International Dairy Journal xxx (2012) 1–7

Contents lists available at SciVerse ScienceDirect

International Dairy Journal

journal homepage: www.elsevier.com/locate/idairyj

Physiological effects of the dietary application of quark produced with enzyme transglutaminase as a sole protein source in growing rats

Jerzy Juskiewicz [a,*], Zenon Zdunczyk [a], Krzysztof Bohdziewicz [b], Maria Baranowska [b]

[a] Institute of Animal Reproduction and Food Research of the Polish Academy of Sciences, 10 Tuwima Street, 10-747 Olsztyn, Poland
[b] Chair of Dairy Science and Quality Management, University of Warmia and Mazury, 7 Oczapowskiego Street, 10-719 Olsztyn, Poland

ARTICLE INFO

Article history:
Received 13 February 2012
Received in revised form
18 April 2012
Accepted 23 April 2012

ABSTRACT

A total of 24 rats were assigned to groups fed for 35 days with the following diets characterised by a similar protein content: a control diet with casein as a sole protein source (C group) and two experimental diets with quark (tvarog) produced with or without the use of transglutaminase as a protein source (groups T and S, respectively). The dietary application of quark T produced with transglutaminase was found to significantly ($P < 0.05$ versus S) decrease caecal ammonia and putrefactive short-chain fatty acid concentrations, increase Ca and P utilisation in the body, and reduce the total cholesterol level in the serum. Incorporating whey proteins into the T quark with the aid of transglutaminase more favourably modified the serum cholesterol level, the Ca and P utilisation, and measurements of fermentation in distal sections of the rats' gastrointestinal tract compared with the dietary quark produced without transglutaminase.

1. Introduction

The idea of incorporating whey proteins into cheese is not new (Hinrichs, 2001), and the methods using enzyme transglutaminase (TG, EC 2.3.2.13) have been recognised as one of the promising approaches (Zhu & Tramper, 2008). Protein crosslinking by transglutaminase has been researched extensively with certain proteins, such as casein, gluten, globulin, myosin and soy proteins, but milk whey polymerisation and its incorporation into dairy products has been less studied (Joo, Kim, & Lee, 2011; Zhu & Tramper, 2008). Even less is known about the physiological consequences (learnt from in vivo experiments) of the consumption of dairy products enriched with whey proteins using microbial transglutaminase. Transglutaminase is used to crosslink whey proteins by crosslinking glutamine and lysine residues; it catalyses the acyl transfer reaction and produces ε-(γ-glutamyl) lysine bonds (Eissa, Bisram, & Khan, 2004). It has been reported that this crosslink formation does not reduce the nutritional quality of the food because the lysine residue remains available for digestion (Seguro, Kumazawa, Kuraishi, Sakamoto, & Motoki, 1996). Our recent study showed that transglutaminase application, apart from initial milk preparation, did not require crucial modifications in quark (tvarog) production and resulted in a 10–15% increased yield (more intensive retention of whey proteins was observed) and some sensory and physicochemical changes compared with the control quark (Bohdziewicz, 2010).

The objective of the present study on Wistar rats was to identify the physiological effects of dietary quark produced with enzyme transglutaminase compared with a dietary treatment with quark obtained via the standard technological process. It was hypothesised that the consumption of quark produced using transglutaminase would be associated with some beneficial physiological changes, including intestinal fermentation, mineral utilisation, and blood biochemistry. To verify this hypothesis, a control, casein-based diet and experimental diets, which contained quark produced with or without transglutaminase as a sole dietary protein source, were applied for 35 days in growing rats.

2. Material and methods

2.1. Dairy products and their characteristics

The experimental quarks were manufactured according to the procedure described by Bohdziewicz (2010). During the production process, one batch of the experimental product was obtained using the preparation Saprona-Reactyn GR 100 new (P.M.T. TRADING Co., Lodz, Poland) containing lactose and enzyme transglutaminase (EC 2.3.2.13) of microbiological origin (*Streptoverticillium mobaraense*); the second batch was produced according to the standard procedures from the same dairy material. In brief, pasteurized milk was introduced to processing tanks, and afterwards the enzymatic

0958-6946/$ – see front matter © 2012 Elsevier Ltd. All rights reserved.
doi:10.1016/j.idairyj.2012.04.006

Please cite this article in press as: Juskiewicz, J., et al., Physiological effects of the dietary application of quark produced with enzyme transglutaminase as a sole protein source in growing rats, *International Dairy Journal* (2012), doi:10.1016/j.idairyj.2012.04.006

transglutaminase preparation (100 U g^{-1} protein; detailed data are part of a patent, patent evaluation pending, patent application No. PCT/PL2011/000122) was added. After 1.5–3.0 h from enzymatic preparation addition, the milk was inoculated with starter cultures of mesophilic lactic fermentation bacteria (Flora Danica, Chr. Hansen, Hørsholm, Denmark). The same starter cultures were applied in both quarks. Once the curd had reached pH values of 4.45–4.65, it was slowly heated to a temperature of 36–38 °C and cut. Gentle stirring and heating resulted in the shrinking of quark grain and in the drip of clear whey. The quark bulk was separated once the whey reached acidity of 28–30 °SH. The pre-dehydrated quark bulk was then pressed to dry matter content of ca. 25–30%. Afterwards, the product was formed, cooled and packed. The following chemical analyses were performed on the casein (Lacpol Co., Murowana Goslina, Poland) used to prepare the control diet and the quark samples, according to the Association of Official Analytical Chemists (AOAC, 2005): moisture (g 100 g^{-1}), ash (g 100 g^{-1}), fat (g 100 g^{-1}), and total protein (g 100 g^{-1}). The lactose content was determined according to the Bertrand method as described by Lesniewicz, Wroz, Wojcik, and Zyrnicki (2010). The non-protein nitrogen content was measured according to a Kjeldahl analysis of the trichloroacetic acid soluble nitrogen, and the Ca content by atomic absorption spectrophotometry (Juskiewicz et al., 2009). Amino acid analysis of dietary protein sources (casein and quarks) was performed in Evonik Degussa GmbH (Essen, Germany) by high performance liquid chromatography (HPLC) (Beckman 6300, Beckman Instruments Corp., Palo Alto, CA, USA) after 24 h of hydrolysis with 6 N hydrochloric acid at 120 °C using the methodology recommended by the National Feed Ingredients Association (NFIA, 1991). For sulphur amino acid analysis, a performic acid oxidation treatment was applied to prevent destruction of cysteine. Phenol was added to prevent partial destruction of phenylalanine, histidine, and arginine. The aforementioned analyses were performed in triplicate. The chemical compositions of the dietary protein components are given in Table 1.

