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ORIGINAL ARTICLE

IMPACT OF PRODUCTS FROM GROUND BUCKWHEAT ADDED TO BALANCED DIETS ON BIOCHEMICAL BLOOD MARKERS IN *WISTAR* RATS

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ABSTRACT

Background. It is believed that buckwheat can be used as a functional food ingredient in the prevention and treatment of diet-related diseases, e.g., atherosclerosis, hypertension, obesity, constipation and cancers. The use of buckwheat protein preparations in the diet for experimental animals had a significant effect on the reduction of cholesterol level in their blood serum, liver and gall bladder, and additionally inhibited the formation of gall stones as a result of changes in cholesterol metabolism. Buckwheat protein extracts have additionally been shown to reduce the level of LDL and VLDL fractions.

Objective. The aim of this study was to evaluate the effects of different products from ground buckwheat nuts (flour, meal and bran) and bread with their addition applied in balanced diets on selected biochemical blood markers in *Wistar* rats.

Material and Methods. The study was conducted with 64 male *Wistar* rats. Animals received a semi-synthetic diet AIN 93M with 20% addition of buckwheat flour, meal or bran and containing 20% rye-buckwheat bread made with 35% buckwheat flour, meal or bran, respectively. The animals were fed the experimental diets for 4 weeks. Whole blood was used to measure hematocrit and hemoglobin concentrations. The contents of glucose, total cholesterol, LDL, HDL, and triglycerides were determined in the blood serum using BioSystem biochemical tests.

Results. There was no significant effect of 20% addition of ground buckwheat products (buckwheat flour, meal and bran) or bread with their addition to balanced diets on hematocrit, hemoglobin concentration in the blood or the concentration of glucose in the blood serum of experimental animals. The study showed a statistically significant beneficial effect of dietary addition of buckwheat or rye-buckwheat bread on the reduction of total cholesterol, LDL cholesterol, and triglycerides (TGC) in the blood serum of rats.

Conclusions. A statistically significant decrease was demonstrated in LDL cholesterol and triglycerides in the blood serum of animals fed diets with 20% addition of ground buckwheat products (buckwheat flour, meal and bran) and rye-buckwheat bread containing these products.

Key words: *buckwheat, cholesterol (TC), triacyloglycerols (TAG), Wistar rats*

STRESZCZENIE

Wprowadzenie. Uważa się, że gryka może znaleźć zastosowanie, jako składnik żywności funkcjonalnej w prewencji i leczeniu chorób dietozależnych, m.in. miażdżycy, nadciśnienia, otyłości, zaparć, a także chorób nowotworowych. Zastosowanie preparatów z gryki w diecie zwierząt doświadczalnych w znaczący sposób wpływa na obniżenie stężenia cholesterolu w surowicy krwi, wątrobie oraz woreczku żółciowym, a ponadto hamuje powstawanie kamieni żółciowych na skutek zmiany metabolizmu cholesterolu. Wykazano, że produkty z gryki obniżają poziom frakcji cholesterolu LDL i VLDL.

Cel. Celem niniejszych badań była ocena wpływu rożnych produktów przemiału orzeszków gryki (mąki, śruty i otrąb) oraz pieczywa z ich udziałem, dodawanych do diet zbilansowanych, na wybrane wskaźniki biochemiczne krwi (hematokryt, stężenie hemoglobiny we krwi oraz stężenie glukozy, cholesterolu całkowitego, cholesterolu HDL i LDL oraz triglicerydów w surowicy krwi) u szczurów rasy *Wistar.*

Materiał i metody. Badania prowadzono z udziałem 64 samców szczurów rasy *Wista*r. Zwierzęta otrzymywały diety AIN 93M zmodyfikowane z 20% dodatkiem odpowiednio - mąki, śruty lub otrąb gryczanych oraz z 20% dodatkiem pieczywa żytnio-gryczanego odpowiednio z 35% udziałem mąki, śruty lub otrąb gryczanych. Zwierzęta karmiono dietami doświad-

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czalnymi przez 4 tygodnie. W krwi pełnej oznaczano: wskaźnik hematokrytu i stężenie hemoglobiny. Zawartość glukozy, cholesterolu całkowitego, cholesterolu LDL, HDL i triglicerydów oznaczano w surowicy krwi.
Wyniki. Nie wykazano istotnego wpływu 20% dodatku produktów przemiału orzeszków gryki (mąki, śruty i otrąb gryczanych) oraz pieczywa z ich udziałem do diet zbilansowanych na wartości hematokrytu, stężenie hemoglobiny we krwi oraz stężenie glukozy w surowicy krwi badanych zwierząt doświadczalnych. Wykazano statystycznie istotny korzystny wpływ dodatku gryki lub pieczywa żytnio – gryczanego na obniżenie zawartości cholesterolu całkowitego, cholesterolu LDL oraz triglicerydów (TGC) w surowicy krwi.
Wnioski. Stwierdzono statystycznie istotne obniżenie stężenia cholesterolu LDL oraz triglicerydów w surowicy krwi zwierząt, karmionych dietami z 20% dodatkiem produktów przemiału orzeszków gryki (mąki, śruty i otrąb gryczanych) oraz pieczywa żytnio - gryczanego z ich udziałem.

