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The effect of pre- and probiotics on the colonic ammonia metabolism in humans as measured by lactose- $^{15}\text{N}_2$ ureide

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Running title: **Pre- and probiotics on colonic ammonia metabolism**

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Background: The evaluation of ammonia detoxification by pre- and probiotics by means of colonic lactose- $^{15}\text{N}_2$ ureide (^{15}N -LU) degradation is of great interest both scientifically and in terms of nutrition physiology.

Objective: Pre- and probiotics were supplemented in healthy adults to evaluate the effect of the ammonia metabolism in the human colon by means of ^{15}N -LU.

Methods: Fourteen subjects aged 20-28 years daily received a regular diet either without (no treatment) or with supplementation of 30 g fibre of potatoes (FP), 30 g wrinkle pea starch (WPS, resistant starch content: 12 and 70%, respectively), and 375 g *Lactobacillus acidophilus* yoghurt (LC1), over a 10-day period in randomised order. After one week, 5.7 mg/kg body weight ^{15}N -LU was administered together with breakfast. A venous blood sample was taken after 6 h. Urine and faeces were collected over a period of 48 and 72 h, respectively. The ^{15}N -abundances were measured by isotope ratio mass spectrometry.

Results: The mean renal ^{15}N -excretion differed significantly between the supplementation of FP and no treatment (32.5 vs 46.3%, $P=0.034$), FP and LC1 (32.5 vs 51.6%, $P=0.001$), and WPS and LC1 (38.5 vs 51.6%, $P=0.048$). The mean faecal ^{15}N -excretion amounted to 42.7% (no treatment), 59.7% (FP), 41.8% (WPS), and 44.0% (LC1). In comparison to no treatment the urinary $^{15}\text{NH}_3$ -enrichment was significantly decreased 16 h after FP supplementation.

Conclusion: The prebiotic intake of FP and WPS lowered the colonic generation and the renal excretion of toxic $^{15}\text{NH}_3$, respectively, when using ^{15}N -LU as a xenobiotic marker.

Keywords:

Ammonia metabolism; human colon, lactose-[¹⁵N₂]ureide; prebiotics; probiotics

Introduction

In recent years, ^{15}N -labelled lactose ureide (^{15}N -LU) has been successfully used for studying colonic nitrogen and ammonia metabolism, respectively, to assess the effects of pre- and probiotics on the human intestinal flora (Cloetens *et al.*, 2008a; De Preter *et al.*, 2006, 2008; De Preter and Verbeke, 2006; Geboes *et al.*, 2006; Jackson *et al.*, 2004). The molecular bond between the sugar moiety and urea of ^{15}N -LU has been shown to resist enzymatic degradation in the small bowel, but to be split by *Clostridium innocuum* in the caecum (Heine *et al.*, 1995; Mohr *et al.*, 1999; Wutzke and Glasenapp, 2004; Wutzke and Schütt, 2007). ^{15}N -LU has the advantage of supplying both the nitrogen and the energy needed for microbial protein synthesis simultaneously and has therefore been used as a carrier for transporting $^{15}\text{NH}_3$ (common abbreviation for the ammonia/ammonium equilibrium $^{15}\text{NH}_3 + \text{H}_3\text{O}^+ \leftrightarrow ^{15}\text{NH}_4^+ + \text{H}_2\text{O}$ shifted to the ammonium side under physiological conditions) into the colon. Probiotics are defined as viable microorganisms that have beneficial effects in the prevention and treatment of specific pathologic conditions in the gut (Fuller, 1989). Human *in vivo* studies have proven that *Lactobacillus johnsonii* formerly, known as *Lactobacillus acidophilus* (La1 and LC1, respectively), supports the physiological function of the gastrointestinal tract and stimulates the immune defence system (Pfeiffer and Rosat, 1999). Prebiotics are defined as non-digestible food ingredients, mainly oligosaccharides, non-digestible polysaccharides, resistant starches, and others that support the growth of certain bacteria such as lactobacilli and bifidobacteria that have various beneficial effects on the gut, and, consequently, for the health of the host (Gibson and Roberfroid, 1995). Prebiotics have the ability of increasing the saccharolytic activity, but contrastingly to decrease the proteolytic activity of the microbiota of the gut (Swanson *et al.*, 2002). The colonic microbiota is responsible for degrading proteins partly to potentially toxic

metabolites such as ammonia, amines and others which are implicated in the pathogenesis of different diseases (Smith and Macfarlane, 1996). It is assumed that ammonia is assimilated systematically by the bacteria, resulting in reduced urinary excretion and corresponding increased faecal excretion. The scientific literature previously provided evidence that the consumption of probiotics and prebiotics containing resistant starch and other non-digestible carbohydrates may influence the bacterial production of toxic metabolic end products, particularly ammonia, in the human colon (Cummings and Macfarlane, 2002; De Preter *et al.*, 2004; Geboes *et al.*, 2005). Thus, the evaluation of ammonia detoxification by pro- and prebiotics, respectively, by means of bacterial ^{15}N -LU degradation to $^{15}\text{NH}_3$ and its subsequent excretion and absorption is still of great interest scientifically, in terms of nutrition physiology, and, with respect to pre- and probiotics, of commercial interest. Therefore, the aim of the present study is to investigate further prebiotics in form of fibre of potatoes (FP) containing a type-1 resistant starch (RS1) of 12% and a total dietary fibre content of 60%, wrinkle pea starch (WPS) containing a type-2 resistant starch (RS2) of 70% and a total dietary fibre content of 1%, and more probiotics in form of *L. acidophilus* yoghurt (LC1), respectively, on the renal and faecal nitrogen excretion, the urinary $^{15}\text{NH}_3$ -enrichment, the absorption, and the potential incorporation in selected blood plasma fractions by using orally administered lactose- $^{15}\text{N}_2$ ureide as a xenobiotic marker.