Table 1
Composition of protein products used as dietary components for the in vivo experiment.[a]

Component	Casein	Quark S[b]	Quark T[b]
Dry matter (%, w/w)	92.58 ± 0.02	26.63 ± 0.01	27.09 ± 0.01
Protein (%, w/w)	89.70 ± 0.04	17.20 ± 0.01	17.72 ± 0.02
Fat (%, w/w)	0.38 ± 0.00	4.5 ± 0.0	4.3 ± 0.0
Lactose (%, w/w)	Not determined	2.7 ± 0.00	3.0 ± 0.0
Non-protein nitrogen (%, w/w)	Not determined	0.11 ± 0.00	0.10 ± 0.00
Ash (%, w/w)	2.50 ± 0.01	1.02 ± 0.01	0.91 ± 0.00
Amino acid composition (%, w/w, of protein)			
Glycine	1.93 ± 0.02^{B}	2.04 ± 0.02^{A}	2.05 ± 0.01^{A}
Alanine	3.07 ± 0.01^{B}	3.36 ± 0.05^{A}	3.53 ± 0.07^{A}
Valine	6.67 ± 0.08	6.70 ± 0.10	6.67 ± 0.05
Leucine	9.70 ± 0.03^{C}	9.96 ± 0.03^{B}	10.20 ± 0.06^{A}
Isoleucine	5.23 ± 0.03^{B}	5.32 ± 0.01^{A}	5.33 ± 0.01^{A}
Proline	11.60 ± 0.03^{A}	11.20 ± 0.11^{B}	10.50 ± 0.07^{C}
Phenylalanine	5.37 ± 0.03	5.30 ± 0.09	5.25 ± 0.05
Tryptophan	1.30 ± 0.02^{C}	1.38 ± 0.02^{B}	1.46 ± 0.01^{A}
Serine	5.87 ± 0.02^{A}	5.71 ± 0.02^{B}	5.73 ± 0.04^{B}
Threonine	4.34 ± 0.01^{C}	4.40 ± 0.03^{B}	4.47 ± 0.01^{A}
Cysteine	0.37 ± 0.00^{C}	0.55 ± 0.01^{B}	0.69 ± 0.01^{A}
Methionine	3.04 ± 0.00^{A}	2.95 ± 0.02^{B}	2.94 ± 0.00^{B}
Methionine + cysteine	3.42 ± 0.01^{C}	3.50 ± 0.03^{B}	3.63 ± 0.01^{A}
Arginine	3.80 ± 0.06	3.80 ± 0.05	3.78 ± 0.05
Histidine	3.12 ± 0.06	3.03 ± 0.04	2.99 ± 0.06
Lysine	8.15 ± 0.05^{C}	8.36 ± 0.02^{B}	8.47 ± 0.02^{A}
Aspartic acid	7.41 ± 0.18^{B}	7.76 ± 0.14^{AB}	7.99 ± 0.13^{A}
Glutamic acid	22.70 ± 0.52	22.20 ± 0.57	22.00 ± 0.29

[a] Results presented as means ± standard errors ($n = 3$ for amino acid composition; $n = 2$ for remaining data). Mean values within a row with unlike superscript letters were significantly different at $P < 0.05$ (statistical analyses were done for amino acid composition of protein).
[b] The experimental quark produced without (S), or with (T), the preparation containing the enzyme transglutaminase.

2.2. Preparation of diets and animal protocol

The animal protocol used in this study was approved by the Local Institutional Animal Care and Use Committee, and the study was performed in accordance with EU Directive 2010/63/EU for animal experiments. The assessment was conducted on 24 male, Wistar rats aged 35 d and weighing 106.3 g (pooled SEM 0.439). The rats were randomly divided into three groups of eight animals each. All animals were housed individually over 35 days in metabolic cages with free access to water and the experimental diets (Table 2). The selection of the animals and their maintenance over the 35 d experiment followed common regulations. The environment was controlled with a 12 h light–dark cycle, a temperature of 21 ± 1 °C, relative humidity of 50 ± 5%, and twenty air changes per hour. In experimental feeding, two types of diets were used: a complete control diet based on casein as the main protein source (supplemented with 0.15% DL-methionine) and the experimental quark-containing diets composed of a supplementary mixture (Table 2) and the quark homogenate (62 and 38%, w/w, respectively). Due to the high water content in quarks and to prevent unintended fermentation, the quark diets were prepared fresh daily by blending quark homogenate and supplementary mixture together in the proportions mentioned above. This procedure allowed for isonitrogenous diets throughout the study. The control diet used a modification of the AIN-93G diet recommended by the American Institute of Nutrition (Reeves, 1997): the dietary protein level was lowered to ca. 11% of dry matter (DM) enabling for the measurement of the protein utilisation rate to determine animal growth. Each diet, as well as faecal and urinal samples (owing to balance experiment; see below) were analysed in duplicate (for each diet and for each rat) for crude protein (AOAC, 2005), Ca and P content (Jankowski, Zdunczyk, Juskiewicz, & Kwiecinski, 2011). The chemical analyses show that the diets were prepared properly: the crude

Table 2
Composition of the control diet given to the control group and the supplementary mixture given to rats as a part of experimental quark diets.

Diet	%
Control diet	
Casein[a]	11.35
DL-methionine	0.15
Cellulose[b]	5.00
Soyabean oil[c]	8.00
Mineral mix[d]	3.50
Vitamin mix[e]	1.00
Maize starch[f]	71.00
Supplementary mixture[g]	
Cellulose[b]	6.00
Soyabean oil[c]	6.50
Mineral mix[d]	4.20
Vitamin mix[e]	1.20
Maize starch[f]	82.10

[a] Casein preparation (Lacpol Company; Murowana Goslina, Poland), coupled with additional DL-methionine (0.15% of a diet), was a sole protein source in the control group.
[b] Sigma–Aldrich (Poznan, Poland).
[c] Kruszwica SA Co. (Kruszwica, Poland).
[d] AIN-93G- MX (Reeves, 1997), tailored as for similar dietary calcium content in all experimental treatments slightly below the rat calcium requirement for undisturbed growth.
[e] AIN-93-VM (Reeves, 1997).
[f] AVEBE; Veendam, Holland.
[g] The experimental quark diet comprised quark homogenate (62%, w/w) and supplementary mixture (38%, w/w).

