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Physiological Effects of Whey- and Milk-Based Probiotic Yogurt in Rats

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Abstract

In an *in vitro* experiment commercially available probiotic products were tested for the survival of bacteria under conditions of simulated human digestion either when used alone or mixed into yogurt. In the *in vivo* experiment the effects of feeding a whey- and milk-based yogurt prepared with the probiotic strain showing adequate survival in the *in vitro* experiment, was measured on body weight, feed consumption and immune response of rats (IgG and IgA level after immunisation), on the composition and volatile fatty acid production of the intestinal microbiota and on the structure of intestinal villi. The *Lactobacillus acidophilus* (LA-15) strain had inadequate surviving ability in rats. *Bifidobacterium animalis* ssp. *lactis* (BB-12) improved the composition of the intestinal microflora, whereas whey-containing product had a mild immunostimulating effect and exerted a favourable influence on the morphology of intestinal villi. The consumption of yogurts increased the depth of crypts in the ileum, which resulted in enhanced secretion and thus softer faeces.

Key words: *Bifidobacterium animalis*, *Lactobacillus acidophilus,* rat intestine physiology, physiological effect of whey and yogurt

Introduction

Probiotics are live microorganisms which, when administered in sufficient quantities, exert a favourable effect on health status (FAO/WHO, 2002). Numerous bacterial species have been suggested to have probiotic effects, but lactic acid producing bacteria (*Lactobacillus*, *Bifidobacterium*, *Streptococcus*), which occur in the body naturally as constituents of the healthy gut microbiota, have gained the widest use in the practice. Some supposed and confirmed effects through which probiotic microbes exert their favourable effects in the intestine are: supporting the mucosal barrier function of the gut, production of antimicrobial substances (*e.g.* bacteriocins), pH reduction, colonisation of the surface of the intestinal epithelium (competitive exclusion), immunostimulatory effects, anti-inflammatory properties (Kumar *et al*., 2015). The proportion of fermented dairy products is increasing steadily on the market of probiotic foods (Özer and Kirmaci, 2010). Commercially available dairy products often contain specific strains of the genera *Lactobacillus* and *Bifidobacterium* that meet the requirements listed above (Gueimonde *et al*., 2004).

Environmentally conscious production is important during milk processing. The disposal of by-products arising in large quantities (including whey, which is the portion of milk that is left behind after removing the casein protein) raises environmental concerns (Chatzipaschali and Stamatis, 2012).

Earlier, whey was used only for animal nutrition, but it can be added to dairy products at a rate of 30–75% without reducing the enjoyment value of the products (Castro *et al.* 2012). The consumption of whey-based products is increasing all over the world (Boynton and Novakovic, 2014). This by-product, which makes up 85–95% of milk, contains valuable substances as it includes 55% of the nutrient content of milk: lactose (45–50 g/l), soluble proteins (6–8 g/l), fats (4–5 g/l), and minerals (8–10% of the dry extract) (Farizoglu *et al*., 2004).

The objective of this series of experiments was to select effective probiotic strains for the development of a whey-based dairy product. In addition, we compared the physiological effects of whey- and milkbased products as well as of probiotic and non-probiotic products in rats.

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Experimental

Materials and Methods

***In vitro* experiment.** Cultures containing *Bifidobacterium animalis* ssp. *lactis*, or *Lactobacillus acidophilus*, or combination of probiotic strains (*Lactobacillus delbrueckii* ssp. *bulgaricus* and *Streptococcus thermophilus*) were tested. A 10 ml suspension from each of the probiotic strains was prepared and the initial germ count was adjusted to 10^8 colony forming units (CFU)/ml. The survival of the probiotic strains was tested in their original condition and subsequently after mixing them into a yogurt product.