Methods

Tracer substance and supplements

^{15}N -LU was synthesized according to our method as described in detail (Wutzke *et al.*, 1997). Lactose was obtained from Merck Darmstadt Germany, whereas $^{15}\text{N}_2$ urea (99 atom%) was obtained from Campro Scientific Berlin Germany. FP and WPS, with a RS1 content of 12% and a RS2 content of 70%, respectively, were provided from the

manufacturer Emsland Group GmbH Emlichheim, Germany, whereas the LC1 yoghurt pure (colony forming units (cfu): 2.6×10^9 /100 g, calorific value: 75 kcal/100 g) was utilised from Nestlé AG Germany. Table 1 shows the detailed composition of both starch products as well as the LC1 yoghurt. Both FP and WPS are commercially available and are commonly used as supplements in bakery as well as in meat products.

Subjects

Fourteen healthy adults (9 female, 5 male, age: 20-28 years, body weight: 51.5-107.3 kg) volunteered for this study. They were in good health throughout the study. None of the subjects were receiving any medication or had a history of gastrointestinal diseases. Furthermore, none of the subjects regurgitated after having received the pre- and probiotics and the ^{15}N -LU, respectively. The volunteers were requested to avoid the additional consumption of pre- and probiotics within the study period. Written consent was obtained from all subjects. The testing protocol was approved by the Committee on Ethics of the Faculty of Medicine of the University of Rostock (Registration Number: II HV 11/2006).

Study protocols

All volunteers received an individually standardised regular diet (carbohydrate, fat, and protein content 55%, 30%, and 15%, respectively) over a time span of 54 days. According to the precursor studies of the Leuven group, no standard diets were imposed (Cloetens *et al.*, 2008b; De Preter *et al.*, 2004; 2006; 2007; 2008; De Preter and Verbeke, 2006). Nevertheless, the volunteers were urged to keep their usual dietary habits, with taken care that their diet remained as constant as possible within the

4 test phases. On the days of ^{15}N -LU administration the breakfast was identical in all 14 subjects. All volunteers received a continental breakfast made up of 1 wheat flour roll, 50 g jam, 50 g cheese, and 1 cup of tea at 7 45. No other food or drink was allowed until 6 h after breakfast. Blood, urine, and faeces samples were taken 30 min prior to the ingestion of ^{15}N -LU to determine the baseline abundance of ^{15}N . ^{15}N -LU was administered in a dosage of 5.7 mg/kg body weight, corresponding to a ^{15}N -excess dose of 0.4 mg/kg, at 8 00 as a single oral pulse strewn on a small area of the jam to ingest the label with one bite. A sample of venous blood was taken 6 h after ^{15}N -tracer administration at 14 00. On the 1st and on the 2nd day the urine was collected quantitatively in 2- and 4-, and in 6-h intervals, respectively, whereas faeces were collected quantitatively over 72 h. After a 4-day wash-out phase, 4 subjects each received either 10 g FP, 10 g WPS (dissolved in 100 ml tea), or 125 g LC1 (the equivalent of one yoghurt cup) together with breakfast, lunch, and dinner at 8 00, 13 00, and 19 00, respectively, in randomised order over a 10-day period. The ^{15}N -LU administration was repeated on the 23rd day followed by the wash-out phase. On the 30th day the randomised cross-over procedure was repeated with the next pre- or probiotic supplement followed by the ^{15}N -tracer administration on the 37th day. After the wash out period the test was repeated with the remaining substance on the 44th day followed by the final ^{15}N -LU administration (Figure 1). The procedure of ^{15}N -LU administration, blood, urine, and faeces collection was identical in all 4 tests. The 10-day supplementation period includes the complete collection of urine and faeces, whereas ^{15}N -LU was administered on the 7th day of the respective pre- or probiotic supplementation (Figure 1).

Sample preparation

The sample preparation prior to isotope ratio mass spectrometry (IRMS) analysis has been recently explained in detail (Wutzke and Sattinger, 2006). After centrifugation of the citrated blood sample, the plasma was treated with sulphuric Na_2WO_4 . The soluble supernatant was removed by centrifugation and was stored at $-20\text{ }^\circ\text{C}$ until analysis (Faust *et al.*, 1981). After defrosting, 0.5 ml supernatant was transferred to tin boats and was dried at $60\text{ }^\circ\text{C}$ for 2 h. Urine volumes and faecal masses scraped from polyvinyl film were homogenized, recorded and stored at $-20\text{ }^\circ\text{C}$ until analysis (Wutzke and Oetjens, 2005). After defrosting, 25 μl urine were transferred to tin boats, whereas 5 mg wet faeces were weighed on tin discs. Urinary ammonia was separated in vessels by micro-diffusion, following alkalization of the urine with NaOH and trapped in boric acid (Faust *et al.*, 1981).