Please cite this article in press as: Juskiewicz, J., et al., Physiological effects of the dietary application of quark produced with enzyme transglutaminase as a sole protein source in growing rats, *International Dairy Journal* (2012), doi:10.1016/j.idairyj.2012.04.006

Table 3
Body weight, diet intake and gastrointestinal tract parameters in rats fed the experimental diets.[a]

Parameter[b]	Diet[c]			*P* value
	C	S	T	
Initial BW (g)	106.2 ± 0.8	106.3 ± 0.8	106.3 ± 0.8	0.993
Final BW (g)	244.8 ± 5.7	232.5 ± 6.4	239.8 ± 5.9	0.361
BW gain (g)	138.6 ± 5.9	126.2 ± 6.4	133.5 ± 5.8	0.364
Diet intake (g)	401.2 ± 13.6	428.4 ± 14.4	424.0 ± 10.3	0.301
PER (g g^{-1})	3.02 ± 0.06^{A}	2.50 ± 0.05^{C}	2.72 ± 0.06^{B}	<0.001
Small intestine				
Full mass (g 100 g^{-1} BW)	2.59 ± 0.08^{B}	3.01 ± 0.10^{A}	2.78 ± 0.12^{AB}	0.027
pH of ileal digesta	6.50 ± 0.05	6.43 ± 0.06	6.48 ± 0.07	0.691
Digesta viscosity (mPa s)	1.38 ± 0.08	1.40 ± 0.03	1.35 ± 0.04	0.765
Mucosal disaccharidase (μmol min^{-1} per g of protein)				
Lactase	0.39 ± 0.04	0.50 ± 0.05	0.42 ± 0.04	0.137
Sucrase	1.61 ± 0.10^{B}	2.35 ± 0.23^{A}	1.89 ± 0.18^{AB}	0.026
Maltase	18.9 ± 1.5	20.8 ± 2.2	16.9 ± 2.1	0.388
Aminopeptidase	40.0 ± 2.6	46.9 ± 3.6	40.1 ± 2.3	0.189
Caecum				
Tissue (g 100 g^{-1} BW)	0.22 ± 0.01	0.26 ± 0.01	0.22 ± 0.01	0.093
Digesta (g 100 g^{-1} BW)	0.54 ± 0.06	0.68 ± 0.05	0.56 ± 0.04	0.134
pH of digesta	7.40 ± 0.06^{A}	7.22 ± 0.04^{B}	7.16 ± 0.03^{B}	0.003
Ammonia (mg g^{-1} of digesta)	0.24 ± 0.01^{A}	0.19 ± 0.02^{B}	0.17 ± 0.01^{C}	<0.001
Colon				
Tissue (g 100 g^{-1} BW)	0.43 ± 0.02	0.46 ± 0.03	0.45 ± 0.02	0.608
Digesta (g 100 g^{-1} BW)	0.26 ± 0.02^{C}	0.44 ± 0.02^{A}	0.33 ± 0.02^{B}	<0.001
pH of digesta	6.99 ± 0.12	6.90 ± 0.05	6.81 ± 0.05	0.301

[a] Results are presented as means ± standard errors of 8 determinations. Mean values within a row with unlike superscript letters were significantly different ($P < 0.05$).
[b] BW, body weight; PER, protein efficiency ratio.
[c] C, control (casein as a sole dietary protein source); S, quark produced with the standard technology as a sole dietary protein source; T, quark produced with technology using transglutaminase as a sole dietary protein source.

protein contents of the C (control), S (containing standard quark) and T (containing quark produced with transglutaminase) diets were 11.44%, 11.74%, and 11.56% of DM, respectively; the dietary P levels were 0.23%, 0.24%, and 0.25% of DM, respectively; and the dietary Ca levels were 0.31%, 0.34%, and 0.35%, respectively. According to the nutrient requirements for growing rats (National Research Council, 1995), the recommended levels of dietary Ca and P are 0.5% and 0.3%, respectively. The analysed Ca and P content (slightly below the recommended levels) in the experimental diets was the prerequisite to proceed with the Ca and P utilisation experiment.

All physiological measurements were done for each animal separately ($n = 8$ for each group). During the study, the digestibility and utilisation tests (balance tests) of nitrogen (N), phosphorus (P), and calcium (Ca) were carried out. After a 10 d preliminary period, faeces and urine were thoroughly collected for 5 d from all rats that were kept in balance cages (Tecniplast Spa, Buguggiate, Italy). The content of total N, P, and Ca in diets, faeces and urine collected in the balance period was assayed using the methods described above. Experimental groups were additionally monitored for body-weight gains (body weight was recorded at the beginning and on termination of the study) and diet intake (daily monitoring), which enabled the calculation of the protein efficiency ratio (PER). At the termination of the experiment, the rats were anaesthetised with sodium pentobarbital according to the recommendations for euthanasia of experimental animals. After laparotomy, blood samples were taken from the caudal vena cava, and then small intestine, caecum, and colon were removed and weighed. The rats' right tibias were removed and cleaned of soft tissue. The bones were weighed and kept deep-frozen (−40 °C) for later mineralization. To determine the content Ca and P, the tibias were dried and mineralized in a VELP DK 20 electric aluminium heating block with selectable temperatures (VELP Scientifica, Usmate Velate, Italy). Analytical samples were prepared together with test samples. The analytical samples were mineralized in a mixture (3:1) of nitric acid and perchloric acid (Merck, Darmstadt, Germany). The P and Ca content of mineralizates was determined by colorimetry and flame atomic absorption spectrometry (acetylene-air flame), respectively (Jankowski et al., 2011).

