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Validation of Twin Oxide as a carcase intervention

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EXECUTIVE SUMMARY

Increasing pressure is on meat processors to investigate antimicrobial decontamination treatments for carcases, in order to comply with United States (US) requirements for minimising the risk of *E. coli* O157:H7 being present on carcases. A number of potential treatments are available overseas, and are emerging in Australia.

Midfield Meats (MMI) and CRF Colac have been investigating the potential for using chlorine dioxide, marketed in Australia under the brand name Twin Oxide®. This report details an in-plant validation study using bobby calf carcases inoculated with a mixed culture of *E. coli* organisms, non-toxic surrogates for *E. coli* O157.

The inoculum was applied to the carcase surfaces to achieve a level of at least 6 $\log_{10}$ *E. coli* cfu/cm². Excision samples were taken from the rump, flank and brisket of each carcase prior to spraying with a 50ppm solution of chlorine dioxide; immediately after spraying; and following overnight chilling. After chilling, samples were also taken for analysis of potential chlorine residues on the carcases.

Prior to spraying, a mean *E. coli* count of 6.71 (range 5.74-7.27) $\log_{10}$ cfu/cm² was achieved. Immediately after spraying, there seemed to be a slight, but not statistically significant, reduction in counts (mean 6.52, range 5.18-7.51 $\log_{10}$ cfu/cm²), while chilling did not cause further reductions (mean 6.57, range 5.60-7.46 $\log_{10}$ cfu/cm²).

No chlorine residues were detected, and there were no observed effects on carcase colour or odour.

However, as a result of the limited antimicrobial efficacy demonstrated, the outcomes of this study do not support the use of 50ppm chlorine dioxide as an effective intervention procedure.

1. INTRODUCTION

In Australia many meat producers use a carcase wash down prior to fat trimming and chilling. This is either done manually or automatically, primarily to remove visible contamination from the carcase. A small, but increasing number of processors also use a hot water rinse to reduce the bacterial load on the carcase prior to chilling. Unfortunately even though the water is recycled and the temperature maintained above 80 degrees there is a substantial risk that many strains of bacteria will survive this process and indeed the wash unit may actually contaminate the meat if there is a breakdown or problem with the unit. In addition, the availability of water in many areas of Australia sometimes precludes processors from using a second, pasteurising step.

Furthermore, processors supplying the United States (US) market are coming under increasing pressure to use carcase decontamination interventions in order to limit the incidence of *E. coli* O157:H7 on carcases. An FSIS (US Food Safety and Inspection Service) document '*E. coli* O157:H7 contamination of beef products', and accompanying guidance documents, state that beef slaughter establishments should consider interventions that can be validated and verified as Critical Control Points (CCPs) for reducing or eliminating *E. coli* O157:H7 (FDA, 2003).

The ongoing issue of *E. coli* O157:H7 carcase contamination within the bobby calf production industry is becoming increasingly concerning as it is now understood the US is in the process of altering its protocol on the management of *E. coli* O157:H7 and specifically the interpretation of this protocol.

This is of significant concern for the Australian bobby calf production industry as the US is the predominant Australian bobby veal market. MMI and CRF collectively process approximately 500,000 animals annually being supplied solely to the US. In 2009 veal producers in general, and CRF in particular, recorded a large number of confirmed positives for *E. coli* O157:H7 on veal carcases leading to reluctance to export veal to the US.

The Australian red meat industry has considered several options for carcass decontamination over recent times e.g. steam, ozone. In the case of steam the effectiveness is well understood however the occupational health and safety concerns and potential damage to the product introduced problems. The use of ozone on the other hand, would seem to be a suitable alternative. It is widely regarded as an effective bactericide and to have a clean image. However it can be difficult to monitor the levels of ozone applied and due to the rapid breakdown of the active component the system requires costly infrastructure to ensure even and effective coverage of the carcass.

Chlorine dioxide (Twin Oxide ®) is being considered due to its reported effectiveness (log 2-3 reduction in *E. coli*), attributed to its powerful oxidative effect, its relatively cheap price, its existing use in the US, and the ability to monitor the concentrations applied and verify that no residue is present.

2. PROJECT AIM

The aim of the investigation is to generate in-plant information on the ability of Twin Oxide (0.3% chlorine dioxide liquid) to reduce the concentration of *E. coli* O157:H7 on

veal carcases; the investigation will provide a validation of the effectiveness of Twin Oxide.

3. METHODS

3.1 Bacterial inoculum

A bacterial culture was prepared from a cocktail of five strains of *E. coli* (EC1604, EC1605, EC1606, EC1607, EC1608) that are non-pathogenic strains that have previously been used by CSIRO in validation trials as surrogate organisms for *E. coli* O157.

Broth cultures of each of the five strains were grown overnight at 37°C in tryptose soya broth (TSB). Equal volumes (1 mL) of the cultures were added to 300 mL of sterile TSB to give an inoculum predicted to contain around 10 million cfu per mL (the inoculum concentration was confirmed by the Petrifilm® *E. coli* method detailed below, giving an actual count of 8.32 $\log_{10}$ cfu/mL .

