

## Influence of Chemically-Modified Potato Starch (RS Type 4) on the Nutritional and Physiological Indices of Rats

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A biological study was undertaken to analyse the metabolic effect of feeding rats with an experimental diet in which cellulose was substituted with 20% contribution of chemically-modified potato starches (subjected to oxidation, esterification, cross-linking and dual modification). Caecum digesta mass was significantly higher in rats fed the experimental potato starch preparations compared to control group. Luminal ammonia concentration and pH of caecal or colonic content were lower as an effect of diets with all the investigated preparations. Compared to the cellulose-containing diet (control), all modified potato starch preparations raised the content of SCFA in caecum digesta when fed to rats. Significant lowering of the levels of triacylglycerols and total cholesterol was noticed for all chemically-modified starch preparations. The activity of  $\beta$ -glucuronidase determined upon the administration of potato starch preparations into rat diets was significantly lower as compared to the control diet. The results indicate that the chemically-modified potato starch preparations are a good substrate for the intestinal microecosystem and may promote the beneficial status of the gastrointestinal tract of rats.

### INTRODUCTION

The most common method for modelling physicochemical and functional properties of starch is chemical modification, which for food purposes is strictly limited in terms of the type of chemical reactions, the kind of modifying agents, the degree of substitution of starch as well as the content of impurities. Common types of chemical reactions for starch modification, for food purposes, include only three types, *i.e.* oxidation, esterification and etherification. The chemical modification of starch may affect the rate and extent of its digestion in the small intestine [Wolf *et al.*, 1999]. It has been stated that the oxidation or dextrinisation of starch, its substitution with hydroxypropyl, acetyl or octenylsuccinate groups as well as crosslinking diminish its digestibility [Wolf *et al.*, 1999; 2001]. From the nutritional point of view, starches subjected to chemical modification are acknowledged as resistant starches type 4 (RS 4). Though resistant starch is not digested and not absorbed in the gastrointestinal tract of man, it may be fermented by the microflora inhabiting the colon. By these means, it may affect a variety of factors responsible for the proper functioning of the intestine, including: accelerating transit and excretion of faeces, increasing faecal bulk, modulating pH of intestinal digesta, reducing contents of ammonia

and bile acids in faecal waters, and increasing concentrations of short-chain fatty acids [Silvester *et al.*, 1995; Lopez *et al.*, 2001]. All these factors may positively affect the intestinal ecosystem and the activity of intestinal microflora. Silvi *et al.* [1999] demonstrates that resistant starch could modify the human gut microflora, particularly stimulating lactic acid bacteria and decreasing potentially pathogenic types such as enterobacteria.

The physicochemical, morphological, thermal and rheological properties of chemically-modified starch preparations have been studied, but there is still a lack of well-done nutritional studies which discuss physiological consequences of their ingestion. The purpose of this *in vivo* study was to investigate the response of the gastrointestinal tract environment and serum lipids of rats to diets containing some types of chemically-modified potato starch (subjected to oxidation, esterification, cross-linking and dual modification) which are commercially permissible for food uses.

### MATERIALS AND METHODS

#### Materials

Native potato starch and its chemically-modified preparations were provided by Luboń SA Co. (WPZZ Luboń, Poland). The following preparations were used in the nutritional experiment: oxidised starch (OS, type of modification: oxidation, International Numbering System (INS) provided

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by Codex Committee on Food Additives and Contaminants, No. 1404), acetylated starch (AS, type of modification: esterification, INS No. 1420), acetylated distarch adipate (ADA, type of modification: dual treatment including esterification with cross-linking, INS No. 1422), distarch phosphate (DP, type of modification: cross-linking, INS No. 1412), and acetylated distarch phosphate (ADP, type of modification: dual treatment including esterification with cross-linking, INS No. 1414).

### Chemical analysis of the material

The AOAC [1990] method was employed to determine contents of ash and proteins. The resistant starch, considered as the starch fraction not hydrolysed *in vitro* by pancreatic  $\alpha$ -amylase (from porcine pancreas, SIGMA, A-3176), was determined according to the method by Champ *et al.* [1999]. The products of hydrolysis were extracted with 80% (v/v) ethanol and the non-digested material was solubilised in 2 mol/L KOH, then hydrolysed with amyloglucosidase (Novozymes, AMG 300L) into glucose. The glucose was quantified with a glucose oxidase/peroxidase analysis kit (Liquick Cor-GLUCOSE 120, Cormay, Poland) and measured spectrophotometrically at 500 nm.

### The *in vitro* hydrolysis

Susceptibility of the studied potato starch preparations to pancreatic  $\alpha$ -amylase was determined *in vitro* according to Soral-Śmietana & Wronkowska [2000] using 200 U of porcine pancreatic  $\alpha$ -amylase (from porcine pancreas, SIGMA, A-3176) per 1 gram of a sample. The sample (500 mg) was suspended in 20 mL of an enzyme solution and the hydrolysis was carried out for 24 h at 37°C. Prior to hydrolysis, isopropanol (100  $\mu$ L) was added to the sample to inhibit the growth of microbes during incubation. The enzyme was inactivated with 95% (v/v) ethanol and after centrifugation at 3,000  $\times$ g/10 min, the samples were dried at a temperature below 30°C.

### Scanning electron microscope (SEM)

Changes in the microstructure of the investigated starches after *in vitro* hydrolysis were analysed by covering dry samples with a gold layer and visualizing at an acceleration of 10 KeV on a scanning electron microscope (JSM 5200, Japan).

