

EFFECT OF RESISTANT STARCH RS4 ADDED TO THE HIGH-FAT DIETS ON SELECTED BIOCHEMICAL PARAMETERS IN *WISTAR* RATS

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ABSTRACT

Background. Resistant starch (RS) is part of potato starch that is not digested in the gastrointestinal tract. RS4 is a chemically modified starch (for example by oxidation and esterification) and physically (by heating).

Objective. The study was aimed at determining the effect of resistant starch on lipid metabolism and activity of hepatic enzymes in *Wistar* strain rats fed high-fat diets containing 15% of lard or 15% of soybean oil.

Material and methods. Four types of diets were administered to the animals (4 groups of males, n=32): control diet (K₁) containing 15% of soybean oil; control diet (K₂) containing 15% of lard as well as two groups receiving the same diets with 10% addition of resistant starch RS4 (K₁S and K₂S).

Results. The mean concentration of total cholesterol was lower in the group of animals fed a diet with vegetable oil (39.9 mg/dl) as compared to that reported in the group of rats fed the lard-supplemented diet (55.2 mg/dl). Compared to the control groups in both groups of animals receiving the diet supplemented with resistant starch RS4 the total cholesterol concentration in serum decreased by ca. 25% (differences were statistically significant). In groups of rats receiving oil- or lard-containing diets with the addition of the resistant starch preparation the concentration of triglycerides in serum decreased by ca. 47% and 10%, respectively.

Conclusions. A beneficial effect of the resistant starch RS4 added to *Wistar* rats diets on the lipid metabolism has been shown. The concentrations of total cholesterol and triglycerides in the serum were lower and concentration of HDL-cholesterol was higher in the rats fed with the diets containing the addition of the RS4 preparation as compared to the control groups. Based on the activity of hepatic enzymes the degree of liver damage was lower in groups of rats fed with diets containing resistant starch RS4 as compared to the control groups.

Keywords: activity of hepatic enzymes, lipid metabolism parameters, RS4 starch, *Wistar* strain rats

STRESZCZENIE

Wprowadzenie. Skrobia oporna (resistant starch, RS) jest to część skrobi ziemniaczanej, która w przewodzie pokarmowym nie ulega strawieniu. Skrobia oporna RS4 to skrobia modyfikowana chemicznie (np. przez oksydację lub estryfikację) oraz fizycznie (przez działanie termiczne).

Cel. Celem badań była ocena wpływu skrobi odpornej RS4 na gospodarkę lipidową oraz aktywność enzymów wątrobowych szczurów rasy *Wistar* karmionych dietami wysokotłuszczowymi zawierającymi 15% smalcu lub 15% oleju sojowego.

Materiał i metody. Zwierzęta (4 grupy samców n=32) karmiono 4 rodzajami diet: kontrolną (K₁) zawierającą 15% oleju sojowego i kontrolną (K₂) zawierającą 15% smalcu oraz odpowiednio 2 grupy takich samych diet z 10% dodatkiem skrobi odpornej RS4 (K₁S i K₂S).

Wyniki. Średnie stężenie cholesterolu całkowitego w grupie zwierząt karmionych paszą z tłuszczem roślinnym było niższe (39,9 mg/dl) w porównaniu do odnotowanego w grupie szczurów karmionych dietą ze smalcem (55,2 mg/dl). W porównaniu do grup kontrolnych, w obu grupach zwierząt karmionych paszą zawierającą olej lub smalec z dodatkiem skrobi RS4 stężenie cholesterolu całkowitego w surowicy krwi obniżyło się o około 25% (różnice były istotne statystycznie). W grupach szczurów karmionych paszą z dodatkiem preparatu skrobi odpornej RS4 stężenie triglicerydów w surowicy krwi obniżyło się odpowiednio o około 47% i 10%.

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Wnioski. Wykazano korzystny wpływ skrobi opornej RS4 dodawanej do diet szczurów *Wistar* na ich gospodarkę lipidową. Stwierdzono zmniejszenie stężenia cholesterolu całkowitego, TGC oraz zwiększenie stężenia cholesterolu HDL w surowicy krwi zwierząt otrzymujących diety z dodatkiem skrobi opornej RS4 w porównaniu do grup kontrolnych. Na podstawie aktywności enzymów wątrobowych stwierdzono niższy stopień uszkodzenia wątroby w grupach szczurów karmionych dietami z dodatkiem skrobi opornej RS4 w porównaniu do grup kontrolnych.

