**FSA Project no. MO 1019: Physical methods readily adapted to existing commercial lines for reducing pathogens, particularly campylobacters, on raw poultry.**

## Extension Project 1

# Commercial trials to investigate the feasibility under commercial conditions of decontamination treatments previously tested in the laboratory.

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### Summary

Four visits were made during 2004 to a poultry processing plant to investigate several physical decontamination methods that had given promising results when investigated on inoculated carcasses in the laboratory. Carcasses naturally contaminated with campylobacters were taken from the production line immediately prior to chilling and treated with steam at atmospheric pressure (100°C), for 10 s, or hot water dip at 80°C for 20 s with or without subsequent crust freezing during chilling, and compared to carcasses only crust frozen or only chilled in the plant chiller. Numbers of campylobacter, Enterobacteriaceae (as indicators for salmonella) and pseudomonads were determined before and after treatments.

Results indicated that reductions were obtained in numbers of all three groups of bacteria, although lower than those observed in the previous laboratory trials. Limitations of the crust freezing rig prevented using more than four carcasses per treatment. In addition, the bacteria (especially the campylobacters, which were the most important target) were present in relatively low numbers, and high variability.

In addition, an investigation was made (Appendix 2) into the effect on appearance of immersing carcasses for 5 s or 10 s in steam or, 10 s or 20 s in hot water (80°C) prior to chilling, portioning and packing as the normal product. Pack appearances were assessed after 5 and 11 days storage at 1°C. The results showed that while a range of appearances were deemed acceptable. The immersion treatments gave better appearance scores than steam treatments. Some portions (drumsticks, skinless breast) were acceptable for all treatments and appearance tended to become more acceptable with time.

Further microbiological investigations were recommended, using only hot water immersion (80°C for 20 s) and examining larger groups of test and control carcasses. Extension Report 2 describes these investigations.

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**Introduction**

Previous laboratory-scale work using chicken carcasses inoculated with *Escherichia coli* and *Campylobacter jejuni* had shown that steam (100$^{o}$C for 12 s) or hot water (80$^{o}$C for 20 s) reduced their numbers by several log cycles, and that subsequent crust freezing enhanced this effect. This extension was designed to investigate whether either of these treatments could be used in a commercial setting, and whether hot water or steam adversely affected the appearance of the carcasses when displayed for retail sale.

## Part 1 Microbiological evaluation

### Materials and Methods

A commercial chicken-processing plant was visited on four occasions for the microbiological trials, and on a fifth occasion to investigate the effect of the treatments on appearance.

**Microbiological trials**

The aim was to try to use carcasses from flocks colonised by campylobacters – psuedomonads and Enterobacteriaceae would be present on the carcasses from any flock. For the first trial (17$^{th}$ August 2004) a flock was chosen that had previously been 'thinned', which usually introduces campylobacters into the shed, and infects the remaining birds. Unfortunately, this flock was not infected, so results were only obtained for Enterobacteriaceae and pseudomonads. Subsequently, samples were taken from as many flocks as possible during one day, and caecal contents were also taken and checked by direct plating to see which flocks were positive for campylobacter. This tactic enabled campylobacter positive flocks to be identified 1-2 days after the visit to the processing plant. All the samples were therefore stored at 3$^{o}\pm$1$^{o}$C until the results from the caecal contents were known, and samples from one campylobacter positive flock examined. Three more trials were conducted on September 8$^{th}$, September 29$^{th}$ and October 14$^{th}$ 2004.

Twenty carcasses were taken from the line immediately pre-chill from each flock examined. Four were treated only by crust freezing ('accelerated in-line maturation' (AIM) chiller rig supplied by Air Products Limited, Basingstoke), four were treated with steam and then by crust freezing, four with hot water and then crust freezing, four with steam only and four with hot water only. In addition, four carcasses were taken from the same flock after chilling in the processing plant chiller.

**Atmospheric steam rig.** A pilot atmospheric steam cabinet (was designed and constructed for project MO 1019, based on the simple cabinet used by James *et al.* (2000)). It consisted of a double-skinned stainless steel treatment chamber supported by two legs. A layer of insulation lay between the two skins and a third skin surrounded the front and sides. The front of the vessel housed a double glazed unit to

allow viewing of product during treatment. The chicken carcass was raised into the chamber on a gambrel that was attached to a pneumatic cylinder mounted on one of the legs and connected via pulleys. This allowed automated movement of the product into the chamber. The pneumatic cylinder was controlled by a relay switch that could be adjusted to a desired treatment time. The only treatment variable was the process time, i.e. the time the chicken remained in the steam environment. The steam was provided by three 2.8 kW boilers operating in tandem. The boilers were placed in a stainless steel box supported on the back of the legs. Steam entered the plenary chamber at the top of the vessel through 7.5 cm pipes and was evenly distributed into the main chamber through a baffle. Pressurised air for the pneumatics was provided by a permanent fixed supply. The control box for the pneumatics and temperature-monitoring equipment was placed on a shelf at the back of the cabinet. Around the bottom of the chamber was a lip to collect condensate from the inner walls of the vessel and drain it into two bottles alongside the boilers. Detailed diagrams of the cabinet are shown in Appendix 1.

**Hot water immersion module** The hot water immersion vessel, designed and constructed for Project MO 1019, utilised two insulated stainless steel skinned water tanks (internal dimensions ca. 500 x 500 x 500 mm). One acted as a heating reservoir from which hot water was pumped to a treatment tank in which the chicken carcass being treated was immersed. Heating was provided by two 2.5 kW heating elements. The pump provided even circulation of the water and substantial insulation ensured that there was little temperature difference (<2°C) between the heating tank and process tank, and that temperature stratification was kept to a minimum. Each chicken carcass was submerged in the process tank in a controlled manner via a pneumatically controlled arm. The rate of immersion was controlled to avoid splashing, and the carcass was held under the water for a set time before being raised in a controlled manner. Detailed diagrams of the module are shown in Appendix 1.

**Crust freezing (AIM) apparatus** An experimental rapid chilling system developed by Air Products plc (UK) was used (Kennedy and Miller, 2004). It was an impingement air chilling system, in which the main force of the air, directed over the breast of the carcass, was designed for the accelerated inline maturation (‘AIM’). of the poultry meat. It was capable of running at air temperatures ranging from ambient to -40°C and operating as a multi-staged system. Previous laboratory studies during MO 1019 had indicated that it enhance heat inactivation of both *E. coli* and *C. jejuni* on inoculated chicken carcasses.

