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Polyphenol-rich Blackcurrant Pomace Counteracts Impaired Antioxidant Status and Serum Lipid Profile in Rabbits Fed a Diet High in Unsaturated Fat

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Abstract

Blackcurrant pomace, a by-product of juice fabrication with promising health benefits, is currently an unutilized nutritional resource. In the presented study an influence of dietary supplementation with blackcurrant pomace, constituting a source of antioxidant phytochemicals and fiber, on selected metabolic biomarkers of rabbits was evaluated. A 28-d experiment carried out on 34-d old rabbits addressed the analysis of physiological properties of two types of diet, standard chow and with additional 10% of lard, each supplemented with unprocessed blackcurrant pomace rich in polyphenols or a processed one, partly deprived of polyphenolic fraction. Twenty rabbits were allocated to four groups fed the following diets: Standard chow +15% of either unprocessed or processed blackcurrant pomace or lard-enriched diet +15% of either unprocessed or processed blackcurrant pomace. Irrespectively of the diet type, inclusion of unprocessed, compared to processed blackcurrant pomace, significantly improved antioxidant status of rabbits expressed by lower level of substances reacting with thiobarbituric acid (TBARS) in the liver and kidneys, as well as higher level of serum total antioxidant status (TAS), integral antioxidant capacities of hydrophilic (ACW) and lipophilic (ACL) substances. Upon lard-enriched diet, unprocessed blackcurrant abundant in polyphenols affected more beneficially serum triacylglycerides (TAG), total cholesterol (TC) and insulin levels. The antioxidant, hypolipidemic and hypoinsulinemic action of the blackcurrant supplement should be ascribed to the polyphenolic constituents present in the pomace.

Keywords: Berries; High-fat diet; Oxidative stress; Atherogenic dyslipidemia

Abbreviations: ACL: Integral Antioxidant Capacity of Lipophilic Substances; ACW: Integral Antioxidant Capacity of Hydrophilic Substances; AI: Atherogenic Index; ANOVA: Analysis of Variance; AOAC: Association of Official Analytic Chemists; BL: Processed Blackcurrant Pomace; BR: Unprocessed Blackcurrant Pomace; BW: Body Weight; CP: Crude Protein; DM: Dry Matter; HFBR: Lard-enriched Diet Supplemented with Unprocessed Blackcurrant Pomace; ELISA: Enzyme-linked Immunosorbent Assay; HFBL: Lard-enriched Diet Supplemented with Processed Blackcurrant Pomace; FFA: Free Fatty Acids; GIP: Gastric Inhibitory Polypeptide; GL: Glucose; HDL-C: HDL Cholesterol; HFD: High-fat Diet; HOMA-IR: Homeostasis Model Assessment for Insulin Resistance; HOMA-β: Homeostasis Model Assessment for Pancreatic Insulin Secretion; HPLC: High-performance Liquid Chromatography; IDF: Insoluble Dietary Fiber; LDL-C: LDL-cholesterol; NS: Not Statistically Significant; SBL: Standard Chow Supplemented with Processed Blackcurrant Pomace; SBR: Standard Chow Supplemented with Unprocessed Blackcurrant Pomace; SD: Standard Deviation; SDF: Soluble Dietary Fiber; TAG: Triacylglyceride; TAS: Total Antioxidant Status; TBARS: Substances Reacting with Thiobarbituric Acid; TC: Total Cholesterol; TDF: Total Dietary Fiber

Introduction

Many authors have proposed that the beneficial activity of polyphenolic compounds present in unrefined diet is linked, to a great extent, with physiological effects of dietary fiber constituting the fiber-polyphenols complexes [1,2]. Some of them have even postulated the necessity of characterizing parameters of the antioxidant status of the body at dietary supplementation with different dietary fibers [2]. Esposito et al. [3] conclude results of their study with a statement that the fiber-polyphenols complex seems to be more beneficial to the consumer's health than the highly-purified medicinal preparation of dietary fiber or antioxidants. The fiber-antioxidants complex seems to be a natural route of delivering components with antioxidative properties to colonic microbiota, thus protecting antioxidants against degradation in the stomach [3,4]. These studies point to potential advantages of diet supplementation with natural preparations containing both functional polysaccharides and polyphenolic compounds, which enables utilizing the physiological properties of both groups of these compounds locally in the gut as well as in internal tissues. Our study showed that the addition of the polyphenolic fraction from chicory root to diets containing prebiotic fructans did not diminish the positive effect of inulin and oligofructose on the ecosystem of the gastrointestinal tract, and triggered positive changes in the blood lipid profile as well as deceleration of pro-oxidative processes in selected tissues [5].

