to a high-fructose diet. This can indicate that some important antioxidants are separated from pomace during the production of apple concentrate.

CONCLUSIONS

In conclusion, this study on rats fed with a saturated fat-rich, high-fructose diet illustrates that the dietary supplementation with a low-pectin fibre preparation obtained from apple pomace as a by-product of apple concentrate processing is still able to favourably affect the gut metabolism. Moreover, the dietary addition of the apple fibre preparation can also ameliorate blood glucose concentration, which seems to be related to the inhibition of mucosal disaccharidase activity. However, the analysed preparation seems to have no influence on the antioxidant status of the organism and can adversely affect cholesterol metabolism.

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REFERENCES

- Anderson J.W., Hanna T.J., Impact of nondigestible carbohydrates on serum lipoproteins and risk for cardiovascular disease. *J. Nutr.*, 1999, 129, 1457S–1466S.
- AOAC (Association of Official Analytic Chemists) (2005) Official Methods of Analysis of the Association of the Official Analytical Chemists, 18th ed. Arlington, VA: AOAC.
- Aprikian O., Duclos V., Guyot S., C Besson C., Manach A., Bernalier C., Morand C., Demigné R., Demigné C., Apple pectin and polyphenol-rich apple concentrate are more effective together than separately on cecal fermentations and plasma lipids in rats. *J. Nutr.*, 2003, 133, 1860–1865.
- Aprikian O., Levrat-Vérny M., Besson C., Busserolles J., Rémésy J., Demigné C., Apple favourably affects parameters of cholesterol metabolism and of anti-oxidative protection in cholesterol-fed rats. *Food Chem.*, 2001, 75, 445–452.
- Bhushan S., Kalia K., Sharma M., Singh B., Ahuja P.S., Processing of apple pomace for bioactive molecules. *Crit. Rev. Biotechnol.*, 2008, 28, 285–296.
- Bonarska-Kujawa D., Sarapuk J., Bielecki K., Oszmiański J., Kleszczyńska H., Anti-oxidant activity of extracts from apple, chokeberry and strawberry. *Pol. J. Food Nutr. Sci.*, 2012, 62, 229–234.
- Buettner R., Parhofer K.G., Woienckhaus M., Wrede C.E., Kunz-Schughart L.A., Scholmerich J., Bollheimer L.C., Defining high-fat-diet rat models: metabolic and molecular effects of different fat types. *J. Mol. Endocrinol.*, 2006, 36, 485–501.
- Colin-Henrion M., Mehinagic E., Renard C.M.G.C., Richomme P., Jourjon F., From apple to applesauce: Processing effects on dietary fibres and cell wall polysaccharides. *Food Chem.*, 2009, 117, 254–260.
- Dahlqvist A., Method for assay of intestinal disaccharidases. *Anal. Biochem.*, 1964, 7, 18–25.
- De Oliveira M.C., Sichieri R., Moura A.S., Weight loss associated with a daily intake of three apples or three pears among overweight women. *Nutrition*, 2003, 19, 253–256.
