Campden BRI Group:
Campden BRI (registered no. 510618)
Campden Technology Limited (registered no. 3836922)
Brewing Research International (registered no. 2690377)

Registered Office:
Station Road ◆ Chipping Campden ◆ Gloucestershire ◆ GL55 6LD ◆ UK

Campden BRI
food and drink innovation

Final Report on FSA Project FS121014B (M01059)

Efficacy, Practicality, and Costs of Using Lactic Acid Solutions, Ozonated Water, or Ozonated Carbon Dioxide Pellets to Reduce *Campylobacter* Contamination in Slaughterhouses

Dean Burfoot, Liz Mulvey, Emma Foy, Rob Turner, Emma Maguire, Keith Jewell
Campden BRI
and
Viv Allen, Dawn Harrison, Victoria Morris, Mike Hutchison
University of Bristol
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SUMMARY

Interventions to reduce *Campylobacter* on chickens at slaughterhouses were tested. Those interventions were spraying carcasses with lactic acid or ozonated water, using ozonated carbon dioxide pellets, or treatment with cold plasma.

Eight trials were carried out to examine the reduction in *Campylobacter* numbers that could be achieved by spraying of lactic acid onto the carcasses. The earliest trials, funded by industry or Campden BRI, applied the acid using a hand sprayer, electrostatic sprayer or an in-line tunnel. Those trials showed that pre-chill spraying was more effective than post-chill spraying. One of the trials reduced presumptive *Campylobacter* counts on carcasses from 2.7 $\log_{10}$ cfu/g to below the limit of detection of10 cfu/g. In all other trials, only confirmed *Campylobacter* were considered and that high level of reduction was not achieved. That early work identified the conditions to be tested in the three FSA-funded trials that used the in-line tunnel. Those trials concluded that applying a 4% solution of lactic acid, buffered using sodium lactate to pH≈3.8, at the rate of 100 g of acid solution per kg of carcass, applied over 7s, produced up to a 0.4-$\log_{10}$ reduction on breast skin and 0.8 $\log_{10}$ reduction on back/neck skin samples. Using an 8% solution buffered to the same pH, produced a 1.9-log reduction in the numbers of *Campylobacter*. During some trials, but not all, a change in the appearance of the *Campylobacter* was caused by the acid treatment; this would explain why presumptive *Campylobacter* was not a good test in the one trial showing very high microbial reductions. Applying an 8% solution adversely affects the appearance of the carcasses causing greying of the leaf fat and skin but use of a 4% solution is acceptable as judged by poultry processors. Further testing using a 5% solution, supported by consumer panelling, has the potential to offer greater microbial reductions.

Pre-chill spraying of ozonated water on to carcasses was carried out in three trials. No evidence was found of a statistically significant reduction in *Campylobacter* numbers due to a 30s treatment and 5.6 kg/min flow rate. The average ozone concentration was 3.7ppm. Increasing the concentration above 6ppm might increase the microbial reduction but it would require specific measures to avoid safety hazards to workers and risks oxidising the materials of the process equipment.

The use of ozonated carbon dioxide pellets offered the potential of *Campylobacter* reductions due to ozidation and temperature reduction. The company due to supply the pellets made the commercial decision not to pursue this technology at the current time.

Treatment of skin-on chicken breast fillets with cold plasma for 20s followed by a 280s holding period produced no statistically significant reduction in *Campylobacter* numbers despite producing a peak ozone concentration of 270 ppm.

Out of the systems tested, the application of lactic acid offers the greatest potential for use as an intervention for *Campylobacter*.

1. INTRODUCTION

Campylobacter species are the most common cause of bacterial foodborne disease in the UK and are most often transmitted by poultry (BBSRC, FSA, defra, 2010). Using interventions at the slaughterhouse is one approach to reducing *Campylobacter* on poultry carcasses. The purpose of this project was to investigate interventions that would require EU approval before they could be used by industry to reduce *Campylobacter* numbers. The interventions included lactic acid, ozonated water, and ozonated carbon dioxide pellets.

In practice, the company that was to produce the ozonated carbon dioxide pellets made the commercial decision to no longer develop that approach. With the agreement of the FSA Project Officer, some of the funding was re-directed to examine the efficacy of using cold plasma.

Each section of this report considers one of the interventions. A summary of the relevant literature, information from industry, and descriptions of each of the trials are included.

2. LACTIC ACID

Several reviews on decontamination methods for poultry, including lactic acid, have appeared over the last 3 years. A literature survey by Loretz *et al*. (2010) reviewed the decontamination treatments for chicken including the use of lactic acid solutions. Burfoot and Mulvey (2010a, 2010b, 2011) appraised the available literature on the use of lactic acid as an intervention in chicken and turkey processing with a view to identifying the most suitable conditions for treatment. The scientific opinion by EFSA (2011) on control options for *Campylobacter* in broiler meat production also reviews the available data on the use of lactic acid. The following comments draw heavily on those recent papers.

Despite microorganisms sometimes throwing up surprises, common sense would suggest that the greatest bactericidal effect with lactic acid would be achieved with: highest concentration of acid; lowest pH and buffered to maintain pH; most acid; applied at all points along the line; longest contact time; highest temperature; and highest carcass temperature. However, to adopt this approach would be expensive, time-consuming, potentially dangerous to operators, and provide an extremely low quality product, and it is for these reasons that the literature was reviewed to identify practical options.

Concentration and pH of lactic solution

None of the papers cited in this sub-section considered the effect of lactic acid on *Campylobacter*: they relate to aerobic plate count (APC). However, several of them provide information on the effect of acid concentration and ph on appearance of treated birds.

Zeitoun and Debevere (1992) used between 0 and 10% lactic acid/sodium lactate buffer (pH=3). The results showed that general microbial reduction increased almost linearly with acid concentration. Buffering enabled the effect of the acid to be maintained during storage.

Van Netten *et al.* (1994) used up to 2% lactic acid and concluded that the higher concentration was most effective. Bactericidal effect increased with reducing pH (tested at 2.6, 3.5, and 4.0). But this was on a meat analogue. This research indicates that pH needs to be dropped below 4.

Pipek *et al.* (1997) used 1 and 2% solutions of lactic acid and found that the higher concentration was more effective in reducing microbial counts.

Purac (2000) recommended 1.5 to 2.5% lactic acid for an optimum effect. Hover (2008), representing Purac, indicated that, based on the literature, 1.5-2.0% solutions gave good results and a 2-unit pH drop was the target.

A MAFF study (1999) concluded that the lactic acid solution needed to be between 2.5 and 5%. Other studies had found that high concentrations of acid can lead to greying of the skin, but this was not found at 5%, perhaps due to using a low dose and rinsing almost immediately after treatment.

Sinhamahapatra *et al.* (2004) found no significant change in the pH of chicken longitudinal sections (not described in detail in the paper) after treating with 2% lactic acid and then storing for up to 48 hours. The pH of the treated birds was 6.3 to 6.4.

In summary, previous research indicates that bactericidal effect, and cost, increases with acid concentration but the use of high concentrations or long contact times can lead to greying of the skin. Discolouration would be most likely where the acid might collect, such as between thighs or wings and breast.

Very low pH would probably adversely affect quality. Buffering the solution, using sodium lactate, would enable a higher pH and may enable greater effectiveness during storage: around a 1.5-log reduction in aerobic plate count (APC) during storage has been reported when a buffer was used. The highest reported microbial reduction (APC) due to a lactic acid treatment was 2.5-log.

The literature indicates that 3.5% solution (pH=3) could be applied to provide an effective treatment on broilers and this liquid would drip off during subsequent stages of processing. In most of the trials carried out in the present study, a 5% commercial solution (FCC80, Purac) with original concentration of 80% lactic acid was used i.e. 4% acid concentration. Tests were also carried with the original pH of the solution (2.0) and with a buffered solution (pH = 3.7 to 3.9).

Application of the acid

None of the papers in this subsection considered the effect on numbers of *Campylobacter*, but they do demonstrate the advantages and disadvantages of spraying and dipping.

Zeitoun and Debevere (1992) and Pipek *et al.* (1997) used spraying alone whereas Sakhare *et al.* (1999) recommended dipping or spraying, based on finding a 0.9-log cfu cm^{-2} reduction in total plate counts using dipping after evisceration, and a 1.0-log cfu cm^{-2} reduction using spraying. A MAFF study (1999) used low volume sprays (15ml/carcass), which may be one of the reasons why a high acid concentration (5%) was recommended. Sinhamahapatra *et al.* (2004) compared the effectiveness of dipping or spraying for 30 seconds with 2% lactic on to chicken. The time of dipping was not defined. After 0, 24 and 48 hours of storage the reductions in total plate counts by dipping were 1.4, 1.2, and 1.4 log cfu cm^{-2} and by spraying the reductions were 1.1, 0.9 and 1.2 log cm^{-2}. Okolocha and Ellerbroek (2005) also specifically compared the effectiveness of spray and dipping and concluded that dipping gave the best overall reductions for the various chemical treatments applied.

However, the results in the table show that for the lactic acid, on the day of treatment, spraying 1-litre of solution, 2- litres of solution, or dipping produced microbial reductions of 0.4, 1.3, or 0.6 log cfu per millilitre of carcass rinse. Three days after treatment the respective reductions were 0.9, 2.0, and 0.7 log, and on Day 6, the respective reductions were 1.0, 1.5, and 2.3 log cfu per millilitre of carcass rinse.

Overall, immersion (dipping) in the acid would be expected to give the best coverage, depending on the dipping time, but it has the potential to create a "microbial soup" associated with some recirculation systems. Deluging is another option but no data is available on its effectiveness for poultry. It would also require collection and treatment of liquid prior to re-use. Spraying generally requires less liquid than other application methods and was used in the trials described in this report. Adding the acid to the water of the inside/outside was considered, but this would have severely diluted the acid due to the large volumes of water used.

Amount of acid solution to be applied

Zeitoun and Debevere (1992) sprayed 600 ml samples of solution onto chicken legs. Assuming a 250 g leg portion, the application rate would have been 600/0.25 = 2400 ml/kg. Sakhare *et al.* (1999) used 50 ml per carcass (= 50/1.5 = 26 ml/kg for a 1.9 kg carcass). However, with a 0.25 % solution this produced less than a 1-log reduction in total viable count (TVC). A MAFF study in 1999 used 15 ml per carcass but used 5% solutions to achieve a significant microbial reduction and, for a 1.9 kg bird, this application rate equates to 15/1.9 = 8 ml/kg. The study reported reductions in TVC between 0.7 and 2.5-log. Applying liquid evenly to carcass surfaces at such small volumes requires many fine spray nozzles that are correctly directed.

Okolocha and Ellerbroek (2005) found that applying 2 litres of lactic acid by spraying gave a greater log reduction in APC than a 1 litre application. The paper is unclear on how these large volumes were applied in the 10 s application time that is quoted: much of the solution would be expected to miss or bounce off the birds. Although bird weight is not specified, the birds are described as intensively reared broilers at 30-35 days old. To estimate the lowest application rate, assuming a 2.2 kg bird (excessively high for a bird of that age) is treated with 1000 ml of acid solution (2%) then the application rate would be 1000/2.2 = 455 ml/kg and the highest application rate would be 2000/1.2 = 1667 ml/kg.

A Meat Industry Services document from Food Science Australia (2006) indicates that 8000 ml of 2% lactic acid is needed to treat 10 pigs. Assuming a surface area of a pig to be 10000 cm^2, and that of a broiler to be 700 cm^2, the amount of acid required to treat one broiler would be 700 x 8000/10000 = 560 ml per bird or 560/1.9 = 295 ml/kg for a 1.9 kg bird.

Hover (2008), a representative of Purac, recommended using 100 ml/kg.

Based on the published data, there is no consensus on the amounts of lactic acid solution required for treating broilers, with amounts varying widely from 8 to 2400 ml/kg. Only the MAFF study specifically tried to reduce the amount of solution required but that appears to have led to the need for higher acid concentrations than used in other studies and used a complex spray arrangement. The figure quoted by Hover (2008), from Purac, is given in units of ml of solution per kg of bird. All other results from the literature have been converted to these units for comparison. Presumably kg are used as the base unit

because the weights of the birds are known. Surface area should be the real basis for scaling-up as it is the surface that is being treated. This point could be important if the process were to be scaled up from broilers to turkeys.

In view of the lack of other data, the initial studies described later in this report applied lactic acid at around 100ml/kg of carcass using a spray. This figure is a compromise between the very high levels used in published research exercises and the low values used in the MAFF study, and agree with Hover.

Location on the process line where the acid should be applied

Sakhare *et al.* (1999) suggested treating the carcasses everywhere at each process along the line, including scalding. The MAFF study (1999) treated the carcasses prior to the inside/outside (I/O) washer but concluded that immediately after the I/O washer would have been preferable. I/O washers operate at high flow rates such that incorporating the lactic acid into the washer would probably be too expensive.

Based on an extensive literature review, Hover (2008) concluded that the acid should be applied on the process line as soon as is practically possible when the microorganisms are still loose. This approach applies to many decontamination methods.

In summary, the greatest microbial reduction would be expected if the acid were applied at each point along the line. Beyond that option, then the acid should be applied while the carcases are still hot. Application of the acid immediately post I/O washer, preferably after water has run-off to avoid dilution, was used in the studies described later.

Temperature and application time of the acid

The study by Van Netten *et al.* (1994) with a meat analogue showed that over the range of 21 to 50°C, the rate of microbial reduction increased with increasing temperature. They also showed that the reduction in *Listeria* was little affected by holding time in a 2% lactic acid solution over times of 0 to 300 s. However, the reduction in *Campylobacter*, *Salmonella* and *Enterobacteriaceae* increased with time. Using 40°C rather than 21°C more than halved the D-value (the time required at a given temperature to reduce the number of organisms by 1 log (90%).

Pipek *et al.* (1997) used acid at 40 to 45°C for an unspecified application time. Presumably this range was used as the highest values without exceeding the bird temperature. Okolocha and Ellerbroek (2005) used a 10 s spray time followed by 5 minutes for dripping. Hover (2008) recommended the application/standing time be as long as possible and the optimum temperature to be 25°C. All of these studies indicate that the highest temperature and longest time should be used. In practice, 20 s is about the longest practical application time available on a poultry line and 40°C the highest temperature. These values were used in preliminary trials described later, but the costsand potential odour problems associated with lactic acid at 40°C were considered significant and further trials were carried out using the acid at room temperature.

2.1 Campden BRI Funded Trial 1 – 2010 (Trial LA1)

Campden BRI carried out a series of trials using, and revising, the conditions identified above to examine the appearance of chicken and turkey carcasses sprayed with lactic acid solution (Burfoot and Mulvey, 2010a, b, 2011). The most appropriate lactic acid application identified from the literature was at 4% concentration, buffered to pH = 3.7, and applied directly after the inside-outside washer at the rate of 100g of acid / kg of carcass. When applied to chicken, this treatment caused a slight paling of chicken skin and slight greying of the leaf fat but this was considered to be commercially acceptable by four poultry processors that examined the treated birds (Burfoot and Mulvey, 2011)..

Burfoot and Mulvey also examined the reduction in aerobic plate count (APC) caused by the acid treatment and found a dependence on the number of days of storage after killing and treating with acid. For chicken (Figure 1), there was a highly significant difference in aerobic plate counts ($p<0.0005$) on control and treated birds on Days 0, 3, 6, and 9, but no difference on Days 13 and 16 when the microbial counts were at the limit of growth. The largest difference between the APC on control and treated birds occurred at Day 6 when the microbial reduction was 2.1 log cfu/g. For turkey (Figure 2), there was a highly significant difference ($p<0.001$) on control and treated birds on Days 2, 3, 6, and 9 but no difference on Days 13 and 16 when the microbial counts were at the limit of growth. The largest difference between the counts on control and treated birds occurred at Day 9, when the microbial reduction was 1.1-log cfu/g. Trials by the industry in conjunction with those studies found that the prevalence of *Campylobacter* on carcasses on the day after slaughter was reduced significantly ($p<0.001$) by the application of the acid (Burfoot and Mulvey, 2010b, 2011). Analysis of data from other trials by the industry showed that the application of lactic acid significantly reduced total plate counts from turkey samples taken from carcasses that had been air ($p<0.001$) or water chilled ($p<0.005$).

In those tests, carcasses were treated with 100 g of acid solution per kg of bird weight. This amount is very large compared to the 0.4 to 2.1% pick-up of solution that was measured, based on the weight of the birds. This finding suggested that less solution, or a better application method, could be used without adversely affecting the microbial reduction.

2.2 Industry Funded Trial 1 (Trial LA2)

Following the Campden BRI-funded trials, the industry funded further work. An electrostatic spray nozzle from Electrostatic Spraying Systems was used in some of these tests with the aim of examining ways to reduce the amount of acid required. The nozzle was a twin fluid design with an inductively charged electrode. Compressed air was supplied to the nozzle at 2 bar and buffered lactic acid solution was supplied at 1 bar from a pressurised Cornelius tank. Preliminary trials were carried out to measure flows rates and visualise the effects of the electrostatic charge on the deposition of liquid onto carcasses. The flow rate was 122 g/min, droplet size (volume median diameter) was 40 microns, and the charge/mass ratio was 2.3 mc/kg. Visualisation was carried out using dyes and this showed some advantage in using electrostatically charged droplets to achieve a more even deposition pattern if small quantities of chemical are to be applied. However, if multiple sprays nozzles can be used, or the product moves relative to the nozzle, or large quantities of liquid are required, then conventional spray nozzles are adequate.

Carcasses were supplied by a poultry processor on day of kill and treated using the electrostatic sprayer on Day 1 with 122g/min of acid solution which equated to 7.7 g of acid per kg of carcass. Thirty-six carcases were treated and 36 left as untreated controls. The buffered acid solution was provided by Purac and diluted to provide a 4% solution of lactic acid. Much further into the series of trials we learned that the solution provided, when diluted as recommended, would only provide 4% of lactate and not 4% of lactic acid buffered to pH=3.7. The solution used in this trial had a pH of 3.9 and calculated lactic acid concentration of 1.9%. The application time was 6s. The carcasses were tested by a microbiology testing laboratory for APC and *Campylobacter* on Days 2 and 8. At Day 2, the reduction in APC was 0.5-$\log_{10}$ ($p<0.001$) and at Day 8 the reduction was 0.2-$\log_{10}$ ($p<0.001$). Insufficient numbers of carcasses tested positive for *Campylobacter* to enable any conclusions to drawn on the effect of the acid on *Campylobacter*. A further trial using hot carcasses was planned.

2.3 Industry Funded Trial 2 (Trial LA3)

In this industry-funded trial carcasses were again treated at the rate of 122g/min using the electrostatic spray nozzle but the treatment was carried out at a process plant and applied to carcasses either just before the chiller or as they left the chiller. The solution consisted of 1.9% lactic acid with a pH=3.9. Carcasses were removed from the line, sprayed for 17s and placed into lined crates until all of the carcasses had been sprayed. They were then returned to the line. The birds were stored in air or a modified atmosphere mixture and tested for APC and *Campylobacter* at Days 0 and 6.

Figure 3 summaries the APC results from this trial. The pre-chill treatment ("Treated hot" in the graph) produced a 0.3-$\log_{10}$ reduction in APC at Day 0 and a 1.2-$\log_{10}$ reduction at Day 6. The effect of using the acid treatment post-chill was not significant at Day 0 but it was significant at Day 6 (0.9-$\log_{10}$ reduction in APC). The pre-chill treatment had a significantly greater effect on APC than the post-chill treatment at both Day 0 and Day 6.

APC on birds treated before chilling and then stored under modified atmosphere packaging (MAP) were 0.6-log less than the APC on birds treated post-chill and then stored under MAP. Again, this shows the advantage of treating with the acid before chilling.

Modified atmosphere storage reduced the APC on untreated birds but it had no significant effect, relative to storage in air, on the APC on acid treated birds.

Figure 4 summaries the *Campylobacter* results from this trial. The *Campylobacter* count on the untreated birds was 2.7-$\log_{10}$ cfu/g at Day 0. None of the birds treated with acid before chilling showed detectable levels of *Campylobacter* at Day 0. The microbiological testing was carried out by a microbiology testing laboratory who reported these results as presumptive *Campylobacter*. After 6 days storage in air, the difference between the *Campylobacter* numbers on the control and treated samples was 1.0-$\log_{10}$. Applying the acid after chilling produced reductions in *Campylobacter* of 1.3-$\log_{10}$ at Day 0 and 0.5-$\log_{10}$ after 6 days of storage in air. These results show that treating the birds with acid before chilling is better than treating with acid after chilling.

The *Campylobacter* counts on the treated birds stored in air increased during storage from undetectable levels to 1.4-$\log_{10}$ cfu per g. *Campylobacter* would not be expected to grow at the low

storage temperature of 4°C. The growth of *Campylobacter* after acid treatment could be due to the organisms not growing soon after treatment but they subsequently recover and then grow.

Presence/absence tests for *Campylobacter* showed no statistically significant evidence of an effect of the lactic acid when applied either before or after chilling. This result is in contrast to the results from the *Campylobacter* enumeration.

After MAP storage, there was some evidence that the *Campylobacter* counts on birds treated before chilling were less than the counts on untreated birds. There was strong evidence that the *Campylobacter* counts were greater on MAP stored birds than those stored in air.

Overall, this study concluded that the acid treatment reduced *Campylobacter* on Day 0 but the numbers were seen to rise during storage, most noticeably in the modified atmosphere environment.

Microbiological testing was also carried for Pseudomonas and the results are summarised in Figure 5. There was no effect of the treatment on Pseudomonas on birds treated pre-chill ("Hot birds") at Day 0 compared to untreated birds.. However, by Day 6, the counts of Pseudomonas were lower on treated birds than untreated birds after storage in air. There was no effect of the treatment on the Pseudomonas counts after modified atmosphere storage.

2.4 Industry Funded Trial 3 (Trial LA4)

Prior to this industry-funded trial, a tunnel was designed and then installed at a processing plant to spray carcasses with lactic acid prior to them entering the chiller. FSA-funding was used to provide the tunnel as the previous trial had indicated that lactic acid could be a useful intervention for reducing the numbers of *Campylobacter*. In designing the tunnel, Spraying Systems Limited was approached to advise on the best type, number and layout of nozzles to achieve the design parameters of an application rate of 20 g of acid/kg of bird, 10000 birds per hour (167 bpm), and average bird weight of 1.5 kg birds. Spraying Systems advice was to use 10 flat fan spray nozzles (Unitip™) with 8 nozzles spraying from the sides (nozzle TPU 8001-SS) and 2 nozzles spraying from the top into the cavity (TPU 6501-SS). Based on the spray angles and typical size of carcass, outline sketches of the proposed layout of nozzles were produced (Figures A1 and A2 in Appendix 1). This arrangement would deliver 12.8 g of acid per kg of bird, consequently, two of these arrangements of nozzles would be required to achieve the required flow rate of 20 g of acid per kg of bird.

Quotations for building a tunnel were obtained from two possible suppliers. Industrial Washing Machines was chosen based on previous experience of working with them. Figures in the Appendix show the computer aided design (CAD) drawings of the tunnel. In this final design, there are 4 vertical spray bars on each side of the tunnel with a sequence of 2 nozzles in one bar with one nozzle in the next bar and this sequence then repeated. There are 8 nozzles spraying downwards onto the birds. The tunnel had to be angled at 45º to follow the rising track of the production line between the inside-outside washer and the chiller at the chosen poultry plant. The appendix shows photographs of the installed tunnel. The liquid flow rate can be adjusted by changing the nozzles or using the valve on the outlet pipe of the tank. Each nozzle is also mounted on a swivel so that it can be adjusted if needed. A filter (300 micron) has been fitted to the inlet pipe to restrict any particles from blocking the fine nozzles. The tunnel was constructed in stainless steel.

In this first trial, the tunnel delivered 38g of acid per kg of carcass and the birds were in the tunnel for 7s. The acid was supplied by Purac and made up to their instructions. This produced a solution with 1.9% lactic acid. Although there was some statistical evidence of a small (0.2-$\log_{10}$) reduction in APC due to the acid treatment at Day 0, 33 of the 36 untreated control birds and 33 of the 36 treated samples had *Campylobacter* counts below the limit of detection (LOD=10 cfu per g). Presence-absence testing showed 18 positive control samples and 19 positive treated samples. No significant effect of the treatment on the numbers of *Campylobacter* could be detected.

Despite the lack of sufficient numbers of *Campylobacter* on the carcasses in this trial, the results from the previous trial were encouraging and three further trials were funded by the FSA and one by Campden BRI.

2.5 FSA Funded Trial 1 (Trial LA5)

The methods and results used in this trial are provided in full because the study was funded by the FSA. The trial was a repeat of that funded by the industry using the spray tunnel.

2.5.1 Methods in Lactic Trial LA5

The tank connected to the spray tunnel was emptied and thoroughly cleaned using warm water. The nozzles were checked for any blockages. The tank width and length were measured and found to be 0.5 m and 1.0 m respectively. The tank was then filled with cold water and operated three times with a 5 minute run time in each case. The depths of water in the tank at the start and end of each 5 minute period were measured to calculate the volume flow rate of liquid.

Prior to the trial, all of the water was removed from the tank and it was then filled with 182 kg of water and 13 kg of buffered lactic acid solution (as supplied by Purac).

A flock was selected that had tested positive at the farm and during the trial these birds were processed at around 9000 birds per hour (150 bpm). 9000 birds per hour equates to a line speed of 9000/(60)(2) feet per minute = 75 ft/min = 75(0.3048) m/min = 22.86/(60) m/s = 0.381 m/s. The tunnel length is 2.5 m, so the birds were in the tunnel for 2.5/0.381 s = 6.56 s ≈ 7s. This value was conformed by measurement.

A 1 minute gap in production was created at the hang-on area. Forty one birds were then allowed along the line followed by another 1 minute gap, another 41 birds, and then another 1 minute gap before the remainder of the flock was processed. The first group of 41 birds were not treated with lactic acid, the tunnel was then operated to apply acid to the next 41 birds passing along the process line (these were the treated birds). The air knives at the end of the tunnel were not used in the trial. The first and last bird in each group (untreated controls and acid treated birds) was tagged using cable ties for identification. Tagging took place in the evisceration room. The temperature of a bird before the sprayer and the room temperature near to the tunnel were measured. No objectionable odours from the lactic acid were detected and no drips from the tunnel were seen.

Birds were removed from the line ex-chill by the legs and placed in trays pre-lined with clean bags. Four birds were placed in each tray, breast side up, without anyone touching the breasts.

Each tray was covered with a liner to protect birds from touching the trays stacked above. Ten trays of controls and 10 trays of treated birds were collected. Birds were weighed, packed in sterile bags which were numbered and sealed with cable tags. Thirty-six controls and 36 treated birds were taken by refrigerated van (4ºC) for microbiological testing of breast skin samples the next day for aerobic plate counts and *Campylobacter* (enumeration and confirmation). The 36 birds selected for microbiological testing were from "within" the 41 bird groups (i.e. not start and end of each group). The remaining birds were held for visual evaluation throughout shelf life.

