

A potential beneficial anti-atherosclerotic effect of supplementing the diet with buckwheat sprouts (*Fagopyrum esculentum* Moench). A study in rats

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Abstract

Cardiovascular diseases are the leading cause of death in Europe. The present study investigated the anti-atherosclerotic effect which the addition of buckwheat sprouts to the lard-fed rats exhibited. Adult male Wistar rats were divided into four groups: control group fed a diet with corn starch, high-fat group which received diet containing 300g/kg of lard, buckwheat group which received lyophilized buckwheat sprouts at the dose of 100 g/kg, and fourth group which received 300g/kg of lard and buckwheat sprouts at the dose of 100 g/kg. Supplementing the diet of rats with buckwheat sprouts resulted in reduced weight gain and protected against weight rise in fat-fed rats. Both dietary factors improved antioxidant protection system and oxidant stress indices. Sprouts ameliorated lipid profile and increased paraoxonase-1 activity in plasma and liver. These findings suggest that buckwheat sprouts supplement to the lard-containing diet had a protective, anti-atherosclerotic effect and may exhibit a significant beneficial health effect in the Western diet-consuming population.

Introduction

High-fat diet-induced obesity increases the risk of many different human disorders, such as cardiovascular diseases, atherosclerosis, hyperlipidemia, hypertension and insulin resistance [1]. High concentration of triglycerides and high ratio of low-density lipoproteins to high density lipoproteins are the most significant risk factors of coronary artery disease. Consumption of high-fat diets also leads to an increase in oxidative stress in plasma and tissues. Therefore, changes in dietary habits could be an important advantage in successful therapy of these diseases and their prevention [2]. Researchers have continually been looking for natural products, which can reverse the harmful effects of consumption of a high-fat diet and possibly reduce the risk of heart disease. Buckwheat (*Fagopyrum esculentum* Moench) is an ancient, highly nutritious grain, containing phenolic acids, flavonoids, anthocyanins, epicatechins, phytosterols and squalene [3–5]. These components make buckwheat a valuable and recommended part of the daily human diet [6]. Numerous studies on buckwheat have demonstrated that diet supplementation with this grain produces antioxidant, anti-inflammatory and antihypertensive effects ([7–10]. Recent studies have indicated that the addition of buckwheat to the diet decreased the total plasma cholesterol level [11–13].

Paraoxonase 1 (PON1) is an enzyme, which takes part in the pathogenesis of cardiovascular disorders. Because of its activity, it is associated with preventing the oxidation of low-density lipoproteins and in this way may play a protective role in the atherogenic process. Previous studies have demonstrated that low serum PON1 activity is associated with coronary heart disease and was noted in obese patients [14, 15]. Perusal of available literature revealed no mention in any paper about the effect of buckwheat sprout consumption on PON1 activity in rats fed a high-fat diet. Choi et al. [16] reported the suppressive effect of buckwheat sprouts on the development of fatty liver in mice fed a high-fat diet. Scarcely any research has been done on the influence of germinated buckwheat seeds on lipid profile and antiatherogenic effect in rats. The present study was conducted to investigate the effects the high-fat diet (30% lard w/w) supplementation with buckwheat sprouts on paraoxonase-1 activity in plasma and liver, and

antioxidant status and lipid profile of plasma in Wistar rats. We report for the first time the impact of buckwheat sprouts on the activity of PON1 in rats, i.a. to confirm the beneficial influence of buckwheat sprouts on reducing the risk factors of atherosclerosis. This study is reported in accordance with ARRIVE guidelines.

Results

In all diets, combined additives (starch, lard, sprouts) constituted 62% of the feed weight. Detailed composition of each diet is shown in Table 1.

Table 1
Composition of experimental diets.

Group	C	CL	BG	BGL
Name of group	Control group	Control group with lard	Buckwheat sprouts group	Buckwheat sprouts with lard
Corn starch (mg/g)	620	320	320	20
Lard (mg/g)	0	300	0	300
Buckwheat grouts (mg/g)	0	0	300	300
Energy ¹ (kcal/g)	3.19	5.89	13.4	20.1
Fat ¹ (mg/g)	53	353	59	357
Protein ¹ (mg/g)	142	142	176	175
Dietary fiber ¹ (mg/g)	53	53	78	77
¹ Calculated on the basis of contributions of constituents based on table data. Each diet contained (in mg/g): casein 200, rapeseed oil 50, chalk 28, calcium monophosphate (CaHPO ₄) 29, soy lecithin 10, sodium chloride 3, potassium sulphate (K ₂ SO ₄) 3.5, magnesium oxide (MgO) 0.7, cellulose 45.8, mixture of vitamins and microelements 10 (Premix LPM, BASF, Poland – vitamins and minerals).				

The effect of buckwheat sprouts on the feeding and growth of rats fed a high-fat diet is presented in Table 2. Significances based on a two-way ANOVA are also shown. Both factors in this study, namely buckwheat sprouts and lard supplement in the diet, influenced independently (with no interaction) the final body weight of rats. Mean final body weight decreased with the presence of sprouts in the diet ($p = 0.049$), and increased when the diet was supplemented with lard ($p = 0.043$). The above-mentioned effects were more evident when the analysis was performed using daily weight gain of rats ($p = 0.035$ and $p = 0.025$ for sprouts and lard in diet, respectively). Water intake was lowered by lard in diet ($p = 0.011$), while sprouts showed no effect. There was also no significant interaction between these variables – however, a weak normalizing trend to the control group cannot be excluded.

