Studies of the Control of *Salmonella* Contamination in Oil Meal and Oil Meal Production Environments

Summary of Doctoral Thesis

Hideki Kitazawa

Graduate School of Veterinary Medicine and Life Science

Nippon Veterinary and Life Science University

(Teacher: Professor Mariko Kobayashi

Introduction

Oil meal is used as an ingredient in animal feed and fertilizer as well as in fermented products such as miso and soy sauce, and plays an important role in the food hygiene and distribution network. There is especially high demand for oil meal as an ingredient in animal feed, in which oil meal has long served as the main source of protein. *Salmonella* contamination has been identified as a major issue affecting oil meal quality, with frequent reports of *Salmonella* being detected in oil meal production environments. To completely control *Salmonella* contamination, it is necessary to reduce the presence of *Salmonella* contamination in production environments.

Therefore, in this study, we evaluated detection methods suitable for oil meal manufacturing plants, with the goal of controlling *Salmonella* contamination in finished oil meal products and oil meal production environments. In addition, we developed measures for controlling *Salmonella* contamination in actual manufacturing plants and in the context of actual production processes.

1. Evaluation of the delayed secondary enrichment (DSE) method in oil meal manufacturing plants

We evaluated the delayed secondary enrichment (DSE) method, which relies on efficient isolation for detection of *Salmonella* in oil meal production environments. In a comparison of an official method for detecting *Salmonella* in animal feed (official method) and the DSE method using selective media amended with Hajna-tetrathionate broth, the DSE method was able to isolate *Salmonella* from two samples that were judged negative for *Salmonella* by the official method. Whereas the official and DSE methods yielded consistent results for environmental swab samples, which contain high numbers of bacterial contaminants, the DSE method showed greater detection sensitivity for oil meal ingredients, which contain low numbers of bacterial contaminants. The DSE method using selective medium amended with either Rappaport-Vassiliadis broth or Tetrathionate-Brilliant-Green broth was able to isolate *Salmonella* from one sample that was judged negative by the official method.

Among the samples that were positive for *Salmonella* using both methods, the proportion of samples in which the O-antigen of the Salmonella isolated differed between the two methods was 10.0-43.8%. Furthermore, in a

number of samples, the DSE method was able to isolate O-antigen strains that were not detected by the official method.

The above results suggest that the DSE method would be useful for epidemiological investigations in oil meal manufacturing plants.

2. State of contamination and measures to control *Salmonella* contamination in oil meal production environments

State of *Salmonella* contamination of oil meal production environments

To clarify the mechanisms of *Salmonella* contamination in oil meal manufacturing plants, we evaluated the state of *Salmonella* contamination of three oil meal factories in Japan and one in India. The contamination rate of the raw materials storage area was substantially higher in the Indian manufacturing plant (60.0%) compared to the three Japanese manufacturing plants. In the Japanese manufacturing plants, the raw materials of oil meal were stored in silos away from everything else; whereas, in the Indian manufacturing plant, the raw materials were stored in jute bags or in open piles in a warehouse. *Salmonella* brought into the oil manufacturing plant with the raw materials is believed to have been spread throughout the raw materials storage area during the carry-in of materials, on workers' clothing or shoes as they moved about, and on the bodies of animal pests.

Inspection of oil meal production areas indicated that the contamination rate tended to be higher in factories handling materials with high oil content such as rapeseed and lower in factories handling materials with low oil content such as soybean. This result reaffirms the relationship between oil content and level of *Salmonella* contamination.

In all four factories, the majority of serotypes detected in the raw materials storage area were also detected in the oil meal production areas. This supports the presumption that a portion of the *Salmonella* brought into manufacturing plants with raw materials contaminates the raw materials storage area and, subsequently, is spread to the oil meal production area via the workers or animal pests. These serotypes are believed to have greater ability to survive than other serotypes and, thus, could become persistent.

Measures to control *Salmonella* contamination in oil meal production environments

To develop a measure to control environmental contamination of oil meal production areas, we evaluated disinfectants that could be used to effectively clean the factory floor. Ten-fold dilutions of surfactants whose main active ingredient is didecyldimethylammonium chloride were found to be superior in terms of disinfection efficacy, safety, and cost performance.

In manufacturing plant B (Japan), we implemented three measures: disinfection of shoe soles, creation of smooth floors using smooth paint, and disinfection the factory floor. Given the large size of the oil meal production area, only the high-oil areas were disinfected approximately once a month to reduce the burden on the workers. When only shoe soles were disinfected, the contamination rate of the high-oil areas was found to be 89.5%. However, when all three measures—disinfection of shoe soles, creation of smooth floors, and disinfection of the factory floor—were implemented, the contamination rate decreased to 25.0%.