Although the technology to obtain blackcurrant juices is highly efficient, from 12% to 25% of processed raw material becomes a by-product and after production is treated as waste mass [6]. The most frequently applied ways of recycling fruit residues are for animal feed and composting or by disposal in dumps; the latter being a

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great waste of beneficial compounds present in the pomace (i.e., fiber, polyphenols), where otherwise these could provide a source of components in health food additives thereby bringing economic profits. Several studies indicate the possibility of using extracts from blackcurrant to positively modulate key markers of the health status of consumer's body [7,8], thus pointing at polyphenols as the most active fraction of fruit pomaces [9,10].

In the present study, it was hypothesized that removing a large part of phenolic fraction from blackcurrant pomace through ethanolic extraction would negatively affect parameters describing the lipoprotein profile and antioxidant status of the host. To verify this statement, two types of rabbits' diet, standard chow and with dietary addition of 10% lard, were supplemented with either natural unprocessed (BR) or processed blackcurrant pomace (BL), deprived of most of polyphenols through extraction.

Methods

Blackcurrant pomaces

Commercial blackcurrant pomace was obtained from the manufacture producing concentrated fruit juice (the ALPEX Company, Łęczeszyce, Poland). Fresh pomace from the Bücher type press was dried in a convection oven at a temperature ≤ 70°C until the moisture content was lower than 5%, and then the dried material was passed through sieves with a mesh of 2 mm. The seedless preparation thus obtained constituted the natural, unprocessed blackcurrant pomace. The processed blackcurrant pomace was subsequently prepared when phenolic compounds were partly removed from pomace using extraction with 45% (v/v) ethanol. This extraction was carried out in a column extractor with contact time. The residue obtained after removal of the ethanol and drying in the air oven constituted the processed blackcurrant pomace.

Chemical analyses of the plant material

The chemical composition of both blackcurrant pomaces are detailed in Table 1. Each pomace was analyzed in duplicate for dry matter (DM), crude protein (CP), fat, ash, total dietary fiber (TDF), and insoluble dietary fiber (IDF) using AOAC methods 934.01, 920.152, 930.09, 940.26, 985.29, and 993.19, respectively. Soluble dietary fiber (SDF) was calculated as the difference between TDF and IDF. High-performance liquid chromatography (HPLC) analysis of phenolic compounds in unprocessed and processed blackcurrant pomaces was also performed. Polyphenol compounds were extracted by a mixture solution of the composition of methanol:water:formic acid at a volumetric ratio of 50:48:2 v/v/v. Before analysis, the extracts were centrifuged at 4,800 × g for 5 min. Anthocyanins and other phenolics were analyzed using KNAUER Smartline chromatograph (Berlin, Germany) equipped with two pumps. The compounds in the phenolic extracts were separated on a 150 mm × 4.6 mm i.d., 5 µm Gemini 5u C18 110A column (Phenomenex Synergi, Torrance, CA, USA) using gradient elution with 10% v/v formic acid in water (A) and 50:40:10 v/v/v acetonitrile:water:formic acid (B). The column temperature was set at 40°C. The flow rate was 1 ml/min and the gradient program was as follows: 0-0.6 min, 12% B; 0.6-16 min, 12-30% B; 16-20.5 min, 30-100% B; 20.5-22 min, 100% B; 22-25 min, 100-12% B, 25-35 min, 12% B. The injection volume was 20 µl. The data was collected by the EuroChrom 2000 program (Knauer GmbH, Berlin, Germany). Quercetin and myricetin glycosides, and their aglycones, were detected at a wavelength of 360 nm, while anthocyanins were assayed at 520 nm. Standards of cyanidin-3-rutinoside, myricetin and kaempferol-3-glucoside were purchased from Extrasyntese Company (Genay, France); quercetin, kaempferol, quercetin-3 rutinoside and (-) epicatechin were purchased from Sigma-Aldrich (Poznań, Poland). To identify anthocyanins and the remaining flavonoids, standards were used, and UV-vis spectra was employed.