Table 2
Food and water intake and related parameters (mean $\pm$ standard deviation).

Experimental groups					ANOVA(p)		
					Sprouts	Lard	
	C	CL	BS	BSL	1	2	1 x 2
Parameters	a	b	c	d			
Body weight, initial (g)	245.3 $\pm$ 9.6	246.0 $\pm$ 7.1	240.8 $\pm$ 5.6	244.8 $\pm$ 5.8	0.348	0.438	0.578
Body weight, final (g)	364.7 $\pm$ 27.7 c(0.10)	375.0 $\pm$ 20.2	342.2 $\pm$ 13.2 a(0.10) d**	365.2 $\pm$ 8.7 ^{c**}	0.049	0.043	0.421
Weight gain (g/day)	18.8 $\pm$ 1.3 ^{b*** c*}	13.9 $\pm$ 0.7 ^{a***} d(0.06)	17.4 $\pm$ 0.5 ^{a* d*}	15.3 $\pm$ 1.5 ^{b(0.06)} c*	0.946	0.000	0.005
Water intake (g/day)	27.1 $\pm$ 3.5 ^{b*}	23.2 $\pm$ 0.7 ^{a* d***}	26.1 $\pm$ 2.2	25.2 $\pm$ 0.5 ^{b***}	0.540	0.011	0.095
Food intake (g/day)	3.41 $\pm$ 0.53 c(0.06)	3.79 $\pm$ 0.63	2.90 $\pm$ 0.28 a(0.06) d*	3.44 $\pm$ 0.37 ^{c*}	0.035	0.025	0.684
Energy intake (kcal/day)	59.9 $\pm$ 4.2 ^{b*** c*}	81.7 $\pm$ 4.2 ^{a***} d(0.07)	55.1 $\pm$ 1.7 ^{a* d***}	90.0 $\pm$ 9.0 ^{b(0.07)} c***	0.444	0.000	0.008
Food efficiency ratio (FER) ^x	0.18 $\pm$ 0.02 ^{b***}	0.27 $\pm$ 0.04 ^{a***} d*	0.17 $\pm$ 0.02 ^{d***}	0.23 $\pm$ 0.03 ^{b*} c***	0.012	0.000	0.166
Food energy efficiency (FEE) ^y	56.9 $\pm$ 7.6 ^{b*}	46.4 $\pm$ 6.6 ^{a* d*}	52.5 $\pm$ 5.0 ^{d***}	38.4 $\pm$ 4.6 ^{b* c***}	0.023	0.000	0.479
^x FER - body weight gain in gram per gram of food intake (g/g); ^y FEE - weight gain per energy intake (g /1000 kcal; g/Mcal). A superscript letter at given mean shows the second group for which difference is significant by one-way ANOVA or is near significance, while asterisks denoted a level of significance: *** for p < 0.001, ** for p < 0.01; * for p < 0.05, An explicit value of p-level was given in parentheses for cases at near significance in the range 0.05–0.10. Lack of superscript letter means lack of significant difference with particular group.							

Food intake was considerably lower for the lard-fed groups (e.g. CL 13.9 vs. C 18.9, p < 0.05), as well as significantly modified by sprouts' addition to the diet (p = 0.005), while being lowered with sprouts as a single factor. There was a significant decrease in food efficiency ratio (FER) for the groups receiving buckwheat sprouts versus control groups (p = 0.012), in spite of no effect of sprouts on the energy intake.

Contrary to the aforementioned effect, FER was higher in lard groups vs. respective non-lard groups ($p < 0.001$). Both dietary factors, i.e. lard and sprouts, showed no significant interaction and changed independently. However, a weak antagonistic effect of sprouts versus the effect of lard added to diet can be speculatively suggested. In spite of no effect of sprouts on the energy intake, sprouts significantly lowered weight gain of rats ($p = 0.035$). The food energy efficiency expressed as weight gain per dietary energy intake (FEE) was the lowest in the group fed with the diet supplemented with lard and sprouts (BSL) suggesting an enhanced exhausting of food energy caused by the high-lard diet or/and poorer availability of fat in the presence of sprouts. These results, namely lowering of weight gain and preventing the weight increase in the presence of high fat content in the diet suggest a significant beneficial effect of sprouts in the diet on weight control of rats.

Based on the analysis of general biochemical characteristics of plasma (Table 3a and Table 3b) it can be concluded that the effect of lard and sprouts either independently or in combination was moderate. Dietary factors studied did not influence the plasma minerals concentration except for magnesium. The increase in magnesium level in the presence of sprouts in the diet vs. control group (BS vs. C) approximated the conventional significance threshold (0.97 vs. 0.83 mmol/L, $p = 0.07$), as determined by a one-way ANOVA. However, this relationship was not noted in the group receiving sprouts + lard combination. For the sprout group (BG), albumin level was depleted, while substantial increase in phosphorus concentration was observed ($p < 0.001$). These effects suggest possible changes in protein metabolism. It is also worth mentioning that glucose concentration in plasma significantly increased when lard was added to the diet, but this increase was much smaller with the presence of sprouts. With the presence of lard in the diet, glucose concentration increased and magnesium level decreased in the rat plasma, and both effects can be considered disadvantageous.

