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Inhibition of *Salmonella* typhimurium by medium-chain fatty acids in an *in vitro* simulation of the porcine cecum

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ABSTRACT

Salmonella typhimurium was responsible for more than half of the reported cases of human salmonellosis in Belgium in 2007 and was the predominant serovar isolated from slaughter pig carcasses. To lower the *Salmonella* contamination of pork meat, measures can be taken at the primary production level, e.g. by reducing the shedding of *Salmonella* through the use of feed additives such as medium-chain fatty acids (MCFAs). An *in vitro* continuous culture system, simulating the porcine cecum, was developed for investigating the effect of MCFAs (sodium caproate, sodium caprylate and sodium caprinate) on the pig intestinal microbial community. The system was monitored by plating on selective media, PCR-DGGE and HPLC analysis of fermentation products. An inoculated *S. typhimurium* strain could be maintained by the system at a population size of about 5 $\log_{10}$ cfu/mL. By the addition of 15 mM caprylate, significant reductions of coliforms and *Salmonella* counts by 4.69 $\log_{10}$ units (95% confidence interval: 4.19–5.18) could be achieved, while other bacterial populations were clearly less affected. This concentration seems economically feasible in pig feed, provided that the substance can reach the cecum without being absorbed. Thus, caprylate, for example in the form of encapsulated beads or as triacylglycerol oil, might have potential as a *Salmonella*-reducing additive in pig feed.

1. Introduction

In 2007, salmonellosis was the second most commonly reported zoonotic disease in the European Union (EFSA, 2009). A quantitative microbial risk assessment (QMRA) from farm-to-fork that was developed to assess the risk for human salmonellosis through consumption of fresh minced pork meat in Belgium estimated the mean number of annual cases as 20,513 (Bollaerts et al., 2009). In Belgium, *Salmonella* typhimurium was responsible for 56.2% of the reported cases of human salmonellosis in 2007 (Anonymous, 2007) and was the predominant serovar (63%) isolated from slaughter pig carcasses (EFSA, 2009). Several risk management strategies aiming to reduce the *Salmonella* contamination in the pork production have been suggested (Goldbach and Alban, 2006; Ojha and Kostrzynska, 2007) such as the use of acidified feed for slaughter pigs. If the gastrointestinal tract (GIT) of the pig can be made an unfavourable environment for *Salmonella* growth, e.g. through the use of feed additives, decreased pathogen shedding will result leading to a reduced *Salmonella* load of the pigs and carcasses. Medium-chain fatty acids (MCFAs) are proposed as a valuable alternative to in-feed antibiotics, used for growth promotion, and even for the preventive and curative treatment of gastrointestinal diseases (Decuypere and Dierick, 2003). For caprylic acid (=octanoic acid), an inhibitory effect on *Salmonella* was obtained in standard bacteriological media (Skrivanova et al., 2004). Although MCFA preparations are commercially available and used in pig farming, their

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effect on the gut bacterial ecosystem remains largely unknown, especially in the hindgut.

In this study, the effect of selected MCFAs on the GIT microbial community was evaluated using an *in vitro* model mimicking the conditions in the porcine cecum, with special emphasis on their inhibitory activity against *S. typhimurium*.

2. Materials and methods

2.1. Bacterial strain

S. typhimurium MB 2184 was originally isolated from pig colon and belongs to phage type DT104. The strain was stored at −80 °C in brain–heart infusion medium (BHI; Oxoid, Basingstoke, UK) supplemented with 15% glycerol as a cryoprotectant (w/v) (Botteldoorn et al., 2003). The strain was cultivated for 24 h on Trypton Soy Agar or on Brain Heart Infusion agar (BHI; Oxoid). Then one colony was picked and transferred to 10 mL of Mueller-Hinton broth (Oxoid) which was incubated overnight at 37 °C. The culture vessel was inoculated with this overnight-grown culture.

2.2. Cecal culture conditions

Cecal growth conditions were mimicked in an *in vitro* system, which operated as a continuous culture system. The fibre and mucin-containing incubation medium for porcine cecum simulation was as described by Dierick et al. (2002), with some modifications. The fibre content was halved to 3.8% and the concentration of the phosphate buffer was lowered to 0.01 M. The medium was acidified to pH 2.0 and kept in autoclaved 13 L Pyrex bottles at 4 °C under constant agitation to prevent fibre precipitation. Equipment for continuous culture consisted of a BioFlo110 unit (New Brunswick Scientific, Edison, NJ, USA) with a 1.3 L fermentor vessel. Fresh medium was added via a peristaltic pump at a constant rate of about 1.8 mL/min and spent culture liquid was wasted at the same rate to maintain a constant working volume of 500 mL (corresponding to 4.6 h retention time). The pH was kept constant at 6.2 with 5N NaOH and the temperature was maintained at 37 °C. Temperature and pH control were performed on-line and moderate agitation at 200 rpm was performed. Anaerobic conditions were maintained by flushing the headspace of the vessel with a nitrogen–carbon dioxide mixture (80 and 20%, respectively) at a flow rate of 20 mL/min. The fermentation was started with pooled cecal content as inoculum. Directly after commercial slaughter, cecal content from twelve pigs at slaughter age (originating from seven different farms) was pooled, homogenized with a kitchen blender, divided in aliquots of 15 mL supplemented with 15% (w/v) glycerol and frozen at −80 °C. *Salmonella* was not detected in the pooled cecum content as black colonies were not observed upon plating on Xylose–Lysine–Desoxycholate agar (XLD). After inoculation with an aliquot of pooled cecum content, the fermentor was operated in batch mode for 24 h. Continuous culture was then started by switching on the peristaltic pumps. Unless otherwise stated, 10 mL of *S. typhimurium* MB 2184 grown overnight in Mueller-Hinton broth (Oxoid) was added after the switch to continuous culture.

