THE DETERMINATION AND CONTROL OF THE MICROBIOLOGICAL QUALITY AND SAFETY OF POULTRY CARCASSES

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ABSTRACT

THE DETERMINATION AND CONTROL OF THE MICROBIOLOGICAL QUALITY AND SAFETY OF POULTRY CARCASSES

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Professors M.W. Griffiths
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Poultry can be a vehicle for the transmission of salmonellosis and the contamination of carcasses is primarily from feces. Bactericidal treatments may be used to correct the problem. A study was conducted to evaluate the effectiveness of several bactericides (i.e., chlorine, trisodium phosphate, Avgard™ and lactic acid). Response surfaces showed that only lactic acid treatments significantly ($p<0.05$) reduced both the total aerobic bacteria and coliform count. In a pilot scale study, lactic acid was used on contaminated carcasses at a concentration of 4.25% (w/w) and pH 3.0 with a dispensing temperature of 40°C. The treatment significantly ($p<0.05$) reduced carcass contamination. To determine the long term effects of lactic acid on viability and recovery of pathogens, *Salmonella hadar* was isolated from poultry and bioluminescent constructs obtained by transformation with the lux (CDABE) cassette. Viability was determined by measuring luminescence following lactic acid treatment of turkey breast and storage at -12, 0, 5°C and 10°C. The ability of the *S. hadar* (lux^+) to recover from the treatment was determined by incubating at 22°C for 10 hrs and monitoring light output. The results showed that metabolic activity was significantly ($p<0.05$) affected by the lactic acid treatment and by storage temperatures of -12, 0 and 5°C. The lowest recovery rate was observed after treatment with lactic acid ($p<0.05$) and storage at 5°C.

The implementation of HACCP programs is proposed as a method of reducing foodborne illness. However, good statistical planning and appropriate measurement techniques are required to verify the control of microbial hazards. Key areas in a poultry processing plant were monitored for levels of hygiene by a conventional swab technique and the ATP (adenosine triphosphate) bioluminescence assay. A statistical design (i.e., randomized incomplete block) was used and the results showed that the hygiene of the process, including the effect of flocks and day of processing, could be predicted.

This thesis demonstrates that implementation of microbiologically-based systems for validation of HACCP and the introduction of effective carcass decontamination techniques will lead to improved safety and quality of poultry meat.

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Table of contents

1.0 Introduction 1

1.1 The plans to improve food safety during processing of poultry 2

1.2 Objectives of Research 3

2.0 Literature Review 5

2.1 Foodborne illnesses and poultry production 5
2.1.1 Pathogens and their impact on foodborne illnesses 7
2.1.1.1 *Salmonella* spp. in poultry products 7
2.1.1.2 *Campylobacter jejuni* in poultry products 10
2.1.1.3 Other foodborne pathogens of interest in poultry products 11

2.2 Current methods to control foodborne illness during poultry production. . . 12
2.2.1 Competitive exclusion and vaccination as a method to control pathogens on the farm 12
2.2.2 Overview of the Meat Inspection Service 13
2.2.3 Current practices of quality assurance by the poultry industry: microbiological analysis 15

2.3 Initiatives to improve the control of foodborne illness during poultry production 16
2.3.1 The Hazard Analysis Critical Control Point (HACCP) program . . 16
2.3.1.1 A Brief Description of HACCP 17
2.3.1.2 Proposed generic HACCP programs for Canadian and American Poultry Processing Plants . 22
2.3.2 The use of food-grade chemicals to improve carcass hygiene . . . 26
2.3.2.1 Chlorine 26
2.3.2.2 Trisodium phosphate and phosphate blends 30
2.3.2.3 Lactic acid 33
2.3.3 The use of temperature and pH as factors to influence the efficacy of bactericidal solutions 36

2.4 Innovative approaches for determining microbial contamination in food products 38
2.4.1 Tests for the rapid identification of pathogens 38

2.4.2 Rapid microbiological testing with the ATP bioluminescence assay 40
2.4.3 Genetically engineered bioluminescent bacteria as a model system to evaluate responses to environmental stimuli . . 43

2.5 The structure of skin and the effects of water immersion during poultry processing 48

3.0 The determination of efficacy of antimicrobial rinses on turkey carcasses using response surface designs 53

3.1 Introduction 53

3.2 Materials and Methods 55
3.2.1 Sample Preparation 55
3.2.2 Microbiological Analyses 56
3.2.3 Experimental Design for the evaluation of bactericidal efficacy . 57
3.2.4 Statistical Analysis of Data 60

3.3 Results and Discussion 61
3.3.1 Chlorine 61
3.3.2 Trisodium Phosphate 68
3.3.3 Avgard™ 71
3.3.4 Lactic Acid 74

3.4 Conclusions 79

4.0 Optimization of lactic acid solution used to treat contaminated poultry surfaces 80

4.1 Introduction 80

4.2 Materials and Methods 82
4.2.1 Microbial efficacy of buffered lactic acid 82
4.2.1.1 Sample preparation 82
4.2.1.2 Microbiological Analyses 82
4.2.1.3 Experimental design for the evaluation of bactericidal efficacy of buffered lactic acid at various temperature and pH combinations 83

4.2.1.4 Statistical Analysis of Data 83

4.3 Results and Discussion 87

4.4 Conclusions 92

5.0 In-plant application of an optimized lactic acid treatment for the improvement of hygiene on fecal contaminated turkey carcasses 93

5.1 Introduction 93

5.2 Materials and Methods 94
5.2.1 On-site laboratory setup for treatment and microbial analysis . . . 94
5.2.2 Types of carcasses used for analysis of hygiene 94
5.2.3 Sample preparation for microbial analysis 96
5.2.4 Microbial Analysis 96
5.2.5 Experimental Design and Statistical Analysis 97

5.3 Results and Discussion 97

5.4 Conclusions 100

6.0 Organoleptic analysis of turkey carcasses treated with lactic acid 102

6.1 Introduction 102

6.2 Materials and Methods 103
6.2.1 Evaluation of colour of skin tissues treated with buffered lactic acid 103
6.2.1.1 Sample preparation 103
6.2.1.2 Experimental design for the evaluation of the discolouration effect of buffered lactic acid over time 104
6.2.1.3 Statistical analysis of data 104
6.2.2 A consumer taste panel survey of turkey carcasses treated with lactic acid and regular washing methods 106
6.2.2.1 Sample preparation and cooking methods 106

6.2.2.2 Taste panel survey comparing control and treated carcasses . . . 107
6.2.2.3 Statistical analysis . . . 107

6.3 Results and Discussion . . . 108
6.3.1 Colour evaluation of turkey skins . . . 108
6.3.2 A consumer taste panel survey of the lactic acid treatment on turkey carcasses . . . 113
6.4 Conclusions . . . 114

7.0 The construction and stability testing of an auto-bioluminescent wild-type *Salmonella* spp. for the purpose of monitoring metabolic activity . . . 115

7.1 Introduction . . . 115

7.2 Materials and Methods . . . 117
7.2.1 Isolation, enrichment and identification of wildtype *Salmonella* spp. from carcasses at a poultry abattoir . . . 117
7.2.2 Transformation of the *lux* (CDABE) genes into target organisms . . . 118
7.2.2.1 Preparation of *E. coli* JM109 for classical transformation with calcium chloride ($CaCl_2$) . . . 118
7.2.2.2 Amplification of the *lux* (CDABE) genes in *E. coli* JM109 . . . 119
7.2.2.3 Electroporation of wildtype *Salmonella* spp. with the *lux* (CDABE) cassette . . . 121
7.2.3 Morphological, biochemical and serotypical characterization of *Salmonella* spp. before and after transformation . . . 121
7.2.3.1 Colonial morphology . . . 122
7.2.3.2 Cellular morphology . . . 122
7.2.3.2 Biochemical testing . . . 122
7.2.3.3 Serological testing . . . 123
7.2.4 Stability studies of bioluminescence output of the successfully transformed *Salmonella* spp. . . . 123
7.2.4.1 Correlation of light output and plate count of *lux*$^+$ transformants . . . 123
7.2.4.2 Growth determination based on conductance and light measurements . . . 124

7.2.4.3 The response of bioluminescent output to lactic acid at different levels of pH 124
7.2.4.4 Temperature stability of transformant at 40°C. 125

7.3 Results and Discussion 136

7.4 Conclusions 139

8.0 The determination of the long-term efficacy of an optimized lactic acid treatment under various storage conditions using a *Salmonella hadar* (*lux*$^+$) 140

8.1 Introduction 140

8.2 Materials and Methods 141
8.2.1 Bacterial strain used for study 141
8.2.2 Preparation of the bioluminescent *Salmonella hadar* (*lux*$^+$) for artificial inoculation onto turkey carcasses 142
8.2.3 Preparation of turkey samples for artificial inoculation 143
8.2.4 Inoculation of turkey samples with *S. hadar* (*lux*$^+$) using a sponge imprint technique 143
8.2.5 An investigation of the viability of *S. hadar* (*lux*$^+$) exposed to different treatments and storage conditions 144
8.2.6 Conditions to investigate the recovery of *S. hadar* (*lux*$^+$) 145
8.2.7 Quantification of light output using a CCD image analyzer (BIQ Bioview, Cambridge Image, Ltd.) 145
8.2.8 Statistical design 146
8.2.8.1 Analysis of data from the viability study of turkey breast samples inoculated with *S. hadar* (*lux*$^+$) 147
8.2.8.2 Analysis of data from the recovery study of turkey samples inoculated with *S. hadar* (*lux*$^+$) 149

8.3 Results and Discussion 151

8.4 Conclusions 164

9.0 Quality assessment of a poultry processing facility using ATP bioluminescence and conventional microbiological techniques . . . 165

9.1 Introduction . . . 165

9.2 Materials and Methods . . . 166
9.2.1 Sampling Protocol . . . 166
9.2.2 Assessment of the microbiological quality of processed carcasses . . . 167
9.2.2.1 Conventional microbiology . . . 167
9.2.2.2 ATP bioluminescence . . . 167
9.2.3 Layout of statistical design and analysis . . . 168

9.3 Results and Discussion . . . 171
9.3.1 The analysis of quality of carcasses at stations along the processing line by conventional microbiology and ATP bioluminescence . . . 171
9.3.2 Analysis of overall quality of poultry slaughtered on individual days by conventional methods and the ATP bioluminescence assay . . . 177
9.3.3 Analysis of quality of chicken flocks on individual days by conventional methodology and the ATP bioluminescence assay . . . 177

9.4 Conclusions . . . 183

10.0 General Discussion . . . 184

11.0 Conclusions . . . 190

12.0 References . . . 192

13.0 Appendices . . . 224

Appendix A: SOB and SOC media . . . 225

Appendix B: *Salmonella* spp. Serotyping Report . . . 226

List of Tables

Table 2.1: The prevalence of pathogens or potential pathogens in poultry products in the United States and other countries 8

Table 3.1: Levels of concentration and pressure used for each bactericide for evaluation of efficacy 58

Table 3.2: Example of an experimental setup of concentration and pressure for response surface design using lactic acid 59

Table 3.3a: Response surface models describing total aerobic count at different concentrations (x_1) and pressures (x_2) for each bactericide 64

Table 3.3b: Response surface models describing coliform count at different concentrations (x_1) and pressures (x_2) for each bactericide 65

Table 3.4: The elimination of *Salmonella* spp. from turkey carcasses by carcass washing. 66

Table 4.1: Levels of pH and temperature used to determine maximum efficacy of the buffered lactic acid/sodium lactate solution for treatment on contaminated carcasses 85

Table 4.2: An experimental setup to determine optimal conditions based on pH and dispensing temperature by a response surface design 86

Table 4.3: Response surface models describing total aerobic count and coliformcount at different temperature (x_1) and pH (x_2) for lactic acid treatments 90

Table 7.1: Summary of physical characteristics of original and transformed (*lux*$^+$) *Salmonella hadar* 125

Table 7.2: Serological testing of unmodified and transformed (*lux*$^+$) *Salmonella hadar* 127

Table 7.3: Results of biochemical testing of the original and transformed *Salmonella hadar* by Vitek Jr. (Biomerieux Vitek) in duplicate . 128

Table 7.3 : Results of biochemical testing of the original and transformed *Salmonella hadar* by Vitek Jr. (Biomerieux Vitek) in duplicate (continued) 129

Table 9.1: Summary of stations evaluated at a poultry pocessing plant 170

Table 9.2: Least Square means of the microbial contamination (i.e., as determined by conventional methods; cfu/mL) of carcasses sampled at different stations at a poultry processing plant for individual days 173

Table 9.3: Least Square means of the microbial contamination (i.e., as determined by the ATP bioluminescence assay; rlu/breast area) of carcasses sampled at different stations at a poultry processing plant for individual days 174

Table 9.4: Least Square means of the microbiological quality (i.e., as determined by conventional methods; cfu/mL) of flocks of poultry processed on individual days 180

Table 9.5: Least Square means of the overall quality (i.e., as determined by the ATP bioluminescence assay; rlu/breast area) of flocks of poultry processed on individual days 180

List of Figures

Figure 2.1: A flowchart of a poultry processing system indicating CCPs as proposed by FSIS (Anon, 1994) 24

Figure 2.2: A flowchart of a poultry processing system indicating CCPs as proposed by CFIA (Anon, 1997) 25

Figure 2.3: Illustration of the Adenosine Triphosphate (ATP) bioluminescence reaction to generate light (Deluca, 1976) 41

Figure 2.4: Organization of the *Vibrio fischeri lux* genes (Hastings *et al.*, 1985) 45

Figure 2.5: Illustration of the reactions involved in the production of light from bacterial bioluminescence (Engebrecht *et al.*, 1983) 46

Figure 2.6a: Cross section of the epidermis of a chicken (Lucas and Stettenheim, 1972) 50

Figure 2.6b: Cross section of the skin and subjacent tissues of a chicken (Lucas and Stettenheim, 1972) 51

Figure 3.1a: Response surface diagram showing the effect of concentration of chlorine and spray pressure on the total count and on turkey carcasses inoculated with intestinal contents. 62