Animal study

The animal protocol used in this study was approved by the local Institutional Animal Care and Use Committee. The study was

	Blackcurrant pomace	
	Unprocessed	**Processed**
Chemical composition, %		
Dry matter	95.5 ± 0.0	90.0 ± 0.1
Crude protein	12.8 ± 0.3	12.5 ± 0.1
Crude fat	3.5 ± 0.2	3.3 ± 0.3
Crude ash	2.8 ± 0.0	2.1 ± 0.0
Total dietary fiber (TDF), including:	66.5 ± 0.8	67.6 ± 0.2
Soluble dietary fiber (SDF)	5.1 ± 0.2	3.4 ± 0.0
Nitrogen-free extract (NFE)	10.0 ± 1.2	4.4 ± 0.3
Polyphenolic compounds (HPLC-DAD), mg/100 g		
Total anthocyanins, including:	512.1 ± 18.9	119.7 ± 3.0
Delphinidin-3-rutinoside†	183.7 ± 6.6	55.3 ± 0.7
Delphinidin-3-glucoside†	156.6 ± 6.3	68.4 ± 1.0
Cyanidin-3-rutinoside†	110.1 ± 3.7	38.6 ± 0.8
Cyanidin-3-glucoside†	48.5 ± 2.0	20.1 ± 0.3
Other anhocyanins	13.3 ± 0.3	9.3 ± 0.1
Total flavonols aglycones, including:	65.5 ± 3.0	49.3 ± 0.2
Myricetin	42.0 ± 2.3	29.1 ± 0.1
Quercetin	17.6 ± 0.5	14.5 ± 0.1
Kaempferol	4.6 ± 0.0	4.3 ± 0.1
Isorhamnetin‡	1.3 ± 0.1	1.4 ± 0.0
Total flavonols glycosides, including:	20.4 ± 0.6	12.1 ± 0.0
Myricetin glycosides¶	10.3 ± 0.4	5.7 ± 0.0
Quercetin glycosides¶	5.3 ± 0.1	3.3 ± 0.0
Kaempferol glycosides¶	2.4 ± 0.2	1.6 ± 0.0
Isorhamnetin glycosides¶	2.3 ± 0.1	1.5 ± 0.0
Total polyphenols	598.0 ± 22.4	253.1 ± 3.2
HPLC-DAD, high-performance liquid chromatography with a diode array etector		
*Data are presented as means ± SD (n=3)		
†The content of the substance calculated on cyanidin-3-rutinoside		
‡The content of the substance calculated on quercetin		
¶The content of glycosides calculated on quercetin-3-rutinoside		

Table 1: Basic chemical (g/100 g) and polyphenolic (mg/100 g) composition of unprocessed and processed blackcurrant pomace*.

conducted on 20 male New Zealand white rabbits aged 34 d and weighing 630 ± 25 g, randomly divided into 4 groups of 5 animals each. They were kept individually in wire net flat deck cages and maintained under standard conditions: Temperature of 19-22°C, relative air humidity of 60-75%, intensive ventilation of rooms, and regulated photoperiod (16-h lighting and 8-h darkness). Feed and tap water were freely available. The detailed composition of the isonitrogenous diets is given in Table 2. For 4 weeks rabbits were subjected to following dietary treatments: groups SBR and SBL were fed standard chow with 15% of either unprocessed or processed blackcurrant pomace, respectively; groups HFBR and HFBL received lard-enriched diet with 15% of either unprocessed or processed pomace, respectively. In the standard diets the energy sources consisted 21% from protein, 7% from fat, and 72% from carbohydrates; in the fat-enriched diets these values were as follows: 17% from protein, 32% from fat, and 51% from carbohydrates.

