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Effect of acid whey-fortified breads on caecal fermentation processes and blood lipid profile in rats

Małgorzata Wronkowska[1]*, Maria Soral-Śmietana[1], Zenon Zduńczyk[2], Jerzy Juśkiewicz[2], Monika Jadacka[1], Anna Majkowska[3] and Fabian J. Dajnowiec[4]

[1]*Department of Chemistry and Biodynamics of Food, Division of Food Science, Institute of Animal Reproduction and Food Research of the Polish Academy of Sciences, Tuwima 10 Str., 10-748 Olsztyn, Poland*
[2]*Department of Biological Function of Food, Division of Food Science, Institute of Animal Reproduction and Food Research of the Polish Academy of Sciences, Tuwima 10 Str., 10-748 Olsztyn, Poland*
[3]*Microbiological Laboratory, Division of Food Science, Institute of Animal Reproduction and Food Research of the Polish Academy of Sciences, Tuwima 10 Str., 10-748 Olsztyn, Poland*
[4]*Department of Process Engineering and Equipment, Faculty of Food Sciences, University of Warmia and Mazury, M. Oczapowskiego 7 Str., 10-957 Olsztyn, Poland*

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Abstract

Two types of diet – standard and atherogenic – were used to study the effect of wheat or wheat–rye breads supplemented with 20 % acid whey concentrate after ultrafiltration on the physiological response of growing rats. The acid whey concentrate after ultrafiltration used in rat diets caused reduced weight gain (for atherogenic diet with wheat bread); growth of caecum tissue and digesta weight; a decrease in the pH of caecum digesta (for atherogenic diet); reduced activity of bacterial glycolytic enzymes; and a significant increase in total SCFA for both types of diet with wheat–rye breads containing acid whey concentrate. For wheat bread with acid whey, in standard diet, a statistically significant increase was found in the population of bifidobacteria. The results showed that the acid whey concentrates could be used as a valuable food ingredient.

Key words: Acid whey: Ultrafiltration: Bread: *In vivo* experiments: Rats

Whey is a product resulting from the technological processes of mature cheese and cottage cheese manufacture. Rennet coagulation of milk is used to produce mature cheese, and the resulting whey is called 'sweet whey' (pH from 6·2–6·6). Acid whey is the by-product obtained only after the acid coagulation of milk during the production of cottage cheese or tvarog (with pH of 4·5–4·7). Whey obtained from these two different processes differs in chemical composition and physicochemical properties. Acid whey has more lactic acid, crude ash and less protein as compared with sweet whey[1]. Currently, acid whey is mostly wasted or used as animal feed[2]. The separation of the liquid part and fractionation of whey solids is now feasible because of the development and implementation of the membrane filtration technique.

Scientists are still looking for food ingredients that would have a positive effect on health and well-being. From our prior investigations, it is known that acid whey contains valuable health components, such as bioactive peptides, lactose and macroelements and microelements[3,4]. Bioactive peptides derived from whey proteins, including β-lactoglobulin, α-lactalbumin and serum albumin, have antibacterial properties[5,6], may reduce blood cholesterol level[7] or prevent hypertension[8–10]. Mannie & Asp[11] showed that the introduction of whey to baking products increased their nutritional value. Acid whey after ultrafiltration could be used for improving the nutritional and sensory quality of baking products, as was presented in our previous study[12].

Changes in the gut microbiota associated with high-fat diets and its contribution to the development of metabolic diseases are the main topics of research in many laboratories. It is well known that high-fat diets, such as the Western-type diet, enhance bile secretion to facilitate lipid digestion. Reaching the large bowel, bile acids undergo some modifications by bacteria, thus increasing secondary bile acid production. The most typical secondary bile acid is deoxycholic acid, which is produced from cholic acid. Earlier studies revealed a remarkable effect of cholic acid on gut microbiota populations and their metabolism[3,13]. The changes in gut microbiota observed in rats fed a diet supplemented with cholic acid were similar to those observed in mice fed high-fat diets[14,15]. The study of Islam *et al.*[16] showed that cholic acid introduced to a rat diet induced phylum-level alterations in the composition of the gut microbiota. They found an increase in the count of Firmicutes and a decrease in the count of Bacteroidetes. Therefore,

* **Corresponding author:** M. Wronkowska, email m.wronkowska@pan.olsztyn.pl

we decided to incorporate cholic acid to a high-fat diet in which the main component was bread made with acid whey.

The objective of the present *in vivo* study was to identify the effects of acid whey-fortified breads on caecal fermentation and blood parameters in growing rats. It was hypothesised that the consumption of breads (wheat or wheat–rye) as the main components of rat diets would be associated with some beneficial changes, including blood lipid profile, caecal microbiota composition and its activity. Two types of diet were used, namely standard diet and cholic acid and cholesterol-supplemented diet (the latter is called atherogenic diet).

