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# Carboxylic acids in the hindgut of rats fed highly soluble inulin and *Bifidobacterium lactis* (Bb-12), *Lactobacillus salivarius* (UCC500) or *Lactobacillus rhamnosus* (GG)

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*Abstract*

***Background***: Propionic and butyric acids are important nutrients for the mucosal cells and may therefore increase the nutritional status and reduce the permeability of the colonic mucosa. These acids have also been suggested to counteract diseases in the colon, e.g. ulcerative colitis and colon cancer. Different substrates lead to different amounts and patterns of carboxylic acids (CAs).
***Objective***: To study the effect of probiotics on CA formation in the hindgut of rats given inulin.
***Design***: The rats were given inulin, marketed as highly soluble by the producer, together with the probiotic bacteria *Bifidobacterium lactis* (Bb-12), *Lactobacillus salivarius* (UCC500) or *Lactobacillus rhamnosus* (GG), or a mixture of all three.
***Results***: Rats fed inulin only had comparatively high proportions of propionic and butyric acids throughout the hindgut. When diets were supplemented with Bb-12 and UCC500, the caecal pool of CAs increased compared with inulin only. In the caecum the proportion of butyric acid generally decreased when the rats were fed probiotics. In the distal colon the proportion of propionic and butyric acid was lower, while that of lactic acid was generally higher. The caecal pH in rats fed GG and Bb-12 was lower than expected from the concentration of CAs. Further, rats fed GG had the lowest weight gain and highest caecal tissue weight.
***Conclusions***: It is possible to modify the formation of CAs by combining inulin with probiotics. Different probiotics had different effects.

Keywords: *Bifidobacterium lactis (Bb-12)*; *carboxylic acids*; *inulin*; *Lactobacillus rhamnosus (GG)*; *Lactobacillus salivarius (UCC500)*; *prebiotics*

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## Introduction

The main substrates reaching the colon for fermentation are indigestible carbohydrates, and their presence in the large intestine supports the growth of the colonic microflora. The metabolic activity in the colon is considerable and these carbohydrates are fermented to carboxylic acids (CAs, mainly acetic, propionic and butyric acid) and gases ($CO_2$, $CH_4$ and $H_2$) (1). Both the type and the amount of CAs in the colon are important from a nutritional point of view. Butyric acid and to some extent also propionic acid serve locally as nutrients for the mucosal cells and provide increased resistance against lesions and reduced permeability of the colonic mucosa (2, 3). Propionic and butyric acid have been suggested to counteract diseases in the colon, e.g. ulcerative colitis (4) and colon cancer (5). Some of the CAs formed may also promote gastrointestinal health by suppressing the growth of potentially pathogenic organisms (6–8). The mechanisms behind such effects may be related either to the low pH as such or to an improved nutritional status of the mucosa, decreasing the risk of translocation of pathogens



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(6, 7). Oligofructose, giving high amounts of butyric acid during fermentation, has been shown to protect hamsters against infection by *Clostridium difficile* (8, 9).

It is of special interest that various indigestible carbohydrates give rise to different amounts and patterns of CAs. Previous studies have shown that the monomeric composition of these carbohydrates and the type of linkages between the monomers can be of great importance (10–12). However, other factors may also have an influence, e.g. the molecular weight and the solubility of the fibre (9) as well as the architecture of the fibre and the amount of resistant starch (11), factors that can all be regulated by processing. Some carbohydrate combinations may also shift the location of fermentation to the distal part of colon, where most colonic diseases occur (12). In principle it would be possible to tailor the CA pattern and the location of fermentation by selecting suitable substrates and optimizing the process conditions. The differences in CA formation may be due to the substrate as such, and/or to the fact that the substrate favours the growth of certain colonic bacteria that produce a specific CA pattern. The composition of the colonic microbiota may also be affected by the intake of probiotic bacteria, i.e. microorganisms providing health benefits to the consumer. Supply of such bacteria to rats has recently been demonstrated to modify the CA pattern (13). Several potential benefits of probiotics per se have been proposed, including increased resistance to infectious diseases, reduction in blood pressure, serum cholesterol concentration and allergy, stimulation of phagocytosis and a reduction in carcinogen or co-carcinogen production (14).