Słowa kluczowe: aktywność enzymów wątrobowych, wskaźniki gospodarki lipidowej, skrobia RS4, szczury rasy *Wistar*

INTRODUCTION

Resistant starch (RS) is a part of potato starch that escapes digestion in the gastrointestinal tract [11, 21]. It constitutes a difference between starch subjected to the activity of amylolytic enzymes and released glucose [12]. RS4 – is the starch modified chemically (e.g. by means of oxidation or esterification) or physically (by means of thermal treatment) [2, 9, 11, 12]. The chemical modification consists on the incorporation of various substituents to starch chains. It results in not only a change in the composition but also in the structure of a molecule, which in turn leads to resistance to the activity of amylolytic enzymes [8].

Products of thermal starch - dextrines, are coming as a result of processes of the depolymerisation, transglucosidation and repolymerisation. During prolonging the dextrinisations process a number of trusses type 1, 3 and 1, 2 - glycosidated between glucose's rests of dextrines increases. Arising free particles of the glucose are joining chains haphazardly. As a result, the new connections in chains of starch absent in natural starch are formed. Creating complexes for its with other elements of the food is other method of increasing the resistance of starch products. Lipid substances can be an example, which penetrating to helices of the amylose for its resistances cause the height [11].

The consumption of resistant starch exerts a positive effect on body functioning, i.e. it limits body mass gains, triggers favorable changes in lipids and saccharides metabolism, and improves the condition of the circulatory system [18, 19]. RS yields similar effects as soluble fractions of dietary fibre, is poorly digested in the small intestine and undergoes fermentation with *Lactobacillus* and *Bifidobacterium* bacteria in the colon. That fermentation results in the generation of gaseous products (hydrogen, methane) and short chain fatty acids - SCFA (propionic, butyric and acetic) [1, 18, 20].

Resistant starch regulates cholesterol metabolism in the liver of humans by transforming higher quantities of cholesterol into bile acids as well as enhances their activity. In addition, it decreases serum concentrations of LDL and VLDL cholesterol [17].

The study was aimed at determining the effect of fat in high-fat diets and resistant starch RS4 added on selected biochemical parameters in *Wistar* rats.

MATERIAL AND METHODS

The experiments were conducted on 32 male *Wistar* strain rats with the initial body weight of 250 - 300 g. The rats were divided at random into four groups (8 rats per each group) based on the type of administered diets. They were kept in an animal laboratory with natural lighting, temp. of ca. 22°C and humidity of ca. 60%. For 28 days the rats were fed the following diets: group 1 – a control diet containing 15% of vegetable oil (soybean oil), without resistant starch addition; group 2 – a diet containing 15% of vegetable oil (soybean oil) with 10% addition of resistant starch RS4; group 3 – a control diet without resistant starch but containing 1% of animal-originated cholesterol and 15% of animal fat (lard); and group 4 – a diet containing 1% of animal-originated cholesterol and 15% of animal fat (lard) supplemented with 10% of resistant starch RS4. The experimental diets were prepared according to AIN – 93 M [15], and their detailed composition was provided in Table 1.

Table 1. Quantitative composition of experimental diets

Dietary component	Diets			
	K ₁ g/kg of diet	K ₂ g/kg of diet	K ₃ g/kg of diet	K ₄ g/kg of diet
cellulose	50.00	50.00	50.00	50.00
cholesterol	-	1.00	-	1.00
choline	2.50	2.50	2.50	2.50
cysteine	1.80	1.80	1.80	1.80
casein	140.00	140.00	140.00	140.00
vegetable fat – soybean fat	150.00	-	150.00	-
saccharose	100.00	100.00	100.00	100.00
minerals- mix AIN-93M-MX	35.00	35.00	35.00	35.00
resistant starch	-	-	327.20	327.20
wheat starch	606.85	605.85	279.65	278.65
animal fat – lard	-	150.00	-	150.00
vitamin E	3.85	3.85	3.85	3.85
liquid vitamins	10.00	10.00	10.00	10.00

Modification of the diet on exchange wheat starch on 30,6% resistant starch. Resistant starch applied in the diet was RS4 potato starch modified chemically through esterification with phosphoric acid salts with industrial method. It was produced at the Department of Agricul-

tural Technology and Storage, Wrocław University of Environmental and Life Sciences, Poland.

