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The Effect of Buckwheat (*Fagopyrum esculentum* Moench) Groats Addition to the Lard Diet on Antioxidant Parameters of Plasma and Selected Tissues in Wistar Rats

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***Abstract*—**Recent studies demonstrated that high-fat diet increases oxidative stress in plasma and in a variety of tissues. Many researchers have been looking for natural products, which can reverse the effect of high fat diet. Recently, buckwheat is becoming common ingredient in functional food because of it properties. In study on buckwheat, it is known that, this plant plays roles as anti-oxidative, anti-inflammatory and anti-hypertensive. Nevertheless still little is known about buckwheat groats. The aim of this study was to investigate the effects of addition of buckwheat groats to the fat diet (30% lard), on some antioxidant and oxidant stress parameters in plasma and selected tissues in Wistar rats. The experiment was carried out with three months old male Wistar rats ca. 250g of body weight fed for 5 weeks with either a high-fat (30% of lard) diet or control diet, with or without addition of buckwheat groats. In plasma biochemistry and the activities of the antioxidant enzymes were measured selected tissues: glutathione peroxidase (GPX), catalase (CAT) and the levels of total and reduced glutathione (GSH), free thiol groups (pSH), antioxidant potential of plasma (FRAP) and oxidant stress indices - proteins carbonyl groups (CO) and malonyldialdehyde concentration (MDA). Activity of catalase (CAT) in plasma of rats was significantly increased in buckwheat groats groups and activity of GPx3 in plasma of rats was decreased in buckwheat groups as compared to control group. The reduced glutathione (GSH) in plasma of rats was significantly increased and protein CO was significantly decreased in buckwheat groups as compared to controls. The lowered concentration of GSH was found in serum of rats fed buckwheat groats addition but it accompanied in 7-fold increase in reduced-to-oxidized glutatione ratio, significant increase in HDL and decrease in nonHDL concentration. Conclusions: Buckwheat groats indicate a beneficial effect in inhibiting protein and lipid peroxidation in rats and improved lipid profile. These results suggest that buckwheat groats exert a significant antioxidant potential and may be used as normal food constituent to ameliorate the oxidant-induced damage in organism.

***Keywords*—**Antioxidant, buckwheat, high-fat diet, rats.

I. Introduction

SEVERAL studies have demonstrated that high-fat diet increases oxidative stress in plasma and in a variety of tissues. Additionally high-fat diet was suggested to be a significant risk factor of cardiovascular disease [1]. High-fat diets increase oxidative stress in plasma and tissues of living organisms. Therefore, changes in dietary habits could be an important factor in successful therapy for that disease. Many researchers look for the natural products which can reverse or prevent against the effects of high fat diet [2], [3].

Recently, buckwheat is becoming a common ingredient of functional food products because of its properties. Buckwheat is grown primarily in Russia, China, but also in the USA, Canada and Europe, including Ukraine and eastern part of Poland [4].

Buckwheat seeds contains: vitamins such as B1, B2 and a factor facilitating the absorption of vitamin B; minerals including K, Mg, Ca, Fe, Se, Zn; and other compounds, such as protein with balanced amino acid composition, phytosterols, soluble carbohydrates, D-chiro-inositol, flavonoids, phenolic acids, tocopherols, inositol phosphates, rutin. Additionally buckwheat is rich in unsaturated fatty acids therefore, consumption of products made from buckwheat is considered to be helpful in preventing human disorders such a cardiovascular diseases, hypercholesterolemia or hypertension [5], [6].

It was shown through numerous studies on buckwheat that, this plant plays an antioxidant, anti-inflammatory and anti-hypertensive role [7].

Nevertheless still little is known about buckwheat groats. Broken or whole buckwheat grain without involucre is called buckwheat groats or kasha [5].

The content of nutrients in buckwheat groats mostly depends on technological processes used for its production, e.g. roasting decreases both antioxidant properties and protein quality [8]. Buckwheat groats in comparison to other groats, such as millet or semolina are characterized by high content of proteins (with balanced amino acid composition), carbohydrates, and fatty acids. It is also a valuable source of minerals and vitamins (potassium, calcium, phosphorus, magnesium, zinc, manganese, vitamins E, and group B vitamins) [9].

Wei et al. showed that the ethanol extract from buckwheat groats may protect DNA from non-site-specific and site-specific hydroxyl radical attack. Thus, buckwheat groats may play also an important role in control of carcinogenesis [10].

Therefore, the aim of this study was to investigate the effect

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of buckwheat groats addition to the 30% lard diet on some antioxidant parameters of plasma and selected tissues in Wistar rats.

II. Materials and Methods

A. Plant Materials

Buckwheat grouts were purchased from the company, Janex" Jan Ciupak, Leszek Ciupak, Spółka Jawna (Poland). A 100g portion of these buckwheat groats contains 350kcal (1503kJ), 13.0g of protein, 70g of digestible carbohydrates, 3.0g of total fat, 5mg of sodium, 0.54mg of thiamine, 52mg of phosphorus, 2.8mg of iron and 218mg of magnesium.

B. Animals and Diets

Twelve weeks old male Wistar rats weighing 244.3 ± 7.6g were used in the experiment. Rats were randomly divided into 6 groups of 6 rats each and acclimatized for 1 week before the main feeding experiments was started. For 5 weeks all rats were kept in stainless steel cages with a plastic bottom in a room with controlled light for 12 hours (8:00-20:00), temperature 22±1°C, humidity (60-62%) and had free access to feed and tap water. The animals were fed a standard diet. The composition of each diet is shown in Table I. Water and food consumption was recorded daily, while body weight was measured every two days in the course of the experiment. These studies were conducted with the approval of the Jagiellonian University Animal Care Committee according to Guidelines for the Care and Use of Experimental Animals.