**Samples.** All samples were taken from the breast skin. Two 10 $cm^2$ samples (one near the head-end and one diametrically opposite, from the tail-end) were taken from each of the first 20 carcasses prior to treatment. Two more samples were taken from opposite sides after treatment. The four carcasses taken from the same flock after chilling in the processing plant chiller were only sampled once, assuming the counts pre-chill to be the mean of the initial counts from the other 20 carcasses. The samples were taken using sterile 10 $cm^2$ templates and sterile forceps and scalpel blades. The pairs of samples were stored in sterile Stomacher-80 bags with built-in filter at 3$^o$C until examination.

**Microbiological examination** The samples (2 x 10 $cm^2$ skin) were homgenised in a Stomacher 80 (Colworth London) for 1 min with 20 ml MRD (maximum recovery diluent, Oxoid CM 733). Decimal dilutions of the suspensions were prepared in

MRD and surface-plated using a spiral plater (WASP, Don Whitley, Shipley, UK) onto VRBG agar for Enterobacteriaceae (Oxoid CM 485, $37^{o}C$ 24 h), CFC agar for *Pseudomonas* spp. (Oxoid CM 559 plus SR 102) and mCCD agar for *Campylobacter* spp. (Oxoid CM 739 plus SR 155) $41.5^{o}C$ 48 h in microaerobic atmosphere). Red/pink colonies on VRBG agar were counted as Enterobacteriaceae. On CFC agar, *Pseudomonas* colonies were confirmed by oxidase test (positive). *Campylobacter* colonies on mCCDA were confirmed by Gram staining and positive oxidase test. The limit of detection was 5 cfu $cm^{-2}$. In trials 2, 3 and 4, presence/absence of campylobacter in 2 ml of the neat homogenate, plus the remnants of the 20 $cm^2$ breast skin was determined by enrichment in Exeter broth ($37^{o}C$ 48h) followed by plating on mCCD agar.

With the exception of Trial 1, ten pairs of caeca were sampled from each flock from which carcasses were taken (but not from the exact carcasses sampled). Caecal contents from each pair of caeca were streaked onto mCCDA. The plates were examined after 24 and 48h incubation in microaerobic atmosphere, confirming colonies as above. The aim was to examine samples only from flocks whose caecal contents were positive for campylobacter.

Numbers of the three bacterial groups were expressed as $log_{10}$ cfu (colony-forming units) $cm^{-2}$ and the results analysed by one-way ANOVA with treatment as a factor.

## Results

### Trial 1

Numbers of campylobacters were all below the limit of detection, Table 1 and Figs 1 and 2 summarize the results for Enterobacteriaceae and *Pseudomonas* spp. respectively. The full results are in Appendix 1.

## Trial 1

Table 1 **Summary of Trial 1 results (mean $\log_{10}$ colony forming units per $cm^2$ chicken breast skin).**

| Carcass nos. | | Before treatment(s) | | After treatment(s) | | Log reduction | |
|---|---|---|---|---|---|---|---|
| | | Enterobacteriaceae | Pseudomonas | Enterobacteriaceae | Pseudomonas | Enterobacteriaceae | Pseudomonas |
| | | | | | | | |
| 1-4 | AIM only | 2.45 | 2.62 | 2.18 | 1.93 | 0.27 | 0.68 |
| 5-8 | Steam + AIM | 2.35 | 2.92 | 1.19 | 1.13 | 1.16 | 1.79 § |
| 9-12 | Hot water + AIM | 2.98 | 2.49 | 1.22 | 1.64 | 1.77 §§ | 0.85 |
| 13-16 | Steam only | 2.29 | 2.16 | 1.56 | 0.91 | 0.73 | 1.25 |
| 17-20 | Hot water only | 2.70 | 2.84 | 0.44 | 0.32 | 2.23 §§ | 2.52 §§ |
| 21-24 | Plant chiller | 2.55* | 2.61* | 2.13 | 1.79 | 0.42 | 0.82 |

*Estimated as mean counts on first 20 carcasses. Reduction significant (§§$P<0.01$, § $P<0.05$)

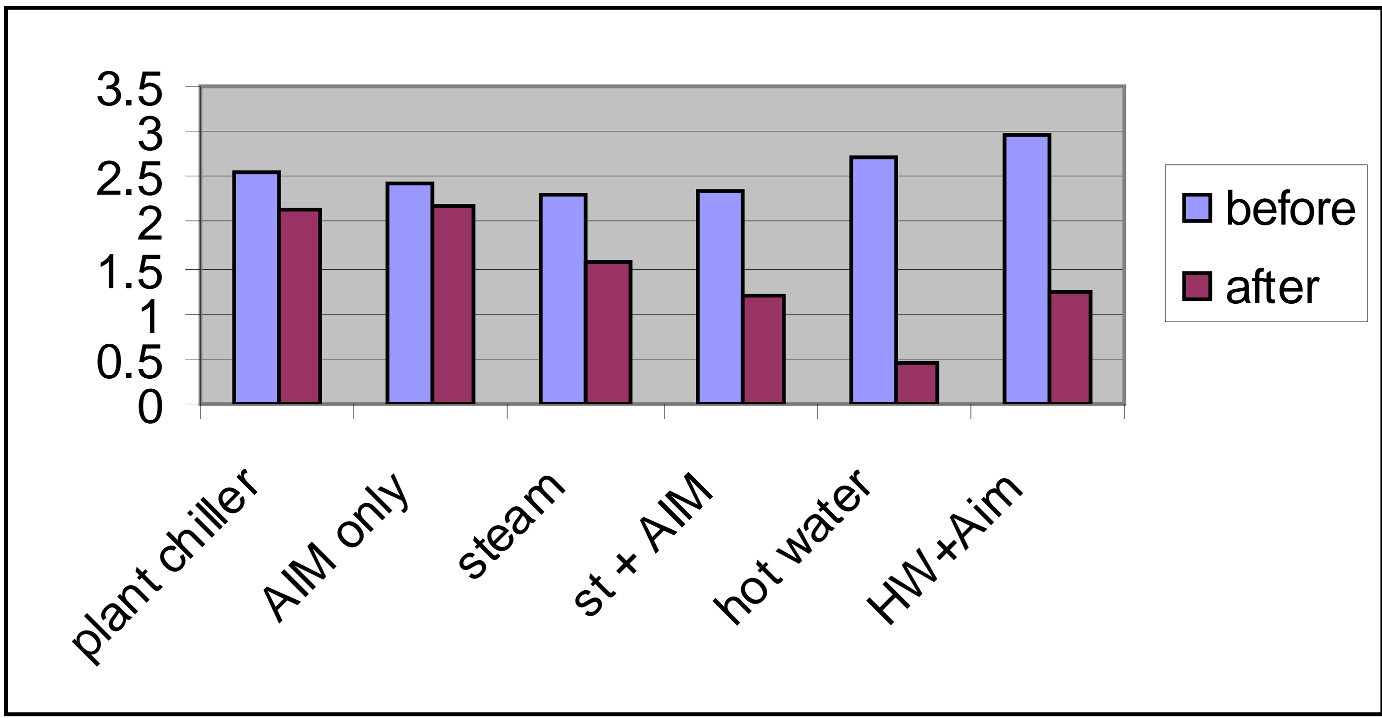


Figure 1 **Trial 1: Mean $\log_{10}$ cfu $cm^{-2}$ chicken breast skin, Enterobacteriaceae before and after various treatments.**