Figure 3.1b: Response surface diagram showing the effect of concentration of chlorine and spray pressure on the coliform count on turkey carcasses inoculated with intestinal contents 63

Figure 3.2a: Response surface diagram showing the effect of concentration of trisodium phosphate and spray pressure on the total aerobic count on turkey carcasses inoculated with intestinal contents 69

Figure 3.2b: Response surface diagram showing the effect of concentration of trisodium phosphate and spray pressure on the coliform count on turkey carcasses inoculated with intestinal contents 70

Figure 3.3a: Response surface diagram showing the effect of concentration of Avgard™ and spray pressure on the total count and coliform count on turkey carcasses inoculated with intestinal contents 72

Figure 3.3b: Response surface diagram showing the effect of concentration of Avgard™ and spray pressure on the coliform count on turkey carcasses inoculated with intestinal contents 73

Figure 3.4a: Response surface diagram showing the effect of concentration of lactic acid and spray pressure on the total count on turkey carcasses inoculated with intestinal contents 75

Figure 3.4b: Response surface diagram showing the effect of concentration of lactic acid and spray pressure on the coliform count on turkey carcasses inoculated with intestinal contents 76

Figure 4.1a: Response surface diagram showing the effect of temperature and pH of lactic acid on the total aerobic count on chicken carcasses inoculated with intestinal contents 88

Figure 4.1b: Response surface diagram showing the effect of temperature and pH of lactic acid spray on the coliform count on chicken carcasses inoculated with intestinal contents 89

Figure 5.1: Least mean squares of aerobic plate count from several carcass categories under various treatment conditions 98

Figure 5.2: Least mean square of coliform count from several carcass categories under various treatment conditions 99

Figure 6.1: The overall effect of buffered lactic acid treatments (4.25% w/w) on Hunter L values (i.e., lightness) on turkey skins. 110

Figure 6.2: The overall effect of buffered lactic acid treatment (4.25% w/w) on Hunter a values (i.e, green-red) on turkey skins 111

Figure 6.3: The overall effect of buffered lactic acid treatment (4.25%w/w) on Hunter b values (i.e., yellow - blue) on turkey skins. 112

Figure 7.1a: Scanning electron microscopy of original *Salmonella hadar* isolated from a poultry abattoir 130

Figure 7.1b: Scanning electron microscopy of the transformed *Salmonella hadar* (*lux*$^+$). 130

Figure 7.2: Relation between plate count and bioluminescence readings of *Salmonella hadar* (*lux*$^+$) 131

Figure 7.3: The effect of dilution (10^0 ■,□; 10^2 ●,○; 10^3 ◆,◇; 10^4 ✱,✳) on light output of the *Salmonella hadar* (*lux*$^+$) over time with (closed symbols) and without (open symbols) ampicillin in LB broth at 37°C. . . . 132

Figure 7.4: Bioluminescence output of *Salmonella hadar* (*lux*$^+$) before treatment and during recovery after exposure to different pH levels 133

Figure 7.5: Plate count of *Salmonella hadar* (*lux*$^+$) 24 hrs after exposure to different pH levels 134

Figure 7.6: Bioluminescence output of *Salmonella hadar* (*lux*$^+$) over time at 40°C 135

Figure 8.1: An example of raw data from the viability study and the subsequent transformation of bioluminescent output 147

Figure 8.2: An example of raw data from the recovery study and the subsequent transformation of bioluminescent output 149

Figure 8.3a: Viability of *Salmonella hadar* (*lux*$^+$) over time (i.e., based on bioluminescence output) after exposure to three different treatments (control ○; water ◇; lactic acid □) and storage at 10°C. 153

Figure 8.3b: Viability of *Salmonella hadar* (*lux*$^+$) over time (i.e., based on bioluminescence output) after exposure to three different treatments (control ○; water ◇; lactic acid □) and storage at 5°C 154

Figure 8.3c: Viability of *Salmonella hadar* (*lux*$^+$) over time (i.e., based on bioluminescence output) after exposure to three different treatments (control ○; water ◇; lactic acid □) and storage at 0°C 155

Figure 8.3d: Viability of *Salmonella hadar* (*lux*$^+$) over time (i.e., based on bioluminescence output) after exposure to three different treatments (control ○; water ◇; lactic acid □) and storage at - 12°C. 156

Figure 8.4: Least Square means on the rates of viability from *Salmonella hadar* (*lux*$^+$) (i.e., based on rates of bioluminescence output) after exposure to different treatments and storage at various temperatures . . 157

Figure 8.5a: Recovery of *Salmonella hadar* (*lux*$^+$) over time (i.e., based on bioluminescence output) from exposure to three different treatments (control ○; water ◇; lactic acid □) and from storage at 10°C. . . 159

Figure 8.5b: Recovery of *Salmonella hadar* (*lux*$^{+}$) over time (i.e., based on bioluminescence output) from exposure to three different treatments (control ○; water ◇; lactic acid □) and from storage at 5°C . . . 160

Figure 8.5c: Recovery of *Salmonella hadar* (*lux*$^{+}$) over time (i.e., based on bioluminescence output) from exposure to three different treatments (control ○; water ◇; lactic acid □) and from storage at 0°C . . . 161

Figure 8.5d: Recovery of *Salmonella hadar* (*lux*$^{+}$) over time (i.e., based on bioluminescence output) from exposure to three different treatments (control ○; water ◇; lactic acid □) and from storage at -12°C . . 162

Figure 8.6: Least Square means on the rates of recovery from *Salmonella hadar* (*lux*$^{+}$) (i.e., based on rates of bioluminescence output) from exposure to different treatments and from various storage temperatures. 163

Figure 9.1: Illustration of the processing steps at a poultry abattoir and stations identified for poultry hygiene assessment during processing . . 169

Figure 9.2: Least Square means of the microbiological quality (i.e., as determined by conventional methods) of carcasses sampled at different stations at a poultry processing plant for the entire study 172

Figure 9.3: Least Square means of the overall quality (i.e., as determined by the ATP bioluminescence assay) of carcasses sampled at different stations at a poultry processing plant for the entire study 174

Figure 9.4: Least Square means of the microbiological quality (i.e., as determined by conventional methods) of carcasses sampled on individual days 178

Figure 9.5: Least Square means of the overall quality (i.e., as determined by the ATP bioluminescence assay) of carcasses sampled on individual days 179

1.0 Introduction

Poultry has been suggested to be a healthy alternative to other types of meat products. As a result, consumption of poultry has been on the rise since the early 1980's. The poultry industry has been able to meet the demands of the public, but there has been a growing concern about the level of food safety of their products.

There are many problems that can lead to the production of low-quality and unsafe foods. Some of these problems are fecal contamination, improper storage temperatures and cross-contamination by workers. The failure to correct these problems during production may lead to foodborne illnesses.

Outbreaks of foodborne illness can have devastating social and economic impacts. This was well demonstrated by the unfortunate incident of *E. coli* O157:H7 tainted hamburgers from "Jack in the Box" restaurants in the United States in 1993. Three young children died due to complications resulting from HUS (haemolytic uremic syndrome) contracted as a result of eating hamburger patties infected with *E. coli* O157:H7. The outbreak had a negative impact on the consumption of meat products and has led to the questioning of the ability of the industry to ensure the production of safe high-quality foods. Although this was an incident associated with beef, it has certainly put the spotlight on all sectors of the meat industry.

Presently, the production of safe foods by the meat industry is monitored through government food inspection services. Based on qualitative assessment, carcasses are examined and evaluated as to their suitability for consumption by the

general public. However, there is increasing debate about the effectiveness and the subjective nature of assessment used by the inspection service.

In an effort to make an impact on the food industry, poultry processors are looking at new approaches to improve meat quality and safety of their products.

1.1 The plans to improve food safety during processing of poultry

Currently, meat inspectors can reject carcasses for a variety of reasons (e.g., disease and other abnormalities) but the most controversial issue is carcasses that have been condemned due to fecal contamination. These carcasses are rejected because feces have spilled onto the surface or within the body cavity of the carcass during evisceration. The chief reason for condemnation of these birds is the potential health risk due to the presence of foodborne pathogens. However, the decision for rejection by government inspectors is based on a qualitative analysis.

Although both the American and Canadian meat inspection agencies have played a vital role in ensuring food safety, there has been some concern that the examination relies purely on subjective assessment. Therefore, research has been directed to find more quantitative means that can address problems associated with contamination of the carcasses during production. Presently, the poultry industry is implementing several strategies to achieve an improvement of poultry carcass hygiene.

One plan is to implement a Hazard Analysis of Critical Control Points (HACCP) program in processing establishments. It is a management program that

addresses food safety through prevention and anticipation of potentially hazardous situations (The National Advisory Committee on Microbiological Criteria for Foods, 1992).

A second plan of the poultry industry is to introduce bactericidal treatments as a method to decontaminate poultry carcasses during processing. The aim is to reduce the microbial levels associated with contaminated birds and improve the quality and safety of the meat. The bactericidal treatment would involve the use of a food-grade chemical(s) that has an antimicrobial effect on food spoilage organisms and pathogens, but would not pose a chemical risk to consumers.

1.2 Objectives of Research

The Canadian poultry industry and the Canadian Food Inspection Agency (CFIA) are looking towards sanitizing carcasses and adopting HACCP programs (ICMSF, 1988). It is expected that these approaches will enable the meat industry to improve product quality and safety. The following experiments were designed to investigate and evaluate processes that can improve the microbiological quality of turkey and chicken carcasses. The investigation focused on two main areas.

First, several bactericides were evaluated as a means to reduce the microbial hazards associated with condemned carcasses. There are numerous food-grade chemicals available that can be used as decontaminating agents. However, research was concentrated on four bactericides for in-plant use. These were Avgard™, trisodium phosphate (TSP), chlorine and lactic acid. Flamingo

Foods, Inc. has played a key role in the development of this process and has supplied the University of Guelph with a prototype machine for carcass treatment. It utilized a simple design of an inside/outside washing device that sprayed the cavity and the surface of the carcass while the carcass was rotated. After identifying the best bactericide(s), further experiments were conducted to optimize the conditions for use of the bactericide and challenge studies were performed to determine the efficacy of the treatment. Bacterial bioluminescence (i.e., *lux*) genes were used to modify a pathogen to become bioluminescent and, thus, act as a "real-time" reporter of the effectiveness of the bactericidal treatment.

Secondly, an observational study was conducted to explore a statistical method to determine baseline levels of hygiene at a poultry establishment during processing. Both conventional microbiology and the ATP (adenosine triphosphate) bioluminescence hygiene test were evaluated as aids to i) monitor contamination of carcass surfaces ii) to verify HACCP programs. In addition, the ATP bioluminescence hygiene test was also evaluated as an alternative to conventional microbiological techniques.

2.0 Literature Review

2.1 Foodborne illnesses and poultry production

Foodborne illness is an international problem. Reports have shown that the incidence of foodborne disease has dramatically increased since the 1970's (Bean and Griffin, 1990; Notermans and Hoogenboom-Verdegaal, 1992). According to the USDA's Economic Research Service report for 1997, the top seven of more than 40 recognized foodborne pathogens caused 3.3 to 12.3 million illnesses and up to 3 900 deaths in the U.S. annually (Buzby and Roberts, 1997). Foodborne illnesses are responsible for large social and economic costs (Roberts and van Ravenswaay, 1989; Shin *et al.*, 1992). In 1997, foodborne pathogens cost society an estimated $35 billion in the United States (Buzby and Roberts, 1997). These costs included medical treatments, productivity loss, pain and suffering of the people involved, food industry losses, and losses within the public health sector.

Reports of the incidence of foodborne diseases show that poultry is an important vehicle of transmission to humans. During 1968 to 1977, poultry contributed to 54% of reported outbreaks in the United States (Bryan, 1980). In 1978, poultry was ranked first or second in foods associated with disease in Australia, Canada, England and Wales and fourth in the United States (Todd, 1978). During 1977 to 1984, poultry was the vehicle for 10% of foodborne disease outbreaks in the United States (Bryan, 1988).

Fecal contamination is one of the major sources of foodborne pathogens

during processing (James *et al.*, 1992) and occurs when intestinal contents and feces spill onto the carcass during the evisceration process (Myers *et al.*, 1984; Prescott and Gellner, 1984; Rosef *et al.*, 1984). Additional contamination may occur during the slaughtering and the cleaning process, where poultry carcasses can come in contact with intestinal and fecal material of other birds (Mulder *et al.*, 1987). According to figures collected by the Canadian poultry industry, the direct loss in revenue due to fecal contamination has been estimated at $8 million annually (Hundt, 1995).

Although the poultry industry tries to ensure the safety and quality of its products by routine examination, there are still instances of improper handling and storage procedures that can contribute to the contamination of products. However, this is not to say that the poultry processors are totally to blame. In fact, the Center for Disease Control (Anon, 1992b) reported that 3% of meat and poultry foodborne illness were caused by manufacturing errors and that 97% were due to the mishandling of poultry products at post slaughter (i.e., insufficient cooking, improper refrigeration, etc.)

Newer policies and methods to improve hygiene may reduce the incidence of foodborne illnesses and increase consumer confidence. In fact, Shin *et al.*, (1992) conducted a consumer survey that indicated participants would pay 55 cents to eliminate *Salmonella* spp. from poultry products and 81 cents to eliminate *Trichinella spiralis* from pork products for each meal that may be contaminated.

2.1.1 Pathogens and their impact on foodborne illnesses

There are 40 pathogens that are known to cause foodborne illness. However, the following information will be focused on the most studied pathogens associated with meat products. A list of common pathogens and their prevalence on poultry are reported in Table 2.1.

2.1.1.1 *Salmonella* spp. in poultry products

Salmonella spp. are small, gram negative rods with or without flagella. They are ubiquitous in nature where the primary reservoirs are humans and animals. All *Salmonella* spp. can be pathogenic and can produce symptoms from mild and discomforting gastroenteritis, to more severe cases of diarrhea, to death. Reports have shown that as little as 100 organisms/g of salmonellae have been recovered from samples of epidemiologically-implicated foods (Blaser and Newman, 1982; D'Aoust and Pivnick 1976). Live poultry can become infected with *Salmonella* spp. by drinking water, eating feed and pecking in soil or litter previously contaminated by infected birds (Anon., 1980).