On termination of the feeding experiment, the animals were anaesthetized with xylazine and ketamine mixture. Blood samples were collected from heart and livers were removed from further preparation. Determinations of the concentration of hemoglobin with the cyanomethemoglobin method and that of hematocrit with the microhematocrit method were carried out in whole blood. Contents of glucose, total cholesterol and its HDL fraction as well as triglycerides were assayed in blood serum with the use of BioSystems kits. In extracts of liver prepared according to the *Folch's* method [7], of extraction depend on fat isolation from homogenized animal tissues with chloroform – methanol (2:1) mixture. Contents of total cholesterol and triglycerides were determined with the use of biochemical kits by BioSystems. Additional assays were performed for activities of hepatic enzymes: alanine aminotransferase (Alat) and aspartate aminotransferase (Aspat), with the kinetic method using pyridoxal phosphate and dehydrogenase-NADH. Blood and liver analyses were conducted immediately after exanguination.

Results obtained were elaborated statistically by calculating arithmetic means and standard deviations with the use of Statistica 6.0 PL software as well as by *Kruskal-Wallis's* tests and *Friedman's* non-parametric rank test for paired samples, for determination of significance of differences between two dependent measurements and *Duncan's* parametric test. Differences occurring at a significance level of $p < 0.05$ were found significant.

The experimental procedure was approved by the Local Ethics Commission for Experiments on Animals at the Wrocław University of Environmental and Life Sciences.

RESULTS AND DISCUSSION

The mean feed intake in groups receiving diets without RS4 addition amounted average 19.84 g/day

and was higher by ca. 35% as compared to the groups administered diets with the addition of RS4, yet the difference was statistically significant. In turn, a significant decrease in body weight was observed in animals receiving diets containing the RS4 preparation (a decline in body weight by ca. 5.2 g) (Table 2).

Table 2. Feed intake and body weight gains from the groups under study

Groups of the rats	Feed intake [g/day]	Weight gains [g/4 week]
	mean± SD	mean± SD
K ₁ (n = 8)	19.99 ± 4.17 ^(a)	63.75 ± 15.06 ^(c)
K ₁ S (n = 8)	14.72 ± 1.91 ^(b)	32.50 ± 10.35 ^(a)
K ₂ (n = 8)	19.69 ± 3.23 ^(a)	48.75 ± 6.41 ^(b)
K ₂ S (n = 8)	14.82 ± 1.91 ^(b)	32.50 ± 10.35 ^(a)

Duncan's test, differences statistically significant at $p < 0.05$; the same letters (a, b or c) denote statistically homogenous groups

In all groups of animals, concentrations of hematocrit ranged from 33.8 to 39.8%. (Table 3). The mean concentrations of hemoglobin in all groups of rats were similar and accounted, on average, for 15.8 mg/dl in groups fed a diet with vegetable oil and for 14.3 mg/dl in those receiving a diet with animal fat. Statistically significant differences were observed in concentrations of Hb and Ht between particular groups of animals. The mean serum concentrations of glucose in rats fed diets without the resistant starch preparation ranged from 117.0 to 147.7 mg/dl, whereas in animals receiving diets with the addition of resistant starch – from 97.4 to 99.0 mg/dl. The RS4 type resistant starch used in the study was demonstrated to exert a significant effect on decreasing glucose concentration in serum of the rats examined.

The mean concentration of total cholesterol in the group of animals fed a diet containing vegetable oil was lower (39.9 mg/dl) than in rats receiving the lard-containing diet (55.2 mg/dl). In groups of animals administered diets with the addition of the RS4 preparation the concentration of total cholesterol in serum decreased by ca. 25% as compared to the respective

Table 3. Hematocrit, hemoglobin, glucose, total cholesterol (TC), HDL-cholesterol, and triglycerides (TGC) determined in blood serum of rats from the groups under study