TABLE I
Composition of Experimental Diets

Group	Corn starch	Lard	BGs	Energy* [kJ/g]	Fat*	Protein*	Dietary fiber*
C	620	0	0	13.9	51	141	47
CL	320	300	0	20.4	351	141	47
BG	320	0	300	13.4	59	176	78
BGL	20	300	300	20.1	357	175	77

C - Control group; CL- Control group with lard; BG- Buckwheat groats group; BGL- Buckwheat groats with lard, BGs - buckwheat groats

Each fodder contained (in mg/g; g/kg): casein 200, rapeseed oil 50, chalk 28, calcium monophosphate ($CaHPO_4$) 29, soy lecithin 10, sodium chloride 3, potassium sulphate (K_2SO_4) 3.5, magnesium oxide (MgO) 0.7, cellulose 45.8, mixture of vitamins and microelements 10 (Premix LPM, BASF, Poland – vitamins and minerals). Potassium (K) and magnesium (Mg) contents in fodder for groups C, CL, BG, BGL were calculated on the basis of contributions of constituents and were: K 0.81; 0.81; 1.76; 1.75mg/g; Mg 0.49; 0.49; 1.15; 1.14mg/g, respectively.

C. Sample Collection and Analysis

At the end of the experiment, after a 16-hour fast, all rats were weighted and euthanized by intraperitoneal injection of sodium thiopental 60mg/kg in compliance with requirements of the Local Ethics Commission. Blood samples were taken from aorta into heparinized tubes and then centrifuged (at 6000xg for 15 minutes at 4°C) to obtain plasma that was immediately analyzed or kept frozen at (-80°C) until further analyses. Animal tissues were rapidly removed, weighed and immediately frozen in liquid nitrogen and stored at -80°C until further analyses.

D. Biochemical and Antioxidant Parameters of Rat Tissues and Plasma

The concentrations of the following biochemical parameters were measured in plasma: total cholesterol, triglycerides, albumin, glucose, urea, uric acid, AST, ALAT, AP using a biochemical analyzer (Alizé) and assay kits from Biomerieux. Results were compared with control serum: normal (Control serum 1 ODC 0003) and pathological (Control serum 2 ODC 0004) from Olimpus.

Antioxidant activity of plasma (FRAP) was estimated using the ferric reducing procedure [11]. 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 (UAFRAP), uric acid activity was subtracted as a doubled micromolar concentration from the measurements after a 60-minute incubation.

Plasma concentrations of reduced glutathione (pGSH), free thiol groups (pSH), protein carbonyl groups (pCO), malondialdehyde (MDA) and activity of antioxidant enzymes catalase (EC 1.11.1.6) and glutathione peroxidase (GPx3; EC 1.11.1.9 were determined essentially according to the same procedures as in [12].

E. 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 (AITCH) based on non-HDL cholesterol was calculated from the modified by us equation by Dornas: AITCH = log (TCH -HDL)/HDL where TCH is the total cholesterol and HDL signifies high-density lipoprotein cholesterol [13]. Logarithmic transformation was applied because we found it better fits to normal distribution as verified by Kolmogorov-Smirnov and chi-square tests. We also calculated triglyceride-based atherogenic index of plasma (AITG) from the formula: AITG = log (TG / HDL), which has been suggested as a significant predictor of atherosclerosis [14]. NonHDL was calculated as a difference between TCH and HDL (NonHDL= TCH -HDL).

F. Statistics

Data were presented as the mean ± 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 were analyzed by a two-way analysis of variance (ANOVA) with buckwheat sprouts and lard as the factors, and their interactions were tested. The Tukey post hoc test was used to determine the differences between the groups when a statistical two-factor interaction was observed. Kruskal-Wallis test was applied to check for any differences between different groups of animals within the whole set of

animals. Dunn's post-hoc test was used to reveal the differences between the paired groups of animals. Differences with $p<0.05$ were considered to be statistically significant.

III. Results

A. Body Weight, Food and Water Consumption

Consumption of buckwheat groats is a statistically significant factor for the weight gain, what was revealed by ANOVA2 for both the final body weight and weight gain. Rats from (BGL) group showed the highest weight at the end of experiment as compared to the other groups. Weight gain was also the highest for groups fed buckwheat groats, despite the fact that food intake in BGL group was significantly lower when compared to the control group. Rats from (C) group ate more feed as compared to (CF) and (BGL) groups. Food and water intake (Table II) were highly statistically significantly dependent on two food components, groats and lard. They acted independently but in opposite directions, fat decreased food intake and groats increased the intake. Similarly, fat and groats acted as independent factors for energy intake and for FER parameter. Food energy efficiency (food energy intake per gram of weight gain) was the least cost-effective for the group consuming groats, which means that this product can help control weight gain.