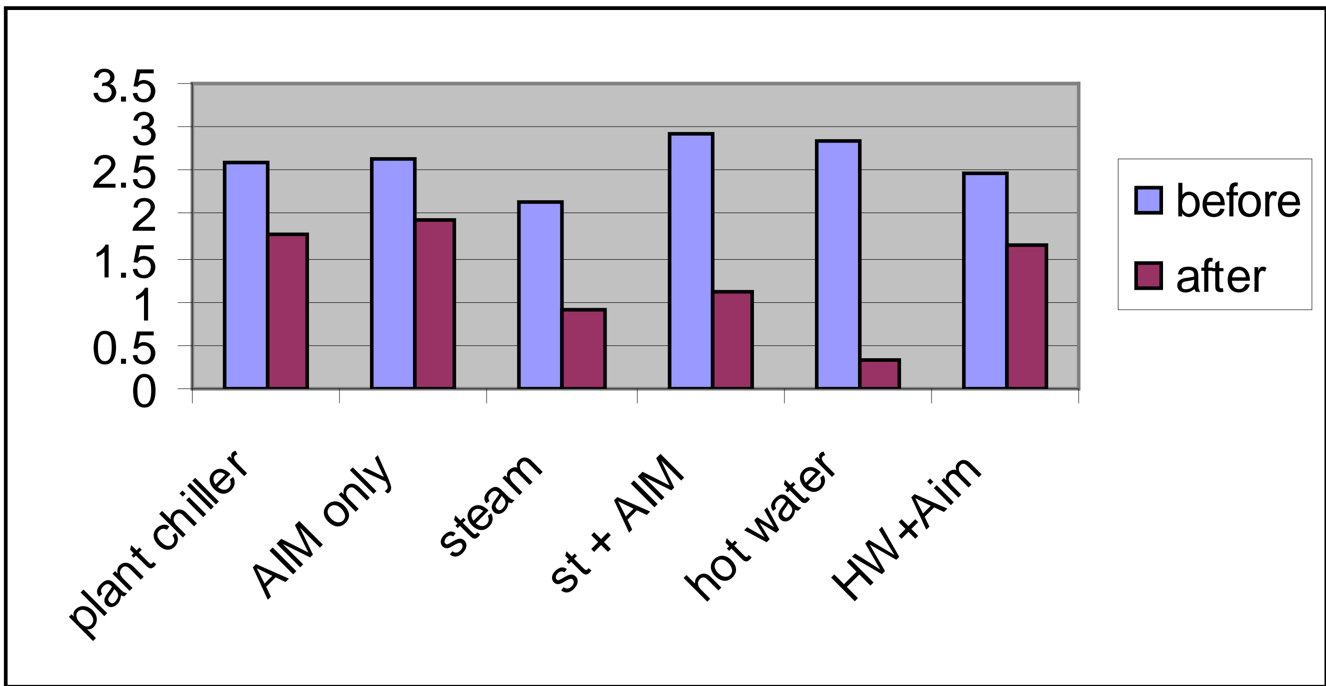


Figure 2 **Trial 1: Mean $\log_{10}$ cfu $cm^{-2}$ chicken breast skin, *Pseudomonas* spp. before and after various treatments.**

Mean numbers of cfu $cm^{-2}$ were lower after all treatments, but no reductions were statistically significant, except that hot water reduced numbers of both pseudomonads and Enterobacteriaceae significantly, hot water plus crust freezing reduced levels of Enterobacteriaceae significantly, and steam plus crust freezing reduced levels of pseudomonads significantly.

**Trials 2, 3 and 4**

Four flocks were sampled and the samples from one *Campylobacter* positive flock (identified by examination of caecal contents) were examined. The results are summarized in Tables 2-4 and Figures 3-14. The full results are in Appendix 1. In trials 2 and 4 both the heat treatments appeared more effective against the Enterobacteriaceae and *Pseudomonas* spp. than they had been in trial 1 (or trial 3), such that all three groups of bacteria were below the level of detection in almost all samples after both types of heat treatment. Numbers of all three groups were reduced following either chill treatment alone, but not below the level of detection, except for *Campylobacter* after crust freezing. However, presence/absence tests for *Campylobacter* demonstrated that viable campylobacters were still present in most samples (Figure 6).

In Trial 3, Enterobacteriaceae and pseudomonads were significantly reduced by steam plus crust freezing, and Enterobacteriaceae additionally by hot water plus crust freezing. In Trial 4, Enterobacteriaceae and pseudomonads were both reduced significantly by steam alone, steam plus crust freezing and hot water alone, while pseudomonads were additionally significantly reduced by hot water plus crust freezing.