Individual feed consumption and body weight (BW) gains of rabbits were determined. After the experiment, rabbits were weighed and anesthetized with sodium pentobarbitone according to recommendations for euthanasia of experimental animals. Blood samples were taken from jugular vein into test tubes, and then serum was prepared by solidification and low-speed centrifugation (350 × g, 10 min, 4°C). Animals were killed by cervical dislocation. After laparotomy, the selected tissues (liver, kidneys, and heart) were removed and weighed.

Lipid peroxidation products in the serum and tissue of the internal organs were assessed by reaction with tiobarbituric acid as TBARS, according to the method of Uchiyama and Michara [11]. The results are expressed as nanomoles of TBARS per gram of tissue or ml of serum. Serum samples were assessed for concentrations of glucose (GL) and lipids, including TC, HDL-cholesterol (HDL-C) and TAG, using direct-measurement assays (Alpha Diagnostic Ltd., Warsaw, Poland). The AI of a diet was calculated for each animal according to the formula AI=log (TAG/HDL-C). The amount of free fatty acids (FFA) present in serum of rabbits was assessed through a coupled reaction to measure non-esterified fatty acid with a commercially available detection kit (Serum/Plasma Non-Esterified Fatty Acids Detection Kit; Zen-Bio, Research Triangle Park, NC, USA). To measure insulin concentration, a validated rabbit insulin ELISA kit was used (Dog, Human and Rabbit Insulin Elisa Kit; Kamiya Biomedical Company, Seattle, USA). Homeostasis model assessments for insulin resistance (HOMA-IR) and pancreatic insulin secretion (HOMA-β) were calculated according to the following formulas: HOMA-IR=[fasting insulin (mU/l) × fasting glucose (mmol/l)/22.5], while HOMA-β=[fasting insulin (mU/l) × 20/ fasting glucose (mmol/l) – 3.5]. TAS of serum was measured using two-reagent assay (TAS Kit; Randox Laboratories Ltd., Crumlin, United Kingdom). The serum ACW and ACL were determined by a photochemiluminescence detection method, using a Photochem analyzer (ACW-Kit and ACL-Kit; Analytik Jena AG, Jena, Germany). Ascorbate and Trolox calibration curves were used in order to evaluate ACW and ACL, respectively, and the results were expressed as mmol ascorbate or Trolox equivalent/ml serum.

Statistical analysis

Values are expressed as means ± SD. The STATISTICA software, version 8.0 (StatSoft Corp., Krakow, Poland), was used to determine whether variables differed among treatment groups. Two-way ANOVA was applied to assess the effects of blackcurrant product processing (unprocessed and processed pomaces differed substantially in polyphenols level), type of diet (standard chow and chow with additional dietary lard) and the interaction between investigated factors (type of supplement × type of diet). When the ANOVA indicated significant treatment effects, means were evaluated using Duncan's multiple range test. Data were checked for normality before statistical analysis was performed. Differences with $P \leq 0.05$ were considered to be significant.

Results

The initial BW of rabbits did not differ between groups (data not shown). During 4 week of feeding, BW gain and daily diet intake

	Group			
	SBR	SBL	HFBR	HFBL
Component, g/100 g of a diet				
Oat	14	14	14	14
Wheat bran	10	10	10	10
Sunflower meal	16.8	16.8	16.8	16.8
Dried sugar beet pulp	5	5	5	5
Grass meal	16	16	1	1
Barley	16.1	16.1	18.1	18.1
Soybean meal	4.1	4.1	7.1	7.1
Unprocessed blackcurrant pomace	15	-	15	-
Processed blackcurrant pomace	-	15	-	15
Lard	-	-	10	10
Additives*	3	3	3	3
Calculated chemical composition, %				
Crude protein	17.5	17.5	17.4	17.4
Crude firber	14.3	14.3	12.6	12.6
Crude fat	2.4	2.4	13.6	13.6
Polyphenols†	0.09	0.04	0.09	0.04