Pasteurized raw material (milk or a 50:50% mixture of milk and whey) was inoculated with the yogurt culture (0.3%) and the propionic strain. Maturation of the product was done at a temperature of 39–42°C and a pH of 4.6–4.8. After the end of the maturation process the yogurts were placed into a blast chiller for 10–16 hours, and subsequently stored in a room of less than 10°C temperature until delivery.

The *in vitro* simulated human digestion process was modelled according to the method of Versantvoort *et al.* (2005).

Study of the physiological effects of milk- and whey-based yogurt made with the selected probiotic strains in rats. Three yogurt products were studied: amilk-based probiotic (MP), a milk-based non-probiotic product (M), and a probiotic one made from the 1:1 mixture of raw milk and whey (WP). The milk was pasteurized at 85–90°C, the fat content was adjusted to 1.5% and then the raw material was cooled down to 40–42°C. The yogurt cultures were added at that temperature (FVV121 culture, 0.01%; *L. acidophilus* culture, 0.01%) during gentle mixing for 5 minutes. Warm maturation was conducted at 38–42°C for 4–5 hours, until the pH reached a value of 4.4. This was followed by cold maturation for 24 hours, during which the temperature of the yogurt was reduced below 10°C.

The probiotic product was made from two commercially available products containing *B. animalis* ssp. *lactis* (BB-12) and *L. acidophilus* (LA-15) strains, respectively.

The yogurts were stored in a refrigerator at +4°C temperature, and their germ counts were checked weekly.

A total of 40 mature male rats (Wistar Crl: WI BR, SPF, Budapest) were housed in individual cages (Techniplast, Buguggiate, Italy) and fed a commercially available rat diet (S8106-S011, ssniff Spezialdiäten GmbH, Soest, Germany) *ad libitum*. Three groups of animals (M, MP, WP, n = 8/group) received yogurt in addition to the rat diet, while the control animals (C, n = 16) were fed only the rat diet throughout the experiment. The rats fed yogurt were offered rat food *ad libitum* during the day, then at 08:00 p.m. the feeders and drinkers were closed down. A 10 ml volume of yogurt was filled into the drinkers and offered to the rats between 08:00 and 09:00 a.m., during which time the control rats were offered water.

On days 1 and 14 of the experiment the rats were inoculated intraperitoneally with 100 µg ovalbumin (OVA; Sigma-Aldrich, Hungary) per animal. Blood samples were taken before vaccination (on day 0), and subsequently on days 14 and 28. Half of the control rats were immunized (Group CI, n = 8), while the other 8 control rats did not receive immunization (Group C, n = 8).

The rats were weighed weekly. Daily feed and yogurt consumption was recorded individually.

At the end of the experiment (on day 28) the rats were decapitated and bled after narcosis with carbon dioxide. Samples were taken from the small intestine and the caecum.

The experimental protocol was authorized by the Food Chain Safety and Animal Health Directorate of the Somogy County Agricultural Office, under permission number RH/162-2/2013.

Parameters examined. Immunoglobulin G (IgG) and immunoglobulin A (IgA) were determined from the blood plasma and the saliva, respectively, on days 0, 14 and 28. For specific IgG detection, microtiter plates (Sigma-Aldrich, Hungary) were coated with 100 µl of OVA solution per each well. Aliquots of 100 µl of two dilutions (1:50 and 1:100) were transferred to the microtiter plate and incubated for 1 hour at room temperature. Wells were washed 3 times with PBS-Tween, and then anti-rat IgG-HRP conjugate (Sigma-Aldrich, 1:10,000) was added to each well, followed by incubation at room temperature for 1 hour. Total secretory IgA (sIgA) from the saliva was measured using a quantitative Rat IgA ELISA kit (Kamiya Biomedical Co., USA) as described by the manufacturer.

From 1 g of small intestinal and caecal content, serial dilutions with 0.9% sterile saline solution were made immediately after sampling and used for microbiological determination. Media used and conditions of incubation are summarised in Table I. Incubation was done under aerobic (Memmert 108 incubator, Memmert, Schwabach, Germany) or anaerobic conditions (Anaerob Jar, Merck, Darmstadt, Germany). Results were expressed in log10 CFU related to 1 g of sample.