Groups of the rats	Hematocrit ** [%]	Hemoglobin** [mg/dl]	Glucose* [mg/dl]	TC** [mg/dl]	HDL* [mg/dl]	TGC** [mg/dl]
	mean± SD	mean± SD	mean± SD	mean± SD	mean± SD	mean± SD
K ₁ (n = 8)	35.7 ± 3.05 ^(a)	16.3 ± 2.84 ^(a)	147.7 ± 31.34 ^(a)	39.9 ± 4.05 ^(b)	18.2 ± 20.12 ^(a)	182.6 ± 55.49 ^(b)
K ₁ S (n = 8)	33.8 ± 7.68 ^(b)	15.3 ± 1.95 ^(a)	97.4 ± 9.14 ^(a)	30.4 ± 12.80 ^(a)	14.0 ± 5.80 ^(ab)	98.6 ± 51.63 ^(a)
K ₂ (n = 8)	39.8 ± 1.18 ^(a)	14.9 ± 1.51 ^(a)	117.0 ± 35.08 ^(ab)	55.2 ± 6.07 ^(c)	14.4 ± 2.03 ^(ab)	176.8 ± 70.07 ^(b)
K ₂ S (n = 8)	39.3 ± 1.58 ^(b)	13.7 ± 0.63 ^(a)	99.0 ± 13.89 ^(ab)	34.5 ± 7.18 ^(b)	22.4 ± 6.5 ^(b)	162.3 ± 60.10 ^(b)

* ANOVA *Kruskal-Wallis's* test, differences statistically significant at $p < 0.05$; the same letters (a or b) denote statistically homogenous groups

** *Duncan's* test, differences statistically significant at $p < 0.05$; the same letters (a or b) denote statistically homogenous groups

control groups, irrespective of the type of fat in the diet. The level of HDL-cholesterol in serum of the analyzed rats fluctuated between 14.0 and 22.4 mg/dl. In turn, in all groups of animals the concentration of triglycerides ranged from 98.6 to 182.6 mg/dl. In groups of rats fed diets with the addition of resistant starch preparation the concentration of triglycerides in serum of animals administered diets containing soybean oil and lard accounted for 47% and 10%, respectively. The applied RS4 preparation was demonstrated to have a statistically significant effect on reducing concentrations of total cholesterol and TGC in all groups of animals. In turn, it had significant effect on the concentration of HDL-cholesterol in the group of rats fed a diet containing vegetable oil.

Table 4 provides results of the mean mass of livers of the animals under study, which accounted for 8.5 g. No statistically significant differences were observed in the mass of that organ between particular groups of rats. The mean concentration of total cholesterol in lipids extracted from livers of animals administered diet without the addition of RS4 was higher (35.65 mg/dl on average) as compared to the rats receiving RS4-supplemented diets (33.95 mg/dl on average). Levels of HDL-cholesterol fraction in livers of the examined animals ranged from 2.56 to 6.5 mg/dl. In groups of animals administered diets with the addition of the resistant starch preparation the concentration of HDL-cholesterol in liver lipids was higher by ca. 30% - in the group fed a diet with soybean oil and by 150% - in the group receiving a diet with lard, as compared to the respective groups receiving diets without the RS4 preparation. The observed differences were statistically significant.

Table 4. Mass of livers and concentration of total cholesterol (TC) and HDL – cholesterol in livers of the animals under study

Groups of the animals	Mass of livers [g]	TC [mg/dl]	HDL [mg/dl]
	mean± SD	mean± SD	mean± SD
K ₁ (n = 8)	8.38 ± 1.33 (a)	35.2 ± 3.48 (a)	4.07 ± 5.75 (a)
K ₁ S (n = 8)	8.60 ± 0.76 (a)	33.2 ± 6.28 (a)	5.15 ± 3.67 (a)
K ₂ (n = 8)	8.99 ± 0.58 (a)	36.1 ± 6.78 (a)	2.56 ± 1,72 (a)
K ₂ S (n = 8)	8.35 ± 0.56 (a)	34.7 ± 7.78 (a)	6.50 ± 4.16 (a)

ANOVA *Kruskal-Wallis*'s test, differences statistically significant at $p < 0.05$; the same letters (a or b) denote statistically homogenous groups

Table 5 presents results obtained for activities of selected hepatic enzymes (ALAT and ASPAT). In the evaluation of liver damage (activity of ALAT and ASPAT enzymes), smaller changes were demonstrated in groups of animals receiving diets with the addition

of RS4 starch. In the case of the diet with vegetable oil, those differences turned out to be statistically significant. In the group of rats fed diets containing lard damage to liver was also smaller, still the differences were not statistically significant.