2.3. Addition of medium-chain fatty acids

After *S. typhimurium* reached steady-state conditions, the blank fresh medium stock was replaced by a stock containing 15 mM of one of the following MCFAs (sodium caproate, sodium caprylate, sodium caprinate); all of Sigma–Aldrich (St. Louis, MO, USA). From that moment on, fermentor liquid was gradually replaced by medium containing one of the MCFAs. Sodium caprylate was also evaluated at a concentration of 5 and 30 mM. Separate fermentation experiments were performed for each condition.

2.4. Analyses

Bacterial populations were monitored by plating on selective media: Reinforced Clostridial Medium (RCM) for total anaerobes, Trypton Soya Agar for total aerobes, de Man, Rogosa & Sharpe medium (MRS) for lactic acid bacteria, Slanetz and Bartley medium for streptococci, MacConkey agar for coliforms, and XLD for *Salmonella*. All media were from Oxoid. For Bifidobacteria, modified Trypticase-phytone-yeast extract (MTPY) was used (Rada and Petr, 2000). For some experiments, a theoretical dilution curve in case of no *Salmonella* growth was calculated based on the hydraulic retention time and *Salmonella* count for the sample taken prior to MCFA addition. Cultivation-independent community analysis was done by 16S rDNA PCR denaturing gradient gel electrophoresis (PCR-DGGE), as described by Boon et al. (2000). PCR-DGGE patterns were numerically compared using the BioNumerics software package version 4.6 (Applied Maths, St. Martens-Latem, Belgium). Organic acids formed during fermentation (formic, acetic, propionic, butyric and lactic acid) were quantified by HPLC analysis using an Aminex HPX-87H column (BioRad Laboratories, Hercules, CA, USA), as described before (Van Coillie et al., 2007).

2.5. Statistical analysis

A two-sample two-sided *t*-test was performed to assess whether the bacterial counts (in $\log_{10}$ cfu/mL) differed before and after the replacement of the blank medium by medium containing one of the MCFAs. Counts were used after reaching an equilibrium (approximately 3 days after replacement of the medium). The *t*-test was performed assuming equality of variances. The significance level α was set at 0.05. All analyses were done in R version 2.7.2 (2008-08-25).

3. Results

In a first experiment, simulating the porcine cecum without any MCFA treatment (blank), the microbial community was left to stabilize for 9 days in continuous culture before *S. typhimurium* MB 2184 was added at a final

concentration of 4.3 $\log_{10}$ cfu/mL. From 42 h after inoculation onwards, a stable population size of this strain was reached, averaging 5.2 ± 0.2 (standard deviation) $\log_{10}$ cfu/mL for at least four operating days (data not shown). In a second blank experiment, *S. typhimurium* MB 2184 was added immediately after switching to continuous culture (data not shown). A mean population density of 4.8 ± 0.8 $\log_{10}$ cfu/mL for at least eight operating days was obtained. Other mean population sizes were 8.7 ± 0.3, 8.5 ± 0.4, 5.5 ± 0.6, 7.2 ± 0.8 $\log_{10}$ cfu/mL for total anaerobes, lactic acid bacteria, coliforms and streptococci, respectively. Organic acid concentrations were 0.7 ± 0.5, 11 ± 2, 94 ± 13, 14 ± 3 and 26 ± 4 mM for lactic, formic, acetic, propionic and butyric acid, respectively. In both experiments, a stable PCR-DGGE pattern (data not shown) was obtained after day 3 of continuous culture.