The temperature, pH, redox-potential, and free and total concentration of the diluted acid solution were measured at the start and end of the trial. The properties of the tap water used to dilute the acid and the properties of the liquid run-off from the spray tunnel were measured.

2.5.2 Results in Lactic Acid Trial LA5

The average flow rate of liquid delivered by the tunnel was 10.4 litres/min (Table 1). The average weights of the control and treated birds were 2.4 and 2.6 kg, respectively (Tables 2a and 2b). These figures show that the average application rate of the acid was 26 g of acid per kg of carcass: a value slightly above the required figure. The bird temperatures measured pre-spray and post-chill were 40 and 3ºC (-0.2°C at tip of neck flap), respectively. The room temperature near to the tunnel was 14ºC. The tap water used to make up the lactic acid solution had a pH of 7.2 and redox potential of 510 to 540 mV (Table 2b). The acid solution in the tank had a temperature of 11ºC, pH of 3.9, redox potential of 690 to 610 mV, and free chlorine concentration of 0.2 ppm to 0.3 ppm. Measurements of chlorine concentration in samples of liquid running out of the tunnel were unreliable as the chlorimeter used for the measurements should not be used in turbid water and we found that adding the DPD tablets to the liquid did not cause the expected change to a purple colour.

Tables 3a-d show the microbial counts on the control and treated samples at Days 1 and 7. The average log aerobic plate count on the control samples was 4.7-log at Day 1. There was evidence that the acid treatment reduced the aerobic plate counts by 0.3-log at Day 1 ($p=0.000$) but there no evidence of an effect at Day 7 ($p=0.620$). The average counts of *Campylobacter* on the untreated carcasses at Days 1 and 7 were 2.3-log and 1.0-log and there was no evidence of the treatment reducing the *Campylobacter* count at either Day 1 ($p=0.127$) or Day 7 ($p=0.402$). All except one of the samples showed *Campylobacter* counts above the limit of detection at Day 1. By Day 7, 20 of the untreated samples and 21 of the treated samples were above the limit of detection: there was no difference in the prevalence of samples below the LOD.

2.5.3 Conclusions from Lactic Acid Trial LA5

The tunnel appeared to provide a good application of acid solution to the birds and at the required flow rate. However, the treatment did not reduce the numbers of *Campylobacter* on the carcasses. Possible reasons for the lack of a reduction were considered by looking at the conditions used in the Campden BRI and industry-funded trials and the *Campylobacter* reductions achieved using those conditions. Upon checking of the composition of the acid provided it was found that in this trial and those funded by the industry, the concentration of lactic acid in the diluted solutions was 1.9% and not the expected 4%. The diluted (buffered) solutions actually contained 4% lactate. Further trials were planned using buffered 4% lactic acid solutions which contained 8% lactate.

2.6 Campden BRI Funded Trial 2 (Trial LA6)

This trial was funded by Campden BRI and was carried out on the same day as Trial 5 to provide additional samples (36 control and 36 treated samples in each trial). Due to differences in weights of the birds in this trial compared to Trial 5, the flow rate of acid applied was different being 26 g of acid per kg of bird.

The results from the two trials, that both used 1.9% acid solutions, were very similar. Trial 6 showed a small (0.4-log) but statistically significant reduction in APC at Day 1 due to the application of the acid but there was no evidence of an effect of the treatment at Day 7. There was no evidence of an effect of the treatment on the numbers of *Campylobacter* at Day 1, however, many of the samples showed *Campylobacter* below the limit of detection (5 cfu/g in this trial and Trial 5). At Day 1, five of the 36 control samples and 8 of the 36 treated samples had *Campylobacter* counts below the LOD. This result shows no evidence of an effect of the treatment. At Day 7, 18 of the control samples and 32 of the treated samples had counts below the LOD thereby showing a significant effect of the treatment.

2.7 FSA Funded Work to Examine Effect on Morphology of *Campylobacter*

Several meetings were held to discuss possible reasons for the large reduction in *Campylobacter* numbers achieved using lactic acid in Trial 3 with no other trial achieving such large reductions. Trial 3 was the only trial when presumptive *Campylobacter*, rather than confirmed, had been reported. Those who had been carrying out the microbiological testing indicated that, on some occasions, the *Campylobacter* on the birds treated with lactic acid were not typical of *Campylobacter*; the lactic acid had possibly changed the morphology of the organisms. For this reason, *Campylobacter* on treated birds might be mistaken as other organisms and not included in the *Campylobacter* count. A visit was made to a processing plant were four carcasses were sprayed with lactic acid (buffered 4% solution, pH =3.8) before the chiller using the hand sprayer used in Trial 1. The birds were returned to the line after treatment and then removed along with four untreated birds as they left the chiller. The samples were taken immediately to the microbiology laboratory in a cool box (1 hour drive) and observations and counts made on the *Campylobacter* on that day and the next day. On this occasion, there was no difference in the appearance of the *Campylobacter* due to the acid treatment. Samples from a later trial did show differences due to the acid treatment and the photographs in Figure 6 are included to illustrate the differences.

2.8 FSA Funded Trial 2 (Trial LA7)

2.8.1 Methods for Trial LA7

In this trial, a range of operating conditions were used that included use of the spray tunnel (Tunnel), electrostatic sprayer with birds treated for 5 or 21 s and then returned immediately to the line (Electro, Quick), or electrostatic sprayer with each bird being treated and then all treated birds returned together to the line (Electro, Slow). There were 20 samples for each treatment and 20 untreated controls. Eight samples for each treatment and 8 controls were sent to one laboratory and 12 samples and 12 controls were sent to another laboratory for testing for APC and *Campylobacter* (enumeration and confirmation). Samples were sent for testing to 2 laboratories to spread the work load.

A suitable batch of birds had been identified prior to the trial by sending boot swabs samples from 10 poultry sheds to AFBI in Northern Ireland for testing by real time PCR. The samples had been collected at the farm around 96 hours before the trial and had been tested around 24 hours before the trial.

At the time of the trial, a gap was created at hang-on, 26 birds were then hung on the line with the first and last birds each tagged with a cable tie. Twenty untreated control samples would later be taken from this group. A further gap of 1 minute was created on the line and a further 26 birds put on the line. Again the first and last birds were tagged. These would be the birds treated in the spray tunnel. Another gap of 1 minute was created to allow the tunnel to drain and identify the end of that group of birds.

After the control birds had passed through the spray tunnel, the spray pump was turned on, the tunnel treated birds were sprayed as they passed through the tunnel and then the pump was turned off after the birds left the tunnel and entered the chiller. Twenty birds were then removed from the line, sprayed with electrostatic sprayer for 21 s and carefully returned immediately to the line by holding the legs.. A suitable gap on the line had been created for the birds to be put on the line. This process was repeated with a further 20 birds but these were each sprayed for 5 s. A further 20 birds were then removed from the line, sprayed for 21 s and put into lined crates. Once all of these birds had been sprayed, they were carefully returned to the line by holding the legs.

The required birds were removed from the process line post-chill and placed in lined crates (5 birds to a crate). The crates were transported by chilled courier (4°C) and then tested the next day and six days later for APC and *Campylobacter* (enumeration and confirmation).

In part of the trial, the carcasses were treated using the tunnel. The flow rate of acid solution was higher than in previous tests because the pump on the liquid supply tank had been damaged and the only replacement pump that was available had a higher flow rate. The acid application rate using the tunnel was 29700 g/min which equated to 104 g of acid per kg of carcass. This was almost the same application rate as that used in Trial 1.

2.8.2 Results for Trial LA7

On average, the level of liquid in the tank fell by 107mm over 108s showing a flow rate of (0.107m x 1m (length of tank) x 0.5m (width of tank)) x 1000000 g/m^3 (approximate liquid density)/ 108s which equates to 29700 g/min. The pH, redox, free chlorine, total chlorine, and chlorine dioxide in the water were 6.5, 465 mV, 0.3 mg/l, 0.3 ,g/l, and 0.4 mg/l. The pH of the acid was 3.9.

Tables 4 and 5 show the microbial counts from samples tested by the 2 laboratories. The tests with the tunnel showed similar reductions in APC due to the acid treatment as those found in Trials 1 and 3. At Day 1, those trials showed reductions in APC of 0.8-log and 0.3-log. Trial 7 showed reductions of 0.3-log and 0.6-log at each of the two laboratories. At Day 7, the previous trials showed reductions in APC of 1.7 log and 1.3 log. Trial 7 found reductions of 1.9-log at one laboratory and 1.2 log at the other. Despite the reductions in APC, no significant reductions in *Campylobacter* were found in Trial 7.

2.8.3 Conclusions from Trial LA7

Despite the carcasses having a residence time of only 7 s in the tunnel, a reduction in APC of 1.2 to 1.9 log was achieved at Day 7. These figures are similar to the 1.7-log reduction achieved using the hand sprayer for 29 s in Trial 1.

No significant reductions in *Campylobacter* were found in this trial. A further trial was planned to examine the effect of using a higher acid concentration and the effect on *Campylobacter* numbers on the back/neck skin. *Campylobacter* counts are higher on the back/neck skin than on the breast samples and this might allow for reductions in *Campylobacter* to be detected more easily.

2.9 FSA Funded Trial 3 (Trial LA8)

2.9.1 Methods for Trial LA8

Carcasses were treated either in the spray tunnel located on the line directly before the chiller or by removing the birds from the line, spraying using a hand sprayer, and returning the carcasses to the line. On the day prior to the trial, the tank of the spray tunnel was cleaned, the nozzles checked for correct operation, and the flow rate determined by measuring the change in liquid level in the tank over a defined time of operating the tunnel (2 minutes). The flow rate was 12500 g/min. On the day of the trial, the flow rate from the hand sprayer was determined by measuring the change in weight of the sprayer over a defined time of operating the sprayer (30 s). The flow rate was 790 g/min.

Testing of boot swabs from farms had been carried out by AFBI to identify *Campylobacter* positive flocks. A batch of birds from a positive shed was chosen. A gap was created on the kill line, then birds were put on the line for one minute, followed by a gap of 20 shackles, followed by 20 birds. These birds were to be the untreated controls and the first and last birds of this group were tagged by a cable tie on one wing. A gap in killing of one minute was then created and a further 30 birds put onto the kill line. These birds were to be treated in the tunnel using lactic acid. The first and last birds of this group were also tagged with a cable tie. Twenty birds from this group of 30 would later be taken for testing. A further gap of 20 shackles was created on the line and 20 birds removed from the line. These birds were to be treated with the hand sprayer. Finally, a 2 minute gap was created on the line to identify the end of the test procedure. Caeca samples were taken from 12 birds from the batch, placed in a sterile bag, and put into cool box with ice packs that had been wrapped in bubble wrap.

Once the 20 untreated control birds had entered the chiller, the spray tunnel was operated and only switched off after the 30 spray tunnel treated birds had entered the chiller. The concentration of acid in the tank was known from the amount of concentrated acid and water put into the tank. The concentration was 4% lactic acid. The pH was measured and found to be 3.88. Chlorine dioxide in the water used to make up the acid was 0.4 ppm, the pH was 7.2, and the temperature was 14.5°C. The concentration of acid used in the hand sprayer was 8% lactic acid and the pH was 3.85. The line speed was 9120 birds per hour. Treatment time in the tunnel was 7 s and with the hand sprayer it was 21 s.

The 20 birds removed from the line pre-chill were weighed and then the back/neck skin removed, put into a sterile bag, and then into a cool box with ice packs with bubble wrap covering. These were the

untreated controls not sent through the chiller. The birds were then sprayed with hand sprayer with an 8% solution of lactic acid and then returned to the line.

The control birds and those treated in the tunnel were in the chiller for 120 minutes. The birds treated by hand spraying were in the chiller for 136 minutes. As the birds left the chiller, they were removed from the line by the legs to avoid contamination of the breast, back or neck. The temperatures of some birds were measured and the average was 1.4°C between the breast skin and flesh and -0.5°C in the neck flap. Each bird was weighed, put into a numbered sterile bag, sealed with cable tie, and put into a cardboard delivery box (6 birds to a box). The boxes were then held in a room at 0°C for 4 hours prior to being transported, along with the cool box, in a refrigerated van (4°C) to the microbiology laboratory. The next day, samples were removed from the carcasses according to plan in Figure 7 and tested for APC and *Campylobacter* (enumeration to LOD of 1 cfu/g and confirmation).

2.9.2 Results for Trial LA8

Table 6 shows that average weights of the birds were 1.8 ± 0.4 kg (untreated), 1.9 ± 0.4 kg (treated with 4% acid in tunnel), and 2.2 ± 0.4 kg (treated with hand sprayer with 8% acid). The birds treated by hand were weighed before chilling and the other birds after chilling, but the difference in average weights of the two groups would not be due to weight loss during chilling. It will also be noted that there was a wide overall variation in bird size from 1.1 to 2.8 kg.

The fours sets of pooled caeca showed *Campylobacter* counts of 9.3, 9.5, 9.5 and 9.7 log cfu per g. Tables 7a to 7i show the microbiological counts and average counts for each group of birds. At Day K+1, the 4% lactic acid treatment reduced the counts of *Campylobacter* from 2.4-log to 2.0-log on the breast skin (0.4-log reduction, p=0.013)) and from 3.4-log to 2.6-log on the back/neck skin (0.8-log reduction, p=0.002). At Day K+1, the 8% lactic acid treatment reduced the counts of *Campylobacter* from 2.4-log to 0.5-log (1.9-log reduction, p=0.000).

At Day K+7, the count of *Campylobacter* on the untreated breast skin samples was 2.1-log and this reduced to 1.7-log (0.4-log reduction, p=0.042) on samples treated with 4% acid and down to 0.2-log (1.9-log reduction, p=0.000) on the samples treated with 8% acid. Most of the samples treated with the more concentrated solution had counts below the limit of detection (1 cfu/g).

The counts of *Campylobacter* on untreated back/neck skins were 3.6-log at pre-chill and 3.4-log at post-chill (no significant difference, p=0.100). Those samples were tested at Day K+1.

Looking at the results from the testing for APC, shows counts, at Day K+1, of 3.5-log on untreated breast skin samples and 3.3-log on samples treated with 4% acid (no significant difference, p=0.102). Untreated back/neck skin samples had APC of 3.8-log and those treated with 4% acid had APC of 3.6-log (no significant difference, p=0.159). At Day K+1, the 8% lactic acid treatment reduced the counts of *Campylobacter* from 3.5-log to 2.9-log (0.6-log reduction, p=0.000).

At Day K+7, the count of *Campylobacter* on the untreated breast skin samples was 7.0-log and on acid treated samples it was 6.5-log (4% solution, no reduction, p=0.106) and 6.0-log (8% solution,1.0-log reduction, p=0.000).

The counts of *Campylobacter* on untreated back/neck skins reduced from 4.5-log at pre-chill to 3.8-log at post-chill (0.7-log reduction, p=0.000). Those samples were tested at Day K+1.

2.9.3 Conclusions from Trial LA8

The 8% acid was very effective in reducing the counts of *Campylobacter* producing a 1.9-log reduction at Days K+1 and K+7. However, the appearance of the birds, see next sub-section, was unacceptable after treatment with this acid concentration which caused greying of the skin. Treatment with 4% acid reduced the *Campylobacter* counts by 0.4-log on breast skins and 0.8-log on back/neck skins. This treatment has a slight effect on appearance of the birds, very slight greying of the leaf fat but in previous work (see Burfoot and Mulvey, 2011) processors considered this to be acceptable.

2.9 FSA Funded Work to Examine Effect on Appearance

This very brief piece of work was carried out to provide some photographic evidence of the effect of buffered 4% and 8% lactic acid treatments on the appearance of poultry. Birds were obtained from a local retailer on 25 and 26 October 2012. They were halved and one half left untreated and the other half sprayed with one of the lactic acid solutions using a hand sprayer. The acid solutions were 4 and 8% lactic acid buffered to pH=3.9. The acid was applied for 25 s to each half. The birds were then stored for 4 or 5 days at 2°C and then (30 October 2012) the appearance was assessed and photographs taken.

Figure 8a shows the appearance of an untreated portion after 5 days of storage. There is much green discolouration around the wing and tail. Figure 8b shows the appearance of another untreated portion after storage for 4 days. This portion had some green discolouration at the wing tip and breast cut line.

Figure 8c shows the appearance of a portion that was bought and treated with 4% lactic acid on 25 October and observed 5 days later. The colour is generally good apart from a slight green discolouration at the bottom of the leg and at the wing tip. Overall the skin colour is acceptable.

Figure 8d shows the appearance of a portion treated with 8% lactic acid and observed 4 days later. There is no green discolouration but there is an unacceptable grey discolouration of the loose skin and at the neck areas and regions of fat.

These observations confirm earlier work by Burfoot and Mulvey (2011) that concentrations of lactic acid over 4% (buffered to pH=3.9) are not an acceptable treatment. However, in view of the good reduction in *Campylobacter* achieved using an 8% lactic acid solution further work has been proposed to the FSA to examine the effect of different lactic acid/sodium lactate mixes on appearance of poultry. That work would include consumer panel testing.

2.10 Practicality and Costs of Using Lactic Acid

Spraying of the birds at typical lines speeds (around 10000 birds per hour) in the tunnel was entirely practical. The tunnel, with a length of 2.5m, could be fitted in most plants between the inside-outside washer and the chiller. No droplets were visibly leaving the tunnel and there was no problem with

odour from the tunnel. Tunnels of similar design, with different nozzles, are used on some lines for washing or wetting of carcasses so the industry is familiar with use of spray tunnels.

For a line operating at 10000 birds per hour with buffered 4% lactic acid solution applied at 100g of acid/bird, a supplier of lactic acid provided a cost of 0.72p per bird. For a plant processing around 100 million birds each year (two process lines), the annual cost of the acid would be £720k.

EFSA (2011, http://www.efsa.europa.eu/en/efsajournal/doc/2317.pdf) considered the spraying or misting of lactic acid at 2 to 5% for the decontamination of beef carcasses, cuts and trimmings. It concluded that "development of enzymatic resistance to therapeutic antimicrobials as a result of exposure to lactic acid and the possibility of mutational changes resulting in the development of resistance to therapeutic antimicrobials are unlikely". Lactic acid is found naturally in animal tissue and is used in a wide range of foods and has a food track record.

EFSA did not carry out an environmental risk assessment because it considered the lactic acid concentration before entering the wastewater treatment system to be negligible. In Section 5 of the EFSA document it reports that "according to the application dossier, it estimated that there is about 10 mg of lactic acid per litre of waste waters just before entering the wastewater treatment plant". That concentration was based on data for water and acid use in a US meat plant. Such an amount of acid was considered to have a negligible effect on pH in the wastewater and the concentration of acid would reduce because lactic acid is biodegradable. The biological oxygen demand of slaughterhouse wastewaters is quoted as being several grams per litre which is 100 to 1000-fold higher than the lactic acid concentration. Trials in this poultry study used 100g of buffered lactic acid per bird at 4% concentration (8% lactate). For a 10000 bph line operating over 16 hours each day, this represents 10000 = x 0.1 g x 0.04 x 16 kg = 640 kg of acid used each day. A typical UK site will send around 2000000 litres of water to its effluent plant over a 24 hour period. The concentration of acid is then 640 x 1000000/2000000 = 320 mg/l. This concentration is much higher than that quoted in the EFSA report relating to beef plants and could at periods of the day be much higher as only average values have been calculated based on water use over a 24 hour period. In this case, an environmental risk assessment is required.

2.11 Conclusions on Use of Lactic Acid

There has been much research on the effectiveness of lactic acid as an antimicrobial. Research into the effect of lactic acid on *Campylobacter* on poultry has focussed on testing inoculated samples, often with application times, concentrations, and pH levels that are not practical in a UK processing plant. Riedel *et al.* (2009) found a 1.7-log reduction in *C. jejuni* on chicken skins immediately after treatment and a 3.9 log reduction after treatment and then at storage at 5°C for 24 hours. They used samples inoculated to 5.4 log cfu/ml, and dipped them for 1 minute in a 2% lactic acid solution with pH of 3.1. The reductions are relative to dipping in water which, by itself, produced a 1-log reduction compared to the initial levels. They also report a yellow discolouration of the skin. Work described earlier in this report caused unacceptable skin discolouration unless the pH of the solution was 3.7 or higher. Cosansu and Ayhan (2009) also inoculated samples, in this case, leg and breast to levels of 4 to 5 log most probable number/cm^2. They also dipped the samples for 10 minutes in lactic acid solutions (1% at pH=2.2, and or 3% at pH=2.0) and compared the effect relative to dipping in water. On leg samples, *C. jejuni* was decreased by 0.4 and 1.1 log , by use of the 1 and 3% solutions.

On breast samples, the reductions were 1.3 and 2.0 log. No mention is made of the appearance of the samples after dipping in solutions at such low pH. The use of a 10 minute treatment time is impractical in existing process plants.

Boulder *et al*. (2006) found a 0.5-log reduction in *Campylobacter* on breast skin samples (compared to the effect of water alone) by dosing a 2% solution of lactic acid at a rate of 45 litres/h into an inside-outside washer. This application rate is lower than used in the present studies however the pH of the solution is not stated. A low pH is known to reduce the numbers of *Campylobacter*, but this can have an adverse effect on appearance.

Table 8 summaries the results from all of the trials with lactic acid. Now that approval has been granted for the use of lactic acid on beef carcasses at concentrations up to 5%, the granting of approval for use on poultry up to the same concentration would be expected to be easier than would have been the case previously. At 4% and 8% concentration, the log reductions in *Campylobacter* on breast skin were 0.4-log and 1.9-log. Very simplistically, assuming a linear change with concentration would show a 0.8-log reduction on breast skin by using a 5% solution. This estimate would likely increase to a 1 log reduction, or more, if back/neck skin samples were used. This suggests that further work to assess the effect on appearance and microbial reductions achievable using a 5% solution should be considered. The pH must be maintained above 3.7 units. The cost of the treatment would increase to 0.9p per bird and the impact on waste water treatment would need to be considered in more detail.

3. OZONATED WATER

No published data have been found on the effects of ozonated water on *Campylobacter*. Unless high pressure systems are used, the concentration of ozone that can be achieved in the water is limited. The highest concentration reported in the literature is 10ppm ozone (Fabrizio *et al.,* 2002). They found a 0.9-log reduction in APC on the day of application when using an immersion treatment, and a 0.55-log reduction (not significant) when using a spray treatment. Wang and Chen (1979) found a 1-log reduction in APC when applying the ozonated water using a bottle dispenser. Sheldon and Brown (1986) applied ozone to chiller water and found no difference on APC on treated and untreated chickens over the 11 days of storage. Although, using inoculated beef samples, Yoder, *et al.* (2012) found no difference in reductions of *Campylobacter* numbers when using ozonated water (3 ppm ozone) and tap water for 15 or 30s: the reductions were between 1 and 2 log cfu/cm^2. Trindade *et al.* (2012) examined the effects using 1.5 ppm ozone or chlorine in an immersion chiller and found similar microbial loads on chicken carcasses after either treatment.

One equipment supplier provided data on the efficacy of their system, which provided water at 4 ppm ozone, when used to treat turkey carcasses (Tables 9 to 11). Analysis of variance of the data in Tables 9 and 10 found weak evidence of an effect of a 10s treatment on the APC (0.2-log reduction) but strong evidence of an effect was seen with a 20s treatment although the average reduction was only 0.4-log. The data in Table 11 show very strong evidence of a reduction in APC due to a 5s treatment with ozonated water with a reduction of 0.6-log. Insufficient numbers of the samples tested positive for *Campylobacter* to provide any evidence of an effect on those organisms. Data was also provided on the APC and presence/absence of *Campylobacter* on 17 control and 17 treated finished product

portions. Strong evidence of an effect of the treatment on APC was found (0.9-log reduction), but none of samples tested positive for *Campylobacter*.

In a further industry-funded trial applying ozonated water at 4 ppm ozone onto turkey carcasses, the average APC on control and treated carcasses were 4.33-log and 3.68 log (0.65-log reduction), respectively. There was very strong evidence of an effect of the treatment on APC ($p<0.001$).

The following sections describe trials carried out to assess the effect of ozonated water on *Campylobacter* numbers on chicken carcasses.

3.1 FSA Funded Trial 1 (Trial OZ1)

3.1.1 Methods Used in Trial OZ1

The ozonated water was produced using a unit (Radical ST4130MWG, provided by Steritrox Ltd., Pershore, UK) that consisted of a mobile stainless steel box (750 mm x 600 mm x 1200 mm) housing a system that concentrates the oxygen in ambient air to 90 to 95%. The oxygen is then converted to ozone using a non-thermal plasma and the ozone is dissolved in water that was sprayed onto the chicken carcasses through a nozzle.

The study was carried out at a chicken plant processing around 60 000 000 birds per year. Four carcasses were removed from the production line and each one was tagged with a cable-tie, weighed, and returned to the production line. These were untreated control birds and the procedure was repeated 6 times to give a total of 25 untreated birds (the last test used 5 not 4 carcasses). A similar procedure was used with the treated birds which were removed from the line, tagged, weighed, sprayed for 30 s with ozonated water or plain water, re-weighed, and returned to the line. Twenty five birds were treated with ozonated water and 25 birds with plain water. All tagged birds were carefully removed from the production line after leaving the chiller and placed in individual sterile bags that were each closed with a cable tie. The bagged birds were then placed in ice boxes (ice blocks covered with bubble wrap) and taken to the microbiology laboratory where they were tested within 24 hours (Day 1) and six days later (Day 7).

As well as weighing the birds, other measurements were made during the study. The liquid flow rate from the nozzle was measured using a bucket and stop watch and the average, based on 8 measurements, was 5.64 kg/min (s.d. = 0.37 kg/min) during the test with ozonated water, and it was 6.53 kg/min (s.d. = 0.29 kg/min), based on 4 measurements when using plain water. The difference was believed to be due to changes in water pressure in the factory. The temperature just under the skin of the first and last carcass of each set of birds (control, ozonated, plain water treated) was measured and the liquid and room temperatures were measured at the same time. The bird temperature was between 37 and 40ºC before spraying and it was between 23 and 34ºC after spraying. The temperature of the ozonated or plain water was between 15 and 18ºC and the room temperature was between 17 and 20ºC. At the time of measuring temperatures, the pH and redox potential of the liquid were measured (Metrohm 825 meter, Metrohm AG, Herisau, Switzerland); the chlorine concentration was measured (Lovibond 2000, Tintometer, Salisbury, UK) and the ozone concentration was measured (Photometer, Palintest Ltd., Gateshead, UK). The average pH, redox potentials, and chlorine concentrations of the ozonated and plain waters were 7.7 and 7.8 (pH),

938 and 762 mV (redox), and 0.5 ppm (chlorine in both waters), respectively. The ozone concentration varied between 3.6 and 3.8 ppm in the ozonated water.