*Per kg of feed: DL-methionine-2 g; L-lysine-2 g; Monocalcium phosphate 22.6%-11 g; Salt-3 g; Vitamin-mineral premix (5 g/kg) providing the following nutrients per kg feed: Vitamin A-10000 IU; Vitamin D-1800 IU, Vitamin E-15 mg; Vitamin K-4.5 mg; Vitamin B1-0.5 mg; Vitamin B2-4 mg; Vitamin B12-0.01 mg; Folic acid-0.1 mg; Pantothenic acid-7 mg; Nicotinic acid-20 mg; I-1 mg; Mn-60 mg; Cu-5.5 mg; Zn-75 mg; Fe-40 mg; Co-0.3 mg; Se-0.08 mg
†Polyphenols originated from blackcurrant pomaces

Table 2: Ingredients (g/100 g) and composition (%) of experimental diets.

increased significantly (P<0.005) in both groups fed diets with 10% of additional fat (Table 3). Final BW was also elevated in HFD-fed animals. The type of blackcurrant pomace had influence neither on weight gains nor on dietary intakes; however, rabbits receiving BL showed tendency to have decreased BW at the end of the experiment (P=0.088).

Irrespective of the polyphenol level in blackcurrant preparations, feeding with HFD caused a significant increase in the relative liver mass ($P \leq 0.001$), and at the same time decreased the relative heart mass (P<0.05), as compared to rabbits receiving standard chow (Table 3). The liver mass was significantly lower in animals given diets with BR pomace than in those fed the BL diets (P<0.05). TBARS concentration in all analyzed tissues (liver, kidney, heart, serum) was higher (P<0.05) in groups on fat-diets in comparison to animals on diets without additional fat. The level of polyphenolic compounds in blackcurrant pomace significantly affected the liver TBARS concentration (BL>BR, P<0.05). Similar effect was observed in the kidney's tissue (P<0.05). For serum concentration of TBARS, the two-way ANOVA showed a significant interaction B × D (P<0.05); in rabbits fed HFD, the dietary addition of BL significantly increased serum TBARS level in HFBL group, compared to HFBR group. No such effect was found for both groups fed standard diet. Regardless of the pomace type, additional fat in the diet caused a significant decrease in the level of serum TAS ($P \leq 0.001$), as well as ACW (P<0.005). Higher values for TAS and ACW were found when rabbits were fed the diet supplemented with polyphenol-rich pomace (P<0.05 for TAS, and $P \leq 0.001$ for ACW). The highest level of ACL was measured in the serum of rabbits fed SBR diet (P<0.05 vs. other treatments), while the lowest in both groups of rabbits fed diets supplemented with BL (see significant interaction B × D).

Serum levels of TAG, TC, insulin and both calculated HOMA indexes were significantly higher in the HFBL rabbits, as compared with other dietary treatments (Table 4). The addition of BR pomace to the HFD significantly reduced the concentrations of TAG, TC, insulin, HOMA-IR and HOMA-β (HFBL>HFBR, P<0.05), but the lowest values for these parameters were observed in both groups fed standard diet (see significant interaction B × D). In comparison to rabbits fed standard diets, feeding with HFD led to a significant increase in the AI value (P<0.05) and serum concentration of circulating FFA (P<0.001), as well as to a significant decrease in the HDL-C/TC profile ($P \leq 0.001$) in rabbits. The presence of BL in both types of diet led to a significant increase in the value of serum AI (P<0.05), as well as a tendency towards lower serum HDL-C/TC profile (P=0.076) in rabbits, in comparison to animals fed diets supplemented with unprocessed pomace, abundant in polyphenols.