The effect of different MCFAs was evaluated in the *in vitro* cecum model upon achieving steady-state conditions in the microbial population. Mean changes in bacterial populations by the addition of MCFAs are given in Table 1. In Fig. 1, the bacterial populations and organic acid production are depicted for the addition of 15 mM sodium caprylate (=sodium octanoate). After 4 days of blank operation, gradual replacement of the medium in the fermentor vessel by 15 mM sodium caprylate-containing medium resulted in a rapid and significant decrease of the *Salmonella* population by on average 4.69 $\log_{10}$ units (from 6.80 to a stable value of 2.11 ± 0.10 $\log_{10}$ cfu/mL after 3.5 days) and the coliform population by on average 3.62 $\log_{10}$ units (from 6.63 to 3.01 ± 0.37 $\log_{10}$ cfu/mL) populations. Small but significant changes were noticed for total anaerobes and lactic acid bacteria (decreases by respectively on average 0.75 and 0.39 $\log_{10}$ units). The fermentation pattern changed after the addition of sodium caprylate: propionic, formic and butyric acid production decreased, while lactic acid production increased. Acetic acid concentrations gradually increased in the course of the experiment. When the feed was switched back to blank medium (after 11 days of operation), no concomitant increase of the *Salmonella* or coliform population was observed while propionic, butyric and lactic acid production returned to their initial levels. Cultivation-independent microbial community analysis via PCR-DGGE for this experiment is shown in Fig. 2. At day 1 (directly after the start of continuous culture) equilibrium was obviously not reached yet, as shown by a community pattern quite distinct from all later samples. From day 3 on, DGGE profiles were all fairly similar, indicating a stable ecosystem. During sodium caprylate treatment, one band (indicated by the arrow in Fig. 2) seemed to be more prominent in the DGGE pattern. On day 15, i.e. 4 days after adding blank medium again, the pattern became as before the switch to sodium caprylate-containing medium.

The fermentation patterns for 15 mM sodium caproate (=sodium hexanoate) are shown in Fig. 3. For the first 7 days, the cecum simulation was run under blank medium conditions, after which the fermentor liquid was gradually replaced by 15 mM sodium caproate-containing medium. The treatment was maintained until the end of the experiment at day 14. The high counts of *Salmonella* and coliforms on day 3 were due to the large inoculum size of strain MB 2184 and the fact that no equilibrium was reached

Table 1
Effect of addition of MCFA on the *in vitro* porcine cecum simulation. Mean changes in bacterial populations in comparison with the initial steady-state bacterial populations, as determined by plating are given after the onset of MCFA addition[a].

MCFA	Conc. (mM)	Bacterial population ($\log_{10}$ cfu/mL)						
		Total anaerobes	Lactic acid bacteria	Total aerobes	*Salmonella*	Coliforms	Bifidobacteria	Streptococci
Sodium caprylate	5	−0.80 [−1.35; −0.24]*	−0.47 [−1.92; 0.98]	−0.44 [−0.99; 0.12]	−2.37 [−2.88; −1.86]***	−2.50 [−2.85; −2.15]***	−0.76 [−1.70; 0.18]	0.51 [−0.66; 1.70]
	5	0.73 [−0.12; 1.59]	0.9 [0.49; 1.24]**	–	−1.95 [−2.20; −1.70]***	−1.08 [−1.52; −0.64]**	–	−0.29 [−2.14; 1.56]
	15	−0.75 [−1.41; −0.11]*	−0.39 [−0.62; −0.16]*	−2.96 [−5.68; −0.24]*	−4.69 [−5.18; −4.19]***	−3.62 [−5.48; −1.77]*	–	–
	30	−1.22 [−7.08; 4.63]	−0.26 [−13.36; 12.85]	−1.44	−3.81 [−3.96; −3.65]***	−3.93 [−4.95; −2.90]*	–	–
Sodium caproate	15	−0.09 [−0.68; 0.50]	−0.42 [−0.70; −0.15]*	−0.25 [−1.21; 0.71]	−0.70 [−1.26; −0.12]*	−0.46 [−1.86; 0.95]	−0.10 [−0.60; 0.38]	−0.87 [−2.42; 0.67]
Sodium caprinate	15	0.04 [−0.69; 0.78]	0.44 [−0.49; 1.38]	–	−0.95 [−2.74; 0.84]	−0.84 [−2.40; 0.72]	–	−0.96 [−2.47; 0.55]

–, not determined.
[a] Positive values indicate an increase in bacterial concentrations, negative values a decrease. The 95% confidence interval (between square brackets) is given, where appropriate.
* Significance level $0.01 < p < 0.05$.
** Significance level $0.001 < p < 0.01$.
*** Significance level, $p < 0.001$.