Methods

Materials

The recipe and technology applied for preparing both types of breads (wheat and wheat–rye) used in this experiment were described in detail by Wronkowska *et al.*[12]. In brief, wheat flour type 750 (Młynomag), tap water, salt and fresh yeast (Lesaffre S.A.) were used for the preparation of wheat bread. Mixed wheat–rye bread was made of a mixture of wheat flour type 750 and rye flour type 720 (3:2, respectively, both flours from Młynomag), tap water, salt and yeast. Acid whey obtained from the industrial production of white fresh cheese (tvarog) was concentrated by ultrafiltration and dehydrated by spray drying. Dried acid whey powder was introduced to the dough as 20 % addition in relation to flour mass. The procedure of acid whey concentration was described by Wronkowska *et al.*[12]. Baked breads after cooling were air-dried, milled and added to the rat diets in powdered form. The following sample abbreviations were used: bread from wheat flour type 750 (W), bread from a mixture of wheat flour type 750 and rye flour type 720 (WR), bread from wheat flour type 750 or from a mixture of wheat flour type 750 and rye flour type 720 with 20 % addition of ultrafiltrated acid whey (WU and WRU).

Preparation of diets and animal protocol

The animal protocol used in this study was approved by the University of Warmia and Mazury Institutional Animal Care and Use Committee (permission no. 24/2008, Olsztyn, Poland). The experiment was conducted on sixty-four Wistar male rats, aged 4 weeks, weighing about 240 g, randomly divided into experimental groups of eight rats each. The required number of experimental animals was determined for each group according to Berndtson[17].

The animals were housed individually in metal cages under standard conditions: temperature 21–22°C, relative air humidity 50–70 %, 12 h light–12 h 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. The content of casein and cellulose was calculated on the basis of the content of protein and carbohydrates in the breads. For the atherogenic diet group, the diet was modified by the addition of lard and cholic acid (4 and 0·2 % of the diet, respectively), and also by increasing the cholesterol content compared with the standard diet (1 *v.* 0·3 %). After the experiment, the rats were anaesthetised with sodium pentobarbital according to the recommendations for euthanasia of experimental animals[18].

Body weight gain and diet intake of rats were determined individually, and the feed conversion ratio (FCR) was calculated as the ratio of diet intake:body weight gain.

After laparotomy, blood samples were taken from the caudal vein and stored in tubes containing heparin. The blood serum was purified by centrifugation at 2500 ***g*** for 15 min at 4°C, and stored at −20°C until analysis. The caecum and colon with contents were removed and weighed. Samples of fresh caecum and colon digesta were used for immediate analysis of DM, pH (microelectrode and a pH/ION meter, model 301; Hanna Instruments), ammonia and SCFA, and the remainder was transferred to tubes and stored at −20°C.

The caecal and colonic walls were flushed clean with ice-cold saline, blotted on filter paper and weighed for tissue mass. DM 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 sulphuric acid[19].

Caecal digesta samples were subjected to an SCFA analysis using GC (Shimadzu GC-2010; Shimadzu). The samples (0·2 g) were mixed with 0·2 ml of formic acid, diluted with deionised water and centrifuged at 10 000 ***g*** for 10 min. The supernatant was loaded onto a capillary column (30 m × 0·53 mm; BP21; SGE Analytical Science) 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 the flame ionisation detector and the injection port were 180 and 85°C, respectively.

Bacterial enzyme activity in the caecal digesta was measured by the rate of *p*- or *o*-nitrophenol release from nitrophenylglucosides according to the method described by Juśkiewicz *et al.*[20]. The following substrates were used: for *β*-glucuronidase, *p*-nitrophenyl-*β*-D-glucuronide; for *α*-galactosidase, *p*-nitrophenyl-*α*-D-galactopyranoside; for *β*-galactosidase, *o*-nitrophenyl-*β*-D galactopyranoside; for *α*-glucosidase, *p*-nitrophenyl-*α*-D-glucopyranoside; and for *β*-glucosidase, *p*-nitrophenyl-*β*-D-glucopyranoside. The reaction mixture contained 0·3 ml of a substrate solution (5 mM) and 0·2 ml of a 1:10 (v/v) dilution of the caecal sample in 100 mM phosphate buffer (pH 7·0) after centrifugation at 10 000 ***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 M cold sodium carbonate. The enzymatic activity of *α*- and *β*-glucosidases, *α*- and *β*-galactosidases and *β*-glucuronidase was expressed as micromoles of product formed per min (unit) per 1 g of fresh caecum digesta.

The concentrations of glucose (no. G6620), TAG (no. T6630), total cholesterol (TC) (no. C6608) and HDL-cholesterol fraction (no. H6421) were analysed in the blood serum, using the kits from Alpha Diagnostics Ltd. Log (TAG/HDL-cholesterol) was calculated as an atherogenic index (AI) of serum.

Determination of bacteria in caecum digesta

Bacteria in caecum digesta were determined according Bielecka *et al.*[21]. Fresh caecum digesta were immediately homogenised

with 1 % peptone water as a diluter. After dispersion, the serial decimal dilutions were made avoiding aeration. The live cell number of bifidobacteria was counted on a modified nutrient Garche's agar medium. Coliforms were identified on MacConkey agar. Other microorganisms were quantitatively determined on selective media: *Lactobacillus* sp., Rogosa Agar (Merck); *Enterococcus* sp., Bile Aesculin Agar (Merck). The identification of bacteria was based on the appearance of colonies; specific morphology of cells was checked under phase contrast with a Microphot FXA microscope (Nikon).

Statistical analysis

Results of the physiological response of the treated animals are expressed as means and standard deviations. Statistical comparisons were done transversely among different dietary groups, for standard and atherogenic diets separately. The effects of the two parameters – kind of bread (B), ultrafiltrated whey concentrate (UWC) or their interactions (B × UWC) – were tested using two-way ANOVA (version 7.1; Statistica). If significance was observed ($P < 0{\cdot}05$), the Duncan's multiple-range test was used to identify differences in the effect of individual diets.