3.1.2 Results from Trial OZ1

The aerobic plate counts and confirmed *Campylobacter* counts for control, ozonated and plain water treated carcasses are shown in Tables 12 to 14. The average (log) aerobic plate counts for the control, ozonated and plain water treated samples were 5.52, 5.22, and 5.29 on Day 1 and 6.93, 6.49, and 6.33 on Day 7. The APC on the control samples were significantly ($p=0.001$) greater than those on the treated samples but there was no evidence of a significant difference between the average log counts on the carcases treated with ozonated or plain water. The APC plate counts increased during storage ($p<0.001$) on control, ozonated and plain water treated samples.

Analysis of the data on *Campylobacter* counts was more complicated as 8 of the 20 control samples had counts below the LOD of 10 cfu/g and 10 of the ozone treated samples were below the LOD. A Fisher's Exact test on the two proportions of the *Campylobacter* positives for the control and ozone-treated samples showed no evidence of the treatment reducing the number of samples with counts above the LOD. Values below the level of detection ($\log_{10}$(10 *Campylobacter* per g)) were replaced with the value log ($10/\sqrt{2}$) following Richard W. Hornung and Laurence D. Reed. "Estimation of Average Concentration in the Presence of Nondetectable Values" Appl. Occup. Environ. Hyg. **5** (1):46-51 (1990). Although the number of values below the limit of detection is large, the means were calculated and an analysis of variance was carried out. The mean confirmed *Campylobacter* counts calculated in this way were 2.35-, 2.04-, and 2.08-log. There was no evidence that the ozonated water produced a different effect on *Campylobacter* counts than the use of plain water. None of the samples, control, ozone or plain water treated, had *Campylobacter* counts above the LOD after 7 days.

3.1.3 Conclusions from Trial OZ1

Although the ozonated water reduced the aerobic plates counts on chicken carcasses, the effect was small (~0.2-log) and there was no evidence that the effect was greater than that produced by plain water. The use of ozonated water was not found to significantly reduce the *Campylobacter* counts. Greater microbial reductions may have been achieved using a longer spray time although the treatment applied in this test did provide a visibly good coverage of each bird. The ozone concentration in the water could have been increased. However, at around 6 ppm the amount of ozone released could be a concern to the health and safety of operators and an enclosed system with extract would be required rather than the use of a simple spray lance as in this preliminary study. A further trial was planned with the aim of having larger numbers of samples with *Campylobacter* above the LOD.

3.2 FSA Funded Trial 2 (Trial OZ2)

3.2.1 Methods used in Trial OZ2

Ozonated water was produced using the same unit as in the previous trial (Radical ST4130MWG, provided by Steritrox Ltd., Pershore, UK). The study was carried out at a chicken processing line that was running at 187 birds per minute. A clearance flock was chosen for the trial. Seventy-six carcasses were removed from the production line after the inside-outside washer and before the chiller and put

into a Dolav bin. Each carcass was then removed from the bin, tagged with a numbered cable-tie, and weighed. Thirty eight of the carcasses were each placed in separate sterile bags and put into crates (6 or 7 carcasses to a crate). These were the untreated control carcasses. Each of the other 38 carcasses was placed on a suspended shackle and sprayed with ozonated water for 30 s. After treatment, each carcass was removed from the shackle directly into a sterile bag and placed in a crate (6 or 7 carcasses to a crate).

The plan was for all 76 carcases to be returned to the process line pre-chiller using the bags to prevent cross contamination during handling. A six minute gap in production had been created to enable the carcasses to be returned carefully to the line. However, the operator of the ozonated water generator noted that the equipment had not been operating correctly throughout all of the trial and he did not know at what time the equipment had begun to operate at a lower than required ozone concentration. Consequently, the birds were not returned to the line. The results presented below show data on the bird weights, flow rates, temperatures, and properties of the ozonated water that were measured. The pH and redox potential of the liquid were measured using a Metrohm 825 meter (Metrohm AG, Herisau, Switzerland); the chlorine and ozone concentrations were measured using meters from Palintest Ltd. (Gateshead, UK).

3.2.2 Results from Trial OZ2

The average weights of the control and treated birds were 1.702 kg (s.d. = 0.267 kg) and 1.654 kg (s.d. = 0.219kg), respectively (Tables 15 and 16). The temperature just under the skin of the untreated control birds was between 31.3 and 33.3ºC and the temperatures of the birds to be treated were between 32.7 and 36.9ºC. The temperatures of the treated birds were measured before those of the untreated controls and that is probably the reason for the differences in temperatures. The room temperature was between 5.3 and 5.6ºC. The liquid flow rate from the nozzle of the ozonated water generator was measured using a bucket and stop watch and the average, based on 3 measurements, was 5.55 kg/min (s.d. = 0.05 kg/min). The temperature of the ozonated water was between 5.6 and 5.8ºC and the pH was around 7.3. The ozone concentration measured at the start of the test was 3.8 ppm.

Figure 9 shows that the redox (oxidation-reduction) potential of the ozonated water reduced from the start to the end of the trial. Figure 10 shows the free and total chlorine values of the tap water used to produce the ozonated water (0.27 and 0.45 ppm) and the values indicated by the chlorine meter at the start, middle and end of the trial when testing the ozonated water. The chlorine levels appear to be higher in the ozonated water than the tap water. The supplier of the chlorine meter was contacted to ask further about the measurements made by the equipment. The colourless liquid being tested is mixed with a reagent (DPD, diethyl-p-phenylene diamine) which changes the liquid to a pink colour and the intensity of the pink colour is proportional to the chlorine concentration. However, ozonated water oxidises the DPD causing a change in the colour and indicating a higher than expected value of the chlorine concentration. Despite this difficulty, the indicated "chlorine" levels do appear to fall and tend towards the values in the tap water. These results, and the downward trend of the redox potential both indicate changes in the ozonated water with time and supports the decision to abort the trial.

3.2.3 Conclusions from Trial OZ2

No samples were taken for microbiological testing because of the uncertainty in the properties of the water. The measurements of redox potential and apparent chlorine indicate changes in the water during the test. The manufacturers of the chlorine meter (Palintest) sent other reagents to enable more reliable assessments of chlorine in ozonated water. A further trial was to be arranged.

3.3 FSA Funded Trial 3 (Trial OZ3)

It was decided not to carry out the third planned trial, as there was no evidence of an effect in Trial OZ1 and the equipment did not work in Trial OZ2.

3.4 Practicality and costs of using Ozonated Water

When applied at the ozone concentration used in this study (~4ppm), ozonated water would not create any concerns over safety and would not have any adverse effect on product quality. The water could easily be applied in a spray tunnel although multiple ozone generators would be required. The reductions in APC were small and no effect on *Campylobacter* numbers was found in these studies. Increasing the concentration of ozone might have a beneficial effect on microbial reductions but this would require further considerations of worker safety, probably using extract systems within spray tunnels. A supplier of ozone generation equipment indicated that a liquid with 8ppm ozone might attack the fabric of process equipment. Applying the ozonated water through the inside-outside washer of a process line was considered but rejected by the plant staff due to not wishing to risk any adverse effect on the equipment even with a 4ppm solution. Dipping birds in ozonated water was considered but rejected as a practical option due to the risk of creating a “microbial soup”.

3.5 Conclusions on Use of Ozonated Water

None of the results from these trials indicate that the spraying of ozonated water would be an effective intervention against *Campylobacter*.

4. OZONATED CO_2 PELLETS

The use of CO_2 pellets containing ozone is a new and novel approach to microbial reduction. A patent application has been submitted by Air Liquide and the trade name ALIGAL Blue Ice has been registered. A conference paper describing investigations by the USDA ARS and Air Liquide into the use of the ozonated pellets on microbial reduction in the air, on surfaces, and on meat products has been published (Fratamico *et al.,* 2011). Challenges studies were performed using *E. coli*, *C. jejuni*, *Salmonella* and *Listeria*. *Campylobacter* and APC have most interest in this project. Pork meat boxed with ozonated pellets showed lower aerobic plate counts between storage days 8 and 13 than pork meat stored with dry ice (Figure 11). Reductions in *Campylobacter* counts on (non-food) surfaces were found to be greater on surfaces treated with ozonated pellets (Aligal BI in Figure 12) than on surfaces treated with dry ice (Figure 6). Reductions in *Campylobacter* counts on inoculated chicken samples showed a 1.3-log reduction on samples treated with ozonated pellets and lower reductions on samples treated with dry ice or wet ice (Figure 13).

A meeting was held with the technical directors of the UK poultry industry and the FSA to discuss how ozonated CO_2 pellets might be utilised in the UK. In the US, the main application was seen as placing pellets into boxes of poultry just before transportation. However, this could lead to freezing of parts of the poultry and this would be unacceptable for poultry to be sold chilled in the UK. There might be options of using the pellets in a chiller or perhaps in the transport vehicle, provided that the pellets do not touch the poultry. At least one food home delivery service in the UK use containers where the food is held in the lower section of the container and dry ice pellets are held in an upper chamber. This enables the food to be kept cold during transport. The group suggested that this type of chamber be used in a trial to assess the effectiveness of the ozonated pellets against *Campylobacter* on poultry. If successful, further discussions should be held as to where would be the best place to use the pellets in the production system.

After looking into the use of the containers applied in the home delivery service, it was decided that these had insufficient capacity and a small chamber was sourced for the purpose of the trials.

Several discussions were held over a 2 year period, but in February 2013, the decision was made by Air Liquide not to produce the ozonated pellets and consequently the proposed trial could not go ahead.

5. COLD PLASMA

Although the trials with ozonated carbon dioxide pellets did not go ahead, an alternative approach using plasma was investigated as this also involves the application of ozone. A plasma is an ionised gas. As energy increases, matter is transformed from solid to liquid to gas, and then ionised gas (Eliezer and Elizier (2001): the latter being called plasma and also known as the "fourth state of matter". The plasma includes atoms, excited species (including ozone), ions and electrons. Although various types of plasma generators exist, the one of most interest to this project is the cold atmospheric pressure plasma generated from air.

Dirks *et al*. (2012) inoculated skinless chicken breast and skin-on thighs with *Campylobacter jejuni* to levels of 10^1 to 10^4 cfu/in^2 and then treated them with cold plasma for 0 to 180s. Reductions ranged from 1.6 to 2.5 log on breast and 1.4 to 3.1 log on skin following a 180 s treatment. Exposure for 30s generally reduced the *Campylobacter* numbers by much less than 1 log and the background microflora by 0.8 log and 0.2 log on breast and skin, respectively.

5.2 FSA Funded Trial (Trial CP1)

5.2.1 Methods used in Trial CP1

Four days before the trial (a Friday), boot swab samples from 10 sheds were sent to a laboratory (AFBI, Belfast, Northern Ireland) for testing for *Campylobacter* using real time PCR. The samples arrived at AFBI at 13:20 on the following Monday and results, available at 16:20, showed that all ten sheds were positive for *Campylobacter*. The following day 30 chickens originally from one of the sheds, were removed from the production line as they left the chiller and were put in to 3 cardboard boxes. The carcasses were then transported by car (1 hour journey) to Campden BRI where the outer

breast portions, with skin attached were removed from each carcass. The portions were placed individually into food trays (Cryovac, BT3-32 Nat Bird Flexi 8160) that were placed inside bags. The bagged portions were then placed in BOC crates and put into a chiller at 2°C for 1 hour.

Twenty chicken breast fillets were then treated in a UVC tunnel for 20 s (methods and results reported in a separate report). Twenty chicken breast fillets were exposed to air for 20 s. Twenty chicken breast samples were treated individually in a cold plasma chamber. The chamber (26 cm x 26 cm x 17 cm) included a polytetrafluoroethylene (PTFE) sheet sandwiched between a copper power electrode and a steel mesh held at a potential of 0 V. This electrode configuration was fixed into the lid of the unit. Applying a voltage to the electrodes generated a plasma discharge between the PTFE and the steel mesh with air as the operating gas. Ozone gas, which is harmful at very low concentrations, was produced by the unit so it was located within a safety cabinet to allow extraction after treatment. The average power consumption of the system had been previously measured as 21W. The chicken samples were located 11.5 cm from the bottom of the chamber and 5.5 cm from the steel mesh. Each sample was treated for 20 s followed by a further holding time of 4 min 40 s when the cold plasma was not operational. Earlier work by Liverpool University had suggested that a longer operation time of the plasma might produce excessive levels of NO active species that would react with the ozone produced by the plasma and reduce the effectiveness of the treatment. A 20 s operation would produce the ozone to a maximum level of 270 ppm and the holding time would be sufficient for the ozone to act.

After treatment, the samples were placed in a chiller at 2°C for 4 hours and then into a cool box containing ice packs covered with bubble wrap. The ice box was transported in a refrigerated van (0.5 hour journey), held overnight in a chiller, and then transported (2 hour journey) to the microbiology laboratory were the samples were tested for aerobic plate counts and *Campylobacter* (enumeration and conformation).

5.2.3 Results from Trial CP1

Tables 17a, b show that the average counts of *Campylobacter* on the untreated and plasma treated samples, were 2.3-log and 2.2 log, respectively. There was no evidence of an effect of the cold plasma treatment on the counts of *Campylobacter*. The average aerobic plate counts from the untreated and plasma treated samples were both 6.7 log showing no effect of the treatment on APC.

The cold plasma treatment had an adverse effect on the odour of the chicken samples causing a sea air/cucumber odour associated with the ozone that is produced by the plasma. This might be due to oxidation of fat in the chicken skin.

5.2.4 Conclusions from Trial CP1 and Use of Cold Plasma

The cold plasma treatment did not reduce the APC or numbers of *Campylobacter*. An adverse odour was produced. The work by Dirks *et al*. (2012) suggests that a longer treatment time might have created reductions in microbial counts but this might also have increased the odour problem. They do not comment on any generation of adverse odour, but say that the system could result in oxidations of lipids and that further research is needed on the impact on acceptability and shelf life.

A further barrier to the use of this technology is the placement of the poultry. Dirk *et al*. placed their plasma surface at 1 to 2 mm from the surface of the chicken. This would not be practical in a processing facility.

6. DISCUSSION AND CONCLUSIONS

The spraying of lactic was the only approach that produced a statistically significant reduction in *Campylobacter* numbers. The approach was tested at full scale and produced a 0.6-log reduction in *Campylobacter* numbers based on the average for breast and back/neck skin samples when applying a buffered 4% solution. This approach would cost around 0.72 p per bird. Further work to assess the effect of using a 5% solution on appearance and microbial reduction would be justified as tests with an 8% showed a 1.9-log reduction. Five percent is likely to be the highest concentration that would be considered by industry in view of the cost, potential impact of waste water treatment and because approval has already been given for its use on beef carcasses. Further work is also needed by the industry to assess the effect of lactic acid treatment on the *Campylobacter* and organisms affecting shelf life when the poultry has been packed in a modified atmosphere. Most of the work described in this report used storage in air. One of the industry funded trials used samples stored in a high CO_2 mixture. However, the industry generally uses a high (80%) O_2 atmosphere. Rajkovic (2010) found that a buffered 10% lactic acid solution reduced *Campylobacter* numbers by 1.8 log and packaging in 80%O_2/20%N_2 produced a further 1.2 log reduction. The change in *Campylobacter* numbers when birds treated with a lower acid concentration and then stored at high O_2 needs to be examined. This might be difficult as the *Campylobacter* numbers reduce considerably when birds are stored at high O_2 and quantifying an additional effect of the acid will require sourcing birds with high initial *Campylobacter* load. This would be difficult to guarantee and is one of the reasons why many studies, including that by Rajkovic *et al*. use inoculation.

The use of ozonated pellets could not be tested so alternative work was carried out. In view of the patent protection held by Air Liquide on the use of ozonated CO_2 pellets, it is unlikely that this potential intervention will become available for several years. The modes of action of the pellets would have been oxidation by the ozone and the effect of the cold temperature. The alternative approach used cold plasma, which produced high levels of ozone. However, these tests did not reduce *Campylobacter* numbers. Further work was carried out immersing samples in liquid nitrogen to produce low temperatures. The results of those tests have been presented in a separate report to the FSA that considers the effects of rapid surface cooling on *Campylobacter* numbers. Reports of this work have not been published as they include commercially sensitive material and are covered by a confidentiality statement.

No evidence was found that spraying with ozonated water would be a practical intervention against *Campylobacter*.

Overall, the use of lactic acid is the only practical option of those tested.

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Figure 1 The effect of buffered lactic acid solution (4% concentration, pH=3.7) on the aerobic plate counts on chicken in Trial LA1 (Burfoot and Mulvey, 2010a)

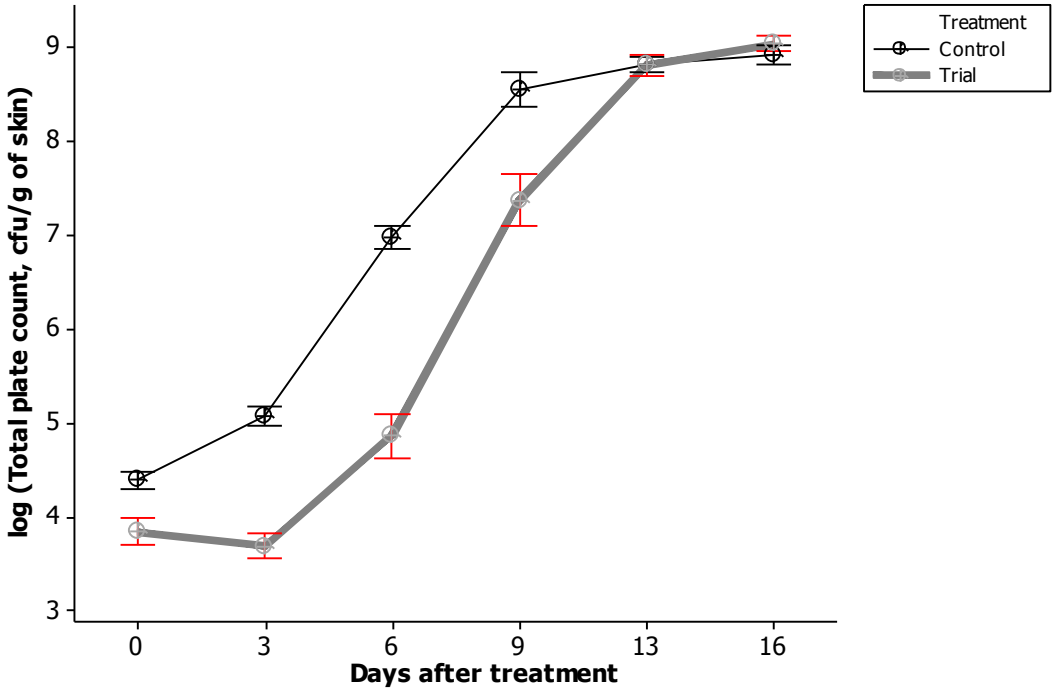


Figure 2 The effect of buffered lactic acid solution (4% concentration, pH=3.7) on the aerobic plate counts on turkey in Trial LA1 (Burfoot and Mulvey, 2010b)

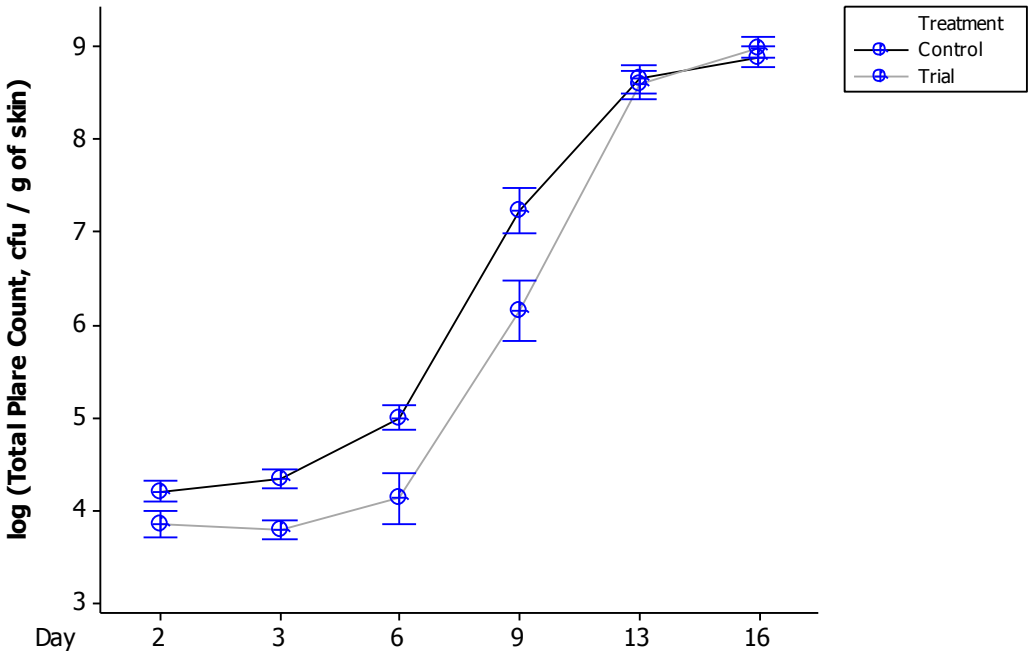


Figure 3 The effect of buffered lactic acid solution (1.9% concentration, pH=3.9) on the APC on chicken carcasses treated post-inside/outside washer (hot carcasses) or post chiller (cold carcasses) and then stored in air or a modified atmosphere for 6 days at 4°C). (Trial LA3)

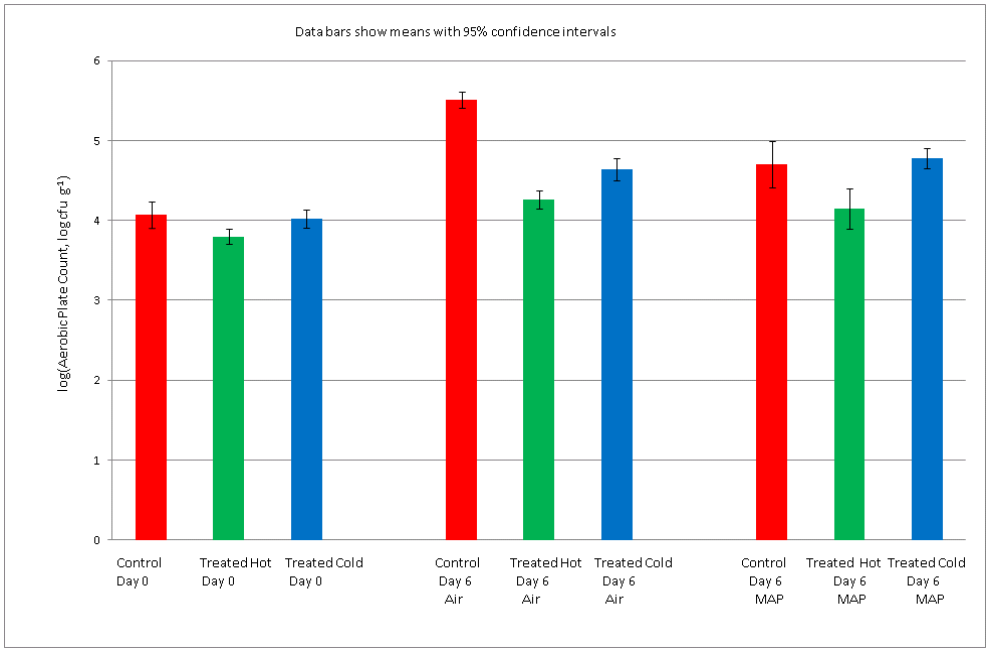


Figure 4 The effect of buffered lactic acid solution (1.9% concentration, pH=3.9) on the counts of *Campylobacter* on chicken carcasses treated post-inside/outside washer (hot carcasses) or post chiller (cold carcasses) and then stored in air or a modified atmosphere for 6 days at 4°C). (Trial LA3)

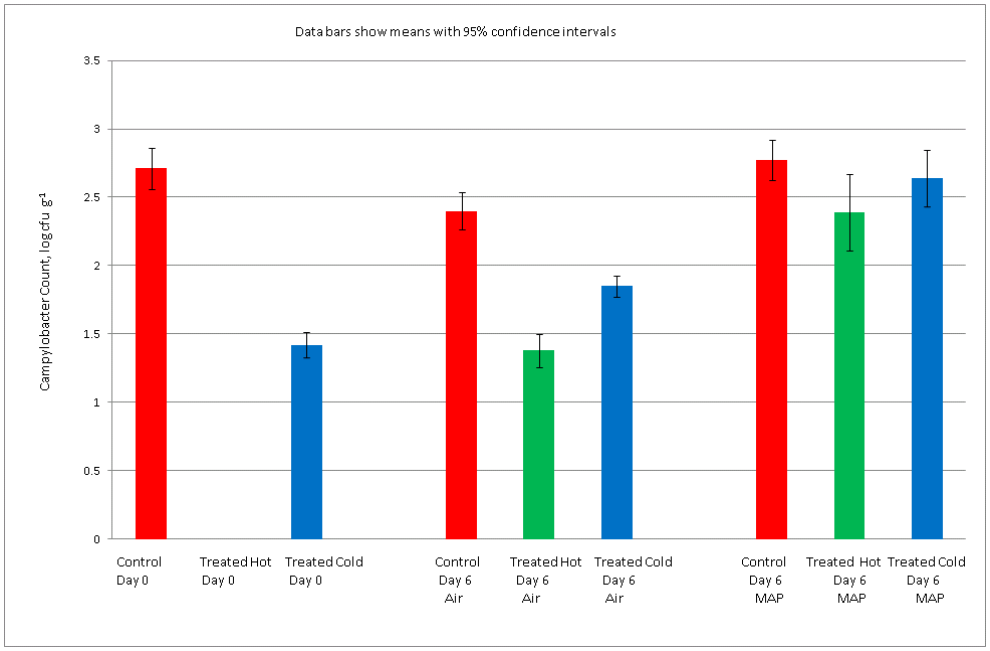


Figure 5 The effect of buffered lactic acid solution (1.9% concentration, pH=3.9) on the counts of Pseudomonas on chicken carcasses treated post-inside/outside washer (hot carcasses) or post chiller (cold carcasses) and then stored in air or a modified atmosphere for 6 days at 4°C). (Trial LA3)

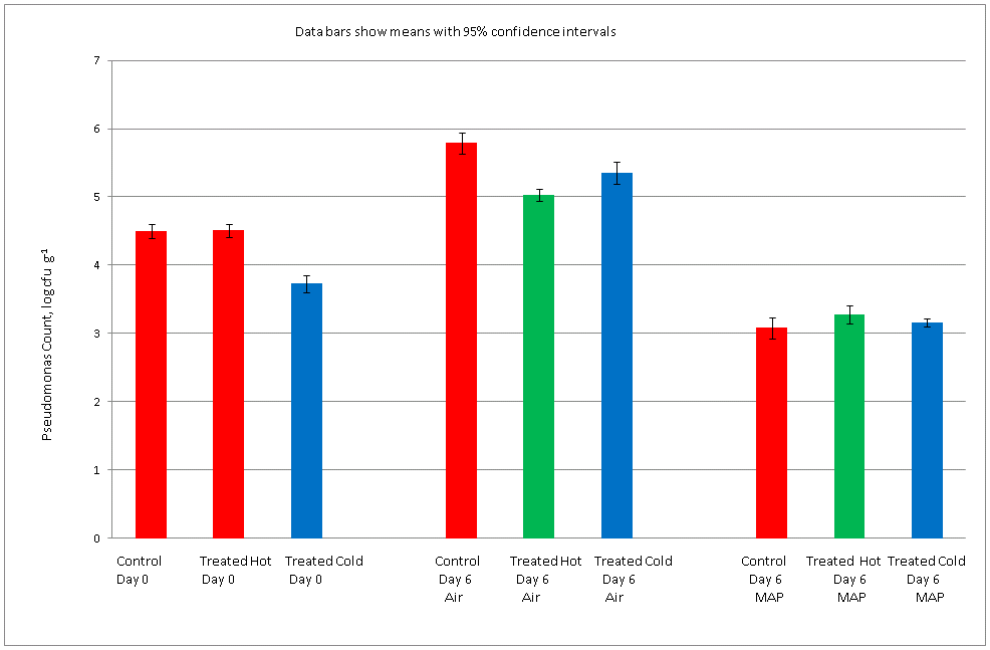
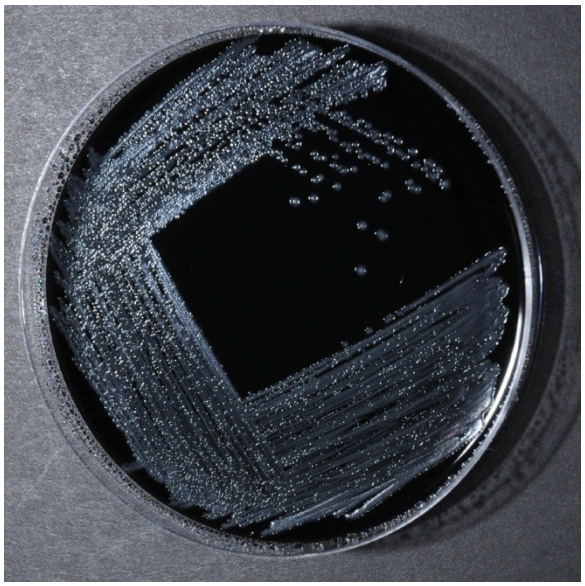
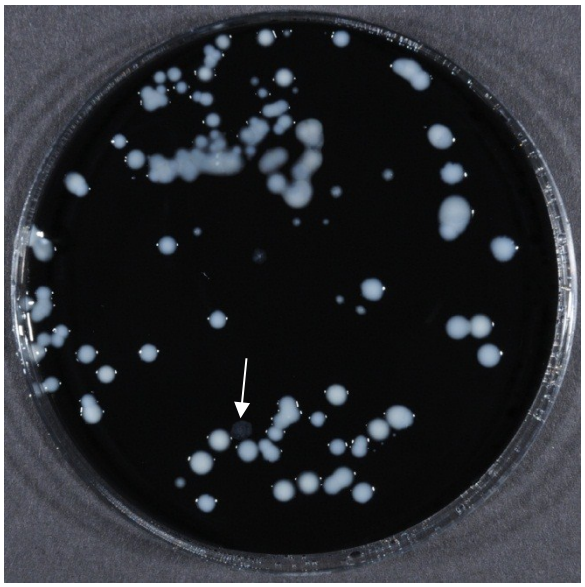


Figure 6 Appearance of *Campylobacter* taken from chicken skin samples treated with lactic acid (4% acid, pH=3.9).