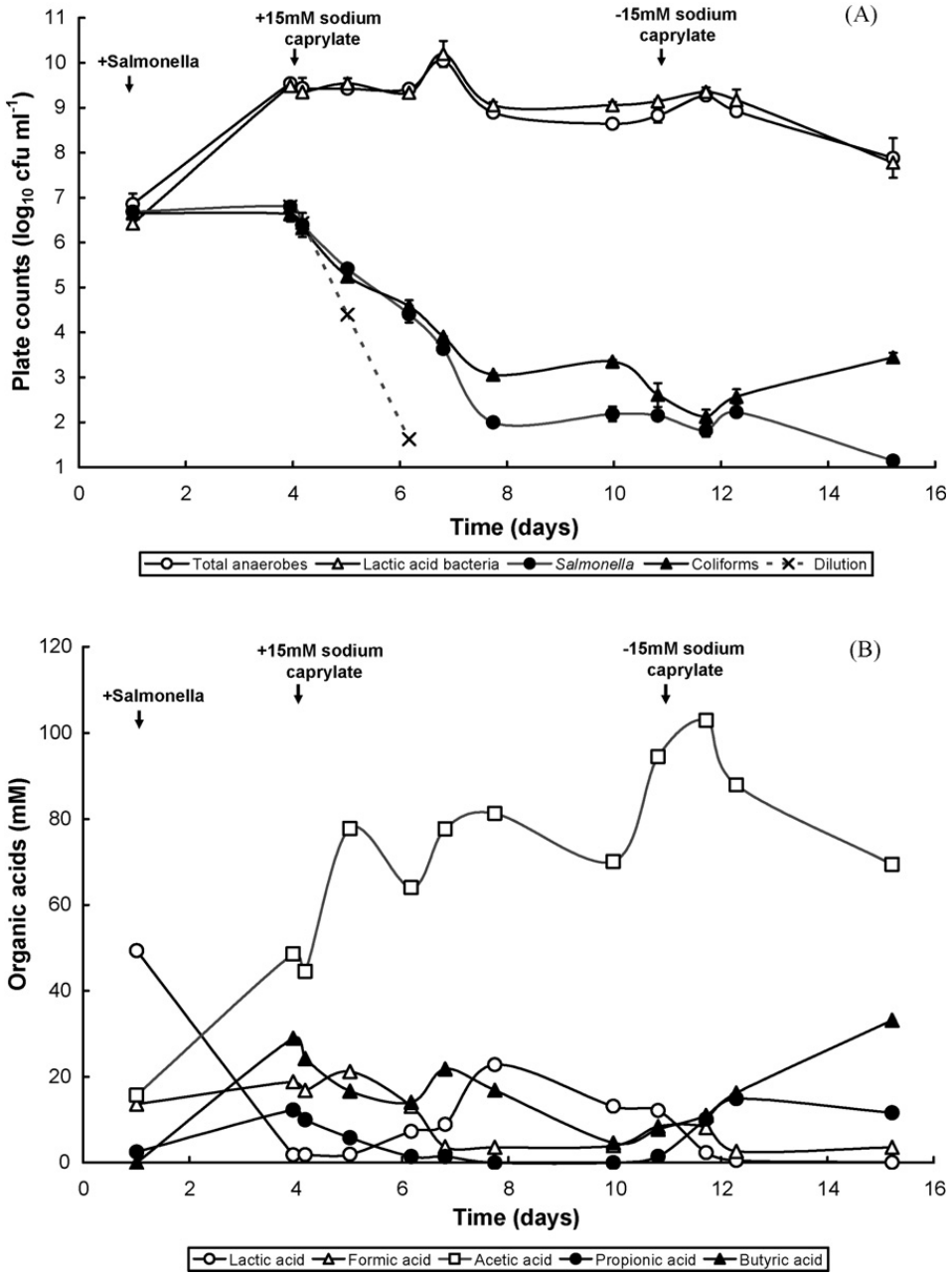

Fig. 1. Effect of 15 mM sodium caprylate on the *in vitro* porcine cecum simulation. Bacterial populations as determined by plating (A) and organic acid production (B) are shown as a function of time. The start of continuous culture, inoculation with *Salmonella*, addition and withdrawal of sodium caprylate are indicated on the graphs.

yet at the time of sampling. From day 6 on and before the addition of 15 mM sodium caproate, bacterial counts and organic acid production remained rather steady. The mean *Salmonella* count was 4.62 ± 0.22 $\log_{10}$ cfu/mL. After addition of 15 mM sodium caproate, no relevant effects on total microbial counts (Table 1) or produced organic acids were observed. *Salmonella* counts were reduced significantly by 0.70 $\log_{10}$ units and averaged 3.92 ± 0.17 $\log_{10}$ cfu/mL upon reaching stable conditions after 3–7 days. Also changes in PCR-DGGE patterns were not introduced (data not shown).

The effect of 15 mM sodium caprinate (=sodium decanoate) addition on the bacterial populations was not very pronounced: streptococci, *Salmonella* and coliform counts decreased during 2–3 days after addition but then stabilized, while total anaerobes and lactic acid bacteria remained steady. Propionic acid production decreased rapidly after sodium caprinate addition, while concentrations of lactic, formic, acetic and butyric acid remained at the same level (Fig. 4). Using PCR-DGGE, it was clear that bacterial community patterns differed in several bands after addition from patterns before addition (data not shown).