Due to an increased consumption of poultry over the years, *Salmonella* spp. have become, and remain, one of the major causes of foodborne illness (Bean and Griffin, 1990; Sharpe, 1990; Todd, 1980). In Canada, *Salmonella* spp. were responsible for the largest number of foodborne illnesses in 1992 (Todd, 1992). It

Table 2.1: The prevalence of pathogens or potential pathogens in poultry products in the United States and other countries

Organism	Type of poultry	Percent positive	Reference
Salmonella spp.	Chicken	0-100	Lammerding *et al.*, 1988; Poppe *et al.*, 1991; Todd, 1992
	Turkey	69	Lammerding *et al.*, 1988
Campylobacter jejuni	Chicken	8-89	Stern *et al.*, 1984; Ternstrom and Molin, 1987
	Turkey	56-64	Rayes *et al.*, 1983
Staphyloccocus aureus	Chicken	41-73	Lillard *et al.*, 1984; Ternstrom and Molin, 1987
Clostridium perfringens	Chicken	0-54	Lillard *et al.*, 1984; Ternstrom and Molin, 1987
Listeria monocytogenes	Chicken	13-56	Bailey *et al.*, 1989; Farber *et al.*, 1989; Genigeorgis *et al.*, 1989
	Turkey	12-18	Genigeorgis *et al.*, 1990

was reported that 50% of 56 foodborne related deaths were caused by *Salmonella* spp. (Todd, 1992). Presently, human salmonellosis continues to be an important public health problem as the annual incidence has increased during the past 20 years (Anon., 1992b). Unfortunately, the rise continues in spite of inspection and control programs.

Between 1973 to 1977 in Canada, *Salmonella typhimurium, Salmonella muenster and Salmonella schwarzengrund* were the major serotypes isolated from broiler chicken and turkey carcasses and were among the predominant causes of foodborne disease in this country (Lammerding *et al.,* 1988). *Salmonella typhimurium* was the most frequently isolated serotype in broiler chicken from 1983 to 1985 (Lammerding *et al.,* 1988). *Salmonella hadar* was the most isolated serotype in broiler flocks in 1991 (Poppe *et al.,* 1991). Recently in the United Kingdom, outbreaks of salmonellosis were attributed to *Salmonella enteritidis* (Powell, 1997)

Numerous studies estimating the incidence of *Salmonella* spp. in North America have been published. In 1987, the United States Department of Agriculture (USDA) documented that 36% of processed broiler carcasses tested were positive for *Salmonella* spp. (Anon., 1992b).Poppe *et al.* (1991) isolated *Salmonella* spp. from samples taken from 76.9% of flocks, 47.4% of litter samples and 12.3% of water samples. In 1983 to 1986, Lammerding *et al.* (1988) collected samples from federally inspected poultry abattoirs across Canada, and isolated *Salmonella* spp. from 60.9% of chicken carcasses and determined *S. typhimurium* to be the most

commonly isolated species.

2.1.1.2 *Campylobacter jejuni* in poultry products

Campylobacter jejuni is a slender, spirally curved rod that possesses a single polar flagellum at one or both ends of the cell. It requires microaerophilic conditions to survive and proliferate. The symptoms of campylobacteriosis usually consist of diarrhea lasting over several days. Other symptoms may include fever, malaise and abdominal pain. Reports show that an infective dose between 10^2 to 10^9 cfu/mL was required to induce these symptoms (Black *et al.*, 1983; Black *et al.*, 1988). Although other *Campylobacter* spp. can produce diarrhea and other gastrointestinal disorders, *Campylobacter jejuni* is by far the most important cause of foodborne illness from poultry.

In the United States between 1982 to 1986, *C. jejuni* was responsible for 5.02 to 7.19 cases per 100 000 people (Tauxe *et al.*, 1988). Surveillance by local and state public health departments suggests that the actual incidence ranges from 30 to 60 cases per 100 000 individuals (Hopkins et al, 1984; Johnson *et al.*, 1985). The prevalence of *C. jejuni* in chicken products has been reported between 8 and 89% (Stern *et al.*, 1984; Terstrom and Molin, 1987). In turkey products, prevalence was 56 to 64%. Studies by Waldroup *et al.*, (1992) showed that *C. jejuni* populations on post chill broiler carcasses from five different plants ranged from 2.4 to 4.2 $\log_{10}$ cfu/carcass.

2.1.1.3 Other foodborne pathogens of interest in poultry products

Staphylococcus aureus is another potentially pathogenic organism that can be found on poultry (Devriese *et al.*, 1975). *S. aureus* is described as a gram positive facultatively anaerobic coccus. They are ubiquitous in nature and can be frequently found as part of the flora in poultry products. Infections caused by *S. aureus* can include gastroenteritis in humans. Studies of the incidence of *S. aureus* in the general population are few. However, an interesting report by Notermans *et al.* (1975) found that the number of *S. aureus* on poultry products can be as low as 10 cells/g, but after processing counts can be as high as 1 000 cells/g. They attributed the increase in counts to the plucking and eviscerating operations. Prevalence of *S. aureus* on poultry has been recorded between 41 and 73% (Lillard *et al.*,1984; Ternstrom and Molin, 1987).

Clostridium perfringens is present in soil and dust and can come in contact with poultry on farms. These bacteria are gram positive, anaerobic, spore-forming rods. The spores can survive inadequate cooking temperatures and initiate growth during cooling of foods (Todd, 1980). *C. perfringens* infections can produce mild discomfort to severe gastroenteritis in humans. Prevalence of *C. perfringens* on poultry has been recorded between 0 and 54% (Lillard *et al.*,1984; Ternstrom and Molin, 1987)

Listeria monocytogenes is also widely distributed in nature. It has been found in soil samples, plants and human and animal feces. The organism is a gram positive, rod-shaped bacterium. This organism also produces gastrointestinal

problems but, the most susceptible individuals include pregnant women and individuals who are immuno-compromised. *L. monocytogenes* is normally associated with ruminant animals (Johnson *et al.*,1988), however, poultry has been reported to be an important vector (Bailey *et al.*,1989). Prevalence of *L. monocytogenes* was determined to be between 13 and 56% and 12 and 18% in poultry (Bailey *et al.*,1989; Farber *et al.*,1989; Genigeorgis *et al.*,1989) and turkey products (Genigeorgis *et al.*,1989), respectively.

2.2 Current methods to control foodborne illness during poultry production.

2.2.1 Competitive exclusion and vaccination as a method to control pathogens on the farm.

Salmonella spp. and other pathogens compete for adherence sites in the intestinal tract. Competitive exclusion is a method where a mixture of nonpathogenic bacteria is given to young chicks and allowed to colonize the intestinal tract. Since the intestinal tract is heavily lined with nonpathogenic bacteria, pathogens are unable to adhere and/or colonize.

Studies have shown that competitive exclusion was successful in preventing or reducing infection by *Salmonella* spp. in broilers and adult breeder birds (Mead and Barrow, 1990). In another study, competitive exclusion in chicks was shown to reduce the level of *Salmonella* spp. during a 48 hrs trial period (Impey and Mead,

1989). Other reports have also confirmed the reduction of *Salmonella* spp. in poultry using this technique. (Pivnick and Nurmi, 1982; Rantala and Nurmi, 1973; Wierup *et al.*,1988).

Vaccination is another method of controlling *Salmonella* spp. on the farm. This involves acquisition of immunity in an animal following exposure to an attenuated pathogen or an antigen of a virulent microorganism. It has been proposed that adherence factors involved with bacterial colonization would be useful antigens for vaccines (Zhao *et al.*,1995). However, this method is in the early stages of development.

2.2.2 Overview of the Meat Inspection Service

A brief description of the meat inspection practices for poultry production will be presented in this thesis. The following is a summary of the information from Wilson (1991) and the Meat Inspection Act (Anon, 1985) on practical meat inspection for poultry.

a) Antemortem inspection

An inspection is performed upon arrival of the flock of poultry at the abattoir. The flock is examined visually for abnormalities. Animals plainly showing symptoms of disease or conditions that would cause condemnation of their carcasses during slaughter are marked for disposal. These visual markers include apparent sickness, abnormal movements, peculiar behaviours and any other abnormalities in

appearance. Upon confirmation by a veterinary inspector, these animals are killed and disposed of in a prescribed manner. If the inspector has reason to rate the animal(s) as unsatisfactory, the birds are quarantined or treated and reassessed once again before slaughter. If the flock cannot be used for slaughter, they are removed from further processing and are disposed of accordingly. If birds cannot be assessed within 24 hrs, carcasses may be inspected one by one on the processing line by a qualified inspector in the unloading area.

b) Post Mortem Inspection

Immediately after defeathering, the external surfaces of the carcasses are examined. Some of the more common characteristics that lead to rejection are: a) obvious systemic disease b) serious injuries c) bruising d) septic wounds e) insufficient bleeding and f) emaciation with pendulous crop.

A second inspection is performed after evisceration of the carcasses. These carcasses are inspected for abnormalities in the viscera, the body cavity (including the anterior air sacs), legs, and body. The major visceral organs examined are the intestines, gizzard, liver, spleen, heart and lungs. Carcasses with abnormalities are removed from the line for salvage or further processing. More serious cases are condemned and removed from further processing. All inspections are performed under the supervision of a veterinary inspector.

c) Final Carcass Inspection

A final inspection is performed after the viscera have been removed and the body cavity has been washed thoroughly. Again, the meat inspector will look for any abnormalities that might have been missed in the previous inspections.

There is some controversy about the inability of visual inspection methods to detect microbial or chemical contamination. All inspections are based on the inspector's organoleptic assessments. One aspect of the controversy is the subjective, non-quantitative nature of inspections and evidence of poor repeatability between inspectors. This has certainly led to questions about the validity of the procedure for determining the quality of poultry carcasses.

Another concern is the inability to detect pathogens by present inspection methodology. Although an attempt is made to remove potentially hazardous foods at the macroscopic level, microscopic assessment of the meat products is not used by the meat inspection service. Presently, meat inspection services are not responsible for any microbial assessment of meat products they analyse at food processing establishments. The onus is placed on the food processor to ensure the safety of their food products.

2.2.3 Current practices of quality assurance by the poultry industry: microbiological analysis

Microbiological analysis is performed on end-products to determine quality

and safety of the product (e.g., packaged poultry carcass pieces). Microbiological analysis of these products is done at either the poultry processing plant or samples are sent to a certified laboratory. The analysis usually consists of classical microbiological techniques where aerobic plate counts are utilized to estimate the total microbial population and coliform levels. Some shelf-life assessment may also be performed using the same techniques. For these microbiological tests, at least 72 hrs is required before results can be interpreted. In some cases, poultry products may be further processed, packaged and shipped to the consumer before the results are analysed thoroughly.

2.3 Initiatives to improve the control of foodborne illness during poultry production

2.3.1 The Hazard Analysis Critical Control Point (HACCP) program

In 1971, the Pillsbury™ company, the U.S. Army Natick Research and Development Laboratories and the National Aeronautics and Space Administration needed to develop a system that would provide astronauts with pathogen-free food (Sperber, 1991). They came to the conclusion that this could not be successfully achieved through the conventional method of "finished-product" testing. They discovered that monitoring "end-products" was not an ideal approach to ensure food safety due to the limitations of sampling regimes.

However, a system of ensuring food safety could be achieved by actively monitoring key areas that could eliminate or control potential hazards within a

production system. This idea of preventative management was named Hazard Analysis Critical Control Points or HACCP. Pillsbury™ saw potential for incorporating HACCP into the food industry and developed HACCP principles for low acid canned foods in 1974.

Presently, the USDA and Agriculture and Agri-food Canada have developed several generic HACCP models for food production systems.

2.3.1.1 A Brief Description of HACCP and its principles

HACCP is a management technique that accomplishes food safety by anticipation and prevention (Tompkin, 1992). It is designed to prevent the occurrence of problems by ensuring that controls are applied at certain points where hazardous situations occur in a food process. The hazards may be physical, chemical or microbiological.

The procedure of HACCP applies rational, objective and systematic methods of identifying hazards and risks in a food process that can be controlled. Ultimately, based on this philosophy, HACCP should be used not only by food processors but also at any point in the food chain (i.e., from the farm to the consumer's plate).

Seven principles must be adopted if HACCP is to be implemented by the food industry. The following is an overview of the principles of HACCP as described in the literature (Bryan, 1991; Sperber 1991; Tompkin, 1992; The National Advisory Committee on Microbiological Criteria for Foods, 1992).

Step I: Identify Hazards associated with the process

All potential hazards in the food chain (i.e., food production/food processing/food preparation) must be identified and evaluated for their severity. Hazards may result from: a) contamination, survival or growth of microorganisms b) chemical contamination c) presence of physical hazards in terms of safety to the consumer (e.g., bone, metal, glass, etc.). This can be illustrated with flowcharts that indicate points of interest along food production systems. Then, a short list is developed of the hazards with descriptions of appropriate preventive measures.

Most important is that the hazard analysis and risk assessment should be geared towards identifying specific points in the process as they relate to production of safe foods. It is imperative that the assessment must differentiate safety issues from concerns of quality of the product.

Step II: Establish Critical Control Points (CCPs)

A critical control point (CCP) is any point in the process where loss of control could result in a health risk of the food product. In designating a CCP in a food process, the severity and risk associated with that area must be high or at least moderate in terms of food safety. Some examples of CCPs are refrigeration during processing, prevention of cross contamination and employee hygiene. The result of monitoring these areas would prevent, or at least minimize, one or more of the potential hazards. Note, if the point within a process cannot be controlled, it must not be designated as a critical control point.

To help in the identification of CCPs, decision trees are available in the literature (The National Advisory Committee on Microbiological Criteria for Foods, 1992).