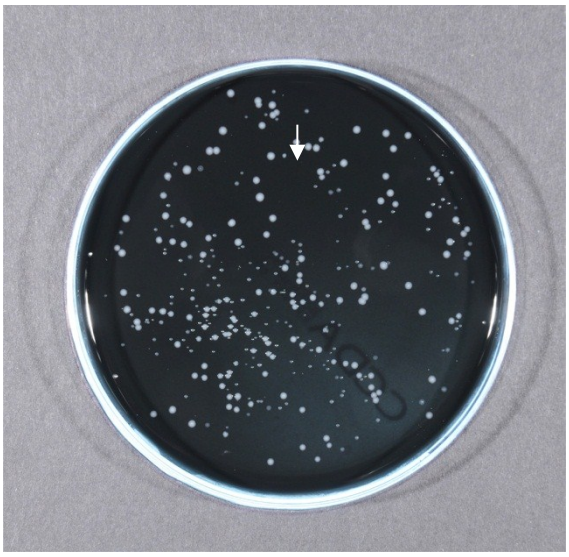


(a) Typical mainly pure culture



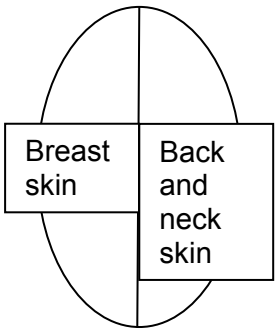
(b) Typical but mixed culture.
The arrow indicates swarming *Campylobacter*

(c) Atypical *Campylobacter* marked with arrow. Mainly pure culture.

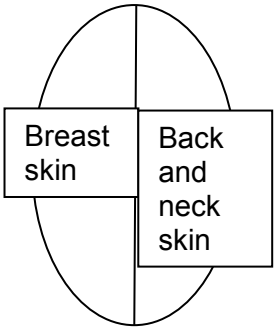


(d) Very mixed culture. Atypical *Campylobacter* marked with arrow.

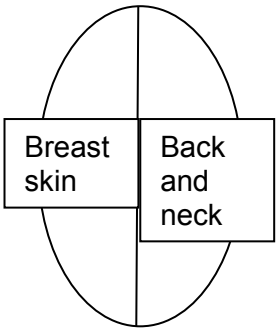
Figure 7 Schematic diagram showing the activities and sampling plan for the Trial LA8



1. Control samples
Remove 20 birds from the line post-chill. Test 20 breast skins for APC and Campy. Test 20 back/neck skins for APC and Campy



2. 4% acid treated
Spray 20 birds using the tunnel on the line. Remove the 20 birds post-chill. Test 20 breast skins for APC and Campy. Test 20 back/neck skins for APC and Campy



3. 8% acid treated
Remove 20 birds from the line pre-chill. Remove the back/neck skin from each bird and put into a cool box with wrapped ice. These are the untreated controls not sent through the chiller. Compared with the other controls, these birds will show the effect of the chiller.
Spray the birds with a hand sprayer and then remove the breast skin samples. Comparison with controls in Part 1 will show the effect of the higher acid conc and the chiller combined. Test samples for APC and Campy.

Figure 8a Appearance of untreated control bought on 25 October 2012 and observed 5 days later.

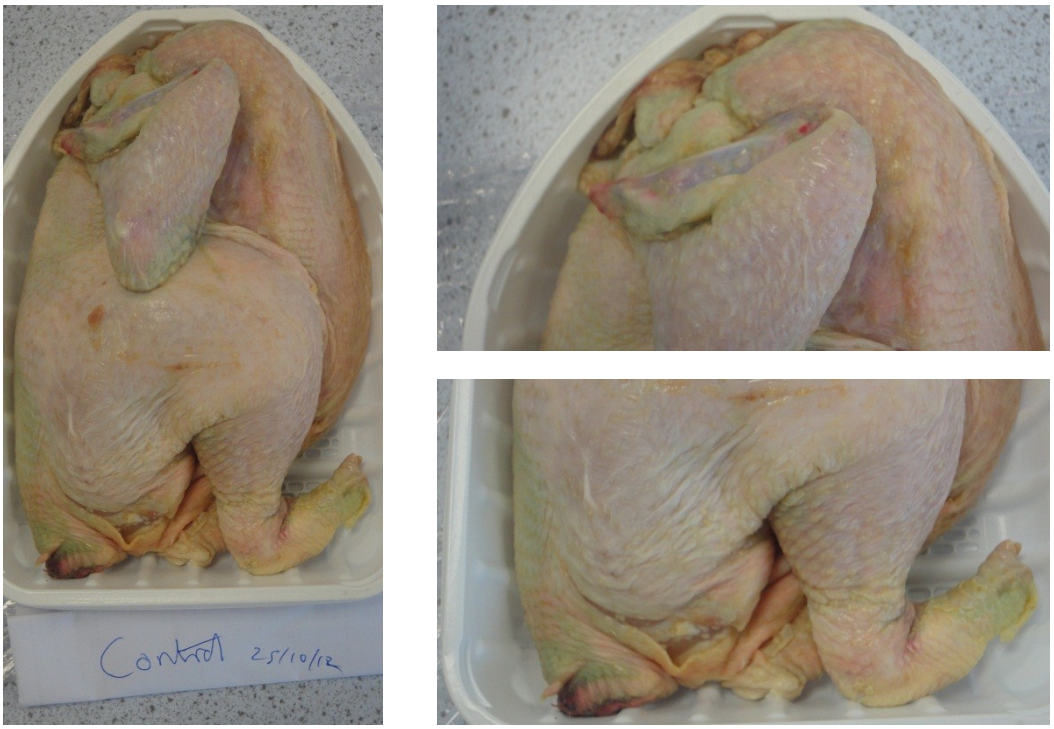


Figure 8b Appearance of untreated controls bought on 26 October 2012 and observed 4 days later

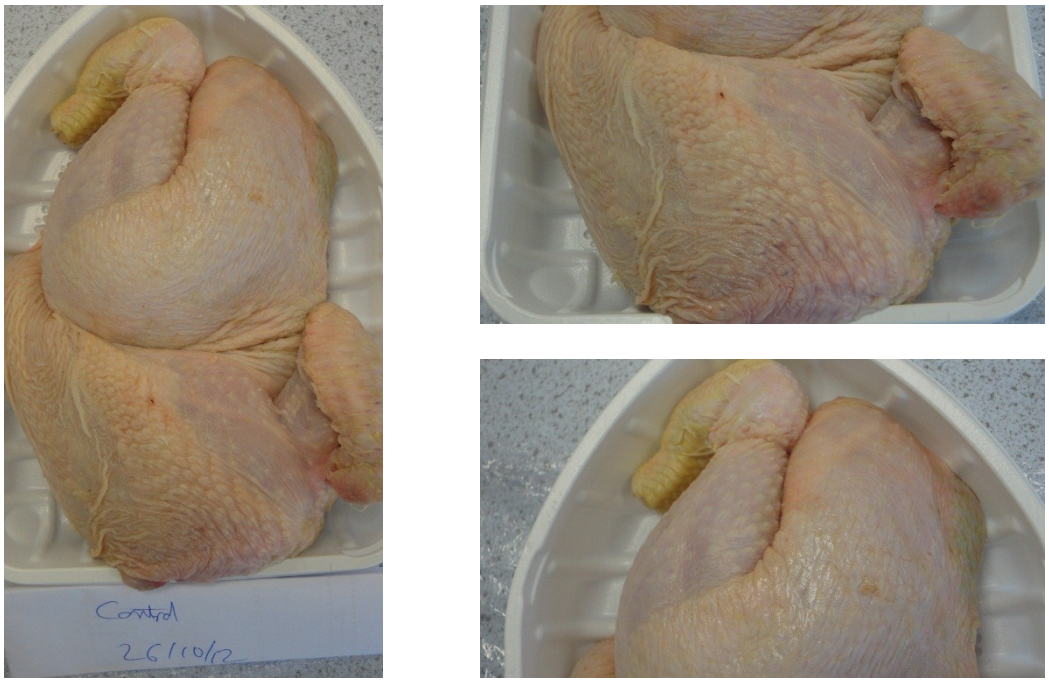


Figure 8c Appearance of bird bought on 25 October 2012, treated with 4% lactic acid (pH=3.9) on that day and then stored for 5 days.

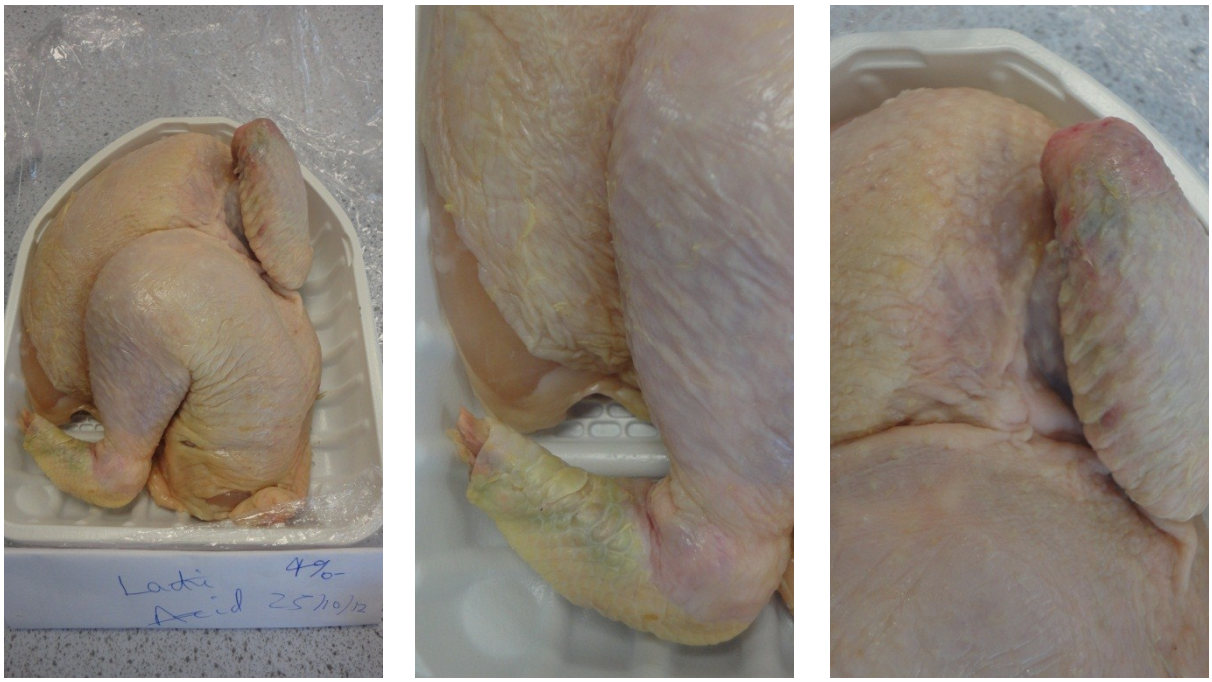


Figure 8d Appearance of bird bought on 26 October 2012, treated with 8% lactic acid (pH=3.9) on that day and then stored for 4 days

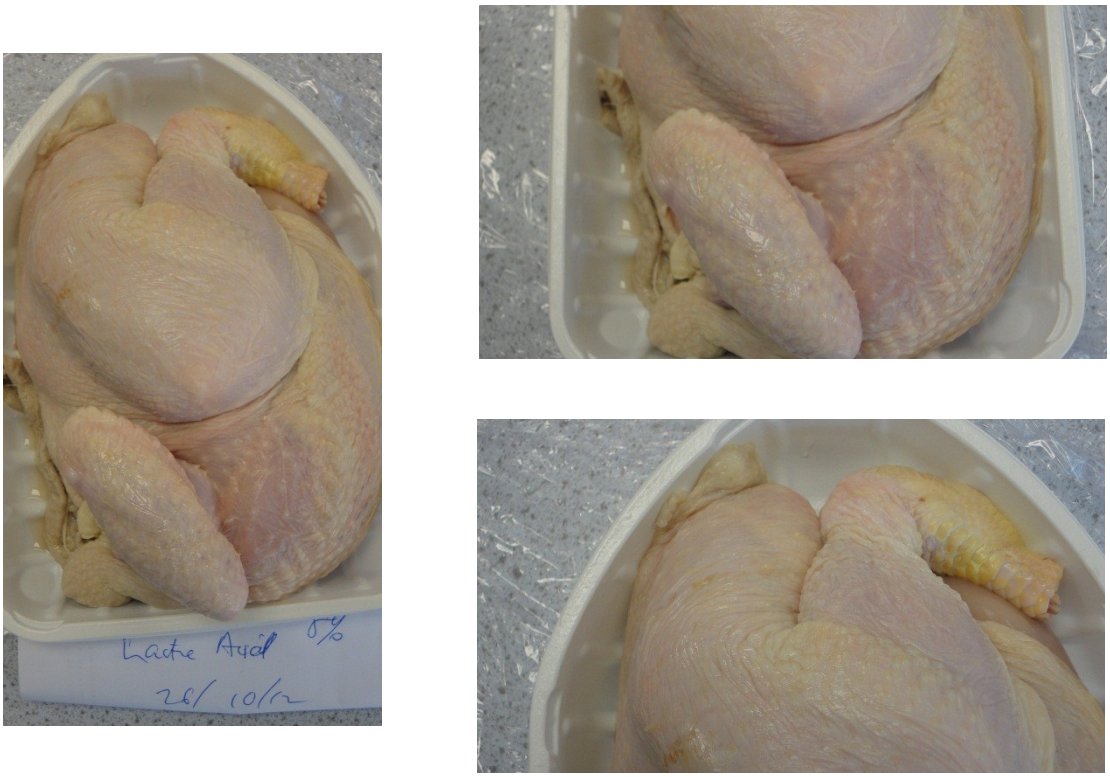


Figure 9 Change in redox (oxidation-reduction) potential of the ozonated water during Trial OZ2

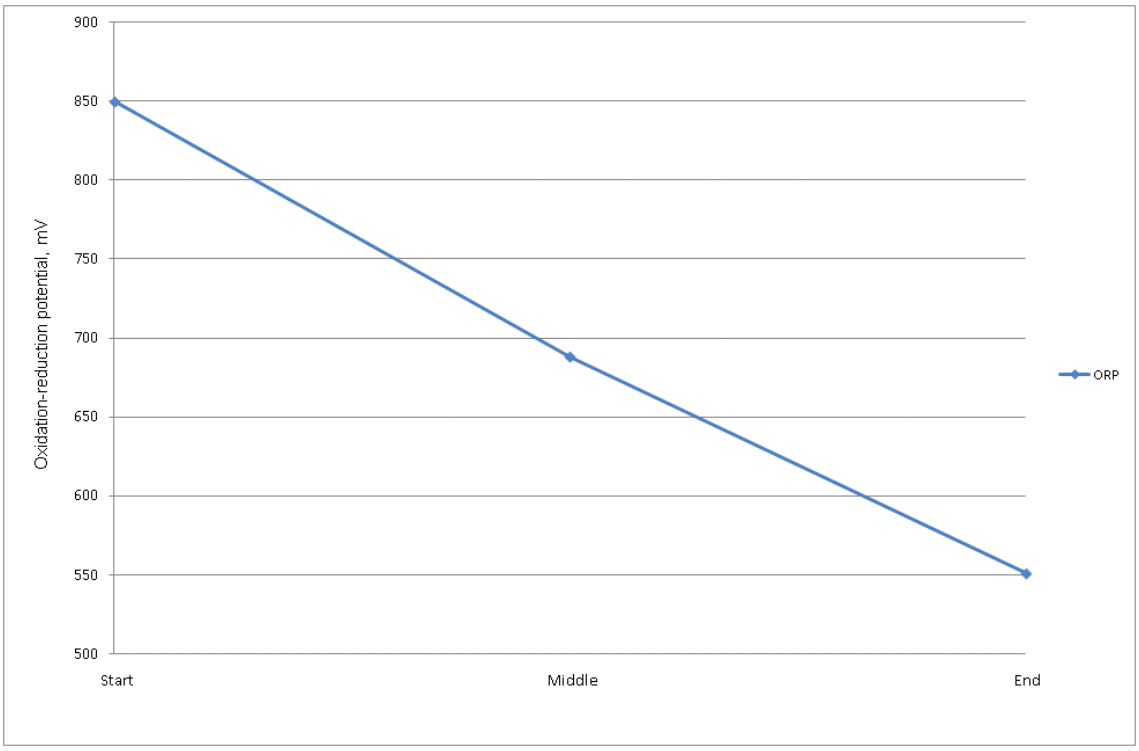


Figure 10 Change in the apparent free chlorine (FC) and total chlorine (TC) concentration of the ozonated water during Trial OZ2. FC and TC of tap water are also shown

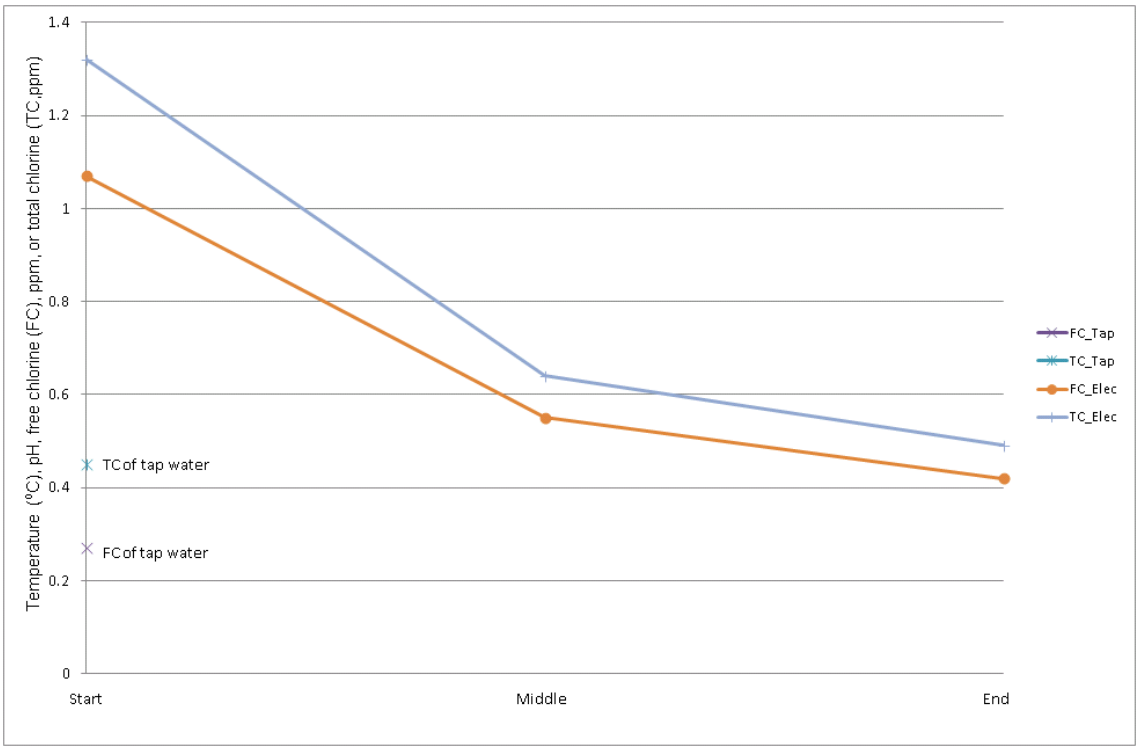


Figure 11 Aerobic plate counts on pork meat stored for up to 18 days with ozonated pellets (Blue Ice) or dry ice (Data provided by Air Liquide)

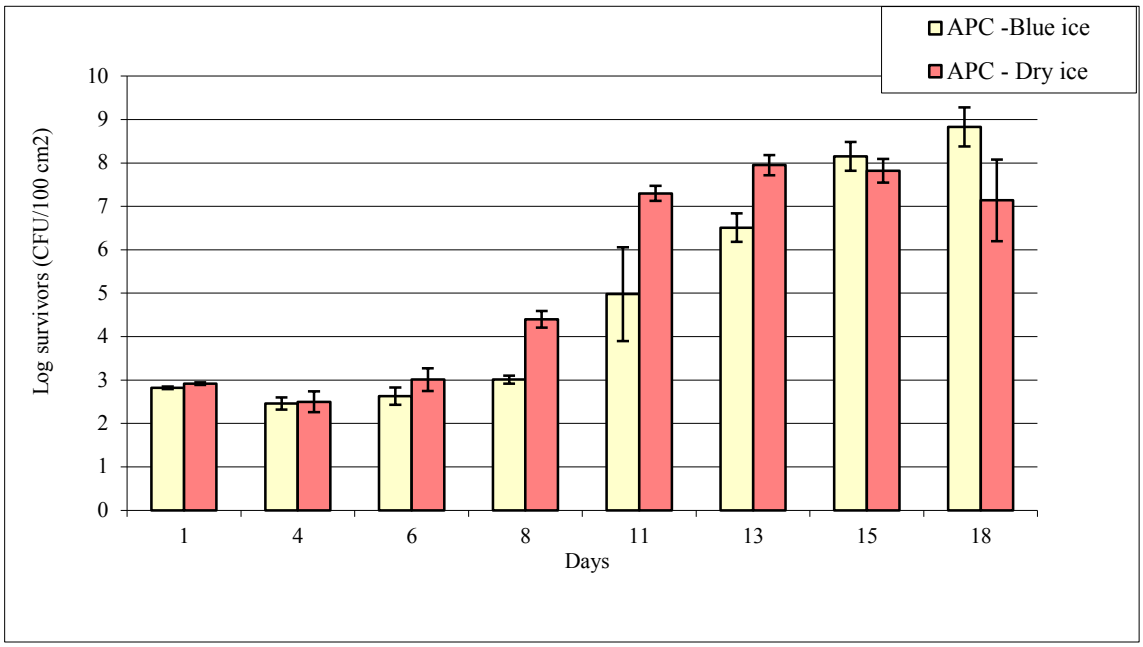


Figure 12 Reductions of C.jejuni and S.typhimurium on non-food surfaces treated with ozonated pellets (Aligal BI) or dry ice (DI) (Data provided by Air Liquide)

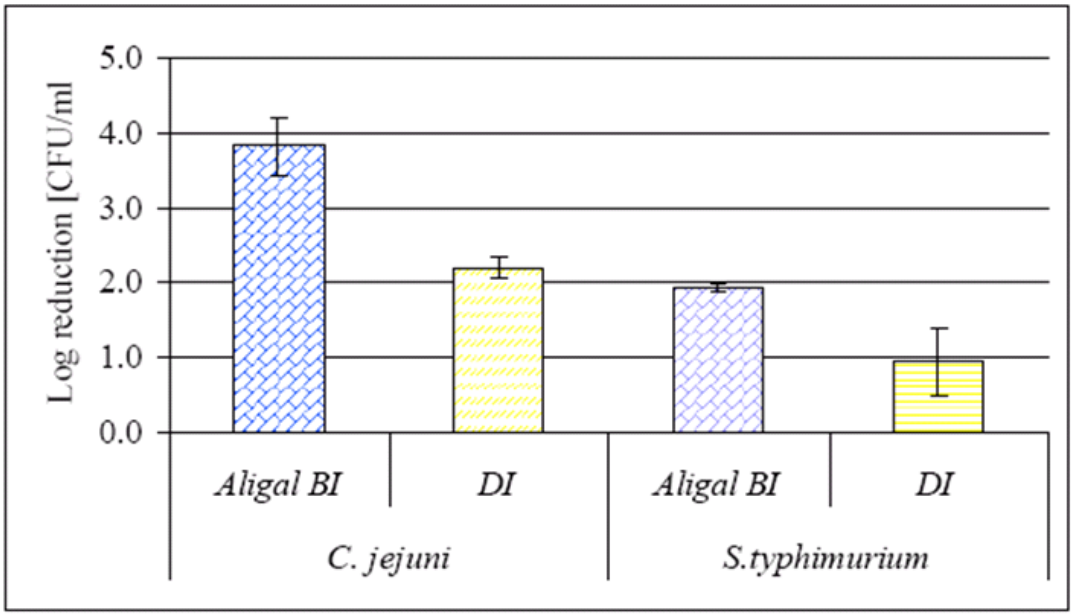


Figure 13 Reductions in C.jejuni on inoculated poultry samples treated with ozonated pellets (Aligal BI), dry ice (DI) or wet ice (WI) (Data provided by Air Liquide)

