An Evaluation of Decontamination of Chicken Carcasses with Acidified Sodium Chlorite

Sexton, M.¹, Holds, G.², Kiermeier, A.², Sumner, J.³

¹ Primary Industries & Resources SA; ² South Australian Research & Development Institute; ³ M&S Food Consultants.

Abstract

A trial was carried out on chicken carcasses in a commercial processing plant to determine the effectiveness of using acidified sodium chlorite (SANOVÀ®) as a dipping treatment, on the prevalence and concentration of Salmonella, Campylobacter, E. coli and Total Viable Count (TVC). A flock was selected which was known to be positive for Campylobacter and Salmonella. The reductions in counts per cm² of carcass after a 20 second dip in acidified sodium chlorite (SANOVÀ®) were as follows: TVC was reduced by log 1.55, E. coli prevalence dropped from 100% to 13% and concentration by log 2.19, Salmonella prevalence was reduced from 90% to 10% with a log 0.05 reduction in concentration and Campylobacter prevalence reduced from 100% to 23% with a log 3.8 reduction in concentration. All of these parameters except Salmonella concentration (which was initially very low) showed statistical significance. These results indicate substantial reductions in both prevalence and concentrations of important foodborne pathogens. The degree of reduction, if achieved commercially, is likely to substantially reduce consumer exposure while providing potential product quality benefits. On this basis a commercial-scale trial is warranted to validate the treatment effect and further examine the shelf-life benefits and cost.

Introduction

As a result of a heightened incidence of Campylobacter- and Salmonella-associated illness in South Australia, the Meat Hygiene Unit (MHU) in the Department of Primary Industries and Resources (PIRSA) has undertaken considerable work with regard to pathogens in the poultry industry.

In 2002, PIRSA commissioned Dr John Sumner to provide an independent risk profile of potential food safety risks from the primary industry sector in South Australia (Sumner, 2002). This report identified Salmonella and Campylobacter as high risks in cooked and further processed poultry.

In November 2002, the MHU carried out a “Microbiological Profile of Poultry processed in SA” (Sumner et al 2004), which demonstrated that 56% of poultry carcasses were positive for Salmonella. There were also indications that there was a lack of technical knowledge in processing controls to minimise carcass contamination. As a result, the MHU commissioned Dr Sumner to develop a “Gold Standard” for processing controls and then survey the plants in SA to assess them against this standard. This led to the development of a national Advisory Interpretive Guideline for the Poultry Processing Standard. Further papers initiated by the MHU which have been published include ‘Which food categories cause salmonellosis in Australia?’(Sumner et al, 2003) and ‘Have changes to meat and poultry food safety regulation in Australia affected the prevalence of Salmonella or salmonellosis?’ (Sumner et al, 2004). These papers identified that poultry was a major cause of salmonellosis and that HACCP and QA, as currently applied during processing, had minimal impact on reducing the risk of foodborne illness.

An issues paper commissioned by the MHU identified other countries which had addressed the problems of Salmonella and Campylobacter in poultry (Sexton and Sumner 2004). This paper identified the strategies that have been carried out in the farming sectors (mainly in Europe) and those carried out in the processing plants (mainly USA).
One of the treatments which appeared promising and has been used with some success in America to reduce the incidence of *Salmonella* on carcases (required to be below 21% by the USDA) was SANOVA®, produced by Ecolab. This is an acidified sodium chlorite solution (using citric acid) which has just been approved for use in Australia. A Steering Committee comprising of the major poultry producers in SA elected to conduct a trial of the product to determine the feasibility and efficacy of its use on Australian produced carcases.

PIRSA assisted in the pilot trial to ensure that it would provide a rigorous assessment of the efficacy of the product with a view to following up with a commercial trial if the results were promising and once equipment had been manufactured and installed.

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**Objective**

To determine the effect of a commercially prepared solution of acidified sodium chlorite (SANOVA®) used as a post screw chill dip on poultry carcases on:

- prevalence and concentration of *Salmonella, Campylobacter*, Total Viable Count (TVC) and *E. coli*;
- visual appearance;
- organoleptic qualities of the carcases; and
- shelf-life of the carcases.

**Materials and Methods**

**Evaluation on chicken carcases**

Approximately 200 grams of fresh caecal and faecal droppings were collected from the floor litter of the sheds into sterile containers at 28 to 33 days of age. These samples were tested for the presence of *Salmonella* and *Campylobacter*. A shed was selected for this trial on the basis that it was positive for *Salmonella* and *Campylobacter*.