Discussion

Hepatic steatosis associated with excessive fat accumulation in hepatocytes, is a condition often present in pathological states related to altered metabolism of the whole organism, which itself may contribute to other illnesses. In the current experiment, dietary administration of blackcurrant polyphenols was accompanied with reduced liver weight in HFD rabbits. As phytochemicals are metabolized by the liver, the organ is directly exposed to these compounds or their metabolic derivatives. Liver weight loss observed in this study may be associated with decreased hepatic fat accumulation and/or storage. In a 12-week experiment on C57BL/6J mice, supplementation of HFD (58% kcal from fat) with rich in cyanidin-3-glucoside extract of purple corn (0.02% of the diet), significantly decreased BW, concentration of TAG, as well as mRNA expression level of genes involved in fatty acid synthesis in the liver [12]. Similar results were obtained in the experiment of Cefalu et al. [13], in which 6 weeks of feeding with HFD supplemented with grape extract containing anthocyanin glycosides, prevented hypertrophy and accumulation of TAG in the liver of mice with genetically induced predisposition to obesity. In the present experiment, cyanidin-3-glucoside present in the diets containing unprocessed pomace constituted 0.007% of the diet, while all the anthocyanins -0.08%. It can therefore be assumed that a significant decrease in the liver weight (g/kg) of rabbits fed diets with unprocessed blackcurrant preparation, as compared to the groups fed diets with processed one, was due to the limited synthesis and accumulation of lipids in hepatocytes resulting from anthocyanin bioactivity.

	Dietary treatments*, n=5				*P* value		
	SBR	SBL	HFBR	HFBL	Pomace (B), n=10	Diet (D), n=10	B × D
Final BW, kg	1.98 ± 0.27	1.78 ± 0.13	2.24 ± 0.13	2.14 ± 0.15	NS	<0.005	NS
BW gain, kg/4 weeks	1.36 ± 0.28	1.16 ± 0.14	1.61 ± 0.13	1.52 ± 0.14	NS	<0.005	NS
Diet intake, g/day	102 ± 14	94 ± 10	114 ± 4	114 ± 8	NS	<0.005	NS
Internal tissues							
Liver mass, g/kg of BW	24.6 ± 3.2	26.7 ± 3.3	28.9 ± 2.1	34.8 ± 4.3	<0.05	≤0.001	NS
TBARS, nmol/g of tissue	73.8 ± 4.0	75.9 ± 3.6	85.7 ± 6.1	91.8 ± 2.3	<0.05	<0.001	NS
Kidney mass, g/kg of BW	6.18 ± 0.22	6.32 ± 0.71	6.36 ± 1.90	6.14 ± 0.47	NS	NS	NS
TBARS, nmol/g of tissue	112 ± 7	117 ± 11	129 ± 8	146 ± 14	<0.05	<0.001	NS
Heart, g/kg of BW	2.53 ± 0.21	2.69 ± 0.43	2.28 ± 0.13	2.39 ± 0.21	NS	<0.05	NS
TBARS, nmol/g of tissue	64.0 ± 3.1	63.7 ± 3.3	66.5 ± 3.7	69.1 ± 2.4	NS	<0.05	NS
Serum							
TBARS, nmol/ml	45.4 ± 3.4^{c}	50.4 ± 3.2^{c}	107 ± 14^{b}	128 ± 13^{a}	≤0.01	<0.001	<0.05
TAS, mmol/l	1.26 ± 0.12	1.17 ± 0.06	1.10 ± 0.08	1.03 ± 0.51	<0.05	≤0.001	NS
ACW, mmol AA/ml	0.081 ± 0.006	0.071 ± 0.006	0.072 ± 0.005	0.061 ± 0.005	≤0.001	<0.005	NS
ACL, mmol Trolox/ml	0.099 ± 0.002^{a}	0.092 ± 0.004^{c}	0.094 ± 0.004^{b}	0.092 ± 0.007^{c}	<0.05	NS	<0.05

ACW: Antioxidant capacity of water-soluble substances in the serum; ACL: Antioxidant capacity of lipid-soluble substances in the serum; BW: Body weight; NS: Not statistically significant; TAS: Total antioxidant capacity; TBARS: Thiobarbituric acid-reactive substances

*Groups SBR and SBL fed standard chow with 15% unprocessed and processed blackcurrant pomace, respectively; groups HFBR and HFBL fed lard-enriched diet with 15% unprocessed and processed pomace, respectively (means ± SD, n=5)

^{a,b,c}Mean values within a row with unlike superscript letters were shown to be significantly different ($P \leq 0.05$) in the case of a statistically significant interaction B × D ($P \leq 0.05$)

Table 3: Body weight and parameters of antioxidant status in rabbits fed experimental diets.