## Trial 2

Table 2 **Trial 2: Summary of results (mean $\log_{10}$ colony forming units per $cm^2$ chicken breast skin).**

| Carcass nos. | | Before treatment (s) | | | After treatment(s) | | | Log reduction | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | Ent. | Ps. | Camp. | Ent. | Ps. | Camp. | Ent. | Ps. | Camp. |
| | | | | | | | | | | |
| 1-4 | AIM only | 2.43 | 2.89 | 1.83 | 2.05 | 1.64 | 0.10 | 0.38 | 1.25 | 1.73 |
| 5-8 | Steam+AIM | 2.43 | 2.84 | 1.50 | 0.10 | 0.10 | 0.10 | 2.33 | 2.74 | 1.40 |
| 17-20 | Hot water+AIM | 1.87 | 2.51 | 1.70 | 0.10 | 0.10 | 0.10 | 1.78 | 2.41 | 1.60 |
| 9-12 | Steam only | 2.47 | 3.01 | 1.87 | 0.10 | 0.10 | 0.10 | 2.37 | 2.91 | 1.77 |
| 13-16 | Hot water only | 2.20 | 3.01 | 2.09 | 0.10 | 0.10 | 0.10 | 2.10 | 2.91 | 1.99 |
| 21-24 | Plant chiller | *2.28 | *2.58 | *1.80 | 1.93 | 1.89 | 1.09 | 0.35 | 0.69 | 0.71 |

*Estimated as mean of counts on first 20 carcasses

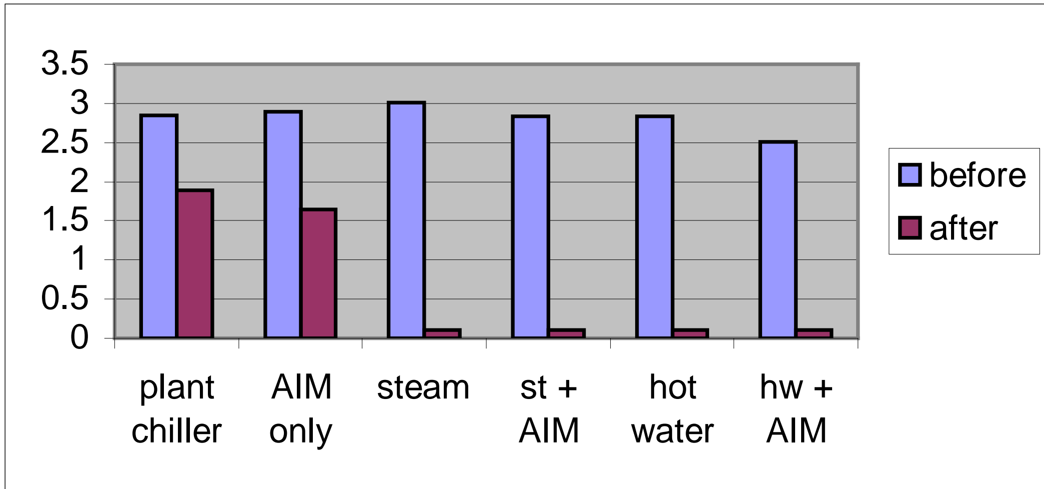


Figure 3 **Trial 2 Mean $log_{10}$cfu $cm^{-2}$ chicken breast skin, Enterobacteriaceae before and after various treatments.**

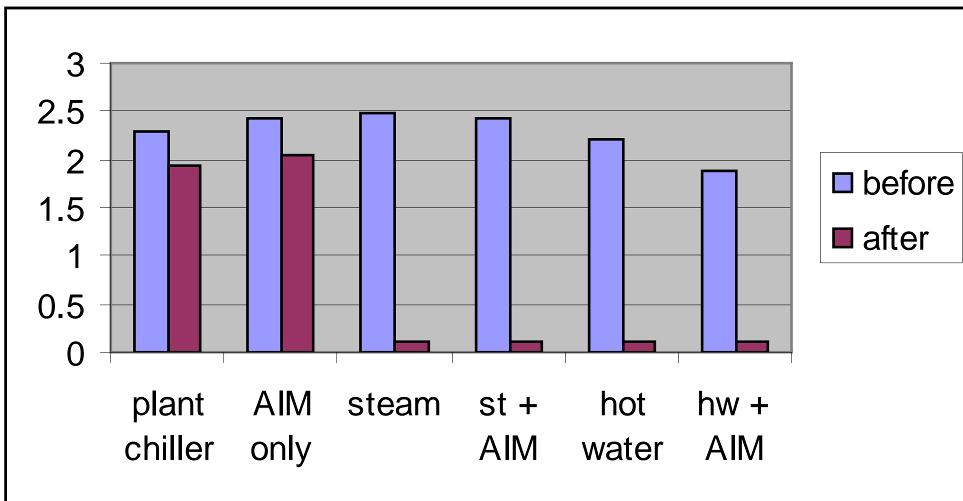


Figure 4 **Trial 2: Mean $log_{10}$ cfu $cm^{-2}$ chicken breast skin, *Pseudomonas* spp. before and after various treatments.**

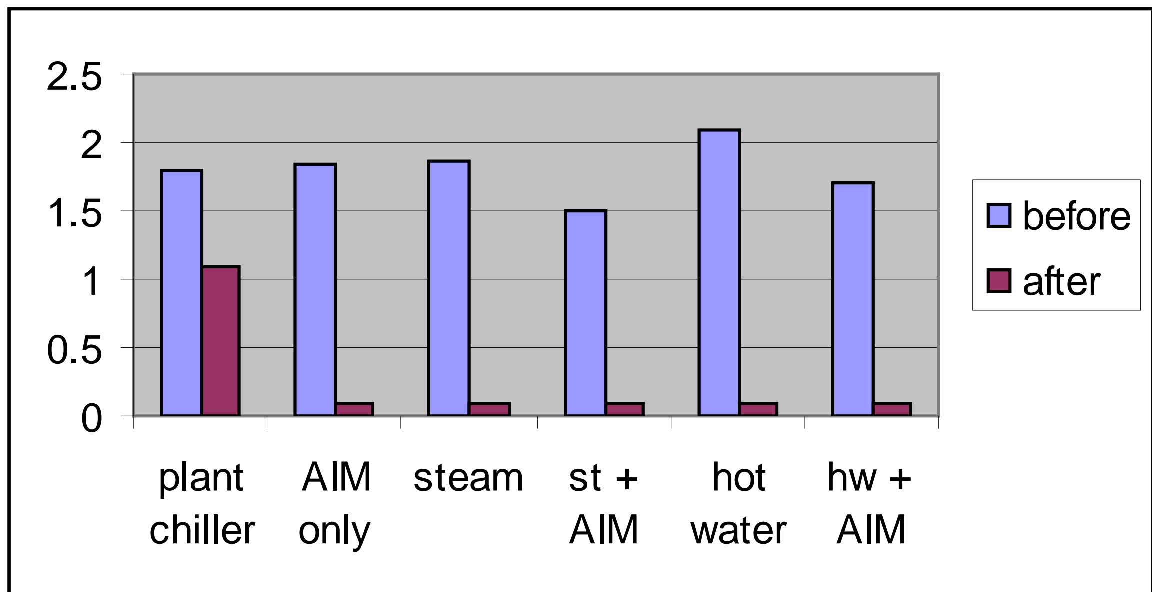


Figure 5 **Trial 2: Mean $\log_{10}$ cfu $cm^{-2}$ chicken breast skin, *Campylobacter* spp. before and after various treatments.**

