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Effect of Formic and Propionic Acid Mixture on *Escherichia Coli* in Fish Meal Stored at 12°C

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Abstract: The purpose of paper was the evaluation of short-chain organic acid effect on *Escherichia coli* in fish meal stored at 12°C. Fish meal samples (n=125) were inoculated with 7×10^7 CFU \times g⁻¹ of *E. coli* ATCC 25922 strain and treated with 0 to 1.2% of formic (35%) and propionic (15%) acid mixture. The treatment resulted in the significant reduction of number of test bacteria, proportional to the concentration of acid added. When applied in mixture, propionic and formic acid appeared to work synergistically against *E. coli*. Accordingly, their application as high-protein feed preservatives seems to be highly appropriate.

Key words: Formic acid, propionic acid, *Escherichia coli*, fish meal

Introduction

The contamination of high-protein animal feeds with *Enterobacteriaceae* significantly increases the health and hygienic risk, since it reaches even 55% of samples controlled (Hofacre *et al.*, 2001; Nabbut *et al.*, 1982; Veldman *et al.*, 1995). The problem is particularly important in case of fish meal used in poultry feeding, due to the presence of *Salmonella* rods. Contamination with the aforementioned bacteria was reported in 14-48% of the samples (Hofacre *et al.*, 2001; Nabbut *et al.*, 1982; Veldman *et al.*, 1995). Fish meal contaminated with salmonellae is the important vector in bacterial transmission to the slaughter and laying birds, since the majority of microorganisms isolated from animal feeds is resistant to commonly used antibiotics (Hofacre *et al.*, 2001). Consequently, economical casualties and the health risk of consumer are considerably extended.

Organic acid addition seems to be the useful technique, improving the microbial status of animal feeds without negative effect on their dietary value (Zawadzki, 1993). The antimicrobial effect of the acids results from the decrease of bacterial cytoplasmic pH. Numerous organic acids were successfully tested against *Enterobacteriaceae* in experimental studies. Their efficacy depended on incubation temperature and the physico-chemical properties of the medium (Malicki and Bruzewicz, 2003; Malicki *et al.*, 2004a, b). *Escherichia coli* exhibits particularly strong acid resistance and

adapts to the low pH conditions faster than the other *Enterobacteriaceae* (Audia *et al.*, 2001; Berry and Cutter, 2000; Brudzinski and Harrison, 1998; Casey and Condon, 2002; Ryu and Beuchat, 1998; Ryu *et al.*, 1999; Samelis *et al.*, 2003; Samelis *et al.*, 2002).

All the references cited deal mostly with *in vitro* or food studies. The relevant literature however lacks data on the antimicrobial efficacy of organic acids added to high-protein animal feeds.

Consequently, the purpose of present paper was defined as the evaluation of organic acid effect on the test strain of *Escherichia coli* in fish meal stored at 12°C. A mixture of propionic and formic acid was chosen, since the experimental data on the antibacterial activity of those acids are very promising (Cherrington *et al.*, 1990; Cherrington *et al.*, 1991; McWilliam Leitch *et al.*, 2002; Shin *et al.*, 2002). Considering literature information, it was assumed that the acid concentrations efficient against the strain studied would be also bactericidal to pathogenic *Enterobacteriaceae*, including salmonellae, being the common reason of food-born infections in animals and human.

Materials and Methods

The experiment was performed on 125 fish meal samples, 10 g each, free from *Escherichia coli* contamination. Prior to investigation the water activity of the samples was measured and the composition of fish

Table 1: Composition of fish meal studied

| | |
|------------------------|--------|
| Dry matter | 95.00% |
| Crude protein | 64.00% |
| Ether extracts | 12.72% |
| Crude ash | 16.94% |
| Crude fibre | 0.87% |
| Nitrogen-free extracts | 0.47% |

meal - the content of dry matter, crude protein, ether extracts, crude ash, crude fibre and nitrogen-free extracts was controlled (Table 1).