Step III: Establish Specifications for each Critical Control Point

Each of the CCPs will have one or several procedures that will prevent, eliminate or significantly minimize the hazard. To ensure control, critical limits (i.e., upper and lower limits) should be set for each designated CCP. Some examples of critical limits that should be established are temperature during storage, available chlorine during chill immersion, final water activity (a_w) of finished products, etc. Critical limits should be set that will reduce or eliminate the hazards. Specifications for each CCP can be constructed by statistical means that would maximize control of the hazards (Anon, 1992a) and should be based on the performance of equipment at each food processing establishment.

Step IV: Monitoring of each Critical Control Point

The actual schedule required for monitoring each of the critical control points is really dependant on the type of food involved. Continuous "real-time" monitoring should be the preferred method of choice to ascertain deviation from specification of CCPs. For example, temperature can be easily monitored with recording charts. If continuous monitoring is not possible, a statistically valid sampling plan for the CCPs must be designed that will reliably identify the loss of control.

Monitoring procedures for CCPs must be able to determine deviation from specifications as accurately and as quickly as possible. This is important because lengthy analytical methods are of little value when results are generated historically. Hence, the consensus of the scientific community is that current microbiological methods are unsuitable as tools for monitoring CCPs. However, this may change with the advancement of the rapid ATP bioluminescence assay for determining microbial loads on meat and poultry products (Bautista *et al.*,1994;1996; Siragusa and Cutter, 1995; Siragusa *et al.*, 1995) and the development of rapid pathogen tests.

Step V: Establish Corrective Action to be taken if deviation occurs at a Critical Control Point

Courses of action must be decided upon whenever the criteria for the maintenance of the Critical Control Point are not met. The course of action should be clearly defined beforehand, and the responsibility for action assigned to an individual on the processing line. Furthermore, these actions must clearly demonstrate that the CPPs have been brought under control.

The products that were produced out of CCP specifications should be removed from the process, clearly identified and placed on hold until the extent of the hazard to the consumer is determined. Then, these products should be dealt with in an appropriate manner (e.g., destroyed or reworked).

Step VI: Establish a Record keeping System

All of the data obtained during monitoring CCPs should be kept on file at food processing establishments. This is for the benefit of the food producer and the regulatory agencies responsible for food safety.

In the case of the food processor, documentation of the HACCP system should provide valuable information regarding the quality of the food product and possible areas for improvement. In the case of agencies, reviews of the food processors can be based on documentation of the HACCP program and CCPs. This would allow food inspectors to streamline safety audits and be certain that food processors are achieving food safety during production.

Step VII: Establish Verification Procedures that the HACCP program is working correctly

Procedures can be developed to determine whether or not the HACCP system developed for the plant is in compliance with the HACCP plan as designed. According to the National Advisory Committee on Microbiological Criteria for Foods (1992), there should be four processes involved in verification of HACCP systems.

a) Science-based approach: A comprehensive objective approach is required to substantiate that the designated CCPs and their critical limits are improving the safety of the food product. Presently, there are no effective and scientific approaches described in the literature that can verify that the HACCP program is working effectively.

b) Overview of the program by the processor: This is a subjective review by the on-site staff to determine whether or not the HACCP program is being followed correctly. Specifically, it relates to the CCPs, monitoring of the CCPs and corrective measures.

c) Revalidation of the HACCP program: Processors are constantly changing formulations and equipment to meet the demands of food production for the public. Therefore, revalidation of the HACCP program is required when a product or the processing system has been modified from the original design. This may also include new information that could lead to the improvement of the existing HACCP program.

d) Government involvement: A third party evaluation of the HACCP program is required to ensure that the HACCP program is functioning properly. This is to ensure that the monitoring programs are performing accurately and being conducted honestly by the processors.

2.3.1.2 Proposed generic HACCP programs for Canadian and American Poultry Processing Plants

Presently, there are two proposed Generic HACCP models for poultry slaughter as presented by the American Food Safety Inspection Service (FSIS) (Anon, 1994) and the CFIA (Anon, 1997b). Only flowcharts of both models with designated CCPs are presented in Figures 2.1 and 2.2. However, both complete HACCP plans are available from the USDA and Agriculture and Agri-Food Canada

(Anon, 1994; Anon, 1997b).

According to the American HACCP plan for poultry slaughter, there are 10 key areas in the poultry processing plant that are considered critical (i.e., CCPs) for maintaining food safety. The Canadian HACCP model for poultry identifies 9 key areas, but each area has been subdivided into several key points along the process. In total, the Canadian HACCP model has 11 key areas critical for maintaining food safety. The agencies used different decision trees when determining CCPs for poultry processing establishments.

Monitoring programs for both models are based on performance of equipment (e.g., temperature regimes during scalding, flow rates during chill immersion, chlorine levels, etc.) and no microbiological evaluations are used for either model. For verification procedures, the American model restricts microbiological analysis to sanitation procedures. Some initiative for microbiological testing of *E. coli* and *Salmonella* spp. is presented with the "Mega-Reg" legislation (Anon, 1997a), but it is not part of the HACCP program.

The Canadian model utilizes microbiological analysis to a larger extent than the American model. It is recommended for evaluation of hygiene of equipment for evisceration, and *Salmonella* spp. and *E. coli* testing in the reprocessing and chilling areas are required. However, the sampling regime for microbial evaluation by the Canadian model is presented as an undefined number tested on a weekly basis. Perhaps, CFIA is still investigating the frequency at which microbial analysis should be performed.

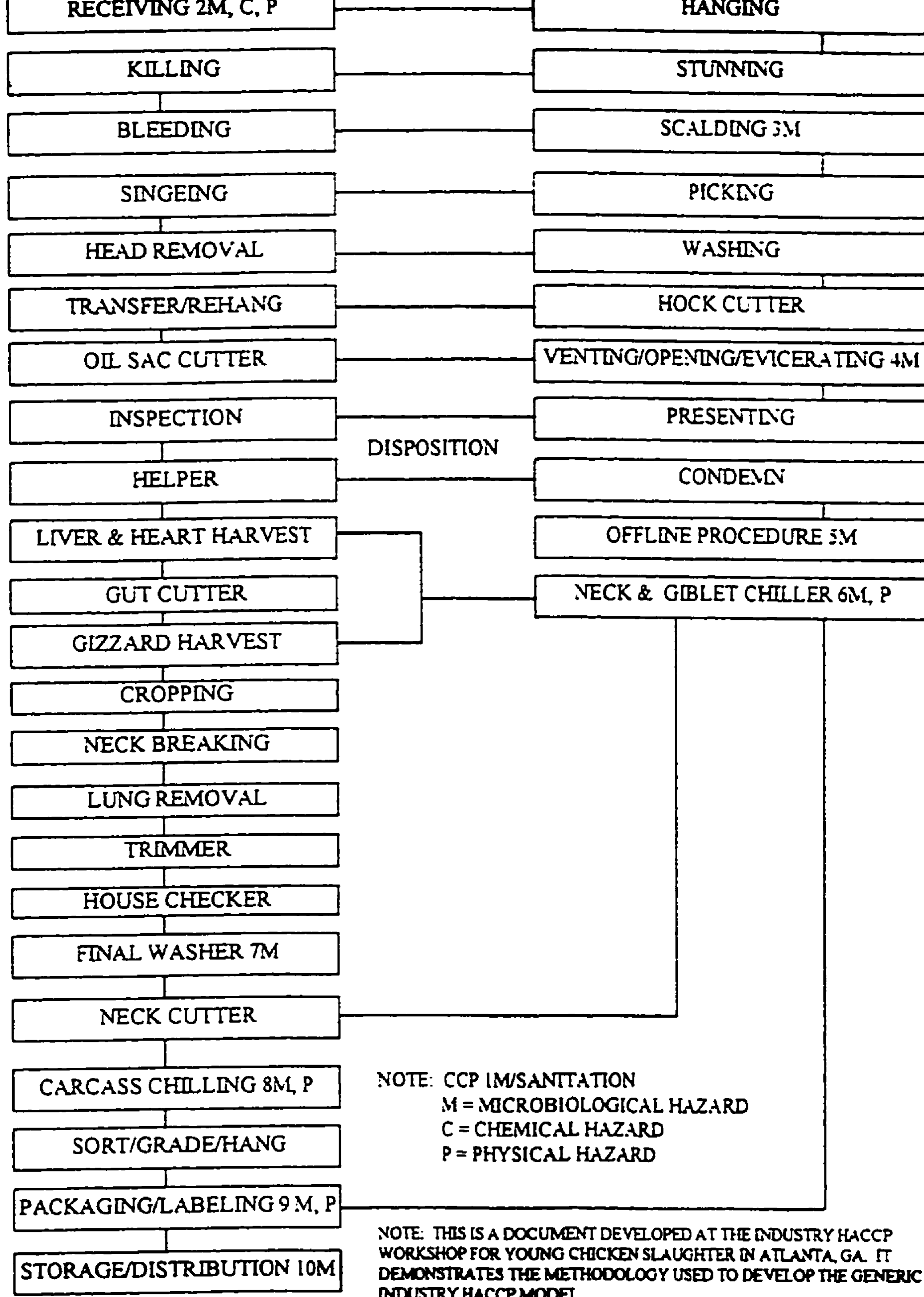


Figure 2.1: A flowchart of a poultry processing system indicating CCPs as proposed by FSIS (Anon, 1994)

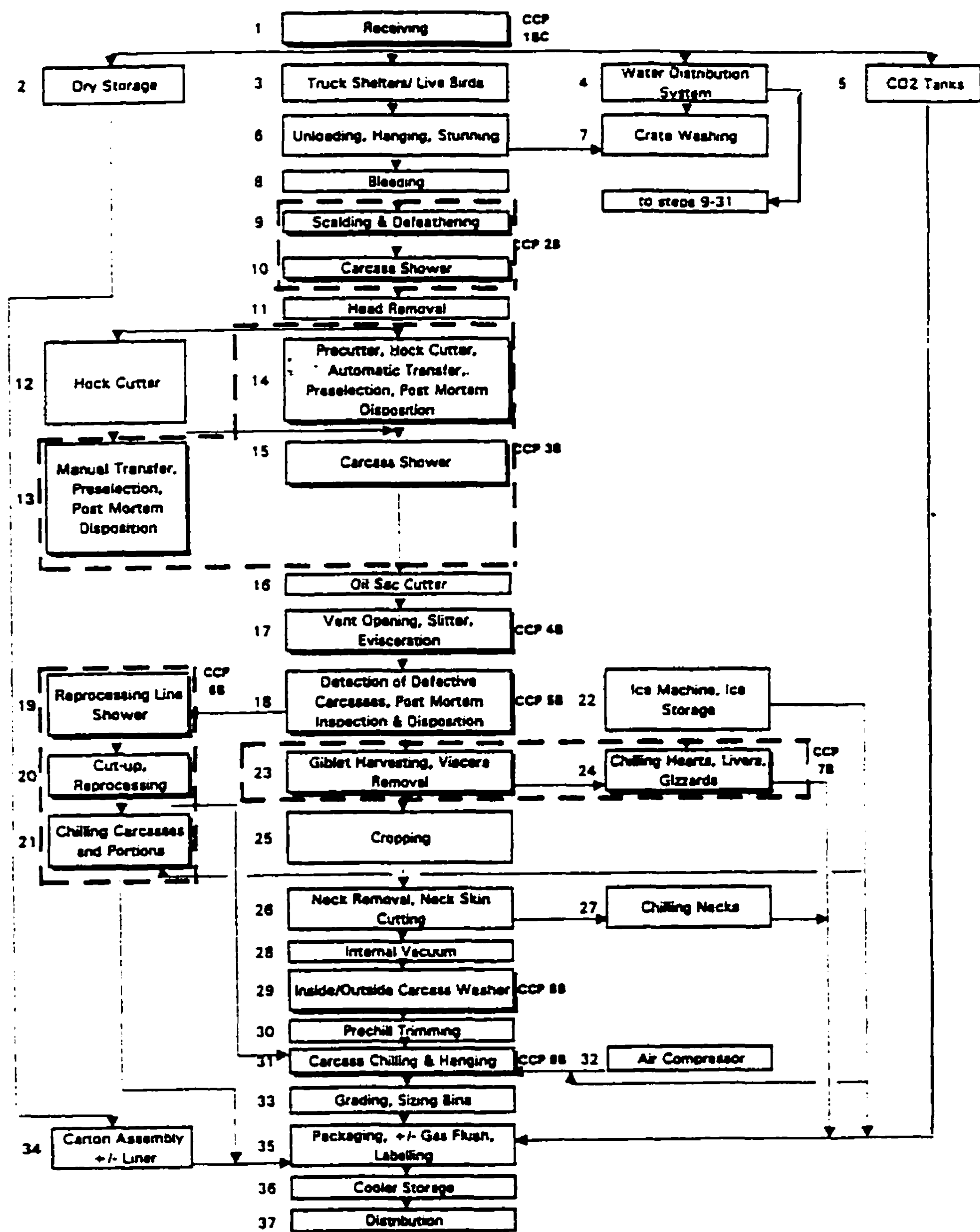


Figure 2.2: A flowchart of a poultry processing system indicating CCPs as proposed by CFIA (Anon, 1997b)

Furthermore, neither model attempts to explain what type of microbiological methods should be used for verification procedures (e.g., conventional or newer methodologies). For this author, it is disconcerting that microbiological techniques play a very small role for evaluating the effectiveness of the proposed HACCP programs.

2.3.2 The use of food-grade chemicals to improve carcass hygiene

It has been proposed that sanitizing carcasses may be a practical solution for the improvement of meat quality and safety (Wabeck, 1994). The use of chemical agents to decontaminate meat surfaces has been thoroughly documented in the literature (Dickson and Anderson, 1992; Snijders *et al.*,1985). The basic requirements of these bactericides are that i) they are food grade and ii) have either bactericidal or bacteriostatic action (or both). Some examples of these agents are phosphates (TSP) (Bender and Brotsky, 1992), chlorine (Mead, 1989) and lactic acid (Smulders *et al.*,1986). Many types of sanitizers have been proposed for a variety of meat and food products and all have claimed to lead to a reduction of pathogenic and spoilage organisms. However, this review is limited to bactericides of special interest to the Canadian poultry industry.