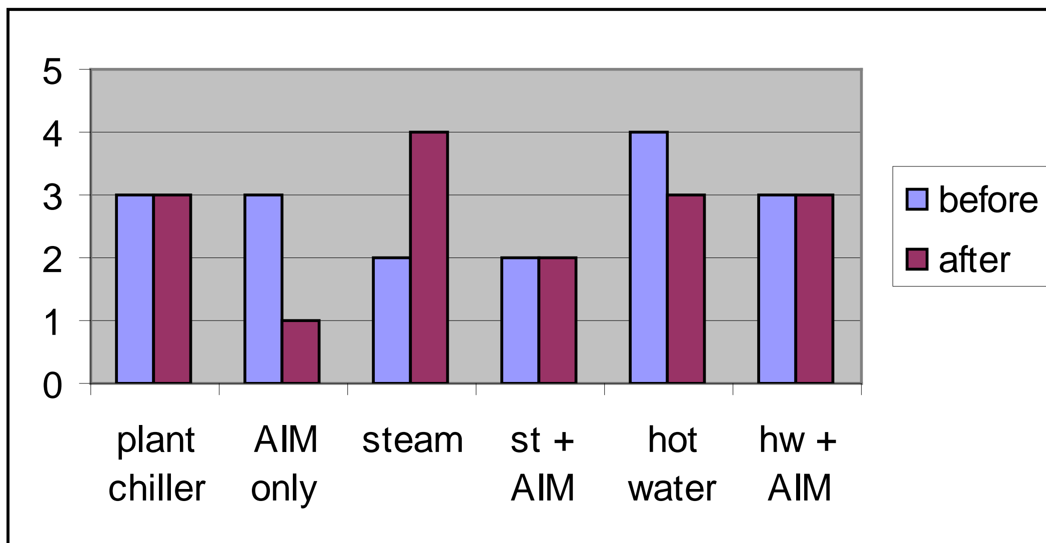


Figure 6 **Trial 2 Number positive out of four presence/absence tests for *Campylobacter* spp. before and after various treatments.**

## Trial 3

Table 3 **Trial 3 Summary of results (mean $\log_{10}$ colony forming units per $cm^2$ chicken breast skin).**

| Carcass nos. | | Before treatment (s) | | | After treatment(s) | | | Log reduction | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | Ent. | Ps. | Camp. | Ent. | Ps. | Camp. | Ent. | Ps. | Camp. |
| | | | | | | | | | | |
| 1-4 | AIM only | 1.53 | 1.67 | 1.18 | 1.36 | 1.84 | 0.40 | 0.17 | -0.34 | 0.78 |
| 5-8 | Steam+AIM | 1.63 | 1.83 | 1.11 | 0.40 | 0.87 | 0.40 | 1.24 | 0.96 | 0.43 |
| 17-20 | Hot water+AIM | 1.57 | 1.53 | 0.89 | 0.68 | 0.55 | 0.40 | 0.89 | 0.99 | 0.49 |
| 9-12 | Steam only | 1.49 | 1.72 | 0.78 | 1.82 | 1.74 | 0.55 | -0.33 | -0.02 | 0.39 |
| 13-16 | Hot water only | 1.70 | 1.55 | 1.02 | 2.28 | 2.73 | 0.82 | -0.58 | -0.83 | 0.20 |
| 21-24 | Plant chiller | *1.58 | *1.66 | *1.00 | 1.77 | 1.82 | 1.16 | -0.19 | -0.16 | -0.16 |

*Estimated as mean of counts on first 20 carcasses.

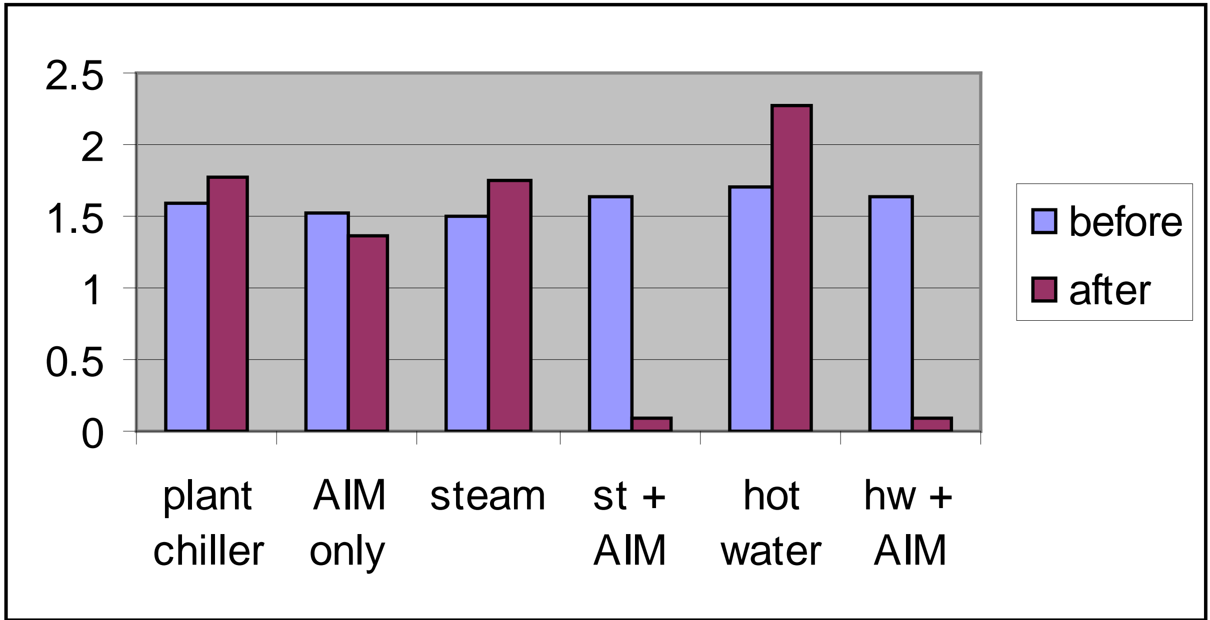


Figure 7 **Trial 3: Mean $log_{10}$ cfu $cm^{-2}$ chicken breast skin, Enterobacteriaceae before and after various treatments.**

