CONTROL OF SALMONELLA IN LIQUID FEEDING SYSTEMS

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Liquid feeding systems could be a challenge as regards Salmonella contamination in pigs, as the feed is often produced from non-controlled feed ingredients. Moreover, there is a possibility of salmonella growth in the feed. Practical systems consist of one or more mixing tanks with raw material feeding equipment, circulation pipes through all stable units fed by the system, a valve for every or every second pen and a computer to control the mixing and dosage systems. As the systems can never be emptied, there is a continuous inoculation of 10 - 50% left-over feed in the tank and the pipes, which leads to a fermentation. The dominating lactic acid bacteria (Lactobacillus and Pediococcus) produce lactic acid and to a minor extent acetic acid, thereby lowering the pH to 4.0 - 5.5. Salmonella growth is possible down to about pH 5.

PURPOSE

Salmonella is susceptible to organic acids, especially in acid environments. The aim of the study was to investigate the possibilities of decontaminating the salmonella added with the feed ingredients by supplementing the in situ produced lactic acid with formic, propionic and acetic acid.

MATERIALS AND METHODS

A. Decontaminating in broth: In a tryptone soy broth buffered with 0.2 M citrate and adjusted with HCl, Sal. enterica serotype 4.12:b- was added with the organic acids alone or in combination. Efficient decontamination is a reduction of 4 log10 in 2 hours at 20 °C.

B. Decontamination in pilot system with fixed pH: A pilot wet feeding system was used to simulate the natural fermentation in the left-over feed. It consists of 6 parallel vessels with continuous registration of pH and temperature, and semi-automatic feed mixing. The physical conditions are simulating conditions in practice. Meal feed mix without additives was mixed 2 times a day with a left-over amount of 25% for 14 days. A starting inoculum of 25% from a functioning system was used. The temperature was app. 20 °C, which is high in relation practice. Salmonella addition was as naturally contaminated rapeseed expeller, coarsely ground. The number of surviving Salmonella spp. was registered by a MPN-variation of NMKL no. 71. Once a day, immediately after feed mixing, the pH was adjusted to the fixed level by addition of formic acid.

C. Decontamination in pilot system with fixed pH: The pilot system and registration are as described in B. A fixed amount of formic or lactic acid was added to the liquid feed once a day. The trial ran for 18 days.

D. Determination of D-values at fixed pH: A menstruum was made on the basis of fermented liquid feed fixed at the chosen pH and added organic acids. All combinations

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were tested with a starting pH of 5.0, 4.5, 4.2, 4.1, and no acid, 0.2% formic acid, 0.3% lactic acid and 0.23% BioAdd (BP Chemicals). As inoculum was either used naturally contaminated cotton seed expeller or a pure culture of Sal. enterica serotype 4.12:b−. The Salmonella number was analysed for naturally contaminated Salmonella by MPN (NMKL no. 71), for pure culture by NMKL no. 71 on ten-fold dilutions (all Salmonella) and by plate spread and counting on Rambach Agar (only Salmonella not sublethally damaged). The decimal reduction time D was calculated.

RESULTS

A. The minimal dosage of organic acids in a buffered TSB including the starting point of the broth and the pH after acid addition are shown in Table 1. In all cases an end pH of 3.8−4.2 is necessary to obtain effective decontamination. The acids in combination did not produce better results, as the needed pH was app. 3.9.

<table>
<thead>
<tr>
<th>Acid</th>
<th>Concentration, %</th>
<th>pH i buffer</th>
<th>pH after addition of acid</th>
</tr>
</thead>
<tbody>
<tr>
<td>Formic acid</td>
<td>0.7</td>
<td>5.0</td>
<td>4.2</td>
</tr>
<tr>
<td></td>
<td>0.7</td>
<td>4.5</td>
<td>4.0</td>
</tr>
<tr>
<td></td>
<td>0.5</td>
<td>4.2</td>
<td>3.8</td>
</tr>
<tr>
<td>Propionic acid</td>
<td>4</td>
<td>5.0</td>
<td>4.2</td>
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<tr>
<td></td>
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<td>3.9</td>
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<td>Acetic acid</td>
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<td>4.0</td>
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</tr>
<tr>
<td></td>
<td>3</td>
<td>4.2</td>
<td>3.8</td>
</tr>
</tbody>
</table>

B. The needed dosage of acid to obtain the fixed pH was (avg. ± std.) 0.11 ± 0.03% to pH 4.1; 0.07 ± 0.03% to pH 4.3 and 0.04 ± 0.02% to pH 4.5. The pH in the control was mostly below 5, reaching app. 4.5 just before new mixing. An exception was day 7, where the pH reached 6. The added natural Salmonella was in average 10 per 100 ml liquid feed. The number determined in the feed is seen in Table 2.

