Lactic acid and acetic acid reduce salmonella in fermented pig feed.

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Introduction

To reduce contamination of pork with salmonella, a reduction of the prevalence of salmonella in the whole pork production chain is needed. At farm level the presence of endemic ('house flora') salmonellae in fattening pigs is recognized as a major risk factor.

Pig feed is considered to be involved in the transmission, survival and multiplication of these salmonellae. Pig farms that supply their pigs with fermented pig feed have a lower prevalence, compared to farms that supply their pigs dry feed (3). In an earlier experiment we have shown that fermentation of feed can reduce the numbers of Salmonella typhimurium in feed. This paper reports the effect of these acids, on the survival of Salmonella typhimurium in brain heart infusion broth. Acid concentrations present after 3 days of fermentation were used in these in vitro experiments.

Materials and Methods

Organisms

Lactobacillus plantarum was used as starter culture. L. plantarum was grown for three days in Man Rogosa Sharp (merck V726761) broth at 20°C.

S. typhimurium was grown on Brain Heart Infusion broth (Oxoid CM255) for 20 hours at 37°C.

Pig feed

A basic dry formulated pig feed was produced (meal) and heated to 80°C for 10 minutes to kill all vegetative bacteria. After cooling, the pig feed was mixed with 2.5 parts sterile water (w/wt). Three flasks of 140 ml pig feed were fermented during three days with L. plantarum at 20°C on a plate shaker (Janke & Kunkel, IKA-Labortechnik).

Acids analysis

Acid analysis was performed by High Liquid Performance Chromatography as reported by Andersson and Hedlund (1). Fermented feed was centrifuged for 10 minutes by 4000 RPM. The supernatant was mixed (1:1) with a 20 mM xylitol-50 mM HCl solution (internal standard). This solution was centrifuged for 10 min. at 14000 RPM and supernatant was injected into HPLC apparatus (pre-column: anion exchanger (chrompack cat. no. 28098) column: organic acid polymeric packing (Chrompack cat. no. 28350); pressure 90 bar; flow 0.6 ml/min.

Acids experiment.

The acids analysed in the fermented feed by HPLC analysis were poured into BHI broth and pH was adjusted to 4.4 as in the fermented feed. Adjustment was achieve with HCl (50mM).

The BHI modified broths were inoculated with 10³ S. typhimurium/ml.

Results

The HPLC analysis of the fermented feed showed that there were 2 acids produced by the L. plantarum: Lactic acid (200 mM) and acetic acid (25 mM).

The effects of these acids in BHI on S. typhimurium is shown in fig 1.

The pH of all BHI broth was adjusted to 4.4 as in the fermented pig feed S. typhimurium multiplied in the control broth at pH 4.4. With 25 mM acetic acid the numbers of S. typhimurium were stable at 5 (log N/ml). S. typhimurium in BHI with 200 mM lactic acid decreased in numbers in 15 hours from 5 (log N/ml) to 3 (log N/ml).

When the 2 acids were together present the bactericidal effect was greater. S. typhimurium could not be detected after 9 hours and onwards either through pre-enrichment nor through direct plating.
Figure 1: Salmonella in Brain Heart Infusion broth with 200 mM lactic acid, acetic acid (25 mM) and combined. Controle is BHI without any acids. All broths had a pH adjusted to 4.4.

Discussion

In farms using fermented pig feed the prevalence for salmonella is less compared to farms using dry feed. In earlier studies (2) we showed that fermented feed had a bactericidal effect on salmonella. In this study we investigated the mechanism of fermented feed on salmonella reduction. After terminating the fermentation only two acids were analysed in fermented feed, lactic acid and acetic acid. The concentration of lactic acid was 200 mM and for acetic acid the concentration was 25 mM.

When we studied these acids in Brain Heart Infusion broth with different pH's than we observe a bacteriostatic effect with 25 mM acetic acid (pH 4.4) and weak bactericidal effect with 200 mM lactic acid (pH 4.4). The bactericidal effect on S. typhimurium of these 2 acids together was stronger than one acid alone.

References