Table 3
Biochemical characteristics of plasma

Experimental groups					ANOVA(p)		
					Sprouts	Lard	
	C	CL	BS	BSL	1	2	1 x 2
Parameter	a	b	c	d			
Ca (mmol/L)	3.13 ± 0.27	2.92 ± 0.28	3.14 ± 0.17	2.99 ± 0.21	0.675	0.071	0.775
Mg (mmol/L)	0.83 ± 0.15 c(0.07)	0.81 ± 0.07	0.97 ± 0.07 a(0.07) d**	0.79 ± 0.09 c**	0.166	0.017	0.069
K (mmol/L)	4.27 ± 0.40 b(0.09)	3.84 ± 0.39 a(0.09)	4.17 ± 0.44	4.14 ± 0.35	0.531	0.168	0.238
Na (mmol/L)	144.9 ± 7.9	143.1 ± 5.7	144.5 ± 6.5	145.1 ± 3.9	0.759	0.820	0.645
P (mmol/L)	3.15 ± 0.26 c(0.11)	2.89 ± 0.48 d*	3.52 ± 0.43 a(0.11)	3.53 ± 0.42 b*	0.007	0.451	0.427
ALB (g/L)	35.1 ± 3.9 c*	35.2 ± 4.0 d**	30.6 ± 2.8 a*	28.9 ± 1.2 b**	0.000	0.520	0.481
PROT (g/L)	48.6 ± 13.6	48.8 ± 2.9	44.5 ± 6.2	47.8 ± 9.9	0.515	0.643	0.681
GL (mmol/L)	9.27 ± 0.86 b**	11.01 ± 0.59 a**	9.55 ± 1.38	10.62 ± 1.10	0.897	0.003	0.431
U (mmol/L)	9.1 ± 2.2	10.1 ± 1.8 d*	11.5 ± 2.7 d**	7.8 ± 0.8 b* c**	0.953	0.115	0.011
UA (µmol/L)	130 ± 64	117 ± 30	161 ± 61	126 ± 24	0.324	0.231	0.560
Mean ± standard deviation; ^x in (mmol/L) if units are not given; Mean ± SD; Ca, Mg, K, Na, P - calcium, magnesium, potassium, sodium and phosphorus, respectively; ALB - albumins; PROT - proteins; GL - glucose; U - urea; UA - uric acid. For explanation of superscripts see legend under Table 2.							

Contrary to intuitive expectation, the TG levels in plasma of rats fed the lard-supplemented diet (Table 4a and Table 4b) was significantly lower than in the control group, irrespective of the presence or the absence of sprouts in the diet. No effect of sprouts on TG level or interaction with the lard factor was found. Sprouts had beneficial effect on both HDL and non-HDL cholesterol levels, however, the differences were of a borderline statistical significance. For the lard groups, there was an increase in HDL level in the presence of sprouts in the diet almost reaching the significance threshold (p = 0.06 for a

one-way ANOVA). A weaker decreasing effect on non-HDL cholesterol level was observed for the sprouts group without lard supplementation. These effects were more pronounced when the analysis was based on the ratio of the aforementioned parameters termed atherogenic index (AITCH), which was significantly lowered in the presence of sprouts in the diet, i.e. in the group BS vs. C ($p < 0.05$) and in the group BSL vs. CL.

Table 4
Plasma lipid profile

Experimental groups						ANOVA(p)		
						Sprouts	Lard	
		C	CL	BS	BSL	1	2	1 x 2
Parameter		a	b	c	d			
TG	mmol/L	1.39 ± 0.45 ^{b**}	0.66 ± 0.19 ^{a**} d(0.05)	1.40 ± 0.43 ^{d*}	0.88 ± 0.14 ^{b(0.05) c*}	0.428	0.000	0.453
TCH	mmol/L	1.99 ± 0.48 ^{c(0.08)}	1.79 ± 0.08	1.59 ± 0.17 ^{a(0.08) d*}	2.03 ± 0.36 ^{c*}	0.530	0.346	0.020
HDL	mmol/L	0.86 ± 0.17	0.69 ± 0.22 ^{d(0.06)}	0.91 ± 0.01 ^{d(0.06)}	0.99 ± 0.05 ^{b(0.06) c(0.06)}	0.052	0.613	0.168
nonHDL	mmol/L	1.14 ± 0.34	1.10 ± 0.20	0.75 ± 0.19	0.94 ± 0.24	0.053	0.566	0.407
AITCH ^x	-	0.11 ± 0.09 ^{c*}	0.22 ± 0.22 ^{d(0.11)}	-0.09 ± 0.10 ^{a*}	-0.03 ± 0.09 ^{b(0.11)}	0.009	0.294	0.785
AITG ^y	-	0.20 ± 0.11 ^{b**}	-0.01 ± 0.11 ^{a**}	0.18 ± 0.15 ^{d(0.11)}	-0.01 ± 0.03 ^{c(0.11)}	0.895	0.003	0.806
Mean ± standard deviation; TG - triacylglycerols; TCH -total cholesterol; HDL - high density lipoproteins; nonHDL cholesterol = TCH - HDL, ^x AITCH: log ₁₀ (TCH / HDL – 1); ^y AITG: log ₁₀ (TG/HDL); For explanation of superscripts see legend under Table 2.								