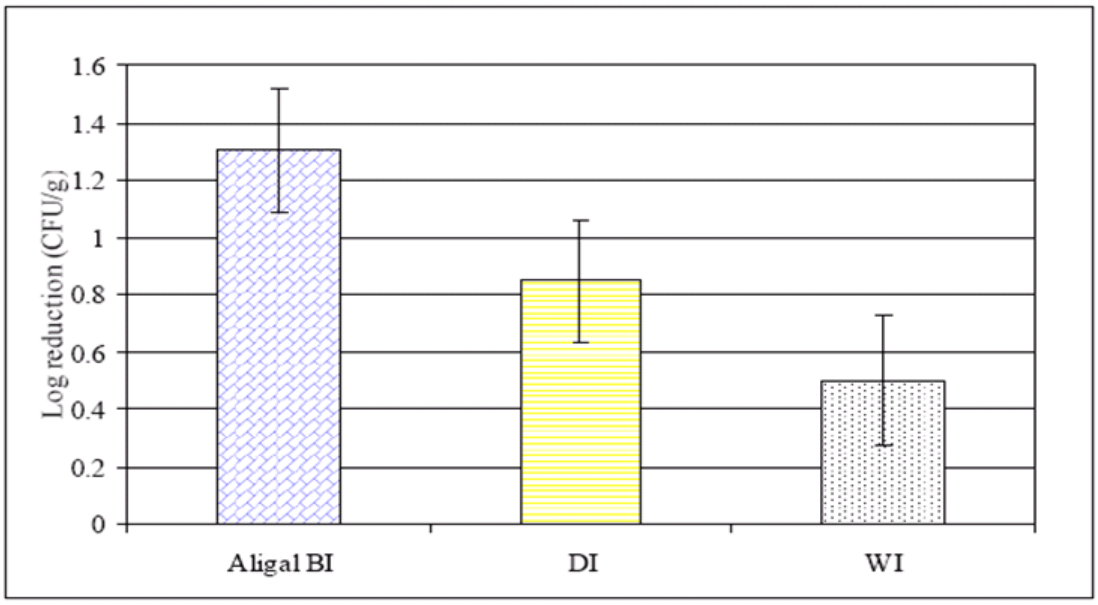


Table 1 Flow rate of liquid sprayed into the tunnel in Trial LA5

Run number	Start depth (cm)	End depth (cm)	Flow rate (litres/min)
1	34	23.6	10.4
2	33.6	23.1	10.5
3	33.8	23.5	10.3
Average	33.8	23.4	10.4
S.D.	0.2	0.3	0.1

Table 2a Weights of the control birds in Trial LA5

Bird No.	Weight after chill-bag weight, g
1	2407.5
2	2114.6
3	2731.2
4	2313.8
5	2092.4
6	2673.4
7	2811.4
8	2073.7
9	2801
10	1959.9
11	2472.7
12	2767.7
13	1681.1
14	2848.2
15	2861.4
16	2558.3
17	2815.8
18	2285.4
19	2628.1
20	1928.8
21	3055.4
22	2585.4
23	2677.7
24	2103.1
25	1778.7
26	1913.1
27	1890.2
28	2325.1
29	1885.9
30	2886.9
31	3037.2
32	2173.3
33	1907.6
34	2123
35	3110.4
36	2788.4
Average	2418.6
S.D.	413.8

Tables 2b Weights of the treated birds and properties of the lactic acid solution and tap water in Trial LA5

Bird No.	Purpose	Temp of liquid applied, C	pH of liquid applied	ORP of liquid applied, mV	Free chlorine in liquid applied, ppm	Total chlorine in liquid applied, ppm	Weight after chill-bag, g
37	Micro	11	3.9	694	0.35	0.43	2629.5
38	Micro						2504.4
39	Micro						2231.1
40	Micro						2498.2
41	Micro						2990.3
42	Micro						2474.7
43	Micro						2840.2
44	Micro						2584.5
45	Micro						2915.3
46	Micro						2260.5
47	Micro						3031.1
48	Micro						2020.8
49	Micro						2323.6
50	Micro						2317.5
51	Micro						2167.3
52	Micro						2128.9
53	Micro						2866
54	Micro						2474.1
55	Micro						2209.4
56	Micro						2318.4
57	Micro						3348.4
58	Micro						2263.6
59	Micro						3073.5
60	Micro						2507.5
61	Micro						2500.8
62	Micro						2822.7
63	Micro						2947.6
64	Micro						3172.2
65	Micro						2605.2
66	Micro						2323.8
67	Micro						2666
68	Micro						2535.6
69	Micro						3149
70	Micro						2818.6
71	Micro						2733.6
72	Micro	11	3.9	611	0.18	0.3	2533.9
Average							2605.2
S.D.							332.6
Plain water tested		Sample 1	7.2	510	0.2	0.33	
at start of trial		Sample 2	7.2	538	0.24	0.4	
		Sample 1	3.9	448	0.4	0.2	
Run-off samples		Sample 2	3.9	412	0.4	0.2	

Table 3a Microbial counts on untreated control samples at Day 1 in Trial LA5

Bird No.	Day	Treatment	Aerobic plate count per g	log(APC)	Campylobacter count per g	log(Campy)
1	K+1	Untreated	85000	4.93	1250	3.10
2	K+1	Untreated	27000	4.43	230	2.36
3	K+1	Untreated	90000	4.95	150	2.18
4	K+1	Untreated	90000	4.95	110	2.04
5	K+1	Untreated	70000	4.85	120	2.08
6	K+1	Untreated	27000	4.43	170	2.23
7	K+1	Untreated	25000	4.40	2400	3.38
8	K+1	Untreated	24400	4.39	90	1.95
9	K+1	Untreated	23000	4.36	150	2.18
10	K+1	Untreated	27500	4.44	100	2.00
11	K+1	Untreated	160000	5.20	150	2.18
12	K+1	Untreated	130000	5.11	370	2.57
13	K+1	Untreated	125000	5.10	410	2.61
14	K+1	Untreated	75000	4.88	300	2.48
15	K+1	Untreated	21000	4.32	20	1.30
16	K+1	Untreated	105000	5.02	70	1.85
17	K+1	Untreated	15500	4.19	<10	0.85
18	K+1	Untreated	30000	4.48	30	1.48
19	K+1	Untreated	155000	5.19	130	2.11
20	K+1	Untreated	24500	4.39	60	1.78
21	K+1	Untreated	85000	4.93	540	2.73
22	K+1	Untreated	45000	4.65	200	2.30
23	K+1	Untreated	60000	4.78	370	2.57
24	K+1	Untreated	25000	4.40	10320	4.01
25	K+1	Untreated	315000	5.50	90	1.95
26	K+1	Untreated	240000	5.38	190	2.28
27	K+1	Untreated	60000	4.78	90	1.95
28	K+1	Untreated	145000	5.16	70	1.85
29	K+1	Untreated	22000	4.34	50	1.70
30	K+1	Untreated	24500	4.39	160	2.20
31	K+1	Untreated	25000	4.40	30	1.48
32	K+1	Untreated	27000	4.43	90	1.95
33	K+1	Untreated	70000	4.85	30	1.48
34	K+1	Untreated	22500	4.35	80	1.90
35	K+1	Untreated	22300	4.35	20	1.30
36	K+1	Untreated	26500	4.42	60	1.78
Average				4.70		2.11
S.D.				0.36		0.59
C.I.				0.12		0.20
N				36		36
n<10						1

Table 3b Microbial counts on treated samples at Day 1 in Trial LA5

Bird No.	Day	Treatment	Aerobic plate count per g	log(APC)	Campylobacter count per g	log(Campy)
37	K+1	Treated	55000	4.74	60	1.78
38	K+1	Treated	13500	4.13	160	2.20
39	K+1	Treated	28100	4.45	280	2.45
40	K+1	Treated	27000	4.43	750	2.88
41	K+1	Treated	20000	4.30	180	2.26
42	K+1	Treated	27000	4.43	170	2.23
43	K+1	Treated	25500	4.41	210	2.32
44	K+1	Treated	23500	4.37	230	2.36
45	K+1	Treated	23500	4.37	220	2.34
46	K+1	Treated	14500	4.16	220	2.34
47	K+1	Treated	12000	4.08	70	1.85
48	K+1	Treated	22800	4.36	120	2.08
49	K+1	Treated	24000	4.38	240	2.38
50	K+1	Treated	19500	4.29	280	2.45
51	K+1	Treated	16500	4.22	200	2.30
52	K+1	Treated	23500	4.37	430	2.63
53	K+1	Treated	25000	4.40	330	2.52
54	K+1	Treated	22500	4.35	70	1.85
55	K+1	Treated	20000	4.30	290	2.46
56	K+1	Treated	14500	4.16	70	1.85
57	K+1	Treated	25000	4.40	1090	3.04
58	K+1	Treated	27500	4.44	150	2.18
59	K+1	Treated	24000	4.38	180	2.26
60	K+1	Treated	20500	4.31	300	2.48
61	K+1	Treated	18000	4.26	90	1.95
62	K+1	Treated	19500	4.29	670	2.83
63	K+1	Treated	22000	4.34	3060	3.49
64	K+1	Treated	135000	5.13	140	2.15
65	K+1	Treated	20000	4.30	80	1.90
66	K+1	Treated	15000	4.18	820	2.91
67	K+1	Treated	15000	4.18	420	2.62
68	K+1	Treated	15500	4.19	80	1.90
69	K+1	Treated	26000	4.41	350	2.54
70	K+1	Treated	27500	4.44	50	1.70
71	K+1	Treated	60000	4.78	370	2.57
72	K+1	Treated	95000	4.98	10	1.00
Average				4.38		2.31
S.D.				0.22		0.44
C.I.				0.07		0.15
N				36		36
n<10						0

Table 3c Microbial counts on untreated control samples at Day 7 in Trial LA5

Bird No.		Treatment	Aerobic plate count per g	log(APC)	Campylobacter count per g	log(Campy)
1	K+7	Untreated	950000	5.98	80	1.90
2	K+7	Untreated	1000000	6.00	<10	0.85
3	K+7	Untreated	5500000	6.74	<10	0.85
4	K+7	Untreated	41000000	7.61	10	1.00
5	K+7	Untreated	2750000	6.44	<10	0.85
6	K+7	Untreated	2575000	6.41	<10	0.85
7	K+7	Untreated	2585000	6.41	20	1.30
8	K+7	Untreated	2240000	6.35	<10	0.85
9	K+7	Untreated	11500000	7.06	<10	0.85
10	K+7	Untreated	17000000	7.23	10	1.00
11	K+7	Untreated	2530000	6.40	40	1.60
12	K+7	Untreated	17000000	7.23	20	1.30
13	K+7	Untreated	425000	5.63	<10	0.85
14	K+7	Untreated	5000000	6.70	<10	0.85
15	K+7	Untreated	2850000	6.45	<10	0.85
16	K+7	Untreated	2650000	6.42	<10	0.85
17	K+7	Untreated	2850000	6.45	<10	0.85
18	K+7	Untreated	40000000	7.60	<10	0.85
19	K+7	Untreated	165000000	8.22	<10	0.85
20	K+7	Untreated	1800000	6.26	<10	0.85
21	K+7	Untreated	9500000	6.98	10	1.00
22	K+7	Untreated	2700000	6.43	<10	0.85
23	K+7	Untreated	22500000	7.35	10	1.00
24	K+7	Untreated	2750000	6.44	20	1.30
25	K+7	Untreated	12000000	7.08	30	1.48
26	K+7	Untreated	850000000	8.93	<10	0.85
27	K+7	Untreated	2500000	6.40	10	1.00
28	K+7	Untreated	1560000	6.19	<10	0.85
29	K+7	Untreated	55500000	7.74	<10	0.85
30	K+7	Untreated	118500000	8.07	10	1.00
31	K+7	Untreated	13000000	7.11	10	1.00
32	K+7	Untreated	405000	5.61	<10	0.85
33	K+7	Untreated	15500000	7.19	<10	0.85
34	K+7	Untreated	360000	5.56	20	1.30
35	K+7	Untreated	1230000	6.09	40	1.60
36	K+7	Untreated	66000000	7.82	60	1.78
Average				6.79		1.04
S.D.				0.77		0.30
C.I.				0.26		0.10
N				36		36
n<10						20

Table 3d Microbial counts on treated samples at Day 7 in Trial LA5

Bird No.	Day	Treatment	Aerobic plate count per g	log(APC)	Campylobacter count per g	log(Campy)
37	K+7	Treated	83000000	7.92	<10	0.85
38	K+7	Treated	2050000	6.31	<10	0.85
39	K+7	Treated	2130000	6.33	10	1.00
40	K+7	Treated	22500000	7.35	10	1.00
41	K+7	Treated	15000000	7.18	<10	0.85
42	K+7	Treated	17500000	7.24	<10	0.85
43	K+7	Treated	2750000	6.44	10	1.00
44	K+7	Treated	1320000	6.12	10	1.00
45	K+7	Treated	1650000	6.22	<10	0.85
46	K+7	Treated	2850000	6.45	10	1.00
47	K+7	Treated	135000000	8.13	<10	0.85
48	K+7	Treated	85000000	7.93	<10	0.85
49	K+7	Treated	1050000	6.02	20	1.30
50	K+7	Treated	7500000	6.88	30	1.48
51	K+7	Treated	2800000	6.45	<10	0.85
52	K+7	Treated	6000000	6.78	<10	0.85
53	K+7	Treated	10000000	7.00	<10	0.85
54	K+7	Treated	1300000	6.11	20	1.30
55	K+7	Treated	1710000	6.23	<10	0.85
56	K+7	Treated	17000000	7.23	<10	0.85
57	K+7	Treated	49000000	7.69	<10	0.85
58	K+7	Treated	2450000	6.39	10	1.00
59	K+7	Treated	1850000	6.27	30	1.48
60	K+7	Treated	2150000	6.33	<10	0.85
61	K+7	Treated	2000000	6.30	<10	0.85
62	K+7	Treated	8500000	6.93	<10	0.85
63	K+7	Treated	11000000	7.04	140	2.15
64	K+7	Treated	2650000	6.42	<10	0.85
65	K+7	Treated	2550000	6.41	<10	0.85
66	K+7	Treated	4000000	6.60	10	1.00
67	K+7	Treated	2800000	6.45	<10	0.85
68	K+7	Treated	6000000	6.78	<10	0.85
69	K+7	Treated	14500000	7.16	10	1.00
70	K+7	Treated	2850000	6.45	<10	0.85
71	K+7	Treated	3000000	6.48	10	1.00
72	K+7	Treated	485000	5.69	10	1.00
Average				6.71		0.99
S.D.				0.58		0.26
C.I.				0.20		0.09
N				36		36
n<10						21

Table 4 Microbial counts on samples tested by Laboratory 1 in Trial LA7

Bird No.	Treatment	Day 1				Day 7			
		Aerobic Plate Count per g	log(APC)	Count of confirmed Campylobacter per g	log(Campy)	Aerobic Plate Counts per g	log(APC)	Count of confirmed Campylobacter per g	log(Campy)
13	Control	7500000	6.88	<5	0.55	840000	5.92	<5	0.55
14	Control	145000	5.16	30	1.48	13500000	7.13	<5	0.55
15	Control	90000	4.95	5	0.70	5000000	6.70	5	0.70
16	Control	225000	5.35	95	1.98	13000000	7.11	<5	0.55
17	Control	60000	4.78	<5	0.55	67500000	7.83	<5	0.55
18	Control	255000	5.41	15	1.18	50000	4.70	<5	0.55
19	Control	35000	4.54	25	1.40	6000000	6.78	5	0.70
20	Control	125000	5.10	220	2.34	27500000	7.44	50	1.70
Average			5.27		1.27		6.70		0.73
S.D.			0.71		0.66		0.98		0.40
C.I.			0.59		0.56		0.82		0.33
N			8		8		8		8
n<5					2				5

Bird No.	Treatment	Day 1				Day 7			
		Aerobic Plate Count per g	log(APC)	Count of confirmed Campylobacter per g	log(Campy)	Aerobic Plate Counts per g	log(APC)	Count of confirmed Campylobacter per g	log(Campy)
33	Tunnel	115000	5.06	95	1.98	485000	5.69	<5	0.55
34	Tunnel	780000	5.89	75	1.88	30000	4.48	<5	0.55
35	Tunnel	90000	4.95	5	0.70	45000	4.65	<5	0.55
36	Tunnel	35000	4.54	<5	0.55	475000	5.68	10	1.00
37	Tunnel	105000	5.02	115	2.06	5000	3.70	<5	0.55
38	Tunnel	70000	4.85	<5	0.55	470000	5.67	<5	0.55
39	Tunnel	50000	4.70	10	1.00	25000	4.40	<5	0.55
40	Tunnel	40000	4.60	75	1.88	70000	4.85	<5	0.55
Average			4.95		1.32		4.89		0.60
S.D.			0.43		0.68		0.73		0.16
C.I.			0.36		0.57		0.61		0.13
N			8		8		8		8
n<5					2				7

Bird No.	Treatment	Day 1				Day 7			
		Aerobic Plate Count per g	log(APC)	Count of confirmed Campylobacter per g	log(Campy)	Aerobic Plate Counts per g	log(APC)	Count of confirmed Campylobacter per g	log(Campy)
53	Hand/Quick/17s	9750	3.99	<5	0.55	60000	4.78	5	0.70
54	Hand/Quick/17s	100000	5.00	135	2.13	70000	4.85	<5	0.55
55	Hand/Quick/17s	15000	4.18	<5	0.55	625000	5.80	<5	0.55
56	Hand/Quick/17s	60000	4.78	10	1.00	5500000	6.74	<5	0.55
57	Hand/Quick/17s	80000	4.90	25	1.40	35000	4.54	<5	0.55
58	Hand/Quick/17s	70000	4.85	15	1.18	40000	4.60	<5	0.55
59	Hand/Quick/17s	75000	4.88	5	0.70	525000	5.72	<5	0.55
60	Hand/Quick/17s	160000	5.20	50	1.70	55000	4.74	<5	0.55
Average			4.72		1.15		5.22		0.57
S.D.			0.42		0.57		0.78		0.05
C.I.			0.35		0.48		0.66		0.04
N			8		8		8		8
n<5					2				7

Bird No.	Treatment	Day 1				Day 7			
		Aerobic Plate Count per g	log(APC)	Count of confirmed Campylobacter per g	log(Campy)	Aerobic Plate Counts per g	log(APC)	Count of confirmed Campylobacter per g	log(Campy)
73	Hand/Quick/4s	120000	5.08	40	1.60	6000000	6.78	<5	0.55
74	Hand/Quick/4s	190000	5.28	445	2.65	320000	5.51	10	1.00
75	Hand/Quick/4s	125000	5.10	85	1.93	410000	5.61	5	0.70
76	Hand/Quick/4s	260000	5.41	90	1.95	11500000	7.06	<5	0.55
77	Hand/Quick/4s	****	****	****	****	****	****	****	****
78	Hand/Quick/4s	910000	5.96	10	1.00	390000	5.59	<5	0.55
79	Hand/Quick/4s	125000	5.10	65	1.81	570000	5.76	15	1.18
80	Hand/Quick/4s	150000	5.18	20	1.30	115000	5.06	<5	0.55
Average			5.34		1.77		5.76		0.75
S.D.			0.33		0.57		0.68		0.27
C.I.			0.34		0.60		0.71		0.28
N			6		6		6		6
n<5					0				3
****	Not enough sample to test								

Bird No.	Treatment	Day 1				Day 7			
		Aerobic Plate Count per g	log(APC)	Count of confirmed Campylobacter per g	log(Campy)	Aerobic Plate Counts per g	log(APC)	Count of confirmed Campylobacter per g	log(Campy)
93	Hand/Slow/17s	490000	5.69	20	1.30	450000	5.65	<5	0.55
94	Hand/Slow/17s	110000	5.04	<5	0.55	105000	5.02	<5	0.55
95	Hand/Slow/17s	175000	5.24	<5	0.55	795000	5.90	<5	0.55
96	Hand/Slow/17s	235000	5.37	20	1.30	205000	5.31	<5	0.55
97	Hand/Slow/17s	330000	5.52	15	1.18	230000	5.36	<5	0.55
98	Hand/Slow/17s	75000	4.88	15	1.18	105000	5.02	<5	0.55
99	Hand/Slow/17s	175000	5.24	65	1.81	10500000	7.02	15	1.18
100	Hand/Slow/17s	150000	5.18	<5	0.55	240000	5.38	<5	0.55
Average			5.21		1.02		5.57		0.64
S.D.			0.21		0.49		0.70		0.24
C.I.			0.19		0.45		0.65		0.22
N			7		7		7		7
n<5					3				6

Sample No.	Caeca	log(Caeca)
1	120000000	8.08
2	180000000	8.26
3	40000000	7.60
4	72000000	7.86
Average		7.95
S.D.		0.28

Table 5 Microbial counts on samples tested by Laboratory 2 in Trial LA7

Bird No.	Treatment	Day 1				Day 7			
		Aerobic Plate Count per g	log(APC)	Count of confirmed Campylobacter per g	log(Campy)	Aerobic Plate Count per g	log(APC)	Count of confirmed Campylobacter per g	log(Campy)
1	Control	11,000	4.04	<5	0.55	25,000	4.40	<5	0.55
2	Control	8,700	3.94	<5	0.55	42,000	4.62	<5	0.55
3	Control	13,000	4.11	5	0.70	79,000	4.90	<5	0.55
4	Control	6,000	3.78	<5	0.55	70,000	4.85	<5	0.55
5	Control	2,100	3.32	100	2.00	15,000	4.18	<5	0.55
6	Control	4,200	3.62	10	1.00	43,000	4.63	<5	0.55
7	Control	2,300	3.36	<5	0.55	470,000	5.67	<5	0.55
8	Control	17,000	4.23	27	1.43	58,000	4.76	100	2.00
9	Control	8,600	3.93	<5	0.55	69,000	4.84	<5	0.55
10	Control	7,700	3.89	<5	0.55	21,000	4.32	<5	0.55
11	Control	8,900	3.95	<5	0.55	45,000	4.65	<5	0.55
12	Control	6,500	3.81	<5	0.55	24,000	4.38	5	0.70
Average			3.83		0.79		4.68		0.68
S.D.			0.28		0.47		0.39		0.42
C.I.			0.18		0.30		0.25		0.27
N			12		12		12		12
n<5					8				10

Bird No.	Treatment	Day 1				Day 7			
		Aerobic Plate Count per g	log(APC)	Count of confirmed Campylobacter per g	log(Campy)	Aerobic Plate Count per g	log(APC)	Count of confirmed Campylobacter per g	log(Campy)
21	Tunnel, 7s	867	2.94	<5	0.55	2,500	3.40	<5	0.55
22	Tunnel, 7s	2,700	3.43	<5	0.55	2,100	3.32	<5	0.55
23	Tunnel, 7s	1,900	3.28	<5	0.55	3,300	3.52	<5	0.55
24	Tunnel, 7s	1,900	3.28	<5	0.55	6,500	3.81	<5	0.55
25	Tunnel, 7s	1,500	3.18	<5	0.55	4,700	3.67	<5	0.55
26	Tunnel, 7s	1,100	3.04	5	0.70	6,500	3.81	<5	0.55
27	Tunnel, 7s	2,500	3.40	<5	0.55	3,500	3.54	<5	0.55
28	Tunnel, 7s	2,100	3.32	<5	0.55	2,800	3.45	<5	0.55
29	Tunnel, 7s	810	2.91	<5	0.55	3,200	3.51	<5	0.55
30	Tunnel, 7s	2,900	3.46	10	1.00	2,500	3.40	<5	0.55
31	Tunnel, 7s	2,600	3.41	<5	0.55	2,100	3.32	<5	0.55
32	Tunnel, 7s	3,500	3.54	<5	0.55	2,500	3.40	<5	0.55
Average			3.27		0.60		3.51		0.55
S.D.			0.21		0.13		0.17		0.00
C.I.			0.13		0.08		0.11		0.00
N			12		12		12		12
n<5					10				12

Bird No.	Treatment	Day 1				Day 7			
		Aerobic Plate Count per g	log(APC)	Count of confirmed Campylobacter per g	log(Campy)	Aerobic Plate Count per g	log(APC)	Count of confirmed Campylobacter per g	log(Campy)
41	Electro, Quick, 17s	4,300	3.63	<5	0.55	6,500	3.81	<5	0.55
42	Electro, Quick, 17s	1,500	3.18	<5	0.55	520,000	5.72	<5	0.55
43	Electro, Quick, 17s	538	2.73	<5	0.55	34,000	4.53	<5	0.55
44	Electro, Quick, 17s	1,700	3.23	100	2.00	38,000	4.58	<5	0.55
45	Electro, Quick, 17s	776	2.89	<5	0.55	75,000	4.88	<5	0.55
46	Electro, Quick, 17s	619	2.79	<5	0.55	54,000	4.73	<5	0.55
47	Electro, Quick, 17s	1,200	3.08	10	1.00	1,600	3.20	<5	0.55
48	Electro, Quick, 17s	1,200	3.08	<5	0.55	6,700	3.83	<5	0.55
49	Electro, Quick, 17s	1,700	3.23	<5	0.55	8,700	3.94	5	0.70
50	Electro, Quick, 17s	1,800	3.26	<5	0.55	15,000	4.18	<5	0.55
51	Electro, Quick, 17s	871	2.94	<5	0.55	6,400	3.81	<5	0.55
52	Electro, Quick, 17s	1,400	3.15	<5	0.55	7,200	3.86	<5	0.55
Average			3.10		0.71		4.25		0.56
S.D.			0.24		0.43		0.67		0.04
C.I.			0.15		0.27		0.42		0.03
N			12		12		12		12
n<5					10				11

Bird No.	Treatment	Day 1				Day 7			
		Aerobic Plate Count per g	log(APC)	Count of confirmed Campylobacter per g	log(Campy)	Aerobic Plate Count per g	log(APC)	Count of confirmed Campylobacter per g	log(Campy)
61	Electro, Quick, 4s	3,800	3.58	<5	0.55	6,800	3.83	<5	0.55
62	Electro, Quick, 4s	2,000	3.30	<5	0.55	2,400	3.38	<5	0.55
63	Electro, Quick, 4s	1,200	3.08	<5	0.55	11,000	4.04	<5	0.55
64	Electro, Quick, 4s	6,100	3.79	<5	0.55	30,000	4.48	<5	0.55
65	Electro, Quick, 4s	2,000	3.30	<5	0.55	2,600	3.41	<5	0.55
66	Electro, Quick, 4s	5,600	3.75	<5	0.55	16,000	4.20	<5	0.55
67	Electro, Quick, 4s	976	2.99	<5	0.55	10,000	4.00	<5	0.55
68	Electro, Quick, 4s	8,500	3.93	<5	0.55	20,000	4.30	<5	0.55
69	Electro, Quick, 4s	2,200	3.34	<5	0.55	72,000	4.86	<5	0.55
70	Electro, Quick, 4s	1,900	3.28	<5	0.55	9,300	3.97	<5	0.55
71	Electro, Quick, 4s	4,700	3.67	<5	0.55	95,000	4.98	<5	0.55
72	Electro, Quick, 4s	914	2.96	<5	0.55	7,500	3.88	<5	0.55
Average			3.41		0.55		4.11		0.55
S.D.			0.32		0.00		0.49		0.00
C.I.			0.21		0.00		0.31		0.00
N			12		12		12		12
n<5					12				12