The ATCC 25922 strain of *Escherichia coli*, replicated in 18 h culture, was employed to the studies. Bacterial cells were thrice spinned and washed with buffered peptone water, prior to fish meal inoculation with 7×10^7 CFU \times g⁻¹ (20 g of culture to 1 kg of the material studied). *Escherichia coli* count in fish meal was measured directly post inoculation and after 24-h adaptation to the material. Subsequently, the mixture of formic (35%) and propionic acid (15%) was added to fish meal in the concentrations of 0.4, 0.6, 0.8, 1 or 1.2% in water solution. Consequently, the final content within the fish meal ranged from 30 to 90 mmol \times kg⁻¹ and from 8 to 24 mmol \times kg⁻¹ for formic and propionic acid, respectively.

The samples studied and the acid-free controls were stored at $12 \pm 1^\circ\text{C}$ for 120 h. The counts of *E. coli*, as well as the water activity and pH of fish meal, were controlled every 24 h. *Escherichia coli* cells were restored on chromogenic solid medium (Chromocult Coliform Agar, Merck). For the purpose of additional identification, all the colonies stained from dark blue to violet were treated with Kovacs reagent for indol. The change of coloration to cherry-red, appearing in a few seconds (positive result of indol test), confirmed the isolation of *E. coli*.

The pH values of the samples were measured with the aid of V 628 pH-meter, type N 517, whereas their water activity was controlled with RTD-33 TH-1-NOVASINA avumeter.

The 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 reduction of the initial bacterial level by 4 log units, were calculated from regression analysis. The importance of the mean value differences was established with the aid of Student's test ($P < 0.05$).

Results

Short chain organic acids, added to fish meal, have not significantly changed the water activity, which ranged from 0.307 to 0.321 in the course of entire experiment. The pH of the material, measured after the different time of storage is presented in Table 2. The pH decrease

from the initial level of 6.11, was proportional to the amount of acid mixture added.

Directly after the inoculation with the test strain of *E. coli*, fish meal contamination amounted to 6.7 log CFU \times g⁻¹, and decreased to 5.9 log CFU \times g⁻¹ after 24-h adaptation (day 0 of experiment).

The counts of test bacteria in control samples have not significantly changed during storage ($P < 0.05$), whereas the acid addition reflected in the important reduction of microorganism studied (Fig. 1). The bacteria were not isolated after 4 days of the storage of fish meal added 1 or 1.2% of acid mixture, and after 5 days in case of 0.8% acid concentration. The rate of *E. coli* reduction increased with the concentration of acid added, as figured out from decreasing T-4D values (Table 3).

Discussion

The rate of the reduction of the test strain of *E. coli* in fish meal was proportional to the amount of short-chain organic acid mixture added. As mentioned in the introduction, organic acids were positively tested as *E. coli* inhibitors in many *in vitro* studies. The data on short-chain acid efficiency against the bacteria however, are sporadically dealt with the available literature.

Cherrington *et al.* (1990, 1991) treated the cultures of *E. coli* with the different concentrations of propionic or formic acid. The addition of 5 mmol \times l⁻¹ of propionic acid resulted in 30-min bacteriostasis, whereas 0.5-0.7 mol \times l⁻¹ of the acid caused the death of 90% of experimental population. The application of 10 mmol \times l⁻¹ of formic acid caused a bacteriostatic effect lasting for 120 min, while the death of 90% of the microorganisms was revealed after the 3-h incubation with 0.5-0.7 mol \times l⁻¹ of acid. In another experiment propionate at the concentrations of 25-40 mmol \times l⁻¹ significantly inhibited *E. coli* strain O157:H7 (Shin *et al.*, 2002). In our experiment, propionic and formic acids were added to fish meal at the concentrations of 8-24 mmol \times kg⁻¹ and 30-90 mmol \times kg⁻¹, respectively. The resulting reduction of test strain was more pronounced than previously described. Consequently, if applied in mixture, propionic and formic acid seem to work probably synergistically against *E. coli*.

The direct bactericidal action of organic acids is widely known and results from pH decrease within bacterial cell. According to Shin *et al.* (2002), the bacteriostatic effect of propionate against *E. coli* was proportional to pH decrease in culture medium. In our studies the pH of fish meal was reduced directly after acid addition and subsequently increased during storage.