Results and discussion

Bread is one of the most widely consumed foods in the world. Wheat–rye bread is one of the most popular baking products in Poland. According to the Food and Nutrition Institute[22], different types of bread were proposed in the everyday diet of healthy humans: dark wheat or rye bread (whole wheat or rye) (about 15 % of all eaten breads), white wheat bread (about 20 % of all eaten breads) and mixed rye–wheat bread or light rye bread (should be about 65 % of all eaten breads). Baking products may be one way for the practical use and application of acid whey in the diet. This is why we have proposed in our research to use a spray-dried acid whey concentrate after ultrafiltration as an ingredient in wheat or wheat–rye bread (20 % addition in relation to flour mass).

In the *in vivo* experiment, Wistar male rats were fed for 4 weeks a standard (containing 0·3 % cholesterol) or atherogenic diet (1 % cholesterol, 4 % lard and 0·2 % cholic acid). The main component of the diet was wheat or wheat–rye bread, which constituted 77 % of the diet. For both types of diets, standard and atherogenic, the breads without acid whey were used as control (Table 1). Animal models for obesity and other metabolic disorders related to CVD are mostly based on different high-fat and/or high-cholesterol diets that can significantly increase body fat accumulation and/or cause dyslipidaemia of the animals. Many factors, such as the quantity and source of fat used in the diet or the length of experimental feeding, may affect the results obtained in this type of *in vivo* experiment[23].

For rats fed the standard diet no difference was found in the body weight gain and diet intake (Table 2). However, for rats administered the atherogenic diet a significant effect of

Table 1 . Diet composition (%)

	W				WR			
	W	WA	WU	WUA	WR	WRA	WRU	WRUA
Bread W	77·0	77·0	–	–	–	–	–	–
Bread WU	–	–	77·0	77·0	–	–	–	–
Bread WR	–	–	–	–	77·0	77·0	–	–
Bread WRU	–	–	–	–	–	–	77·0	77·0
Casein	6·2	6·2	6·1	6·1	8·2	8·2	8·0	8·0
DL-Methionine	0·3	0·3	0·3	0·3	0·3	0·3	0·3	0·3
Soya oil*	8·0	4·0	8·0	4·0	8·0	4·0	8·0	4·0
Lard†	–	4·0	–	4·0	–	4·0	–	4·0
Cholesterol	0·3	1·0	0·3	1·0	0·3	1·0	0·3	1·0
Cholic acid	–	0·2	–	0·2	–	0·2	–	0·2
Mineral mix	3·0	3·0	3·0	3·0	3·0	3·0	3·0	3·0
Vitamin mix	1·0	1·0	1·0	1·0	1·0	1·0	1·0	1·0
Maize starch	2·7	1·8	2·8	1·9	0·9	-	1·1	0·2
Cellulose	1·5	1·5	1·5	1·5	1·3	1·3	1·3	1·3
Total	100	100	100	100	100	100	100	100
Macronutrients								
Carbohydrates	57·7	56·8	48·8	47·9	55·6	54·7	47·1	46·2
Proteins	18·1	18·1	18·8	18·8	18·2	18·2	18·4	18·4
Fats	9·4	10·3	9·1	10·0	9·5	10·4	9·3	10·1
Fatty acid profile								
SFA	1·47	2·65	1·42	2·60	1·47	2·65	1·45	2·63
MUFA	1·88	2·92	1·81	2·89	1·97	3·01	1·94	2·99
PUFA	5·32	3·27	5·30	3·24	5·36	3·31	5·22	3·28
n-6	4·86	3·00	4·80	2·99	4·87	3·01	4·78	3·00
n-3	0·45	0·27	0·39	0·25	0·49	0·30	0·47	0·28

W, bread from wheat flour type 750; WR, bread from mixture of wheat flour type 750 and rye flour type 720 (3:2); WU and WRU, bread from wheat flour type 750 and from mixture of wheat flour type 750 and rye flour type 720 with 20 % addition of acid whey concentrated by ultrafiltration and dehydrated by spray drying; WA, WUA, WRA and WRUA, atherogenic diet with bread W, WR, WU and WRU.

* Fatty acid profile: C16 : 0 (12·1 %); C18 : 0 (4·1 %); C18 : 1 *n*-9 (22·1 %); C18 : 2 *n*-6 (55·4 %); C18 : 3 *n*-3 (5·3 %); C20 : 0 (0·4 %); C22 : 0 (0·5 %).

† Fatty acid profile: C14 : 0 (1·6 %); C16 : 0 (28·1 %); C18 : 0 (14·2 %); C18 : 1 *n*-9 (48·2 %); C18 : 2 *n*-6 (8·9 %); C18 : 3 *n*-3 (0·6 %); C20 : 0 (2·2 %).