Table 5. Activities of selected hepatic enzymes (ASPAT and ALAT) of the animals under study

Groups of the animals	ASPAT* [U/l]	ALAT** [U/l]
	mean± SD	mean± SD
K ₁ (n = 8)	85.55 ± 15.8 (b)	48.89 ± 28.74 (a)
K ₁ S (n = 8)	39.07 ± 43.04 (a)	37.28 ± 31.30 (a)
K ₂ (n = 8)	108.03 ± 12.86 (b)	46.73 ± 24.15 (a)
K ₂ S (n = 8)	38.77 ± 19.14 (a)	38.41 ± 26.22 (a)

* *Duncan*'s test, differences statistically significant at $p < 0.05$; the same letters (a or b) denote statistically homogenous groups

** ANOVA *Kruskal-Wallis*'s test, differences statistically significant at $p < 0.05$; the same letters (a or b) denote statistically homogenous groups

In the reported study, the effect of 10% addition of RS4 type resistant starch to high-fat diets on lipid metabolism and activity of selected hepatic enzymes was evaluated in *Wistar* rats. In rats fed a diet with the addition of resistant starch body weight values were demonstrated to decrease. The intake of the analyzed RS4 type resistant starch was positively correlated with the improvement of the lipid profile of the body, thus evoking a significant decrease in the concentration of triglycerides and an increase in the serum concentration of HDL-cholesterol fraction. The influence of resistant starch on levels of cholesterol and lipids in blood of experimental animals is, however, not unambiguous. Most of biological analyses indicate that the above parameters are subject to a decrease upon RS [1, 5, 13, 21].

Results obtained in this presented study are similar to those reported in literature that confirm the beneficial effect of RS on lipid metabolism, including that of total cholesterol, LDL, HDL, VLDL and triglycerides, in experimental animals [4, 10, 16, 18]. In a research by *Younes et al.* [22], the addition of RS to diets for rats caused a decrease in the serum concentration of cholesterol (by 32%) and triglycerides (by 29%). Alike results were obtained by *Lopez et al.* who also observed a significant reduction in serum concentrations of cholesterol and triglycerides in rats fed a diet supplemented with 20% addition of starch from raw potato and high-amylose maize starch. The beneficial effect of RS on the reduction of triglyceride levels in the examined experimental animals was also confirmed in investigations carried out by *Verbeek et al.* and *Kishid et al.* [cit. after 14].

In a study by *Delahaye et al.* [6], who applied a high-fat diet (14% of maize oil and 14% of palm oil), the addition of resistant starch resulted in a decreased

serum concentrations of cholesterol (by 36.6%) and triglycerides (by 35.6%) as compared to the control group. Investigations of *Younes et al.* [22] demonstrated that 25% addition of crude potato starch to a diet for *Wistar* strain rats decreased serum concentrations of both total cholesterol and triglycerides by 22-32% and 29-42%, respectively. Results obtained in the presented study for the concentration of total cholesterol in liver are consistent with data reported by other authors. The research by *Cheng et al.* [3] demonstrated statistically significant differences in concentrations of total cholesterol in livers of rats, namely in the group of animals administered a diet with the addition of resistant starch the concentration of cholesterol was lower by 6.6% than in the control group.

Results obtained in this study indicate the possibility of applying such type of starch as a health-promoting food additive.

CONCLUSIONS

1. In animals receiving diets with the addition of RS4 modified starch preparation, irrespective of the type of fat, a tendency was observed for diminishing body weight gains as compared to the control group.
2. Irrespective of the type of fat in the diet, a statistically significant decrease was reported in the concentrations of total cholesterol and glucose in sera of animals fed the RS4-containing diet when compared to the respective control groups.
3. Again, irrespective of the type of fat in the diet, the study demonstrated a significant increase in the serum concentration of HDL-cholesterol fraction in rats administered diets with the addition of RS4 as compared to the respective control groups.
4. In animals fed diets containing lard and oil as well as 10% addition of modified resistant starches, the serum concentration of TGC was statistically significantly lower (by ca. 10 – 47%) as compared to the respective control groups.
5. In animals receiving the diet with vegetable oil and RS4 starch, damage of liver turned out to be significantly smaller than in the control rats.

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