The latter effect indicates a lower risk of LDL oxidation and thus sprouts should be considered protective against cardiovascular diseases. Atherogenic index of plasma (AITG), estimated as the TG-to-HDL ratio, was significantly decreased by the high-lard diet ($p = 0.003$). However, this effect was mainly related to the substantial decrease in TG level in the lard groups vs. respective control lard-free groups. Taking together, dietary sprouts did not influence TG levels but modified cholesterol fractions and relative

markers of atherogenicity. For lard diets, the changes in cholesterol fractions were not significant. However, the diminishing effect of lard on TG levels in plasma was observed. Although this can be considered controversial, the above observation suggested temporary beneficial influence of lard in the diet of rats on lipid profile.

Both factors in the study did not influence liver weight and relative liver weight vs. rat body weight (Table 5a and 5b). Lard and sprouts in the diet produced significant positive effects on liver glycogen and both factors interacted antagonistically ($p = 0.001$). Similar effects were found for fat in the liver, although no significant interaction was observed between both factors. Hepatic enzymes were generally not affected by both factors under study, except for plasma ALP activity which was increased by the lard-supplemented diet. This effect was attenuated by dietary sprouts ($p = 0.014$ for interaction). Plasma PON activity (pPON) was strongly increased by sprouts in the diet. For lard in diet the effect was lesser, and significant antagonistic interaction between both these factors was observed ($p < 0.001$). In the liver, the effect of sprouts on PON activity increase was lower and statistically insignificant and only lard-supplemented diet significantly enhanced its activity. Both factors interacted antagonistically when combined.

Table 5
Characteristics of liver function

Experimental groups		ANOVA(p)						
		Sprouts		Lard				
		C	CL	BS	BSL	1	2	1 x 2
Parameter		a	b	c	d			
Liver wt.	g	13.0 ± 2.3	12.8 ± 1.2	12.8 ± 1.0	12.8 ± 0.4	0.884	0.874	0.927
Liver wt. %	g/100gb.wt.	3.54 ± 0.44	3.41 ± 0.17	3.75 ± 0.28 d(0.06)	3.50 ± 0.09 c(0.06)	0.205	0.117	0.596
Glycogen	g/100g	1.68 ± 1.05 ^{b**} c***	3.58 ± 0.83 ^{a**}	4.05 ± 0.67 ^{a***}	3.52 ± 0.42	0.002	0.044	0.001
Liver fat	g/100g	21.6 ± 3.9 ^{b* c*}	27.1 ± 2.0 ^{a*} d(0.07)	28.0 ± 3.5 ^{a*}	30.7 ± 3.8 b(0.07)	0.002	0.008	0.318
ALP	U/L	284 ± 95 ^{b*} c(0.06)	432 ± 79 ^{a*}	371 ± 38 a(0.06)	363 ± 59	0.754	0.026	0.014
ALT	U/L	37.1 ± 7.0 b(0.07) c*	47.3 ± 9.9 a(0.07)	50.7 ± 11.1 ^{a*}	45.8 ± 8.7	0.127	0.492	0.061
ASP	U/L	121 ± 56	154 ± 53	173 ± 46	148 ± 28	0.249	0.832	0.146
pPON	U/mg p.	336.3 ± 64.0 b(0.10) c***	386.6 ± 23.1 a(0.10) d**	592.3 ± 74.8 a*** d***	258.5 ± 73.7 b** c***	0.021	0.000	0.000
Liver PON	U/mg p.	10.8 ± 1.7 ^{b***}	20.9 ± 2.2 ^{a***} d**	14.7 ± 5.9	13.0 ± 3.8 ^{b**}	0.212	0.013	0.001
Mean ± standard deviation; ALP - alkaline phosphatase activity; ALT - alanine aminotransferase activity; AST - aspartate aminotransferase activity; pPON -plasma paraoxonase activity; Liver wt. - liver weight as percent of body weight; For explanation of superscripts see legend under Table 2.								

Sprouts in the diet did not affect plasma FRAP or uric acid-free ferric ion reducing capacity of plasma, (FRAPf) – Table 6a and Table 6b. In spite of the above result, sprout-supplemented diet significantly lowered carbonyl group (CO) level in plasma and MDA levels in plasma.