Table 2. Body weight gain and diet intake of rats fed the experimental diets (Mean values and standard deviations; *n* 8)

	Initial body weight (g)		Body weight gain (g)		Diet intake (g)		FCR	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Standard diets								
W	243·0	9·0	116·4	19·8	547·6	25·0	4·79	0·64
WU	242·8	9·6	112·2	13·1	543·8	19·2	4·90	0·58
WR	242·4	9·2	109·6	13·0	544·5	22·6	5·04	0·78
WRU	242·9	9·3	104·2	10·7	533·7	20·4	5·16	0·45
ANOVA								
B	NS		NS		NS		NS	
UWC	NS		NS		NS		NS	
B × UWC	NS		NS		NS		NS	
Atherogenic diets								
WA	242·6	10·1	122·2^{A}	8·5	559·5	24·4	4·59	0·35
WUA	242·5	8·7	105·2^{B}	13·9	527·2	24·9	5·08	0·66
WRA	242·3	9·8	105·2^{B}	11·9	521·7	15·2	5·01	0·53
WRUA	242·5	8·7	99·3^{B}	13·5	518·2	20·2	5·23	0·60
ANOVA								
B	NS		***		NS		NS	
UWC	NS		***		NS		NS	
B × UWC	NS		NS		NS		NS	

FCR, feed conversion ratio; W, bread from wheat flour type 750; WR, bread from mixture of wheat flour type 750 and rye flour type 720 (3:2); WU and WRU, bread from wheat flour type 750 and from mixture of wheat flour type 750 and rye flour type 720 with 20 % addition of acid whey concentrated by ultrafiltration and dehydrated by spray drying; WA, WUA, WRA and WRUA, atherogenic diet with bread W, WR, WU and WRU.

^{A,B} Mean values within a column with unlike superscript letters were significantly different ($P < 0·05$) in Duncan's test.

*** Significant effects by kind of bread (B), ultrafiltrated whey concentrate (UWC) or their interactions (B × UWC) at $P < 0·05$.

bread kind and acid whey concentrate used was observed. A significant decrease of body weight gain was noticed for rats fed the bread with 20 % addition of acid whey concentrated by ultrafiltration. It should be noted, however, that diet intake for all diets, standard and atherogenic, was almost the same. The FCR for all diets containing acid whey was higher compared with the control diet with only wheat or wheat–rye bread, but these results were not statistically significant. Tranberg *et al.*[24,25] found that whey reduced early weight gain in high-fat-fed young mice, but they also showed that whey had an acute but not chronic effect on weight gain. For healthy aged rats an increase of whey protein intake was effective in slowing lean body mass loss[26]. Kopeć *et al.*[27] showed that the body weight gain was significantly higher in rats fed bread with sourdough and whey proteins compared with control diets. In turn, Masarwi *et al.*[28] suggested that in growing male rats whey protein led to slower bone growth with reduced weight gain compared with casein protein. Whey protein isolate and a free amino acid mixture simulating the amino acid composition of the whey protein isolate had a similar effect on satiety of normal-weight women as presented by Chungchunlam *et al.*[29]. Wright *et al.*[30] did not find an influence of 9-month whey protein supplementation on bone quantity (bone mineral density or content) in overweight and obese adults.

In the case of the standard diets, there were no differences in the parameters analysed for the small intestine, as the mass and pH of digesta were not affected by the type of bread used (Table 3). However, results obtained for the atherogenic diets showed that the addition of acid whey to wheat bread caused a significant increase in small intestinal mass. In addition, acid whey in the diet significantly decreased the pH of small intestinal digesta in rats fed both types of bread.

Acid whey concentrate after ultrafiltration significantly increased the mass of caecal tissues and digesta in rats fed either the standard or atherogenic diets (Table 3). Control wheat–rye bread in the standard diet significantly decreased the pH of the caecal and colon digesta, whereas in the case of the atherogenic diets a significant decrease in the pH of caecal digesta was found for rats fed diets with acid whey concentrate. Generally, pH of colon digesta was not related to the type of diet used. Only for the WRU diet was a significant increase in colon pH observed compared with the WR diet. The ammonia level in caecal digesta did not change under the influence of acid whey. This effect is beneficial because, as shown by Nousiainen[31], ammonia can destroy cells and reduce villus height and once absorbed must be excreted as urea with the loss of energy. When quark was used as an ingredient of rat diets a significant decrease of caecal pH value and caecal ammonia concentration was observed by Juśkiewicz *et al.*[32]. The acidification of caecal digesta for rats fed the atherogenic diet with acid whey can be considered a beneficial influence of acid whey on fermentative processes in the hind gut[33].

The enzyme activity determined in the caecal digesta is presented in Table 4. Generally, for both types of diets a decrease was found in the activity of all analysed enzymes as affected by acid whey concentrate. This effect was more pronounced for diets with wheat bread than for those with wheat–rye bread. Jurgoński *et al.*[34] found that a decrease in enzyme activity could indicate a reduction in the content of easily fermentable components in the caecum. In the standard diet, acid whey concentrate caused no reduction in β-galactosidase activity, but in the atherogenic diet it suppressed β-galactosidase activity. As shown previously, breads with acid whey are a good source of lactose[12]. According to the literature, lactose that is undigested and unabsorbed in the small

Table 3. Gastrointestinal tract parameters of rats fed the experimental diets
(Mean values and standard deviations; *n* 8)