Słowa kluczowe: *gryka, cholesterol, triglicerydy, szczury Wistar*

INTRODUCTION

In the food industry, buckwheat nuts are a valuable material for the formulation of functional foods. Buckwheat seeds contain from 8.5 to 19.0% of protein, the biological value of which is higher than that of wheat, barley or even pork. The value of buckwheat protein is similar to the biological value of egg protein, which is a protein standard in terms of the nutritional value. Starch is a dominant carbohydrate in buckwheat seed composition, the content of which totals 60 – 70%. Buckwheat nuts are also a source of sucrose (2.0 – 2.5%) and dietary fiber (5.0 – 11.0%). In addition, buckwheat seeds contain 2.5 – 3.0% of fat and 2.0 – 2.2% of ash, the content of which includes iron, calcium, phosphorus, potassium, magnesium, zinc, nickel, copper, cobalt, boron and iodine. Buckwheat grain is also a rich source of B-group vitamins: thiamine, riboflavin, PP, pantothenic acid, as well as organic acids such as citric, oxalic, malic, and folic acids [5, 8].

Studies in laboratory animals have shown beneficial effects of buckwheat nuts addition to feed on lipid metabolism. In rats fed buckwheat-supplemented feed, a decrease in the content of serum cholesterol was found resulting from increased fecal excretion of neutral steroids and bile acids [18]. The use of buckwheat protein preparations in the diet for experimental animals had a significant effect on the reduction of cholesterol level in their blood serum, liver and gall bladder, and additionally inhibited the formation of gall stones as a result of changes in cholesterol metabolism. Buckwheat protein extracts have additionally been shown to reduce the level of LDL and VLDL fractions [11]. It is believed that buckwheat can be used as a functional food ingredient in the prevention and treatment of diet-related diseases, e.g., atherosclerosis, hypertension, obesity, constipation and cancers [6, 8].

The aim of this study was to evaluate the effects of different products from ground buckwheat (flour, meal and bran) and bread with their addition applied in balanced diets on selected biochemical blood markers (hematocrit ratio, blood hemoglobin and glucose concentration, total cholesterol, HDL and LDL cholesterol, and triglycerides in the blood serum) in *Wistar* rats.

MATERIALS AND METHODS

The study was conducted with 64 male *Wistar* rats with an initial body weight of 150-250 g. Animals were housed under the conditions specified in the requirements of the second Local Ethics Committee for Experiments on Animals of the Wroclaw University of Environmental and Life Sciences (Resolution No. 112/2012).

Experiments on the effect of products from ground buckwheat and bread supplemented with these products, added to the modified AIN 93M diets, on selected biochemical blood parameters of *Wistar* rats, were carried out in two stages. In each stage of the study 32 rats were divided into 4 groups (8 individuals/group). Composition of the experimental diets is shown in Table 1. During the experiment, groups of animals (K1, K2, D1, D2, D3, D4, D5, D6) were fed appropriately-modified semi-synthetic diets AIN-93M for laboratory rodents [16]. The control groups (K1 and K2) received a semi-synthetic diet AIN 93M. At the first stage of the study, D1, D2 and D3 groups were administered the modified AIN 93M diets with 20% addition of buckwheat flour, meal or bran, respectively. At the second stage of the study, groups D4, D5 and D6 received the modified AIN 93M diets containing 20% rye-buckwheat bread made with 35% buckwheat flour, meal or bran, respectively. The ground buckwheat products and bakery products with their addition were prepared at the Department of Fruit, Vegetable and Cereals Technology, Wroclaw University of Environmental and Life Sciences. The animals were fed the experimental diets for 4 weeks. Every other day, feed and water intake was controlled. Once a week, body weight of test animals was measured. In a state of anesthesia, blood was collected from the heart for further testing. Biological material was studied at the Department of Human Nutrition and the