Table 6
Plasma redox status

Experimental groups						ANOVA(p)		
						Sprouts	Lard	
		C	CL	BS	BSL	1	2	1 x 2
Parameter		a	b	c	d			
FRAP	μmol/L	237 ± 54	306 ± 111	255 ± 27 ^{d**}	314 ± 35 ^{c**}	0.647	0.026	0.846
FRAPf	μmol/L	279 ± 108	375 ± 157	219 ± 95 ^{d*}	351 ± 72 ^{c*}	0.366	0.022	0.695
MDA	μmol/L	1.42 ± 0.15 ^{b*}	1.16 ± 0.20 ^{a*} ^{d**}	1.12 ± 0.40 ^{d(0.05)}	0.74 ± 0.12 ^{b**} ^{c(0.05)}	0.002	0.005	0.535
CO	nmol/mg p.	6.57 ± 1.94 ^{c***}	6.01 ± 0.68	1.95 ± 0.40 ^{a*** d**}	4.92 ± 1.63 ^{c**}	0.000	0.038	0.004
CAT	U/mg p.	467 ± 56 ^{b*** c***}	678 ± 33 ^{a*** d***}	782 ± 34 ^{a*** d***}	532 ± 64 ^{b*** c***}	0.001	0.362	0.000
GPX3	U/mg p.	10.1 ± 1.1 ^{b*} ^{c(0.08)}	9.0 ± 0.4 ^{a*} ^{d**}	8.4 ± 1.9 ^{a(0.08)}	6.8 ± 1.4 ^{b**}	0.002	0.019	0.692
GPX3/CAT	kU/U	22.1 ± 4.5 ^{b*** c***}	13.3 ± 1.1 ^{a***}	11.4 ± 2.3 ^{a***}	12.8 ± 1.5	0.000	0.003	0.000
SH	μmol/L	112 ± 36	145 ± 72	148 ± 132	139 ± 10	0.658	0.715	0.534
GSHT	mmol/L	25.4 ± 3.4 ^{b(0.10)} ^{c(0.07)}	29.5 ± 4.2 ^{a(0.10)} ^{d*}	29.1 ± 2.8 ^{a(0.07)} ^{d*}	35.0 ± 4.3 ^{b* c*}	0.007	0.004	0.550
Mean ± standard deviation; FRAP - ferric ion reducing activity at 4 minute incubation;; FRAPf - uric acid activity free FRAP, ferric ion reducing activity at 60 minutes incubation minus doubled uric acid concentration(UA); pCAT, pGPX3 - catalase and glutathione peroxidase activity in plasma; MDA - malondialdehyde; CO - plasma carbonyl groups; CAT- catalase activity; GPX3 - glutathione peroxidase activity, GPX3/CAT - GPX3 and CAT enzyme activities ratio, SH - thiol groups, GSHT - total glutathione concentration; GSHT - reduced glutathione; GSH_RO - reduced to oxidized glutathione ratio. For explanation of superscripts see legend under Table 2.								

Experimental groups						ANOVA(p)		
						Sprouts	Lard	
GSHR	μmol/L	115 ± 13 ^{b***} c(0.10)	190 ± 22 ^{a***} d***	129 ± 15 ^{a(0.10)} d***	376 ± 93 ^{b***} c***	0.000	0.000	0.000
GSH_RO	mmol/mol	4.62 ± 0.91 ^{b**}	6.55 ± 0.84 ^{a** d**}	4.48 ± 0.58 ^{d***}	10.83 ± 2.20 ^{b**} c***	0.001	0.000	0.000
Mean ± standard deviation; FRAP - ferric ion reducing activity at 4 minute incubation;; FRAPf - uric acid activity free FRAP, ferric ion reducing activity at 60 minutes incubation minus doubled uric acid concentration(UA); pCAT, pGPX3 - catalase and glutathione peroxidase activity in plasma; MDA - malondialdehyde; CO - plasma carbonyl groups; CAT- catalase activity; GPX3 - glutathione peroxidase activity, GPX3/CAT - GPX3 and CAT enzyme activities ratio, SH - thiol groups, GSHT - total glutathione concentration; GSHR - reduced glutathione; GSH_RO - reduced to oxidized glutathione ratio. For explanation of superscripts see legend under Table 2.								

The influence of lard was not uniform. FRAP and FRAPf was surprisingly enhanced by lard in the diet. Lard in the diet significantly lowered MDA concentration while it increased CO level in plasma. This effect was antagonistically related the effect of sprouts in diet (p = 0.004).

Catalase in plasma (CAT) was significantly increased by sprouts in the diet, while an opposite effect was observed for glutathione peroxidase (GPX3). The later effect was also produced by lard in the diet. From the analysis of GPX3-to-CAT ratio, it is evident that both factors influenced enzyme activities in plasma and there was significant interaction between sprouts and lard.

Although both diets under study had no effect on thiol group levels in plasma, their effect was significant if analyzed for both reduced (GSHR) and total glutathione concentration (GSHT). GSHR and its relative parameter, i.e. reduced-to-oxidized glutathione ratio (GSH_RO), was affected by sprouts and lard in the diet, and strong interaction was observed between these factors. The increase in GSHR and GSH_RO was especially pronounced when the diet contained a combination of sprouts and lard. The above observations suggest existing strong synergistic interaction of both dietary factors in enhancing the endogenous antioxidant defense system. Among these effects, lard strengthened antioxidant defense system for lipids, while sprouts enhanced protection of protein fraction of the rat organism.

Discussion

In many developed countries cardiovascular diseases are the leading cause of death. Many studies have indicated that the increased concentration and oxidation of plasma low-density lipoproteins (LDL) are the main risk factors of atherosclerosis [17, 18]. Recently, much attention has been focused on looking for natural substances in daily diets, which would be able to prevent hypercholesterolemia and to slow the progression of atherosclerosis, particularly when supplemented to a high-fat diet rich in saturated

fatty acids. Researchers have focused their attention on searching for dietary components, which could lower lipid and cholesterol concentrations and possess antioxidant activity. The enzyme PON1 (paraoxonase-1) can be a target of such dietary intervention since it is an agent having antioxidant capacity and inhibiting oxidation of low-density cholesterol [19].