Since sodium caprylate was the most effective MCFA in reducing *Salmonella* at a concentration of 15 mM, a higher (30 mM) and lower (5 mM) concentration were evaluated. In comparison with 15 mM sodium caprylate, very similar observations could be made using 30 mM sodium caprylate (Table 1). Mean *Salmonella* counts decreased significantly by

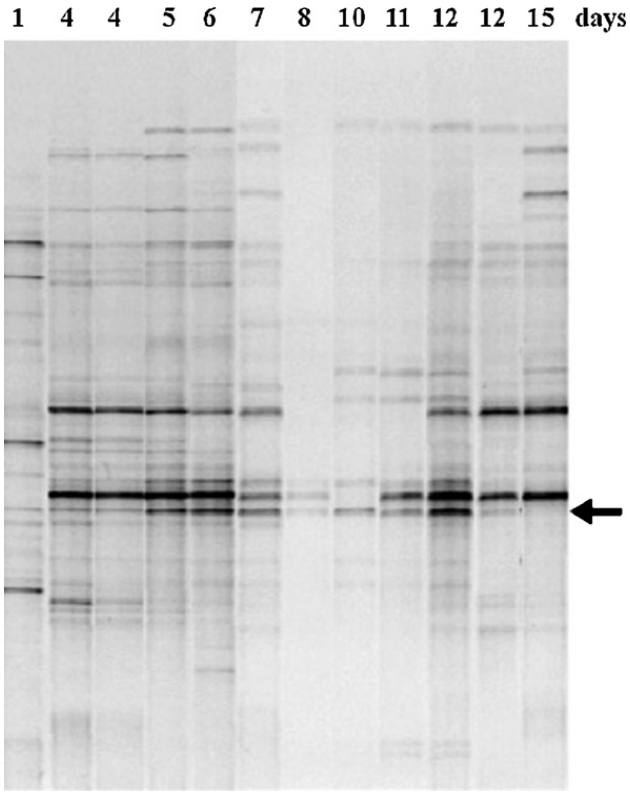

Fig. 2. Denaturing gradient gel electrophoresis patterns of the microbial populations in the experiment described in Fig. 1. The arrow indicates the position of a band that seems to be correlated with the presence of sodium caprylate in the incubation medium.

3.81 $\log_{10}$ units (from 5.72 to 1.91 ± 0.01 $\log_{10}$ cfu/mL) and mean coliform counts by 3.93 $\log_{10}$ units (from 5.88 to 1.95 ± 0.07 $\log_{10}$ cfu/mL). Likewise, propionic and butyric acid production decreased rapidly after sodium caprylate addition while lactic concentration increased. The effect of 5 mM sodium caprylate was evaluated in duplicate. In the first experiment, *Salmonella* counts significantly decreased by 2.37 $\log_{10}$ units (from 6.03 to 3.66 ± 0.19 $\log_{10}$ cfu/mL) and coliform counts by 2.50 $\log_{10}$ units (from 6.30 to 3.80 ± 0.17 $\log_{10}$ cfu/mL) upon addition of 5 mM sodium caprylate. In the second experiment, *Salmonella* counts significantly decreased by 1.95 $\log_{10}$ units (from 4.99 ± 0.06 to 3.04 ± 0.09 $\log_{10}$ cfu/mL) and coliform counts by 1.1 $\log_{10}$ unit (from 5.3 ± 0.2 to 4.2 ± 0.1 $\log_{10}$ cfu/mL) upon addition of 5 mM sodium caprylate. Counts for other bacterial groups remained relatively constant in both experiments (Table 1). The shift in fermentation profile, as observed with higher sodium caprylate concentrations, was less pronounced or even absent when a 5 mM dose was used (data not shown).

Considering the data of all experiments, the original pooled cecal content inoculum, derived from pigs at slaughter age, evolved in the *in vitro* model within 3–4 days after the onset of continuous culture into a relatively stable microbial ecosystem with stable fermentation characteristics, as observed from plating results, PCR-DGGE analysis and organic acid production. When operated under blank conditions, population densities of total anaerobic bacteria, lactic acid bacteria, *Salmonella*, and coliforms stabilized to a mean value of 8.7 ± 0.5, 8.1 ± 0.7, 5.3 ± 0.9, 5.6 ± 0.8 (mean of 8 fermentations) $\log_{10}$ cfu/mL, respectively. For total aerobes, streptococci and bifidobacteria stabilization to a mean value of 7.9 ± 1.1 (mean of 6 fermentations), 7.1 ± 0.7 (mean of 5 fermentations), and 8.90 ± 0.05 (mean of 3 fermentations) $\log_{10}$ cfu/mL, respectively was obtained. Mean organic acid concentrations of 8 fermentations were 2.2 ± 2.8, 11 ± 11, 84 ± 21, 17 ± 16 and 26.5 ± 3.2 mM for lactic, formic, acetic, propionic and butyric acid, respectively.

4. Discussion

The use of an *in vitro* model facilitates the study of the GIT microbial community and circumvents animal-to-animal variation encountered in animal trials. *In vitro* techniques can play an important role as fast screening methods for potential antimicrobial feed additives and feed ingredients before *in vivo* trials are planned. A review is given in Williams et al. (2005). Existing continuous culture GIT models are often operated on particle free medium with a chemical composition that is quite distinct from actual chymus. In our model, we mimicked the physiological conditions in the porcine cecum as closely as possible through the use of a cellulose (non-digestible fibre) and mucin (polysaccharides isolated from the porcine stomach)-containing incubation medium. The cecum was specifically selected, because the large intestine (comprising the colon and cecum) is the most heavily colonized region of the GIT (Williams et al., 2001) and also because the cecum is the site where *Salmonella* is predominantly colonizing and invading.