Analytical techniques

Supernatant, urine, and faeces samples were combusted in the elemental analyser SLTM (SerCon, Crewe, U.K.) using copper oxide at $900\text{ }^\circ\text{C}$ and subsequently reduced to nitrogen gas using copper at $600\text{ }^\circ\text{C}$. Thereafter, the ^{15}N -abundances were measured by isotope ratio mass spectrometry (IRMS, Tracer mass 20-20TM, SerCon, Crewe, U.K.) as detailed previously (Wutzke *et al.*, 1997). A capillary interface is included for connecting the 20-20TM with the SLTM. The precision (mean %RSD or CV) for ^{15}N is 0.2‰. The data were expressed either as ^{15}N -enrichment in atom percent excess (atom%exc), or as percentage cumulative urinary and faecal ^{15}N -excretion.

Percentage of cumulative urinary and faecal ^{15}N -excretion

The ^{15}N -excess dose (D) of ^{15}N -LU is the product of the ^{15}N -enrichment in atom%exc ($\text{APE}_{^{15}\text{N-LU}}$) times the intake (I) times the number of labelled ^{15}N -atoms ($n=2$) divided by the molecular weight ($\text{MW}=403.4\text{ g/mol}$) and 100.

$$D = APE_{15N-LU} \cdot I \cdot n / 100 \cdot MW.$$

The ^{15}N -excretion (E) in a specified urine or faeces sample is the product of the measured ^{15}N -enrichment in atom%exc (APE_{sample}) times the urine volume (vol) or the faecal mass (m) in ml or g, respectively, times the N-concentration (c_N) of the urine or faeces sample in mg/ml or mg/g, respectively, divided by 100.

$$E = APE_{sample} \cdot vol(m) \cdot c_N / 100.$$

The percentage of cumulative ^{15}N -excretion (PCE) is the product of the urinary or faecal ^{15}N -excretion (E) in mmol, respectively, times 100 divided by the ^{15}N -excess dose (D) in mmol, respectively (Wutzke and Oetjens, 2005).

$$PCE = E \cdot 100 / D.$$

$$^{15}ND = 0.0267 \text{ mmol } ^{15}N/\text{kg body weight}.$$

Statistical method

The Kolmogorov-Smirnow test was used for testing the distribution normality. Then, the factorial one- and two-way analysis of variance (ANOVA) using a post hoc Bonferroni correction for multiple comparisons on program SPSS 15.0 for Windows were used for statistical analysis of the total N-, the faecal ^{15}N -, and the urinary ^{15}N -excretion, respectively, (Table 2-4) whereas the Kruskal-Wallis and the Mann-Whitney U-test were used for the urinary $^{15}NH_3$ -enrichment (significance $P \leq 0.01$). All results are quoted as means $\pm$ s.d.

Results

The maximum ^{15}N -enrichments in urinary ammonia were reached 12 h after ^{15}N -LU administration in all 4 tests (no treatment, FP, WPS, and LC1 supplementation) averaging 0.027 ± 0.019 , 0.018 ± 0.024 , 0.023 ± 0.019 , and 0.029 ± 0.018 , atom%exc, respectively (Figure 2). After 48 h the mean renal percentage cumulative excretion

amounted to 46.3 ± 11.1 , 32.5 ± 8.0 , 38.5 ± 12.9 , and $51.6 \pm 16.9\%$ (Table 2), respectively, whereas after 72 h the mean total faecal excretion amounted to 42.7 ± 20.6 , 59.7 ± 24.4 , 41.8 ± 26.9 , and $44.0 \pm 21.7\%$, respectively (Table 3). The urine fractionation in intervals of 0-6 h, 6-24 h, and 24-48 h revealed that the major part of ^{15}N was excreted between 6-24 h (Table 2). The resulting total ^{15}N -excretion (urine + faeces) shows that 89.0%, 92.2, 80.2, and 95.6%, respectively, of the ingested dose were excreted. The measured ^{15}N -enrichments in the supernatant amounted to 0.036 ± 0.015 , 0.040 ± 0.024 , 0.041 ± 0.017 , and 0.036 ± 0.020 atom%exc, respectively, whereas the respective numbers of the plasma protein ranged below the detection limit of the IRMS of 0.001 atom%. With the exception of the urinary $^{15}\text{NH}_3$ -enrichment values, the Kolmogorov-Smirnow test showed that by the majority, the data were normally distributed. When using ANOVA, between no treatment and the 3 different supplementation regimes statistically significant differences of the total urinary ^{15}N -excretion (0-48 h) were observed: no treatment vs FP, FP vs LC1, and WPS vs LC1, respectively, (Table 2), whereas no significant differences could be found neither for the respective faecal ^{15}N -excretion nor for the ^{15}N -enrichment of the supernatant of the blood plasma. Furthermore, significant differences of the fractional urinary ^{15}N -excretion were observed between 6-24 h and 24-48 h as well as for the $^{15}\text{NH}_3$ -enrichment between no treatment and FP supplementation ($P=0.008$) 16 h after ^{15}N -LU ingestion. The total urinary N-excretion measured over the 2-day period after ^{15}N -LU administration (25.1, 22.0, 23.9, and 29.4 g N/2 days) yielded no statistically significant differences between the 4 tests phases (Table 4).