In this study, a long-chain fructo-oligosaccharide with high solubility was combined with three probiotic strains (*Bifidobacterium lactis*, *Lactobacillus salivarius* and *Lactobacillus rhamnosus*) to investigate whether the CA formation in the hindgut of rats could be modified. The inulin used reaches the colon, is highly fermented and has been shown to give high amounts of propionic acid compared with other fructo-oligosaccharides (9). Inulin also stimulates the growth of bifidobacteria specifically and has been classified as a prebiotic substrate (15), i.e. a colonic substrate that specifically increases the number of bacteria that have beneficial physiological effects. As it has been suggested that a combination of probiotics may have more pronounced effects in the colon than a single strain, a mixture of the three strains was also investigated (16).

## Materials and methods

### *Materials*

The inulin used (Raftiline® HP; Orafti, Tienen, Belgium), was derived from chicory root and has an average degree of polymerization (DP) of 23 (10–60). This inulin is obtained by removing fructo-oligosaccharides of a low DP from natural inulin (17). It has a high solubility (20 g $l^{-1}$ at 25°C) compared with another inulin (Raftiline® HPX 1 g $l^{-1}$ at 25°C) with the same DP also marketed by Orafti.

Three bacterial strains were included in the study: *Bifidobacterium lactis* (*B. lactis* Bb-12, Chr. Hansen, Hørsholm, Denmark), *Lactobacillus salivarius* (UCC500; University College, Cork, Ireland) and *Lactobacillus rhamnosus* (GG; Valio, Helsinki, Finland). The three strains were delivered freeze-dried.

### *Experimental design, animals and diets*

Five diets were prepared: one reference diet containing inulin only and four test diets including inulin together with either one of the probiotic bacteria (GG, UCC500 or Bb-12) or a mixture of the three bacteria (Table 1). Male Wistar rats, 3–4 weeks old, with an initial weight of $86.1 \pm 1.4$ g (mean $\pm$ SE), were then randomly divided into groups of seven and assigned one of the five diets. All diets contained casein (Sigma Chemical Co., St Louis, MO, USA) as protein source, sucrose (Danisco Sugar, Malmö, Sweden), maize oil (Mazola, Bestfoods Nordic A/S, Copenhagen, Denmark), DL-methionine (Sigma), choline chloride (Aldrich Chemie, Steinheim, Germany), a mineral mixture (Apoteket, Malmö, Sweden), a vitamin mixture (Apoteket) and wheat starch (Lundbergs, Malmö, Sweden). Wheat starch was used to adjust the dry matter content and can be expected to be completely digested and absorbed before the colon and thus does not contribute to any CA formation (18). The inulin was added at a level of 80 g $kg^{-1}$ diet [dry weight (dw)] and the probiotic strains were included to give each rat an amount of Bb-12, GG and UCC500 corresponding to $1 \times 10^{10}$, $1 \times 10^{10}$ and $1 \times 10^{9}$ cfu per day, respectively. In the diet containing a mixture of the probiotic strains one-third of these amounts from each strain was added.

*Table 1.* Composition of reference and test diets (g $kg^{-1}$ dry weight)

| Component | Reference diet | Test diets |
|---|---|---|
| Inulin | 80.4[a] | 80.4[a] |
| Bacteria[b] | – | 1–7 |
| Casein | 120 | 120 |
| DL-Methionine | 1.2 | 1.2 |
| Maize oil | 50 | 50 |
| Mineral mixture[c] | 48 | 48 |
| Vitamin mixture[d] | 8 | 8 |
| Choline chloride | 2 | 2 |
| Sucrose | 100 | 100 |
| Wheat starch | 590.4 | 583.4–589.4[e] |

[a] Corresponding to 80 g indigestible carbohydrate $kg^{-1}$ diet (dry weight).

[b] Corresponding to $1.0 \times 10^9$, $1.0 \times 10^{10}$ and $1.0 \times 10^{10}$ cfu per day for UCC500, Bb-12 and GG, respectively. The mixture contained one-third of each strain.