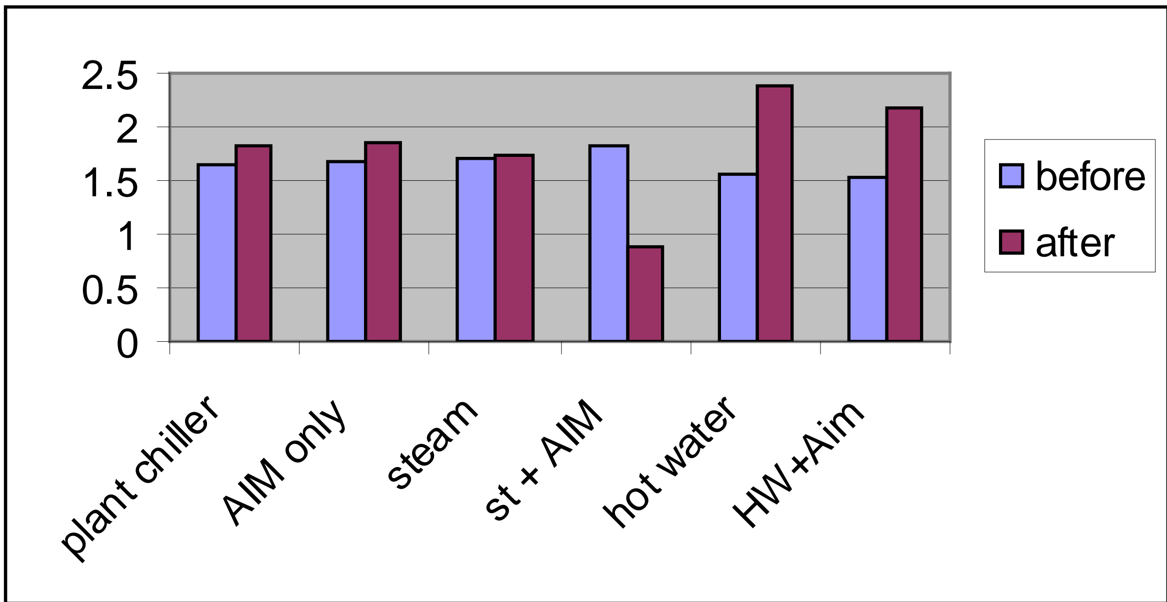


Figure 8 **Trial 3: Mean $log_{10}$ cfu $cm^{-2}$ chicken breast skin, *Pseudomonas* spp. before and after various treatments.**

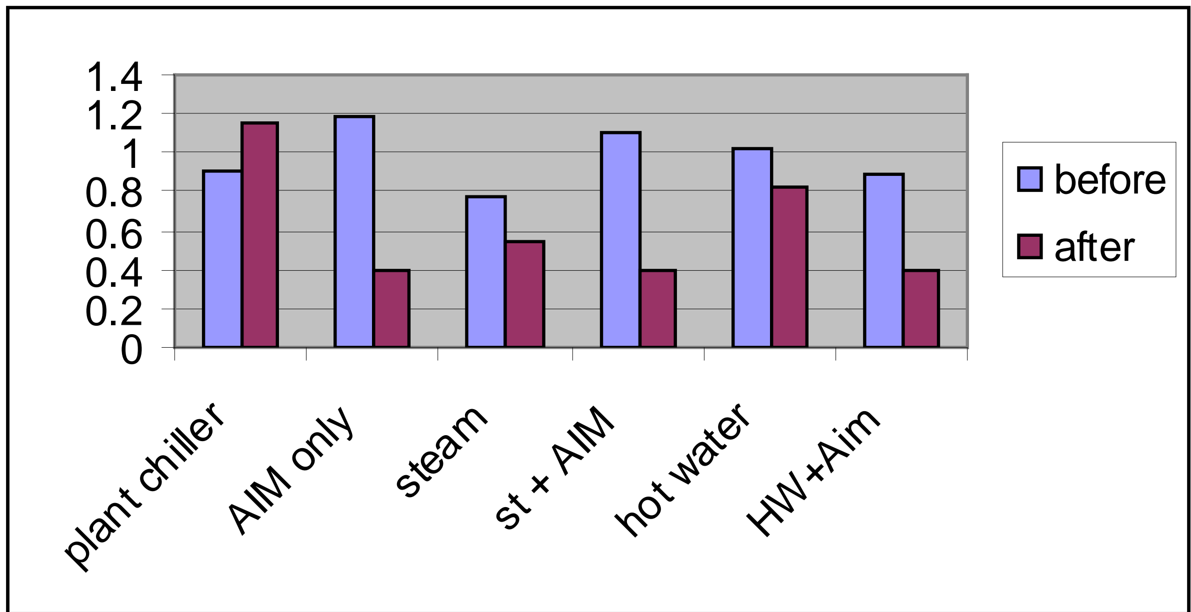


Figure 9 **Trial 3: Mean $\log_{10}$ cfu $cm^{-2}$ chicken breast skin, *Campylobacter* spp. before and after various treatments.**

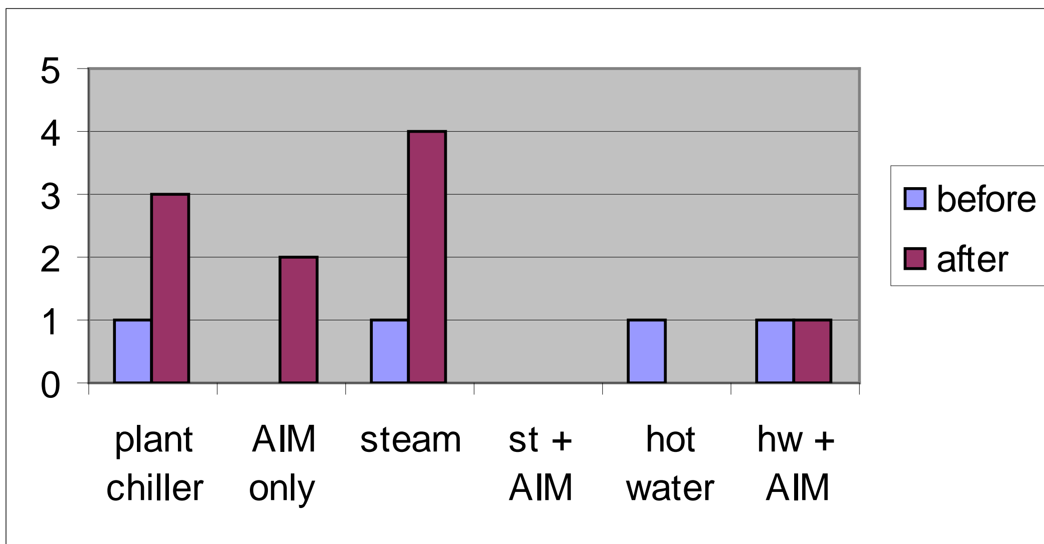


Figure 10 **Trial 3 Number positive out of four presence/absence tests for *Campylobacter* spp. before and after various treatments.**