At 49 days of age the birds were caught, transported to the processing plant and slaughtered under normal commercial procedures, the flock being the last processed on the day. As the birds moved through the screw chiller the free available chlorine levels of the water were tested using a Lovibond Comparator and recorded.

As the carcases exited the screw chiller and were conveyed to the rehang area, carcases which were beside one another were paired. Operators wearing clean gloves picked up one of the birds by the wing, drained the cavity and placed it in a clean labelled plastic bag (control), the other bird was picked up by the wing, drained and placed in a clean plastic crate. The sampling continued until 6 birds had been collected in each group.

The crated birds were lowered into a 600 litre solution of acidified sodium chlorite (SANOVA®) which had been prepared with a concentration of 900-1000 ppm of sodium chlorite and a pH of 2.5 to 2.6. The crate was gently agitated to ensure complete immersion and then removed from the solution after 20 seconds. Each bird was lifted by the wing, drained and placed in a clean, labelled plastic bag using freshly gloved hands. The procedure was repeated until 30 birds for the treatment and 30 untreated birds had been collected. The acidified sodium chlorite (SANOVA®) solution was tested for base concentration and pH before, during and after the treatment. Each of the bagged carcases, including the controls, were weighed and 450mL of sterile buffered peptone water (1%) was poured into each bag, which was then closed and shaken for 2 minutes. Each carcase was then removed from the bag in a sterile manner allowing the rinse to drain. The bag was then resecured.
and placed in a chilled esky. Samples were transported to the laboratory within 2 hours. The deep muscle temperature of each carcase was recorded after removal from the bag using a temperature probe.

**Microbiological testing**

The rinses were tested as per AS 1766.3.2(1990) except that the rinse volume was 450mL (commercially available) instead of 500mL. The tests carried out were as follows:

- **TVC** – petrifilm with $10^{-1}$ to $10^{-3}$ mL of rinse, incubation at 25°C.
- **E. coli** – petrifilm with $10^{0}$ to $10^{-1}$ mL of rinse, incubation at 37°C.
- **Salmonella** Most Probable Number (MPN) – 3x10mL of double strength (DS), 3x1.0mL of single strength (SS), 3x0.1mL (SS) Buffered Peptone Water, enrichment Rappaport–Vassiliadis Medium and selective agar media Xylose Lysine Deoxycholate. Limits of detection <3 to >1100 colony forming units (cfu) per 100mL rinse.
- **Campylobacter** direct plate –0.1mL rinse and 0.1mL of 1:10 of rinse spread on Campylobacter Blood Free Agar and incubated microaerophilically at 41.5°C. Limit of detection <10cfu per mL of rinse.
- **Campylobacter** MPN (carried out on the acidified sodium chlorite (SANova®) treated carcases) – 3x10mL(DS), 3x1.0mL(SS) and 3x0.1mL(SS) Preston Broth without blood. All additives included prior to inoculation due to difficulties with adding small volumes to MPN tubes. Limits of detection <3 to >1100 cfu per 100mL rinse.

**Evaluation of shelf life**

A further 6 carcases were sampled as 3 untreated and 3 treated carcases to indicate the effect of acidified sodium chlorite (SANova®) on shelf-life. A temperature probe attached to a data logger was inserted into the breast meat of one untreated and one treated bird. All birds were transported in plastic bags surrounded by ice in lined cartons to the laboratory and held in a cool room which was kept at 3.5°C to 4°C. The condition of the carcases was assessed organoleptically and the shelf-life subjectively determined to be the day prior to the identification of distinct “off” odours. One carcase from each group was also viewed and photographed at day 7 to determine if there were any visual differences.

The processor also treated extra carcases (some for 20 seconds dip and some at 60 seconds) which were visually assessed, cooked and taste tested 5 days later and compared with untreated carcases.

**Results**

**Process control**

The FAC (free available chlorine) in the screw chiller during the passage of carcases for the trial was 1.5 mg/kg

<table>
<thead>
<tr>
<th></th>
<th>Sodium Chlorite</th>
<th>pH</th>
</tr>
</thead>
<tbody>
<tr>
<td>Before trial</td>
<td>960 ppm</td>
<td>2.5</td>
</tr>
<tr>
<td>During trial</td>
<td>960 ppm</td>
<td>2.5-2.6</td>
</tr>
<tr>
<td>After trial</td>
<td>949 ppm</td>
<td>2.6</td>
</tr>
</tbody>
</table>

Deep muscle temperatures of the carcases after peptone water rinse ranged between 8°C and 16°C, averaging 13°C.
**Microbiological results for carcases**

Microbiological results for the 30 untreated control carcases and 30 acidified sodium chlorite (SANOMA®) carcases are presented in Table 2.