If the probiotic strains could be detected from the small intestinal and caecal chyme pure cultures were obtained from the *Lactobacillus* and *Bifidobacterium* cultures by a single passage on the surface of the above-mentioned selective media and studied by molecular genetic tools. Partial sequences of the 16S rRNA coding gene of colonies picked up from pure cultures were determined by the use of universal bacterial primers (M13F-27F: TGTAAAACGACGGC-

Table I
Microbiological cultivation methods

Bacteria	Media	Conditions of incubation
In vitro experiment		
B. animalis ssp. *lactis*	TOS-propionate agar medium[1] supplemented with lithium-mupirocin	37°C, 72 h, anaerobic
L. acidophilus	MRS agar	35°C, 72 h, anaerobic
L. delbrueckii ssp. *bulgaricus*	MRS agar	35°C, 72 h, anaerobic
Streptococcus thermophilus	M-17 agar supplemented with 10% lactose solution[1]	42°C, 24 h, anaerobic
In vivo experiment		
Total aerobic bacteria	blood agar	30°C, 72 h, aerobic
Total anaerobic bacteria	blood agar	37°C, 48 h, anaerobic
E. coli	Chromocult differentiation medium[2]	37°C, 24 h, aerobic
Bifidobacterium	TOS-propionate agar medium[1]	37°C, 72 h, anaerobic
Bacteroides	Schaedler's agar[3] supplemented with esculin[2], neomycin[2] and iron ammonium citrate[3]	37°C, 96 h, anaerobic
Lactobacillus sp.	MRS agar[3]	37°C, 72 h, anaerobic

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CAGTCAGTC-AGAGTTTGATYMTGGCTCAG and M13R-338R: CAGGAAACAGCTATGACCCAT-GCTGCCTCC CGTAGGAGT). The 5' ends of the universal bacterial primers had M13 sequences to facilitate the sequencing reaction described later. A colony was directly used in a PCR reaction. After amplification (96°C, 5 min; 35 cycles of 94°C 30 sec, 62°C 45 sec, 68°C 45 sec followed by 72°C 2 min), the PCR product was checked on 4% MetaPhor™ (Lonza, USA) agarose gel (6 min 6 V cm^{-1}, 8 min 9 V cm^{-1}). Successful PCR reactions were treated with ExoSAP-IT™ (USB, USA) at 37°C for 15 min and at 80°C for 15 min to inactivate unconsumed dNTPs, primers and ExoSAP itself. This mixture was directly used in the sequencing reaction (Big Dye Direct Sequencing kit, Life Technologies, USA). Conditions of the sequencing reaction were: 37°C 15 min, 80°C 2 min, 96°C 1 min; 25 cycles 96°C 10 sec, 50°C 5 sec, 60°C 4 min. Primers were complements of M13 sequences presented on the 5' regions of the previously described M13F-27F and M13R-338R primers. The product of the sequencing reaction was purified by BigDye XTerminator™ (Life Technologies, USA). The sequence was determined on an ABI-3500 fragment analyser. Sequences were identified on the basis of a public 16S ribosomal RNA sequence database (http://blast.ncbi.nlm.nih.gov/Blast.cgi?PROGRAM=blastn&BLAST_SPEC=WGS&BLAST_PROGRAMS=megaBlast&PAGE_TYPE=BlastSearch).

The concentration of volatile fatty acids (VFA) was measured by gas chromatography (Shimadzu GC 2010, Japan; Nukol 30 m × 0.25 mm × 0.25 µm capillary column, Supelco, Bellefonte, PA, USA; FID detector, 1:50 split ratio, 1 µl injected volume, helium 0.84 ml /min^{-1}). Detector conditions: air 400 ml/min, hydrogen 47 ml/min, temperature: injector 250°C, detector 250°C, column 150°C). 2-ethyl-butyrate (FLUKA Chemie GmbH, Buchs, Switzerland) was used as internal standard.