### Feeding experiment

The protocol used in this animal study was approved by the Institutional Animal Care and Use Committee, at the University of Warmia and Mazury, Olsztyn, Poland. The experiment was conducted on 56 male Wistar rats, aged 4 weeks, divided into experimental groups of 8 rats each. The animals were housed individually under standard conditions: temperature 21–22°C, relative air humidity 50–70%, 12-h light:dark cycle, intensive ventilation (air turnover 10/h), and *ad libitum* access to water and feed. The nutritional experiment lasted 4 weeks. The composition of experimental diets is presented in Table 1. Cellulose (200 g/kg) was added as a source of dietary fibre. In the experimental treatments, the whole dietary cellulose was substituted with native or chemically-modified potato starch preparations. After the experiment, the rats were anaesthetized with sodium pentobarbitone according to

TABLE 1. Composition of experimental diets (g/100 g diet).

	Diets*						
	C	NS	OS	AS	ADA	DP	ADP
Cellulose (C)	20.0						
Native potato starch (NS)	–	20.0	–	–	–	–	–
Oxidised starch (OS)	–	–	20.0	–	–	–	–
Acetylated starch (AS)	–	–	–	20.0	–	–	–
Acetylated distarch adipate (ADA)	–	–	–	–	20.0	–	–
Distarch phosphate (DP)	–	–	–	–	–	20.0	–
Acetylated distarch phosphate (ADP)	–	–	–	–	–	–	20.0
Casein	14.0	14.0	14.0	14.0	14.0	14.0	14.0
DL-methionine	0.2	0.2	0.2	0.2	0.2	0.2	0.2
Soy oil	8.0	8.0	8.0	8.0	8.0	8.0	8.0
Cholesterol	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Mineral mix <sup>a</sup>	3.5	3.5	3.5	3.5	3.5	3.5	3.5
Vitamin mix <sup>b</sup>	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Corn starch	52.8	52.8	52.8	52.8	52.8	52.8	52.8

<sup>a</sup>AIN-93G-MX [Reeves, 1997]; <sup>b</sup>AIN-93G-VM [Reeves, 1997]; \*Diets: C – control with cellulose; NS – with native potato starch; OS – with oxidised starch; AS – with acetylated starch; ADA – with acetylated distarch adipate; DP – with distarch phosphate; ADP – with acetylated distarch phosphate. The diets were isoenergetic and isonitrogenous.

the recommendations for euthanasia of experimental animals followed by the 12 h of fasting [Close *et al.*, 1997].

### Indices of diets intake and utilization

Body weight gain of rats and feed intake were determined individually. Coefficients of apparent nitrogen digestibility and utilisation were calculated in the first 5-day period of experimental feeding, from daily N intake and N excretion in faeces or in faeces and urine, respectively. Faeces and urine were collected from rats in each group for 5 days, preceded by a 10-day preliminary period. Nitrogen in the samples was determined for each rat according to the Kjeldahl method [AOAC, 1990].

### Physiological parameters of the caecum

After laparotomy, blood samples were taken from the tail vein, and then the caecum and colon with contents were removed and weighed. Samples of fresh digesta were used for immediate analysis of dry matter, ammonia and short chain fatty acids (SCFA), and the remainder was transferred to tubes and stored at -70°C. The caecal and colonic walls were flushed clean with ice-cold saline, blotted on filter paper and weighed for tissue mass. The caecal and colonic pH was measured using a microelectrode and a pH/ION meter (model 301, Hanna Instruments, Vila do Conde, Portugal). Dry mass of caecal contents was determined after primary drying at 50–60°C for 24 h, with a secondary drying at 105°C to determine the constant mass. In fresh caecal digesta, ammonia was extracted and trapped in a solution of boric acid in Conway's dishes, and determined by direct titration with sulfuric acid [Hofirek & Haas 2001].

Bacterial enzyme activity in the caecal digesta was measured by the rate of *p*- or *o*- nitrophenol release from nitrophenylglucosides according to the method of Djouzi & Andrieux [1997], modified by Juśkiewicz & Zduńczyk [2004]. The following substrates were used: for  $\beta$ -glucuronidase, *p*-nitrophenyl- $\beta$ -D-glucuronide; for  $\alpha$ -galactosidase, *p*-nitrophenyl- $\alpha$ -D-galactopyranoside; for  $\beta$ -galactosidase, *o*-nitrophenyl- $\beta$ -D galactopyranoside; for  $\alpha$ -glucosidase, *p*-nitrophenyl- $\alpha$ -D glucopyranoside; and for  $\beta$ -glucosidase, *p*-nitrophenyl- $\beta$ -D glucopyranoside. The reaction mixture contained 0.3 mL of a substrate solution (5 mmol/L) and 0.2 mL of a 1:10 (v/v) dilution of the caecal sample in 100 mmol/L phosphate buffer (pH 7.0) after centrifugation at  $10,000 \times g$  for 15 min. Incubation was carried out at 37°C and *p*- or *o*-nitrophenol was quantified at  $\lambda=400$  nm and at  $\lambda=420$  nm, respectively, after addition of 2.5 mL of 0.25 mol/L cold sodium carbonate. The enzymatic activity of  $\alpha$ - and  $\beta$ -glucosidases,  $\alpha$ - and  $\beta$ -galactosidases, and  $\beta$ -glucuronidase, was expressed as micromoles of product formed per minute (unit) per 1 g of digesta in the fresh caecal sample. Caecal digesta samples were subjected to an SCFA analysis using gas chromatography (Shimadzu GC-2010; Shimadzu, Kyoto, Japan). The samples (0.2 g) were mixed with 0.2 mL of formic acid, diluted with deionised water and centrifuged at  $10,000 \times g$  for 10 min. Supernatant was loaded onto a capillary column (SGE BP21, 30 m x 0.53 mm) using an on-column injector. The initial oven temperature was 85°C, and was raised to 180°C by 8°C/min and held for 3 min. The temperatures of flame ionization detector and the injection port were 180°C and 85°C, respectively. The sample volume for a gas chromatography analysis was 1  $\mu$ L. The caecal SCFA pool was calculated as the concentration of SCFA in the caecum ( $\mu$ mol/g) multiplied by the mass of the caecal contents (g) and was expressed in micromoles per 100 g of body weight.

