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Effect of Water Activity on the Heat Resistance of *Salmonella* in "Dry" Materials

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It is well known that the availability of water has a pronounced influence on the heat resistance of microorganisms. The resistance of completely dry organisms is many times higher than the resistance of the same organisms in a dilute suspension which has a water activity close to 1.0. There is, however, very little information about the resistance of vegatative bacterial cells at the intermediate water activities found in "dry" foods and feeds, and in foods containing moderate concentrations of sugar. This problem has important practical implications in relation to the survival of salmonellae in "dry" foods and feeds.

Banwart and Ayres (1) observed that the survival of salmonellae in dry egg white at 50 to 70 C decreased rapidly when the water content of the egg white was increased from 3 to 5% to 6 to 12%. However, the water activity of the products was not recorded.

Rasmussen et al. (4) observed that wet *Salmonella* cells added to dry meat and bone meal were reduced by a factor of 10^6 by heating for 15 min at 68 C; however, heating for 1 hr at 82 C was required to kill salmonellae in naturally contaminated meal. The water activity was not recorded. Mossel and Koopman (3) found a rapid 10^5-fold reduction in viable cells when a liquid culture of *Salmonella* was mixed into dry meat and bone meal, but additional storage for 5 days caused only a 10-fold decrease in the number of survivors. The water activity of the meat and bone meal was 0.46. J. Taylor (*personal communication*) reported that salmonellae appear to survive indefinitely at room temperature in bone meals, meat meals, dried egg, etc., when the water content is below 10 to 12%.

The aim of this study was to measure the heat resistance of salmonellae at water activities in the range from 0.60 to 1.0. Samples of meat and bone meal with adjusted water contents were used in most of the experiments.

Salmonella typhimurium and *Salmonella senftenberg* 775W were grown in a heat-sterilized suspension of meat and bone meal (1:5) in water. The cultures were centrifuged, the supernatant fluid was discarded, and the wet residue, which contained large numbers of *Salmonella*, was dried at 40 C under vacuum for 12 hr. It was then stored at room temperature over concentrated H_2SO_4 in a sealed container. This dry material, which contained approximately 10^8 viable salmonellae per gram, was mixed into meat and bone meal for heat resistance studies.

Various amounts of distilled water were added to commercial meat and bone meal and the water activity was determined according to the method of Landrock and Procter (2) after equilibration for 2 weeks in sealed mason jars that were kept at room temperature. The water activity of the meat and bone meal at different moisture levels are shown in Table 1.

Salmonellae-containing dry residue (5 g) was mixed into 100-g samples of meat and bone meal conditioned at various water activities, and the inoculated samples were equilibrated for 1 to 2 weeks at room temperature. A 100-fold decrease in the numbers of viable Salmonellae was observed during the first 3 days of equilibration when the water content of the meal was high (13 to 16%). The counts remained constant after this initial decline. No multiplication of salmonellae was observed during storage of meal samples that contained up to 16% water. The high water activity in meal with 13 or 16% water would be expected to permit growth, but growth arrest may be caused by inhibitory solutes present in the meal. These hypothetical inhibitors are diluted to a noneffective level when a 1:5 suspension of meal is prepared in water. Heat resistance tests were carried out by heating 2-g samples of inoculated equilibrated meal in screw-cap tubes in a thermostatically controlled water bath. The heating times were corrected for temperature lag. The correction was based on heat transmission measurements.

Five ml of cold phosphate buffer (M/15, *p*H 7.0) that contained 0.1% peptone was added to each tube after heating and mixed with the meal on a Vortex Junior Mixer. Survivors were counted on Brilliant Green agar by the agar layer technique. Each plate was layered with 1 ml of semisolid Brilliant Green agar that contained a dilu-

TABLE 1. *Water activity of meat and bone meal at different moisture levels*

Per cent water content (w/v)	Water activity
4	0.62
7	0.77
10	0.87
13	0.99

TABLE 2. *Summary of results obtained from heat-temperature tests with S. typhimurium*

Temperature (C)	Water activity in meal	Heating time (min)	Reduction in no. of viable cells
55	0.99	40	10-fold
75	0.77	40	10-fold
75	0.88	40	10^2-fold
90	0.77	40	10^4-fold
90	0.88	20	10^6-fold
100	0.62	20	10^3-fold

tion of the meal. The plates were incubated for 24 to 48 hr at 37 C. Tryptose agar gave the same counts as Brilliant Green agar when inoculated with sterile meal that contained only *Salmonella*.

Low numbers of *Salmonella* were determined by a most-probable-number (MPN) technique in selenite cystine broth, and followed by confirmation. It is possible that higher recoveries could have been obtained by using preenrichment in a nonselective medium. However, the MPN counts in selenite cystine broth showed good agreement with plate counts.

The heat resistance showed that the strain of *S. typhimurium* that was used had a higher heat resistance than the so-called heat-resistant strain of *S. senftenberg* 775W. The data in Table 2 summarize the results obtained with *S. typhimurium*. The initial counts were 10^5 to 10^7 *Salmonella* per g of meal (after equilibration for 2 weeks).

These results indicate that a drastic reduction in viable *Salmonella* can be obtained by heating meat and bone meal to 90 C for a relatively short time after the meal has been conditioned to a water activity of about 0.9. The "natural" water activity of meat and bone meal is generally 0.6 to 0.7.

Furthermore, it was observed that the addition of 5% lactic acid to meal with a water activity of 0.9 increased the kill of *Salmonella* at 75 C by a factor of more than 10^4. Results similar to those obtained with meat and bone meal were found when *Salmonella* was heated in a liquid laboratory medium after the water activity had been adjusted by adding sucrose.

Storage of meal at room temperature caused a 10^5- to 10^6-fold reduction in the numbers of viable salmonellae in 4 to 6 weeks (not counting the 100-fold decrease which took place in the first few days after inoculation of the meal) if the water activity was 0.9 or higher. Meat and bone meal with this water activity still has the physical characteristics of a dry feed, but there is a risk that mold will grow during prolonged storage. Therefore, meat and bone meal which has been pasterurized at a high water activity should be dried.

Pelleting, followed by storage at low air humidity may be a convenient method for drying. Pelleting will in itself cause considerable destruction of salmonellae if the water activity of the meal is high and if enough heat is generated in the pelleting process.

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