The original pooled cecal content inoculum, derived from pigs at slaughter age, evolved in the *in vitro* model within 3–4 days after the onset of continuous culture into a relatively stable microbial ecosystem with stable fermentation characteristics, as observed from plating results, PCR-DGGE analysis and organic acid production. Bacterial counts as well as organic acid concentrations in our model corresponded well with literature data available for the porcine cecum (Mikkelsen et al., 2004). It should be remarked, however, that the same steady-state conditions were not always reached. Concentrations of formic (from 1 to 34 mM) and propionic acid (from 0 to 37 mM) varied most widely among different experiments, while within a single experiment typically much less variation was found. For repetitions of experiments, different PCR-DGGE profiles were obtained that typically showed similarity to other profiles in only a few (1–3) bands. It was not our aim to identify the PCR-DGGE bands but PCR was used to demonstrate the change in the fermentation pattern after the addition of MCFA. It has already been observed in continuous-flow bioreactor cultures simulating the chicken cecal bacterial community, that there are significant differences in bacterial concentrations and variations in bacterial diversity in replicate bioreactors initiated from the same stock, but no significant differences in bacterial concentrations of repetitive samples from the same bioreactor occurred (Crippen et al., 2008). Since *Salmonella* was not detected prior to the deliberate inoculation with *S. typhimurium* MB 2184, the *in vitro* simulation can be used to specifically monitor the

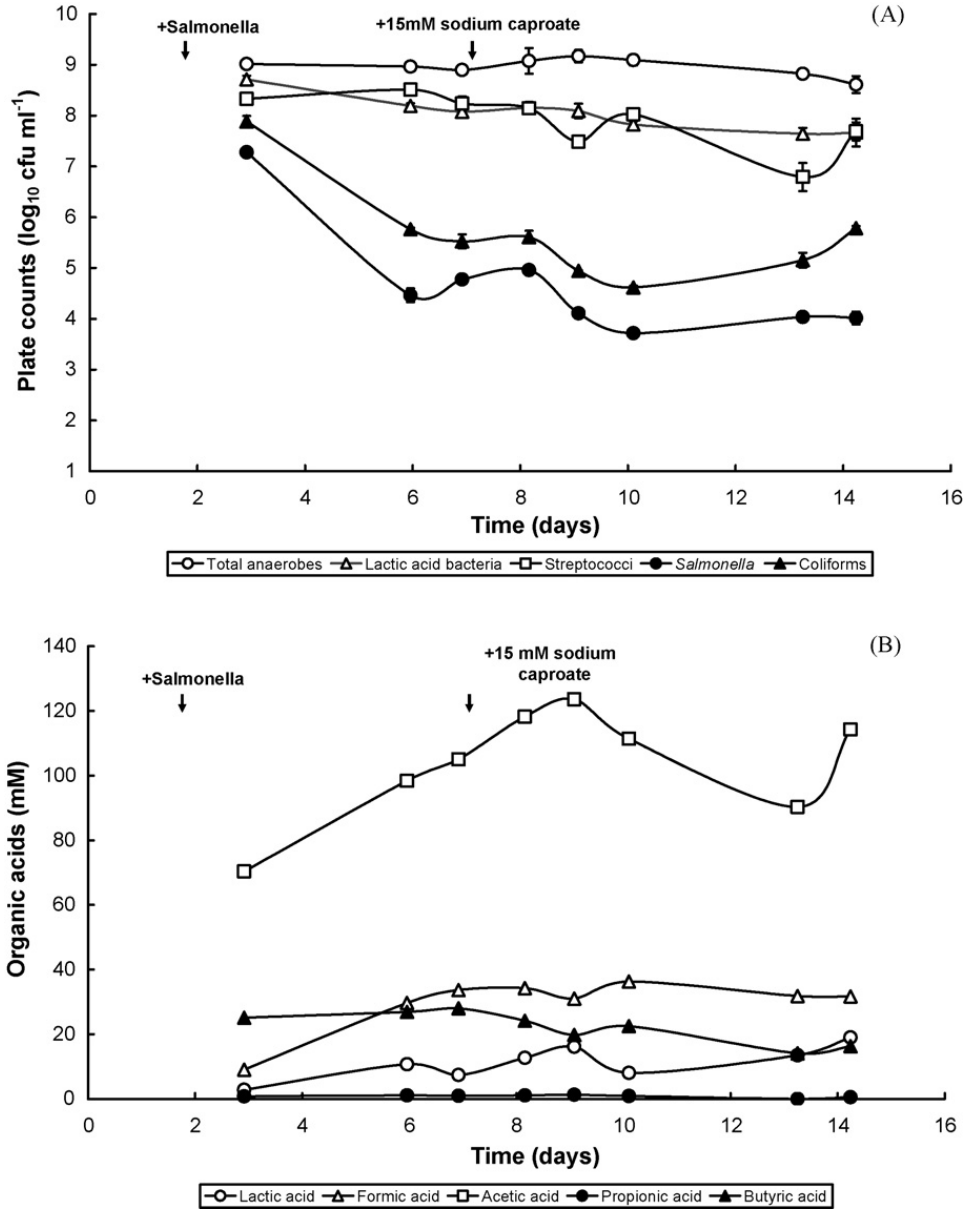

Fig. 3. Effect of 15 mM sodium caproate on the *in vitro* porcine cecum simulation. Bacterial populations as determined by plating (A) and organic acid production (B) are shown as a function of time. The start of continuous culture, inoculation with *Salmonella*, and the onset of sodium caproate addition are indicated on the graphs.

behaviour of the inoculated strain via enumeration on XLD agar plates.