**Trial 4**

Table 4 **Trial 4 Summary of results (mean $\log_{10}$ colony forming units per $cm^2$ chicken breast skin).**

| Carcass nos. | | Before treatment (s) | | | After treatment(s) | | | Log reduction | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | Ent. | Ps. | Camp. | Ent. | Ps. | Camp. | Ent. | Ps. | Camp. |
| | | | | | | | | | | |
| 1-4 | AIM only | 2.06 | 1.41 | 0.17 | 2.15 | 2.24 | 0.52 | -0.09 | -0.83 | -0.35 |
| 5-8 | Steam+AIM | 1.95 | 1.62 | 0.30 | 0.10 | 0.32 | 0.10 | 1.85 | 1.30 | 0.20 |
| 17-20 | Hot water+AIM | 1.79 | 1.38 | 0.29 | 1.20 | 0.10 | 0.10 | 0.59 | 1.28 | 0.19 |
| 9-12 | Steam only | 1.65 | 1.62 | 0.60 | 0.10 | 0.10 | 0.10 | 1.55 | 1.52 | 0.50 |
| 13-16 | Hot water only | 1.86** | 2.24 | 0.66 | 0.10 | 0.10 | 0.10 | 1.76 | 2.14 | 0.56 |
| 21-24 | Plant chiller | 1.86** | 1.65* | 0.36* | 2.20 | 1.62 | 0.47 | -0.34 | 0.03 | -0.11 |

*Estimated as mean of counts on first 20 carcasses;
** Estimated as mean of counts on first 16 carcasses.

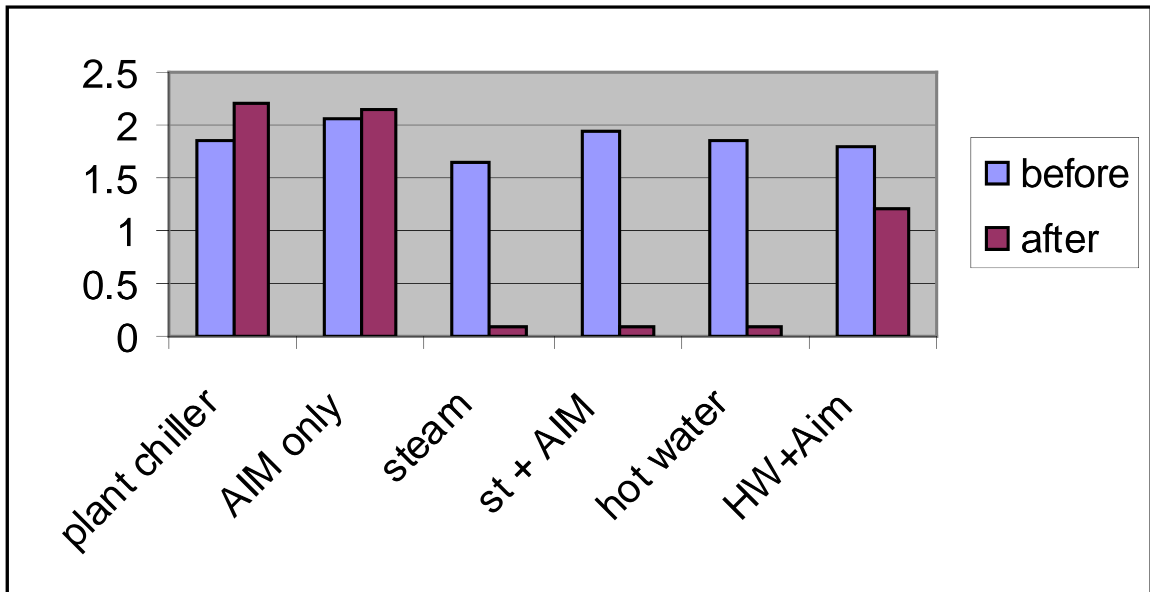


Figure 11 **Trial 4. Mean $\log_{10}$cfu $cm^{-2}$ Enterobacteriaceae before and after various treatments.**

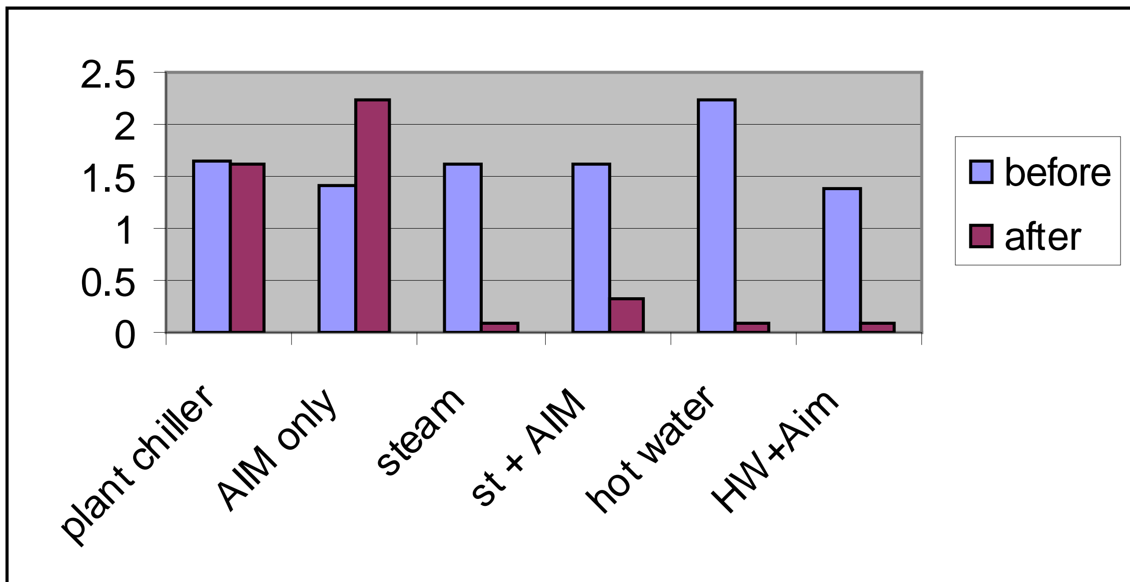


Figure 12 **Trial 4. Mean $\log_{10}$cfu $cm^{-2}$ *Pseudomonas* spp. before and after various treatments.**