The broth culture was transferred to a sterile plastic screw-top container. In turn this container was placed within a secure insulated container for transfer to the location on the slaughter floor where the test sides were located.

3.2 Preparation, sampling and treatment of test carcasses

Twenty bobby calf carcases were used for the trial.

Three sites were used for the evaluation –butt, flank, and brisket or as close to the ESAM sites as was accessible. For each of the sites on each test side, an area measuring approximately 20 cm x 10 cm was painted with the bacterial inoculum. Protective gloves were used and care taken to avoid runoff of the suspension to areas outside the test sites.

The test carcasses were held for approximately 20 minutes in the detained chiller at CRF to allow attachment of the bacteria to the painted surfaces and to simulate the interval between the first opportunity for contamination of meat surfaces and sides reaching the carcase wash unit.

An excision sample measuring 10 cm² of surface area, approximately 1mm thick was taken from each of the inoculated rump, flank and brisket.

The test carcasses were sprayed with chlorine dioxide solution (Twin Oxide®) using a hand-held electric sprayer (Hills Garden Products) with a 3 Litre reservoir. The applied concentration was 50ppm, measured using Chlorine Dioxide Test Strips (Waterworks, Rock Hill SC, USA - part code 480031), to leave an estimated residual concentration of 10ppm on the carcase surface.

Immediately after treatment, the three sites were again sampled for each of the 20 carcasses, with care being taken to sample locations adjacent to that sampled prior to treatment.

The test carcasses were located together in a chiller, separate to non-treated carcasses.

After the test carcasses were chilled overnight, the three sites were again sampled for each of the twenty carcasses, at sites adjacent to the previous two excision samples.

One untreated carcase and two treated carcases were sampled for analysis for the presence of residues. From each of these carcases, an area 10cm x10cm and depth 5mm, was excised from the surface of the carcase, using a sterile knife. The samples were placed in individual, labelled, sealed plastic bags and frozen. Samples were sent overnight to the testing laboratory (Levay & Co. Environmental Services, Ian Wark Research institute, University of South Australia) and tested for chlorite and chlorate using ion chromatography (Method *IC-2*) assay.

Test carcases were destroyed following the trial, and did not proceed to processing for human consumption, in case of residual *E. coli* presence.

3.3 Microbiological testing

The samples were tested for *E. coli* using *standard* Petrifilm® methods. Briefly, 50mL of Maximum Recovery Diluent (MRD, Oxoid) was added to each sample, and this was stomached for 1 minute. A decimal dilution series was prepared in MRD, and 1 mL aliquots of appropriate dilutions were put onto Petrifilm in duplicate and incubated at 37°C for 48 h. Counts were expressed as $\log_{10}$ units. Mean counts and standard deviations for each sample site at each time point were calculated.

3.4 Chemical analysis

Chemical analysis was carried out by Levay & Co, South Australia. The modified ion chromatography method involved sample preparation, which included water extraction of the meat sample, followed by clean-up of the water extract to remove any material such as proteins which may have interfered with the analysis. As part of the quality control, measurements of the standards (spiked blank recovery) to validate the chromatographic method and measurements of recovery of each analyte from a spiked sample (spiked recovery) were included. The spiked samples were taken through the full method, including sample storage, extraction and chromatographic analysis.

As yet, there is no standard method (Australian nor international) for testing chlorite and chlorate in meat tissue. Using the modified ion chromatography procedure in this study, the spiked meat samples (Table 2) showed recoveries of 0% for chlorite and 99% for chlorate. Chlorite is a particularly unstable compound, and rapidly decomposes, thus, it is not unexpected that it could not be recovered.

Chlorous acid and chlorine dioxide were not included in the analyses as these compounds readily oxidise with air and are difficult to detect even immediately after the reaction. Considering that the meat tissue was excised 24 hours after treatment, and the samples were then transported frozen to the laboratory, it is even more unlikely to detect chlorine dioxide or chlorous acid residues.

4. RESULTS

4.1 Antimicrobial Efficacy

Application of the inoculum to the carcases resulted in a mean count of 6.71 log_{10} cfu/cm² (Table 1), range 5.74 to 7.27. Counts on brisket were marginally higher than counts on rump. Following application of Twin Oxide, a slight, but not statistically significant, decrease in counts was seen, to an overall mean of 6.52 (range 5.18 to 7.51) log_{10} cfu/cm², but the counts on brisket seemed to rise (mean count before treatment 6.97 log_{10} cfu/cm²; mean count after treatment 7.08 log_{10} cfu/cm²). Chilling did not induce a further decline.

Table 1: Mean (± standard deviation) *E. coli* counts (log_{10} cfu/cm²) on bobby calf carcases at successive points before and after spraying with Twin Oxide, and after chilling.