The effect of MCFA on *Salmonella* and other microbial populations was investigated in the *in vitro* cecum model. At a dose of 15 mM (corresponding to a practically and economically feasible concentration in pig feed), caproate only had a small effect, while a clear *Salmonella*-inhibiting effect was found for caprylate. Earlier, Skrivanova et al. (2004) found caprylic acid to be the only *Salmonella*-inhibiting compound among the 15 fatty acids tested in liquid cultures. Inhibition (measured as a 50% decrease in glucose utilisation) by caprylic acid occurred at a dose of 6 mM, while caproic and capric acid did not show any effect at 30 mM for three *Salmonella* strains (*Salmonella enterica* serovar Enteritidis, *Salmonella* infantis and *S.* typhimurium). On the contrary, Van Immerseel et al. (2004a) found significant bacteriostatic effects on a *S. enteritidis* strain at concentrations as low as 5 mM (for caproic and capric acid) or 10 mM (for caprylic acid), as assessed by performing growth curves in Luria-Bertani broth. Our results seem to be more in line with those of Skrivanova et al. (2004), although strain-specific or methodological differences should not be ruled out. Also in our experiments, cecal flora was present while pure *Salmonella* cultures were used in both cited studies. In our experiments, doubling the caprylate dose from 15 to 30 mM did not result in an improved *Salmonella* inhibition. However, when comparing the actual decrease of *Salmonella* counts with the theoretical dilution curve, i.e. considering *Salmonella* as a non-growing particle in the continuous culture, for the different caprylate concentrations evaluated, a slower decrease is noticed when lower caprylate concentrations were applied. This could be