	Dietary treatments†, n=5				*P* value		
	SBR	SBL	HFBR	HFBL	Pomace (B), n=10	Diet (D), n=10	B × D
GL, mmol/l	6.03 ± 0.76	6.11 ± 0.91	5.96 ± 0.32	5.88 ± 0.60	NS	NS	NS
TAG, mmol/l	1.10 ± 0.20^{c}	1.02 ± 0.23^{c}	1.26 ± 0.13^{b}	1.44 ± 0.25^{a}	NS	<0.01	<0.05
TC, mmol/l	2.02 ± 0.26^{c}	1.92 ± 0.23^{c}	2.28 ± 0.32^{b}	2.55 ± 0.28^{a}	NS	<0.005	<0.05
HDL-C, mmol/l	1.08 ± 0.14	0.98 ± 0.17	1.06 ± 0.15	1.04 ± 0.11	NS	NS	NS
HDL-C/TC, %	53.8 ± 4.2	51.0 ± 3.7	46.5 ± 5.2	41.2 ± 5.3	0.076	≤0.001	NS
AI‡	0.004 ± 0.111	0.013 ± 0.131	0.077 ± 0.081	0.136 ± 0.039	<0.05	<0.05	NS
FFA, mmol/l	425 ± 127	402 ± 111	1002 ± 119	1110 ± 247	NS	<0.001	NS
Insulin, pmol/l	54.0 ± 2.5^{c}	55.6 ± 2.3^{c}	67.1 ± 1.6^{b}	77.9 ± 1.6^{a}	0.088	<0.001	<0.05
HOMA-IR¶	2.08 ± 0.5^{c}	2.17 ± 0.3^{c}	2.56 ± 0.6^{b}	2.93 ± 0.6^{a}	0.062	<0.05	≤0.05
HOMA-β§	61.5 ± 3.5^{c}	61.3 ± 4.4^{c}	78.5 ± 6.9^{b}	94.3 ± 3.6^{a}	0.072	≤0.05	<0.05

AI: Atherogenic index; FFA: Free fatty acids; GL: Glucose; HDL-C: HDL cholesterol; HOMA-IR: Homeostasis model assessment for insulin resistance; HOMA-β: Homeostasis model assessment for pancreatic insulin secretion; NS: Not statistically significant; TAG: Triacylglyceride; TBARS: Thiobarbituric acid-reactive substances; TC: Total cholesterol

*Blood samples were taken in overnight food-deprived animals

†Groups SBR and SBL fed standard chow with 15% unprocessed and processed blackcurrant pomace, respectively; groups HFBR and HFBL fed lard-enriched diet with 15% unprocessed and processed pomace, respectively (means ± SD, n=5).

‡AI=log(TAG/HDL-C)

¶HOMA-IR=[fasting insulin (mU/l) × fasting glucose (mmol/l)/22.5]

§HOMA-β=[fasting insulin (mU/l) × 20/fasting glucose (mmol/l)-3.5]

^{a,b,c} Mean values within a row with unlike superscript letters were shown to be significantly different ($P \leq 0.05$) in the case of a statistically significant interaction B × D ($P \leq 0.05$)

Table 4: Biochemical indices of the serum in experimental rabbits*.

It is well established that elevated levels of TAG and LDL-cholesterol (LDL-C) are risk factors for cardiovascular diseases and serum concentration of HDL-C is inversely related to that risk. In dyslipidemic patients, pure anthocyanins derived from bilberry and blackcurrant produced a dual beneficial effect on lipoprotein profile, which included a decrease in LDL-C and an increase in HDL-C serum levels [14]. In the present experiment different dietary treatments did not affect HDL-C concentration in rabbits' serum; however, among HFD-fed animals, supplementation of the diet with BR decreased elevated HDL-C/TC profile. In these animals, beneficial changes were also observed for the serum level of TAG, TC and calculated value of AI index. In numerous studies on animal models, as well as those using in vitro systems, attempts have been made to clarify the possible mechanisms involved in the regulatory effect exerted by polyphenols from berries on the absorption and metabolism of lipids. It is suggested that the hypolipidemic effect of dietary polyphenols may be associated with the inhibition of lipid absorption from the gastrointestinal tract. It was shown that polyphenols from berries have the ability to inhibit the activity of lipolitic enzymes (including pancreatic lipase), which greatly handicap the uptake of fat from the intestinal lumen [12].