#### Blood serum analysis

Blood samples collected from tail veins were left for 1 h at a room temperature to aggregate red blood cells. Blood serum was purified by centrifugation at  $2,500 \times g$  for 15 min at 4°C, and stored at -70°C after freezing with liquid nitrogen. Concentrations of the following indices were determined in the blood serum: triacylglycerols (TG) (ChF Reagent, catalog No. 220–200), glucose (ChF Reagent, catalog No. 210–200), total cholesterol (TC) (ChF Reagent, catalog No. 150–200), and high-density lipoprotein (HDL-C) cholesterol fraction (ChF Reagent, catalog No. 160–100). Log (TG/HDL-C) was calculated as an atherogenic index of serum.

#### Statistical analysis

Results of the physiological response of the treated animals are expressed as means and pooled standard error (SEM). Statistical comparisons were done transversely among different dietary groups. Data were analysed by one-way ANOVA, with one factor (diet). If significance was observed ( $p < 0.05$ ), the Duncan's multiple range test was used to identify differences in the effect of individual diets. Calculations were made with STATISTICA 6.0 software (StatSoft Corporation, Kraków, Poland).

### RESULTS AND DISCUSSION

The content of resistant starch in native and chemically-modified potato starch preparations was: native potato starch – 77.2% dry matter; ADA – 76.5% d.m.; AS – 75.6% d.m.; OS – 74.5% d.m.; DP – 14.5% d.m. and ADP – 13.5% d.m. The modification of potato starch, namely esterification, changed slightly the RS content in acetylated starch (AS) and acetylated distarch adipate (ADA) preparations. Also in oxidised starch (OS) the content of resistant starch did not change significantly compared to native potato starch. The next type of modification, *i.e.* cross-linking, which was applied to obtain distarch phosphate (DP), caused a significant reduction in RS content. In turn, dual modification, *i.e.* a combination of substitution and cross-linking which was used to produce acetylated distarch phosphate (ADP), resulted in the lowest RS content among the starch preparations applied. Protein and ash contents in all potato starch preparations were almost similar and reached from 0.20 to 0.37% d.m. for proteins and from 0.23 to 0.39% d.m. for ash.

The addition of starch preparations applied in the study to the rat diets was stipulated based on our previous investigations and some literature data according Wurzburg [1986] and Bird *et al.* [2006]. According to recommendation of the World Health Organization [FAO Report, 1998], the daily intake of the dietary fibre should be at the range of 20–40 g/day. Therefore in our study the control diet contained 20% of cellulose and 20% of investigated starches in the experimental diet.

Body weight gains of the rats and diets intake in experimental groups are presented in Table 2. Both, diet intake and final body weight gain of the rats from the ADA group turned out to be the lowest as compared to the control and NS groups ( $p < 0.05$ ). Hodgkinson *et al.* [1982] demonstrated that feeding animals with the addition of acetylated waxy maize starches evoked an increase in their body weight

TABLE 2. Diet intake, body weight gain and nitrogen apparent digestibility of rats fed experimental diets.

	Diets*							SEM
	C	NS	OS	AS	ADA	DP	ADP	
Diet intake (g/28 days)	562.1 <sup>a</sup>	531.5 <sup>ab</sup>	523.4 <sup>abc</sup>	514.5 <sup>bc</sup>	487.0 <sup>c</sup>	531.9 <sup>ab</sup>	529.5 <sup>abc</sup>	5.7
Body weight gain (g/28 days)	134.1 <sup>ab</sup>	132.4 <sup>ab</sup>	136.7 <sup>ab</sup>	131.0 <sup>ab</sup>	117.4 <sup>b</sup>	143.8 <sup>a</sup>	130.7 <sup>ab</sup>	2.5
Nitrogen apparent digestibility (%)	82.1 <sup>a</sup>	80.4 <sup>ab</sup>	81.7 <sup>ab</sup>	81.4 <sup>ab</sup>	79.9 <sup>b</sup>	80.1 <sup>b</sup>	80.9 <sup>ab</sup>	0.2

\*Diets: C – control with cellulose; NS – with native potato starch; OS – with oxidised starch; AS – with acetylated starch; ADA – with acetylated distarch adipate; DP – with distarch phosphate; ADP – with acetylated distarch phosphate; <sup>a,b,c</sup> – values in the same row with different letters are significantly different ( $p < 0.05$ ); SEM – pooled standard error of the means (standard deviation for all rats divided by square root of rat number, n=56).

gains by ca. 5% as compared to the animals not administered modified starches. In turn, in a model study conducted on rats Wronkowska *et al.* [2002] showed that, as compared to the control diet containing 10% of cellulose, an experimental diet in which cellulose was substituted with 10% of physically-modified potato starch preparation elicited a statistically significant increase in body weight gain of the animals.