[c] Containing (g $kg^{-1}$) 0.37 $CuSO_4$ $5H_2O$, 1.4 $ZnSO_4$ $7H_2O$, 332.1 $KH_2PO_4$, 171.8 $NaH_2PO_4$ $2H_2O$, 324.4 $CaCO_3$, 0.068 KI, 57.2 $MgSO_4$, 7.7 $FeSO_4$ $7H_2O$, 3.4 $MnSO_4$ $H_2O$, 0.020 CoCl $6H_2O$ and 101.7 NaCl.

[d] Containing (g $kg^{-1}$) 0.62 menadion, 2.5 thiamin hydrochloride, 2.5 riboflavin, 1.25 pyridoxin hydrochloride, 6.25 calcium pantothenate, 6.25 nicotinic acid, 0.25 folic acid, 12.5 inositol, 1.25 *p*-aminobenzoic acid, 0.05 biotin, 0.00375 cyanocobalamin, 0.187 retinol palmitate, 0.00613 calciferol, 25 *d*-α-tocopheryl acetate and 941.25 maize starch.

[e] Depending on the concentration of bacteria in the added powder.

The diets containing the probiotics were kept refrigerated until fed to the rats (Table 1).

To obtain a similar feed intake, the amount was restricted to 12 g dw per day and distributed to the rats daily. Water was given *ad libitum*. The rats were allowed to adapt to the diet for 7 days and then a 5 day experimental period followed, during which faeces and feed residues were collected daily. The faeces were stored at −20°C and then freeze-dried and milled before being analysed with regard to remaining inulin. During the following 24 h of the experiment, fresh faeces were collected on dry ice for CA determination. After the animals had been killed using carbon dioxide narcosis, the caecum and proximal and distal colon were removed. Caecal tissue weight, content and pH were measured directly, and the different parts of the hindgut were frozen and stored at –40°C until analysis of the CAs. The Ethics Committee for Animal Studies at Lund University approved the experiments.

### Analysis

*Carboxylic acids.* A gas–liquid chromatographic method was used to analyse the amount of CAs (formic, acetic, propionic, isobutyric, butyric, iso-valeric, valeric, caproic and heptanoic acid). Other CAs quantified with this method were lactic acid and succinic acid (19). The intestinal content and the faecal samples were homogenized (using a Polytron®; Kinematica, Switzerland) together with an internal standard (2-ethylbutyric acid, Sigma Chemical Co., St Louis, MO, USA). Hydrochloric acid was added to protonize the CAs so that they could be extracted in diethyl ether. After being silylated with *n*-(tert-butyldimethylsilyl)-*n*-methyl trifluoroacetamide (MTBSTFA; Sigma), the samples were allowed to stand for 48 h for complete derivatization. Samples were then injected onto an HP-5 column (GLC, HP 6890; Hewlett Packard, Wilmington, DE, USA). Chem Station software (Hewlett Packard) was used for the analysis.

*Inulin.* The amount of inulin in the raw material was assumed to be the value determined by the manufacturer (995 g $kg^{-1}$ dw). The amount of inulin in faeces from rats fed the reference diet was quantified with AOAC method 999.03 (20). In this method fructo-oligosaccharides are treated with fructanase (exo-inulinase) and the amount of fructose is quantified with the *R*-hydroxy-benzoic acid hydrazine (PAHBAH) reducing-sugar method. Only small amounts of inulin were detected in the faeces of these rats (0.070 g per 5 days, corresponding to 1.5% of the ingested amount), and as the faecal weights were similar in rats fed the diets containing probiotics it was assumed that inulin had been almost completely fermented also in these rats.

### Calculations and statistical evaluation

The design of the experiment resulted in a reference diet containing inulin only and four test diets, which also contained GG, Bb-12, UCC 500 or a mixture of the three strains. Data from rats given diets containing the probiotics were compared with those fed the reference diet with no probiotics. All analyses were performed at least in duplicate. The maximum error in the analyses was less than 5%. The dry matter digestibility (DMD) was calculated as: 1–(g dry matter in faeces/g dry matter ingested). The caecal pools of CAs were calculated as the concentration of each acid (μmol $g^{-1}$) multiplied by the weight of the caecal content (Table 2). The values were extrapolated to complete intake of indigestible carbohydrates (4.8 g) and thus corrected for the small amounts of feed residues. Weight gain was calculated per gram consumed