2.3.2.1 Chlorine

Chlorine has been routinely used to sanitize equipment and food-contact

surfaces in the meat and food industry. Several reports (Dawson *et al.*,1956; Ranken *et al.* 1965) have described the use of chlorine to reduce bacterial levels on poultry products. Presently, chlorine is permitted for use in waters for chill immersion at 20 ppm during processing.

Properties of Chlorine

Chlorine has a molecular weight of 35.45. The element is unstable and is highly reactive. The most stable forms of chlorine used in food industrial applications are chlorine gas (Cl_2), chlorine dioxide (ClO_2) and sodium hypochlorite (Na-O-Cl). All of these forms are readily soluble in water. However, the most widely used form is sodium hypochlorite (Na-O-Cl). It is quite economical in comparison to other bactericidal agents.

Mechanisms of inhibition

Hypochlorite solution acts as a strong oxidizer. This characteristic can cause chemical changes, poisoning and/or destruction of microorganisms (Tastyre and Holley, 1986). Secondly, there exists a lethal derivative of sodium hypochlorite in aqueous solutions. Mariott (1985) determined that the hypochlorous acid form is capable of disrupting protein synthesis, interferes with the functionality of proteins along the cell membrane and can denature DNA (deoxyribonucleic acid) sequences within the cell.

Studies using chlorine on meat surfaces

Chlorine has been studied as an antimicrobial agent on a variety of meat surfaces (e.g., beef, lamb, pork, poultry). Unfortunately, the majority of results indicate that chlorine applications are less effective than other bactericidal treatments.

Most reports indicate that chlorine produces between 1 to 2 $\log_{10}$ cfu/mL reduction in count on carcasses. For example, Kotula *et al.* (1974) washed beef carcasses with 200 mg/L chlorine solutions at several temperatures and pH levels, and found that the best reduction they could achieve was 1 to 2 $\log_{10}$ cfu/mL. Skelley *et al.* (1985) used 200 mg/L sodium hypochlorite solution for 10 min. and reported a 1.5 $\log_{10}$ reduction of psychrotrophic bacteria on pork carcasses. Smith *et al.* (1976) reported only a 1 $\log_{10}$ cfu/mL reduction with a 200 mg/L chlorine application on lamb carcasses. Emswiler *et al.* (1976) used 100 to 400 mg/L chlorine application that was electrically generated. They reported a reduction of 1.5 to 1.8 $\log_{10}$ cfu/mL of total aerobic bacteria on beef samples.

Other reports show that the use of chlorine is not effective at all in reducing microbial levels in comparison with other methods. Stevenson *et al.* (1978) reported no difference in the populations of coliforms, staphylococci, or total aerobic bacteria on beef carcasses sprayed with 200 mg/L chlorine and control treatments. Titus *et al.* (1978) reported an initial reduction in plate count with 200 mg/L chlorine, but no significant difference was evident after 8 days of storage. Johnson *et al.* (1979) did not find any significant difference in total plate count and lactic acid bacteria

between treated and control beef samples. Cutter and Siragusa (1995) used 50, 100, 250, and 500 ppm chlorine applications on beef samples and found that the reductions of aerobic bacteria were less than 1 log_{10} cfu/mL.

For chlorine to be effective, higher concentrations need to be applied to meat surfaces. For example, chlorine at 1 200 ppm was required to achieve a 99% kill of microorganisms on poultry products (Wabeck, 1994). However, chlorine at higher levels can cause certain undesirable organoleptic qualities (Teotia and Miller, 1975). Furthermore, chlorine can be absorbed into poultry products. A report by Wabeck (1994) showed that poultry can absorb 21.8 ppm chlorine into the skin and 4.4 ppm into meat tissues after one hour of chill immersion in water containing 50 ppm chlorine (Wabeck, 1994).

General Comments

Generally, hypochlorite solutions are not effective in the presence of high concentrations of organic residues (Tastayre and Holley, 1986). This is due to inactivation of the available chlorine when it becomes bound to organic residues (Siragusa, 1995). Wabeck (1994) reported that the total available chlorine was reduced to half the original concentration after 60 mins during the chill-immersion process. Therefore, due to the high organic material found on poultry carcasses and generated during processing, chlorine may not be the antimicrobial agent of choice for decontamination of poultry carcasses.

Furthermore, chlorine solutions have been reported to cause deterioration

of equipment by causing pitting in the metal (Wabeck, 1994). Another issue is the health concern associated with the buildup of organochlorines from the combination of chlorine with proteins during processing. This effect may be reduced with chlorine dioxide applications. However, chlorine dioxide is more expensive and is no more effective than chlorine solutions (Baran *et al.* 1973; Cutter and Dorsa, 1995).

2.3.2.2 Trisodium phosphate and phosphate blends

Phosphates are frequently used in the food industry as processing aids or additives. Phosphates have been approved for use in meat products to protect flavour, increase water binding capacity of meat tissues and to retain moisture in a variety of food products. In addition, a number of reports have indicated that phosphates have antimicrobial properties (Bender and Brotsky, 1992; Kim *et al.* 1994a; 1994b).

Due to the frequent use of phosphates in meat products and their antimicrobial properties, several studies have investigated the use of this chemical agent as an indirect method of controlling microorganisms. In October 1992, trisodium phosphate was approved by FSIS and the USDA as a post chill antimicrobial treatment for poultry products (Giesse, 1993).

Properties of trisodium phosphate and phosphate blends

Trisodium phosphate (TSP, Na_3PO_4) has a molecular weight of 163.94. It

has GRAS (Generally Regarded As Safe) status. It is available as a powder and the maximum solubility of trisodium phosphate in water is approximately 10 % w/w. The pH of a trisodium phosphate solution proportionally increases with increasing concentration.

Phosphate blends have the same components and characteristics as trisodium phosphate but have more complex structures.

Mechanisms of inhibition

In aqueous solutions, trisodium phosphate is a strong base which can rapidly destroy organic matter associated with microorganisms. As a result, cellular functions become disrupted and the cell membrane breaks down (Tastayre and Holley, 1986). In addition, trisodium phosphate is a surfactant which could be useful in detaching microorganisms from food surfaces (Kim *et al.* 1994a; 1994b).

Overall, very little information is available about the mechanism of inhibition by trisodium phosphate and other closely related compounds.

Studies using trisodium phosphate on meat surfaces

Trisodium phosphate has been shown to be effective in reducing the numbers of *Salmonella* spp. on poultry without compromising flavour characteristics (Bender and Brotsky, 1992). *Escherichia coli* O157:H7 and *Salmonella typhimurium* were reduced by 0.51 and 1.39 $\log_{10}$ cfu/mL, respectively, with 10% solution of trisodium phosphate (Kim *et al.*, 1994a). In another study, Kim *et al.* (1994b)

reported that *Salmonella* spp. were reduced up to 8% with applications of 10% trisodium phosphate. Broiler carcasses treated with at least 4% trisodium phosphate solution showed a reduction in the incidence of *Salmonella* spp. (Bender and Brotsky, 1991). Dickson and Anderson (1992) observed an overall reduction of 1 to 1.5 $\log_{10}$ cycles of gram-negative pathogens with trisodium phosphate concentrations between 8 to 10%. However, these results suggest that phosphates are no more efficacious than chlorine treatments.

Reports also show that the effectiveness of phosphate treatments can depend on the type of organism present. Slavik *et al.* (1994) reported that, because of immediate recovery of *Campylobacter* spp. after treatment, there was no difference between control poultry samples and poultry samples treated with 10% trisodium phosphate. Dickson and Anderson (1992) observed that populations of *Listeria monocytogenes* on beef samples were relatively unaffected by trisodium phosphate at concentrations between 8 to 10%. A similar observation was reported by Flores *et al.* (1996) for the same organism in sausages.

General Comments

A problem that must be addressed is the issue of spent trisodium phosphate solutions at the end of the production day. As a raw effluent, trisodium phosphate is an environmental concern (Wabeck, 1994). A second problem is the selective nature of its effect on microorganisms. This could lead to an imbalance in the microbial population on foods which could allow the proliferation of microorganisms

resistant to trisodium phosphate. It is especially a concern if certain foodborne pathogens are able to withstand the effects of the chemical.

2.3.2.3 Lactic acid

Lactic acid has been used for a variety of purposes in food products (Shelef, 1994). It has GRAS status and has no known deleterious long term effects on human health. Lactic acid has been evaluated as an antimicrobial agent that is capable of effectively reducing contamination in food products. Presently, the Canadian poultry industry and Agriculture and Agri-Food Canada are evaluating the use of lactic acid treatments in poultry applications (Hundt, 1996, personal communication).

Properties of Lactic Acid

Lactic acid is a carboxylic acid with a molecular weight of 90.08. It is a colourless, nonvolatile, viscous liquid that is highly soluble in water. The pK_a of lactic acid is approximately 3.86 at 22° C and it is highly corrosive. It can corrode stainless steel unless certain precautionary measures are performed (e.g., plastic coatings or high grade stainless steel). The pH of lactic acid will be proportional to its concentration in solution (e.g., a 1% solution has a pH of 2.50; a 10% solution has a pH of 2.20).

Lactic acid is commercially available as an aqueous solution at concentrations between 80 to 90% (w/w).

Mechanisms of Inhibition of Bacteria

There are two main theories describing the effects of lactic acid on bacterial populations. The first theory is related to the direct effects of the pH of lactic acid solutions. The second theory is related to the lactate moiety of the organic acid, which is thought to exert a disrupting effect on intracellular mechanisms.

Generally, acidic solutions are corrosive and can attack organic matter resulting in a disruption of cellular functions associated with the cell membrane (Eklund,1980; Tastayre and Holley, 1986). This effect has been documented for various concentrations of lactic acid on bacterial populations (Ingram and Coppock. 1956). Other reports have also indicated that exposure to low pH will sensitize microorganisms, induce a longer lag phase (Mountney and O'Malley, 1965; Smulders and Woolthuis, 1985) and eventually result in the death of the organisms (Levine and Feller, 1940; Van Netten *et al.*,1984).

Another interesting effect of lactic acid is its ability to decrease the intracellular pH of microorganisms. When using lactic acid, or salts of lactic acid, undissociated forms of the lactic acid are able to penetrate into the interior of the cell (Gould *et al.*,1983). If the environment surrounding the bacteria is more acidic than the interior of the cell, the undissociated acid becomes unstable and releases protons. The consequence is a decrease in the intracellular pH and disruption of metabolic pathways (Smulders *et al.*,1986). The bacteria will try to compensate for the change in intracellular pH by actively removing protons by pumping mechanisms found on the cell membranes. Since the bacteria will expend most of

their energy stabilizing the intracellular pH, this will hamper all other cellular functions (Gould *et al.*,1983) and, eventually, result in cell starvation. This effect seems to be most pronounced at pH values below 5.5 (Smulders *et al.*,1986).

Studies using lactic acid in food products

As a bactericide, lactic acid has been widely studied on a variety of food surfaces (Abul-Raouf *et al.*,1993; Anderson and Marshall, 1990b; Epling *et al.*,1993; Patterson *et al.*,1984; Snijders *et al.*,1985; Van der Marel *et al.*,1988). These reports have indicated a significant reduction of microbial populations by low concentrations of lactic acid (approximately 1 to 2%). However, most of these reports indicate only a 1 $\log_{10}$ cfu/mL reduction of aerobic bacteria. In a report by Greer and Jones (1991), a lactic acid (1%) spray was applied on the longissimus dorsi muscle of beef carcasses which was subsequently vacuum packaged. The treatment was only able to reduce the bacterial levels by 0.7 $\log_{10}$ cfu/mL and did not have an effect on survival of beef spoilage *Pseudomonas* spp. It has been suggested that these studies need to be reevaluated for their practical application on food surfaces, especially, for the meat industry.

Some reports indicate that lactic acid can be more effective when combined with other bactericides. Some examples are acetic acid or other organic acids (Anderson and Marshall, 1990a), potassium sorbate (Patterson *et al.*,1984) and sodium lactate buffer (pH 3.0) (Zeitoun and Debevere, 1991). These were used on a variety of meat surfaces. However, such treatments have reduced microbial

counts only by 1 to 2 log_{10} cfu/mL.

General Comments

It has been reported that lactic acid treatments at high concentrations (>3%) will cause a notable discolouration of meat surfaces. However, this effect can be minimized by a post treatment process. The most practical method would be to use water to rinse off the remaining lactic acid. Another disadvantage is that high organic material may have adverse effects on the activity of lactic acid (Cherrington *et al.*,1992). This may pose a problem during application on certain foods at processing establishments. Therefore, it is important that the correct concentration be used to overcome the effects of the organic material.

Unlike other organic acids, lactic acid is one of the fundamental components found in muscle tissues and blood. It neither has a strong odour nor produces any off flavours in meat products (Zeitoun and Debevere, 1991).

2.3.3 The use of temperature and pH as factors to influence the efficacy of bactericidal solutions

The effectiveness of the sanitizing action of bactericides is related to the application temperature. Earlier reports have indicated that treatment of poultry carcasses with hot water can control *Salmonella* spp., reduce total microbial contamination and extend shelf life (Avens and Miller, 1972; Cox *et al.*,1974; Dawson *et al.*,1963; Pickett and Miller, 1966; Teotia and Miller, 1972; Thompson

et al.,1979)

Several recent attempts have been made to increase the efficacy of bactericidal treatments by increasing the temperature during application. In a report by Prasai *et al.* (1991), hot (55°C), 1% lactic acid was sprayed on beef carcasses. They reported a > 90% reduction in plate count at the time of treatment. However, this only constitutes a reduction of 1 log_{10} cfu/mL. In another report, a significant reduction of microbial levels was observed when hot beef carcasses were sprayed before chilling (Snijders *et al.*,1985). Anderson and Marshall (1990) observed that aerobic plate counts were increasingly reduced as the temperature of a mixed acid sanitizer (2% acetic, 1% lactic, 0.25% citric and 0.1% ascorbic acids) increased from 25 to 70°C. They observed the greatest reduction (2.3 log_{10} cfu/mL) of *Salmonella typhimurium* count with a solution temperature of 70°C. Cherrington (1992) observed no change in total count at 20 and 30°C with acetic acid (0.1%) treatments, but a 4 log_{10} cfu/mL reduction at 40 and 50°C. In the same study, lactic acid was found to reduce *Salmonella* spp. by 6 log_{10} units. Kelly *et al.* (1982) observed that application of water rinses at 80°C on lamb carcasses was as effective in reducing microbial levels as a chlorine treatment at a concentration of 95 mg/L. They also observed greater reductions of bacteria with 30 mg/L chlorine with an application temperature of 65 and 80°C.