Samples for histological examination were taken from the duodenum (1 cm distal to the pylorus), the proximal part of the jejunum and the ileum (1 cm proximal to the ileocaecal valve opening). The samples were fixed in 10% buffered formaldehyde solution then embedded in paraffin and stained with haematoxylin and eosin. The general condition of the intestinal mucosa, the epithelial layer covering the intestinal villi, the structure of the *stratum villosum*, quantitative and qualitative composition of the cells were evaluated. The cytomorphology of the gut-associated lymphoid tissue (GALT) in the ileum was also examined. The length of the intestinal villi and the depth of the intestinal glands (crypts) were measured by a histometric method (using an ocular micrometer).

The effect of treatment or treatment and age was determined by one-way and multi-factor analysis of variance, respectively (SPSS 10.0, 2002). The significance of between-group differences was tested by the LSD *post-hoc* test.

Results and Discussion

Survival of the probiotic strains after *in vitro* simulated digestion. Probiotic bacterial strains could be cultured in a germ count of 10^6–10^7 magnitude, with the *B. animalis* ssp. *lactis* strains having the highest

initial germ count. After *in vitro* digestion the colony-forming unit counts of the *B. animalis* ssp. *lactis* bacteria decreased by 4 and 5 logs. The *L. acidophilus*, *L. delbrueckii* ssp. *bulgaricus* and *S. thermophilus* strains survived the conditions of simulated digestion in very low numbers (only 10^1 CFU/ml).

In the yogurt the initial germ count of probiotic strains was 10^8–10^9, during the digestion the germ counts decreased by 2–6 logs.

Selection and testing of the appropriate probiotic strains require extreme circumspection. These bacteria have to survive the bactericidal effects occurring in the stomach and small intestine (gastric acid, pH, bile salts, proteolytic enzymes) in order to colonize the small or the large intestine. For this reason, it is not enough to test whether the probiotic strain can be cultured from a given product, since – as shown by our results – the germ count can decrease significantly even after an *in vitro* simulated digestion. The type and chemical composition of the food raw material selected for the production of probiotic products have a decisive influence on colonization of the gut by these bacteria. Yogurt has proved to be an ideal material (Ranadheera *et al*., 2010). This has been demonstrated also by our experiment, as the probiotic strains mixed in yogurt survived *in vitro* digestion in germ counts of 10^3–10^6, whereas their survival in their original condition was minimal: lactobacilli and bifidobacteria could be cultured only in germ counts as low as 10 to 1000.

Probiotic microbial strains used in combination may exert their beneficial effect more effectively than a single strain used alone (Timmerman *et al*., 2004). This effect probably occurs with bacteria capable of complementing the metabolism of one another, and is manifested also in the higher germ count of bacteria surviving digestion. In this experiment, in the yogurt product containing both the *Lactobacillus delbrueckii* ssp. *bulgaricus* and the *Streptococcus thermophilus* strains the bacteria survived the digestion procedure only in a germ count of 10^3–10^4.

Despite the fact that some strains of the genus *Bifidobacterium* can survive in yogurt for a short period of time (Roy, 2005), *Bifidobacterium animalis* ssp. *lactis* was the bacterium that survived the simulated human digestion procedure in the highest germ count (5.3×10^7). As we obtained favourable survival results (10^6 CFU/ml) for *L. acidophilus* strains as well, we decided on the combined use of *L. acidophilus* and *B. animalis* ssp. *lactis* strains in the rat experiment. Strains belonging to the genera *Lactobacillus* and *Bifidobacterium* are often included in commercially available dairy products (Masco *et al*., 2005).