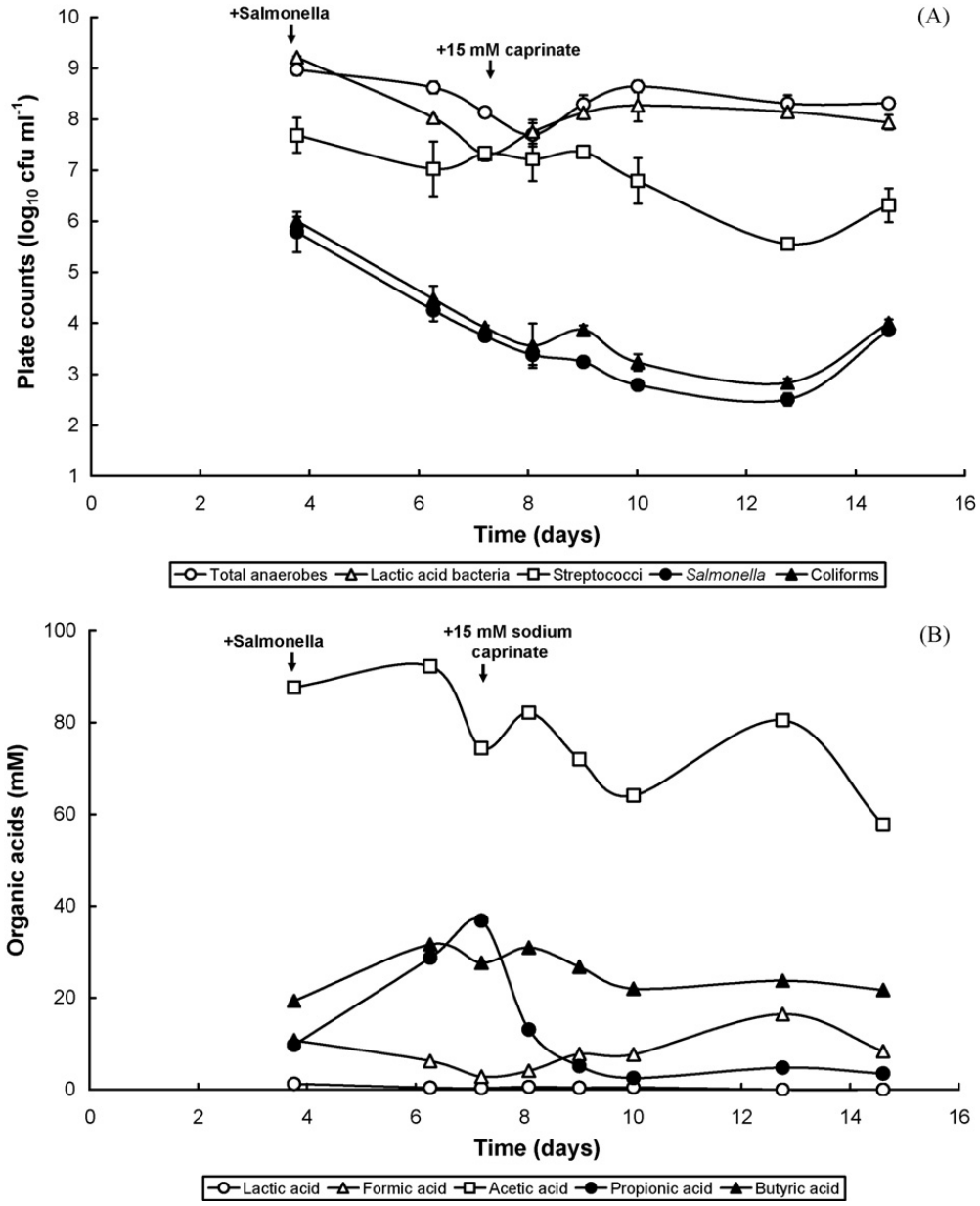

Fig. 4. Effect of 15 mM sodium caprinate on the *in vitro* porcine cecum simulation. Bacterial populations as determined by plating (A) and organic acid production (B) are shown as a function of time. The start of continuous culture, inoculation with *Salmonella*, and the onset of sodium caprinate addition are indicated on the graphs.

explained by a certain lag time before an effective caprylate concentration is reached in the fermentor (with lower concentrations in the fresh medium stock, this process takes longer) and by partial inhibition of *Salmonella*, i.e. decreased growth rate as opposed to zero growth. At a concentration of 5 mM, the inhibitory effect of sodium caprylate is clearly lower and more delayed, but nevertheless a significant reduction of *Salmonella* with about two $\log_{10}$ units was achieved. With none of the evaluated caprylate concentrations, *Salmonella* counts dropped below the detection level. This means that *S. typhimurium* MB 2184 is still able to grow in the system, albeit at a much reduced growth rate. The observed effect does not seem to be *Salmonella*-specific, since coliform counts showed a similar inhibition in response to caprylate (Fig. 1 and Table 1). Other bacterial groups, such as lactic acid bacteria, were only marginally affected by the addition of MCFAs. This is advantageous because there is a delicate balance between beneficial and pathogenic bacteria in the GIT, and many symbiotic and competitive interactions occur between them (Williams et al., 2001). MCFAs may increase the lactic acid bacteria/*Escherichia coli* ratio, which is considered as one of the best gut health criteria for the pig gut. The susceptibility of bacteria to MCFAs is most probably related to their membranes. It is suggested that at low pH the outer lipopolysaccharide membrane of the Gram+ bacteria become less dense and allow the MCFAs to penetrate into the cell membrane. Recent studies show that minimal inhibitory concentration (MIC) values depend on chain length, decrease with decreasing pH and are lower for Gram− than Gram+ bacteria (Sun et al., 2002; Thormar and Hilmarsson, 2007).

As judged from PCR-DGGE patterns, caprylate addition in concentrations of 5, 15 or 30 mM had a visual effect on the composition of the microbial community in the *in vitro* cecum simulation. However, PCR-DGGE might be a too coarse method to detect minor shifts in abundance, since the decline of the *Salmonella* population escaped detection as well with this molecular cultivation-independent method. The observed shift in fermentation pattern as induced by caprylate (lower propionic and butyric acid production and higher lactic acid production) and caprinate (decreased propionic acid production) addition might therefore be due to minor changes in abundance of certain bacterial groups that escaped detection by PCR-DGGE or – perhaps more likely – to metabolic shifts occurring in an unaltered microbial community. It has been shown that butyrate decreases *Salmonella* invasion in epithelial cell lines (Van Immerseel et al., 2003) and lowers *Salmonella* colonisation in chickens (Van Immerseel et al., 2004b). It would be interesting to evaluate in *in vivo* pig trials whether the beneficial *Salmonella*-inhibiting effect of caprylate would not be counterbalanced by an increased colonisation due to a lower cecal butyric acid concentration resulting from caprylate application.

In conclusion, an *in vitro* continuous culture model for the microbial community in the porcine cecum was developed and validated. The model can therefore be used in future studies on the composition of cecal flora in response to several parameters such as feed composition and -additives, pH, presence of probiotic strains, etc. In the present study, caprylate was found to selectively inhibit *Salmonella* and coliforms at cecal concentrations that seem economically feasible, provided that the substance can reach the cecum without being absorbed. Therefore, caprylate, for example in the form of encapsulated beads (Van Immerseel et al., 2004b) or as triacylglycerol oil (Dierick et al., 2002), might have potential as a *Salmonella*-reducing additive in pig feed. Although MCFA oils are absorbed in the proximal regions of the digestive tract (stomach 25%, ileum remainder), significant effects on the flora and metabolism at the distal regions (cecum, colon) are possible (Dierick et al., 2004). The use of MCFA oils with lipases has additional advantages over the use of MCFA oils solely (Dierick et al., 2002).

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