The pH of lactic acid solution has also been investigated as a means of improving bactericidal efficacy. Mendonca *et al.* (1994) indicated a possible relationship between high pH and moderate temperatures (up to 45°C). Teo *et al.*

(1996) determined that the combination of high pH and high temperatures resulted in a significant ($p = 0.0001$) interaction effect on the rapid death of both *E. coli* O157:H7 and *S. enteritidis*. Their observations were based on a sodium-bicarbonate sodium hydroxide buffer (NaHCO3-NaOH).

Buffered pH systems have also been used to improve the efficacy of various bactericides. For example, lactic acid solutions can be used in conjunction with sodium lactate to produce a stable buffer (Zeitoun and Debevere, 1990; 1991). The buffering capacity will also help maintain the pH of the food product during storage. Zeitoun and Debevere (1991) reported that best results occurred with samples treated with 10% lactic acid/sodium lactate buffer and stored under modified atmosphere packaging (MAP).

2.4 Innovative approaches for determining microbial contamination in food products

2.4.1 Tests for the rapid identification of pathogens

Identification of bacteria by conventional microbiological techniques can take several days before results can be obtained. This may not be suitable for the food industry where immediate corrective action may be necessary to resolve problem situations. Therefore, there is a need for faster methods of identifying pathogens in food products.

For convenience, researchers have miniaturized conventional

microbiological/biochemical techniques into compact and "easy to use" diagnostic kits. The basic components are multiple inoculation devices and containers to house the biochemical tests. Some examples of rapid microbiological/biochemical diagnostic kits are API, Enterotube, R/B, Minitek, MicroID, Vitek jr., Biolog and IDS. More detailed reviews can be found in Feng (1992) and Fung (1994).

Serological methods have also been developed to aid in the rapid identification of microorganisms. Basically, these rely on the specific interaction of antibodies with corresponding antigens on target organisms. The enzyme-linked immunosorbent assay (ELISA) developed by Organon Teknika (Durham, NC) uses two monoclonal antibodies specific for *Salmonella* spp. (Fung, 1994). Another system by Tecra (International BioProducts, Redmond, WA), uses polyclonal antibodies to detect *Salmonella* spp. (Fung, 1994). Several immunoassays for the detection of *Salmonella* spp. and other pathogens are available commercially.

In the area of molecular biology, Polymerase Chain Reaction (PCR) technology and nucleic acid fingerprinting have been investigated as a possible means of identifying organisms. Using probes targeted for specific DNA (deoxyribonucleic acids) sequences, the target DNA can be amplified into millions of copies. With the aid of gel electrophoresis, a DNA fingerprint of the sequence can be produced and the pattern used to determine the presence of the target organism. It is anticipated that PCR and DNA fingerprinting will become increasingly used in the food industry (Fung, 1994).

Phage-based assays are also being studied for detection and identification

of organisms. The assay is based on the ability of a phage to attach and infect a specific host (e.g., pathogenic bacteria). This characteristic is useful for the identification of bacteria. Bioluminescence genes (Baker *et al.*,1992), fluorescent dyes (Hennes and Suttle, 1995) and ATP bioluminescence (Griffiths, 1996) can be used to enable phage interaction with host bacteria to be detected and, thus, provide a way for detecting pathogens.

Although many of these tests are regarded as "rapid methods", most of them still rely on cultural methods for resuscitation of injured cells and selective media for the amplification of target organisms.

2.4.2 Rapid microbiological testing with the ATP bioluminescence assay

As stated earlier, the major problem associated with conventional microbiological techniques is the incubation time required before the results can be interpreted (i.e., up to 72 hrs). This technology is inadequate for the food industry and needs to be reevaluated as an indicator of the microbiological quality and safety of food products.

An alternative for hygiene assessment that may be widely applicable to the food industry is the ATP bioluminescence assay. ATP is a universal energy transfer molecule that is found in all living cells. Levels of ATP on food-contact surfaces can be used as an indicator of the levels of hygiene and effectiveness of sanitation procedures. The assay relies on the generation of light from a reaction catalysed by the luciferase-luciferin complex found in firefly tails (*Photinus pyralis*).

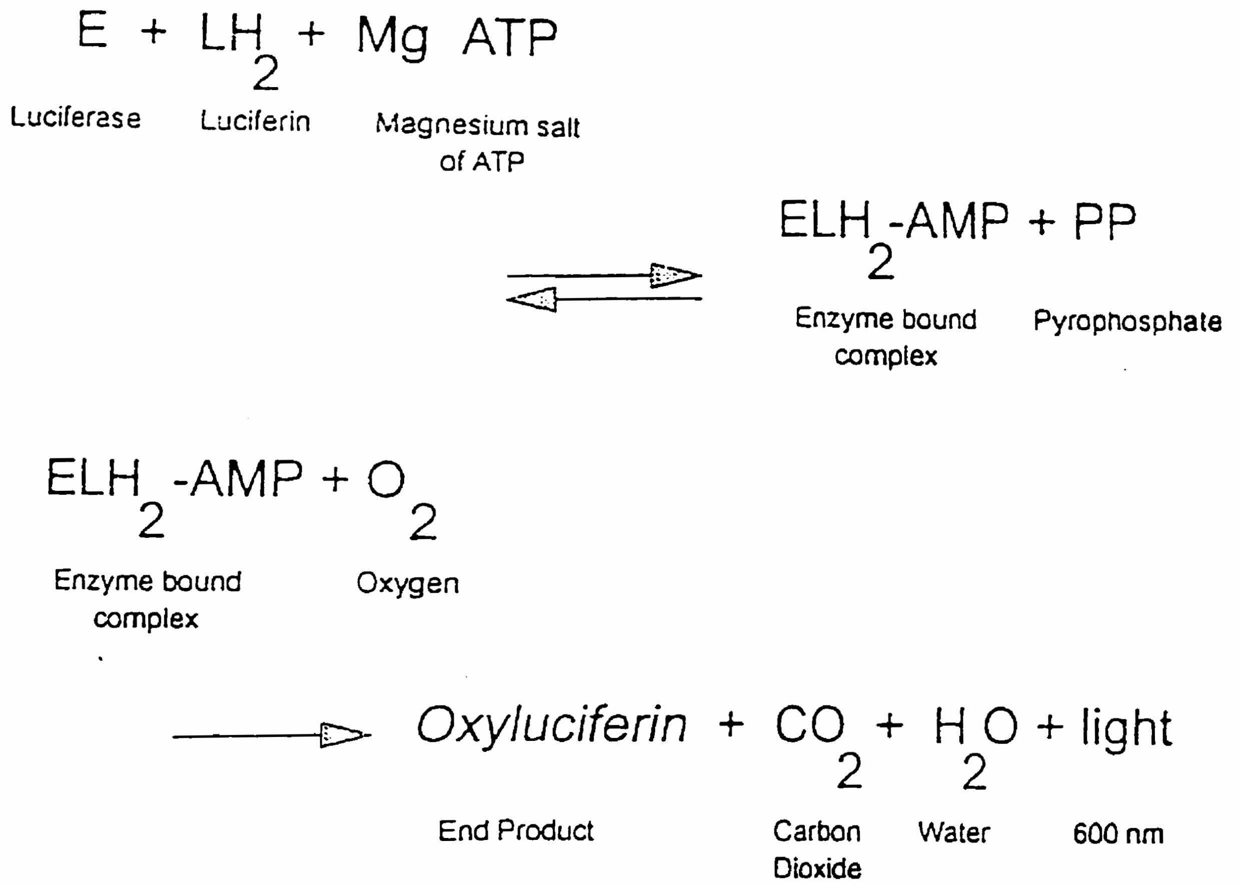


Figure 2.3: Illustration of the Adenosine Triphosphate (ATP) bioluminescence reaction to generate light (Deluca, 1976)

In detail, the enzyme, luciferase, catalyzes the reaction of luciferin, and ATP to form an excitable enzyme-bound luciferyl-adenylate molecule (McElroy and DeLuca, 1981). This enzyme bound complex will react with oxygen to form an oxyluciferin, carbon dioxide, water and light at 600 nm. An illustration of the reaction can be found Figure in 2.3. The amount of light produced is proportional to the amount of available ATP (McElroy and Deluca 1981; Stanley, 1989). Light emission can be easily measured with a device called a luminometer.

Several commercial ATP bioluminescence assays are available to determine hygiene at food processing establishments. The assay is based on the association of free ATP molecules and level of cleanliness. For example, unclean surfaces will be contaminated with microorganisms and food residues that both contain ATP. Cleaner surfaces would contain little or no ATP from microorganisms or food residues.

Bautista *et al.* (1992) and Seeger and Griffiths (1994) were able to demonstrate the usefulness of the ATP bioluminescence hygiene test for food processing and food service establishments. Both studies evaluated overall ATP content on food contact surfaces to determine efficacy of sanitation procedures. Due to the speed of the test (i.e., < 2 mins), the researchers stated that the ATP bioluminescence hygiene test was a useful indication of plant hygiene. Presently, the ATP bioluminescence hygiene test is routinely used in Europe and increasingly used in North America. Unfortunately, the hygiene test does not differentiate between ATP from microbial and other sources (e.g., food residues).

Other studies have modified the hygiene test to determine only the microbial levels on food products. By incorporating extraction methods (Bautista *et al.*,1992; 1994, Griffiths, 1991; Siragusa *et al.*,1995; Stanley, 1989), bacteria can be separated from the sample and then, their ATP extracted. The extracted microbial ATP can be assayed with the luciferin/luciferase complex to generate a light output proportional to the level of microorganisms in the sample.

Several researchers have shown that the ATP bioluminescence method for bacterial assessment can be used effectively to enumerate microorganisms (Brovko *et al.*,1991, Lundin, 1989, Stanley, 1989; Ugarova *et al.*,1991). Numerous reports have shown the success of employing the modified ATP bioluminescence assay for bacterial enumeration in food products. Bautista *et al.* (1992;1994) were able to produce a good correlation ($r > 0.80$) with plate count techniques for milk, poultry and beef samples. Siragusa *et al.* (1995) were able to use the ATP bioluminescence assay to determine microbial loads with better correlations ($r > 0.90$) for beef and pork products.

2.4.3 Genetically engineered bioluminescent bacteria as a model system to evaluate responses to environmental stimuli

Microorganisms can be genetically modified to produce certain characteristic(s) that are not normally exhibited. This is accomplished by inputting a genetic sequence into the host microorganism by transduction or transformation

techniques. For example, organisms have been genetically engineered to induce the production of toxic protein for biological control (Gawron Burke and Baum, 1991), and improve rumen fermentation (Forsberg *et al.*,1986).

This same technology has also been used to engineer organisms to produce auto-bioluminescence (Ulitzur and Kuhn, 1987). Bacterial bioluminescence is under the control of an operon (*lux*) that includes two structural genes that encode for two protein subunits of the luciferase enzyme, as well as genes that encode for enyzmes involved in the production of substrates necessary for the reaction. An illustration of the complete *lux* operon is illustrated in Figure 2.4. The *lux* genes can be isolated from a variety of organisms such as *Vibrio fischeri* (Engebrecht *et al.*,1983), *Vibrio harveyi* (Cohn *et al.*,1983) and *Xenorhabdus luminescens* (Meighen and Szittner, 1992).

The bioluminescence reaction involves the oxidation of a long chain aldehyde and reduced riboflavin phosphate in the presence of the luciferase enzyme. Light (wavelength 490 nm) is produced by the enzyme with the concomitant conversion of the aldehyde to the corresponding fatty acid (Baker *et al.*,1992). A schematic of the reaction is illustrated in Figure 2.5. More importantly, this system can be incorporated into other bacteria capable of expressing these genes.

Chen and Griffiths (1996) and Loessner *et al.* (1996) have both successfully constructed bioluminescent phenotypes of *Salmonella* spp. and *Listeria* spp.,

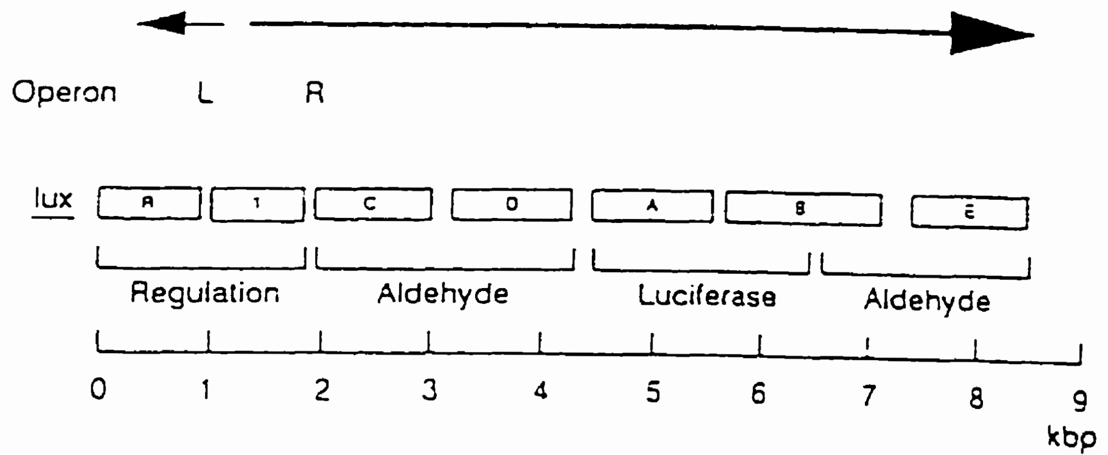


Figure 2.4: Organization of the *Vibrio fisheri* lux genes (Hastings et al., 1985).