	Small intestine				Caecum										Colon					
	Full mass (g/100 g BW)		pH		Tissues (g/100 BW)		Digesta (g/100 BW)		pH		DM of digesta (%)		Ammonia (mg/g digesta)		Tissues (g/100 BW)		Digesta (g/100 BW)		pH	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Standard diets																				
W	2·20	0·22	7·13	0·38	$0·30^{c}$	0·04	$0·99^{c}$	0·17	$6·73^{a}$	0·15	$19·75^{a}$	1·53	0·25	0·07	0·33	0·06	$0·29^{b}$	0·08	$6·72^{a}$	0·27
WU	2·24	0·19	6·98	0·23	$0·35^{b}$	0·04	$1·39^{b}$	0·19	$6·59^{a}$	0·22	$17·64^{b}$	1·05	0·25	0·02	0·36	0·03	$0·46^{a}$	0·06	$6·67^{a}$	0·20
WR	2·37	0·15	7·31	0·46	$0·39^{a,b}$	0·05	$1·50^{b}$	0·27	$6·12^{c}$	0·12	$16·03^{c}$	1·39	0·26	0·04	0·35	0·03	$0·44^{a}$	0·17	$6·11^{c}$	0·13
WRU	2·32	0·22	7·29	0·17	$0·43^{a}$	0·04	$1·96^{a}$	0·26	$6·31^{b}$	0·17	$16·56^{b,c}$	1·93	0·25	0·05	0·38	0·05	$0·44^{a}$	0·10	$6·44^{b}$	0·21
ANOVA																				
B	NS		NS		***		***		***		***		NS		NS		NS		***	
UWC	NS		NS		***		***		NS		NS		NS		NS		***		NS	
B × UWC	NS		NS		NS		NS		***		***		NS		NS		NS		***	
Atherogenic diets																				
WA	$2·38^{B}$	0·21	$7·24^{B}$	0·15	$0·37^{C}$	0·04	$1·43^{C}$	0·20	$6·31^{A}$	0·18	$17·95^{A}$	1·32	0·27	0·04	$0·35^{B}$	0·07	$0·42^{B}$	0·08	6·31	0·26
WUA	$2·64^{A}$	0·27	$6·86^{C}$	0·24	$0·53^{A}$	0·08	$2·80^{B}$	0·41	$5·97^{B,C}$	0·28	$16·81^{A,B}$	1·26	0·28	0·07	$0·43^{A}$	0·05	$0·58^{A}$	0·11	6·25	0·31
WRA	$2·65^{A}$	0·17	$7·46^{A}$	0·15	$0·47^{B}$	0·06	$2·65^{B}$	0·59	$6·08^{B}$	0·16	$15·50^{B}$	1·17	0·27	0·03	$0·41^{A}$	0·04	$0·63^{A}$	0·15	6·09	0·17
WRUA	$2·83^{A}$	0·18	$6·94^{C}$	0·28	$0·59^{A}$	0·04	$3·44^{A}$	0·34	$5·84^{C}$	0·21	$15·85^{B}$	2·06	0·28	0·03	$0·39^{A,B}$	0·04	$0·52^{A,B}$	0·12	6·11	0·25
ANOVA																				
B	***		NS		***		***		***		***		NS		NS		NS		NS	
UWC	***		***		***		***		***		NS		NS		NS		NS		NS	
B × UWC	NS		NS		NS		NS		NS		NS		NS		***		***		NS	

BW, body weight; W, bread from wheat flour type 750; WR, bread from mixture of wheat flour type 750 and rye flour type 720 (3:2); WU and WRU, bread from wheat flour type 750 and from mixture of wheat flour type 750 and rye flour type 720 with 20 % addition of acid whey concentrated by ultrafiltration and dehydrated by spray drying; WA, WUA, WRA and WRUA, atherogenic diet with bread W, WR, WU and WRU.

^{A,B,C,a,b,c} Mean values within a column with unlike superscript letters were significantly different ($P < 0·05$) in Duncan's test.

*** Significant effects by kind of bread (B), ultrafiltrated whey concentrate (UWC) or their interactions (B × UWC) at $P < 0·05$.

Table 4. Bacterial enzyme activity in the caecum digesta
(Mean values and standard deviations; *n* 8)

	α-Glucosidase (μmol/min per g of digesta)		β-Glucosidase (μmol/min per g of digesta)		α-Galactosidase (μmol/min per g of digesta)		β-Galactosidase (μmol/min per g of digesta)		β-Glucuronidase (μmol/min per g of digesta)	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Standard diets										
W	15·73[a]	2·91	11·67[a]	4·01	16·41[a]	6·47	39·96	10·63	11·94[a]	4·63
WU	8·06[b]	1·68	6·07[b]	2·04	8·14[b]	1·98	38·35	12·13	3·85[a,b]	1·13
WR	23·84[a]	9·90	7·49[a,b]	1·15	13·36[a]	2·90	34·13	6·37	4·32[a,b]	2·37
WRU	7·44[b]	3·56	6·73[b]	1·13	7·82[b]	3·81	31·62	8·73	3·44[b]	1·32
ANOVA										
B	NS		***		NS		NS		***	
UWC	***		***		***		NS		***	
B × UWC	***		***		NS		NS		***	
Atherogenic diets										
WA	14·63[A]	4·12	8·20[A]	2·15	11·90[A]	2·92	25·94[A]	2·93	3·59[A]	1·25
WUA	6·87[B]	2·04	3·58[C]	1·24	5·18[B]	2·86	13·64[C]	4·54	2·06[B]	1·04
WRA	9·95[A,B]	1·33	6·83[A,B]	1·87	8·02[A,B]	2·77	19·32[B]	5·29	3·29[A]	1·00
WRUA	6·86[B]	2·10	5·34[B,C]	2·96	6·86[B]	1·83	18·86[B,C]	7·53	2·68[A,B]	0·72
ANOVA										
B	***		NS		NS		NS		NS	
UWC	***		***		***		***		***	
B × UWC	***		***		***		***		NS	

W, bread from wheat flour type 750; WR, bread from mixture of wheat flour type 750 and rye flour type 720 (3:2); WU and WRU, bread from wheat flour type 750 and from mixture of wheat flour type 750 and rye flour type 720 with 20 % addition of acid whey concentrated by ultrafiltration and dehydrated by spray drying; WA, WUA, WRA and WRUA, atherogenic diet with bread W, WR, WU and WRU.