As soon as possible after euthanasia (ca. 10 min), the small intestinal, caecal and colonic pH values were measured (pH meter model 301, Hanna Instruments, Vila do Conde, Portugal). The small intestinal viscosity was determined using a Brookfield LVDV-II+ cone–plate rotational viscometer (CP40; Brookfield Engineering Laboratories Inc., Stoughton, MA, USA) at 37 °C and a shear rate of 60 min^{-1}. Disaccharidase activity (expressed as μmol disaccharide hydrolysed min^{-1} per g of protein) in the jejunal mucosa was analysed by the method of Messer and Dahlqvist (1966) with small modifications as described previously (Juskiewicz et al., 2011a). Aminopeptidase-N (E.C. 3.4.11.2) was assayed using L-alanine-*p*-nitroanilide as a substrate, absorbance was measured at 384 nm, and activity was determined using a *p*-nitroaniline standard curve (Maroux, Louvard, & Baratti, 1973). In the fresh caecal digesta, ammonia (NH_3) was determined by micro-diffusion analysis in Conway's dishes and short-chain fatty, acids (SCFA) were analysed using a gas chromatograph (Shimadzu GC-2010, Kyoto, Japan) equipped with a capillary column (SGE BP21, 30 m × 0.53 mm, SGE Europe Ltd., Kiln Farm Milton Keynes, UK) as described previously (Juskiewicz et al., 2011a). The activity of selected bacterial enzymes (α- and β-glucosidase, α- and β-galactosidase and β-glucuronidase) released into the caecal environment was measured by the rate of *p*- or *o*-nitrophenol release from their nitrophenylglucosides, according to the method described elsewhere (Juskiewicz & Zdunczyk, 2002).

Please cite this article in press as: Juskiewicz, J., et al., Physiological effects of the dietary application of quark produced with enzyme transglutaminase as a sole protein source in growing rats, *International Dairy Journal* (2012), doi:10.1016/j.idairyj.2012.04.006

Serum concentration of calcium, phosphorus, creatinine, urea, glucose, triacylglycerols (TAG), total cholesterol (TC) and its high-density lipoprotein (HDL) fraction were estimated with diagnostic sets from Alpha Diagnostics Limited (Warsaw, Poland).

2.3. Statistical analyses

Results were analysed statistically using one-way analysis of variance (ANOVA), and the significance of differences between groups was determined using Duncan's multiple-range test at a significance level of $P < 0.05$. Data are expressed as mean values (for physiological measurements, $n = 8$ for each group) and standard error of the mean (SEM). Calculations were made with STATISTICA 8.0 (StatSoft Corporation, Krakow, Poland).

3. Results

The quark products applied in the study had no significant effect on either body-weight gain or dietary intake (Table 3). The addition of both test quarks decreased the PER (protein efficiency ratio) value significantly compared with the control group fed a casein-based diet ($P < 0.001$). Moreover, the S treatment was associated with a significantly lower PER value relative to the T group fed a diet containing quark produced with transglutaminase. The experimental treatments with both test quarks had no significant effect on either the ileal pH value or the viscosity rate of small intestine digesta. In the S group, the relative full mass of the small intestine and the mucosal sucrase activity were found to be significantly higher compared with the controls ($P < 0.05$). The 35-day administration of the S and T diets resulted in a significant decrease in the caecal pH value and caecal ammonia concentration compared with the C diet. The lowest caecal ammonia level was observed for the T treatment ($P < 0.05$ versus C and S). The highest and the lowest relative colonic digesta mass were found in the S and C groups, respectively (in both cases $P < 0.05$ versus other treatments).

The caecal bacterial β-glucuronidase activity of control rats was found to be higher than in other groups ($P < 0.05$), and the control rats showed significantly higher bacterial α-galactosidase activity compared with the S group (Table 4). The dietary S treatment was also associated with significantly lower bacterial β-galactosidase activity compared with the C and T groups. The dietary administration of both test quarks did not significantly affect the activity of either bacterial α- or β-glucosidase in the caecum. The total caecal concentration of SCFA was not affected by any of the dietary treatments, which resulted mainly from the unchanged concentrations of acetic and butyric acid. A significant decrease in caecal propionic acid concentration followed the experimental feeding with standard quark (group S) compared with groups C and T ($P = 0.003$), while the caecal concentration of three putrefactive SCFA (PSCFA; iso-butyric, iso-valeric and valeric acids) was significantly reduced following the 35-day dietary treatment with the T quark, compared with the control rats. As a result, the lowest concentration of total caecal PSCFA was found in the T group, while the highest was found in the control group (in both cases $P < 0.05$ versus other treatments). The profile analysis of three major fatty acids showed that the treatment with the quark produced without transglutaminase was characterised by a significantly lower propionic acid:total SCFA ratio when compared with the controls and the group fed a diet containing the S quark.

The addition of both test quarks increased faecal N excretion significantly ($P < 0.05$ versus the other groups) and the highest value of the apparent N digestibility index was noted in the C group (Table 5). The quantity of N excreted with urine was significantly

Table 4
Bacterial enzyme activity, as well as concentration and profile of SCFA[a] in the caecal digesta.[b]

	C[c]	S[c]	T[c]	*P* value
Enzyme activity (μmol min^{-1} per g digesta)				
α-Glucosidase	0.45 ± 0.05	0.43 ± 0.04	0.49 ± 0.02	0.531
β-Glucosidase	0.10 ± 0.01	0.08 ± 0.01	0.10 ± 0.01	0.400
α-Galactosidase	0.29 ± 0.04^{A}	0.21 ± 0.03^{B}	0.27 ± 0.02^{AB}	0.050
β-Galactosidase	1.53 ± 0.09^{A}	1.13 ± 0.15^{B}	1.47 ± 0.07^{A}	0.032
β-Glucuronidase	0.63 ± 0.05^{A}	0.39 ± 0.02^{B}	0.30 ± 0.04^{B}	<0.001
SCFA (μmol g^{-1} digesta)				
Acetic acid	39.1 ± 1.9	38.7 ± 2.0	39.7 ± 1.2	0.932
Propionic acid	8.54 ± 0.38^{A}	6.78 ± 0.38^{B}	8.40 ± 0.30^{A}	0.003
Iso-butyric acid	0.83 ± 0.04^{A}	0.74 ± 0.03^{AB}	0.64 ± 0.04^{B}	0.004
Butyric acid	7.69 ± 0.52	7.11 ± 0.38	9.05 ± 0.62	0.189
Iso-valeric acid	0.71 ± 0.04^{A}	0.61 ± 0.04^{AB}	0.55 ± 0.03^{B}	0.034
Valeric acid	1.18 ± 0.08^{A}	1.02 ± 0.04^{AB}	0.87 ± 0.02^{B}	0.003
Total PSCFA[d]	2.73 ± 0.14^{A}	2.36 ± 0.08^{B}	2.07 ± 0.08^{C}	0.001
Total SCFA	58.1 ± 2.7	56.0 ± 2.5	59.2 ± 1.5	0.615
Profile (% SCFA total)				
Acetic acid	67 ± 1	69 ± 1	67 ± 1	0.185
Propionic acid	15 ± 0^{A}	12 ± 1^{B}	14 ± 1^{A}	0.003
Butyric acid	13 ± 1	15 ± 1	15 ± 1	0.151