**Table 2 Prevalence and counts/cm^2 of carcase with statistical significance**

<table>
<thead>
<tr>
<th></th>
<th>Untreated Controls</th>
<th>Acidified Sodium Chlorite (SANOMA®)</th>
<th>P-value (statistical significance)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean log TVC</td>
<td>2.78 (602)</td>
<td>1.23 (17)</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td><em>E. coli</em> prevalence</td>
<td>30/30 (100%)</td>
<td>4/30 (13%)</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td><em>E. coli</em> log mean positives</td>
<td>1.55 (35)</td>
<td>-0.64 (0.23)</td>
<td>0.0003</td>
</tr>
<tr>
<td><em>Salmonella</em> prevalence</td>
<td>27/30 (90%)</td>
<td>3/30 (10%)</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td><em>Salmonella</em> log mean positives</td>
<td>-1.80 (0.016)</td>
<td>-1.85 (0.014)</td>
<td>0.8858</td>
</tr>
<tr>
<td><em>Campylobacter</em> prevalence</td>
<td>30/30 (100%)</td>
<td>7/30 (23%)</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td><em>Campylobacter</em> log mean positives</td>
<td>1.59 (39)</td>
<td>-2.21 (0.006)</td>
<td>&lt; 0.0001</td>
</tr>
</tbody>
</table>

- Arithmetic counts are in brackets
- *E. coli* and *Campylobacter* prevalence and counts are per 1mL of rinsate
- *Salmonella* prevalence and counts are per 100mL of rinsate
- Log mean of the positives for *E. coli*, *Salmonella* and *Campylobacter* is calculated by adding up the log counts on positive samples and dividing by the number of positive samples
- Counts/cm^2 of carcase surface are calculated according to the formula in the Microbiological Testing Guidelines for Poultry which accompany the Australian Standard for Construction of Premises and Hygienic Production of Poultry Meat for Human Consumption (A.S. 4465:2001) and are also described in A.S. 1766.3.2.6.2.

P values of less than 0.05 are significant, therefore all comparisons except the *Salmonella* log mean positives are statistically significant. The TVC (Gram positive and Gram negative organisms) reduction is 1.5 log (>90%) but the reduction in the specific Gram negative organisms is substantial. *E. coli* were reduced from 100% to 13% with mean counts falling from 35/cm^2 to 0.23/cm^2, a 2 log reduction. *Salmonella* were reduced from a prevalence of 90% to 10% however the mean concentrations of the positives were at a low level on the birds prior to treatment. They were reduced by 0.05 log (not significant). *Campylobacter* were reduced from 100% prevalence to 23% and the mean log of the positives was reduced by 3.8 log (from a level of 39/cm^2 to 0.006/cm^2). This equates to the count on a 1.5 kg carcase dropping from 75,660 to 12 cfus.

**Salmonella serotypes**

The original flock samples were serotyped for *Salmonella* and were positive for *S. Sofia* and *S. Infantis*. On the carcase rinses 3 colony picks from each dilution of the positives (up to a maximum of 9) were selected for typing. This gave the following results:

**Table 3 Salmonella serovar prevalence on control and treated carcases**

<table>
<thead>
<tr>
<th></th>
<th>Untreated Controls</th>
<th>Acidified Sodium Chlorite (SANOMA®)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>S. Sofia</em></td>
<td>16/30 (53%)</td>
<td>3/30 (10%)</td>
</tr>
<tr>
<td><em>S. Infantis</em></td>
<td>5/30 (17%)</td>
<td>0/30 (0%)</td>
</tr>
<tr>
<td><em>S. Sofia &amp; S. Infantis</em></td>
<td>6/30 (20%)</td>
<td>0/30 (0%)</td>
</tr>
<tr>
<td><strong>Total Sofia</strong></td>
<td>22/30 (73%)</td>
<td>3/30 (10%)</td>
</tr>
<tr>
<td><strong>Total Infantis</strong></td>
<td>11/30 (37%)</td>
<td>0/30 (0%)</td>
</tr>
</tbody>
</table>