[A,B,C,a,b] Mean values within a column with unlike superscript letters were significantly different ($P < 0·05$) in Duncan's test.

*** Significant effects by kind of bread (B), ultrafiltrated whey concentrate (UWC) or their interactions (B × UWC) at $P < 0·05$.

intestine passes into the colon, where in large part it is used as a substrate for microbial anaerobic digestion, and its residues are excreted with faeces[35]. Juśkiewicz *et al.*[32] found an increase in the activity of β-galactosidase in the caecal digesta of rats fed quark, which could indicate improved use of lactose that is not digested in the upper parts of the gastrointestinal tract. The suppressed activity of β-galactosidase after the administration of the atherogenic diet with whey concentrate may indicate a lower content of undigested lactose reaching the caecum. This could confirm the hypothesis that for high-fat diets lactose is used in larger amounts in the upper parts of the gastrointestinal tract[36–38]. Special attention should be paid to the potentially adverse activity of enzymes, such as β-glucuronidase. The level of β-glucuronidase activity is a marker of pathogenic microflora activity leading to metabolic changes in a pro-carcinogenic direction. Bacterial β-glucuronidase is able to release toxins, drugs and steroids, previously bound in the liver with glucuronic acid. A decrease in β-glucuronidase activity caused by the addition of acid whey concentrates to the bread should be considered as a positive effect for consumer health[39].

Generally, acid whey concentrate after ultrafiltration used in the standard rat diet increased the total content of SCFA in caecum digesta (Table 5). In the case of the atherogenic diet, the increase in total SCFA content was statistically significant only for wheat–rye bread with acid whey. Particularly noteworthy is the significant increase of butyric acid content as a result of incorporation of the acid whey concentrate after ultrafiltration. Breads used in this study were a source of lactose[12], which could be a substrate for intestinal microflora. The final bacterial metabolites of lactose fermentation are the SCFA (mainly acetic, propionic and butyric acids) and gas (H, carbon dioxide). Thus, the higher concentration of SCFA observed for the diet with bread with acid whey concentrate after ultrafiltration in relation to the control group could result from increased contents of protein and lactose in the analysed breads, and these components could be used as potential substrates for intestinal microbiota[35,40].

For rats fed the atherogenic diets, the total SCFA concentrations in caecal digesta were only about half of those observed in rats fed the standard diets. Literature data demonstrate that fat enhances the absorption of lactose and carbohydrate by slowing gastric emptying and elongating intestinal transit[36–38,40]. This leads to prolonged contact of lactase with the intestinal contents and with lactose. This could be the reason for the lower total content of SCFA observed for the atherogenic groups compared with the standard groups. Juśkiewicz *et al.*[32] found no changes in total SCFA content in rats fed a diet containing quark compared with the standard diet, and only a significant decrease in propionic acid concentration was observed for that group.

Biochemical indices in blood serum of rats fed the experimental diets are shown in Table 6. A statistically non-significant decrease was observed in serum glucose and TAG levels of rats fed both types of diet with wheat bread fortified by acid whey concentrate. Only the TC concentrations in blood serum of rats fed the atherogenic diets differed significantly. Introduction of acid whey into these diets caused a significant increase of TC. There was no effect of experimental diets on the AI value. Juśkiewicz *et al.*[32] studied the physiological effect of quark produced with or without transglutaminase in growing rats, and reported that quark produced with transglutaminase exerted more favourable modifications to serum cholesterol levels, as well as Ca and P utilisation, compared with quark produced without this enzyme. Formigoni *et al.*[41] found a lowering of plasma glucose concentrations for pigs fed liquid whey. A diet with

Table 5. Concentration and profile of SCFA in the caecum digesta
(Mean values and standard deviations; *n* 8)