[a] SCFA, short-chain fatty acids.
[b] Results are presented as means ± standard errors of 8 determinations. Mean values within a row with unlike superscript letters were significantly different ($P < 0.05$).
[c] C, control (casein as a sole dietary protein source); S, quark produced with the standard technology as a sole dietary protein source; T, quark produced with technology using transglutaminase as a sole dietary protein source.
[d] PSCFA, putrefactive short-chain fatty acids (the sum of iso-butyric, iso-valeric and valeric acids).

Table 5
Nitrogen, calcium and phosphorus excretion patterns, as well as calcium and phosphorus concentration in the femur in rats fed with the experimental diets.[a]

	C[b]	S[b]	T[b]	*P* value
Nitrogen (N)				
N intake (g 5 d^{-1})	1.19 ± 0.05	1.32 ± 0.05	1.24 ± 0.02	0.117
N in faeces (mg 5 d^{-1})	89.1 ± 4.2^{B}	114 ± 4^{A}	111 ± 5^{A}	0.002
N faecal (% N intake)	7.46 ± 0.17^{B}	8.67 ± 0.30^{A}	8.99 ± 0.35^{A}	0.003
N in urine (mg 5 d^{-1})	319 ± 23^{B}	507 ± 20^{A}	462 ± 14^{A}	<0.001
N urinary (% N intake)	26.5 ± 1.1^{B}	38.4 ± 1.2^{A}	37.3 ± 0.9^{A}	<0.001
N digestibility (%)	92.5 ± 0.2^{A}	91.3 ± 0.3^{B}	91.0 ± 0.3^{B}	0.003
N utilisation (%)	66.0 ± 1.1^{A}	53.0 ± 1.3^{B}	53.8 ± 0.8^{B}	<0.001
Calcium (Ca)				
Ca intake (mg 5 d^{-1})	202 ± 8^{B}	240 ± 9^{A}	234 ± 4^{A}	0.004
Ca in faeces (mg 5 d^{-1})	34.0 ± 3.6^{C}	112 ± 7^{A}	83.0 ± 5.4^{B}	<0.001
Ca faecal (% N intake)	16.5 ± 1.2^{C}	46.4 ± 1.9^{A}	35.3 ± 2.0^{B}	<0.001
Ca in urine (mg 5 d^{-1})	1.39 ± 0.29	1.32 ± 0.12	1.74 ± 0.34	0.503
Ca urinary (% N intake)	0.68 ± 0.14	0.55 ± 0.04	0.75 ± 0.15	0.515
Ca digestibility (%)	83.5 ± 1.2^{A}	53.6 ± 1.9^{C}	64.6 ± 2.0^{B}	<0.001
Ca utilisation (%)	82.8 ± 1.3^{A}	53.0 ± 1.9^{C}	63.9 ± 1.9^{B}	<0.001
Phosphorus (P)				
P intake (mg 5 d^{-1})	150 ± 6^{B}	169 ± 7^{A}	167 ± 3^{A}	0.041
P in faeces (mg 5 d^{-1})	27.5 ± 1.8^{C}	76.2 ± 3.5^{A}	57.4 ± 3.2^{B}	<0.001
P faecal (% N intake)	18.3 ± 0.7^{C}	45.0 ± 0.9^{A}	34.2 ± 1.7^{B}	<0.001
P in urine (mg 5 d^{-1})	29.3 ± 1.9^{B}	34.8 ± 2.0^{A}	31.8 ± 1.5^{AB}	0.049
P urinary (% N intake)	19.6 ± 1.2	20.7 ± 1.2	19.0 ± 0.8	0.534
P digestibility (%)	81.7 ± 0.7^{A}	55.0 ± 0.9^{C}	65.8 ± 1.7^{B}	<0.001
P utilisation (%)	62.1 ± 1.6^{A}	34.3 ± 1.2^{C}	46.8 ± 1.9^{B}	<0.001
Femur				
P (% of dry matter)	10.2 ± 0.1	10.0 ± 0.1	10.2 ± 0.2	0.350
Ca (% of dry matter)	22.0 ± 0.3^{A}	21.3 ± 0.2^{B}	21.9 ± 0.1^{A}	0.034

[a] Results are presented as means ± standard errors of 8 determinations. Mean values within a row with unlike superscript letters were significantly different ($P < 0.05$).
[b] C, control (casein as a sole dietary protein source); S, quark produced with the standard technology as a sole dietary protein source; T, quark produced with technology using transglutaminase as a sole dietary protein source.