- Décorde K., Teissèdre P., Auger C., Cristol J.P., Rouanet J.M., Phenolics from purple grape, apple, purple grape juice and apple juice prevent early atherosclerosis induced by an atherogenic diet in hamsters. *Mol. Nutr. Food Res.*, 2008, 52, 400–407.
- Demigné C., Morand C., Levrat M.A., Besson C., Moundras C., Rémésy C., Effect of propionate on fatty acid and cholesterol synthesis and on acetate metabolism in isolated rat hepatocytes. *Br. J. Nutr.*, 1995, 74, 209–219.
- Dongowski G., Sembries S., Bauckhage K., Willand F., Dietrich H., Degradation of apple cell wall material by commercial enzyme preparations. *Nahrung-Food*, 2002, 46, 105–111.
- Fung K.Y., Cosgrove L., Lockett T., Head R., Topping D.L., A review of the potential mechanisms for the lowering of colorectal oncogenesis by butyrate. *Br. J. Nutr.*, 2012, 108, 820–831.
- Ghibu S., Decea N., Morgovan C., Mogosan C., An experimental model to induce metabolic syndrome in rats. The fructose-enriched diet. *Farmacia*, 2013, 61, 420–426.
- Howard M.D., Kerley M.S., Sunvold G.D., Reinhart G.A., Source of dietary fiber fed to dogs affects nitrogen and energy metabolism and intestinal microflora populations. *Nutr. Res.*, 2000, 20, 1473–1484.
- Hyson D.A., Comprehensive review of apples and apple components and their relationship to human health. *Adv. Nutr.*, 2011, 2, 408–420.
- Illman R.J., Topping D.L., McIntosh G.H., Trimble R.P., Storer G.B., Taylor M.N., Cheng B.Q., Hypocholesterolaemic effects of dietary propionate: studies in whole animals and perfused rat liver. *Ann. Nutr. Metab.*, 1988, 32, 95–107.
- Jayani R.S., Saxena S., Gupta R., Microbial pectinolytic enzymes: a review. *Process Biochem.*, 2005, 40, 2931–2944.
- Jurgoński A., Juśkiewicz J., Zduńczyk Z., An anthocyanin-rich extract from Kamchatka honeysuckle increases enzymatic activity within the gut and ameliorates abnormal lipid and glucose metabolism in rats. *Nutrition*, 2013, 29, 898–902.
- Jurgoński A., Juśkiewicz J., Zduńczyk Z., Ingestion of black chokeberry fruit extract leads to intestinal and systemic changes in a rat model of prediabetes and hyperlipidemia. *Plant Foods Hum. Nutr.*, 2008, 63, 176–182.
- Juskiewicz J., Zdunczyk Z., Jurgonski A., Brzuzan L., Godycka-Klos I., Zary-Sikorska E., Extract of green tea leaves partially attenuates streptozotocin-induced changes in antioxidant status and gastrointestinal functioning in rats. *Nutr. Res.*, 2008, 28, 343–349.
- Juśkiewicz J., Żary-Sikorska E., Zduńczyk Z., Król B., Jarosławska J., Jurgoński A., Effect of dietary supplementation with unprocessed and ethanol-extracted apple pomaces on caecal fermentation, antioxidant and blood biomarkers in rats. *Br. J. Nutr.*, 2012, 107, 1138–1114.