The above results demonstrate that, when combined, these three measures can effectively prevent *Salmonella* from becoming persistent. It was also reaffirmed that the *Salmonella* is spread from the high-oil to the low-oil areas by workers. Accordingly, continued implementation of these measures will likely be effective in preventing the spread of *Salmonella*.

3. Development of techniques for controlling *Salmonella* in oil meal production

We assessed the contamination of residues accumulated in the production line after the heat treatment stage. High contamination rates were found at the entrances and exits of horizontal conveyors and at the bottoms of vertical conveyors. In the fines collection line, high contamination rates were found in horizontal duct sections and the cyclone exhaust chute, while no contamination was detected in vertical duct sections. The locations where high contamination rates were recorded were also locations where large amounts of residue from the production process tended to accumulate, suggesting that *Salmonella* is able to survive for an extended period of time in residue. Based on the discovery of *Salmonella* in the production line, residue in the production line after the heating stage and highly-contaminated fines, which are conventionally added to oil meal later in the production process, were removed from the product line. No *Salmonella* was detected in finished oil meal produced after the removal of

residues. Although the contamination rate of fines did decline after the removal of residues, the difference in contamination rates before and after residue removal was not significant ($p > 0.05$).

Because fines collection line are typically designed to have few openings, the complete removal of residue or *Salmonella* surviving in residue is difficult. Accordingly, the most effective method for reducing *Salmonella* contamination of oil meal would be to intensively and regularly clean and remove residues from the production line, especially that accumulated at the entrances and exits of horizontal conveyors and at the bottoms of vertical conveyors. Furthermore, the above results suggest that fines should be removed and discarded to prevent the introduction of *Salmonella* from fines.

4. Rapid, easy method for detecting *Salmonella* in oil meal

In many cases, Japanese oil meal manufacturing plants lack sufficient product storage capacity, and finished products are often shipped without being inspected for *Salmonella*. For this reason, rapid, easy-to-use PCR-based detection methods that can provide results in approximately 24 h are widely used in oil meal manufacturing plants. However, the time required is still too long. What is needed is a *Salmonella* detection method that can provide results in approximately 10 h.

To reduce the time required for pre-enrichment culture, we compared MP medium culture medium with Buffered Peptone Water (BPW), Enterobacteriaceae Enrichment Mannitol broth, and Lactose broth. The bacterial count after 7 h of incubation was significantly higher for MP than for the other culture media ($p < 0.001$). Incubating enrichment cultures from artificially and naturally contaminated samples for 18 h in BPW and for 14 h in MP yielded the same detection rate. The above results suggest that the time required for pre-enrichment culture can be reduced to 14 h by using MP culture medium.

Next, to reduce the time required for secondary enrichment after pre-enrichment as well as that for lysis, we evaluated QuickGene-mini80 benchtop DNA extraction system. We evaluated the performance of QuickGene-mini80 by comparing the QUALIBAX™ system (BAX method) and a combination of QuickGene-mini80 and the QUALIBAX™ system (QuickGene-BAX method) using *Salmonella* suspensions. The

QuickGene-BAX method showed greater detection than the BAX method alone. In trials using naturally contaminated materials, the QuickGene-BAX method was able to detect *Salmonella* in one sample that was judged negative using the BAX method.

The above results demonstrate that use of QuickGene-mini80 can increase the detection sensitivity of the BAX method and suggests that the time required for pre-enrichment culture can be shortened. Accordingly, when we shortened the pre-enrichment time in MP culture medium to 7 h, the detection rate in artificially contaminated samples (10^0 cfu/25 g) using the QuickGene-BAX method was 100%. The same 7-h pre-enrichment and QuickGene-BAX method showed the same detection rate for naturally contaminated samples. These results demonstrate that the time required for pre-enrichment can be shortened to 7 h by using the QuickGene-BAX method.

Summary

To prevent *Salmonella* contamination in oil meal, it is necessary to reduce *Salmonella* contamination in the production environment. To this end, it is important to efficiently isolate *Salmonella* from the production environment using DSE. The present study also identified various measures that are effective in preventing contamination of the production environment, including 1) segregated storage of raw materials in silos; 2) separating (as much as possible) raw materials storage and oil meal production areas and limiting movement of workers and animal pests between areas; and 3) thorough removal of oil, especially in production plants that handle raw materials with high-oil content for oil meal. In addition, the study demonstrated that implementation of all three of the following measures is effective in reducing contamination of production environments: 1) disinfection of shoe soles; 2) creation of smooth floors using smooth paint; and 3) regular disinfection of high-oil areas of the factory floor by spraying with effective disinfectants. Finally, the present study identified the following two measures as being effective in reducing *Salmonella* contamination of oil meal: 1) removal of residue in the production line and 2) removal of fines. The efficacy of these measures in reducing *Salmonella* contamination of production environments and oil meal were verified in actual manufacturing plants and production lines. Accordingly, they are

applicable to not only oil meal factories, but also other animal feed and food-production plants that handle powders.