Effects of the milk- and whey-based yogurt prepared with the selected probiotic strains in rats. The germ count of probiotic yogurts was 4×10^8 ml^{-1} for

Table II

Body weight (g), solid feed (g) and yogurt (ml) consumption of the animals (means ±SD)

Parameter	Group[1]				Date				P		
	CI	WP	MP	M	week 1	week 2	week 3	week 4	group	date	group × date
Body weight	398 ± 23	410 ± 39	411 ± 38	403 ± 38	365 ± 16^{A}	391±21^{B}	436 ± 25^{C}	445 ± 28^{C}	NS	< 0.05	NS
Solid feed consumption	27.8 ± 4.6	28.6 ± 4.9	28.6 ± 5.2	28.8 ± 4.9	28.0 ± 4.9	30.0 ± 6.1	26.4 ± 5.4	28.0 ± 4.1	NS	NS	NS
Yogurt consumption	0	5.8 ± 3.9^{b}	4.1 ± 3.0^{a}	4.2 ± 3.6^{a}	4.3 ± 2.9^{A}	5.0 ± 3.9^{AB}	5.3 ± 3.9^{B}	5.1 ± 3.4^{AB}	< 0.05	< 0.05	NS
Total consumption2	28.3 ± 4.4^{a}	34.0 ± 6.3^{b}	32.9 ± 5.8^{b}	32.8 ± 6^{b}	30.9 ± 6.1	33.0 ± 7.2	30.2 ± 7	31.4 ± 6.3	< 0.05	NS	NS

Different indices mean significant difference between ^{a,b} groups and ^{A,B,C} dates.

[1] n = 8 in each group

[2] total consumption means solid feed + yogurt intake (g)

L. acidophilus and 1.6×10^8 for *B. animalis* ssp. *lactis*, and storage at +4°C did not decrease the viability of probiotic bacteria. The rats drank about 3 – 8 ml of yogurt each, which represented an average daily yogurt intake of 2 dl for a human of average body weight (60 kg). The result of testing under *in vitro* conditions does not necessarily mean the survival of the probiotic strain in the living organism. In our animal experiment from the small and large intestinal samples of rats of groups WP and MP the *B. animalis* ssp. *lactis* (YIT4121) strain administered as a probiotic could be identified, whereas the *L. acidophilus* (ATCC 700396) strain also used as a probiotic could not be detected by molecular genetic methods. The poor survival of lactobacilli was indicated by the bacterial counts cultured from the small and the large intestine, which did not differ significantly according to whether probiotic (WP, MP) or non-probiotic (M) yogurt was fed (see later). Survival may be different under *in vitro* and *in vivo* conditions, as in the living organism the survival of bacteria is influenced also by factors as the interaction between the probiotic strains and the natural intestinal microbiota, the antimicrobial substances produced by microbes, the motility of the intestine and the local immune response (Holzapfel and Schillinger, 2002).

As the results suggested that probiotic lactobacilli were not present in the small and large intestine in sufficient numbers to exert such effects, the physiological effects found in the experiment can be attributed to bifidobacteria.

Body weight, diet and yogurt drink consumption. The body weight of the experimental animals increased approximately by 20% during the four weeks of the experiment, and there was no significant difference between the groups (Table II). Although the rats consuming the probiotic product weighed about 10 g more, the difference was not statistically significant. Yogurt consumption increased by the second week and remained roughly on the same level subsequently. The whey-containing probiotic yogurt was consumed by the rats in the highest quantity. Adding up the average daily feed and yogurt consumption of the rats, it can be seen that this was substantially higher than the feed consumption of the control rats, which means that the experimental rats consumed the yogurt in addition to the feed that they ate in the same amount as the control rats.