Please cite this article in press as: Juskiewicz, J., et al., Physiological effects of the dietary application of quark produced with enzyme transglutaminase as a sole protein source in growing rats, *International Dairy Journal* (2012), doi:10.1016/j.idairyj.2012.04.006

Table 6
Biochemical indices of blood serum in rats fed with the experimental diets.[a]

Parameter[b]	C[c]	S[c]	T[c]	*P* value
Creatinine (mg dL^{-1})	0.47 ± 0.02	0.48 ± 0.02	0.43 ± 0.01	0.110
Urea (mg dL^{-1})	23.0 ± 0.6^{B}	27.9 ± 1.0^{A}	26.6 ± 1.2^{A}	0.005
Glucose (mg dL^{-1})	191 ± 5	179 ± 5	177 ± 6	0.166
TAG (mg dL^{-1})	187 ± 10	170.7 ± 5.5	177.0 ± 7.5	0.355
TC (mg dL^{-1})	67.7 ± 2.0^{AB}	73.1 ± 3.6^{A}	64.7 ± 3.3^{B}	0.050
HDL-TC (mg dL^{-1})	48.0 ± 2.2^{AB}	54.2 ± 2.3^{A}	45.9 ± 3.6^{B}	0.048
HDL-TC (% TC)	70.9 ± 2.8	74.1 ± 1.1	70.3 ± 2.3	0.441
P (mg dL^{-1})	5.22 ± 0.14^{B}	5.72 ± 0.12^{A}	6.09 ± 0.20^{A}	0.003
Ca (mg dL^{-1})	9.96 ± 0.08	10.0 ± 0.1	9.92 ± 0.04	0.772

[a] Results are presented as means ± standard errors of 8 determinations. Mean values within a row with unlike superscript letters were significantly different ($P < 0.05$).
[b] TAG, triacylglycerols; TC, total cholesterol; HDL-TC, high-density lipoprotein fraction of total cholesterol; P, phosphorus; Ca, calcium.
[c] C, control (casein as a sole dietary protein source); S, quark produced with the standard technology as a sole dietary protein source; T, quark produced with technology using transglutaminase as a sole dietary protein source.

lower in the control rats than in those fed experimental diets containing quarks. The retention N index, which considers the loss of N in both faeces and urine, was found to be significantly lower in both the S and T groups compared with the control group. The rats fed both test cheese diets excreted significantly higher quantities of faecal calcium and phosphorus within 5 days of the balance study than that recorded in the C group. Moreover, the highest quantities of excreted faecal Ca and P were found in the S treatment. When Ca and P losses in urine were expressed as a percentage of ingested Ca and P, no differences were observed between the C group and the experimental groups. Considering both minerals (Ca and P), the apparent digestibility indices, as well as the retention indices, were lowest in the group fed a diet containing quark produced without transglutaminase and highest in the control treatment group (in all cases $P < 0.05$ versus the other groups). The Ca concentration (expressed as the percentage of dry matter) in the tibia bone was significantly lower in the S treatment group compared with the other groups ($P < 0.05$ versus C and T dietary treatments). The tibia P concentration did not differ among the groups.

The control group was characterised by the lowest serum urea and P concentrations ($P < 0.05$ versus the other groups) (Table 6). The serum concentrations of total cholesterol and HDL-cholesterol declined significantly in the T treatment with respect to the S treatment, which resulted in no differences in the HDL-cholesterol/total cholesterol profile between all treatments.

4. Discussion

To the best of our knowledge, there have been no reports on in vivo experiments evaluating nutritional and health promoting properties of quark products produced with the use of enzyme transglutaminase. As expected, the amino acid composition analyses of the dietary protein sources proved that to some extent whey proteins were incorporated into the quark T (Table 1); compared with the quark S and casein, the quark T was characterised by a higher content of essential amino acids, including leucine, threonine, tryptophan, lysine, and cysteine (and the sum of cysteine and methionine; please note that methionine can be synthesised from cysteine with high efficiency when need demands; McIntosh et al., 1998). It is well known that β-lactoglobulin, the main protein in whey, is a major source of branched-chain amino acids and is composed largely of leucine (approximately 14.5%). Nolles, Verreijen, Koopmanschap, Verstegen, and Schreurs (2009) observed that native or peptide-bound leucine, as opposed to free-form leucine, may be utilised more efficiently. As expected, the rats fed a control diet (casein supplemented with 0.15% methionine as a dietary protein source) exceeded both groups that were fed the quark diets (no supplementation with amino acids) in nitrogen utilisation (Table 5), and the control diet was characterised by the highest protein efficiency ratio (Table 3). Ample animal and human studies provide evidence that even slightly imbalanced dietary proteins enhance nitrogen excretions and disturb new protein synthesis (Ha & Zemel, 2003). In this regard, interesting results were obtained in our study for both experimental quark treatments; although both quark groups did not differ when the N excretion routes were considered (5-day balance experiment), a significantly higher PER value (calculated for the whole duration of the study—35 days) followed experimental feeding with a diet containing the quark produced with transglutaminase (group T) compared with the treatment S (the standard quark). This may be, at least in part, explained by a higher content of leucine in the T quark.

In the present study, the dietary application of test quarks had no negative effect on jejunal disaccharidase activity, which indicates undisturbed absorption of carbohydrates from the upper intestine (Juskiewicz et al., 2011a). The standard quark, but not the T quark, was found to increase the mucosal sucrase activity compared with the control diet. Although these differences were within normal physiological ranges, the effect of the T treatment on sucrase activity (no significant changes to control) is especially desirable if, for instance, the host has problems with increased postprandial glycaemia (Juskiewicz et al., 2008). In the present experiment, however, there were no differences between treatments with respect to blood serum glucose concentrations (Table 6). As for small intestine measurements, there were no significant differences between groups in the digesta viscosity values; enhanced viscosity negatively affects nutrient absorption (Dikeman, Murphy, & Fahey, 2006).

The present experiment demonstrated the beneficial influence of dietary quarks on fermentative processes in the hind gut, as characterised by the acidification of the caecal digesta (Kosmala, Kolodziejczyk, Zdunczyk, Juskiewicz, & Boros, 2011). This pH decline could be explained by significantly lower concentrations of caecal ammonia in the S and T groups. Moreover, with reference to the proposed hypothesis, the application of quark produced with transglutaminase triggered a desirable, additional reduction in caecal ammonia levels compared with the S group. The proper balance between ammonia production and absorption is crucial for maintaining the ammonia concentration in the intestine, which at high levels is detrimental to the intestinal epithelium (Juskiewicz, Zdunczyk, & Frejnagel, 2007). One cannot assume that the higher ammonia concentration in the control group in the present study was caused by an increased amount of undigested dietary protein entering the caecum; ammonia could have also been derived from blood urea, leading to the lowering of blood urea. In fact, the lowest serum urea concentration was found in the control rats (Table 6). Compared with the standard quark diet (group S), more efficient quark protein utilisation in group T was also confirmed by decreased caecal concentration of putrefactive short-chain fatty acids (PSCFA, Table 4). The enhanced production of PSCFA may point to more intensive anaerobic bacterial fermentation of polypeptides and amino acids originating from undigested dietary proteins (Juskiewicz, Zary-Sikorska, Zdunczyk, Krol, & Jurgonski, 2011b). The lowest caecal ammonia and PSCFA concentrations as well as a significantly higher PER value (versus group S) found in the T group support the accepted hypothesis that consumption of quark enriched with whey proteins via the use of transglutaminase provides additional positive effects to the nutritional and health status of the body. The present experiment demonstrated a significant difference between the quark groups (T > S; $P < 0.05$) in propionic acid concentration and the propionate:total SCFA ratio in the