In this study, the effects of supplementation of buckwheat sprouts to the diet containing fat (30% lard) on food intake and related parameters, as well as the selected biochemical parameters of plasma, lipid profile of plasma, antioxidant status, and paraoxonase-1 activity in plasma and liver in Wistar rats were investigated. As shown in the study, the addition of fat to the diet of rats always resulted in the decreased food intake, for instance from 18.8 g in group C to 13.9 g/day in group CL, respectively. This effect was statistically significant (two-way ANOVA, $p < 0.001$). The food intake was the lowest in the fat-fed group (CL, control + lard) in comparison to other groups. Fat as an independent factor resulted also in higher FER values ($p < 0.001$) in the group with lard supplement. This result is similar to the finding reported by Lee et al. [11], who observed that the food intake was lower in animals fed high-fat diets when compared to the group of rats fed the diet with addition of buckwheat leaf and flower. In contrast, these results were opposite to the effect observed by Warwick & Weingarten [20] who reported that high-fat diet enhanced food intake relative to the high-carbohydrate diet. The present study demonstrated that diet supplementation with buckwheat sprouts, a product rich in polyphenols, had a marginal effect on food intake. However, different effect was observed by Oliveira de Souza et al. [21], who found that products rich in polyphenols could modulate appetite and lower the food intake in rats. The weight gain, expressed per one megacalorie of dietary energy intake (FEE), was the lowest in the group fed the diet with lard and sprouts (BSL), suggesting an enhanced exhausting of food energy caused by high-lard diet and/or poor availability of nutrients in the presence of sprouts. Buckwheat sprouts had an anorectic-like effect on the weight gain of rats. The low FER and the low food intake and nutrient availability were suggested to be related to the high contents of fiber and polyphenols in buckwheat sprouts [22, 23].

The results obtained in our study allow for suggestion of a significant beneficial effect of sprouts in the diet of rats, due to lowering of weight gain and keeping it within the normal range even in the presence of high fat content in the diet.

From the typical set of biochemical characteristics of plasma, the highest level of magnesium was observed in the sprouts-fed rats. Addition of the fat to the diet with buckwheat sprouts caused a significant reduction of serum magnesium concentration ($p < 0.05$). This effect was confirmed by the results obtained by Watkins et al. [24] who confirmed that the presence of saturated fats in the diet reduced the absorption of magnesium.

Some authors reported that elevated blood level of phosphorus could be associated with an increased risk of cardiovascular diseases, but this is particularly true in patients with renal impairment. In the report of Foley [25], it was suggested that higher serum level of phosphorus may be due to i.a. a lower body weight and lower BMI. In our study, rats fed buckwheat sprouts had the lowest weight after the

experiment, and the lowest weight gain in comparison to other groups of rats. Additionally this effect was consistent with significantly lower albumin level in both buckwheat sprouts-fed groups ($p < 0.001$).

Urea level is a potential marker of renal dysfunction, but in this study, there was no significant increase in it in rats fed buckwheat sprouts compared to control. However, fat addition to the sprouts fed rats resulted in a significant decrease in the urea level (BSL vs. BS, $p < 0.05$). Similarly, sprout addition to the control fat-fed group significantly reduced plasma urea (BSL vs. CL, $p < 0.05$). This interaction, i.e. the fact that sprouts decreased plasma urea concentration in the presence of high lard content in the diet was confirmed by two-way ANOVA ($p = 0.011$). Normal urea level excludes renal insufficiency as a potential cause of the elevated phosphorus level mentioned above.

In our experiment, feeding the rats the high-lard diet caused an increase in blood glucose level, regardless of the presence of buckwheat sprouts in the diet, which is consistent with the results of other researchers [26]. The above result was consistent with the reports of Oliveira et al.) [21], who suggested that fat in diet was an independent pro-diabetic factor.

In the present study, it was found that the consumption of lard decreased the plasma TG level ($p < 0.001$) but buckwheat sprouts did not. Oliveira et al. [21] reported that the addition of fat to the diet resulted in a decrease in TG when rats were administered cholesterol at a dose of 10 g/kg of feed. Similar effect was observed by Turbino-Ribeiro, et al. [27], who noted that rats fed the hypercholesterolemic diet showed a significant decrease in triacylglycerol level in serum. Park et al. [28] observed in a rat model that rutin was capable of reducing serum cholesterol and triglycerides by inhibiting the activity of acyl-CoA - cholesterol acyltransferase. Despite a high level of rutin in buckwheat sprouts, in this study the hypolipidemic activity of buckwheat sprouts was not confirmed as they did not influence TG level in plasma of rats.

There were no significant differences in plasma HDL cholesterol and non-HDL cholesterol concentrations between the groups, unlike in the works of Choi, et al. and Park, et al. [16, 28], who stated that the presence of rutin and phytosterols in buckwheat sprouts reduced the concentration of different fractions of cholesterol. Phytosterols evoked clinically relevant reductions of serum lipids by lowering the solubility of cholesterol in micelles and thus diminishing its absorption. In contrast to the studies by Choi, Seog, Park, Kim & Choi and Park, et al. [16, 28], we did not note a significant difference in HDL and non-HDL cholesterol levels between the groups. Our results confirmed that buckwheat sprouts increased HDL and decreased other cholesterol fractions, however, these effects did not reach statistical significance. Additionally, the addition of buckwheat sprouts to a diet, especially when supplemented with fat, produced a significant effect on the reduction of AIC, which may suggest that buckwheat sprouts have anti-atherosclerotic effects, which was especially pronounced since was observed regardless of the addition of fat to the feed of rats (two-way ANOVA, $p = 0.01$). Our results clearly suggest that buckwheat sprouts induced a decrease in the non-HDL cholesterol-dependent atherogenic index (AIC). Lard impact was observed for the TG-dependent atherogenic index (AIT), which was significantly reduced.