*Table 2.* Carboxylic acids in the hindgut of rats fed diets containing inulin supplemented with *Lactobacillus salivarius* (UCC500), *Bifidobacterium lactis* (Bb-12), *Lactobacillus rhamnosus* (GG) or a mixture of the three probiotics

| | Inulin (%)[b] | +UCC500 (%) | +Bb-12 (%) | +GG (%) | +Mixture (%) |
|---|---|---|---|---|---|
| Caecum | | | | | |
| Formic | 1±0 | 1±0 | 1±0 | 2±0*** | 2±0*** |
| Acetic | 50±2 | 57±2 | 51±3 | 52±1 | 57±1 |
| Propionic | 27±2 | 18±1*** | 26±2 | 26±1 | 22±1 |
| Butyric | 14±1 | 8±1** | 11±2 | 9±1* | 7±0** |
| Lactic | 3±0 | 6±1* | 7±1** | 5±0 | 4±0 |
| Succinic | 3±1 | 8±1*** | 1±0 | 3±0 | 5±1 |
| Minor[c] | 2±0 | 2±0 | 3±1 | 3±1 | 3±0 |
| Total[d] ($\mu$mol g$^{-1}$) | 79.1±3.6 | 74.1±5.1 | 75.5±10.8 | 52.7±5.3* | 41.5±9.1** |
| Total[d] ($\mu$mol) | 155±13 | 264±29* | 356±46*** | 175±30 | 135±17 |
| Proximal colon | | | | | |
| Formic | 2±1 | 4±1 | 7±1*** | 8±1*** | 7±1** |
| Acetic | 49±3 | 49±2 | 39±2** | 40±2** | 45±2 |
| Propionic | 18±2 | 9±1** | 14±2 | 11±1* | 12±1* |
| Butyric | 8±1 | 6±1 | 8±2 | 5±1 | 4±1 |
| Lactic | 20±4 | 22±2 | 28±4 | 29±3 | 27±3 |
| Succinic | 1±0 | 8±1*** | 2±1 | 5±1*** | 3±1 |
| Minor[c] | 2±0 | 2±0 | 2±0 | 2±0 | 2±0 |
| Total[d] ($\mu$mol g$^{-1}$) | 66.6±4.1 | 75.8±3.3 | 62.0±3.7 | 58.8±2.0 | 50.4±3.6** |
| Distal colon | | | | | |
| Formic | 2±1 | 4±1 | 4±1 | 5±1* | 4±0 |
| Acetic | 43±3 | 49±2 | 42±2 | 47±2 | 52±2* |
| Propionic | 26±1 | 15±2*** | 18±1*** | 15±1*** | 19±2** |
| Butyric | 20±2 | 11±2** | 10±1*** | 8±2*** | 10±1** |
| Lactic | 6±2 | 13±2 | 21±3*** | 21±2*** | 12±1 |
| Succinic | 1±1 | 6±1*** | 3±1 | 3±1 | 2±1 |
| Minor[c] | 2±0 | 2±0 | 2±0* | 1±0** | 1±0** |
| Total[d] ($\mu$mol g$^{-1}$) | 98.8±5.4 | 101.3±3.9 | 78.8±10.1 | 84.4±5.3 | 84.4±6.8 |

Values are means±SEM ($n=7$).

[b]% of total carboxylic acids.

[c] Isobutyric, valeric, isovaleric, caproic and heptanoic acid.

[d] Formic, acetic, propionic, butyric, lactic, succinic, isobutyric, valeric, isovaleric, caproic and heptanoic acid.

Asterisks indicate significant differences from rats fed diets without bacteria: ***$p<0.001$, **$p<0.01$, *$p<0.05$.

feed, while caecal content, faecal wet and dry weights were calculated per gram ingested inulin (Table 3).