<table>
<thead>
<tr>
<th>Day of trial</th>
<th>Control</th>
<th>Formic acid to pH 4.1</th>
<th>Formic acid to pH 4.3</th>
<th>Formic acid to pH 4.5</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>&lt;1</td>
<td>&lt;1</td>
<td>&lt;1</td>
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<tr>
<td>7</td>
<td>38</td>
<td>&lt;1</td>
<td>1.6</td>
<td>1.6</td>
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<td>10</td>
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<tr>
<td>14</td>
<td>8.4</td>
<td>2.4</td>
<td>&lt;1.2</td>
<td>&lt;1</td>
</tr>
</tbody>
</table>
C. The fixed pH was in general not obtained by the used dosages of acid and a lack of correlation between the dosage used and the obtained pH was observed. The pH obtained is indicated in Table 3. The Salmonella contamination added was app. 5 per 100 ml and the number analysed appears from Table 3.

Table 3. Approximative pH obtained throughout the period and number of Salmonella (MPN) determined in liquid feed after addition of app. 4 per 100 g. Trial C

<table>
<thead>
<tr>
<th>Day of trial</th>
<th>Control</th>
<th>Formic acid 0.34% to pH 4.1</th>
<th>Formic acid 0.24 % to pH 4.3</th>
<th>Formic acid 0.17 % to pH 4.5</th>
<th>Lactic acid, 0.22 % to pH 4.3</th>
</tr>
</thead>
<tbody>
<tr>
<td>App. pH obtained</td>
<td>4.8</td>
<td>4.6</td>
<td>4.6</td>
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<td>4.5</td>
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<tr>
<td>4</td>
<td>17</td>
<td>&lt;1</td>
<td>1.6</td>
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<td>14</td>
<td>&lt;1</td>
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<td>&lt;1</td>
</tr>
<tr>
<td>18</td>
<td>&lt;1</td>
<td>&lt;1</td>
<td>&gt;960</td>
<td>1.6</td>
<td>&lt;1</td>
</tr>
</tbody>
</table>

D. Only when using pure culture for inoculation and starting pH at and below 4.2, reduction in counts over time were obtained. Determinations of all viable cells (10-fold dilutions of pure cultures and MPN of natural contamination, both by NMKL no. 71) showed no reduction. Statistical analysis showed that only in one out of four trials there was a significant effect of the supplementary addition of acids, whereas the treatment time was significant (p< 0.1 %) in 3 out of 4 cases. In the 3 cases where reduction over time was seen, the reduction per hour was between 0.29 and 0.75 (log 10), leading to a D-value of 1.3 – 3.4. This only relates to sublethal damage.

DISCUSSION

High levels of organic acids corresponding to a pH of about 4 are necessary to obtain an efficient Salmonella decontamination in a buffered liquid. In spontaneously fermented liquid feed a reduction of added naturally contaminating Salmonella is probable, although the uneven distribution of the organisms in the material prohibits a clear conclusion. Sporadic findings – sometimes in elevated numbers – underlines feed as a risk of salmonella contamination. The lack of correlation between acid dosage and final pH – all other factors equal – illustrates the mutual relations between fermentation and added acids – that the added acid inhibits the fermentation and / or the microflora degrades the added acids. The conclusion of this work is, that it is not possible to obtain a reliable Salmonella decontamination by the combination of fermentation and added organic acids. Nevertheless results from practice indicate that liquid feed has a protective effect against subclinical salmonellosis. This effect is possibly due to an impact of the fermentation products on the pig health. Therefore, the work is continued on the topic of controlling the fermentation in practical liquid feeding systems.

REFERENCES