For the control group (C), nitrogen apparent digestibility was the highest (not statistically significant) in comparison with groups receiving the investigated potato starch preparations (Table 2). The lowest values of that indicator were noticed when the diets contained acetylated distarch adipate (ADA) and distarch phosphate (DP). Results of this study proved a reduction in nitrogen concentration in faeces and urine of animals (data not shown). The characteristics of nitrogen metabolism is strictly linked with the type of dietary carbohydrates [Pastuszewska *et al.*, 2000]. Fermentable carbohydrates enhance faecal N excretion by promoting bacterial proliferation and also by accelerating the digestive transit time [Younes *et al.*, 1995]. Krupa-Kozak *et al.* [2010] found a statistically significant decrease in nitrogen apparent digestibility of rats fed diets in which 30% of corn starch was substituted with bean or pea starches.

Results of the analysis of caecum and colon parameters were provided in Table 3. As compared to the control diet, in which the non-digestible constituent was cellulose, all diets with the examined starches caused a statistically significant ( $p < 0.05$ ) increase in caecal tissue mass and caecal digesta bulk. The highest mass of caecal digesta was found for the diet containing distarch phosphate (DP). In turn, the diets were not observed to evoke analogous changes in colonic parameters (Table 3). Literature data presents that preparations containing different types of RS significantly influence the metabolism and morphology of the gastrointestinal tract, including hypertrophy of large intestinal tissue and bulking effect [Ebihara *et al.*, 1998; Wronkowska *et al.*, 2002; Annison *et al.*, 2003]. All types of RS are fermentable, so their bulking action is rather variable and much less than that of wheat

bran which is thought as a most effective faecal bulking agent [Topping & Clifton, 2001].

In groups C and OS, a significantly higher dry matter content in the caecal digesta was found as compared to the other groups (Table 3). Both, pH values and the concentration of ammonia in the caecal digesta of the control group were significantly higher than in all experimental groups (C vs. the other treatments,  $p < 0.05$ ). The lowest pH value of the caecal digesta was determined in OS group. The pH of colonic content was lower for all experimental groups compared with the control group. Microflora metabolizes nitrogenous compounds that enter the large intestine into putrefactive catabolites, such as ammonia and phenols, which may negatively affect gastrointestinal tract health. Fermentable carbohydrates may decrease the concentration of putrefactive compounds by providing gut microflora with an additional energy [Younes *et al.*, 1995]. Cermak *et al.* [2002] demonstrated that luminal ammonia inhibited sodium and chloride absorption in the distal colon of rats. Til *et al.* [1986] showed that feeding hydroxypropyl distarch phosphate from potato starch led to an increase in tissue mass of the caecum and colon of rats. In turn, Kishida *et al.* [2000] found an increase in the caecal wall and contents after the addition of gelatinized hydroxypropyl distarch phosphate from tapioca starch to the rats diet.

A statistically significant ( $p < 0.05$ ) increase in the pool of SCFA in the caecal content was observed in all groups fed diets with the addition of the native potato starch and its modified preparations (Table 4). In respect of the control group, all experimental groups were characterised by a significant increase in concentrations of acetic, propionic, butyric and valeric acids as well as by considerably diminished concentrations of iso-butyric and iso-valeric acids. The significant increase in the total content of SCFA in the caecum was due to significant acidification of the caecal and colonic contents (Table 3) observed for all types of the investigated potato starch. Acetylated starches could be used to raise the SCFA level in the large bowel of rats as presented by An-

TABLE 3. Caecal and colonic parameters of rats fed experimental diets.

	Diets*							SEM
	C	NS	OS	AS	ADA	DP	ADP	
Caecum								
Mass of tissue (g/100 g BW)	0.29 <sup>c</sup>	0.69 <sup>a</sup>	0.73 <sup>a</sup>	0.64 <sup>ab</sup>	0.57 <sup>b</sup>	0.68 <sup>a</sup>	0.65 <sup>ab</sup>	0.02
Mass of digesta (g/100 g BW)	0.96 <sup>d</sup>	3.23 <sup>ab</sup>	3.59 <sup>ab</sup>	3.12 <sup>ab</sup>	2.33 <sup>c</sup>	3.87 <sup>a</sup>	2.87 <sup>bc</sup>	0.15
Dry matter of digesta (%)	28.2 <sup>a</sup>	24.0 <sup>b</sup>	28.1 <sup>a</sup>	23.0 <sup>b</sup>	24.4 <sup>b</sup>	24.5 <sup>b</sup>	22.8 <sup>b</sup>	0.40
Ammonia (mg/g digesta)	0.26 <sup>a</sup>	0.19 <sup>b</sup>	0.20 <sup>b</sup>	0.20 <sup>b</sup>	0.21 <sup>b</sup>	0.19 <sup>b</sup>	0.19 <sup>b</sup>	0.01
pH of caecal contents	6.88 <sup>a</sup>	5.21 <sup>bc</sup>	5.12 <sup>c</sup>	5.32 <sup>bc</sup>	5.38 <sup>b</sup>	5.16 <sup>bc</sup>	5.34 <sup>bc</sup>	0.08
Colon								
Mass of tissue (g/100 g BW)	0.65	0.64	0.65	0.64	0.61	0.63	0.61	0.01
Mass of digesta (g/100 g BW)	0.67	0.72	0.76	0.62	0.62	0.70	0.62	0.02
pH of colonic contents	6.64 <sup>a</sup>	5.08 <sup>b</sup>	5.07 <sup>b</sup>	5.07 <sup>b</sup>	5.06 <sup>b</sup>	5.04 <sup>b</sup>	4.95 <sup>b</sup>	0.08

\*Diets: C – control with cellulose; NS – with native potato starch; OS – with oxidised starch; AS – with acetylated starch; ADA – with acetylated distarch adipate; DP – with distarch phosphate; ADP – with acetylated distarch phosphate; <sup>a,b,c</sup> – values in the same row with different letters are significantly different ( $p < 0.05$ ); BW – body weight; SEM – pooled standard error of the means (standard deviation for all rats divided by square root of rat number,  $n = 56$ ).