Minitab® statistical software (Release 13.32) was used for statistical evaluation of the results, and a general linear model (ANOVA) followed by

*Table 3.* Body weight gain, caecal content, pH and tissue weight, faecal dry weight and dry matter digestibility (DMD) in rats fed diets containing inulin and inulin with *Lactobacillus salivarius* (UCC500), *Bifidobacterium lactis* (Bb-12), *Lactobacillus rhamnosus* (GG) or a mixture of the three probiotic strains

| Diet | Body weight gain (g g$^{-1}$) | Caecal content (g g$^{-1}$) | Caecal pH | Caecal tissue weight (g) | Faecal dry weight (g g$^{-1}$) | DMD |
|---|---|---|---|---|---|---|
| Inulin | 0.29±0.02 | 0.33±0.04 | 6.8±0.1 | 0.77±0.06 | 0.59±0.03 | 0.95±0.00 |
| +UCC500 | 0.30±0.02 | 0.51±0.06 | 6.6±0.2 | 0.79±0.05 | 0.45±0.06 | 0.97±0.00* |
| +Bb-12 | 0.29±0.02 | 0.61±0.13 | 6.1±0.2* | 0.85±0.07 | 0.40±0.05 | 0.98±0.00** |
| +GG | 0.21±0.02* | 0.73±0.15* | 6.2±0.1* | 1.02±0.09* | 0.45±0.08 | 0.97±0.01** |
| +Mixture | 0.25±0.02 | 0.66±0.10 | 6.4±0.2 | 0.86±0.06 | 0.43±0.05 | 0.97±0.00* |

Values are means±SEM ($n=7$).

Asterisks indicate significant differences from rats fed diets without any probiotic bacteria: **$p<0.01$, *$p<0.05$.

Dunnett's procedure was used to compare the test diets ($p < 0.05$) with the reference diet.

## Results

### *Body weight gain, caecal content and pH, and faecal weights*

The rats remained in good condition throughout the study. The daily feed intake was almost complete and ranged between 10.7 and 11.7 g dw per day. With GG in the diet, the rats gained less in body weight per gram of feed intake (0.21 g $g^{-1}$ versus 0.29 g $g^{-1}$ for the control diet, $p < 0.05$) (Table 3).

The caecal content increased when probiotics were included in the diet, from 0.33 g $g^{-1}$ inulin consumed in rats fed the reference diet to $0.63 \pm 0.05$ g $g^{-1}$ inulin consumed in rats fed the diets containing probiotics (Table 3). The highest caecal content was obtained in rats fed GG (0.73 g $g^{-1}$ consumed fibre, $p < 0.05$). Caecal pH decreased (from 6.8 to an average of 6.3) when probiotics were added to the diets and Bb-12 and GG supplementation caused the most pronounced decrease (to 6.1 and 6.2, respectively, $p < 0.05$). Rats consuming probiotics also had a higher caecal tissue weight (mean 0.88 g versus 0.77 g without any added probiotics), with GG providing the highest weight (1.02 g, $p < 0.05$). No effects on faecal dry weights were seen with probiotics, while the DMD increased ($p < 0.05$–$0.01$). There was a correlation between caecal content and caecal tissue weight ($r = 0.78$, $p < 0.05$), but not between caecal pool of CAs and caecal tissue weight ($r = 0.40$).

### *Carboxylic acids*

*Caecal pool size.* The caecal pool of CAs was higher when the rats were fed diets containing Bb-12 ($p < 0.001$) and UCC500 ($p < 0.05$) compared with inulin only (Table 2).

*Concentrations and proportions of carboxylic acids.* The caecal concentrations of CAs were lower in rats fed GG ($p < 0.05$) and the mixture of probiotics ($p < 0.01$) than inulin only (Table 2). The same tendencies were seen in the proximal and distal colon, although they were only significant in the proximal colon of rats fed the mixture. No effects were seen with the other probiotics at any place in the hindgut. A correlation was found between distal and faecal concentrations of acetic, propionic and butyric acid ($r = 0.99$–$1.00$, $p < 0.05$, data not shown), although the faecal concentrations were somewhat higher than those in distal colon. However, when lactic acid was included in the calculation there was no longer any significant correlation ($r = 0.86$, $p = 0.14$).