TABLE II
Food and Water Intake and Related Parameters

Parameter	Experimental groups				ANOVA(p)		
	Control		Groats		Groats	Lard	
	C	CL	BG	BGL	1	2	1 x 2
	a	b	c	d			
Rat weight, initial (g)	245.3±9.6	246.0±7.1	239.7±4.8	242.5±9.6	0.178	0.600	0.745
Rat weight, final (g)	364.7±27.7 ^{c*}	375.0±20.2 ^{d*}	398.8±14.2 ^{a*}	403.2±17.4 ^{b*}	0.001	0.392	0.724
Weight gain (g/day)	3.41±0.53 ^{c**}	3.79±0.63 ^{d*}	4.55±0.35 ^{a**}	4.59±0.27 ^{b*}	0.000	0.274	0.380
Water intake (g/day)	27.1±3.5 $^{b*c(0.10)}$	23.2±0.7 ^{a*d***}	29.9±1.5 $^{a(0.10)\ d**}$	26.2±1.4 ^{b***c**}	0.002	0.000	0.880
Food intake (g/day)	18.8±1.3 ^{b***}	13.9±0.7 ^{a***d*}	19.7±0.1 ^{d***}	14.7±0.0 ^{b*c***}	0.012	0.000	0.948
Energy intake (kJ/day)	251±18 ^{b***}	342±18 ^{a***d*}	263±1 ^{d***}	363±1 ^{b*c***}	0.005	0.000	0.402
Food efficiency ratio (FER)[x]	0.18±0.02 ^{b***c**}	0.27±0.04$^{a***d(0.05)}$	0.23±0.02 ^{a**d***}	0.31±0.02$^{b(0.05)\ c***}$	0.001	0.000	0.615
Food energy efficiency (FEE; g/MJ)[y]	13.6±1.8 ^{b*c**}	11.1±1.6 $^{a*d(0.05)}$	17.3±1.4 ^{a**d***}	12.6±0.7 $^{b(0.05)\ c***}$	0.000	0.000	0.081

Mean ± standard deviation; [x] FER - food weight efficiency, body weight gain in gram per gram of food intake (g/g); [y] FEE - weight gain per energy intake (g /1000 kJ; g/MJ). Significant difference of means (one-way ANOVA) was indicated by superscript letter assigned to second group, followed by asterisks or p-value; where *** denotes $p<0.001$, ** $p<0.01$; * $p<0.05$, and p-level in the range 0.05-0.10 is given in parentheses

B. The Effect of Buckwheat Groats Addition to the High-Fat Diet on Plasma Biochemical Parameters in Rats

Addition of lard to the diets, particularly with buckwheat groats, statistically significantly decreased the plasma calcium level. Plasma magnesium level was the highest in the group of rats on the kasha diet; addition of lard decreased this parameter to the level close to that for the control group, although these differences were not statistically significant.

The decrease in plasma potassium levels was the effect of addition of lard to the diet, and the difference in concentration of this element between the groups CL and C was statistically significant, what is more, buckwheat groats addition to high-fat diet increased the plasma magnesium concentration (BGL vs. CL, $p<0.001$). A similar relationship as for magnesium occurred for phosphorus, adding kasha to the fat-containing diet increased the phosphorus level (BGL vs. CL, $p<0.05$).

Plasma albumin concentration in buckwheat groats-fed group was significantly diminished; both with the addition of groats as well as groats and with a high-fat combination, however, the level of proteins did not change.

TABLE III
Biochemical Characteristics of Plasma

Parameter[x]	Experimental groups				ANOVA(p)		
	Control		Sprouts		Groats	Lard	
	C	CL	BG	BGL	1	2	1 x 2
	a	b	c	d			
Ca	3.13±0.27	2.92±0.28	3.15±0.12 ^{d*}	2.93±0.14 ^{c*}	0.876	0.022	0.931
Mg	0.83±0.15	0.81±0.07	0.93±0.10 $^{d(0.10)}$	0.82±0.10 $^{c(0.10)}$	0.212	0.144	0.376
K	4.27±0.40 $^{b(0.09)}$	3.84±0.39 $^{a(0.09)\ d**}$	4.29±0.63	4.54±0.25 ^{b**}	0.056	0.628	0.075
Na	144.9±7.9	143.1±5.7	140.2±2.8	146.7±16.0	0.895	0.543	0.296
P	3.15±0.26	2.89±0.48 ^{d*}	3.36±0.71	3.73±0.65 ^{b*}	0.031	0.814	0.176
ALB	35.1±3.9 $^{c(0.05)}$	35.2±4.0 $^{d(0.08)}$	31.4±1.3 $^{a(0.05)}$	31.7±1.8 $^{b(0.08)}$	0.009	0.880	0.931
PROT	48.6±13.6	48.8±2.9	49.6±18.1	51.5±3.9	0.690	0.823	0.856
GL(g/L)	9.27±0.86 ^{b**}	11.01±0.59 ^{a**d*}	10.65±2.49	10.04±0.67 ^{b*}	0.720	0.330	0.052
U	9.1±2.2 ^{c*}	10.1±1.8	12.3±1.9 ^{a*d*}	9.9±1.0 ^{c*}	0.047	0.337	0.033
UA (μmol/L)	130±64$^{c(0.07)}$	117±30	236±111$^{a(0.07)d(0.06)}$	135±29 $^{c(0.06)}$	0.037	0.052	0.123
ALP (U/L)	284±95 ^{b*}	432±79 ^{a*d*}	319±26	343±52 ^{b*}	0.350	0.006	0.038
ALT (U/L)	37.1±7.0 $^{b(0.07)}$	47.3±9.9 $^{a(0.07)}$	40.5±2.1	42.0±4.6	0.719	0.042	0.123
ASP (U/L)	121±56 ^{c*}	154±53	190±39 ^{a*}	178±24	0.019	0.572	0.233

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. Significant difference of means (one-way ANOVA) was indicated by superscript letter assigned to second group, followed by asterisks or p-value; where *** denotes $p<0.001$, ** $p<0.01$; * $p<0.05$, and p-level from the range 0.05-0.10 is given in parentheses