TABLE 4. Caecal digesta total short chain fatty acids (SCFA) pool of rats fed experimental diets.

SCFA pool ( $\mu\text{mol}/100\text{ g BW}$ )	Diets*							SEM
	C	NS	OS	AS	ADA	DP	ADP	
Acetic	63.2 <sup>d</sup>	339.0 <sup>ab</sup>	324.4 <sup>ab</sup>	307.2 <sup>ab</sup>	221.0 <sup>c</sup>	384.8 <sup>a</sup>	282.1 <sup>bc</sup>	16.1
Propionic	12.10 <sup>b</sup>	73.20 <sup>a</sup>	71.91 <sup>a</sup>	96.72 <sup>a</sup>	74.437 <sup>a</sup>	79.90 <sup>a</sup>	77.00 <sup>a</sup>	4.61
Iso-butyric	0.61 <sup>a</sup>	0.05 <sup>b</sup>	0.04 <sup>b</sup>	0.14 <sup>b</sup>	0.08 <sup>b</sup>	0.09 <sup>b</sup>	0.04 <sup>b</sup>	0.03
Butyric	7.85 <sup>c</sup>	51.41 <sup>a</sup>	40.58 <sup>a</sup>	25.61 <sup>b</sup>	25.38 <sup>b</sup>	47.59 <sup>a</sup>	40.43 <sup>a</sup>	2.58
Iso-valeric	0.74 <sup>a</sup>	0.12 <sup>b</sup>	0.14 <sup>b</sup>	0.54 <sup>a</sup>	0.13 <sup>b</sup>	0.14 <sup>b</sup>	0.08 <sup>b</sup>	0.05
Valeric	0.92 <sup>c</sup>	10.28 <sup>a</sup>	7.93 <sup>b</sup>	6.09 <sup>b</sup>	5.97 <sup>b</sup>	10.35 <sup>a</sup>	6.01 <sup>b</sup>	0.55
Total	85.4 <sup>d</sup>	474.9 <sup>ab</sup>	444.1 <sup>ab</sup>	436.0 <sup>ab</sup>	327.5 <sup>c</sup>	522.2 <sup>a</sup>	406.0 <sup>bc</sup>	22.1

\*Diets: C – control with cellulose; NS – with native potato starch; OS – with oxidised starch; AS – with acetylated starch; ADA – with acetylated distarch adipate; DP – with distarch phosphate; ADP – with acetylated distarch phosphate; <sup>a,b,c,d</sup> – values in the same row with different letters are significantly different ( $P < 0.05$ ); BW – body weight; SEM – pooled standard error of the means (standard deviation for all rats divided by square root of rat number,  $n = 56$ ).

nison *et al.* [2003]. In a human study Clarke *et al.* [2007] found that the application of acetylated starches was a potentially effective method for delivering significant quantities of specific SCFAs to the colon. However, not all RS of type 4 appear to be equal in their effects on large bowel SCFA. Ebihara *et al.* [1998] reported that the ingestion of hydroxy-propylated starches increased faecal bulk in rats but did not affect changes in SCFA. In our study, the results indicated strongly that the type of industrial processing itself, rather than the RS content affected the effects of the preparations applied on the caecal SCFA yield. In the investigated potato starch preparations, dextrans of different molecular mass can be formed during starch degradation and these degradation products are supposed to have effect on the physiological processes in the animals examined. It is in line with suggestions provided by several authors that the physiological effects of chemically-modified starches are affected by the type and extent of modification [Ebihara *et al.*, 1998; Ferguson & Jones, 2000; Nugent, 2005].

A significant indicator of the physiological effect is the activity of bacterial glycolytic enzymes analysed in caecal digesta (Table 5). In comparison to the control diet, a statistically significant ( $p < 0.05$ ) increase was observed in the activities of  $\alpha$ -glucosidase and  $\beta$ -galactosidase upon the administration of all experimental diets under study. Distinguishing

activities of  $\beta$ -glucosidase as well as  $\alpha$ - and  $\beta$ -galactosidase were noted upon the application of the diet with acetylated starch (AS). Yet, worthy of special attention is a diminished activity of  $\beta$ -glucuronidase as affected by the addition of native potato starch and chemically-modified starches to diets, except for ADP (Table 5). The increase in  $\beta$ -galactosidase and  $\alpha$ -glucosidase activities and the decrease in that of  $\beta$ -glucuronidase could be considered beneficial for the host.  $\beta$ -Galactosidase and  $\alpha$ -glucosidase activities may improve the fermentation of lactose and resistant starch, leading to the production of SCFA and lactic acid which are a source of energy for colonic tissues [Cummings & Macfarlane, 1991]. The increase in  $\beta$ -glucosidase activity is more ambiguous because its hydrolytic activity is responsible both for the generation of toxins [Mallet & Rowland, 1988], and for the production of bacterial glucoside derivatives which are assumed to be responsible for protection against chemically-induced cancer [Rowland & Tanaka, 1993].  $\beta$ -Glucuronidase is involved in the generation of toxic and carcinogenic metabolites in the hindgut [Reddy *et al.*, 1992]. Wronkowska *et al.* [2002] reported the decreasing of  $\beta$ -glucuronidase activity in caecal digesta of rats fed diets with 10% of physically-modified starch preparations obtained from wheat, potato or pea starch, as compared with diets containing native starches. The previous *in vivo* study [Krupa-Kozak *et al.*, 2010] con-

TABLE 5. Caecal bacterial enzyme activity of rats fed experimental diets.