Table 1. Composition of experimental diets

Diet components	Experimental diets							
	K1	D1	D2	D3	K2	D4	D5	D6
	Stage I of the study				Stage II of the study			
	(g/kg of diet)							
wheat starch	620.7	420.7	420.7	420.7	620.7	420.7	420.7	420.7
buckwheat flour	-	200	-	-	-	-	-	-
buckwheat meal	-	-	200.0	-	-	-	-	-
buckwheat bran	-	-	-	200.0	-	-	-	-
bread with 35% content of buckwheat flour	-	-	-	-	-	200.0	-	-
bread with 35% content of buckwheat meal	-	-	-	-	-	-	200.0	-
bread with 35% content of buckwheat bran	-	-	-	-	-	-	-	200.0
casein	140.0	140.0	140.0	140.0	140.0	140.0	140.0	140.0
saccharose	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
cellulose	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0
vegetable oil-soybean oil	40.0	40.0	40.0	40.0	40.0	40.0	40.0	40.0
mineral mix AIN – 93M -MX	35.0	35.0	35.0	35.0	35.0	35.0	35.0	35.0
vitamin mix AIN – 93M -VX	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0
choline	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5
L - cysteine	1.8	1.8	1.8	1.8	1.8	1.8	1.8	1.8

Department of Internal Medicine and Clinic of Diseases of Horses, Dogs and Cats of the Wroclaw University of Environmental and Life Sciences. Whole blood was used to measure hematocrit and hemoglobin concentrations. The contents of glucose, total cholesterol, LDL, HDL, and triglycerides were determined in the blood serum using BioSystem biochemical tests.

Statistical analysis was performed using the Statistica PL 10.0 software (StatSoft). *Shapiro-Wilk* test was used to check parametric distribution of variables obtained. To assess the significance of differences between groups of animals, one-way analysis of variance ANOVA was applied with the use of Fisher LSD test for multiple comparisons. *Kruskal-Wallis* test was used for the distribution of non-parametric results. The differences between results of the groups were determined at a significance level of $p < 0.05$. Small letters a, b, c in tables indicate statistically significant differences between groups.

Table 2. The average feed intake and average body weight gain of *Wistar* rats

Group of animals	Feed intake* $\bar{x}$±SD (g/day/rat)	Body weight gain* $\bar{x}$±SD (g/4 weeks/rat)
K1 (n=8)	13.45 ± 2.8 a	132.5 ± 26.7 a
D1 (n=8)	15.12 ± 2.3 a	161.2, 18.07 bc
D2 (n=8)	14.09 ± 5.6 a	157.5, 21.8 abc
D3 (n=8)	16.00 ± 2.7 a	166.2, 29.2 c
K2 (n=8)	13.36 ± 2.3 a	137.5, 12.8 ab
D4 (n=8)	14.94 ± 1.0 a	143.7, 13.0 abc
D5 (n=8)	11.19 ± 4.8 a	132.5 ± 13.8 a
D6 (n=8)	14.16 ± 0.3 a	143.7, 18.4 abc

$\bar{x}$ ± SD – mean ± standard deviation
**ANOVA, Fisher's exact test,* statistically significant differences at $p < 0.05$; the same letter (a, b or c) indicates statistically homogeneous group

RESULTS AND DISCUSSION

The four-week-long experiment evaluated the impact of diet supplementation with various products from buckwheat nuts milling and rye-buckwheat bread containing these products on selected biochemical blood markers of *Wistar* rats. During the experiment, no adverse changes were observed in the appearance nor behavior of the animals. Table 2 shows the average feed intake and average body weight gain of animals in particular feeding groups. No statistically significant differences were observed in feed intake that ranged from 11.19 to 16.00 g/day/rat. In contrast, statistically significant differences were shown in mean body weight gains of rats in the analyzed groups that ranged from 132.5 to 166.3 g/4 weeks/rat. The lowest body weight gain (132.5 g/4 weeks/rat) was found in the following groups: control group K1 and group D5, in which the animals were fed a diet with 20% addition of bread containing 35% of buckwheat meal. The highest body weight gain was observed in the group D3, administered a feed mixture with 20% of buckwheat bran (166.2 g/4 weeks/rat). In groups D1, D2, and D3, fed diets with 20% addition of ground buckwheat products (buckwheat flour, meal and bran, respectively), the body weight gains were higher by 16.5% on average when compared to the control group K1.