	Acetic acid (μmol/g of digesta)		Propionic acid (μmol/g of digesta)		Iso-butyric acid (μmol/g of digesta)		Butyric acid (μmol/g of digesta)		Iso-valeric acid (μmol/g of digesta)		Valeric acid (μmol/g of digesta)		Total SCFA (μmol/g of digesta)		C2 Profile (% of total SCFA)		C3 Profile (% of total SCFA)		C4 Profile (% of total SCFA)	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Standard diets																				
W	51·12^{a}	13·79	16·78	4·59	0·23^{a,b}	0·05	4·55^{b}	0·78	0·20	0·12	0·74^{a}	0·32	73·6^{a,b}	16·1	68·60^{a}	6·86	23·18^{b}	5·01	6·54^{b}	2·27
WU	54·73^{a}	10·42	16·27	2·38	0·27^{a}	0·06	10·69^{a}	2·65	0·28	0·08	0·79^{a}	0·14	83·0^{a}	12·2	65·60^{a,b}	3·95	19·80^{b}	2·86	12·96^{a}	2·77
WR	36·69^{b}	9·04	18·47	2·98	0·15^{c}	0·11	4·37^{b}	2·32	0·21	0·19	0·37^{b}	0·14	60·3^{b}	12·6	60·46^{b}	3·12	31·18^{a}	4·02	7·06^{b}	2·80
WRU	50·62^{a}	11·92	15·50	4·13	0·17^{b,c}	0·05	10·47^{a}	5·03	0·19	0·07	0·48^{b}	0·19	77·4^{a}	15·2	65·05^{a,b}	6·16	20·37^{b}	5·66	13·49^{a}	5·74
ANOVA																				
B	***		NS		***		NS		NS		***		NS		***		***		NS	
UWC	***		NS		NS		***		NS		NS		***		NS		***		***	
B × UWC	NS		NS		NS		NS		NS		NS		NS		NS		***		NS	
Atherogenic diets																				
WA	29·22^{A}	4·69	16·34^{B}	2·57	0·17	0·06	1·65^{A,B}	0·49	0·21	0·12	0·06	0·06	47·6^{A}	5·1	61·09^{A}	5·18	34·44^{B}	5·07	3·53	1·33
WUA	28·94^{A}	7·73	16·74^{B}	4·01	0·13	0·05	1·77^{A}	0·82	0·24	0·11	0·09	0·11	47·9^{A}	9·3	60·06^{A}	7·57	35·38^{B}	7·98	3·59	1·33
WRA	18·93^{B}	4·28	18·04^{A,B}	2·58	0·14	0·04	0·90^{C}	0·85	0·20	0·08	0·04	0·02	38·2^{B}	6·7	49·11^{A,B}	2·86	47·55^{A}	3·94	2·30	2·02
WRUA	24·22^{A,B}	4·52	21·73^{A}	5·07	0·14	0·05	1·07^{B,C}	0·38	0·20	0·08	0·06	0·05	47·4^{A}	8·9	51·15^{B}	2·90	45·54^{A}	3·57	2·41	1·16
ANOVA																				
B	***		***		NS		***		NS		NS		NS		***		***		NS	
UWC	NS		NS		NS		NS		NS		NS		NS		NS		NS		NS	
B × UWC	NS		NS		NS		NS		NS		NS		***		NS		NS		NS	

W, bread from wheat flour type 750; WR, bread from mixture of wheat flour type 750 and rye flour type 720 (3:2); WU and WRU, bread from wheat flour type 750 and from mixture of wheat flour type 750 and rye flour type 720 with 20 % addition of acid whey concentrated by ultrafiltration and dehydrated by spray drying; WA, WUA, WRA and WRUA, atherogenic diet with bread W, WR, WU and WRU.

^{A,B,C,a,b,c} Mean values within a column with unlike superscript letters were significantly different ($P < 0·05$) in Duncan's test.

*** Significant effects by kind of bread (B), ultrafiltrated whey concentrate (UWC) or their interactions (B × UWC) at $P < 0·05$.

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Table 6. Biochemical indices in blood serum of rats fed the experimental diets (Mean values and standard deviations; *n* 8)

	Glucose (mmol/l)		TAG (mmol/l)		TC (mmol/l)		HDL-TC (mmol/l)		HDL-TC (% TC)		AI	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Standard diets												
W	7·36	0·62	1·46	0·27	1·97	0·05	1·05	0·11	53·19	5·87	0·90	0·21
WU	7·29	0·90	1·02	0·26	2·09	0·32	1·04	0·09	50·07	4·31	1·01	0·18
WR	7·08	0·19	1·35	0·32	1·82	0·11	0·98	0·15	53·30	5·49	0·89	0·21
WRU	7·36	0·22	1·06	0·28	1·82	0·36	0·97	0·24	53·39	5·54	0·89	0·17
ANOVA												
B	NS		NS		NS		NS		NS		NS	
UWC	NS		NS		NS		NS		NS		NS	
B × UWC	NS		NS		NS		NS		NS		NS	
Atherogenic diets												
WA	7·80	0·93	1·00	0·23	3·59^{A,B}	0·97	0·96	0·10	28·06	5·08	2·67	0·65
WUA	7·05	0·66	0·81	0·34	3·98^{A}	0·49	1·14	0·32	28·27	6·30	2·70	0·83
WRA	7·01	0·77	0·88	0·23	2·84^{B}	0·38	0·96	0·14	35·02	9·81	2·03	0·73
WRUA	7·74	0·93	0·74	0·18	3·19^{A,B}	0·47	1·03	0·10	32·42	2·26	2·10	0·21
ANOVA												
B	NS		NS		***		NS		NS		NS	
UWC	NS		NS		NS		NS		NS		NS	
B × UWC	NS		NS		NS		NS		NS		NS	

TC, total cholesterol; HDL-TC, HDL fraction of total cholesterol; AI, atherogenic index (TC-HDL/HDL); W, bread from wheat flour type 750; WR, bread from mixture of wheat flour type 750 and rye flour type 720 (3:2); WU and WRU, bread from wheat flour type 750 and from mixture of wheat flour type 750 and rye flour type 720 with 20 % addition of acid whey concentrated by ultrafiltration and dehydrated by spray drying; WA, WUA, WRA and WRUA, atherogenic diet with bread W, WR, WU and WRU.
^{A,B} Mean values within a column with unlike superscript letters were significantly different ($P < 0·05$) in Duncan's test.
*** Significant effects by kind of bread (B), ultrafiltrated whey concentrate (UWC) or their interactions (B × UWC) at $P < 0·05$.