	Diets*							SEM
	C	NS	OS	AS	ADA	DP	ADP	
$\alpha$ -Glucosidase (U/g c.c.)	0.13 <sup>b</sup>	1.29 <sup>a</sup>	1.33 <sup>a</sup>	1.55 <sup>a</sup>	1.42 <sup>a</sup>	1.33 <sup>a</sup>	0.13 <sup>b</sup>	0.07
$\beta$ -Glucosidase (U/g c.c.)	0.04 <sup>c</sup>	0.09 <sup>abc</sup>	0.07 <sup>bc</sup>	0.14 <sup>a</sup>	0.12 <sup>ab</sup>	0.07 <sup>bc</sup>	0.04 <sup>c</sup>	0.01
$\alpha$ -Galactosidase (U/g c.c.)	0.06 <sup>b</sup>	0.05 <sup>b</sup>	0.06 <sup>b</sup>	0.09 <sup>a</sup>	0.07 <sup>ab</sup>	0.04 <sup>b</sup>	0.06 <sup>b</sup>	0.00
$\beta$ -Galactosidase (U/g c.c.)	0.35 <sup>d</sup>	0.99 <sup>ab</sup>	0.66 <sup>c</sup>	1.21 <sup>a</sup>	0.99 <sup>ab</sup>	0.88 <sup>bc</sup>	0.35 <sup>d</sup>	0.05
$\beta$ -Glucuronidase (U/g c.c.)	0.16 <sup>a</sup>	0.07 <sup>b</sup>	0.08 <sup>b</sup>	0.10 <sup>b</sup>	0.10 <sup>b</sup>	0.10 <sup>b</sup>	0.16 <sup>a</sup>	0.01

c.c. – caecum contents; \*Diets: C – control with cellulose; NS – with native potato starch; OS – with oxidised starch; AS – with acetylated starch; ADA – with acetylated distarch adipate; DP – with distarch phosphate; ADP – with acetylated distarch phosphate; <sup>a,b,c</sup> – values in the same row with different letters are significantly different ( $p < 0.05$ ); SEM – pooled standard error of the means (standard deviation for all rats divided by square root of rat number,  $n = 56$ ).

firmed the significant decrease of  $\beta$ -glucuronidase activity in caecal digesta when rats were fed a diet containing dietary legume starches, bean and pea or their microwaved starch preparations.

The addition of distarch phosphate (DP) and acetylated distarch adipate (ADA) to the diets was found to decrease glucose concentration ( $p < 0.05$ ) in the rat blood serum (Table 6). All chemically-processed potato starch preparations led to a significant drop in the serum level of triacylglycerols (TG). On the other hand, all starch treatments caused a significant decrease in the total cholesterol and also a significant increase in the ratio of HDL-C/total cholesterol in the serum. However, the calculated log (TG/HDL-C) index of serum atherogenicity was the highest in the control group and its significant reduction was found in all experimental treatments. It should also be emphasized that the lowest log (TG/HDL-C) index was observed in the DP group ( $p < 0.05$  vs. all other groups).

De Deckere *et al.* [1993] try to explain the effect of RS on serum total cholesterol and triacylglycerols concentrations: (1) serum cholesterol might have been decreased by RS as a result of an increased faecal excretion of sterols and inhibited *via* SCFA of cholesterol synthesis in the liver; (2) serum TAG concentration may be lowered due to the effects on lipid absorption and hepatic fatty acid synthesis. It has been reported in an *in vivo* test that acetylated potato starch in a meal improved the glycemic, insulinemic and satiating properties, because the acetylation decreased the susceptibility of starch to  $\alpha$ -amylase [Raben *et al.*, 1997]. Some researchers have observed that the cross-linking modification may reduce the rate of starch digestion, however in this regard the type and extent of cross-linking seem to be of paramount importance [Woo & Seib, 2002]. Several studies have shown that high amylose corn starch (RS type 2) reduces serum cholesterol and triacylglycerols concentration in rats [Sacquet *et al.*, 1983] and hamsters [Ranghotra *et al.*, 1997]. In an *in vitro* study, acetylated potato starch was characterised by the highest cholesterol binding capacity in comparison with other investigated chemically-modified potato starches [Wronkowska *et al.*, 2008]. Kishida *et al.* [2002] found that high amylose corn starch exerted a hypocholesterolemic effect in caecotomized rats fed a cholesterol-free diet, and this effect was most

likely mediated through the enlargement of the bile acids pool in the intestine and increased faecal excretion of bile acids, proving that various ways leading to the hypocholesterolemic effect are attributable to RS preparations.

For in-depth recognition of the course of hydrolysis of the chemically-modified preparations of potato starch investigated in this study, an analysis was conducted of the microstructure of these starch preparations subjected to 24-h hydrolysis with pancreatic  $\alpha$ -amylase (Figure 1). After 24-h activity of that enzyme, a surface-damaging effect could be observed, being typical of granules of the native potato starch (NS) [Gallant *et al.*, 1992]. In contrast, the modified preparations revealed a significant effect of the destruction of granule structure, especially in the case of the oxidised starch (OS), distarch phosphate (DP) and acetylated distarch phosphate (ADP). The study proved that upon the process of modification, those preparations were hydrolysed by pancreatic  $\alpha$ -amylase by the formation of pin-like channels from the surface to the interior of the granules, which is typical of cereal starches with A type crystalline structure. In contrast, in the acetylated preparation (AS), observations have shown the radial mode of amylolysis only in more damaged fragments of the granules and collapse of the external structure in the inter-crystalline space. Generally, more or less intensive superficial erosion was observed in the AS and ADA preparations, which is typical course of the amylolysis of potato starch.