Immune response. There was no difference between the treatments in specific IgG level at the end of the experiment (Fig. 1A). It can be seen, however, that in rats fed the whey-containing probiotic product the IgG level increased faster and was significantly higher than in the rats fed the non-probiotic milk-based yogurt. Orally ingested whey had a more expressed beneficial effect on the local immune response as sIgA concentration of the saliva on day 14 was significantly higher in WP rats than in rats of all the other groups (Fig. 1B). Enzymatic degradation of the whey proteins results in the formation of numerous peptides of immunomodulatory effect, which have been shown to increase the concentration of specific IgG and intestinal anti-CT IgA after immunization of mice (Gauthier *et al*., 2006). In the present experiment, although the concentration of the tested antibodies increased, the consumption of neither milk nor whey had a detectable influence on the quantitative and qualitative composition of the lymphoid tissue (GALT) of the intestinal mucosa.

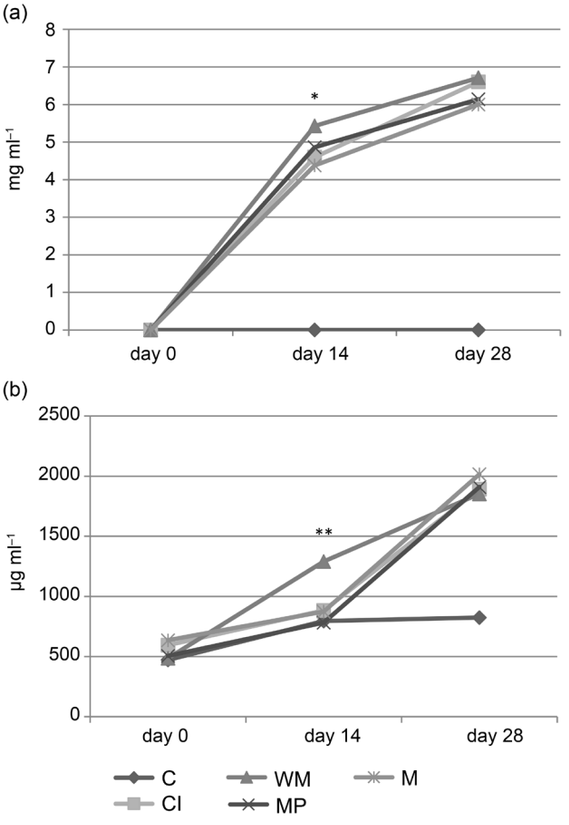

Fig. 1A. Level of OVA-specific IgG measured in the blood plasma and Fig. 1B. sIgA measured in the saliva 14 days after the first (on day 0) and second (day 14) immunisation

*WP significantly different from M

*WP significantly different from all other groups

Microbiota and fermentation. One of the main beneficial digestive-physiological effects of probiotics is stabilization of intestinal eubiosis. As had been expected, in our experiment the probiotic dairy products (WP and MP) significantly increased the counts of bifidobacteria in the large intestine, while in the small intestine a significant effect was exerted only by the milk-based yogurt (MP) (Table III). In addition to the also important Bacteroidetes (usually making up about 10–50% of the flora) and Firmicutes strains (accounting

Table III
Composition of the small intestinal and caecal microbiota (log_{10} CFU[1]/g), pH, dry matter and VFA content of the caecal chyme (means ± SD)