Discussion

The primary metabolic function of the microbiota is the fermentation of non-digestible food ingredients that have passed the small bowel such as resistant starch, non-

digestible polysaccharides, non-digestible oligosaccharides and proteins and others. During this passage, these substrates are exposed to the differing conditions of the gastrointestinal tract and are then found nearly indigested in the faeces of the large bowel (Wutzke and Oetjens, 2005). The outcome of the subsequent complex metabolic activity of the colon is the supply of energy and the retrieval of reusable substrates for the microbiota itself, and, consequently, for the host. The protein degradation in the large bowel leads to the generation of amino acids (which can be further partly deaminated to ammonia) and of other potentially toxic metabolic end products such as phenols, indoles, amines and others (Smith and Macfarlane, 1996). *In vivo* studies have been shown that the ammonia generation and accumulation, respectively, can be reduced by lowering the protein supply and by the colonic fermentation of suitable non-digestible carbohydrates of the food (Geboes *et al.*, 2005). In contrast to polysaccharides, preferably butyric acid is generated in the colon during the fermentation of resistant starch. However, the resulting generation of both short chain fatty acids (SCFA) and butyric acid lead to a decrease of the pH-value in the colon, and, consequently, to an inhibition of the protease activity, whereas the additional energy supply increases the total bacterial mass (Macfarlane and Cummings, 1991). In combination, these effects lead to an increased incorporation of both amino acid nitrogen and ammonia by the microbiota. The Leuven group recently demonstrated that after ^{15}N -LU administration the supplementation of type-3 resistant starch (RS3) in comparison to baseline significantly reduced the renal ^{15}N -excretion (Cloetens *et al.*, 2008b). Furthermore, the study revealed that the major part of ^{15}N was excreted between 6 and 24 h after ^{15}N -LU ingestion suggesting that the $^{15}\text{NH}_3$ had not reached the distal colon, and, therefore, it only justifies the evaluation of the degradation processes in the proximal colon. When considering the results of the present study, our data are in the same order of magnitude of those of the Leuven studies and confirm the

tendency of lowering the urinary ^{15}N -excretion after supplementation with prebiotics when using ^{15}N -LU (Cloetens *et al.*, 2008b; De Preter *et al.*, 2004; Geboes *et al.*, 2005). In the present study, the mean renal ^{15}N -excretion 48 h after ^{15}N -LU ingestion differed significantly between the supplementation of FP and baseline, FP and LC1, and WPS and LC1 (Table 2). Similar to the findings obtained after RS3 supplementation, we could confirm that the major part of ^{15}N was excreted in urine between 6 and 24 h after ^{15}N -LU ingestion, suggesting that the $^{15}\text{NH}_3$ had not reached the distal colon (Cloetens *et al.*, 2008b, Table 2). In contrast, the urinary ^{15}N -excretion between 0 and 6 h mainly reflects the glucose- $^{15}\text{N}_2$ ureide absorption deriving from the enzymatic degradation of ^{15}N -LU by β -galactosidase in the small bowel (Wutzke and Glasenapp, 2004). RS3 is mainly retrograded amylose formed after cooking and cooling, whereas RS2 occurs relatively dehydrated in uncooked natural granular form (Sajilata *et al.*, 2006). When considering the results obtained after RS3 supplementation containing 54% RS3 and the data of our WPS supplementation containing 70% RS2 the outcome becomes obvious. In contrast to the findings of Cloetens *et al.*, the decrease in the urinary ^{15}N -excretion in the present study was less-pronounced, indicating that, in comparison to RS3, less fermentable RS2 had reached the colon (Heijnen and Beynen, 1997). However, the corresponding findings after the supplementation of the mainly dietary fibre-containing FP were similar to those after WPS supplementation, indicating that the anaerobic bacterial degradation of cellulose to SCFA in the human colon is likewise responsible for decreasing the urinary ^{15}N -excretion (Figure 2 and Table 2). The observed significant difference of the urinary $^{15}\text{NH}_3$ -enrichment between no treatment and FP, 16 h after ^{15}N -LU ingestion confirm the decreased urinary ^{15}N -excretion after FP supplementation in comparison to no treatment conditions and clearly reflect the microbial degradation of the ^{15}N -LU (Birkett *et al.*, 1996, Table 1). The observed significant ^{15}N -enrichments of the mainly urea-containing supernatant fraction of the blood plasma (0.035 atom%exc on average)