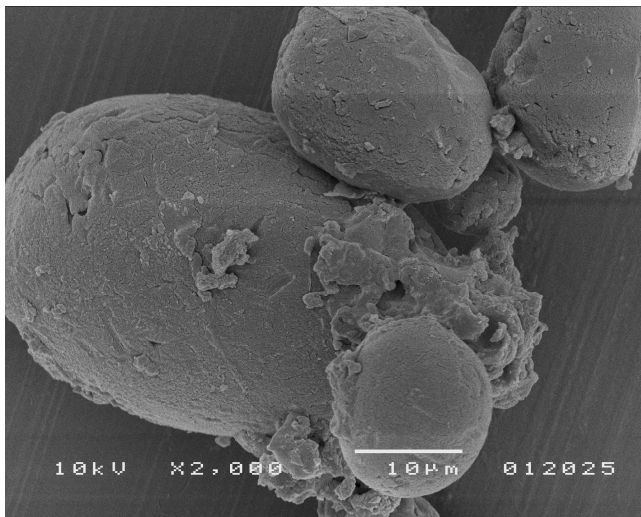
Results of the microstructural study seem to be especially interesting in respect of the DP and ADP preparations, in which RS content was determined to be low. Microphotographs of these preparations proved the significance of the physical status of potato starch preparations and also the type of modification used, provided as a substrate for intestinal microflora. The digestion of starch granules is a complex process, consisting from a few stages, *i.e.*: adsorption of the enzyme by starches; penetration of the enzyme through a substrate, which is linked with porosity of granules; and at the end – the process of hydrolysis. Potato starch and other starches with type B crystalline structure are digested superficially, whereas cereal starches are hydrolysed through pores and channels that may be used by the enzyme [Lehmann & Robin, 2007]. Our research proved that the process of hy-

TABLE 6. Serum indices of rats fed experimental diets.

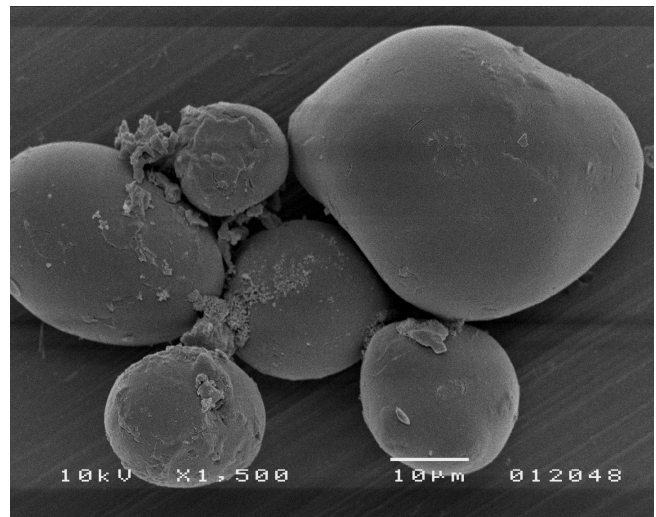
	Diets*							SEM
	C	NS	OS	AS	ADA	DP	ADP	
Glucose (mmol/L)	12.19 <sup>a</sup>	11.58 <sup>a</sup>	12.27 <sup>a</sup>	11.27 <sup>ab</sup>	10.30 <sup>b</sup>	10.45 <sup>b</sup>	11.69 <sup>a</sup>	0.15
TG (mmol/L)	1.54 <sup>a</sup>	1.39 <sup>ab</sup>	1.26 <sup>bcd</sup>	1.33 <sup>bc</sup>	1.14 <sup>de</sup>	1.02 <sup>c</sup>	1.18 <sup>cde</sup>	0.03
TC (mmol/L)	2.81 <sup>a</sup>	2.24 <sup>b</sup>	2.18 <sup>b</sup>	2.32 <sup>b</sup>	2.21 <sup>b</sup>	2.15 <sup>b</sup>	2.26 <sup>b</sup>	0.04
HDL-C (mmol/L)	1.01 <sup>b</sup>	1.24 <sup>a</sup>	1.04 <sup>b</sup>	1.18 <sup>ab</sup>	1.07 <sup>ab</sup>	1.15 <sup>ab</sup>	1.15 <sup>ab</sup>	0.02
HDL-C/TC (%)	36.02 <sup>b</sup>	55.23 <sup>a</sup>	47.58 <sup>a</sup>	51.01 <sup>a</sup>	48.44 <sup>a</sup>	53.23 <sup>a</sup>	51.08 <sup>a</sup>	1.24
Log (TG/HDL-C)	0.18 <sup>a</sup>	0.05 <sup>b</sup>	0.09 <sup>b</sup>	0.05 <sup>b</sup>	0.03 <sup>bc</sup>	-0.05 <sup>d</sup>	0.01 <sup>c</sup>	0.00

\*Diets: C – control with cellulose; NS – with native potato starch; OS – with oxidised starch; AS – with acetylated starch; ADA – with acetylated distarch adipate; DP – with distarch phosphate; ADP – with acetylated distarch phosphate; <sup>a,b,c,d</sup> – values in the same row with different letters are significantly different ( $p < 0.05$ ); SEM – pooled standard error of the means (standard deviation for all rats divided by square root of rat number,  $n = 56$ ); TC – total cholesterol; TG – triacylglycerol; HDL-C: HDL fraction of cholesterol.

