PRESSURIZATION EFFECT ON *Salmonella* sp. 
WITHIN THE FISH MEAL

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Received for publication January 21, 2005.

Abstract

The experiment was performed on 130 fish meal samples inoculated with $10^7$ CFU x g\(^{-1}\) of *S. Enteritidis* or *S. Hadar* and exposed on HHP (100-400 MPa) for 15 min. The treatment resulted in significant reduction of the bacteria, ranging from 0.7 to 4.9 log CFU x g\(^{-1}\), depending on the strain and the amount of pressure applied. Our results suggest that fish meal pressurization could be the profitable alternative for currently admitted thermal treatment, enhancing the microbiological safety of the feed, plausibly without resulting decrease in its nutritional value.

Key words: fish meal, *Salmonella*, high hydrostatic pressures.

High hydrostatic pressures (HHP) gain the increasing interest as a potential non-thermal technique for food preserving. Consequently, they were subjected to numerous studies either *in vitro* or in food systems. The results suggest that pressurization can be also applied for eradication of pathogenic and spoilage microorganisms from animal feeds, especially from the high-protein ones. According to the actual legislation the fish meal constitutes virtually the only source of protein of animal origin among the feeds (3). The main microbiological problem connected with that material is its contamination with germs of *Enterobacteriaceae* family, and particularly with *Salmonella* sp. Despite of the obligatory treatment, the bacteria are present, according to different authors, in 14 to 48% of the fish meal samples (5, 7, 11). Fish meal contaminated with salmonellae is the important vector of bacterial transmission to the farm animals, significantly affecting their health and productivity and enhancing the health risk of consumer.

High hydrostatic pressure effects on salmonellae were examined in several experiments. It was revealed that the species discussed is relatively susceptible to pressurization and the pressures of 500-700 MPa significantly reduce the test strain counts (1, 4, 6, 8-10, 12-14).

Consequently, the purpose of our experiment was the practical evaluation of HHP application for *Salmonella* sp. eradication from the fish meal.

Material and Methods

The studies were performed on 130 fish meal samples, each of 10 g, free from *Salmonella* sp. contamination. The composition of the fish meal was controlled prior to the investigation (Table 1).

<table>
<thead>
<tr>
<th>Component</th>
<th>Mean (%)</th>
<th>SD (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dry matter</td>
<td>95.00</td>
<td>3.215</td>
</tr>
<tr>
<td>Crude protein</td>
<td>64.00</td>
<td>1.230</td>
</tr>
<tr>
<td>Ether extracts</td>
<td>12.72</td>
<td>0.009</td>
</tr>
<tr>
<td>Crude ash</td>
<td>16.94</td>
<td>0.014</td>
</tr>
<tr>
<td>Crude fiber</td>
<td>0.87</td>
<td>0.003</td>
</tr>
<tr>
<td>Nitrogen-free extracts</td>
<td>0.47</td>
<td>0.001</td>
</tr>
</tbody>
</table>
The poultry strains of *Salmonella* Enteritidis and *Salmonella* Hadar, kindly provided by the National Veterinary Research Institute in Puławy, were subjected to the experiment. They were replicated in 18-h culture, three times spinned, washed with the buffered peptone water and calibrated with saline. Each strain was inoculated to the fish meal in the amount of $7 \times 10^7$ CFU x g$^{-1}$ (20 ml of the culture was injected to 1 kg of the material studied and mixed carefully). The counts of *S*. Enteritidis and *S*. Hadar within the samples were measured directly after the inoculation and after 24-h adaptation to the material.

Subsequently, the fish meal was treated with HHP (100, 200, 300 or 400 MPa) for 15 min at room temperature (ca 20°C). The fluid system with manually generated pressure was applied. The survived bacteria and not exposed to HHP controls were restored on BGA medium (37°C, 24 h).

Logarithmic transformation of bacterial counts and their statistical analysis were done with the aid of Microsoft® Excel 2000 and Statistica 5, Version 97 software. The T-4D values, time required for the reduction of the initial bacterial level by 4 log units, were calculated from the regression analysis. The importance of the mean value differences was established with the aid of Student’s test.

**Results**

The pressurization caused significant reduction of both the test strains of *Salmonella* sp. (P<0.05). Depending on the amount of pressure applied, the reduction 15-min treatment ranged from 1.9 to 4.9 log CFU x g$^{-1}$ for *S*. Enteritidis and from 0.7 to 4.2 log CFU x g$^{-1}$ for *S*. Hadar (Fig. 1).

Analysis of T-4D values, time required for the reduction of the initial bacterial level by 4 log units, revealed that the efficiency of pressurization increases with the applied amount of HPP. Four-log reduction of both the strains was achieved only after 15-min treatment with 400 MPa (Table 2).

The differences between *S*. Enteritidis and *S*. Hadar in resistance to HHP were not statistically significant for any pressure applied (P<0.05).

![Fig. 1](image-url)  
**Fig. 1.** Counts of *S*. Enteritidis and *S*. Hadar in fish meal treated for 15 min with different pressures. Bars represent standard deviations; A-D = P<0.05

**Table 2**

Values of T-4D (min) for fish meal pressurized with 100-400 MPa

<table>
<thead>
<tr>
<th>Pressure (MPa)</th>
<th><em>S</em>. Enteritidis</th>
<th><em>S</em>. Hadar</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>Confidence range (95%)</td>
</tr>
<tr>
<td>100</td>
<td>32.3</td>
<td>32.2-32.4</td>
</tr>
<tr>
<td>200</td>
<td>30.3</td>
<td>30.1-30.5</td>
</tr>
<tr>
<td>300</td>
<td>16.6</td>
<td>16.0-17.2</td>
</tr>
<tr>
<td>400</td>
<td>12.3</td>
<td>11.9-12.7</td>
</tr>
</tbody>
</table>
Discussion

Our experiment proved the application of pressurization as non-thermal process for preserving the fish meal. According to our knowledge, HHP were not considered to animal feeds so far. Antibacterial effect of pressurisation results mainly from the modifications of protein and lipid composition within the microbial membranes (9).

We have chosen two species of Salmonella as the test strains in our study since the bacteria of this genus constitute the particular microbiological threat in high protein animal feeds (5, 7, 11). According to the results of in vitro experiments, salmonellae are relatively susceptible to HHP comparing to other pathogenic bacteria (1, 4, 12-13). We have achieved the satisfactory level of salmonellae reduction (4 log units), treating the fish meal with the pressure of 400 MPa for 15 min. Analysis of T-4D values revealed that the expected action of pressures 100-300 MPa requires longer duration of the treatment, connected with higher energy consumption. It should be also considered that the strains of variable resistance to HHP occur within every bacterial species (2).

The reduction of the salmonellae, observed in our experiment, was higher than that previously described for in vitro studies (10, 13). The phenomenon probably reflects the presence of several inhibitory factors supporting the action of HHP against salmonellae within the stock of animal origin. There exist the experimental data on synergy of pressurization with decreased pH (1, 8-10), elevated temperatures (1, 6), or lactoperoxidase system addition (4) on death of salmonellae. The evidence of interactions of that kind seems particularly important, considering the number of sublethal salmonella injuries caused by HPP alone (13-14).

Concluding, the pressurization of fish meal seems to be the profitable alternative, or at least the supplement for actually admitted thermal treatment, since it enhances the microbiological safety, probably without resulting decrease in nutritional value of the feed. Consequently, the practical consideration of the technique described seems desirable.

References