Table 3 shows the mean and median (depending on the distribution of variables) values of biochemical blood parameters of the rats. There were no statistically

Table 3. Values of biochemical blood marker measured in *Wistar* rats

Group of animals	Biochemical blood markers						
	Hematocrit* [%] $\bar{x}$±SD	Hemoglobin* [mg%] $\bar{x}$±SD	Glucose* [mg/dL] $\bar{x}$±SD	Total cholesterol* [mg/dL] Me ± Q	LDL cholesterol** [mg/dL] Me ± Q	HDL Cholesterol* [mg/dl] $\bar{x}$±SD	Triglycerides** [mg/dL] Me ± Q
K1 (n=8)	44.0 ± 3.8 a	8.9 ± 1.2 a	140.5±9.8 a	111.9±8.6 a	42.6±5.6 a	59.9 ± 5.1 a	178.5±12.4 a
D1 (n=8)	45.0 ± 2.9 a	9.3 ± 0.9 a	107.9±11.3 a	98.8±9.5 a	35.7±6.1 b	54.9 ± 4.8 a	125.9±10.5 b
D2 (n=8)	45.0 ± 2.3 a	9.2 ± 1.3 a	118.3±8.6 a	92.3±12.7 a	35.5±8.4 b	54.8 ± 4.9 a	128.3±9.8 b
D3 (n=8)	45.0 ± 2.7 a	9.2 ± 1.6 a	129.9±9.4 a	95.2±7.6 a	34.6±6.3 b	59.3 ± 6.5 a	90.5±10.1 b
K2 (n=8)	41.0 ± 2.8 a	8.4 ± 0.9 a	141.0±11.2 a	112.9±12.4 a	44.2±5.9 a	61.7 ± 7.2 a	182.2±13.4 a
D4 (n=8)	44.0 ± 3.4 a	8.9 ± 1.3 a	142.7±8.4 a	103.8±6.7 a	33.8±7.3 b	56.8 ± 6.3 a	101.9±11.6 b
D5 (n=8)	46.0 ± 3.2 a	9.4 ± 1.5 a	133.5±11.5 a	99.7±9.3 a	34.2±9.1 b	55.7 ± 4.9 a	112.6±10.3 b
D6 (n=8)	45.0 ± 2.9 a	9.3 ± 0.9 a	145.3±12.8 a	97.0±10.5 a	36.5±5.1 b	57.7 ± 7.0 a	101.5±12.1 b

Me ± Q – median ± quartile deviation
$\bar{x}$± SD – mean ± standard deviation
**ANOVA, Fisher's exact test,* statistically significant differences at p <0.05; the same letter (a or b) indicates statistically homogeneous group
***Kruskal-Wallis test,* statistically significant differences at p <0.05; the same letter (a or b) indicates statistically homogeneous group

significant differences in hematocrit and mean hemoglobin levels between the analyzed groups of animals. Hematocrit ranged from 41% to 46%, whereas hemoglobin concentration ranged from 8.4 to 9.4 mg% and both were within the range of physiological standards. No statistically significant differences were observed in the serum level of glucose in any of the groups of animals that ranged from 107.9 to 145.3 mg/dL. In the groups of rats fed diets containing 20% of buckwheat flour, meal and bran (D1, D2, D3, respectively) the concentration of glucose was lower by about 16% compared to the control group K1.

The concentration of glucose in the blood is a function of diet composition, the rate of glucose penetration into cells and its metabolism in the cells. The primary hormone responsible for blood glucose regulation is insulin. The rate of glucose metabolism in a cell depends also on the activity of enzymes involved in the metabolic process [9]. Due to the presence of resistant starch in buckwheat nuts, which may contribute to the reduction of glucose concentration in the blood serum, results obtained were compared with the studies that involved rats fed a diet with the addition of resistant starch. The study by *Ding* et al. [7] showed a decrease in the glucose concentration of about 20% in the blood of rats fed a diet containing resistant starch (6 g/kg of feed). A 21% reduction in glucose concentration was also found in the blood serum of animals fed a diet containing 12% wheat resistant starch (RS1) as compared to the control group [2].

Table 3 shows the mean and median concentrations of total cholesterol, LDL cholesterol, HDL cholesterol and triglycerides (TGC) in the blood serum of the analyzed animals. There were no statistically significant differences in total cholesterol content between any groups of the rats. Median of the total cholesterol level ranged from 92.3 to 112.9 mg/dL in all groups of animals. In the groups fed diets containing products from ground buckwheat, the total cholesterol was lower by about 11 - 15% as compared to control groups K1 and K2.