6 h after ^{15}N -LU ingestion clearly demonstrates the appearance of ^{15}N -urea and possibly of certain free ^{15}N -amino acids in the metabolic pool of the blood plasma indicating the release of $^{15}\text{NH}_3$ after ^{15}N -LU degradation, its subsequent absorption and the following re-synthesis to ^{15}N -urea and partly to ^{15}N -amino acids. Nevertheless, the supernatant fraction of the blood plasma can also be potentially contaminated by the absorption of glucose- ^{15}N ureide deriving from the enzymatic ^{15}N -LU degradation. The urinary ^{15}N -excretion observed within the first 6 hours after ^{15}N -LU administration could confirm this assumption. According to the kinetics of the urinary urea and ammonia enrichment after ^{15}N -LU administration described in an earlier published paper, the sample of venous blood was deliberately taken 6 h after ^{15}N -LU administration, since we assumed the highest ^{15}N -enrichment in the blood plasma fraction around this time point (Wutzke *et al.*, 1997). The significant ^{15}N -enrichments of the urinary ammonia fraction and of the supernatant of the blood plasma, respectively, indicate the immediate excretion, and, in particular, the absorption of ^{15}N deriving from the bacterial degradation of ^{15}N -LU in the human colon. However, the respective ^{15}N -abundances of the plasma protein precipitate ranged below the detection limit of the IRMS of 0.001 atom%. The measurement of the ^{15}N -enrichments in urinary ammonia as well as in the supernatant of the blood plasma when using ^{15}N -LU as a xenobiotic marker is a novelty. The acidification of the colon caused by the fermentation to SCFA could reduce the enzymatic cleavage of ^{15}N -LU and the conversion of $^{15}\text{N}_2$ urea to $^{15}\text{NH}_3$, respectively, resulting in an increased faecal ^{15}N -excretion. After supplementation of FP and LC1, the resulting faecal ^{15}N -excretions (59.7 and 44.0%, respectively) tend to result in an increase in comparison to no treatment (42.7%) but without a statistical significance. The comparatively high standard deviations were presumably caused by an incomplete homogenization of the faeces prior to combustion in the SLTM. De Preter *et al.*, 2004 observed that the supplementation of *Lactobacillus casei* to healthy adults in

comparison to no treatment revealed no statistical significant differences of the urinary ^{15}N -excretion. Furthermore, a short term intake of *Bifidobacterium breve* and *L. casei* as well neither influenced the urinary nor the faecal ^{15}N -excretion, respectively (De Preter *et al.*, 2007). Similar to these findings, we also did not observe a decrease of the urinary ^{15}N -excretion after the supplementation of LC1 yoghurt in comparison to no treatment. We deliberately applied a portion of 3 yoghurt cups per day (375 g, equivalent to 9.8×10^9 cfu) to mimic common usage consumption (Sanders and Huis In't Veld, 1999). The constant total urinary N-excretion over the 2-day period after ^{15}N -LU administration clearly argues for the ingestion of a standardised diet within the 4 test phases (Table 4). We assume that the relatively short 10-day period of our LC1 supplementation and the 7-day period prior to ^{15}N -LU administration, respectively, is adequate for achieving steady state conditions. Nevertheless, our findings clearly demonstrate that commercially available LC1 yoghurt in the chosen dosage led to identical results as under no treatment conditions and has no immediate impact on the colonic ammonia metabolism when using ^{15}N -LU. When summarising our findings, we are convinced that lactose- $^{15}\text{N}_2$ ureide is a valid tool for investigating nitrogen and ammonia metabolism in the colon. Furthermore, we suggest that the prebiotic intake lowered the colonic generation of $^{15}\text{NH}_3$ and its corresponding urinary ^{15}N -excretion and tends to enhance the $^{15}\text{NH}_3$ uptake by the microbiota. Seen in this light, the process of ammonia detoxification in the colon can be interpreted as an interplay of the microbiota with certain supplements establishing appropriate conditions for their growth and proliferation. In this sense, the lowering of the proteolytic generation and accumulation of ammonia in the colon and the resulting noxious effects for the host by suitable supplementations of pre- and probiotics, particularly in subjects suffering from certain diseases such as kidney insufficiency, hepatic encephalopathy, inflammatory bowel

disease, and colon cancer is understandably of great interest for biochemists, physicians, dieticians and in particular the patients as well.

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Conflict of interest

The authors declare no conflict of interest.

References

- Birkett A, Muir J, Phillips J, Jones G, O'Dea K (1996). Resistant starch lowers fecal concentrations of ammonia and phenols in humans. *Am J Clin Nutr* **63**, 766-72.
- Cummings JH, Macfarlane GT (2002). Gastrointestinal effects of prebiotics. *Br J Nutr* **87**, Suppl 2:145-151.
- Cloetens L, De Preter V, De Loor H, Rutgeerts P, Verbeke K (2008a). Does the biomarker ¹⁵N-lactose ureide allow to estimate the site of fermentation of resistant starch? *Eur J Nutr* **47**, 217-223.
- Cloetens L, De Preter V, Swennen K, Broekaert WF, Courtin CM, Delcour JA, Rutgeerts P, Verbeke K (2008b). Dose-response effect of arabinoxyloligosaccharides on gastrointestinal motility and on colonic bacterial metabolism in healthy volunteers. *J Am Coll Nutr* **27**, 512-518.
- De Preter V, Geboes K, Verbrugghe K, De Vuyst L, Vanhoutte T, Huys G, Swings J, Pot B, Verbeke K (2004). The in vivo use of the stable isotope-labelled biomarkers lactose-[¹⁵N]ureide and [²H₄]tyrosine to assess the effects of pro- and prebiotics on the intestinal flora of healthy human volunteers. *Br J Nutr* **92**, 439-446.