TABLE IV
PLASMA LIPID PROFILE

Parameter	Experimental groups				ANOVA(p)		
	Control		Groats		Groats	Lard	
	C	CL	BG	BGL	1	2	1 x 2
	a	b	c	d			
TG[&]	1.39±0.45 ^{b**}	0.66±0.19 ^{a**}	1.38±0.26 ^{d***}	0.75±0.19 ^{c***}	0.764	0.000	0.680
TCH	1.99±0.48	1.79±0.08	2.02±0.25	1.80±0.22	0.885	0.101	0.962
HDL	0.86±0.17 ^{c***}	0.69±0.22 ^{d*}	1.30±0.14 $^{a***d(0.05)}$	1.05±0.24 $^{b*c(0.05)}$	0.000	0.018	0.589
nonHDL	1.14±0.34 ^{c*}	1.10±0.20 ^{d**}	0.71±0.23 ^{a*}	0.76±0.17 ^{b**}	0.001	0.971	0.701
AITCH [x]	0.11±0.09 ^{c***}	0.22±0.22 ^{d*}	-0.28±0.16 ^{a***}	-0.14±0.17 ^{b*}	0.000	0.090	0.803
AITG [y]	0.20±0.11 ^{b**c*}	-0.01±0.11 $^{a** d(0.07)}$	0.02±0.10 ^{a*d*}	-0.15±0.12 $^{b(0.07) c*}$	0.002	0.000	0.617

Mean ± standard deviation; [&] in mmol/l; 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); Significant difference of means (one-way ANOVA) was indicated by superscript letter assigned to second group, followed by asterisks or p-value; where *** denotes p<0.001, ** p<0.01; * p<0.05 and p-level from the range 0.05-0.10 is given in parentheses

TABLE V
CHARACTERISTICS OF ANTIOXIDANT DEFENSE STATUS

Parameter		Experimental groups				ANOVA(p)		
		Control		Groats		Groats	Lard	
		C	CL	BG	BGL	1	2	1 x 2
		a	b	c	d			
FRAP4	µmol/L	237±54 ^{c*}	306±111	323±52 ^{a*}	309±15	0.141	0.348	0.165
UAFRAP	µmol/L	279±108	375±157	149±209 $^{d(0.07)}$	328±49 $^{c(0.07)}$	0.146	0.029	0.482
pCAT	U/mg p.	467±56 ^{b***c***}	678±33 ^{a***}	691±73 ^{a***}	645±42	0.000	0.001	0.000
pGPx3	U/mg p.	10.13±1.11 ^{b*c*}	8.98±0.45 ^{a*d***}	8.12±1.30 ^{a*d*}	6.76±0.53 ^{b***c*}	0.000	0.003	0.790
pGPx3/pCAT	kU/U	22.1±4.5 ^{b***c***}	13.3±1.1 ^{a***d***}	12.1±0.4 ^{a***d**}	10.5±0.9 ^{b***c**}	0.000	0.000	0.002
CAT_KD	U/mg p.	219.8±86.0	171.4±91.6	199.3±60.8	154.5±59.2	0.552	0.147	0.954
CAT_LG	U/mg p.	7.5±1.7 ^{c***}	8.8±2.6 ^{d*}	14.8±2.7 ^{a***}	12.6±2.1 ^{b*}	0.000	0.653	0.072
CAT_HR	U/mg p.	26.3±11.8 ^{c**}	22.2±8.0	6.3±4.1 ^{a**d*}	23.7±14.9 ^{c*}	0.043	0.136	0.021
CAT_SP	U/mg p.	76.3±41.9$^{b(0.06)c*}$	119.4±26.8 $^{a(0.06)d***}$	4.8±1.8 ^{a*d*}	32.2±17.1 ^{b***c*}	0.000	0.015	0.555
CAT_BR	U/mg p.	0.17±0.22	0.42±0.46	0.31±0.33	0.45±0.45	0.588	0.214	0.718
CAT_PN	U/mg p.	9.60±1.98 ^{c***}	14.30±6.86 ^{d*}	3.39±0.89 $^{a***d(0.08)}$	5.63±2.64 $^{b* c(0.08)}$	0.000	0.038	0.441
CAT_MS	U/mg p.	1.34±0.86 $^{c(0.08)}$	0.98±0.50 ^{d***}	2.47±1.15 $^{a(0.08)d*}$	4.23±1.43 $^{b*** c*}$	0.000	0.117	0.022
CAT_TS	U/mg p.	12.0±1.6 ^{b***c***}	2.34±0.70 ^{a***d***}	10.5±1.8 ^{c***}	6.93±2.84 ^{b**}	0.152	0.062	0.190
GPX_KD	U/mg p.	0.76±0.36	0.58±0.24	0.74±0.21	0.54±0.20	0.757	0.092	0.933
GPX_LG	U/mg p.	0.21±0.02 $^{c(0.06)}$	0.22±0.04	0.25±0.05$^{a0.06)d0.08}$	0.22±0.02 $^{c(0.08)}$	0.139	0.288	0.076
GPX_HR	U/mg p.	0.39±0.10	0.35±0.09 $^{d(0.09)}$	0.26±0.16	0.25±0.11 $^{b(0.09)}$	0.022	0.619	0.832
GPX_SP	U/mg p.	0.44±0.11	0.43±0.11 ^{d***}	0.50±0.18 ^{d**}	0.90±0.18 ^{b***c**}	0.000	0.005	0.003
GPX_BR	U/mg p.	0.15±0.06	0.13±0.07	0.12±0.01 ^{d*}	0.09±0.02 ^{c*}	0.047	0.290	0.628
GPX_PN	U/mg p.	0.34±0.04 $^{b* c***}$	0.52±0.15 ^{a*d**}	0.11±0.03$^{a***d(0.10)}$	0.19±0.10$^{b**c(0.10}$	0.000	0.004	0.239
GPX_MS	U/mg p.	0.06±0.02 ^{c*}	0.07±0.02 ^{d*}	0.10±0.03 ^{a*}	0.11±0.02 ^{b*}	0.001	0.165	0.966
GPX_TS	U/mg p.	0.36±0.12 ^{b***c**}	0.09±0.01 ^{a***d***}	0.21±0.04 ^{a*}	0.18±0.09 ^{b*}	0.664	0.571	0.052