Table 7. Effect of experimental diets on caecum microbiota populations (Mean values and standard deviations; *n* 8)

	Lactobacillus (log CFU/g of digesta)		*Bifidobacterium* (log CFU/g of digesta)		Coliforms (log CFU/g of digesta)		*Enterococcus* (log CFU/g of digesta)	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Standard diets								
W	8·80	0·61	8·54^{c}	0·51	6·73	1·24	6·89	0·13
WU	8·92	0·33	9·61^{a}	0·52	6·55	1·11	6·66	0·34
WR	9·00	0·33	9·08^{b}	0·43	7·21	1·42	7·07	0·49
WRU	8·76	0·33	9·43^{a,b}	0·28	5·81	1·62	6·73	0·35
ANOVA								
B	NS		NS		NS		NS	
UWC	NS		***		NS		NS	
B × UWC	NS		***		NS		NS	
Atherogenic diets								
WA	8·58	0·22	8·55	0·34	7·96	0·54	6·76	0·29
WUA	8·71	0·35	8·77	0·50	8·30	1·09	6·76	0·34
WRA	8·32	0·64	8·45	0·36	8·34	0·60	6·92	0·40
WRUA	8·54	0·79	8·86	0·65	8·63	0·59	6·67	0·49
ANOVA								
B	NS		NS		NS		NS	
UWC	NS		NS		NS		NS	
B × UWC	NS		NS		NS		NS	

CFU, colony-forming units; W, bread from wheat flour type 750; WR, bread from mixture of wheat flour type 750 and rye flour type 720 (3:2); WU and WRU, bread from wheat flour type 750 and from mixture of wheat flour type 750 and rye flour type 720 with 20 % addition of acid whey concentrated by ultrafiltration and dehydrated by spray drying; WA, WUA, WRA and WRUA, atherogenic diet with bread W, WR, WU and WRU.
^{a,b,c} Mean values within a column with unlike superscript letters were significantly different ($P < 0·05$) in Duncan's test.
*** Significant effects by kind of bread (B), ultrafiltrated whey concentrate (UWC) or their interactions (B × UWC) at $P < 0·05$.

whey protein supplements administered to rats reduced liver lipid content but only when lipids were increased with a high-cholesterol diet as presented by Nagaoka *et al.*[42]. In humans, a decrease of postprandial blood glucose levels under the influence of whey protein was observed by Petersen *et al.*[43].

The content of selected groups of bacteria in rats caecum digesta was analysed and the results are presented in Table 7. Acid whey concentrate after ultrafiltration caused favourable, but not statistically confirmed, growth of lactobacilli and bifidobacteria. Only for the standard diet with wheat bread fortified with acid whey was a

statistically significant increase of bifidobacteria observed. The counts of *Enterococcus* and coliforms decreased, insignificantly, for the standard diets with acid whey concentrate. However, for the atherogenic diets with acid whey concentrate, a statistically insignificant increase was found in the count of coliforms. Wronkowska *et al.*[12] showed that wheat and wheat–rye bread supplemented with 20 % acid whey concentrate after ultrafiltration were sources of lactose. Lactose that is undigested and unabsorbed from the small intestine could be used as a substrate for anaerobic microbial fermentation in the colon[35].

A high-fat diet seems to be strongly involved in the phylogenic changes of gut microbiota composition in obese individuals, probably because of the overflow of dietary fat to the distal intestine[44,45]. Costabile *et al.*[46] found a significant increase in bifidobacteria count after *in vitro* fermentation (with human faeces) of bread produced using a sourdough process compared with breads produced with yeasted dough.

Summary

The effect of wheat or wheat–rye breads supplemented with 20 % of acid whey concentrate after ultrafiltration on the physiological response of growing rats was studied. Generally, the acid whey concentrate used in both types of bread had a more positive influence on rats fed the atherogenic diet compared with standard diets. A significant decrease in body weight gain and an increase in small intestinal mass were observed for rats fed the atherogenic diet with wheat bread supplemented by acid whey. For the atherogenic diets with acid whey concentrate, a significant decrease was found in caecal digesta pH. In addition, for the high-fat diet, a significant decrease in the activity of β-glucuronidase caused by the addition of acid whey concentrate should be considered as a positive effect for consumer health. Generally, acid whey concentrate after ultrafiltration increases the total content of caecal digesta SCFA. The significant increase in butyric acid content as a result of incorporation of the acid whey concentrate after ultrafiltration is particularly noteworthy. Advantageous effects of both types of bread with the acid whey concentrate used in the standard diet were confirmed by the increase in the population of bifidobacteria.

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M. S.-Ś. managed the grant and all authors had full access to all the data in the study and take responsibility for the integrity of the data and the accuracy of analyses. M. S.-Ś. and Z. Z. were involved in the conception and design of the study. A. M. designed and performed microbiological analyses. F. J. D. designed and prepared acid whey concentrate. M. W and J. J. were involved in the collection and assembly of data, and the analysis and interpretation of the data. J. J. and M. J. performed the statistical analyses. M. J. was involved in carrying out most of the chemical analyses. Critical revision of the manuscript for important intellectual content was completed by all the authors. This research was used as a part of the PhD thesis of M. J.

The authors declare that there are no conflicts of interest.

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