- De Preter V, Verbeke K (2006). Evaluation of the necessity of induction for lactose- $[^{15}\text{N}, ^{15}\text{N}]$ -ureide to study the colonic ammonia metabolism. *Scand J Gastroenterol* **41**, 396-400.
- De Preter V, Vanhoutte T, Huys G, Swings J, Rutgeerts P, Verbeke K (2006). Effect of lactulose and *Saccharomyces boulardii* administration on the colonic urea-nitrogen metabolism and the bifidobacteria concentration in healthy human subjects. *Aliment Pharmacol Ther* **23**, 963-974.
- De Preter V, Vanhoutte T, Huys G, Swings J, De Vuyst L, Rutgeerts P, Verbeke K (2007). Effects of *Lactobacillus casei* Shirota, *Bifidobacterium breve*, and oligofructose-enriched inulin on colonic nitrogen-protein metabolism in healthy humans. *Am J Physiol Gastroenterol Liver Physiol* **292**, G358-G368.
- De Preter V, Vanhoutte T, Huys G, Swings J, Rutgeerts P, Verbeke K (2008). Baseline microbiota activity and initial bifidobacteria counts influence responses to prebiotic dosing in healthy subjects. *Aliment Pharmacol Ther* **27**, 504-513.
- Faust H, Bornhak H, Hirschberg K, Jung K, Junghans P, Krumbiegel P, Reinhardt R (1981). Klinisch-chemische und isopenanalytische Methoden zur Untersuchung des Stickstoff-Stoffwechsels mit N-15 beim Menschen. In: Wetzel K (ed). *Methodenkatalog. Zfl-Mitteilungen*. vol. 36. Akademie der Wissenschaften: Leipzig. pp 115-132.
- Fuller R (1989). Probiotics in man and animals. *J Appl Bacteriol* **66**, 365-378.
- Geboes KP, De Preter V, Luybaerts A, Bammens B, Evenepoel P, Ghos Y, Rutgeerts P, Verbeke K (2005). Validation of lactose- $[^{15}\text{N}, ^{15}\text{N}]$ ureide as a tool to study colonic nitrogen metabolism. *Am J Physiol Gastrointest Liver Physiol* **288**, G994-999.
- Geboes KP, De Hertogh G, De Preter V, Luybaerts A, Bammens B, Evenepoel P, Ghos Y, Geboes K, Rutgeerts P, Verbeke K (2006). The influence of inulin on the

- absorption of nitrogen and the production of metabolites of protein fermentation in the colon. *Br J Nutr* **96**, 1078-1086.
- Gibson GR, Roberfroid MB (1995). Dietary modulation of the human colonic microbiota: introducing the concept of prebiotics. *J Nutr* **125**, 1401-1412.
- Heijnen ML, Beynen AC (1997). Consumption of retrograded (RS3) but not uncooked (RS2) resistant starch shifts nitrogen excretion from urine to feces in cannulated piglets. *J Nutr* **127**, 1828-1832.
- Heine WE, Berthold HK, Klein PD (1995). A novel stable isotope breath test: ^{13}C -labeled glycosylureides used as noninvasive markers of intestinal transit time. *Am J Gastroenterol* **90**, 93-98.
- Jackson AA, Gibson NR, Bundy R, Hounslow A, Millward DJ, Wootton SA (2004). Transfer of ^{15}N from oral lactose-ureide to lysine in normal adults. *Int J Food Sci Nutr* **55**, 455-462.
- Macfarlane GT, Cummings JH (1991). The colonic flora, fermentation and large bowel digestive function. In: Phillips SF, Pemberton H, Shorter RG (eds). *The large intestine: physiology, pathophysiology and disease*. Raven: New York. pp 51-92.
- Mohr C, Heine WE, Wutzke KD (1999). *Clostridium innocuum*: a glucose ureide splitting inhabitant of the human microbial flora. *Biochim Biophys Acta* **1472**, 550-554.
- Pfeiffer A, Rosat JP (1999). Probiotics in alimentation: clinical evidence for their enhancement of the natural immunity of the gut. In: Hanson LA, Yolken RH (eds). *Probiotics, other nutritional factors and intestinal microflora*. Nestlé nutrition workshop series. vol. 42. Lippincott – Raven: Philadelphia. pp 243-255.
- Sanders MA, Huis In't Veld J (1999). Bringing a probiotic-containing functional food on the market: microbiological, product, regulatory and labeling issues. *Antonie Van Leeuwenhoek* **76**, 293-315.

- Sajilata MG, Singhal RS, Kulkarni PR (2006). Resistant starch – a review. *Compr Rev Food Sci Food Saf* **5**, 1-17.
- Smith EA, Macfarlane GT (1996). Enumeration of human colonic bacteria producing phenolic and indolic compounds: effects of pH, carbohydrate availability and retention time on dissimilatory aromatic amino acid metabolism. *J Appl Bacteriol* **81**, 288-302.
- Swanson KS, Grieshop CM, Flickinger EA, Bauer LL, Chow J, Wolf BW, Garleb KA, Fahey GC Jr (2002). Fructooligosaccharides and *Lactobacillus acidophilus* modify gut microbial populations, total tract nutrient digestibilities and fecal protein catabolite concentrations in healthy adult dogs. *J Nutr* **132**, 3721-3731.
- Wutzke KD, Heine WE, Plath C, Leitzmann P, Radke M, Mohr C, Richter I, Gülzow U, Hobusch D (1997). Evaluation of oro-coecal transit time: a comparison of the lactose-[¹³C, ¹⁵N]ureide ¹³CO₂- and the lactulose H₂-breath test in humans. *Eur J Clin Nutr* **51**, 11-19.
- Wutzke KD, Glasenapp B (2004). The use of ¹³C-labelled glycosyl ureides for evaluation of oro-caecal transit time. *Eur J Clin Nutr* **58**, 568-572.
- Wutzke KD, Oetjens I (2005). ¹³C- and ¹⁵N-incorporation of doubly stable isotope labelled *Lactobacillus johnsonii* in humans. *Eur J Clin Nutr* **59**, 1167-1172.
- Wutzke KD, Sattinger V (2006). ¹⁵N-excretion of heat-killed *Lactobacillus casei* in humans. *Eur J Clin Nutr* **60**, 847-852.
- Wutzke KD, Schütt M (2007). The duration of enzyme induction in oro-caecal transit time measurements. *Eur J Clin Nutr* **61**, 1161-1166.