Bird No.	Treatment	Day 1				Day 7			
		Aerobic Plate Count per g	log(APC)	Count of confirmed Campylobacter per g	log(Campy)	Aerobic Plate Count per g	log(APC)	Count of confirmed Campylobacter per g	log(Campy)
81	Electro, Slow, 17s	971	2.99	<5	0.55	23,000	4.36	<5	0.55
82	Electro, Slow, 17s	2,300	3.36	5	0.70	3,900	3.59	<5	0.55
83	Electro, Slow, 17s	2,200	3.34	<5	0.55	8,800	3.94	<5	0.55
84	Electro, Slow, 17s	1,800	3.26	<5	0.55	41,000	4.61	<5	0.55
85	Electro, Slow, 17s	5,400	3.73	<5	0.55	34,000	4.53	<5	0.55
86	Electro, Slow, 17s	3,300	3.52	<5	0.55	86,000	4.93	<5	0.55
87	Electro, Slow, 17s	5,200	3.72	<5	0.55	250,000	5.40	<5	0.55
88	Electro, Slow, 17s	2,000	3.30	<5	0.55	74,000	4.87	5	0.70
89	Electro, Slow, 17s	8,000	3.90	<5	0.55	23,000	4.36	<5	0.55
90	Electro, Slow, 17s	2,200	3.34	<5	0.55	110,000	5.04	<5	0.55
91	Electro, Slow, 17s	1,500	3.18	5	0.70	9,300	3.97	<5	0.55
92	Electro, Slow, 17s	2,900	3.46	<5	0.55	110,000	5.04	<5	0.55
Average			3.42		0.57		4.55		0.56
S.D.			0.26		0.06		0.54		0.04
C.I.			0.16		0.04		0.34		0.03
N			12		12		12		12
n<5					10				11

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Table 6 Weights of untreated birds and those treated with 4% lactic acid or 8% lactic acid in Trial LA8

Bird No.	Weight, g	Treatment	Bird No.	Weight, g	Treatment	Bird No.	Weight, g	Treatment
1	2253	Untreated	21	1970	Tunnel 4%	41	2029	Hand 8%
2	1679	Untreated	22	2288	Tunnel 4%	42	1662	Hand 8%
3	1158	Untreated	23	1920	Tunnel 4%	43	2431	Hand 8%
4	1642	Untreated	24	1319	Tunnel 4%	44	1854	Hand 8%
5	2014	Untreated	25	1540	Tunnel 4%	45	2144	Hand 8%
6	1927	Untreated	26	1872	Tunnel 4%	46	2764	Hand 8%
7	2558	Untreated	27	1957	Tunnel 4%	47	1698	Hand 8%
8	1147	Untreated	28	2084	Tunnel 4%	48	2033	Hand 8%
9	2093	Untreated	29	1422	Tunnel 4%	49	2322	Hand 8%
10	2011	Untreated	30	1782	Tunnel 4%	50	2409	Hand 8%
11	1954	Untreated	31	2502	Tunnel 4%	51	1878	Hand 8%
12	1603	Untreated	32	1714	Tunnel 4%	52	2660	Hand 8%
13	1120	Untreated	33	2542	Tunnel 4%	53	2741	Hand 8%
14	1938	Untreated	34	1756	Tunnel 4%	54	2261	Hand 8%
15	1507	Untreated	35	1643	Tunnel 4%	55	1954	Hand 8%
16	2241	Untreated	36	1876	Tunnel 4%	56	1698	Hand 8%
17	1824	Untreated	37	1961	Tunnel 4%	57	2414	Hand 8%
18	2205	Untreated	38	1652	Tunnel 4%	58	2827	Hand 8%
19	1810	Untreated	39	2760	Tunnel 4%	59	2609	Hand 8%
20	1877	Untreated	40	1474	Tunnel 4%	60	2285	Hand 8%
Average	1828		Average	1902		Average	2234	
S.D.	386		S.D.	383		S.D.	375	
Min	1120		Min	1319		Min	1662	
Max	2558		Max	2760		Max	2827	
N	20		N	20		N	20	

Table 7a Microbial counts on untreated breast skin samples at Day K+1 (samples taken post-chill) in Trial LA8

Sample No.	Bird No.	Day	Location	Treatment	Sample	Aerobic Plate Count per g	log(APC)	Campylobacter count per g	log(Campy)
1	1	K+1	Post-chill	Untreated	Breast	1640	3.21	271	2.43
2	2	K+1	Post-chill	Untreated	Breast	9000	3.95	174	2.24
3	3	K+1	Post-chill	Untreated	Breast	2180	3.34	359	2.56
4	4	K+1	Post-chill	Untreated	Breast	3020	3.48	320	2.51
5	5	K+1	Post-chill	Untreated	Breast	2340	3.37	252	2.40
6	6	K+1	Post-chill	Untreated	Breast	2780	3.44	211	2.32
7	7	K+1	Post-chill	Untreated	Breast	2880	3.46	149	2.17
8	8	K+1	Post-chill	Untreated	Breast	1770	3.25	81	1.91
9	9	K+1	Post-chill	Untreated	Breast	40000	4.60	1192	3.08
10	10	K+1	Post-chill	Untreated	Breast	10500	4.02	1520	3.18
11	11	K+1	Post-chill	Untreated	Breast	10000	4.00	262	2.42
12	12	K+1	Post-chill	Untreated	Breast	1720	3.24	47	1.67
13	13	K+1	Post-chill	Untreated	Breast	900	2.95	167	2.22
14	14	K+1	Post-chill	Untreated	Breast	3120	3.49	281	2.45
15	15	K+1	Post-chill	Untreated	Breast	1990	3.30	401	2.60
16	16	K+1	Post-chill	Untreated	Breast	11000	4.04	244	2.39
17	17	K+1	Post-chill	Untreated	Breast	2040	3.31	163	2.21
18	18	K+1	Post-chill	Untreated	Breast	1620	3.21	194	2.29
19	19	K+1	Post-chill	Untreated	Breast	8000	3.90	614	2.79
20	20	K+1	Post-chill	Untreated	Breast	555	2.74	80	1.90
Average							3.52		2.39
S.D.							0.44		0.36
C.I.							0.21		0.17
N							20		20
n<1									0

Table 7b Microbial counts on untreated back/neck skin samples at Day K+1 (samples taken post-chill) in Trial LA8

Sample No.	Bird No.	Day	Location	Treatment	Sample	Aerobic Plate Count per g	log(APC)	Campylobacter count per g	log(Campy)
21	1	K+1	Post-chill	Untreated	Back/Neck	3100	3.49	7000	3.85
22	2	K+1	Post-chill	Untreated	Back/Neck	2680	3.43	1500	3.17
23	3	K+1	Post-chill	Untreated	Back/Neck	16500	4.22	1110	3.05
24	4	K+1	Post-chill	Untreated	Back/Neck	720	2.86	2750	3.44
25	5	K+1	Post-chill	Untreated	Back/Neck	2020	3.31	2000	3.30
26	6	K+1	Post-chill	Untreated	Back/Neck	1510	3.18	1440	3.16
27	7	K+1	Post-chill	Untreated	Back/Neck	22000	4.34	77750	4.89
28	8	K+1	Post-chill	Untreated	Back/Neck	2460	3.39	1056	3.02
29	9	K+1	Post-chill	Untreated	Back/Neck	15500	4.19	1622	3.21
30	10	K+1	Post-chill	Untreated	Back/Neck	15000	4.18	10000	4.00
31	11	K+1	Post-chill	Untreated	Back/Neck	11500	4.06	1202	3.08
32	12	K+1	Post-chill	Untreated	Back/Neck	12000	4.08	1114	3.05
33	13	K+1	Post-chill	Untreated	Back/Neck	16000	4.20	1750	3.24
34	14	K+1	Post-chill	Untreated	Back/Neck	12000	4.08	928	2.97
35	15	K+1	Post-chill	Untreated	Back/Neck	14500	4.16	1500	3.18
36	16	K+1	Post-chill	Untreated	Back/Neck	13500	4.13	4500	3.65
37	17	K+1	Post-chill	Untreated	Back/Neck	14500	4.16	2750	3.44
38	18	K+1	Post-chill	Untreated	Back/Neck	2500	3.40	14750	4.17
39	19	K+1	Post-chill	Untreated	Back/Neck	21500	4.33	1218	3.09
40	20	K+1	Post-chill	Untreated	Back/Neck	1430	3.16	1526	3.18
Average							3.82		3.41
S.D.							0.47		0.49
C.I.							0.22		0.23
N							20		20
n<1									0

Table 7c Microbial counts on breast skin samples at Day K+1 after treatment with 4% lactic acid (samples taken post-chill) in Trial LA8

Sample No.	Bird No.	Day	Location	Treatment	Sample	Aerobic Plate Count per g	log(APC)	Campylobacter count per g	log(Campy)
41	21	K+1	Post-chill	4% tunnel	Breast	1055	3.02	118	2.07
42	22	K+1	Post-chill	4% tunnel	Breast	785	2.89	184	2.26
43	23	K+1	Post-chill	4% tunnel	Breast	1220	3.09	83	1.92
44	24	K+1	Post-chill	4% tunnel	Breast	2550	3.41	1748	3.24
45	25	K+1	Post-chill	4% tunnel	Breast	850	2.93	79	1.90
46	26	K+1	Post-chill	4% tunnel	Breast	3780	3.58	179	2.25
47	27	K+1	Post-chill	4% tunnel	Breast	850	2.93	64	1.81
48	28	K+1	Post-chill	4% tunnel	Breast	11000	4.04	4	0.60
49	29	K+1	Post-chill	4% tunnel	Breast	1880	3.27	11	1.04
50	30	K+1	Post-chill	4% tunnel	Breast	960	2.98	44	1.64
51	31	K+1	Post-chill	4% tunnel	Breast	1020	3.01	53	1.72
52	32	K+1	Post-chill	4% tunnel	Breast	1930	3.29	15	1.18
53	33	K+1	Post-chill	4% tunnel	Breast	17000	4.23	223	2.35
54	34	K+1	Post-chill	4% tunnel	Breast	980	2.99	110	2.04
55	35	K+1	Post-chill	4% tunnel	Breast	9000	3.95	100	2.00
56	36	K+1	Post-chill	4% tunnel	Breast	1320	3.12	63	1.80
57	37	K+1	Post-chill	4% tunnel	Breast			834	2.92
58	38	K+1	Post-chill	4% tunnel	Breast	1580	3.20	370	2.57
59	39	K+1	Post-chill	4% tunnel	Breast	1340	3.13	271	2.43
60	40	K+1	Post-chill	4% tunnel	Breast	2820	3.45	35	1.54
Average							3.29		1.96
S.D.							0.40		0.62
C.I.							0.19		0.29
N							19		20
n<1									0

Table 7d Microbial counts on back/neck skin at Day K+1 after treatment with 4% lactic acid (samples taken post-chill) in Trial LA8

Sample No.	Bird No.	Day	Location	Treatment	Sample	Aerobic Plate Count per g	log(APC)	Campylobacter count per g	log(Campy)
61	21	K+1	Post-chill	4% tunnel	Back/Neck	6000	3.78	2000	3.30
62	22	K+1	Post-chill	4% tunnel	Back/Neck	1080	3.03	396	2.60
63	23	K+1	Post-chill	4% tunnel	Back/Neck	27000	4.43	24250	4.38
64	24	K+1	Post-chill	4% tunnel	Back/Neck	1740	3.24	46	1.66
65	25	K+1	Post-chill	4% tunnel	Back/Neck	2960	3.47	23	1.36
66	26	K+1	Post-chill	4% tunnel	Back/Neck	2720	3.43	2000	3.30
67	27	K+1	Post-chill	4% tunnel	Back/Neck	7500	3.88	15000	4.18
68	28	K+1	Post-chill	4% tunnel	Back/Neck	43500	4.64	4000	3.60
69	29	K+1	Post-chill	4% tunnel	Back/Neck	2340	3.37	750	2.87
70	30	K+1	Post-chill	4% tunnel	Back/Neck	14500	4.16	1424	3.15
71	31	K+1	Post-chill	4% tunnel	Back/Neck	2360	3.37	438	2.64
72	32	K+1	Post-chill	4% tunnel	Back/Neck	2820	3.45	21	1.32
73	33	K+1	Post-chill	4% tunnel	Back/Neck	1360	3.13	19	1.28
74	34	K+1	Post-chill	4% tunnel	Back/Neck	1280	3.11	356	2.55
75	35	K+1	Post-chill	4% tunnel	Back/Neck	1390	3.14	300	2.48
76	36	K+1	Post-chill	4% tunnel	Back/Neck	1980	3.30	434	2.64
77	37	K+1	Post-chill	4% tunnel	Back/Neck	1760	3.25	151	2.18
78	38	K+1	Post-chill	4% tunnel	Back/Neck	2560	3.41	1500	3.18
79	39	K+1	Post-chill	4% tunnel	Back/Neck	1425	3.15	103	2.01
80	40	K+1	Post-chill	4% tunnel	Back/Neck	86000	4.93	154	2.19
Average							3.58		2.64
S.D.							0.55		0.88
C.I.							0.26		0.41
N							20		20
n<1									0

Table 7e Microbial counts on untreated back/neck skin samples at Day K+1 (samples taken pre-chill) in Trial LA8

Sample No.	Bird No.	Day	Location	Treatment	Sample	Aerobic Plate Count per g	log(APC)	Campylobacter count per g	log(Campy)
81	41	K+1	Pre-chill	Untreated	Back/Neck	24500	4.39	2250	3.35
82	42	K+1	Pre-chill	Untreated	Back/Neck	11000	4.04	1846	3.27
83	43	K+1	Pre-chill	Untreated	Back/Neck	166000	5.22	43500	4.64
84	44	K+1	Pre-chill	Untreated	Back/Neck	36000	4.56	1572	3.20
85	45	K+1	Pre-chill	Untreated	Back/Neck	53000	4.72	2250	3.35
86	46	K+1	Pre-chill	Untreated	Back/Neck	30000	4.48	1632	3.21
87	47	K+1	Pre-chill	Untreated	Back/Neck	118000	5.07	8250	3.92
88	48	K+1	Pre-chill	Untreated	Back/Neck	2060	3.31	7250	3.86
89	49	K+1	Pre-chill	Untreated	Back/Neck	86000	4.93	1454	3.16
90	50	K+1	Pre-chill	Untreated	Back/Neck	3200	3.51	1514	3.18
91	51	K+1	Pre-chill	Untreated	Back/Neck	14000	4.15	32500	4.51
92	52	K+1	Pre-chill	Untreated	Back/Neck	40000	4.60	10250	4.01
93	53	K+1	Pre-chill	Untreated	Back/Neck	40000	4.60	3000	3.48
94	54	K+1	Pre-chill	Untreated	Back/Neck	15000	4.18	15000	4.18
95	55	K+1	Pre-chill	Untreated	Back/Neck	53000	4.72	6250	3.80
96	56	K+1	Pre-chill	Untreated	Back/Neck	49000	4.69	6750	3.83
97	57	K+1	Pre-chill	Untreated	Back/Neck	128000	5.11	4500	3.65
98	58	K+1	Pre-chill	Untreated	Back/Neck	41000	4.61	2250	3.35
99	59	K+1	Pre-chill	Untreated	Back/Neck	74500	4.87	6250	3.80
100	60	K+1	Pre-chill	Untreated	Back/Neck	10000	4.00	2250	3.35
Average							4.49		3.65
S.D.							0.50		0.44
C.I.							0.24		0.21
N							20		20
n<1									0

Table 7f Microbial counts on untreated breast skin samples at Day K+1 after treatment with 8% lactic acid (samples taken post-chill) in Trial LA8

Sample No.	Bird No.	Day	Location	Treatment	Sample	Aerobic Plate Count per g	log(APC)	Campylobacter count per g	log(Campy)
101	41	K+1	Post-chill	8% hand	Breast	880	2.94	29	1.46
102	42	K+1	Post-chill	8% hand	Breast	325	2.51	3	0.48
103	43	K+1	Post-chill	8% hand	Breast	4170	3.62	5	0.70
104	44	K+1	Post-chill	8% hand	Breast			4	0.60
105	45	K+1	Post-chill	8% hand	Breast	780	2.89	<1	-0.15
106	46	K+1	Post-chill	8% hand	Breast	920	2.96	<1	-0.15
107	47	K+1	Post-chill	8% hand	Breast	940	2.97	4	0.60
108	48	K+1	Post-chill	8% hand	Breast	630	2.80	2	0.30
109	49	K+1	Post-chill	8% hand	Breast	350	2.54	1	0.00
110	50	K+1	Post-chill	8% hand	Breast	760	2.88	49	1.69
111	51	K+1	Post-chill	8% hand	Breast	540	2.73	2	0.30
112	52	K+1	Post-chill	8% hand	Breast	345	2.54	29	1.46
113	53	K+1	Post-chill	8% hand	Breast	9000	3.95	2	0.30
114	54	K+1	Post-chill	8% hand	Breast	1410	3.15	2	0.30
115	55	K+1	Post-chill	8% hand	Breast	1780	3.25	6	0.78
116	56	K+1	Post-chill	8% hand	Breast	1580	3.20	5	0.70
117	57	K+1	Post-chill	8% hand	Breast	680	2.83	1	0.00
118	58	K+1	Post-chill	8% hand	Breast	680	2.83	<1	-0.15
119	59	K+1	Post-chill	8% hand	Breast	1160	3.06	<1	-0.15
120	60	K+1	Post-chill	8% hand	Breast	25	1.40	<1	-0.15
Average							2.90		0.45
S.D.							0.51		0.57
C.I.							0.25		0.27
N							19		20
n<1									5

Table 7g Microbial counts on untreated breast skin samples at Day K+7 (samples taken post-chill) in Trial LA8

Sample No.	Bird No.	Day	Location	Treatment	Sample	Aerobic Plate Count per g	log(APC)	Campylobacter count per g	log(Campy)
1	1	K+7	Post-chill	Untreated	Breast	3850000	6.59	257	2.41
2	2	K+7	Post-chill	Untreated	Breast	3200000	6.51	109	2.04
3	3	K+7	Post-chill	Untreated	Breast	17500000	7.24	49	1.69
4	4	K+7	Post-chill	Untreated	Breast	1200000	6.08	85	1.93
5	5	K+7	Post-chill	Untreated	Breast	2300000	6.36		
6	6	K+7	Post-chill	Untreated	Breast	700000	5.85	193	2.29
7	7	K+7	Post-chill	Untreated	Breast	410000000	8.61	734	2.87
8	8	K+7	Post-chill	Untreated	Breast			158	2.20
9	9	K+7	Post-chill	Untreated	Breast	9900000	7.00	190	2.28
10	10	K+7	Post-chill	Untreated	Breast	12800000	7.11	198	2.30
11	11	K+7	Post-chill	Untreated	Breast	14500000	7.16	96	1.98
12	12	K+7	Post-chill	Untreated	Breast	450000	5.65	161	2.21
13	13	K+7	Post-chill	Untreated	Breast	7700000	6.89	98	1.99
14	14	K+7	Post-chill	Untreated	Breast	8350000	6.92	27	1.43
15	15	K+7	Post-chill	Untreated	Breast	36600000	7.56	24	1.38
16	16	K+7	Post-chill	Untreated	Breast	37600000	7.58	377	2.58
17	17	K+7	Post-chill	Untreated	Breast	2150000	6.33	74	1.87
18	18	K+7	Post-chill	Untreated	Breast	145000000	8.16	185	2.27
19	19	K+7	Post-chill	Untreated	Breast	100000000	8.00	38	1.58
20	20	K+7	Post-chill	Untreated	Breast	7400000	6.87	259	2.41
Average							6.97		2.09
S.D.							0.78		0.39
C.I.							0.38		0.19
N							19		19
n<1									0

Table 7h Microbial counts on breast skin samples at Day K+7 after treatment with 4% lactic acid (samples taken post-chill) in Trial LA8

Sample No.	Bird No.	Day	Location	Treatment	Sample	Aerobic Plate Count per g	log(APC)	Campylobacter count per g	log(Campy)
41	21	K+7	Post-chill	4% tunnel	Breast	14600000	7.16	19	1.28
42	22	K+7	Post-chill	4% tunnel	Breast	2200000	6.34		
43	23	K+7	Post-chill	4% tunnel	Breast	2700000	6.43	48	1.68
44	24	K+7	Post-chill	4% tunnel	Breast	2250000	6.35	4350	3.64
45	25	K+7	Post-chill	4% tunnel	Breast	37600000	7.58	61	1.79
46	26	K+7	Post-chill	4% tunnel	Breast	45000	4.65	85	1.93
47	27	K+7	Post-chill	4% tunnel	Breast	1250000	6.10	27	1.43
48	28	K+7	Post-chill	4% tunnel	Breast	127000	5.10	62	1.79
49	29	K+7	Post-chill	4% tunnel	Breast	2900000	6.46	40	1.60
50	30	K+7	Post-chill	4% tunnel	Breast	6700000	6.83	37	1.57
51	31	K+7	Post-chill	4% tunnel	Breast	500000	5.70	49	1.69
52	32	K+7	Post-chill	4% tunnel	Breast	19100000	7.28	68	1.83
53	33	K+7	Post-chill	4% tunnel	Breast	3650000	6.56	9	0.95
54	34	K+7	Post-chill	4% tunnel	Breast	3150000	6.50	37	1.57
55	35	K+7	Post-chill	4% tunnel	Breast	1350000	6.13	6	0.78
56	36	K+7	Post-chill	4% tunnel	Breast	1050000	6.02	29	1.46
57	37	K+7	Post-chill	4% tunnel	Breast	5400000	6.73	15	1.18
58	38	K+7	Post-chill	4% tunnel	Breast	14400000	7.16	1350	3.13
59	39	K+7	Post-chill	4% tunnel	Breast	55000000	7.74	59	1.77
60	40	K+7	Post-chill	4% tunnel	Breast	105000000	8.02	31	1.49
Average							6.54		1.71
S.D.							0.83		0.67
C.I.							0.39		0.32
N							20		19
n<1									0

Table 7i Microbial counts on breast skin samples at Day K+7 after treatment with 8% lactic acid (samples taken post-chill) in Trial LA8

Sample No.	Bird No.	Day	Location	Treatment	Sample	Aerobic Plate Count per g	log(APC)	Campylobacter count per g	log(Campy)
101	41	K+7	Post-chill	8% hand	Breast	4250000	6.63	4	0.60
102	42	K+7	Post-chill	8% hand	Breast	1100000	6.04	1	0.00
103	43	K+7	Post-chill	8% hand	Breast	160000	5.20	<1	-0.15
104	44	K+7	Post-chill	8% hand	Breast	160000	5.20	<1	-0.15
105	45	K+7	Post-chill	8% hand	Breast	13600000	7.13	1	0.00
106	46	K+7	Post-chill	8% hand	Breast	3350000	6.53	<1	-0.15
107	47	K+7	Post-chill	8% hand	Breast	1350000	6.13	<1	-0.15
108	48	K+7	Post-chill	8% hand	Breast	400000	5.60	<1	-0.15
109	49	K+7	Post-chill	8% hand	Breast	8750000	6.94	<1	-0.15
110	50	K+7	Post-chill	8% hand	Breast	650000	5.81	<1	-0.15
111	51	K+7	Post-chill	8% hand	Breast	273000	5.44	<1	-0.15
112	52	K+7	Post-chill	8% hand	Breast	2350000	6.37	<1	-0.15
113	53	K+7	Post-chill	8% hand	Breast	650000	5.81	<1	-0.15
114	54	K+7	Post-chill	8% hand	Breast	600000	5.78	2	0.30
115	55	K+7	Post-chill	8% hand	Breast	3850000	6.59	1	0.00
116	56	K+7	Post-chill	8% hand	Breast	79000	4.90	6	0.78
117	57	K+7	Post-chill	8% hand	Breast	600000	5.78	<1	-0.15
118	58	K+7	Post-chill	8% hand	Breast			63	1.80
119	59	K+7	Post-chill	8% hand	Breast	130000	5.11	20	1.30
120	60	K+7	Post-chill	8% hand	Breast	8000000	6.90	4	0.60
Average							5.99		0.19
S.D.							0.67		0.56
C.I.							0.32		0.26
N							19		20
n<1									11

Table 8 Summary of microbial reductions and conditions used in the eight trials to examine the efficacy of lactic acid solutions in reducing numbers of *Campylobacter*