	CI	WP	MP	M
Small intestine				
Aerobic bacteria	7.92 ± 0.56	8.05 ± 0.09	8.04 ± 0.19	8.07 ± 0.45
Anaerobic bacteria	8.69 ± 0.33	9.05 ± 0.15	8.78 ± 0.20	8.87 ± 0.41
E. coli	4.99 ± 0.66	5.77 ± 0.66	4.82 ± 0.35	5.25 ± 0.56
Bifidobacterium sp.	3.31 ± 0.47^{a}	4.38 ± 0.59^{ab}	4.74 ± 0.40^{b}	4.38 ± 0.64^{ab}
Lactobacillus sp.	8.35 ± 0.16	8.56 ± 0.13	8.28 ± 0.18	8.31 ± 0.24
Ceacum				
pH value	6.35 ± 0.20	6.4 ± 0.1	6.24 ± 0.1	6.5 ± 0.2
Dry matter %	24.1 ± 2.2^{b}	22.4 ± 1.4^{ab}	21.9 ± 0.9^{ab}	21.0 ± 1.5^{a}
Aerobic bacteria	9.07 ± 0.21	9.58 ± 0.21	9.20 ± 0.36	9.21 ± 0.42
Anaerobic bacteria	9.78 ± 0.06^{a}	10.4 ± 0.13^{b}	10.2 ± 0.08^{b}	10.2 ± 0.31^{b}
E. coli	6.66 ± 0.45	6.67 ± 0.39	6.66 ± 0.33	7.24 ± 0.52
Bifidobacterium sp.	5.61 ± 0.21^{a}	7.40 ± 0.41^{b}	7.21 ± 0.66^{a}	6.38 ± 0.97^{ab}
Bacteroides sp.	8.51 ± 0.54	8.82 ± 0.31	8.47 ± 0.54	8.76 ± 0.53
Lactobacillus sp.	8.89 ± 0.16^{a}	9.34 ± 0.21^{b}	9.37 ± 0.22^{b}	9.28 ± 0.25^{b}
Total VFA (mmol/kg)	87.7 ± 26.1	96.9 ± 28.0	78.5 ± 17.1	98.6 ± 37.6
Acetic acid (%)	57.1 ± 1.2	61.1 ± 5.8	56.5 ± 5.4	64.1 ± 3.2
Propionic acid (%)	10.1 ± 1.2^{b}	11.6 ± 2.8^{bc}	13.0 ± 1.4^{c}	6.2 ± 0.2^{a}
Butyric acid (%)	32.6 ± 2.1	27.3 ± 3.9	30.4 ± 1.8	29.1 ± 3.5

n = 8/group; [1] CFU = colony forming unit; [a, b, c] significant difference between groups ($P < 0.05$)

for as much as 75%), *Bifidobacterium* strains constitute about 10% of the large intestinal microflora. These microorganisms have numerous favourable physiological effects; their exopolysaccharides facilitate the survival of bacteria in the gastrointestinal tract, they favourably influence the composition of the microbiota and the metabolic processes of microorganisms, and may have a beneficial effect on the immune response (Scott *et al.,* 2014). The dairy products did not have an effect on the counts of lactobacilli in the small intestine, while they increased it in the large intestine, irrespective of the probiotic *Bifidobacterium* content. Milk (M) increased the propagation of *E. coli* in the large intestine ($P > 0.05$), which was decreased by the probiotic (WP, MP), and thus in these animals an *E. coli* count similar to that found in the control rats not consuming the dairy product was demonstrated. Because of their easily fermentable lactose content, milk and whey serve as ideal substrates for lactobacilli and *E. coli*, while the other bifidobacteria and lactobacilli exert a competitive antagonistic effect on *E. coli*.

Yogurt-fed rats had slightly softer faeces containing less dry matter, but the difference from the control was statistically significant only for rats of group M (Table II). Within the total quantity of the volatile fatty acids (VFA), the proportion of propionic acid was the lowest in group M and the highest in group MP.

Bifidobacteria produce acetic acid and lactic acid in a ratio of 3:2; however, the increase in their colony-forming unit counts as compared to the control did not have a notable effect on the quantity and proportion of the volatile fatty acids produced. Only the non-probiotic milk-based yogurt resulted in a low propionic acid production. The lower water content of the intestinal content in the yogurt-consuming animals (7–15% as compared to the control) is consistent with the results obtained by other researchers: the feeding of a lactobacillus-containing probiotic increased the water content of the faeces by 7–20% in rats (Wang *et al*., 2009). In the present experiment, this could be observed also in the rats fed a non-probiotic yogurt (group M), which is possibly attributable to the fact that the number of colony-forming lactobacilli was significantly higher in these animals as well. Increased water secretion may also be attributable to the increased depth of crypts between the intestinal villi (see later), as these results in an increase in the number of secretory cells located in the crypts, leading to enhanced electrolyte and water excretion (Nabuurs and Hoogendoorn, 1993). Despite the laxative effect, the rats did not show signs of diarrhoea.