Table 1

Percentage composition of fibre of potatoes, wrinkle pea starch, and *Lactobacillus acidophilus*

constituents	fibre of potatoes	wrinkle pea starch	<i>L. acidophilus</i>
total starch	28 ⁺	98 ⁺	-
RS1	12 ⁻	-	-
RS2 (amylose)	-	70 ⁻	-
total dietary fibre (cellulose)*	60 ⁻	> 1	-
protein	7 ⁺	0.5 ⁺	4.5
carbohydrate	88	98	6.5
fat	0.5 ⁺	0.5 ⁺	3.5
moisture	10 ⁺	14 ⁺	85.5

⁺: maximal, ⁻: minimal

* minor amounts of hemicellulose, xylose, arabinose, pectin and lignin

Table 2

Mean percentage urinary ^{15}N -excretion between 0-6 h, 6-24 h, 24-48 h, and 0-48 h, respectively, after the supplementation of fibre of potatoes (FP), wrinkle pea starch (WPS), *Lactobacillus acidophilus* (LC1), and no treatment (NT) when using ^{15}N -LU

sub- ject	0-6 hours				6-24 hours				24-48 hours				0-48 hours			
	NT	FP	WPS	LC1	NT	FP	WPS	LC1	NT	FP	WPS	LC1	NT	FP	WPS	LC1
1	6.87	7.24	6.35	6.4	33.95	19.5	23.58	33.52	3.46	5.44	5.46	8.95	44.29	32.19	35.39	48.87
2	9.1	4.71	4.95	3.63	27.01	20.11	34.35	42.05	11.18	3.89	12.16	12.0	47.29	28.71	51.47	57.67
3	11.82	10.57	11.2	8.06	36.56	25.73	12.63	27.41	20.89	9.5	12.75	26.56	69.27	45.8	36.58	62.02
4	4.66	5.1	5.46	3.09	18.52	10.47	14.13	23.87	9.35	1.61	4.47	9.31	32.53	17.18	24.06	36.27
5	6.21	9.48	12.34	11.04	31.13	12.18	18.57	40.58	3.84	0.32	3.39	5.94	41.17	21.98	34.31	57.56
6	16.78	14.1	41.69	15.51	40.55	19.89	24.43	30.79	7.75	4.57	2.74	2.69	65.08	38.56	68.86	48.99
7	10.35	5.56	5.2	5.16	19.22	18.46	25.66	32.81	13.4	7.97	16.98	12.98	42.97	32.0	47.84	50.95
8	9.63	7.07	8.26	5.57	18.92	15.33	20.77	28.43	10.28	5.64	4.59	4.86	38.83	28.04	33.61	38.86
9	2.9	3.5	2.55	4.52	18.67	18.34	13.61	27.3	10.22	8.39	7.69	6.69	31.78	30.23	23.85	38.5
10	7.25	7.86	5.48	4.7	37.81	21.62	22.6	31.24	9.28	6.76	7.87	12.05	54.35	36.23	35.96	47.98
11	4.22	6.59	6.42	6.16	34.86	23.6	32.62	27.05	11.96	8.52	14.69	11.01	51.03	38.71	53.73	44.21
12	10.7	6.91	6.66	7.88	19.23	16.24	13.54	16.87	8.28	5.11	3.34	4.85	38.2	28.26	23.53	29.6

13	5.5	5.39	5.41	6.37	20.98	29.29	20.72	39.01	12.77	10.88	8.56	16.3	39.25	45.56	34.71	61.69
14	4.99	5.49	3.82	4.84	34.42	19.8	24.1	75.06	12.16	6.19	6.48	19.43	51.57	31.48	34.39	99.32
mean	7.93	7.11	8.98	6.64	27.99¹	19.32²	21.52³	34.0⁴	10.34⁵	6.06⁶	7.94⁷	10.97⁸	46.26⁹	32.49¹⁰	38.45¹¹	51.61¹²
s.d.	3.7	2.7	9.8	3.3	8.3	5.0	6.8	13.6	4.3	2.9	4.5	6.5	11.1	8.0	12.9	16.9

significance $P \leq 0.05$:

² vs ⁴: 0.001, ³ vs ⁴: 0.004, ⁶ vs ⁸: 0.049, ⁹ vs ¹⁰: 0.034, ¹⁰ vs ¹²: 0.001, ¹¹ vs ¹²: 0.048