	Before treatment	***After treatment***	***After chilling***
Rumps	6.26 ± 0.25	5.85 ± 0.51	6.14 ± 0.36
Flanks	6.89 ± 0.35	6.64 ± 0.55	6.58 ± 0.37
Briskets	6.97 ± 0.37	7.08 ± 0.28	7.01 ± 0.31
All samples	6.71 ± 0.45	6.52 ± 0.68	6.57 ± 0.49

4.2 Chemical residue analysis

A total of three meat tissue samples were sent for chemical analysis for chlorite and chlorate, one from an untreated carcase (designated sample A) and two from treated carcases (designated B and C). Chlorite and chlorate concentrations were below the level of detection using the modified ion chromatography procedure for all meat tissue samples tested (Table 2).

Table 2: Analysis of chlorite and chlorate in meat trimmings by ion chromatography (Method IC-2).

Sample	***Chlorite (mg/kg wet wt)***	***Chlorate (mg/kg wet wt)***
A (untreated)	< 2.0	< 5.0
B (treated)	< 2.0	< 5.0
C (treated)	< 2.0	< 5.0
Detection Limit	2.0	5.0
Blank[a]	<0.01 mg/L	<0.02 mg/L
Spiked blank recovery[b]	100%	97%
Spiked recovery[c]	0%	99%

a Blank – water only
b Known quantity of the analyte (chlorite or chlorate) added to the blank (0.1 mg/L)
c Known quantity of the analyte (chlorite or chlorate) added to the meat tissue sample (10 mg/Kg)

5. DISCUSSION

Antimicrobial treatments for carcases have been extensively researched, and now range from simple water washes and stream pasteurisation through a number of organic and inorganic chemical solutions (Castillo *et al*, 2002). Chlorine dioxide at a concentration of 200ppm has been investigated alone (Stivarius et al 2002) and in conjunction with trisodium phosphate (Pohlman et al 2002a) or cetylpyridinium chloride (Pohlman et al 2002b). Stivarius et al reported a statistically significant reduction in *E. coli* (0.71 $\log_{10}$ cfu/g) and *Salmonella* Typhimurium (0.61 $\log_{10}$ cfu/g) counts on beef trimmings using 200ppm Chlorine dioxide alone, while Pohlman et al reported a similar reduction in *E. coli* (0.61 $\log_{10}$ cfu/g) on beef trimmings when used in combination with 10% trisodium phosphate, and a 1.13 $\log_{10}$ cfu/g reduction when used in combination with 0.5% cetylpyridinium chloride. These reported reductions, although statistically significant in the studies performed, are likely in practice not to be significant under commercial conditions. Previous criteria used when assessing potential interventions under commercial conditions have been that reductions in microbial counts of 1-2 $\log_{10}$ cfu/cm² or more would be appropriate (MLA project PRMS.083, 2006), because the natural variation in counts on carcases can be in the order of 0.5-1 $\log_{10}$ cfu/cm².

In the current investigation, the antimicrobial efficacy of 50ppm TwinOxide® sprayed onto a total of 20 bobby calf carcases was tested. No significant reduction in inoculated *E. coli* levels was demonstrated. However, when all data was combined, a slight reduction was observed immediately after spraying.

Residual chlorine compounds were not detected in the tissue samples analysed. Chlorine dioxide may be used as a processing aid in meat production, providing that residual chlorite, chlorate, chlorous acid and chlorine dioxide cannot be quantified in the final product (Food Standards Code, Standard 1.3.3, Clause 14; FSANZ, 2006). The “natural” level of chlorite and chlorate in beef tissue should be zero as these analytes are not a nutrient for cattle and there are no natural sources to which the animals are likely to be exposed, and this was backed up by the results from the untreated sample (A).

6. CONCLUSION

As a result of the limited antimicrobial efficacy demonstrated, the outcomes of this study do not support the use of 50ppm chlorine dioxide as an effective intervention procedure.

REFERENCES

Castillo, A., Hardin, M. D., Acuff, G. R., Dickson, J. S. (2002) Reduction of microbial contaminants on carcasses. In: Control of Foodborne Microorganisms (Eds) V. K. Juneja & J. N. Sofos, (pp 351-381) New York: Marcel Dekker AG.

FDA (2003) Code of Federal Regulations Title 21, Government Printing Office, USA

FSANZ (2006) Australia New Zealand Food Standards Code, consolidated version including amendment 85. http://www.foodstandards.gov.au/foodstandardscode/ Accessed 8th March 2006.

MLA Project PRMS.083 Review of new and emerging technologies for red meat safety (June 2006).

Pohlman, F.W., Stivarius, M.R., McElyea, K.S., Johnson, Z.B., Johnson, M.G. (2002a) The effects of ozone, chlorine dioxide, cetylpyridinium chloride and trisodium phosphate as multiple antimicrobial interventions on microbiological, instrumental color, and sensory color and odor characteristics of ground beef. *Meat Science* 61: 307-313

Pohlman, F.W., Stivarius, M.R., McElyea, K.S., Johnson, Z.B., Johnson, M.G. (2002b) Reduction of microorganisms in ground beef using multiple intervention technology. *Meat Science* 61: 315-322

Stivarius, M.R., Pohlman, F.W., McElyea, K.S., Apple, J.K. (2002) Microbial, instrumental color, and sensory color and odor characteristics of ground beef produced from beef trimmings treated with ozone or chlorine dioxide. *Meat Science* 60: 299-305