Two serovars were isolated from the flock after processing: *S. Sofia* and *S. Infantis*. The former is a regular contaminant of poultry carcases in Australia but is only rarely associated with salmonellosis (22 cases in 2003). *S. Infantis*, by contrast, is invariably in the top-10 serovars causing salmonellosis (201 cases in 2003).
**Organoleptic observations**

The deep muscle temperatures of both carcases held in the cool room ranged between 3.5°C and 4°C. The control carcases had a shelf life of 12 to 13 days and the acidified sodium chlorite (SANOVA®) treated carcases had a shelf life of 14 days. The only difference in visual appearance was a darkening of the extremity of the wingtips (1 mm) on one acidified sodium chlorite (SANOVA®) treated carcase. Taste testers were unable to detect any differences between treated and control carcases, nor could they detect any visual differences before or after cooking. It was noted that the carcases appeared bleached immediately after treatment with acidified sodium chlorite (SANOVA®) but the pink colouration returned within a day and the carcases were indistinguishable from those that were untreated.

**Discussion**

A flock known to be positive for *Salmonella* and *Campylobacter* was selected for the trial and processed as the last flock through the processing plant so as to provide carcases with the maximum amount of contamination seen in normal processing. The trial dipping tank was set up where it would be placed within the normal process system if it were incorporated as a whole bird dip tank. The results of the trial confirm the widely held view that flocks positive for *Salmonella* at farm level are associated with a high prevalence of contaminated birds.

The results of treatment with acidified sodium chlorite (SANOVA®) indicate that a statistically significant reduction in pathogenic organisms can be achieved with this product. Prevalence of *Salmonella* was reduced from 90% to 10%, *Campylobacter* was reduced from 100% to 23% and *E. coli* was reduced from 100% to 13% and although the levels of *Salmonella* were low the counts of *Campylobacter* were reduced by log 3.8. As an infective dose (I.D.50) of *Campylobacter* is considered to be approximately 1000 organisms (Anon. 2001) the reduction of the total carcase count from 75,000 to 10 is likely to significantly reduce the possibility of poultry causing illness.

While the data indicate a relatively high prevalence of a serovar commonly associated with human illness (*S. Infantis*), these data cannot be extrapolated to industry. However, the elimination of this pathogenic serovar from a relatively high prevalence indicates the potential public health benefit of this treatment.

From the qualitative observations, there was a detectable bleaching of the treated product, however, the pink colouration returned to the carcases within a day and it was impossible to detect which carcases had been treated. There was also no detectable odour difference between the control and treated birds either initially or throughout the shelf-life. A wide range of people tasted the birds after they had been cooked and were unable to detect any differences in taste, smell or texture.

These results indicate the use of acidified sodium chlorite (SANOVA®) can lead to substantial reductions in both the prevalence and concentration of important foodborne pathogens. The degree of reduction, if achieved commercially, is likely to substantially reduce consumer exposure while providing potential product quality benefits. On this basis, a commercial-scale trial is warranted to validate the treatment effect and further examine the shelf-life benefits and cost.

Although this is only one step in the process of pathogen reduction it would suggest that it is worth trialling a commercial system and following product through further processing stages (such as boning, portioning and marinating) to determine if the significant reduction in pathogens can be achieved on further processed products. Rosenquist (2003) predicts (using QRA) it may be possible to produce a 30 fold reduction in the incidence of campylobacteriosis associated with consumption of chicken meals by introducing a 2 log reduction of the number of *Campylobacter* on chicken carcases.
References


Acknowledgements

Thanks to the staff of Gourmet Poultry and personnel from Ecolab and the S.A. Meat Hygiene Unit for their assistance in this study.

Appendix

Statistical Analysis

Test for difference in prevalences were performed using Fisher’s exact test, which is preferable to the Chi-squared test when sample sizes are small.

Tests for differences in mean logs were performed using the Welch two-sample test, which unlike the standard t-test, allows for different variability in the two groups.

- For E. coli only the four non-zero cells were used in the acidified sodium chlorite (SANOVÁ®) group (as logs of zero counts are not possible). Clearly, this results in a large loss of efficiency as we are ignoring 26 very valuable pieces of information. A better way is to deal with the original counts directly and fit a “generalized linear model” which can incorporate all data points. The resulting P-value was < 0.0001 – clearly the differences here are so large that it is irrelevant.

- For Campylobacter and Salmonella a value such as “<3” has been treated as “3”. This results in positively biased means, but has no practical significance – the differences are still large. For the purpose of prevalence testing these values have been treated as no-detects.