The influence of fatty acids on the expression of virulence genes of *Salmonella Typhimurium* and the colonization of pigs

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**Abstract**

*Salmonella Typhimurium* infections in pigs are a major source of human foodborne salmonellosis. To reduce the number of infected pigs, acidified feed or drinking water can be administrated. A study was carried out to evaluate the use of short-chain fatty acids (SCFA) and medium-chain fatty acids (MCFA) for the control of *Salmonella Typhimurium* infections in pigs. Short-chain fatty acids formate, acetate, propionate and butyrate (pH 6, osm 600, conc 10mM) and medium-chain fatty acids caproic, caprylic and capric acid (pH6, osm 600, conc 2mM) were used. First, the effect of these acids on the invasion rate of *Salmonella Typhimurium* in porcine intestinal epithelial cells and on the expression of the virulence genes *fimA* and *hilA* was assessed in *vitro*. The expression of *hilA* was decreased by butyrate, propionate, caproic and caprylic acid. Caproic and caprylic acid also decreased the expression of *fimA*. Contact of bacteria with butyrate, caprylic and caproic acid decreased invasion in porcine intestinal epithelial cells in *vitro*. Since butyrate and caprylic acid resulted in the strongest attenuation of *Salmonella in vitro*, these acids were further tested in an *in vivo* trial. In order to reach the large intestine, the tested acids were coated. Coated butyrate showed a strong reduction of *Salmonella* excretion (approximately 100 times lower on average compared to the control group). Coated caprylic acid also showed a reduction of shedding (10 times lower on average compared to the control group). Neither butyrate nor caprylic acid decreased the colonization of the tonsils by *Salmonella Typhimurium*. In conclusion, coated butyrate lowers the shedding of *Salmonella Typhimurium* by pigs. Coated butyrate and, to a lesser extent coated caprylic acid, decreased the colonization of the intestinal tract but did not affect the colonization of tonsils.

**Introduction**

*Salmonella Typhimurium* is known as one of the most important zoonotic organisms and causes many cases of human salmonellosis each year worldwide. An important source of salmonellosis in humans is the consumption of *Salmonella* contaminated pork. The use of SCFA and/or MCFA could contribute to control the colonization of pigs by *Salmonella Typhimurium* by influencing the expression of virulence genes, such as *fimA* (2) and *hilA* (1). Both genes are essential for an efficient colonization in pigs by *Salmonella Typhimurium* (1, 2). In this study, the influence of SCFA and MCFA on the expression of the virulence genes *fimA* and *hilA* and the invasion rate of *Salmonella Typhimurium* in porcine intestinal epithelial cells was determined in *vitro*. Those acids which attenuated the *Salmonella Typhimurium* strain to the highest degree were selected for an *in vivo* trial. In this trial, the effect of the selected acids on the colonization of pigs by *Salmonella Typhimurium* was determined.

**Material and methods**

1) Influence of SCFA and MCFA on the expression of the *Salmonella Typhimurium* virulence genes *hilA* and *fimA*

*Salmonella Typhimurium* strain MB 2486, isolated from a pig stool sample was used in all *in vitro* experiments. Strains of MB 2486 carrying plasmids containing a *fimA-luxCDABE* or a *hilA-luxCDABE* transcriptional fusion were used to determine the gene expression. These transcriptional fusions result in light production when *fimA* or *hilA* is transcribed. Therefore, light production can be a marker for gene expression (4).
Light production was measured in an Ascent luminometer. The expression of fimA and hilA was compared between *Salmonella* Typhimurium grown in LB-broth with or without acid supplementation. Osmolarity and pH were corrected after the addition of the acids. Short-chain fatty acids formate, acetate, propionate and butyrate (pH 6, osm 600, conc 10mM) and medium-chain fatty acids caprylic, caprylic and capric acid (pH6, osm 600, conc 2mM) were used.

2) Influence of SCFA and MCFA on invasion of *Salmonella* Typhimurium in porcine intestinal epithelial cells

The effect of SCFA and MCFA on the invasion capacity of *Salmonella* Typhimurium in a porcine intestinal epithelial cell line (IPI-21) was determined in a standard gentamicin protection assay.