Median LDL cholesterol level ranged from 33.8 to 44.2 mg/dL in the investigated groups of animals. A statistically significant decrease of approximately 20% of the LDL cholesterol was found in the blood serum of animals receiving the modified diets with ground buckwheat products and rye-buckwheat bread compared to the control group. There were no statistically significant differences in the mean concentrations of HDL cholesterol in the blood serum in the test groups of animals, and it ranged from 54.8 to 61.7 mg/dL. In groups of animals fed diets supplemented with 20% of buckwheat flour, meal and bran (D1, D2, D3, respectively), and a 20% addition of rye-buckwheat bread (D4, D5, D6), statistically significant lower medians (by about 40%) were observed in the TGC content in the serum, compared to the median TGC concentration of the serum in the control rats (Table 3).

The present study showed a statistically significant beneficial effect of dietary addition of buckwheat or rye-buckwheat bread on the reduction of total cholesterol, LDL cholesterol, and triglycerides (TGC) in the blood serum of rats. There are very few studies of other authors concerning the influence of ground buckwheat products added to diets, on lipid metabolism in laboratory animals. The protein content of buckwheat grains ranges from 8.5 to 19.0%. Buckwheat protein is rich in lysine, an amino acid limiting the biological value of other cereal proteins. Low ratios of lysine:arginine and methionine:arginine amino acids may be the cause of decreasing cholesterol levels in the blood serum of animals fed buckwheat-supplemented diets [6, 8, 18].

The work of *Kayashita* et al. [11] showed a decrease of approximately 15% in serum level of cholesterol in rats fed a diet supplemented with a buckwheat protein extract in comparison to the control group that was receiving casein.

The study of *Tomotake* et al. [18] demonstrated that the addition of a buckwheat protein extract or buckwheat flour to the diets for rats evoked a 30 - 33% reduction in contents of cholesterol and triglycerides (TGC) in the animals' serum when compared to the control group. The beneficial effect of buckwheat grains, added to the diets of experimental rats, was also confirmed by other authors [12, 17, 19].

Buckwheat grains contain resistant starch, which constitutes 33 - 38% of the total starch. Experiments with laboratory animals have demonstrated a positive influence of resistant starch added to diets on their lipid metabolism [2]. Investigations with hamsters showed a statistically significant decrease in HDL cholesterol and serum TGC in the animals tested. The groups receiving a feed mixture containing extruded cassava starch with the addition of 9.9% oat fiber, had by 18% lower TGC concentration compared to the control group. In the groups of animals administered a feed mixture with extruded cassava starch with 9.7% addition of resistant starch RS4, levels of TGC were observed to decrease by about 9% compared to the control group [14].

Similar effects of various types of resistant starch (RS) were observed by other authors. In the study of Cheng et al. [4] TGC content in the blood serum of animals fed a diet supplemented with rice RS decreased by 10%, whereas *Younes* et al. [21] found that 25% addition of raw potato starch to a diet for *Wistar* rats caused a 29 - 42% decrease of triglycerides.

A research conducted in rats fed a diet supplemented with 5% of resistant starch of *Kintoki* beans demonstrated about 20% increase of HDL cholesterol compared to the control diet [10]. A similar study was conducted with *Wistar* rats administered a diet supplemented with 30% high-amylose corn starch where a decrease in total cholesterol and serum TGC was found in experimental animals (29% and 54%, respectively) compared to control groups [13].

Buckwheat is also a good source of dietary fiber (5 - 11%), particularly its soluble fraction and plant sterols (198 mg/100 g), which beneficially reduce cholesterol concentration in the body. The buckwheat grain contains a number of important antioxidants, e.g., rutin, quercetin, orientin and others [3, 5, 8].

Studies with experimental rats showed that rutin, supplemented with the diet, suppressed the activity of myeloperoxidase (MPO) [15, 20]. The MPO is involved in the inflammatory process destabilizing atherosclerotic plaque and enhances the oxidation of LDL cholesterol, which in turn accelerates atherogenesis and the occurrence of acute coronary syndromes [1].

The use of ground buckwheat for the production of new cereal products such as rye-buckwheat bread would ensure a high nutritional value of food products the long-term consumption of which could favorably affect lipid metabolism of the body.

CONCLUSIONS

1. There was no significant effect of 20% addition of ground buckwheat products (buckwheat flour, meal and bran) or bread with their addition to balanced diets on hematocrit, hemoglobin concentration in the blood or the concentration of glucose in the blood serum of experimental animals.
2. A statistically significant decrease was demonstrated in LDL cholesterol and triglycerides in the blood serum of animals fed diets with 20% addition of ground buckwheat products (buckwheat flour, meal and bran) and rye-buckwheat bread containing these products.

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Conflict of interest
The authors declare no conflict of interest.

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