24. Kabbert R., Goworek S., Kunzek H., Preparation of single-cell material from enzymatically disintegrated apple tissue – changes in structure and functional properties. *Z. Lebensm. Unters. Forsch.*, 1997, 205, 380–387.
25. Kashyap D.R., Vohra P.K., Chopra S., Tewari R., Applications of pectinases in the commercial sector: a review. *Biores. Technol.*, 2001, 77, 215–227.
26. Kim Y., Kim Y., Bae I.Y., Lee H.G., Lee S., Preparation of dietary fibre-enriched materials from preharvest dropped apples and their utilisation as a high-fibre flour substitute. *J. Sci. Food Agric.*, 2013, 93, 1974–1978.
27. Krzysik M., Grajeta H., Prescha A., Weber R., Effect of cellulose, pectin and chromium(III) on lipid and carbohydrate metabolism in rats. *J. Trace. Elem. Med. Biol.*, 2011, 25, 97–102.
28. Lahouar L., Pochart P., Hichem B.S., El Felah M., Mokni M., Magne F., Mangin I., Suau A., Pereira E., Hammami M., Achour L., Effect of dietary fibre of barley variety ‘Rihane’ on azoxymethane-induced aberrant crypt foci development and on colonic microbiota diversity in rats. *Br. J. Nutr.*, 2012, 108, 2034–2042.
29. Maffei F., Tarozzi A., Carbone F., Marchesi A., Hrelia S., Angeloni C., Forti G.C., Hrelia P., Relevance of apple consumption for protection against oxidative damage induced by hydrogen peroxide in human lymphocytes. *Br. J. Nutr.*, 2007, 97, 921–927.
30. Nishina P.M., Freedland R.A., Effects of propionate on lipid biosynthesis in isolated rat hepatocytes. *J. Nutr.*, 1990, 120, 668–673.
31. O’Keefe S.J.D., Ou J., Aufreiter S., O’Connor D., Sharma S., Sepulveda J., Fukuwatari T., Shibata K., Mawhinney T., Products of the colonic microbiota mediate the effects of diet on colon cancer risk. *J. Nutr.*, 2009, 139, 2044–2048.
32. Ohkami H., Tazawa K., Yamashita I., Effects of apple pectin on fecal bacterial enzymes in azoxymethane-induced rat colon carcinogenesis. *Jpn. J. Cancer Res.*, 1995, 86, 523–529.
33. Poudyal H., Campbell F., Brown L., Olive leaf extract attenuates cardiac, hepatic, and metabolic changes in high carbohydrate-, high fat-fed rats. *J. Nutr.*, 2010, 140, 946–953.
34. Robertson A.M., Lee S.P., Lindop R., Stanley R.A., Thomsen L., Tasman-Jones C., Biliary control of beta-glucuronidase activity in the luminal contents of the rat ileum, cecum, and rectum. *Cancer Res.*, 1982, 42, 5165–5166.
35. Sembries S., Dongowski G., Mehrlander K., Will F., Dietrich H., Dietary fiber-rich colloids from apple pomace extraction juices do not affect food intake and blood serum lipid levels, but enhance fecal excretion of steroids in rats. *J. Nutr. Biochem.*, 2004, 15, 296–302.
36. Sudha M.L., Baskaran V., Leelavathi K., Apple pomace as a source of dietary fiber and polyphenols and its effects on the rheological characteristics and cake making. *Food Chem.*, 2007, 104, 686–692.
37. Suni M., Nyman M., Eriksson N.A., Björck L., Björck I., Carbohydrate composition and content of organic acids in fresh and stored apples. *J. Sci. Food Agr.*, 2000, 80, 1538–1544.
38. Takahashi T., Karita S., Ogawa N., Goto M., Crystalline cellulose reduces plasma glucose concentrations and stimulates water absorption by increasing the digesta viscosity in rats. *J. Nutr.*, 2005, 135, 2405–2410.
39. Topping D.L., Clifton P.M., Short-chain fatty acids and human colonic function: roles of resistant starch and nonstarch polysaccharides. *Physiol. Rev.*, 2001, 81, 1031–1064.
40. Topping D.L., Short-chain fatty acids produced by intestinal bacteria. *Asia Pacific J. Clin. Nutr.*, 1996, 5, 15–19.
41. Uchiyama M., Mihara M., Determination of malonaldehyde precursor in tissues by thiobarbituric acid test. *Anal. Biochem.*, 1978, 86, 271–278.
42. Visek W.J., Diet and cell growth modulation by ammonia. *Am. J. Clin. Nutr.*, 1978, 31, 216–220.
43. Wong J.M., de Souza R., Kendall C.W., Emam A., Jenkins D.J., Colonic health: fermentation and short chain fatty acids. *J. Clin. Gastroenterol.*, 2006, 40, 235–243.
44. Younes H., Demigne C., Behr S., Remesy C., Resistant starch exerts a lowering effect on plasma urea by enhancing urea N transfer into the large intestine. *Nutr. Res.*, 1995, 15, 1199–1210.
45. Zagrodzki P., Joniec A., Gawlik M., Gawlik M., Krosniak M., Folta M., Barton H., Pasko P., Chlopicka J., Zachwieja Z., High fructose model of oxidative stress and metabolic disturbances in rats. part I. Antioxidant status of rats’ tissues. *Bull. Vet. Ist. Pulawy*, 2007, 51, 407–412.

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