3) Effect of SCFA and MCFA on the colonization of pigs by *Salmonella* Typhimurium

This study was conducted on 24 weaned piglets, from a serologically negative breeding herd, that were negative for *Salmonella* at faecal sampling. The piglets were randomly divided into 3 groups: a control group, a group that received feed supplemented with coated butyrate (10 mM) and a group that received feed supplemented with coated caprylic acid (2mM). Acids were coated to avoid absorption in the proximal intestine. Twelve days after the piglets were given the different feeds, the animals were orally inoculated with 5x10^7 CFU of an invasive nalidixic acid resistant derivative of *Salmonella* Typhimurium strain MB 2486. Fecal samples were collected daily and animals were monitored for clinical disease signs. At 4 days post infection (p.i.), all piglets were euthanized and samples were taken from various organs for bacteriological analysis.

Results

1) Influence of SCFA and MCFA on the expression of the *Salmonella* Typhimurium virulence genes *hilA* and *fimA*

*Salmonella* Typhimurium grown in LB-broth containing butyrate, propionate, caproic or caprylic acid showed a significantly (p<0.01) lower expression of the gene *hilA* compared to *Salmonella* Typhimurium grown in plain LB-broth. The expression of *fimA* was significantly (p<0.01) lower when bacteria were grown in LB-broth containing caproic or caprylic acid compared to *Salmonella* Typhimurium grown in LB-broth without acid supplementation. These results are summarized in figures 1 and 2.

![Figure 1](image1.png)  
**Figure 1**: Expression of *hilA* of *Salmonella* Typhimurium grown in acidified LB-broth relative to *Salmonella* Typhimurium grown in plain LB-broth

![Figure 2](image2.png)  
**Figure 2**: Expression of *fimA* of *Salmonella* Typhimurium grown in acidified broth relative to *Salmonella* Typhimurium grown in plain LB-broth

2) Influence of SCFA and MCFA on invasion of *Salmonella* Typhimurium in porcine intestinal epithelial cells.

Contact of *Salmonella* Typhimurium with butyrate, caprylic acid or caproic acid resulted in a significant (p<0.01) decrease of invasion capacity in the porcine intestinal epithelial cells *in vitro*. These results are presented in figure 3.

![Figure 3](image3.png)  
**Figure 3**: Effect of SCFA and MCFA on invasion of *Salmonella* Typhimurium in porcine intestinal epithelial cells.

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Figure 3: Invasion rates in the IPI-21 cell line of *Salmonella Typhimurium* grown in acidified LB-broth relative to *Salmonella Typhimurium* grown in LB-broth.

3) Effect of SCFA and MCFA on the colonization of pigs by *Salmonella Typhimurium*

In the *in vivo* trial, coated butyrate showed a strong decrease of *Salmonella* excretion at days 2 and 3 p.i. (approximately 100 times lower on average compared to the control group). Moreover, the colonization of the internal organs was also decreased when coated butyrate was supplemented (p<0.1). Coated caprylic acid showed a reduction of *Salmonella* shedding (10 times lower compared to the control group), mainly at day 2 p.i. Neither butyrate nor caprylic acid influenced the colonization of the tonsils. The results are summarized in figures 4, 5 and 6.

Figure 4: Fecal shedding of *Salmonella Typhimurium* at 3 days post infection.

Figure 5: *Salmonella Typhimurium* colonization in tonsils, liver and spleen.
Figure 6: *Salmonella* Typhimurium colonization of the intestinal organs

Discussion

The pronounced lower *hilA* expression of *Salmonella* Typhimurium grown in LB-broth supplemented with butyrate, caproic or caprylic acid and the lower *fimA* expression of *Salmonella* Typhimurium grown in LB-broth supplemented with caproic or caprylic acid were reflected in the gentamicin protection assay, in which bacteria grown in LB-broth supplemented with the same acids showed a reduced invasion capacity. Supplementation of feed with coated butyrate or coated caprylic acid was also efficient in reducing fecal shedding of *Salmonella* Typhimurium. Application of fatty acids is thus one of the possible measures to decrease the number of infected pigs. However, a combined approach with other different control measures (hygiene, vaccination...) is necessary to control the *Salmonella* problem in pigs.

Conclusions

In this study, coated butyrate and to a lesser extent coated caprylic acid have proven to be useful tools for decreasing fecal shedding and colonization of the intestinal organs by *Salmonella* Typhimurium in pigs. Colonization of the tonsils was, however, not influenced.

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References

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