Please cite this article in press as: Juskiewicz, J., et al., Physiological effects of the dietary application of quark produced with enzyme transglutaminase as a sole protein source in growing rats, *International Dairy Journal* (2012), doi:10.1016/j.idairyj.2012.04.006

caecal digesta. Propionate is claimed to be a cholesterol-lowering agent causing impaired acetate utilisation, especially under enhanced faecal losses of steroids (Deminge et al., 1995). The above changes in the caecal SCFA were consistent with the lipid profiles of rats from the T and S groups; the total cholesterol level in the S group was found to be elevated compared with rats fed the T quark. A similar effect of the dietary quarks on HDL-cholesterol fraction was observed, thus the HDL:total cholesterol ratio remained unaffected by the consumed quarks.

With regards to the experimental hypothesis, there was a tendency ($P = 0.091$; T versus S) towards diminished β-glucuronidase activity in the caecal digesta of rats from group T. Moreover, the T treatment did not significantly reduce the activity of glycolytic enzymes α- and β-galactosidase compared with the control animals, as it was observed in the S group. These changes in the caecal bacterial enzymatic activity patterns of the rats from group T could be interpreted as beneficial; decreased levels of the β-glucuronidase activity is of particular importance because this enzyme is associated with intestinal activation of pro-cancer substances (Wroblewska, Juskiewicz, & Wiczkowski, 2011). In this regard, the T quark-fed rats were clearly less subject to the influence of undesired metabolites. The enhanced activity of bacterial β-galactosidase may improve the utilisation of lactose not digested in the small intestine, whereas α-galactosidase may affect the digestion of raffinose-family oligosaccharides (Mikulski et al., 2011). Some authors have reported the beneficial effects of dietary whey protein (lactoferrin; β-lactoglobulin) against the development of colon cancer in young and mature rats, via diminished passage of putative tumour precursors through the hind gut wall (McIntosh et al., 1998). These authors suggest that the mechanism behind the whey proteins' apparent anti-cancer activity might be related to their high sulphur amino acid content.

Compared with the S group, the incorporation of whey proteins into the T quark enhanced Ca and P utilisation, thus supporting the hypothesis of the study. As a result, the rats fed the standard quark diet had lower calcium concentrations in the femur bone, while similar femoral calcium levels were observed among the control and T rats. Hara, Suzuki, Kasai, Aoyama, and Ohta (1999) reported that propionic acid concentration is a more important factor in Ca absorption in the large intestine of rats than other factors, such as soluble Ca and caecal pH; the latter factors may be indirectly correlated with Ca absorption. A study on human volunteers (Trinidad, Wolever, & Thompson, 1999) confirmed that Ca absorption in the distal colon and the rectum is enhanced by acetate and propionate. The results obtained from the S group in the present study also suggest a link between lower production of caecal propionate and decreased Ca utilisation in the body. Increased Ca utilisation observed for the quark produced with transglutaminase treatment may also play an important role in human weight management. Dietary Ca is claimed to play a paramount role in the regulation of energy metabolism and obesity risk (Zemel, 2005). It has been observed that reasonably high levels of dietary Ca may attenuate body fat accumulation and increase fat breakdown during periods of over-consumption of an energy-dense diet in humans. In this case, dairy sources of Ca are claimed to be more effective than supplemental Ca in reducing body fat (Xue, Greenberg, Kraemer, & Zemel, 2001). The components of dairy products, specifically the whey fraction, have been proposed as the active factors for this augmentation (Zemel, 2005).

5. Conclusion

Concerning the present study's experimental hypothesis, the 35-day consumption of quark enriched with whey proteins produced with transglutaminase exerted more favourable modifications to serum cholesterol levels, Ca and P utilisation, and measurements of fermentation in distal sections of the gastrointestinal tract in rats, compared with the dietary treatment with quark produced without transglutaminase. Therefore, quarks could be considered suitable delivery vehicles for dietary whey protein that may benefit human health.

References

AOAC. (2005). *Official methods of analysis* (18th ed.). Washington, DC, USA: Association of Official Analytical Chemists.

Bohdziewicz, K. (2010). The influence of transglutaminase on production process, yield and quality of curd cheeses. *Przeglad Mleczarski, 2*, 4–9, (in Polish).

Deminge, C., Morand, C., Levrat, M. A., Besson, C., Moundras, C., & Remesy, C. (1995). Effect of propionate on fatty acid and cholesterol synthesis and on acetate metabolism in isolated rat hepatocytes. *British Journal of Nutrition, 74*, 209–219.

Dikeman, C. L., Murphy, M. R., & Fahey, G. C. (2006). Dietary fibers affect viscosity of solutions and stimulated human gastric and small intestinal digesta. *Journal of Nutrition, 136*, 913–919.

Eissa, A. S., Bisram, S., & Khan, S. A. (2004). Polymerization and gelation of whey protein isolates at low pH using transglutaminase enzyme. *Journal of Agricultural and Food Chemistry, 52*, 4456–4464.

Ha, E., & Zemel, M. B. (2003). Functional properties of whey, whey components, and essential amino acids: mechanisms underlying health benefits for active people. *Journal of Nutritional Biochemistry, 14*, 251–258.

Hara, H., Suzuki, T., Kasai, T., Aoyama, Y., & Ohta, A. (1999). Ingestion of guar gum hydrolysate, a soluble fiber, increases calcium absorption in totally gastrectomized rats. *Journal of Nutrition, 129*, 39–45.

Hinrichs, J. (2001). Incorporation of whey proteins in cheese. *International Dairy Journal, 11*, 495–503.