Mean ± standard deviation; FRAP - ferric ion reducing activity at 4 minute incubation in micromol Fe(II)/L, UAFRAP - uric acid activity free FRAP, ferric ion reducing activity at 60 minutes incubation minus doubled uric acid concentration (UA in micromol/L) , pCAT, pGPX3 - catalase and glutathione peroxidase activity in plasma; CAT_ GPX_ - activities in tissues: KD - kidney, LG - lang, HR - heart, SP - spleen, BR-brain, PN-pancreas, MS-muscle, TS-testicle; Significant difference of means (one-way ANOVA) was indicated by superscript letter assigned to second group, followed by asterisks or p-value; where *** denotes p<0.001, ** p<0.01; * p<0.05 and p-level from the range 0.05-0.10 is given in parentheses

TABLE VI
PLASMA REDOX STATUS

Parameter		Experimental groups				ANOVA(p)		
		Control		Groats		Groats	Lard	
		C	CL	BG	BGL	1	2	1 x 2
		a	b	c	d			
MDA	µmol/l	1.42±0.15 ^{b*}	1.16±0.20 ^{a*d**}	1.39±0.59 ^{d*}	0.73±0.24 ^{b**c*}	0.113	0.004	0.161
pCO	nmol/mg p.	6.57±1.94 ^{c***}	6.01±0.68 ^{d***}	1.34±0.37 ^{a***d*}	2.19±0.64 ^{b***c*}	0.000	0.756	0.130
pSH	µmol/l	2.35±0.55	2.94±1.36	2.72±1.49	2.32±0.79	0.783	0.833	0.291
GSHT	mmol/l	25.4±3.4 $^{b(0.10) c(0.07)}$	29.5±4.2 $^{a(0.10) d***}$	29.2±3.2 $^{a(0.07) d***}$	10.6±2.7 ^{b***c***}	0.000	0.000	0.000
GSHR	µmol/l	115±13 ^{b***c***}	190±22 ^{a***d***}	286±61 ^{a***}	335±66 ^{b***}	0.000	0.004	0.502
GSH_RO	mmol/mol	4.62±0.91 ^{b**c***}	6.55±0.84 ^{a**d***}	9.91±2.04 ^{a***d***}	34.37±9.46 ^{b***c***}	0.000	0.000	0.000
GSH_RT	mmol/mol	4.60±0.90 ^{b**c***}	6.51±0.83 ^{a**d***}	9.81±2.00 ^{a***d***}	33.16±8.89 ^{b***c***}	0.000	0.000	0.000

Mean±standard deviation; MDA - malondialdehyde; CO - carbonyl groups; SH - thiol groups, GSHT - total glutathione concentration; GSHR - reduced glutathione; GSH_RO - reduced to oxidized glutathione ratio, GSH_RT - reduced to total glutathione ratio. Significant difference of means (one-way ANOVA) was indicated by superscript letter assigned to second group followed by asterisks or p-value; where *** denotes p<0.001, ** p<0.01; * p<0.05 and p-level from the range 0.05-0.10 is given in parentheses

The level of glucose was higher in the lard group in comparison to the control group, what is more, addition of groats to the high fat diet caused a decrease in plasma glucose levels in rats (CL vs. BGL, p <0.05).

Plasma of rats eating buckwheat groats was characterized by statisticaly higher levels of urea and uric acid in comparison to control group. Besides, lard did not show a significant decreasing effect on the above-mentioned parameters, there

was found a significant interaction (at least for uric acid in plasma), and rats eating groats together with the fat have reduced these parameters to values similar to those of the control group.

Activity of alkaline phosphatase (ALP) and alanine aminotransferase (ALT) were the higest in the lard group. In the case of the ALP there was a statistically significant interaction between buckwheat groats and lard, weakening its high rise with lard. Groats produced an adverse effect on plasma aspartate aminotransferase activity, increasing the activity of this enzyme (Table III).

C. The Effect of Buckwheat Groats Addition to the 30% Lard Diet on Plasma Lipid Profiles

The addition of lard to the control (C) and buckwheat groats (BGL) diets resulted in a statistically significant ($p < 0.001$) decrease in plasma triacylglycerol concentration. Lard was the factor always decreasing the level of triacylglycerols, independently of the presence of BG. No BG effect on TG levels was observed.

Buckwheat groats in the diets influenced plasma HDL and nonHDL cholesterol level, and also AITC and AITG. Group of rats eating BG had the highest HDL cholesterol levels and the lowest nonHDL cholesterol concentration in plasma. The presence of lard in the diet caused a decrease in plasma HDL cholesterol concentration; however, buckwheat groats addition to the lard group counteracted the adverse changes. Lard had no effect on nonHDL cholesterol levels and such an interaction was not observed.

In accordance to the above, atherogenic index of plasma related to TCH (AITCH) and TG (AITG) more clearly showed a significant decreasing effect of the buckwheat groats diets (Table IV). Lard exhibited a decreasing effect only on the AITG, acting additively with BG. For the TCH related AI, possible there was an increasing tendency produced by lard (BGL vs. BG, $p<0.05$).

D. The Effect of BUCKWHEAT Groats Addition to the 30% Lard Diet on Antioxidant Defense System

The FRAP value, an indicator for total antioxidant ability of plasma (FRAP4) was increased by buckwheat groats added to the diet (BG vs. C, $p < 0.05$) while urate-free FRAP (UAFRAP) was increased by lard in the diet (ANOVA2, $p=0.029$).