Histology. The intestinal mucosa of rats receiving different treatments showed a normal histological picture characteristic of the species and the age, with no

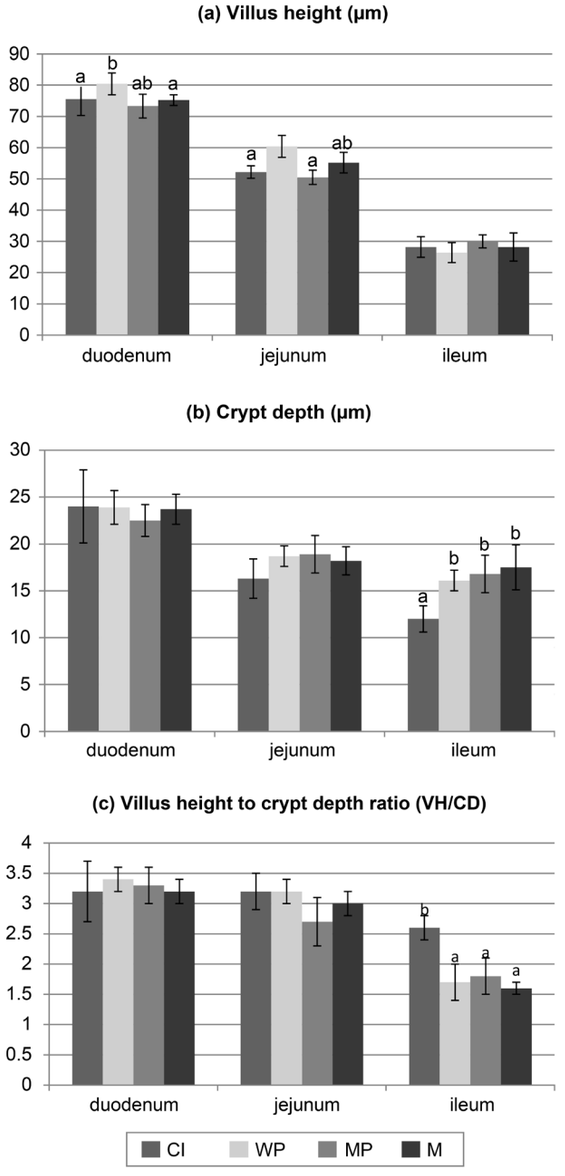

Fig. 2A. Villus height (μm), Fig. 2B. crypt depth (μm) and Fig. 2C. their ratio (VH/CD) along the small intestine.
[a, b] different indices mean significant difference between groups ($P < 0.05$)

difference in the quantitative and qualitative composition of the lymphoid tissue of the intestinal mucosa (GALT). The intestinal villi of the duodenum and jejunum were higher in group WP as compared to the other three treatments, but this did not cause a significant deviation in the calculated villus height/crypt depth (VH/CD) value (Fig. 2). The crypts in the ileum of control rats were significantly shallower, which resulted in a significantly higher VH/CD value as compared to the rats consuming yogurt.

Presumably owing to the bioactive peptides contained by it, milk whey resulted in higher intestinal villi in the duodenum and jejunum. This is indicative of a more intensive proliferation or a slower apoptosis, and results in a larger absorptive surface and higher brush border enzyme activity (Pluske *et al.*, 1996). In weaned piglets challenged with lipopolysaccharide, Xiao *et al.* (2016) studied the effect of a whey protein concentrate (WPC) on intestinal integrity, and found a greater intestinal VH and, in association with the former, a better intestinal barrier function.

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Conflict of interest

The authors declare that they have no conflict of interest.

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