Jankowski, J., Zdunczyk, Z., Juskiewicz, J., & Kwiecinski, P. (2011). The effect of different dietary sodium levels on the growth performance of broiler chickens, gastrointestinal function, excreta moisture and tibia mineralization. *Journal of Animal and Feed Sciences, 20*, 93–106.

Joo, S.-I., Kim, J.-E., & Lee, S.-P. (2011). Physicochemical properties of whole soybean curd prepared by microbial transglutaminase. *Food Science and Biotechnology, 20*, 437–444.

Juskiewicz, J., Jankowski, J., Zdunczyk, Z., Lecewicz, A., Przybylska-Gornowicz, B., & Zieba, M. (2009). Effect of diets with different contents of soybean α-galactosides and crude fibre on modification of duodenal microstructure and selected parameters of nutrient utilization in young turkeys. *Polish Journal of Veterinary Sciences, 12*, 455–463.

Juskiewicz, J., Zary-Sikorska, E., Zdunczyk, Z., Krol, B., & Jurgonski, A. (2011b). Physiological effect of chicory preparations with various levels of fructan and polyphenolic fractions in diets for rats. *Archives of Animal Nutrition, 65*, 74–87.

Juskiewicz, J., & Zdunczyk, Z. (2002). Lactulose-induced diarrhoea in rats: effects on caecal development and activities of microbial enzymes. *Comparative Biochemistry and Physiology A, 133*, 411–417.

Juskiewicz, J., Zdunczyk, Z., & Frejnagel, S. (2007). Caecal parameters of rats fed diets supplemented with inulin in exchange for sucrose. *Archives of Animal Nutrition, 61*, 201–210.

Juskiewicz, J., Zdunczyk, Z., Jurgonski, A., Brzuzan, L., Godycka-Klos, I., & Zary-Sikorska, E. (2008). Extract of green tea leaves partially attenuates streptozotocin-induced changes in antioxidant status and gastrointestinal functioning in rats. *Nutrition Research, 28*, 343–349.

Juskiewicz, J., Zdunczyk, Z., Zary-Sikorska, E., Krol, B., Milala, J., & Jurgonski, A. (2011a). Effect of dietary polyphenolic fraction of chicory root, peel, seed and leaf extracts on caecal fermentation and blond parameters in rats fed diets containing prebiotic fructans. *British Journal of Nutrition, 105*, 710–720.

Kosmala, M., Kolodziejczyk, K., Zdunczyk, Z., Juskiewicz, J., & Boros, D. (2011). Chemical composition of natural and polyphenol-free apple pomace and the effect of this dietary ingredient on intestinal fermentation and serum lipid parameters in rats. *Journal of Agricultural and Food Chemistry, 59*, 9177–9185.

Lesniewicz, A., Wroz, A., Wojcik, A., & Zyrnicki, W. (2010). Mineral and nutritional analysis of Polish infant formulas. *Journal of Food Composition and Analysis, 23*, 424–431.

Maroux, S., Louvard, D., & Baratti, J. (1973). The aminopeptidases from hog intestinal brush-border. *Biochimica et Biophysica Acta, 321*, 282–295.

McIntosh, G. H., Royle, P. J., Le Leu, R. K., Regester, G. O., Johnson, M. A., et al. (1998). Whey proteins as functional food ingredients? *International Dairy Journal, 8*, 425–434.

Messer, M., & Dahlqvist, A. (1966). A one-step ultramicro method for the assay of intestinal disaccharidases. *Analytical Biochemistry, 14*, 376–392.

Mikulski, D., Juskiewicz, J., Skowronska, A., Sosnowska, E., Jankowski, J., Lecewicz, A., et al. (2011). Response of the caecal microflora of turkeys fed diets with a different content of high-fibre sunflower meal. *Annals of Animal Science, 11*, 143–155.

National Research Council. (1995). *Nutrient requirements of laboratory animals* (4th ed.). Washington, DC, USA: National Academy Press.

Please cite this article in press as: Juskiewicz, J., et al., Physiological effects of the dietary application of quark produced with enzyme transglutaminase as a sole protein source in growing rats, *International Dairy Journal* (2012), doi:10.1016/j.idairyj.2012.04.006

NFIA. (1991). *Amino acids, special purpose, miscellaneous products*In *Laboratory methods compendium, Vol. 3*. West Des Moines, IA, USA: National Feed Ingredients Association.

Nolles, J. A., Verreijen, A. M., Koopmanschap, R. E., Verstegen, M. W. A., & Schreurs, V. V. A. M. (2009). Postprandial oxidative losses of free and protein-bound amino acids in the diet: interactions and adaptation. *Journal of Animal Physiology and Animal Nutrition, 93*, 431–438.

Reeves, P. G. (1997). Components of the AIN-93 diets as improvements in the AIN-76A diet. *Journal of Nutrition, 127*, S838–S841.

Seguro, K., Kumazawa, Y., Kuraishi, C., Sakamoto, H., & Motoki, M. (1996). The ε-(γ-glutamyl)lysine moiety in crosslinked casein is an available source of lysine for rats. *Journal of Nutrition, 126*, 2557–2562.

Trinidad, T. P., Wolever, T. M. S., & Thompson, L. U. (1999). Effects of calcium concentration, acetate, and propionate on calcium absorption in the human distal colon. *Nutrition, 15*, 529–533.

Wroblewska, M., Juskiewicz, J., & Wiczkowski, W. (2011). Physiological properties of beetroot crisps applied in standard and dyslipidemic diets for rats. *Lipids in Health and Disease, 10*, 178–185.

Xue, B., Greenberg, A. G., Kraemer, F. B., & Zemel, M. B. (2001). Mechanism of intracellular calcium inhibition of lipolysis in human adipocytes. *FASEB Journal, 15*, 2527–2529.

Zemel, M. B. (2005). The role of dairy foods in weight management. *Journal of the American College of Nutrition, 24*, S537–S546.

Zhu, Y., & Tramper, J. (2008). Novel applications for microbial transglutaminase beyond food processing. *Trends in Biotechnology, 26*, 559–565.

Please cite this article in press as: Juskiewicz, J., et al., Physiological effects of the dietary application of quark produced with enzyme transglutaminase as a sole protein source in growing rats, *International Dairy Journal* (2012), doi:10.1016/j.idairyj.2012.04.006