The addition of buckwheat groats significantly enhanced by 48% ($p < 0.001$) plasma catalase activity. Lard in the diet led to an increase in plasma catalase activity (pCAT) versus the control group (C) ($p < 0.001$), while both nutritional factors acting together counteracted these effects. The similar effects were observed for GPX/CAT ratio. In contrast, significant but opposite and independent effects (no interaction) were observed for the GPX activity.

Buckwheat groats consumption resulted in an enhanced antioxidant enzyme activity in the lungs (CAT), spleen (GPX), pancreas (GPX) and muscle (CAT, GPX). A decreasing effect of BG was observed in the spleen (CAT), pancreas (CAT), heart (CAT, GPX) and weakly in the brain (GPX). Lard increased activity of both enzymes in the spleen and pancreas. The adverse effect of lard on CAT in the heart was completely abolished by BG added to the diet what indicated a significant interaction ($p=0.021$). Similar effect on CAT was observed in muscle. A possible synergistic interaction could also be noted in the case of GPX in the spleen.

E. Antioxidant Status and Oxidant Stress Parameters

The addition of buckwheat groats did not influence MDA levels, however, a decreasing effect of lard was observed. A strong, significant decreasing effect on MDA concentration in plasma was observed with the lard and buckwheat groats combination diet.

Buckwheat particularly afforded protection against oxidation of proteins, as demonstrated by three- to five-fold lower levels of carbonyl groups (pCO) in the plasma of animals in comparison with the control groups.

Positive effects of both nutrients were also observed on the total glutatione level (GSHT), reduced glutatione level (GSHR, 2.5-fold increase) and reduced-to-oxidized (GSH_RO, 2-fold increase) and reduced-to-total level (GSH_RT, 2-fold increase) ratios. The both latter parameter values were significantly higher in rats fed the buckwheat groats and lard diet in combination (7-fold increase); however, it was accompanied by a decrease in the total GSH concentration. These observations suggest that both factors acting together significantly shift redox status into an advantageous direction.

IV. Discussion

High fat-fed rats are considered to be one of many models, which have been developed to reflect an increased cholesterol level and lipid peroxidation. Fat feeding can induce a greater free radical formation and reduce antioxidant enzyme activity. Malheiros et al found evidence indicating that activities of antioxidant enzymes were lower in animals fed a high-fat diet [15]. In addition, many disorders as well cerebrovascular as cardiovascular can be induced by oxidative stress [16].

Results of several studies indicate a significant role of antioxidants in prevention of disorders to which a high-fat diet has contributed [17]-[19].

Therefore, the role of antioxidants in diseases induced by a high-fat diet may be very important. Buckwheat is recognized as a rich source of antioxidants, therefore, in our experiment; we investigated the effect of buckwheat groats addition to the 30 % lard diet on antioxidant parameters in plasma and selected tissues in Wistar rats.

Final weight of rats fed buckwheat groats was statistically significantly higher as compared to the control diet. On the contrary, rats fed tartary buckwheat bran extract in addition to the high fat diet (in 0.5g/kg and 1g/kg of body weight dose) took on weight as compared to control and control fat diet [20]. Besides, enrichment of a high fat and cholesterol meal with 2.5% buckwheat seed or 2.5% buckwheat sprouts affected hamster weight in a similar way as the control diet [21].

Rats in the buckwheat groats (BG) groups consumed statistically significantly more fodder than rats from the buckwheat groats plus lard group (BGL) group. On the other hand, fodder consumption in the control (C) group was

statistically significantly higher as compared to the control fat (CL) diet and the buckwheat groats plus fat diet (BGL) groups. In a study on the effects of mulberry (*Morus alba* L.) ingredients on obesity, it was observed that mice fed a high fat diet enriched with mulberry-leaf powder or purified quercetin (one of the major components of the leaves of this plant) also consumed more food as compared to animals fed a high fat diet only [22]. Similar influence on feed intake was also observed in the studies on saffron addition to the high fat diet [23].

In our study, we did not observe any significant differences in concentration of magnesium, potassium and sodium in plasma among the tested animals. Only the level of calcium in plasma of rats fed buckwheat groats and lard in combination decreased in comparison to rats from group that ate only buckwheat groats. Addition of lard, which is a saturated fat slows down the absorption of elements. Saturated fatty acids in particular may alter the calcium absorption [24].

For the buckwheat groats group, we observed the depletion of albumin level and increase in the phosphorus level, which caused changes in protein metabolism.

In our experiment, feeding of rats the high-lard diet caused an increase in blood glucose level, regardless of the presence of buckwheat groats in the diet, which is consistent with the results of other researchers. The above result was in line with the reports of Oliveira et al. in 2010, which suggested that fat in the diet was an independent pro-diabetic factor. [25]. Buckwheat sprouts-supplemented diet lowered blood glucose level in diabetic mice. These properties of buckwheat sprouts may be associated not only with the presence of flavonoid compounds but also cyanidin 3-rutinoside [26].

Similar observation was made for Tartary buckwheat bran, which is a source of D-chiro-inositol. This component induces the decrease in glucose level in diabetic mice [27]. Therefore, the influence of buckwheat on blood glucose levels in diabetic mice is different than in rats fed the 30% fat diets.

Uric acid is a potent antioxidant and an increase in its concentration could be associated with the elevated level of cholesterol, triglycerides or glucose [28], [29].