Results of the present study demonstrate that polyphenols from blackcurrant pomace exert advantageous influence on insulin secretion and action, reducing dietary-induced hyperinsulinemia as well as pancreatic insulin secretion and systemic insulin resistance (manifested by lower HOMA-β and HOMA-IR values, respectively). Within the animals fed a diet with 10% fat content, rabbits, whose diet was enriched with unprocessed blackcurrant preparation, compared to HFBL group, had lower values of all aforementioned biomarkers in the serum at the end of the experiment. This indicates the potential use of blackcurrant polyphenols in the control of insulinemia. Insulin resistance is often proposed to be a major causative factor in fatty liver development. Due to the fact that, in comparison to the animals fed processed pomace, rabbits fed diets supplemented with BR had most likely lower fat accumulation in their livers (which was indirectly demonstrated by the lower weight of this organ in SBR and HFBR groups), as well as preferable characteristic of serum lipids, possible regulatory effect of blackcurrant polyphenols on lipid absorption and/or metabolism may account for an improved insulinemia observed in these animals. Polyphenolic compounds, especially anthocyanins, seem to influence the cascade of reactions associated with insulin secretion and signaling [12,13,15].

Free radicals and products of their reaction with biomolecules are the leading risk factors for developing atherosclerosis, some cancers, as well as accelerated aging processes [16,17]. The severity of their appearance leads to a condition called oxidative stress and it has been postulated for a long time that the health-promoting activity of phenolic compounds mainly relays on their ability to reduce oxidative stress. Polyphenols are potent scavengers of reactive forms of oxygen and nitrogen, able to inhibit the activity of enzymes and chelate metal ions involved in catalyzation of oxidation reactions [18]. Although ORAC analysis showed that the antioxidant capacity of lipophilic antioxidants derived from berries is rather small [19], numerous studies have demonstrated beneficial effects of whole berries or polyphenolic fraction extracted from these fruits on oxidation of blood or internal organ lipids [9,10,20,21]. In the current study, blackcurrant polyphenols were shown to inhibit lipid peroxidation induced by dietary fat (lower level of TBARS in the liver, kidneys and serum as well as serum ACL in rabbits from HFBR, compared to HFBL group). The suppression of lipid peroxidation observed in the liver and kidneys of rabbits fed diet enriched with unprocessed pomace, is probably the effect of polyphenolic compounds that may increase filtration in these organs and inactivate free radicals. The significantly higher overall activity of endogenous antioxidant capacity observed in serum of rabbits fed diet with natural pomace (demonstrated by higher TAS, ACW and ACL values), may suggest that large amount of dietary polyphenols exerted stimulating and/or protective effect towards the activity of endogenic antioxidant compounds found in serum (i.e., glutathione, vitamin E). It was shown that polyphenols may interact synergistically with other antioxidants (both endogenous and delivered with a diet), thereby affecting the final antioxidant effect [22,23]. It should be noted that dietary fiber may also influence the antioxidant properties of phenolic compounds, i.e., by providing protection for the bioactive substances against the acidic environment of the stomach and/or digestive enzymes [4].

Our study showed that the higher content of polyphenolic compounds in the diet preferably affected the antioxidant status and blood lipid profile of rabbits. In order to affect the function of cells and tissues beyond the gastrointestinal tract, bioactive components of the diet need to be absorbed and reach the peripheral circulation. It has been postulated that delivering polyphenolics together as a complex with dietary fiber in natural products is a good approach enabling utilization of antioxidant properties of phenolic compounds by the intestinal flora. Colonic fermentation of these both constituents yields physiologically active metabolites with potential systemic effects. Apart from polyphenols, both preparations tested in the present study contained reasonable amounts of dietary fiber. An additive or even synergistic effect of dietary fiber and polyphenols in counteracting poor metabolic outcomes caused by the HFD is highly possible, and cannot be excluded when interpreting the results of the study.

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