McWilliam Leitch and Stewart (2002) claimed that propionate was less efficient against *E. coli* at 20 and 5°C than at 37°C, probably due to the lower rapidity of pH

Table 2: Values of pH of acid-treated fish meal stored at 12°C (initial pH prior acid treatment = 6.11, s - standard deviation)

| Acid addition [%] | Storage time [hours] | | | | | | | | | |
|-------------------|----------------------|------|------|------|------|------|------|------|------|------|
| | 24 | | 48 | | 72 | | 96 | | 120 | |
| | pH | s | pH | s | pH | s | pH | s | pH | s |
| 0 | 5.98 | 0.08 | 6.02 | 0.08 | 6.23 | 0.04 | 5.91 | 0.06 | 6.40 | 0.03 |
| 0.4 | 5.72 | 0.06 | 5.69 | 0.07 | 5.74 | 0.06 | 5.82 | 0.04 | 5.74 | 0.05 |
| 0.6 | 5.46 | 0.08 | 5.42 | 0.05 | 5.37 | 0.07 | 5.53 | 0.06 | 5.24 | 0.03 |
| 0.8 | 5.39 | 0.09 | 5.37 | 0.06 | 5.34 | 0.06 | 5.43 | 0.05 | 5.41 | 0.02 |
| 1.0 | 5.35 | 0.06 | 5.32 | 0.03 | 5.34 | 0.05 | 5.27 | 0.02 | 5.21 | 0.02 |
| 1.2 | 5.22 | 0.04 | 5.21 | 0.04 | 5.27 | 0.06 | 5.31 | 0.03 | 5.40 | 0.01 |

Table 3: Values of T-4D (time required for reduction of initial *E. coli* level by 4 log units) for acid-treated fish meal stored at 12°C

| Acid addition [%] | T-4D (hours) | 95% confidence range |
|-------------------|--------------|----------------------|
| 0.4 | 103.2 | 102.2-104.2 |
| 0.6 | 91.8 | 90.5-93.1 |
| 0.8 | 77.1 | 76.4-77.8 |
| 1.0 | 69.8 | 69.6-70.0 |
| 1.2 | 68.5 | 67.9-69.1 |

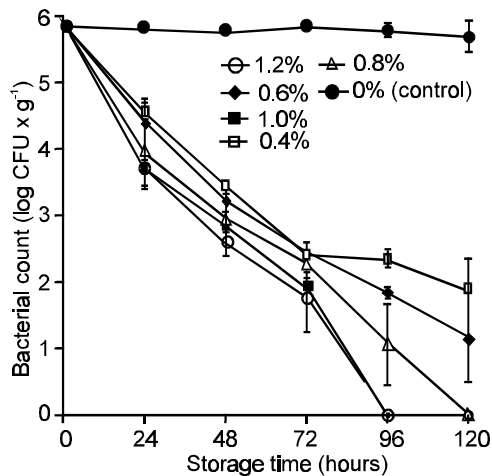


Fig. 1: Reduction of the ATCC 25922 strain of *E. coli* in fish meal treated with the different concentrations of formic and propionic acid mixture, stored at 12°C (error bars represent standard deviations)

decrease and reduced membrane potential at lower temperatures. In a present study the mixture of propionic and formic acid showed satisfactory efficiency against the test strain within fish meal stored at 12°C. The action

of acids on the material kept at lower temperatures was not studied to avoid the overlapping effect of a cold shock. It is widely known that under refrigeration the doubling time of bacteria is significantly prolonged despite any chemical inhibitor addition.

Escherichia coli is more resistant to pH decrease than the other *Enterobacteriaceae*. Consequently, it is very likely that short-chain organic acid mixture, causing the death of test strain of *E. coli* in our experiment, would be also bactericidal to *Salmonella* rods, one of the main reasons for food-borne infections in animals and human. Accordingly, the preservation of high-protein feeds with short-chain organic acids seems to be highly appropriate.

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