The proportion of acetic acid in the caecum and distal colon was generally not affected by supplementation with probiotics, an exception being rats fed the mixture of bacteria. These rats had a higher proportion of acetic acid in the distal colon ($p < 0.05$) (Table 2). Further, there was a lower proportion of acetic acid in the proximal colon when rats were fed Bb-12 and GG. The proportion of propionic acid was lower all along the hindgut when rats were fed UCC500 ($p < 0.05$–$0.001$) compared with inulin only. No effects were seen on this acid in the caecum of rats fed the other probiotics, while the proportions were generally lower in the proximal and distal colon. The proportion of butyric acid was lower in the caecum and the distal colon when the rats were fed probiotics ($p < 0.05$–$0.01$), the only exception being in the caecum of rats fed Bb-12. The proportion of lactic acid was generally higher, but reached significance only in the caecum of rats fed UCC500 ($p < 0.05$) and Bb-12 ($p < 0.01$) and in the distal colon of rats fed Bb-12 ($p < 0.001$) and GG ($p < 0.001$). The proportion of succinic acid was higher ($p < 0.001$) all along the hindgut when the rats were fed UCC500 than in rats fed inulin only. No other effects were seen on this acid, except for an increase in the proportion in the proximal colon when the rats were fed GG. Notably high proportions of formic acid were found in the proximal colon of rats fed probiotics, an exception being UCC500.

## Discussion

The present study shows that it is possible to modify the formation of CAs in rats fed inulin with a high solubility, by supplementing the diet with probiotic bacteria. Different types of probiotic strains had different effects.

The caecal pool of CAs in rats fed UCC500 and Bb-12 was higher than with inulin only. Concerning Bb-12, there was a similar increase in acetic, propionic and butyric acid pools and the proportion of lactic acid increased. Fructo-oligosaccharides, such as inulin and oligofructose, have been reported to be preferred substrates for bifidobacteria and increase the number of these bacteria in

the faeces when fed to humans (15, 21) and rats (22). This may explain the considerably higher caecal formation of CAs in rats fed Bb-12 than inulin only, which gave low amounts of CAs compared with many other substrates (10). In a previous study on inulin with low solubility the caecal formation of CAs also increased when rats were fed Bb-12, but in that study there was a specific increase in propionic acid (13). One reason for the difference could be that the insoluble type of inulin used gave considerably lower caecal amounts of CAs including propionic acid. In the present study the proportion of propionic acid was already very high without the addition of Bb-12 and it might be questioned whether it is possible to increase the proportion further, or whether a maximum had already been reached. Thus, the substrate is of crucial importance for CA formation, which was also shown in a previous study (13). This is important to keep in mind when designing food products with specific health effects, not least when probiotics are added. UCC500 gave a higher caecal pool of CAs when fed to rats, which to some extent could be ascribed to succinic acid. This is interesting, as this acid has been associated with decreased/changed bacterial activity, such as after some types of antibiotic treatment (23). Low numbers of certain bacteria, such as bacteroides and propionibacteria, have been reported to decrease the formation of propionic acid, while that of succinic acid increased (24). It may be speculated whether UCC500 together with inulin modifies the composition/activity of certain organisms, resulting in an increase in the amount of succinic acid formed. The production of propionic acid from succinic acid by certain microorganisms has been shown to be dependent on vitamin $B_{12}$ (25), and if the $B_{12}$-producing microorganism are reduced by, for example, antibiotic treatment, the synthesis of this vitamin would decline. In a previous study on inulin with a low solubility, no increase in the CA formation and only minor effects on the proportions of CAs could be seen in the hindgut of rats when UCC500 was added to the diet. These discrepancies, with different types of inulin, indicate that the outcome of the CA formation depends to a great extent on physicochemical properties of the substrate and can also be modified by addition of probiotics.

The caecal concentrations of CAs were lower with GG and the mixture of probiotic strains in the diet than with the reference diet. This was partly due to the higher weight of the caecal content. Another explanation could be an increased adhesion of GG to the mucosa, causing an increased absorption of CAs.