In this study, the above dependence was observed in rats fed the fodder supplemented with buckwheat groats (BG). In our experiment, we observed that buckwheat groats addition to basic diet increased the concentration of uric acid in plasma of rats. Similar results were obtained in the Pre'stamo et al., study on the role of buckwheat as prebiotic and healthy food [30]. Nevertheless, in the control fat (CL) group, the concentration of uric acid was decreased as compared to the control (C) group. The opposite effect was reported by Lin et al., which indicates that a high-fat diet not only produces disorders in lipid metabolism, but also affects uric acid metabolism [21] In addition, in our results, enrichment of the 30% fat diet with buckwheat groats contributed to lowering of uric acid levels, bringing the value to those obtained in the control (C) group. Similar dependence was obtained in a study associated with G. Cambogia [29].

In our study, the concentration of urea in plasma of rats fed the high-fat diet (CL) increased slightly as compared to animals fed the control diet (C). In studies on the effects of Garcinia Cambogia, it was observed also that a high-fat diet caused an increase in the concentration of that parameter as compared to the normal diet while Garcinia Cambogia supplement to a high fat diet also reduced urea levels as compared to the normal diet [29]. The same dependence was obtained in the studies on *Syzygium aromaticum* (Gaertn) Linn in hyperlipidemic rats [31].

Aspartic and alanine aminotransferase (AST, ALT) are enzymes produced by the liver. Therefore, their activity in serum illustrates state of liver cells. In our study, we did not observe significant differences. However, rats in the control fat (CL) group had a higher activity of ALT as compared to the control (C) group. These results demonstrate hepatotoxicity of the high-fat diet [32]. Enrichment of the fat diet with buckwheat groats (BGL) decreased only slightly the levels of these parameters as compared to the control fat (CL) group. On the other hand, enrichment of a fat diet with flavonoid extract obtained from dried leaves of *Litsea coreano* significantly decreased concentration of both parameters. This plant is used in Chinese medicine in hyperlipidemia and similarly to buckwheat is rich in flavonoids. Therefore, Wang's et al confirmed antihyperlipidemic properties of *Litsea coreano* [33]. Similar results were obtained also in research on *Syzygium aromaticum* (Gaertn.) Linn. (cloves) in a rich fat diet [31]. Alkaline phosphatase (ALP) is a parameter reflecting state of the liver, as well. Its increased activity indicates organ damage [30]. In this study, ALP was significantly increased in the control fat (CL) group as compared to the control (C) group. Buckwheat groats (BG) increased ALP activity as compared to the control diet (C) but the differences were not significant. Further, in the studies on buckwheat as a prebiotic, enrichment of the control diet with buckwheat caused a decrease in ALP activity as compared to the control diet, also insignificantly, though [30]. Therefore, further studies are required to understand buckwheat influence on this enzyme's activity. However, in the studies on one of the major green tea flavonoid, epigallocatechin gallate, animals fed a high fat diet with epigallocatechin gallate had even slightly more increased activity of alkaline phosphatase as compared to the fat diet group [34].

In the present study, we found that the consumption of lard decreased the plasma TG level ($p<0.001$) but buckwheat groats did not. Oliveira et al. [1] reported that the addition of fat to the diet resulted in a decrease in TG when rats were administered cholesterol at a dose of 10g/kg of feed, similar effect was observed by Turbino-Ribeiro, et al. [35] who noted that rats fed the hypercholesterolemic diet showed a significant decrease in triacylglycerol level in serum. Park et al. 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 groats, in our study we did not confirm the hypolipidemic activity of buckwheat groats since they did not influence TG level in plasma of rats [36].

There were no significant differences in plasma TCH cholesterol concentrations between the groups, unlike in the works of [36] and [37] 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 of [36], [37], we did not note a significant difference in TCH cholesterol levels between the groups. Our results confirmed that buckwheat groats significantly increased HDL in comparison to the control group. Additionally, the addition of buckwheat groats to fodder, especially supplemented also with fat, produced a significant effect on the reduction of AITCH, which may suggest that buckwheat groats have anti-atherosclerotic effects, which was especially pronounced since it 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 groats induced a decrease in the non-HDL cholesterol-dependent atherogenic index (AITCH). Lard impact was observed for the TG-dependent atherogenic index (AITG), which was significantly reduced. According to results reported by Lee et al. [2] diet with addition of buckwheat in the form of powder of leaves and flowers lowered plasma cholesterol levels in animals both on normal and high-fat diet groups. A similar interaction was also observed with triglycerides. However, the dose of buckwheat was important in establishing the effect of buckwheat in animals on high-fat diet [38]. Moreover, the variety of buckwheat and the form in which it is consumed affects its beneficial properties.

Ferric reducing antioxidant power (FRAP) was determined as an indicator of the total antioxidant capacity of plasma. We did not observe any significant differences among tested animal groups. However, rats in buckwheat groats (BG) group and buckwheat groats plus fat diet (BGF) group had a higher antioxidant capacity than control (C) and control fat (CF) groups. This could be due to the fact that among pseudocereal, buckwheat showed the highest antioxidant capacity what is associated with the highest total polyphenol content [39]. However, re-examination of this study is necessary. Possibly the dose of buckwheat groats used in that research was not high enough to induce a significant effect.