There is a growing amount of evidence indicating that the decreased PON1 activity is a significant factor associated with an increased risk of coronary atherosclerosis [19, 29, 30]. The decreased serum PON1 activity was reported to accompany other health conditions associated with atherosclerosis, including diabetes, hypercholesterolemia and renal diseases [31–33]. On the other hand, the decreased PON1 activity in serum of rats fed the high-lard diet could be explained by an imbalance in the antioxidant capacity caused by high amount of fat. Sutherland et al. [34] reported the reduced postprandial serum PON1 activity after a meal rich in reused cooking fat. PON1 activity may reflect the antioxidant and anti-atherogenic capacity [35, 36]. There is considerable evidence that the antioxidant activity of high-density lipoproteins (HDL) is largely due to the paraoxonase-1, being its component [37]. Our research shows that the germinated buckwheat itself significantly increased the plasma PON1 activity by almost two times. A similar effect was observed in the work of Canales et al. [38] where the addition of walnut-enriched meat to the human diet also increased significantly the activity of PON1 in the plasma.

The addition of buckwheat sprouts to rats' diet resulted in a significant increase in plasma PON1 compared to the control group. However, lard added to that diet abolished this effect. PON1 activity in the liver highly increased under the influence of fat supplement to the control diet. For diet containing buckwheat sprouts, in spite of the increased PON activity by 40%, statistical significance was not achieved. Furthermore, fat addition to the sprouts-supplemented diet had no effect on PON1 activity in the rat liver suggesting an antagonistic interaction. This was confirmed by a two-way ANOVA, which indicated that lard in the diet significantly modified the activity of hepatic PON and sprouts significantly affected this effect.

The addition of buckwheat sprouts to the high-fat diet caused an excessive reduction of damage caused by lipid peroxidation, expressed by the plasma MDA level. It was observed that the addition of the germinated buckwheat to the diet resulted in a decrease in the concentration of MDA relative to the control groups ($p = 0.002$, two-way ANOVA). Buckwheat sprouts greatly decreased CO-group concentration in comparison to control group, which indicates that they are capable of antioxidant protection of proteins.

Catalase activity was increased by the addition of buckwheat sprouts to the diet ($p < 0.001$). The high-fat diet promoted the absorption of lipophilic antioxidants. FRAP activity after the addition of fat to the diet was higher by 29% and 23% in the control group and in the group fed the diet containing sprouts, respectively. Similar correlations were found in the uric acid-free ferric reducing activity (FRAPf).

Conclusions

To summarize, these results indicate a significant beneficial effect of supplementing the diet of rats with buckwheat sprouts which resulted in reduction of weight gain and its normalization in rats fed a high-lard diet. In addition, buckwheat sprouts would be beneficial for improving the lipid profile and protection against atherosclerosis. These findings may suggest that buckwheat sprouts, a little known dietary component in Europe and rare ingredient of European cuisine, could be introduced into the Western diet

because of their potential ability to control weight gain and obesity, and of their anti-atherosclerotic effects. These effects are worth a further investigation.

Materials and Methods

Plant material

Buckwheat seeds (*Fagopyrum esculentum* Moench, variety “Luba”) were purchased from the Station of Plant Breeding in Palikije, Poland, in 2008. Voucher specimens were deposited in the Department of Food Chemistry and Nutrition, Faculty of Pharmacy, Medical College, Jagiellonian University, Ref. No. Nr/PP/PL 1038. In our previous paper the detailed information about antioxidant capacity of the tested material [39] was described. Buckwheat seeds were soaked in water for 6 hours at room temperature, then the wetted seeds were spread onto special glass vessels. They were germinated at room temperature (25°C) and 96% humidity, while being rinsed with tap water 3 times every day. Buckwheat sprouts were harvested 8 days after seeding, freeze-dried at -50 °C (FreeZone 4.5 Liter Console Freeze Dry System, Labconco, Kansas City, USA) and powdered.

Chemicals and solvents

Paraoxon-ethyl (diethyl-p-nitrophenylphosphate, Pestanal) was of analytical standard (CAS No 311-45-5, Fluka Analytical), all other reagents were of analytical grade and were obtained from Sigma Aldrich Chemical Company (Steinheim, Germany).

Animals

The selection of appropriate animal species and the number of animals in the group was performed in accordance with the ARRIVE guidelines. Ten-week-old male Wistar rats weighing approximately 250 g were used in the experiment. Animals were kept for 5 weeks in stainless steel cages with plastic bottom in a room with controlled conditions: 12-hour light/dark cycles; temperature $22 \pm 1^\circ\text{C}$; humidity 60–62% and had free access to feed and tap water. During the experiment, water and food consumption were recorded daily, while body weight of rats was measured three times a week. The study was conducted with the approval of the Jagiellonian University, under the Guidelines for the Care and Use of Experimental Animals (nr 80/2009).