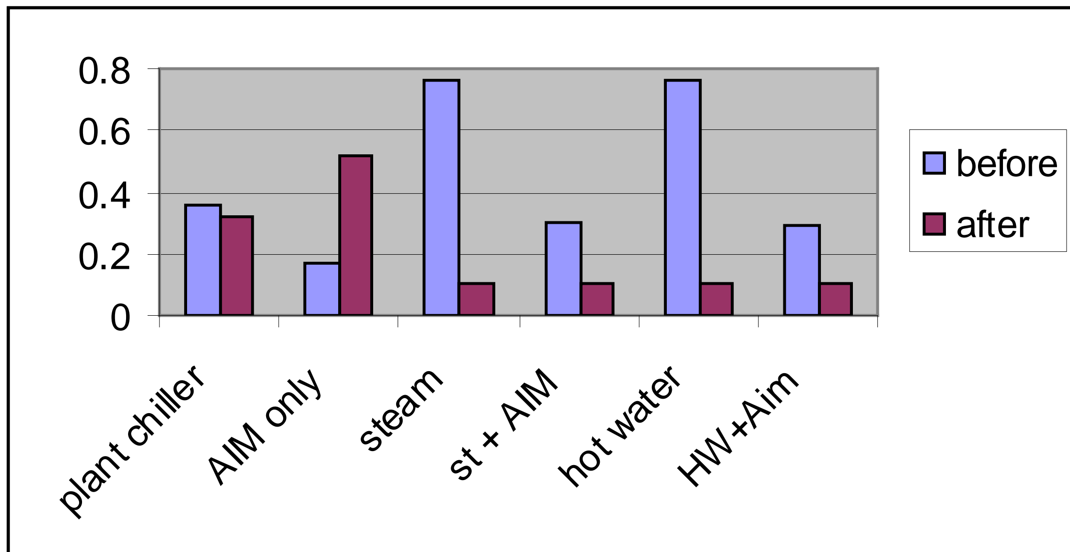


Figure 13 **Trial 4. Mean $\log_{10}$cfu $cm^{-2}$ *Campylobacter* spp. before and after various treatments.**

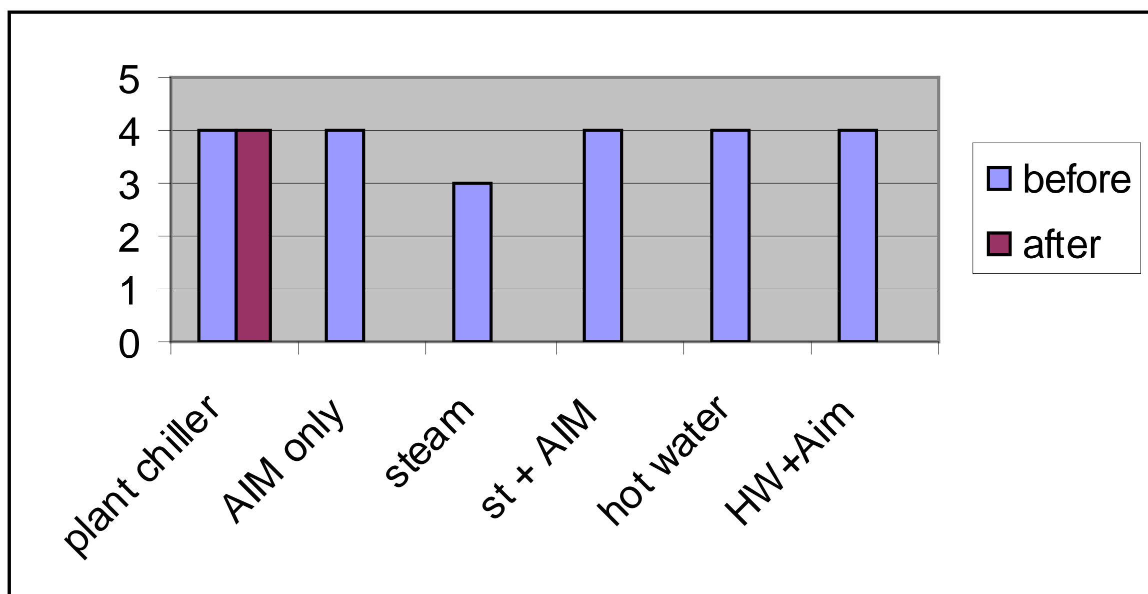


Figure 14 **Trial 4. Number positive out of four presence/absence tests for *Campylobacter* spp. before and after various treatments.**

## Discussion and Conclusions

Numbers of all groups of bacteria enumerated before and after the various treatments were low, especially numbers of campylobacters, and the range of results obtained for duplicate results was high, making it difficult to compare different heat treatments – e.g. steam versus hot water. The results of the presence/absence tests showed that none of the treatments reduced the target bacteria below the level of detection, as would have been expected from the laboratory-scale studies carried out on inoculated

carcasses. However, there was a trend for heat treatment to reduce numbers of all three groups of bacteria. On some occasions crust freezing seemed to enhance the effect of the heat treatments, and on others had no effect, or appeared to make matters worse (e.g. trial 4 pseudomonads Fig. 12). In general crust chilling alone yielded lower counts than chilling in the plant chiller, but this could have been due to there being more opportunities for carcasses passing through the plant chiller to be contaminated from the environment and other carcasses. Comparison of the total number of campylobacter positive samples obtained by enrichment plating (presence/absence) from all three trials on campylobacter positive carcasses (Figures 6, 10 and 14) ranks the treatments in the following order (best first):

1. steam plus crust freezing (2/12);
2. hot water alone (3/12);
3. crust freezing alone (3/12);
4. hot water plus crust freezing (4/12);
5. steam alone (7/12);
6. plant chiller (10/12).

At the conclusion of this study the crust freezer pilot plant was no longer available. The pilot crust freezer had also previously limited the number of replicate carcasses that could be treated at one time. With this in mind, and on the basis of the results of the investigation of the effect of steam and hot water on carcass appearance, it was decided to carry our further trials using hot water only (80$^{o}$C 20 s) on larger groups of carcasses from campylobacter positive flocks. Chilling would be done in the plant chiller, but on separate racks, to minimise possible cross contamination from the environment and from untreated carcasses.

## References

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Kennedy, C., Miller, J., 2004. A new chilling technique for processing chicken. Food Science and Technology 18, 30-33.