Table 3

Mean percentage faecal ^{15}N -excretion 3 days after the supplementation of fibre of potatoes, wrinkle pea starch, *Lactobacillus acidophilus*, and no treatment when using ^{15}N -LU

subjects	no treatment	fibre of potatoes	wrinkle pea starch	<i>L. acidophilus</i>
1	28.05	60.88	12.93	36.68
2	37.39	55.58	16.68	37.4
3	43.04	36.42	73.43	53.62
4	21.18	55.95	42.42	30.2
5	57.45	85.25	70.07	61.4
6	35.68	92.91	59.91	92.53
7	49.92	69.9	1.47	57.91
8	30.78	92.76	97.51	53.14
9	46.48	54.92	33.07	36.29
10	8.26	59.14	28.32	12.33
11	78.68	12.07	19.15	68.52
12	84.58	87.8	33.34	31.2
13	33.04	37.96	56.35	18.5
14	43.43	33.64	41.04	26.35
mean	42.71	59.66	41.84	44.01
s.d.	20.6	24.4	26.9	21.7

No significant data were found.

Table 4

Mean urinary total N-excretion in [g/2days] after the supplementation of fibre of potatoes, wrinkle pea starch, *Lactobacillus acidophilus*, and no treatment when using ^{15}N -LU

subjects	no treatment	fibre of potatoes	wrinkle pea starch	<i>L. acidophilus</i>
1	24.57	18.72	16.96	20.89
2	37.36	28.03	47.22	61.31
3	27.01	22.6	26.83	26.52
4	18.45	14.33	14.56	18.68
5	24.38	19.42	27.61	43.78
6	32.15	28.63	27.6	22.9
7	24.21	20.77	20.78	22.03
8	19.09	21.92	19.32	24.74
9	23.15	20.68	18.32	19.86
10	25.11	17.57	23.8	21.09
11	32.49	22.93	24.72	23.71
12	22.32	23.66	16.52	20.73
13	10.84	20.67	21.3	21.43
14	30.32	27.92	28.79	64.4
mean	25.07	22,0	23.88	29.4
s.d.	6.7	4.1	8.1	15.4

No significant data were found.

Figure legends

Figure 1: Time frame of the study protocol

Figure 2: Mean urinary $^{15}\text{NH}_3$ -enrichment over a 24-h time span after the supplementation of fibre of potatoes, wrinkle pea starch, *Lactobacillus acidophilus*, and no treatment when using ^{15}N -LU

Figure 1

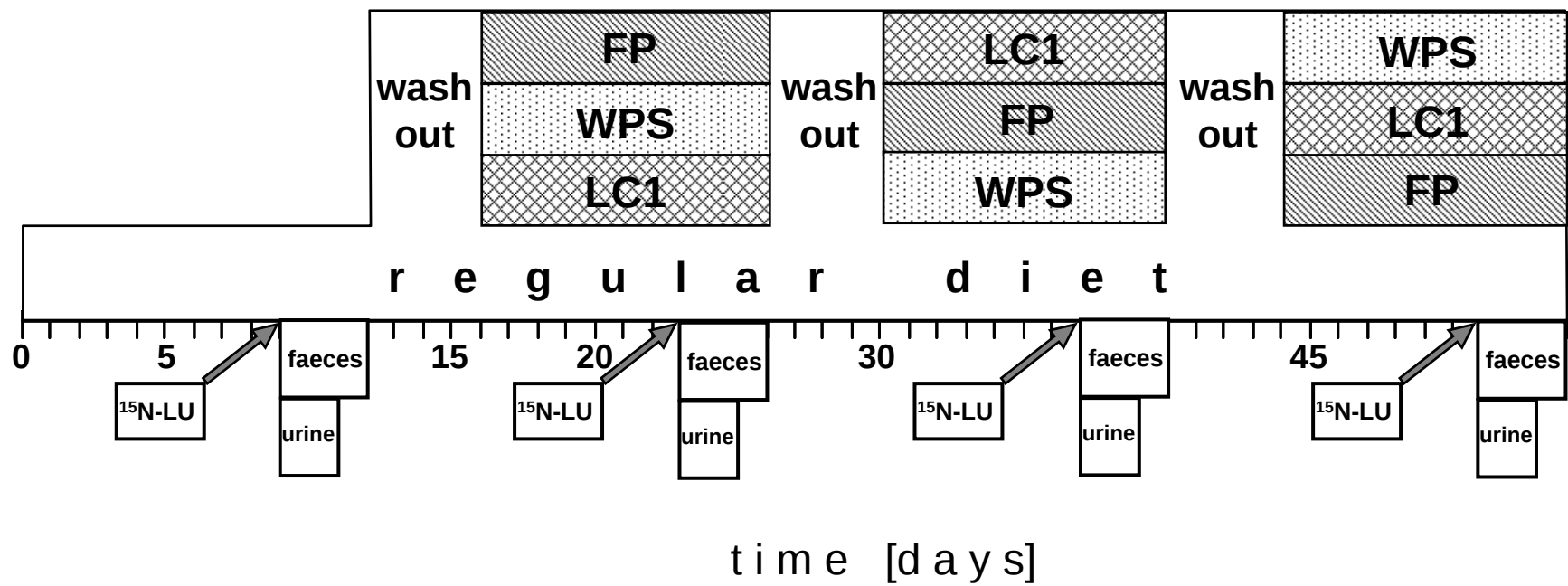


Figure 2