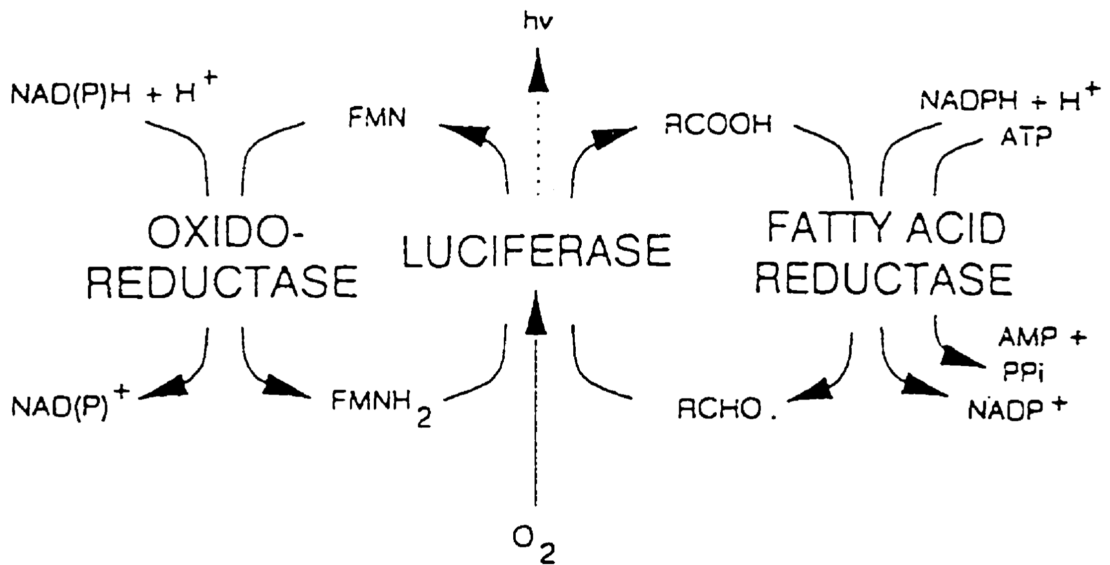


Figure 2.5: Illustration of the reactions involved in the production of light from bacterial bioluminescence (Engebrecht *et al.*, 1983)

respectively. Both groups of researchers spliced only the *lux* AB subunits of the bioluminescence sequence into the genes of host specific bacteriophages. Bioluminescent bacteria were produced by allowing the modified phages to adsorb onto target organisms and inject the *lux* genes. Providing the reducing powers are present, the successful result is the expression of bioluminescence from the target organism. In both studies, however, a substrate, decanal (~ 1% w/w in ethanol), was required to instigate bioluminescence output since the substrate genes were not incorporated into the phage. The goal of both research groups was to identify and detect the presence of organisms in samples.

Other researchers used transformation techniques to insert the genetic sequence into target organisms. This was easily accomplished by splicing the *lux* AB genes onto a plasmid that bacteria could transcribe (Ellison *et al.*,1991; Ellison *et al.*,1994; Meighen and Szittner, 1992; Szittner and Meighen, 1990). Furthermore, studies have shown that the bioluminescence output of the engineered bacteria can be influenced by the environmental conditions (Lampinen *et al.*, 1995; Walker *et al.*,1992). Specifically, the light output will decrease per unit area, or population of modified microorganisms, in times of stress. The opposite will hold true when the organism is grown under favourable conditions. Therefore, by monitoring the level of light output, one can determine whether or not the organism is responding positively or negatively to certain stimuli.

Several reports have described the use of bioluminescence (i.e., *lux* AB subunits only) to interpret response to stimuli. Lampinen *et al.* (1995) used

controlled luciferase expression to monitor chemicals affecting protein synthesis in *E. coli* K-12. Both Ellison *et al.* (1994) and Duffy *et al.* (1995) have used bioluminescent *Salmonella typhimurium* constructs to evaluate thermal inactivation. In another report, Ellison *et al.* (1991) were able to successfully monitor, in "real time", the injury and recovery of a bioluminescent *Salmonella typhimurium* during and after freezing.

Bacterial bioluminescence can be useful to evaluate chemical or physical conditions that may influence the survival of microorganisms. Where it might prove to be most useful, is in the studies of the behaviour of pathogens in food products during storage. Both Hudson *et al.* (1996) and Tomicka *et al.* (1996) have used luminescence to monitor survival in fermented foods.

2.5 The structure of poultry skin and the effects of water immersion during processing

The main functions of the skin are to aid in the prevention of water loss and protect the animal from disease-producing organisms. It is composed of two main layers, the epidermis and the dermis. During processing, chill immersion can cause structural tissue changes within these layers which may affect skin colour and product safety.

The epidermis is the outermost skin layer and is composed of stratified squamous epithelial tissues (Figure 2.6a). The top layer (i.e., stratum corneum) of the epidermis consists of hardened dead cells (i.e., laminae) that are constantly being sloughed off. The layer just below it (i.e., stratum intermedium) consists of new cells which take the place of cells that are lost. These new cells are produced from the actively growing and dividing cells of the stratum basale. The membrana baslis is a basement membrane that lies beneath the stratum basale.

The dermis is located below the epidermis (Figure 2.6b). Blood vessels run through the dermis layer to supply oxygen and required nutrients to the live cells of the epidermis layer. It is primarily composed of connective tissue and it is this layer that binds the skin to the body. It is also comprised of several parts. The stratum superficiale and the stratum profundum are the top and bottom layers of the dermis, respectively. The stratum profundum is sub-divided into the stratum compactum, stratum laxum and lamina elastica.

Poultry carcasses are chilled by immersion in cold water (5°C) to reduce the risk of microbial growth. Some changes occur that can affect skin tissues during chill immersion. For example, the skin tissues can become lighter in colour (Lyon and Cason, 1995) and may increase in mass due to water absorption (Lillard, 1986; Thomas and McMeekin, 1984; Veerkamp *et al,* 1973). Another important change

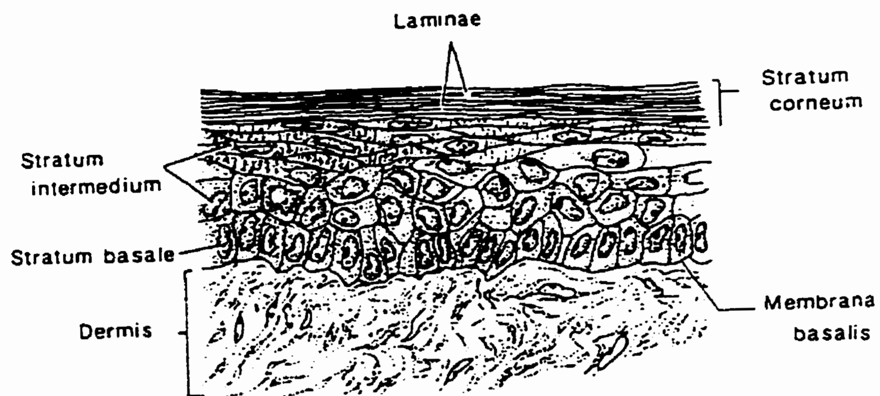


Figure 2.6a: Cross section of the epidermis of a chicken X1000

(Lucas and Stettenheim, 1972)

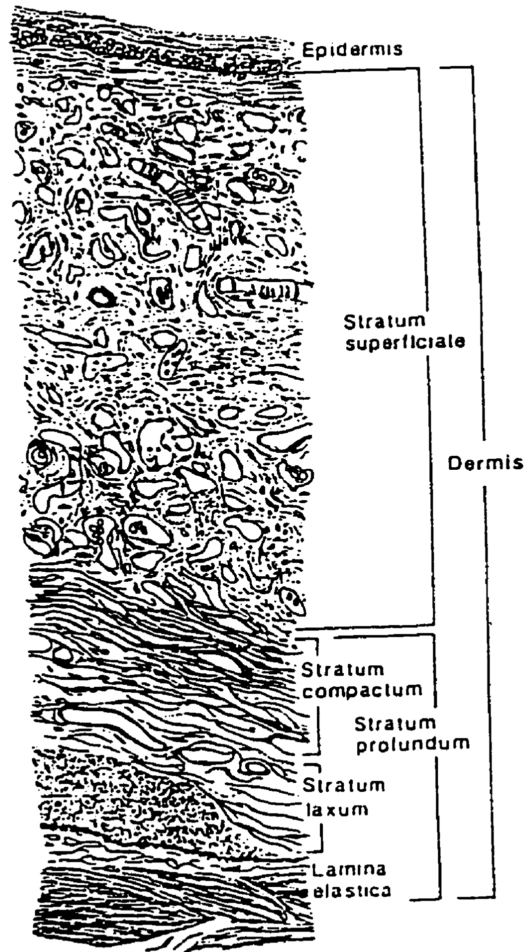


Figure 2.6b: Cross section of the skin and subjacent tissues of a chicken X500 (Lucas and Stettenheim, 1972)

is an increased swelling of the skin tissues and subsequently, the exposure of the deep channels and crevices along the surface (Thomas and McMeekin, 1982).

At the microscopic level, the changes in skin microtopography due to water absorption may play a negative role during chill immersion (Lillard, 1980; Thomas and McMeekin, 1980). The exposed skin channels and crevices may allow bacteria from the processing chill water to penetrate deep into the skin surface (Notermans and Kampelmacher, 1974). Bacteria can become lodged within these spaces and may be difficult to remove from carcasses (Thomas and McMeekin, 1984). This is of importance in regards to food spoilage organisms and/or foodborne pathogens.

During scalding (i.e., hot water temperature at 60°C), some of the top layers of the epidermis is removed from the carcass. This may compromise the protective features of the skin during further processing.

3.0 The determination of efficacy of antimicrobial rinses on turkey carcasses using response surface designs

3.1 Introduction

Carcass contamination can occur at several points throughout the processing of poultry. For example, the immersion of birds during the scalding and chilling operations may provide an opportunity for cross-contamination (Mead, 1990; Mulder, 1994). The defeathering process has also been mentioned to contribute to cross contamination by aerial dispersion of microorganisms (Mead, 1990; Mulder, 1994). Hudson and Mead (1989) reported that cross contamination from evisceration equipment led to 50% of carcasses becoming contaminated with *Listeria monocytogenes*. Primary processing, in general, has also been reported to increase the frequency of contamination among carcasses (Lammerding *et al.*,1988; Butzler and Oosterom, 1991; Slavik *et al.*,1991).

Several measures have been developed to reduce "in-process" microbial contamination of poultry (Dickson and Anderson, 1992). Most of the current methods focus on washing and sanitizing procedures that can reduce the bacterial levels on the surface of carcasses (Dickson and Anderson, 1992). However, the sanitizers must be nontoxic and active against microorganisms under routine processing conditions (Cherrington *et al.*,1992; Slavik *et al.*,1991).

Lactic acid is of particular interest since the mechanism of inhibition has been thoroughly studied (Smulders *et al.*,1986; Shelef, 1994). It has a broad

specificity (Snijders *et al.,*1985; El-Khateib *et al.,*1993; Van der Marel *et al.,*1989, Zeitoun and Debevere, 1990) and has GRAS status (Smulders, 1987). Van der Marel *et al.* (1988) observed that 2% lactic acid was effective in inhibiting post-treatment colonization by *Enterobacteriaceae* during refrigerated storage.

Chlorine was reported to be effective in controlling microbial levels (Mead and Thomas, 1973a; 1973b) and cross contamination (Mead, 1989) in poultry chill tanks. Baran *et al.* (1973) recorded residual effects of chlorine dioxide in reducing total and coliform counts in the chiller.

Phosphates were shown to reduce spoilage and rancidity in broiler carcasses (Thompson *et al.,*1979). In particular, trisodium phosphate (TSP) has been reported to be an effective bactericide for whole bird carcass washing and post-chill treatment for reducing *Salmonella* contamination (Bender and Brotsky, 1991; 1992). On October 1992, TSP was approved for use in poultry processing by the USDA (Giesse, 1993). With the approval of TSP for poultry production, other commercial phosphate blends, such as Avgard™, have been introduced (Giesse 1992; 1993).

Water sprays, with and without bactericides, have also been investigated at various pressure, temperature and concentration combinations for decontaminating food surfaces (Epling *et al.,*1993; Dickson and Anderson, 1992; Smulders and Woolthuis, 1985). Results indicate that there may be some mechanical effects of spraying to remove bacteria from contaminated food surfaces. Overall, very little information has been published that illustrates the significant combination effect

between concentration and spraying of bactericides to improve hygienic quality of food surfaces.

The objective of this study was to determine the effects of pressure, concentration and their synergistic effects during bactericidal application on contaminated turkey carcasses. Response surface designs were used to evaluate these effects. This comprehensive statistical approach was used to effectively evaluate the microbiological quality and safety of contaminated poultry carcasses after bactericide application. Carcass washing procedures were based on spraying technology with short contact time. Antimicrobial agents were limited to lactic acid, chlorine, trisodium phosphate (TSP) and a commercial phosphate blend (i.e. Avgard™).

3.2 Materials and Methods

3.2.1 Sample Preparation

During the experimental period,washed, post-eviscerated, pre-chilled turkey carcasses (n = 15) were collected daily from a local commercial processing plant. Carcasses were weighed, pre-rinsed with water and inoculated with 2 mL of a fecal slurry (1:10 dilution with sterile water) originally collected from the intestines of five different carcasses on each day of the experiment. Anderson and Marshall (1990a) observed that bacteria from fresh manure were more susceptible to acid treatments than organisms grown in broth. Therefore, the fecal slurry used in this study may accurately reflect a poultry processing scenario.

Inoculum was administered to two areas of the carcass. The areas were the inside of the carcass cavity towards the keel bone and on the exterior breast area. A seam roller (5.08 cm; A. Richard Ltd., Bertherville, Que. Product # 724) was used to distribute the inoculum on the surface of the carcass. To evaluate bactericidal treatments, inoculated birds were suspended on a rotating spool in a spray chamber. Each carcass was simultaneously sprayed inside and outside with the bactericidal treatment at 22°C for 10 sec. Treated carcasses were immediately placed in a large plastic bag and rinsed with 500 mL sterile water in a mechanical shaker (McNab *et al.*,1993). The rinse water was retrieved for microbiological analyses. Control samples consisted of the uninoculated carcasses and the carcasses inoculated with the fecal slurry. The number of microorganisms in the slurry itself was evaluated on each day.