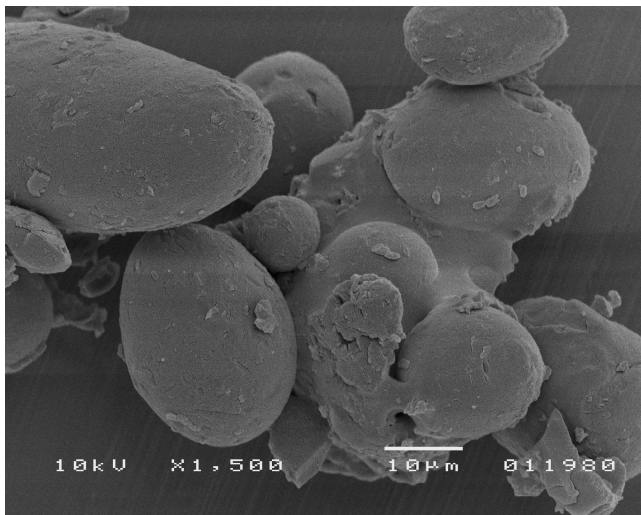
NS



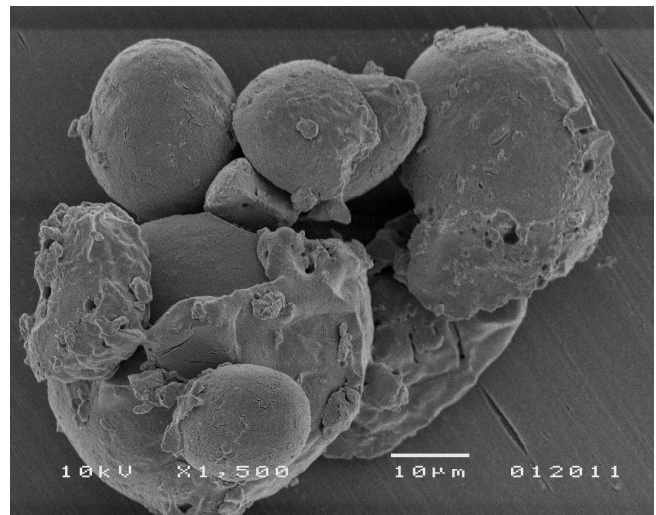
ADA



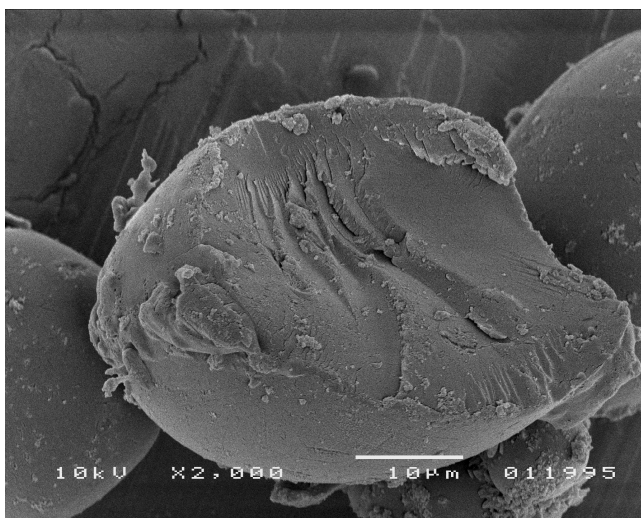
OS



DP



AS



ADP

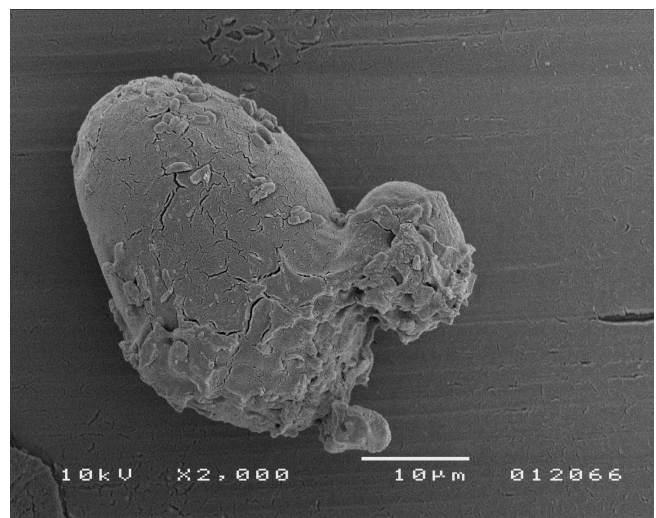


FIGURE 1. Microphotographs (SEM) of the potato starch preparations subjected to 24-h hydrolysis with pancreatic  $\alpha$ -amylase. NS – native potato starch; OS – oxidised starch; AS – acetylated starch; ADA – acetylated distarch adipate; DP – distarch phosphate; ADP – acetylated distarch phosphate.

drolisis of potato starch subjected to various treatments of chemical modification might proceed in a diversified mode, untypical of type B tuber starches, which was additionally reflected in the present *in vivo* study.

#### SUMMARY

Chemically-modified potato starches containing RS type 4 are widely used in the food industry rather for technological than nutritional reasons. In summary, the present study provides data regarding the effect of diets supplemented by different kinds of chemically-modified potato starch on small mammals (rats). Partial substitution of rats diets by the experimental starches caused a significant increase in body weight gain compared with the control animals. For all investigated starch preparations, a statistically significant increase of the total SCFA (especially butyric acid) in the caecal digesta was found. As an effect of diets with all the investigated preparations, the significant lowering of luminal ammonia concentration, pH of caecal or colonic content, triacylglycerols, total cholesterol and activity of  $\beta$ -glucuronidase in caecum content were noticed compared to the control rats. The positive physiological results obtained *in vivo* in a model experiment with rats should, however, be verified in a human model research.

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