The change in caecal pH in rats fed inulin was in accordance with findings in previous studies (10). However, when Bb-12 and GG were included in the diet, the pH was significantly lower (6.1 and 6.2, $p < 0.05$) than expected from the CA concentration. The reason for this is not known, but might be ascribed to components other than CAs. Ammonia, for example, is formed during colonic fermentation of protein (26) and it may be speculated that smaller amounts are formed in the presence of GG and Bb-12. Bifidobacteria have been shown to decrease the number of ammonia-producing bacteria such as clostridia, resulting in a decreased production of ammonia and possibly explaining the low pH (27). It may be that GG and Bb-12 are especially prone to inhibit the growth of pathogens. Further, subjects with impaired liver function have an increased production of ammonia (27), and it has been shown that probiotics can decrease this production (28).

When the rats were fed probiotics the proportion of butyric acid was lower and that of lactic acid generally higher in both the caecum and the distal colon. Further, the proportion of propionic acid was lower in the distal colon than in rats fed inulin without probiotics. Both *Lactobacillus* and *Bifidobacterium* have been reported to form high amounts of lactic acid (29) and when the number of these bacteria increases others are suppressed, leading to a modified profile of CAs and increased proportions of lactic acid. These probiotic effects were especially seen in the distal part of the colon. Similar results have been obtained in a previous study and the property of a probiotic strain to form a specific CA seemed to depend, to a great extent, on the amount of substrate available (13). Thus, with limited amounts of substrate in relation to the number of bifidobacteria there was an increased formation of lactic acid, while at abundant amounts the properties of the substrate seem to regulate the CA formation.

Rats fed GG had the highest caecal content and tissue weight. The increased weight of the caecal

content may be due to an increased formation of CAs, increased dry matter content or the addition and/or proliferation of bacteria as such. However, since the caecal concentrations of CAs were lower and the DMD values higher in rats fed GG compared with those fed inulin only, this could not be an explanation. Further, the amount of GG added was only 4 g $kg^{-1}$ diet (corresponding to 0.05 g per day per rat) and could thus not contribute to the increased caecal content per se. A more likely explanation could be that GG increased the bacterial activity/metabolism, leading to an increase in bacterial mass and thus also higher amounts of water in the caecum (30), which in turn may explain the higher caecal tissue weight. This has been found in previous studies (9) and explained by increased mechanical stress. A correlation between caecal content and tissue weight was also found ($r = 0.78$, $p < 0.05$) in this study, whereas there was no correlation between the pool of CAs and caecal tissue weight ($r = 0.40$, $p < 0.05$). Rats fed GG had significantly higher caecal tissue weights than rats fed the reference diet, but similar caecal pools of CAs. One explanation for this fact could be that this strain in some way increases the absorption of CAs. In this respect, probiotic bacteria have been shown to increase calcium uptake in human intestinal-like Caco-2 cells (31). The caecal tissue weight and content were only significantly increased in rats fed GG, although the same tendencies were seen in rats fed the other probiotic strains. A longer study duration could have shown more pronounced differences. However, the rat model used has been shown to yield stable fermentation of different types of dietary fibre after an adaptation time of 5–7 days (32). This has been confirmed by others, concerning both total fermentation of dietary fibre and the profile of CAs formed from resistant starch (33, 34). To the authors' knowledge, no studies have investigated whether adaptation time is of importance when probiotics are included in the diet.

There are suggestions that the microflora may affect lipid metabolism and that some probiotic strains may offer protection against obesity if included in the diet (35, 36). The present study lasted for 13 days only, and the energy intake was similar for all rats. Nevertheless, the weight gain was lower in rats fed GG ($p < 0.05$) than in rats fed the reference diet. The reason for the lower weight gain is not known, but the caecal concentrations of CAs were lower in rats fed this strain. Ley et al. (35) found that there were considerable differences in the composition of the gut microbiota in obese mice compared with lean mice, suggesting that intentional manipulation of the gut microbiota may be useful for regulating energy balance in obese individuals.

In conclusion, it is possible to modify the formation of CAs by combining inulin with probiotics. In the caecum of rats the probiotics had effects on total concentrations and pools of CAs, while the proportions were affected to a great extent in the distal part of the colon. The weight gain was lower in rats fed GG than inulin only, which needs to be elucidated further. The caecal pH in rats fed GG and Bb-12 was lower than expected from the concentration of CAs, indicating that these strains decrease the formation of alkaline components, e.g. ammonia. None of the probiotics investigated increased the proportion of butyric acid and propionic acid at any place in the hindgut of rats.

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