High-fat diet impairs the activity of the enzymatic antioxidant SOD and the non-enzymatic antioxidant GSH in serum. Subsequently, the decreased antioxidant capacity, leads to cell injury as a result of peroxidation [40]. In our experiment, the following parameters that influence antioxidant capacity were examined: catalase (CAT) activity, glutathione peroxidase (GPX3) activity, total and reduced glutathione (GSH) concentration and the level of carbonyl and thiol groups. Activity of CAT in plasma of rats was statistically significantly increased in the buckwheat groats (BG) groups as compared to the control (C) group ($p<0.001$). Addition of lard to the basic diet also increased the activity of this enzyme. The activity of glutathione peroxidase (GPX3) in plasma of rats was another evaluated parameter that determines antioxidant capacity. It was observed that GPX3 activity was significantly decreased in the buckwheat groats plus fat diet (BGL) groups as compared to the control diet (C). In the Wang's study, the effect of fat diet enriched in extract from Tartars Buckwheat on glutathione peroxidase activity was dependent on the dose of bran extract. Its low and average dose (0.2g/kg and 0.5g/kg of body weight) further increased GPX3 activity while a high dose of this extract (1g/kg of body weight) caused a decrease in this parameter in serum in comparison to the control group [38] Therefore, in that study reducing activity of glutathione peroxidase influenced by buckwheat groats could be the result of too high dose of them. Influence of buckwheat on changes in glutathione peroxidase activity could be due to its composition – mainly flavonoids that have antioxidant properties [38]. Our results also revealed a slight reduction of GPX3 activity in plasma of the control fat (CL) group as compared to the control (C) group. It follows from the fact that the fat diet is a factor contributing to the increase in oxidative stress. The lowest concentration of GSH was obtained in serum of rats in the buckwheat groats plus fat diet (BGL) group. This difference was statistically significant as compared to the control group with fat (CL). These results could indicate an important role of buckwheat groats in regulating the level of GSH. In Asdag's et al. study, saffron and crocin despite antioxidant properties also resulted in lower levels of GSH in plasma of rats fed on a high-fat diet enriched in them as compared to the control high-fat diet [23]. This observation confirms our results particularly in the case of the buckwheat groats plus fat diet group (BGL).

The concentration of free thiol groups (-SH) is an indicator of damaged protein molecules. One of many functions of -SH groups is related to the activation of enzymes, including antioxidant enzymes, which participate in inactivation of free radicals. High-fat diet contributed to reduction of -SH groups content in liver. Enrichment of the fat diet with saffron and crocin corrected concentration of this parameter [23]. Acai (*Euterpe oleracea* Mart.) enhanced body's antioxidant ability. This plant is rich in polyphenols, and contains unsaturated fatty acids. Therefore, a high fat diet enriched with Acai pulp was favorable and produced a decrease in oxidative stress by lowering the concentration of carbonyl groups in serum and increasing concentration of thiol-free radical scavengers [25]. The above dependence was noted among rats fed on fodder with buckwheat groats (BG) as compared to the control group (C). Moreover, in rats of this group a significant increase in activity of catalase in relation to (C) group was observed. These results evidence a considerable role of thiol groups in activation of antioxidant enzymes.

The level of protein carbonyl groups (PC) is another indicator of protein damage and thus oxidative stress [41]. In our experiment, buckwheat groats statistically significantly decreased the concentrations of PC in plasma as compared to control (C) and control fat (CL) and buckwheat sprouts plus fat diet (BSL) groups. Therefore, our results indicate a beneficial effect of buckwheat groats in inhibiting protein peroxidation. Beneficial effect of buckwheat groats on decline of oxidative stress may be associated with its composition. It is known that buckwheat is also a potential rich source of polyphenol compounds [39]. Similarly, Acai (*Euterpe oleracea* Mart.) is rich in polyphenols and what is interesting both hypercholesterolemic diet and control diet enriched with acai reduced the levels of carbonylated proteins in serum [25] which

is consistent with our results.

We also observed a decrease in MDA levels in plasma of rats fed the groats plus fat diet (BG) as compared to the control diet (C). These results were not statistically significant, though. Antioxidant properties of buckwheat are strongly related to the presence of rutin and flavonoid. However, the content of these ingredients depends on the species of buckwheat. According to Jiang et al. *Fagopyrum esculentum* contains the least of them in comparison to *Fagopyrum tataricum* and *Fagopyrum homotropicum*. As a result *Fagopyrum esculentum* provided the least protection against LDL peroxidation in plasma of rabbits fed the 0.5% cholesterol-supplemented diet [42]. Therefore, no statistically significant differences in our results might be related to the species of buckwheat which we used. Also the dose of buckwheat is important, because a significant lowering of MDA in plasma was observed by addition of only the lowest dose of extract from Tartary Buckwheat bran to the fat diet [38].

Many authors observed that the oxidant factors, like streptozotocin, ethanol, cisplatin or CCl_4 caused a decrease in the GPX activity in the testes [43] kidneys [44], [45] hearts [46], lungs [47], livers [48], spleen [49] and pancreas [50]. The administration of buckwheat groats increased GPX in the lung, spleen and muscle; these results suggest the antioxidant properties of buckwheat groats.

Catalase is an antioxidant enzyme that is involved in the reduction of free radicals level, representing the first line of antioxidant protection in organisms. The decline in the activities of catalase may be related with high susceptibility of tissue to oxidative damage [51]. Many reports indicate that dietary compounds with high antioxidant activity, potentially increasing the activity of catalase could protect the body against free-radical-induced damage [52]-[54].

In our investigations, in all determined tissues supplementation of the diets in buckwheat increased the CAT activity and counteracted the fructose-induced decrease in activity of this enzyme. Buckwheat groats increased CAT activities in plasma, lung and muscles of rats.

V. Conclusions

High fat diet-fed rats constitute a model of the increased cholesterol level and lipid peroxidation. Fat feeding can induce a greater free radical formation and reduce activity of antioxidant enzymes. Our results show that the buckwheat groats consumption may protect against dyslipidemia by decreasing plasma triglyceride and low-density lipoprotein cholesterol and increasing HDL cholesterol, thus lowering the indexes of atherogenicity. Buckwheat consumption resulted in the increase in antioxidant enzyme activities and antioxidant defense indices, which consequently led to improved health.

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