Diets and experimental design

The rats were randomly divided into four groups of six animals each. The first group was fed a diet with corn starch and served as the control group (C), the second group (CL) received diet containing 300g/kg of lard added as corn starch replacement and served as the control high-fat diet. The third group (BS) consisted of rats fed a diet containing lyophilized buckwheat sprouts at the dose of 100 g/kg, and the

fourth group (BSL) received 300g/kg of lard and buckwheat sprouts at the dose of 100 g/kg. In all diets, combined additives (starch, lard, sprouts) constituted 62% of the feed weight. Detailed composition of each diet is shown in Table 1.

Chemicals and solvents

At the end of the five-week experimental period, after 16-hour fasting, all rats were weighted. Animals were euthanized by a sodium thiopental overdose (60 mg/kg) administered by intraperitoneal injection in compliance with requirements of the Local Ethics Commission. In order to determine the levels of plasma components, blood samples were taken from aorta into heparinized tubes and then centrifuged (at 3000 rpm for 15 minutes at 4°C). For paraoxonase activity determination, a serum aliquots were stored at – 80°C. Animal liver and major tissues were rapidly removed, weighed and immediately frozen in liquid nitrogen and stored at -80°C until further analyses.

Analytical procedures

Major biochemical parameters of the rat plasma were measured using biochemical analyzer Alizé with assay kits from Biomerieux. Normal serum level 1 (Olympus System Reagent, Control ODC0003) and pathological serum level 2 (Olympus System Reagent, Control ODC 0004) were used for quality control. Antioxidant activity of plasma (FRAP) was estimated using ferric reducing procedure [40]. The kinetic measurements were performed during 1 hour on 48-well microplates using spectrophotometric reader Synergy 2 (Biotek, U.S.A.). The results were expressed in micromoles of Fe(II)/L of plasma after 4- and 60- minute incubation. For estimation of uric acid-free antioxidant capacity of plasma (FRAPf), uric acid activity was subtracted as a doubled micromolar concentration from the measurements after a 60-minute incubation.

Paraoxonase 1 (PON1; EC 3.1.8.1) enzyme activity in the plasma and liver homogenates was determined by measuring hydrolysis of paraoxon stimulated in the presence of sodium chloride (NaCl) and monitoring spectrophotometrically the rate of increase in absorbance at 412 nm at 25°C, according to the modified method of Eckerson et al. [41]. The amount of generated p-nitrophenol was calculated using the molar extinction coefficient $18.29 \times 10^3 \text{ M}^{-1} \text{ cm}^{-1}$ at pH 8.0. Paraoxonase activity was expressed as U/mg protein. Plasma concentrations of reduced glutathione (pGSH), free thiol groups (pSH), protein carbonyl groups (pCO), malondialdehyde (pMDA) and antioxidant enzyme activity catalase (CAT; EC 1.11.1.6) and glutathione peroxidase (GPx3; EC 1.11.1.9) were determined essentially according to the same procedures as in our previous paper [42]. Fat content in the liver was estimated from the difference between the mass of freeze-dried samples before and after fat extraction. Dry powdered freeze-dried samples of the liver of about 0.3 g (accuracy 0.001g) were weighted in a dry paper filter thimble and were extracted in Soxhlet apparatus with petroleum ether for 3 hours. Residual sample was dried on a filter paper to constant weight. Fat content was expressed in milligrams per gram of dry liver sample. The content of glycogen in the liver was determined by hydrolysis of tissue (1 g) with KOH

(300g/L) in boiling water bath during 30 minute. Glycogen was precipitated by addition of methanol and the sediment was dried. Glycogen content in the liver was expressed in grams per 100g of wet tissue.

Other parameters

Food efficiency ratio (FER) was calculated as a ratio of body weight gain expressed per one gram of food intake. Atherogenic index of plasma (AI) based on non-HDL cholesterol was calculated from the modified equation by Dornas: $AITCH = \log(TCH / HDL - 1)$ where TCH is the total cholesterol and HDL signifies high-density lipoprotein cholesterol [43]. Logarithmic transformation was applied as it was found to better fit normal distribution as verified by Kolmogorov-Smirnov and chi-square tests. Triglyceride based atherogenic index of plasma (AITG) was also calculated from the formula: $AITG = \log(TG/HDL)$, which has been suggested as a significant predictor of atherosclerosis [44].

Statistics

Data is presented as the mean $\pm$ standard deviation (SD). The statistical analysis was conducted using the STATISTICA 5.1 PL software (StatSoft, Inc.). A type of distribution for the analyzed variables was tested by the chi-square test. Outliers were removed from groups based on the Grubbs test. Data with a normal distribution was analyzed by a two-way analysis of variance (ANOVA) with buckwheat sprouts and lard as the factors, and their interactions were tested. The general critical significance level was set at $p < 0.05$

Declarations

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Author Contributions statement

Conceptualization, Ch.J. and F.R.; methodology, Ch.J., F.R.; software, B.H., F.S.; validation, B.M., F.R. P.H.; formal analysis, B.M., K.M., F.R.; investigation, Ch.J., P.H.; writing—review and editing, Ch.J., F.R., P.H., B.B

Data Availability Statement

The datasets used and/or analysed during the current study available from the corresponding author on reasonable request

All authors have read and agreed to the published version of the manuscript. The authors declare no conflicts of interest.

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