Trial No.	Day	Control log(APC)	APC reduction	Control log(Campy)	Campy reduction	pH	% lactic acid	% lactate	Method	Flow g/min	Flow g/kg carcass	Time, s	Funder
1	1	4.6	0.8	****	****	3.7	4.0	8.3	Hand	310	100	29	RA
1	7	7.6	1.7	****	****	3.7	4.0	8.3	Hand	310	100	29	RA
2	2	4.7	0.5	NF	NF	3.7	1.9	4.0	Electro, Cold	122	7.7	6	Ind
2	8	6.1	0.2	NF	NF	3.7	1.9	4.0	Electro, Cold	122	7.7	6	Ind
3	0	4.1	0.3	2.7	2.7	3.9	1.9	4.0	Electro,Slow	122	20	17+hold	Ind
3	6	5.5	1.3	2.4	1.0	3.9	1.9	4.0	Electro,Slow	122	20	17+hold	Ind
4	1	3.0	0.2	NF	NF	4.0	1.9	4.0	Tunnel	10300	38	7	Ind
4	7	5.0	-0.1	NF	NF	4.0	1.9	4.0	Tunnel	10300	38	7	Ind
5	1	4.7	0.3	2.1	-0.2	3.9	1.9	4.0	Tunnel	10400	26	7	FSA
5	7	6.8	0.1	1.0	0.1	3.9	1.9	4.0	Tunnel	10400	26	7	FSA
6	1	3.8	0.4	1.4	-0.1	3.9	1.9	4.0	Tunnel	10400	29	7	RA
6	7	5.6	0.3	1	0.3	3.9	1.9	4.0	Tunnel	10400	29	7	RA
7	1	5.3	0.3	1.3	-0.1	4.0	4.0	8.3	Tunnel	29700	104	7	FSA
7	7	6.7	1.9	0.7	0.1	4.0	4.0	8.3	Tunnel	29700	104	7	FSA
7	1	5.3	0.5	1.3	0.1	4.0	4.0	8.3	Electro,Quick	184	29	21	FSA
7	7	6.7	1.5	0.7	0.2	4.0	4.0	8.3	Electro,Quick	184	29	21	FSA
7	1	5.3	-0.1	1.3	-0.5	4.0	4.0	8.3	Electro,Quick	184	29	5	FSA
7	7	6.7	0.9	0.7	0	4.0	4.0	8.3	Electro,Quick	184	29	5	FSA
7	1	5.3	0.1	1.3	0.2	4.0	4.0	8.3	Electro,Slow	184	29	21+hold	FSA
7	7	6.7	1.1	0.7	0.1	4.0	4.0	8.3	Electro,Slow	184	29	21+hold	FSA
7	1	3.8	0.6	0.8	0.2	4.0	4.0	8.3	Tunnel	29700	104	7	FSA
7	7	4.7	1.2	0.7	0.1	4.0	4.0	8.3	Tunnel	29700	104	7	FSA
7	1	3.8	0.7	0.8	0.1	4.0	4.0	8.3	Electro,Quick	184	29	21	FSA
7	7	4.7	0.4	0.7	0.1	4.0	4.0	8.3	Electro,Quick	184	29	21	FSA
7	1	3.8	0.4	0.8	0.2	4.0	4.0	8.3	Electro,Quick	184	29	5	FSA
7	7	4.7	0.6	0.7	0.1	4.0	4.0	8.3	Electro,Quick	184	29	5	FSA
7	1	3.8	1.4	0.8	0.2	4.0	4.0	8.3	Electro,Slow	184	29	21+hold	FSA
7	7	4.7	0.1	0.7	0.1	4.0	4.0	8.3	Electro,Slow	184	29	21+hold	FSA
8	1	4.5	0.2	2.4	0.4	3.9	4.0	8.3	Tunnel	12500	43	7	FSA
8	7	7.0	0.4	2.1	0.4	3.9	4.0	8.3	Tunnel	12500	43	7	FSA
8	1	4.5	0.6	2.4	1.9	3.9	8.0	16.6	Hand	790	124	21	FSA
8	7	8.0	1.0	2.1	1.9	3.9	8.0	16.6	Hand	790	124	21	FSA
8	1	4.8	0.2	3	0.7	3.9	4.0	8.3	Tunnel (Back/neck)	12500	43	7	FSA

Table 9 Industry data on the effect of a 10 s treatment with ozonated water (4 ppm ozone) on the TVC and *E. coli* counts on turkey carcasses

Samp Date	Sample		Pre-ozone TVC	log(pre-ozone TVC)	Post-ozone TVC (10s)	log(post-zzone TVC)	Pre_ozone E.coli	log(pre-ozone Ecoli)	Post-ozone E.coli (10s)	log(post-ozone Ecoli)
			cfu/swab						cfu/swab	
28/09	1a	PRE-OZONE	630	2.80			10	1.00		
28/09	1/10	10 SEC OZONE			440	2.64			0	0.85
28/09	2a	PRE-OZONE	1100	3.04			110	2.04		
28/09	2/10	10 SEC OZONE			460	2.66			10	1.00
28/09	3a	PRE-OZONE	960	2.98			60	1.78		
28/09	3/10	10 SEC OZONE			1400	3.15			0	0.85
28/09	4a	PRE-OZONE	1800	3.26			30	1.48		
28/09	4/10	10 SEC OZONE			2400	3.38			10	1.00
28/09	5a	PRE-OZONE	12000	4.08			40	1.60		
28/09	5/10	10 SEC OZONE			3500	3.54			0	0.85
28/09	6a	PRE-OZONE	330	2.52			10	1.00		
28/09	6/10	10 SEC OZONE			400	2.60			0	0.85
28/09	7a	PRE-OZONE	620	2.79			0	0.85		
28/09	7/10	10 SEC OZONE			280	2.45			0	0.85
28/09	8a	PRE-OZONE	910	2.96			30	1.48		
28/09	8/10	10 SEC OZONE			400	2.60			0	0.85
28/09	9a	PRE-OZONE	800	2.90			60	1.78		
28/09	9/10	10 SEC OZONE			550	2.74			0	0.85
28/09	10a	PRE-OZONE	2100	3.32			110	2.04		
28/09	10/10	10 SEC OZONE			390	2.59			0	0.85
Mean				3.07		2.84		1.50		0.88
S.D.				0.42		0.38		0.43		0.06
C.I.				0.30		0.27		0.31		0.05
N				10		10		10		10

Table 10 Industry data on the effect of a 20 s treatment with ozonated water (4 ppm ozone) on the TVC and *E. coli* counts on turkey carcass

Sample Date	Sample	Carcass	Pre-Ozone TVC	log(pre-ozone TVC)	Post-ozone TVC (10s)	log(post-zzone TVC)	Pre_ozone E.coli	log(pre-ozone Ecoli)	Post-ozone E.coli (10s)	log(post-ozone Ecoli)
			cfu/swab						cfu/swab	
28/09	1b	PRE-OZONE	670	2.83			0	0.85		
28/09	1/20	20 SEC OZONE			640	2.81			0	0.85
28/09	2b	PRE-OZONE	440	2.64			30	1.48		
28/09	2/20	20 SEC OZONE			400	2.60			10	1.00
28/09	3b	PRE-OZONE	1000	3.00			10	1.00		
28/09	3/20	20 SEC OZONE			260	2.41			10	1.00
28/09	4b	PRE-OZONE	1000	3.00			0	0.85		
28/09	4/20	20 SEC OZONE			70	1.85			0	0.85
28/09	5b	PRE-OZONE	990	3.00			30	1.48		
28/09	5/20	20 SEC OZONE			230	2.36			20	1.30
28/09	6b	PRE-OZONE	480	2.68			10	1.00		
28/09	6/20	20 SEC OZONE			410	2.61			10	1.00
28/09	7b	PRE-OZONE	1000	3.00			280	2.45		
28/09	7/20	20 SEC OZONE			360	2.56			0	0.85
28/09	8b	PRE-OZONE	1000	3.00			0	0.85		
28/09	8/20	20 SEC OZONE			300	2.48			0	0.85
28/09	9b	PRE-OZONE	850	2.93			0	0.85		
28/09	9/20	20 SEC OZONE			300	2.48			0	0.85
28/09	10b	PRE-OZONE	460	2.66			0	0.85		
28/09	10/20	20 SEC OZONE			340	2.53			0	0.85
Mean				2.87		2.47		1.16		0.94
S.D.				0.16		0.25		0.52		0.15
C.I.				0.11		0.18		0.37		0.10
N				10		10		10		10

Table 11 Industry data on the effect of a 5 s treatment with ozonated water (4 ppm ozone) on the TVC and *E. coli* counts and the presence/absence of *Campylobacter* on turkey carcass

Sample Date	Sample		Pre-ozone TVC	log(pre-ozone TVC)	Post-ozone TVC (10s)	log(post-zzone TVC)	Pre_ozone E.coli	log(pre-ozone Ecoli)	Post-ozone E.coli (10s)	log(post-ozone Ecoli)	Campy-pre	Campy-post
			cfu/swab				cfu/swab				Abs/Pres	Abs/Pres
28/10	1a/5	PRE-OZONE	0				600	2.78			0	
28/10	1/5	5 SEC OZONE			130	2.11			0			0
28/10	2a/5	PRE-OZONE	1100	3.04			50	1.70			0	
28/10	2/5	5 SEC OZONE			160	2.20			30	1.48		0
28/10	3a/5	PRE-OZONE	220	2.34			10	1.00			0	
28/10	3/5	5 SEC OZONE			20	1.30			0			0
28/10	4a/5	PRE-OZONE	250	2.40			80	1.90			0	
28/10	4/5	5 SEC OZONE			30	1.48			0			0
28/10	5a/5	PRE-OZONE	280	2.45			70	1.85			0	
28/10	5/5	5 SEC OZONE			240	2.38			0			0
28/10	6a/5	PRE-OZONE	30	1.48			20	1.30			0	
28/10	6/5	5 SEC OZONE			130	2.11			0			0
28/10	7a/5	PRE-OZONE	340	2.53			20	1.30			0	
28/10	7/5	5 SEC OZONE			190	2.28			0			0
28/10	8a/5	PRE-OZONE	700	2.85			0				0	
28/10	8/5	5 SEC OZONE			120	2.08			0			0
28/10	9a/5	PRE-OZONE	280	2.45			30	1.48			0	
28/10	9/5	5 SEC OZONE			150	2.18			10	1.00		0
28/10	10a/5	PRE-OZONE	240	2.38			80	1.90			0	
28/10	10/5	5 SEC OZONE			70	1.85			10	1.00		0
28/10	11a/5	PRE-OZONE	210	2.32			110	2.04			0	
28/10	11/5	5 SEC OZONE			20	1.30			0			0
28/10	12a/5	PRE-OZONE	160	2.20			0				0	
28/10	12/5	5 SEC OZONE			190	2.28			0			0
28/10	13a/5	PRE-OZONE	170	2.23			20	1.30			0	
28/10	13/5	5 SEC OZONE			150	2.18			0			0
28/10	14a/5	PRE-OZONE	190	2.28			0				0	
28/10	14/5	5 SEC OZONE			160	2.20			0			0
28/10	15a/5	PRE-OZONE	290	2.46			30	1.48			0	
28/10	15/5	5 SEC OZONE			30	1.48			0			0
28/10	16a/5	PRE-OZONE	950	2.98			60	1.78			0	
28/10	16/5	5 SEC OZONE			50	1.70			0			0
28/10	17a/5	PRE-OZONE	140	2.15			0				0	
28/10	17/5	5 SEC OZONE			160	2.20			0			0
28/10	18a/5	PRE-OZONE	580	2.76			10	1.00			0	
28/10	18/5	5 SEC OZONE			170	2.23			0			0
28/10	19a/5	PRE-OZONE	130	2.11			0				0	
28/10	19/5	5 SEC OZONE			50	1.70			0			0
28/10	20a/5	PRE-OZONE	170	2.23			20	1.30			0	
28/10	20/5	5 SEC OZONE			440	2.64			0			0
28/10	21a/5	PRE-OZONE	290	2.46			0				0	
28/10	21/5	5 SEC OZONE			30	1.48			0			0
28/10	22a/5	PRE-OZONE	2000	3.30			30	1.48			0	
28/10	22/5	5 SEC OZONE			130	2.11			0			0
28/10	23a/5	PRE-OZONE	450	2.65			10	1.00			0	
28/10	23/5	5 SEC OZONE			100	2.00			0			0
28/10	24a/5	PRE-OZONE	13000	4.11			0				0	
28/10	24/5	5 SEC OZONE			30	1.48			0			0
28/10	25a/5	PRE-OZONE	450	2.65			0				0	
28/10	25/5	5 SEC OZONE			110	2.04			0			0
28/10	26a/5	PRE-OZONE	2900	3.46			10	1.00			0	
28/10	26/5	5 SEC OZONE			0				0			1
28/10	27a/5	PRE-OZONE	1400	3.15			10	1.00			0	
28/10	27/5	5 SEC OZONE			0				0			0
28/10	28a/5	PRE-OZONE	50	1.70			50	1.70			0	
28/10	28/5	5 SEC OZONE			160	2.20			0			0
28/10	29a/5	PRE-OZONE	360	2.56			10	1.00			0	
28/10	29/5	5 SEC OZONE			450	2.65			10	1.00		0
28/10	30a/5	PRE-OZONE	780	2.89			70	1.85			0	
28/10	30/5	5 SEC OZONE			300	2.48			0			0
28/10	31a/5	PRE-OZONE	2200	3.34			20	1.30			0	
28/10	31/5	5 SEC OZONE			120	2.08			0			0
28/10	32a/5	PRE-OZONE	6300	3.80			110	2.04			0	
28/10	32/5	5 SEC OZONE			30	1.48			0			0
28/10	33a/5	PRE-OZONE	4300	3.63			20	1.30			0	
28/10	33/5	5 SEC OZONE			720	2.86			0			0
28/10	34a/5	PRE-OZONE	1100	3.04			0				0	
28/10	34/5	5 SEC OZONE			30	1.48			0			0
28/10	35a/5	PRE-OZONE	330	2.52			50	1.70			0	
28/10	35/5	5 SEC OZONE			120	2.08			0			0
28/10	36a/5	PRE-OZONE	180	2.26			40	1.60			1	
28/10	36/5	5 SEC OZONE			60	1.78			0			0
28/10	37a/5	PRE-OZONE	2800	3.45			230	2.36			0	
28/10	37/5	5 SEC OZONE			110	2.04			0			0
28/10	38a/5	PRE-OZONE	130	2.11			40	1.60			0	
28/10	38/5	5 SEC OZONE			20	1.30			0			0
28/10	39a/5	PRE-OZONE	13000	4.11			760	2.88			1	
28/10	39/5	5 SEC OZONE			110	2.04			0			1
28/10	40a/5	PRE-OZONE	0				3400	3.53			0	
28/10	40/5	5 SEC OZONE			140	2.15			30	1.48		0
28/10	41a/5	PRE-OZONE	27000	4.43			3200	3.51			1	
28/10	41/5	5 SEC OZONE			30	1.48			20	1.30		0
28/10	42a/5	PRE-OZONE	130	2.11			20	1.30			0	
28/10	42/5	5 SEC OZONE			130	2.11			0			0
28/10	43a/5	PRE-OZONE	4400	3.64			400	2.60			1	
28/10	43/5	5 SEC OZONE			290	2.46			30	1.48		0
28/10	44a/5	PRE-OZONE	220	2.34			10	1.00			0	
28/10	44/5	5 SEC OZONE			140	2.15			0			0
28/10	45a/5	PRE-OZONE	660	2.82			20	1.30			0	
28/10	45/5	5 SEC OZONE			120	2.08			0			0
28/10	46a/5	PRE-OZONE	90	1.95			20	1.30			0	
28/10	46/5	5 SEC OZONE			240	2.38			20	1.30		0
28/10	47a/5	PRE-OZONE	0				900	2.95			0	
28/10	47/5	5 SEC OZONE			0				560	2.75		0
28/10	48a/5	PRE-OZONE	2000	3.30			260	2.41			0	
28/10	48/5	5 SEC OZONE			430	2.63			0			0
28/10	49a/5	PRE-OZONE	0				10	1.00			0	
28/10	49/5	5 SEC OZONE			0				0			0
28/10	50a/5	PRE-OZONE	220	2.34			0				0	
28/10	50/5	5 SEC OZONE			150	2.18			10	1.00		0
Mean				2.73		2.02		1.72		1.38		
S.D.				0.65		0.39		0.69		0.53		
C.I.				0.19		0.12		0.22		0.38		
N				46		46		40		10		

Table 12 Aerobic plate counts and confirmed *Campylobacter* counts on control (untreated) carcasses on Days 1 and 7 after kill in Trial OZ1

		Day 1				Day 7			
	Treatment	Aerobic Plate Count per g	log(APC)	Count of confirmed Campylobacter per g	log(Confirm)	Aerobic Plate Count per g	log(APC)	Count of confirmed Campylobacter per g	log(Confirm)
1	Control	710,000	5.85	24000	4.38	2200000	6.34	<10	
2	Control	3,500,000	6.54	2000	3.30	10000000	7.00	<10	
3	Control	400,000	5.60	1600	3.20	3800000	6.58	<10	
4	Control	140,000	5.15	36	1.56	4800000	6.68	<10	
5	Control	380,000	5.58	<10	0.85	25000000	7.40	<10	
6	Control	400,000	5.60	<10	0.85	1500000	6.18	<10	
7	Control	6,300,000	6.80	<10	0.85	26000000	7.41	<10	
8	Control	780,000	5.89	1400	3.15	20000000	7.30	<10	
9	Control	95,000	4.98	60000	4.78	27000000	7.43	<10	
10	Control	320,000	5.51	120000	5.08	25000000	7.40	<10	
11	Control	230,000	5.36	<10	0.85	26000000	7.41	<10	
12	Control	170,000	5.23	<10	0.85	79000000	7.90	<10	
13	Control	500,000	5.70	<10	0.85	8900000	6.95	<10	
14	Control	410,000	5.61	10000	4.00	19000000	7.28	<10	
15	Control	120,000	5.08	2600	3.41	11000000	7.04	<10	
16	Control	130,000	5.11	66	1.82	1600000	6.20	<10	
17	Control	250,000	5.40	3400	3.53	650000	5.81	<10	
18	Control	130,000	5.11	90	1.95	6500000	6.81	<10	
19	Control	100,000	5.00	<10	0.85	1800000	6.26	<10	
20	Control	200,000	5.30	<10	0.85	19000000	7.28	<10	
Mean			5.52		2.35		6.93		
S.D.			0.48		1.52		0.55		
C.I.			0.23		0.71		0.26		

Table 13 Aerobic plate counts and confirmed *Campylobacter* counts on carcasses treated with ozonated water and microbiologically tested on Days 1 and 7 after kill in Trial OZ1

		Day 1				Day 7			
	Treatment	Aerobic Plate Count per g	log(APC)	Count of confirmed Campylobacter per g	log(Campy)	Aerobic Plate Count per g	log(APC)	Count of confirmed Campylobacter per g	log(Confirm)
26	Ozonated	440,000	5.64	<10	0.85	21000000	7.32	<10	
27	Ozonated	150,000	5.18	<10	0.85	1800000	6.26	<10	
28	Ozonated	170,000	5.23	<10	0.85	35000000	7.54	<10	
29	Ozonated	110,000	5.04	<10	0.85	3700000	6.57	<10	
30	Ozonated	51,000	4.71	<10	0.85	19000000	7.28	<10	
31	Ozonated	140,000	5.15	<10	0.85	460000	5.66	<10	
32	Ozonated	24,000	4.38	<10	0.85	2200000	6.34	<10	
33	Ozonated	340,000	5.53	<10	0.85	800000	5.90	<10	
34	Ozonated	740,000	5.87	<10	0.85	260000	5.41	<10	
35	Ozonated	390,000	5.59	21000	4.32	270000	5.43	<10	
36	Ozonated	43,000	4.63	44	1.64	8300000	6.92	<10	
37	Ozonated	160,000	5.20	22000	4.34	3500000	6.54	<10	
38	Ozonated	150,000	5.18	30	1.48	310000	5.49	<10	
39	Ozonated	410,000	5.61	12000	4.08	5100000	6.71	<10	
40	Ozonated	220,000	5.34	100	2.00	3200000	6.51	<10	
41	Ozonated	430,000	5.63	18000	4.26	3100000	6.49	<10	
42	Ozonated	210,000	5.32	1500	3.18	23000000	7.36	<10	
43	Ozonated	150,000	5.18	<10	0.85	10000000	7.00	<10	
44	Ozonated	230,000	5.36	800	2.90	6300000	6.80	<10	
45	Ozonated	45,000	4.65	14000	4.15	1500000	6.18	<10	
Mean			5.22		2.04		6.49		
S.D.			0.39		1.46		0.66		
C.I.			0.18		0.69		0.31		

Table 14 Aerobic plate counts and confirmed *Campylobacter* counts on carcasses treated with plain tap water and microbiologically tested on Days 1 and 7 after kill in trial OZ1

		Day 1				Day 7			
	Treatment	Aerobic Plate Count per g	log(APC)	Count of confirmed Campylobacter per g	log(Campy)	Aerobic Plate Count per g	log(APC)	Count of confirmed Campylobacter per g	log(Confirm)
51	Plain	110,000	5.04	440	2.64	280000	5.45	<10	0.85
52	Plain	52,000	4.72	<10	0.85	5800000	6.76	<10	0.85
53	Plain	130,000	5.11	2300	3.36	2900000	6.46	<10	0.85
54	Plain	210,000	5.32	<10	0.85	270000	5.43	<10	0.85
55	Plain	74,000	4.87	<10	0.85	2500000	6.40	<10	0.85
56	Plain	280,000	5.45	10	1.00	31000000	7.49	<10	0.85
57	Plain	120,000	5.08	56	1.75	2400000	6.38	<10	0.85
58	Plain	24,000	4.38	12	1.08	980000	5.99	<10	0.85
59	Plain	350,000	5.54	<10	0.85	1700000	6.23	<10	0.85
60	Plain	26,000	4.41	1400	3.15	4500000	6.65	<10	0.85
61	Plain	850,000	5.93	840	2.92	4700000	6.67	<10	0.85
62	Plain	360,000	5.56	1400	3.15	8200000	6.91	<10	0.85
63	Plain	450,000	5.65	16000	4.20	490000	5.69	<10	0.85
64	Plain	1,400,000	6.15	60000	4.78	27000000	7.43	<10	0.85
65	Plain	460,000	5.66	<10	0.85	410000	5.61	<10	0.85
66	Plain	360,000	5.56	<10	0.85	260000	5.41	<10	0.85
67	Plain	810,000	5.91	<10	0.85	300000	5.48	<10	0.85
68	Plain	39,000	4.59	78	1.89	4900000	6.69	<10	0.85
69	Plain	290,000	5.46	3400	3.53	17000000	7.23	<10	0.85
70	Plain	210,000	5.32	148	2.17	2000000	6.30	<10	0.85
Mean			5.29		2.08		6.33		
S.D.			0.50		1.29		0.67		
C.I.			0.24		0.61		0.31		

Table 15 Temperatures and weights of the untreated control carcasses in Trial OZ2

OZONATED WATER - Dec 2011 (CONTROL BIRDS)				
Weight of 10 tags, g =			15.3	
Bird No.	**Purpose**	**Bird temp before spray, C**	**Weight with tag, g**	**Weight without tag, g**
1	Micro	32.0	1796	1794
2	Micro		2024	2022
3	Micro		1427	1425
4	Micro		1546	1544
5	Micro		1820	1818
6	Micro		1692	1690
7	Micro		1816	1814
8	Micro		2250	2248
9	Micro		1670	1668
10	Micro		1316	1314
11	Micro		1548	1546
12	Micro		1943	1941
13	Micro		1425	1423
14	Micro		1105	1103
15	Micro		1202	1200
16	Micro		1871	1869
17	Micro		2061	2059
18	Micro	33.3	1502	1500
19	Micro		2152	2150
20	Micro		1759	1757
21	Micro		1728	1726
22	Micro		1776	1774
23	Micro		1504	1502
24	Micro		1443	1441
25	Micro		1644	1642
26	Micro		1833	1831
27	Micro		1717	1715
28	Micro		1623	1621
29	Micro		2129	2127
30	Micro		1820	1818
31	Micro		1371	1369
32	Micro		1729	1727
33	Micro		1979	1977
34	Micro		1920	1918
35	Micro		1442	1440
36	MIcro	31.3	1730	1728
S1	Photo*		2081	
S2	Spare		1269	
Mean				1702
S.D.				267

* Photograph beside treated bird on Days 0, 3, and7

Table 16 Temperatures and weights of the treated carcasses and properties of the ozonated water in Trial OZ2

OZONATED WATER - Dec 2011 (Treated birds)											
Weight of 10 tag, gs =					15.3						
Weight of liquid collected over 30 seconds at start, g					2502	2564	2595				
Av flowrate		2553.667		sd flowrate		47.35328					
Bird No.	Purpose	Bird temp before spray, C	Temp of liquid applied, C	pH of liquid applied	ORP of liquid applied, mV	Free chlorine, ppm	Total chlorine, ppm	Weight with tag before spray, g	Bird temp after spray, C	Room temp, C	Ozone concentration
37	Micro	36.9	5.6	7.35	850	1.07	1.32	1890		5.7	3.8
38	Micro							1747			
39	Micro							1357			
40	Micro							1350			
41	Micro							1314			
42	Micro							1420			
43	Micro							1934			
44	Micro							1664			
45	Micro							1970			
46	Micro							1726			
47	Micro							1854			
48	Micro							1449			
49	Micro							1628			
50	Micro							1475			
51	Micro							1488			
52	Micro							1404			
53	Micro							1536			
54	Micro	35.4	5.8	7.3	688	0.55	0.64	1244		5.5	
55	Micro							1768			
56	Micro							2030			
57	Micro							1469			
58	Micro							1741			
59	Micro							1631			
60	Micro							1303			
61	Micro							1700			
62	Micro							1892			
63	Micro							1703			
64	Micro							1925			
65	Micro							1828			
66	Micro							1530			
67	Micro							1708			
68	Micro							1535			
69	Micro							1703			
70	Micro							1990			
71	Micro							1830			
72	Micro	32.7	5.6	7.25	551	0.42	0.49	1809		5.3	
S3	Photo*										
S4	Reserve										
Mean								1654			
S.D.								219			

* Photograph beside control bird on Days 0, 3, and7

Table 17a Microbial counts on untreated chicken breast skin when tested at Day K+1 in Trial CP1

Sample No.	Bird No.	Side	Treatment	Temperature, °C	Flux, mW/cm²	Aerobic Plate Count per g	log(APC)	Campylobacter count per g	log(Campy)
21	1	Left	Untreated			3540000	6.55	105	2.02
22	2	Right	Untreated			7100000	6.85	290	2.46
23	3	Left	Untreated			2680000	6.43	585	2.77
24	4	Right	Untreated			6820000	6.83	85	1.93
25	5	Left	Untreated			4160000	6.62	140	2.15
26	6	Right	Untreated			6240000	6.80	295	2.47
27	7	Left	Untreated			3800000	6.58	270	2.43
28	8	Right	Untreated			4180000	6.62	85	1.93
29	9	Left	Untreated			4020000	6.60	225	2.35
30	10	Right	Untreated			10240000	7.01	325	2.51
31	11	Left	Untreated			3990000	6.60	95	1.98
32	12	Right	Untreated			4660000	6.67	130	2.11
33	13	Left	Untreated			3920000	6.59	170	2.23
34	14	Right	Untreated			6200000	6.79	150	2.18
35	15	Left	Untreated			4940000	6.69	335	2.53
36	16	Right	Untreated			6200000	6.79	160	2.20
37	17	Left	Untreated			5880000	6.77	240	2.38
38	18	Right	Untreated			6320000	6.80	525	2.72
39	19	Left	Untreated			4290000	6.63	130	2.11
40	20	Right	Untreated			6340000	6.80	115	2.06
Average							6.70		2.28
S.D.							0.14		0.25
C.I.							0.06		0.12
N							20		20
n<1									0

Table 17b Microbial counts on chicken breast skin treated with cold plasma and then tested at Day K+1 in Trial CP1

Sample No.	Bird No.	Side	Treatment	Temperature, °C	Flux, mW/cm²	Aerobic Plate Count per g	log(APC)	Campylobacter count per g	log(Campy)
41	21	Right	Plasma			4640000	6.67	90	1.95
42	21	Left	Plasma			3800000	6.58	105	2.02
43	22	Left	Plasma			4220000	6.63	115	2.06
44	22	Right	Plasma			8020000	6.90	275	2.44
45	23	Right	Plasma			6720000	6.83	180	2.26
46	23	Left	Plasma			11280000	7.05	350	2.54
47	24	Left	Plasma			7000000	6.85	400	2.60
48	24	Right	Plasma			6790000	6.83	820	2.91
49	25	Right	Plasma			5540000	6.74	635	2.80
50	25	Left	Plasma			4320000	6.64	865	2.94
51	26	Left	Plasma			4800000	6.68	70	1.85
52	26	Right	Plasma			4220000	6.63	120	2.08
53	27	Right	Plasma			3980000	6.60	170	2.23
54	27	Left	Plasma			4420000	6.65	150	2.18
55	28	Left	Plasma			4620000	6.66	5	0.70
56	28	Right	Plasma			5190000	6.72	35	1.54
57	29	Right	Plasma			5220000	6.72	6450	3.81
58	29	Left	Plasma			5900000	6.77	965	2.98
59	30	Left	Plasma			4440000	6.65	20	1.30
60	30	Right	Plasma			3880000	6.59	25	1.40
Average							6.72		2.23
S.D.							0.12		0.70
C.I.							0.06		0.33
N							20		20
n<1									0

APPENDIX 1 Design of the lactic acid spray tunnel

Figure A1 Sketch of nozzle layout shown as side view of the tunnel

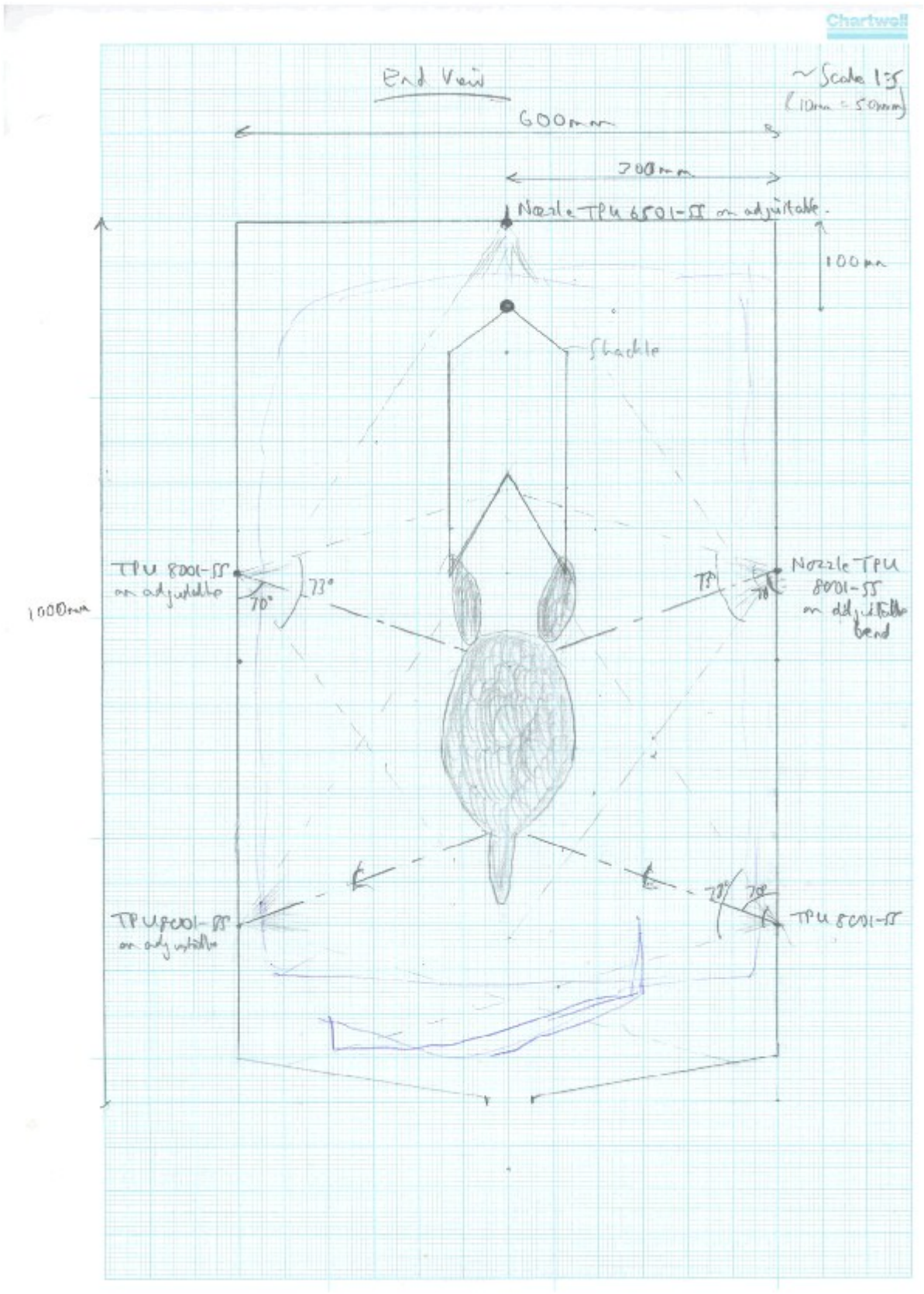

Figure A2 Sketch of nozzle layout for nozzles located in the top of the tunnel

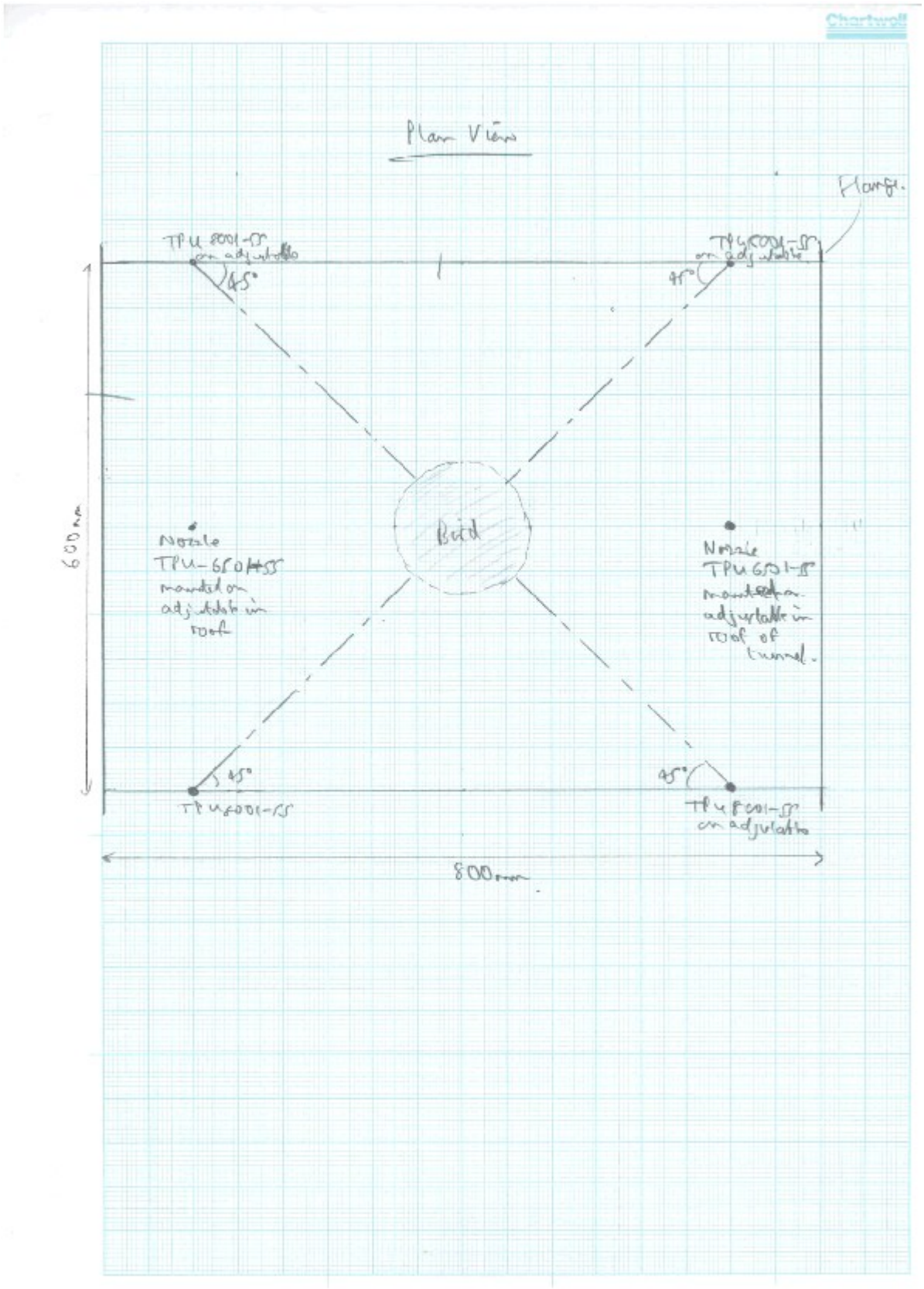

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Figure A3 CAD drawing showing perspective view of the tunnel

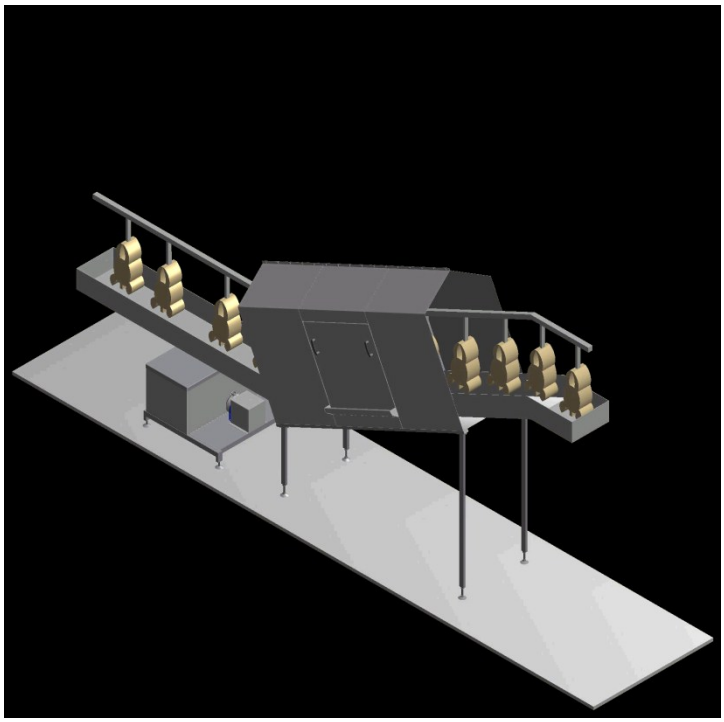


Figure A4 CAD drawing showing side view of the tunnel

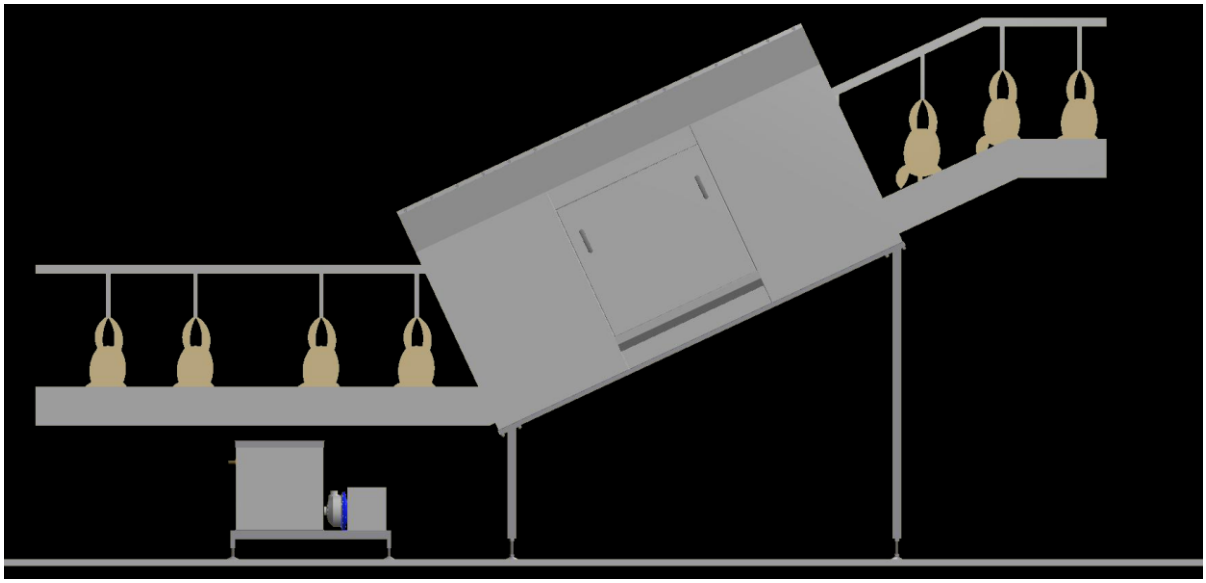


Figure A5 CAD drawing showing key features of the tunnel

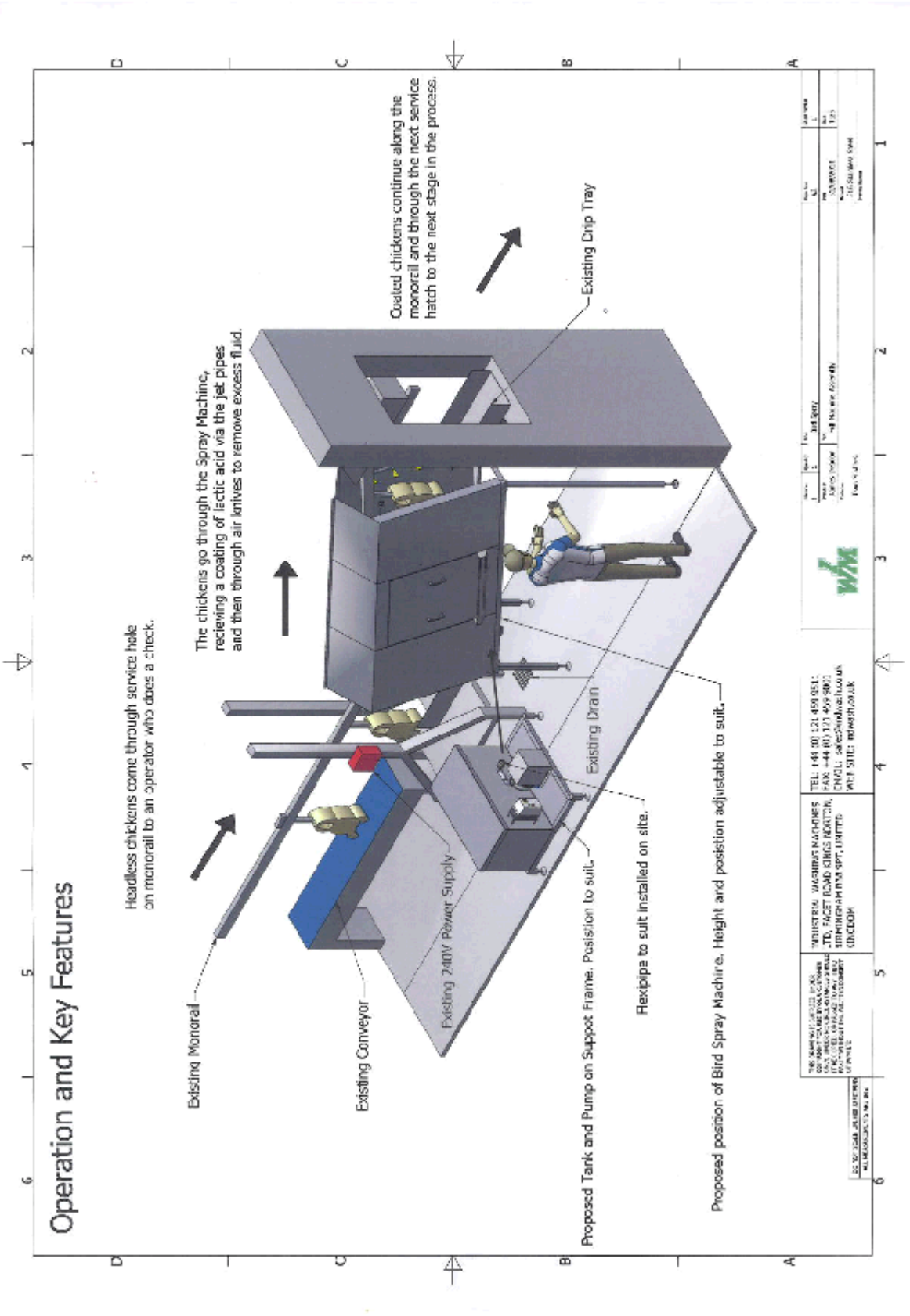

Operation and Key Features
Headless chickens come through service hole on monorail to an operator who does a check.
The chickens go through the Spray Machine, recieving a coating of lactic acid via the jet pipes and then through air knives to remove excess fluid.
Coated chickens continue along the monorail and through the next service hatch to the next stage in the process.
Existing Chip Tray
Existing Monorail
Existing Conveyor
Existing 240V Power Supply
Existing Drain
Proposed Tank and Pump on Support Frame. Position to suit.
Flexipipe to suit installed on site.
Proposed position of Bird Spray Machine. Height and position adjustable to suit.

Figure A6 CAD drawing showing dimensions of the tunnel

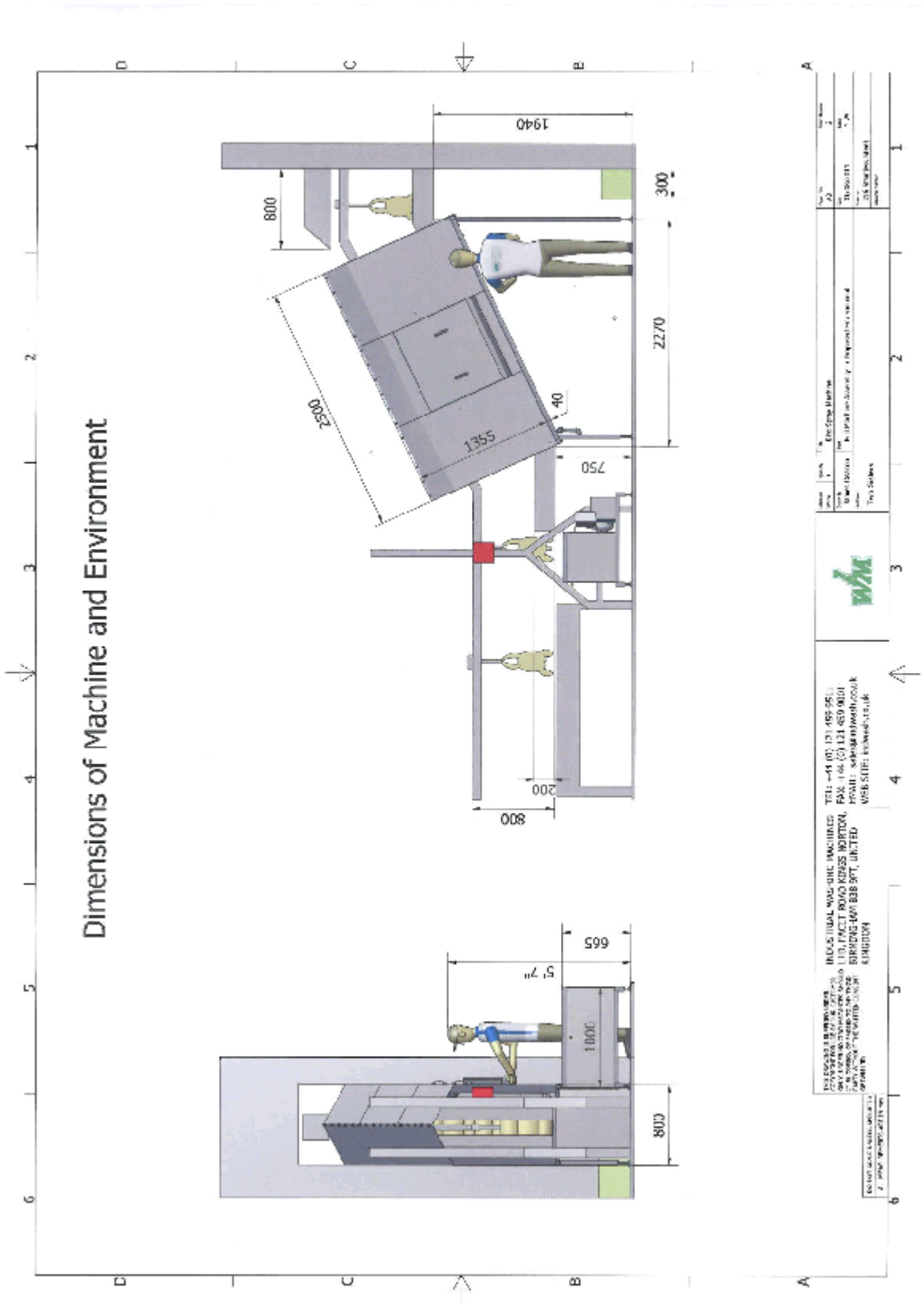


Figure A7 Photograph of side view of tunnel showing access panel

Figure A8 Photograph of side view of tunnel

Figure A9 View inside tunnel showing spray bars on one side of the tunnel (a similar arrangement exists on the other side)

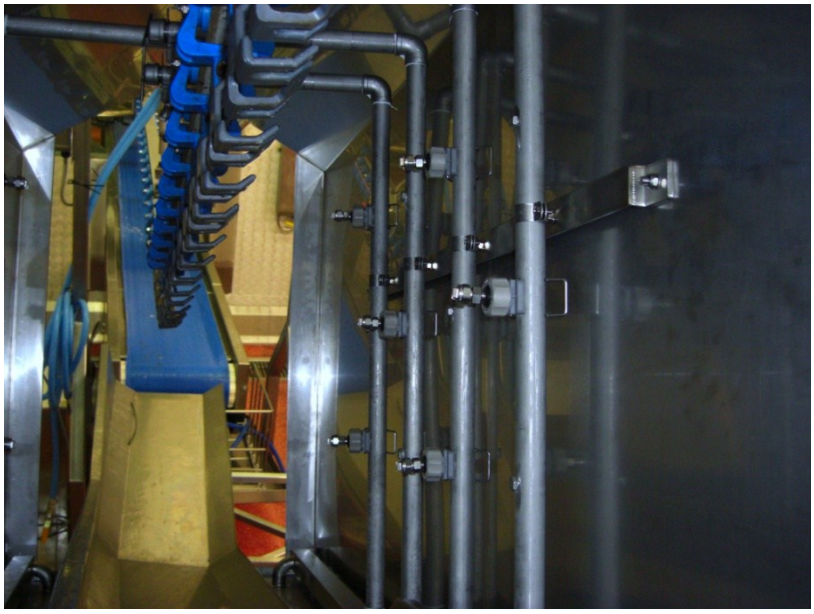


Figure A10 Spray nozzles located in the top of the tunnel for spraying liquid onto the top surface and into the body cavity of each chicken

Figure A11 Air knives (yellow) used to restrict the movement of aerosols out of the end of the tunnel

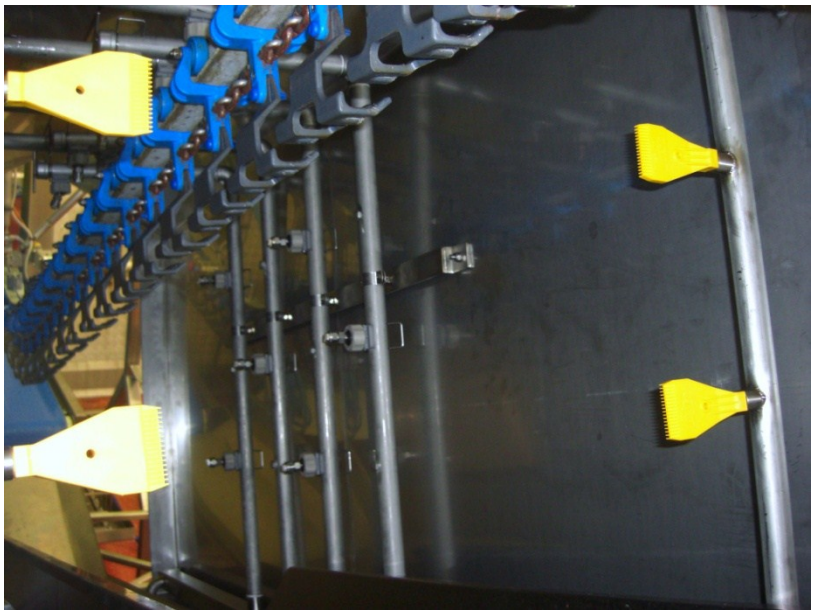
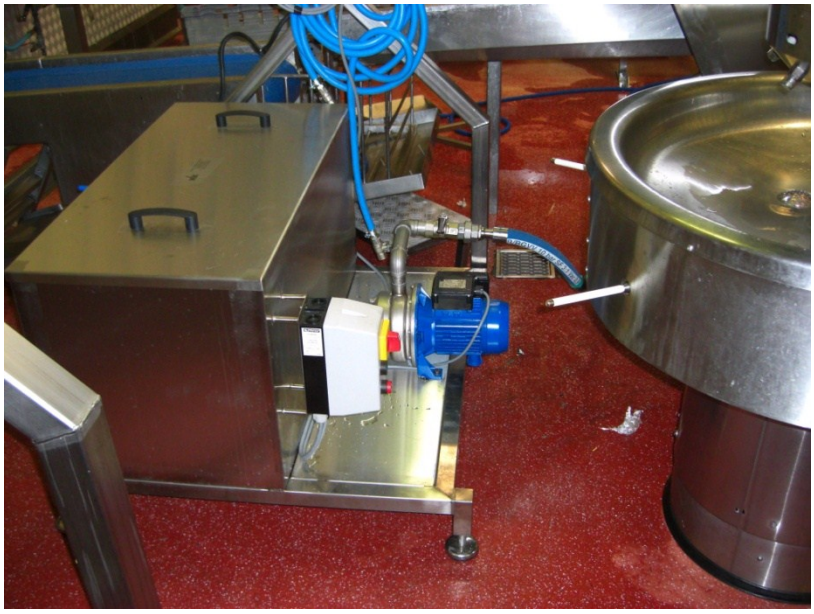


Figure A12 The tank and pump delivering liquid to the spray bars within the tunnel



CALCULATION OF FLOW RATES FROM NOZZLES

Nozzle type TPU 8001-SS when operated at 2 bar delivers a flow rate of 0.32 l/min and spray angle of 73^0 (based on information from Spraying Systems Limited and reproduced in the Table A1 and Figure A1).

Nozzle type TPU 6501-SS when operated at 2 bar delivers a flow rate of 0.32 l/min and spray angle of 67^0 (based on information from Spraying Systems Limited and reproduced in the Table A1 and Figure A1).

Ten nozzles when operating at a flow rate of 0.32 l/min provide a total flow rate of 3.2 l/min (=192000g/h).

Line speed = 10000 birds /hour = 15000 kg of bird per hour (assuming 1.5kg bird).

Therefore, the application rate of chemical = 192000/15000 = 12.8 g of acid per kg of bird.

The use of 20 nozzles would deliver 25.6 g acid/kg bird which is just over the target of 22 g acid/kg bird.

Acknowledgements

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