3.2.2 Microbiological Analyses

Serial dilutions (11:99 mL of rinse water in 0.1% Peptone Water; Difco Labs., Detroit, MI) were prepared. Undiluted and 10^{-2} dilutions were plated onto Standard Plate Count and MacConkey Agars (Oxoid Ltd., Basingstoke, UK) for the determination of total and coliform counts, respectively, using the Spiral Plater Model D (Spiral Biotech, Inc., Bethesda, MD). Plates were incubated at 37°C for 18 to 24 hrs. Inhibitory effects of the antimicrobial agents remaining in the carcass rinse were minimal because of the dilution used and the small volume (0.0490 mL) required by the Spiral plater to inoculate the plates. The pH of the plating medium

did not change following inoculation with the rinse washes.

Detection of *Salmonella* spp. involved pre-enrichment of 11 mL of the rinse solution in 99 mL of 1.0% Peptone Water incubated at 37°C for 18 to 24 hrs for resuscitation of sublethally injured cells. The pre-enriched sample (0.1 mL) was pipetted onto Modified Semisolid Rappaport Vassiliadis agar (MSRV; Difco Labs.) containing 1 mL/L of 2% novobiocin. Plates were incubated at 37°C for 18 to 24 hrs. The presence of *Salmonella* spp. was indicated by a zone of migration, resulting in a clearing of the MSRV media around the inoculum.

MSRV has been shown to be an excellent medium for the isolation and detection of *Salmonella* spp. (Dusch and Altwegg, 1995).

3.2.3 Experimental Design for the evaluation of bactericidal efficacy

The experiment was conducted using a response surface central composite design (Cochran and Cox, 1957). The treatments were based on four bactericidal treatments applied at five concentrations and five pressures (Table 3.1). Two individual replicates were performed at different occasions. Bactericides used were a hypochlorite solution (i.e., chlorine; Naschem Inc., Mississauga, ON), lactic acid (Fisher Scientific, Fair Lawn, NJ), trisodium phosphate (Na_3PO_4; TSP; Griffith Labs., Toronto, ON) and a commercial phosphate blend (Avgard™, Rhone-Poulenc Food Ingredients, Cranbury, NJ). For each bactericide, there were 13 pressure by concentration treatment combinations, including 5 centre points (Table 3.2).

Table 3.1: Levels of concentration and pressure used for each bactericide for evaluation of efficacy

Level	Lactic acid (% w/w)	Chlorine (ppm)	TSP\Avgard™ (% w/w)	Pressure (psi)
1	0.00	0.00	0.00	40.00
2	1.24	7.32	2.93	47.00
3	4.25	25.00	10.00	65.00
4	7.26	43.00	17.07	83.00
5	8.50	50.00	20.00	90.00

Table 3.2: Example of an experimental setup of concentration and pressure for response surface design using lactic acid

	Combination of levels based on response surface designs		Treatment used	
Treatment number	Concentration	Pressure	Concentration (% w/w)	Pressure (psi)
1	2	2	1.24	47.00
2	2	4	1.24	83.00
3	4	2	7.26	47.00
4	4	4	7.26	83.00
5	1	3	0.00	65.00
6	5	3	8.50	65.00
7	3	1	4.25	40.00
8	3	5	4.25	90.00
9	3	3	4.25	65.00
10	3	3	4.25	65.00
11	3	3	4.25	65.00
12	3	3	4.25	65.00
13	3	3	4.25	65.00

3.2.4 Statistical Analysis of Data

Data were analyzed using the General Linear Model of SAS® (SAS Institute, 1988) after employing $\log_{10}$ transformation to counts. Estimates for the linear, quadratic and interaction effects of each parameter (i.e., concentration and pressure) were developed to fit the following equation:

Equation 1:

$$y = \beta_0 + \beta_1 x_1 + \beta_2 x_2 + \beta_{11} x_1^2 + \beta_{22} x_2^2 + \beta_{12} x_1 x_2 + \varepsilon$$

y= bacterial level at the certain concentration and pressure
x_1 = concentration of bactericide
x_2 = spray pressure used to apply bacteride
β_0 - estimate for the y - intercept
β_1 - estimate for the linear effect of bacteride concentration
β_2 - estimate for the linear effect of pressure used to administer bactericide
β_{11} -estimate for the quadratic effect of bactericide concentration
β_{22} -estimate for the quadratic effect of pressure used to administer bactericide
β_{12} - estimate for the interactive effect between concentration and pressure used to administer bactericide
ϵ = error term

All response surfaces were generated using a software graphics package, Graftools (3-D Vision Corporation: Release 3.3) for response surface modeling.

3.3 Results and Discussion

3.3.1 Chlorine

The effect of concentration and pressure was not significantly different ($p>0.20$) at any chlorine level used in this study (Tables 3.3 a and b). A chlorine concentration of 50 ppm resulted in less than a 1 $\log_{10}$ cfu/mL reduction in both the total count and coliform count (Figures 3.1a and 3.1b). This result is comparable with results obtained by Baran *et al.* (1973) using chlorine dioxide. However, Kotula *et al.* (1974) obtained a similar reduction of bacteria at lower spraying pressures but at a concentration of 200 ppm chlorine and 45 min of application. Emswiler *et al.* (1976) observed a 1 to 2 $\log_{10}$ cfu/mL reduction in total and coliform counts using chlorine dioxide in chill tank water.

The antimicrobial effect of chlorine is based on the availability of chlorine during treatment. Kotula *et al.* (1974) and Emswiler *et al.* (1976) observed residual bactericidal effects of chlorine on beef carcasses after 45 min and 24 hrs of treatment. These effects may be due to a film of chlorinated wash water that was formed on the carcasses (Emswiler *et al.*, 1976). These findings imply that storing

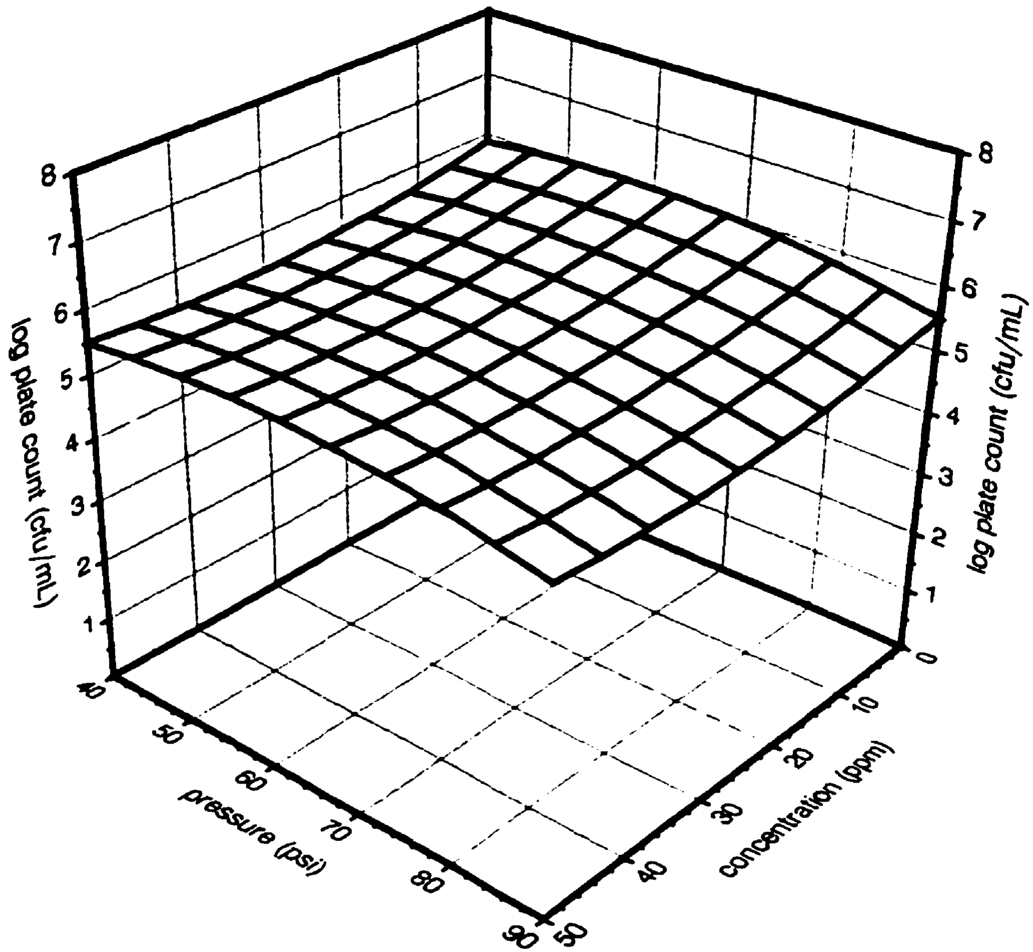


Figure 3.1a: Response surface diagram showing the effect of concentration of chlorine and spray pressure on the total count and on turkey carcasses inoculated with intestinal contents.

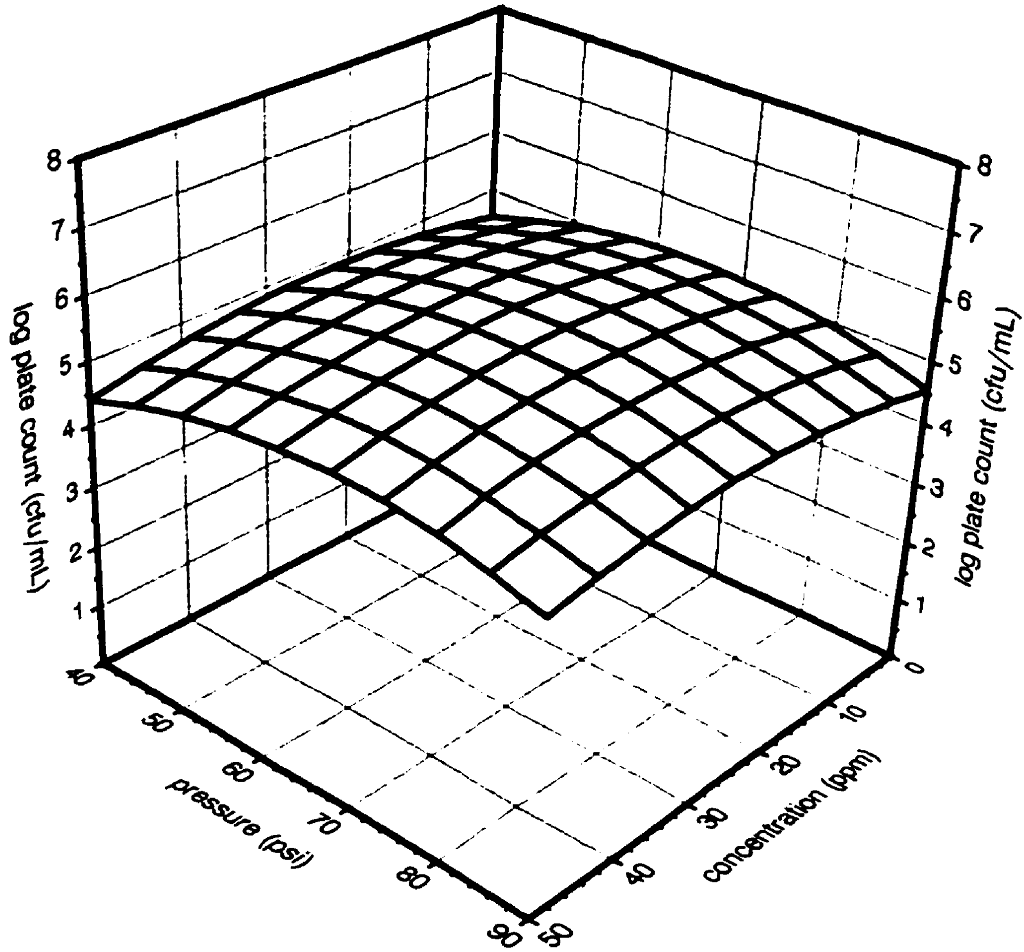


Figure 3.1b: Response surface diagram showing the effect of concentration of chlorine and spray pressure on the coliform count on turkey carcasses inoculated with intestinal contents.

Table 3.3a: **Response surface models describing total aerobic count at different concentrations (x_1) and pressures (x_2) for each bactericide**

Treatment	Model
(i) Coefficients for response surface models	
Chlorine	$y = 4.35^{a} - 0.0275x_1 + 0.0580x_2 + 0.0004x_1^2 - 0.0005x_2^2 + 0.0000x_1x_2$
TSP	$y = 4.52^{a} + 0.0195x_1 + 0.0912x_2 + 0.0015x_1^2 - 0.0006x_2^2 - 0.0015x_1x_2$
Avgard™	$y = 4.80^{a} - 0.0734x_1 + 0.0361x_2 + 0.0022x_1^2 - 0.0003x_2^2 - 0.0001x_1x_2$
Lactic acid	$y = 9.70^{a} - 1.2329x_1 - 0.0678x_2 + 0.1041x_1^2 + 0.0004x_2^2 + 0.0004x_1x_2$

(ii) Significance (i.e., p values) of estimates of response surface model coefficients

Treatment	*y*-intercept[a]	Linear effect of		Quadratic effect of		Interactive
		Concentration	Pressure	Concentration	Pressure	effect
Chlorine	0.9129	0.6115	0.4265	0.4406	0.3431	0.9820
TSP	0.1215	0.8853	0.2122	0.6592	0.2780	0.3992
Avgard™	0.9470	0.5883	0.6199	0.5206	0.5459	0.9551
Lactic acid	0.1215	0.0002	0.3526	0.0001	0.4429	0.9148

[a] *y*-intercepts are biased and are not unique estimators for the model

Table 3.3b: Response surface models describing coliform count at different concentrations (x_1) and pressures (x_2) for each bactericide

Treatment	Model
(i) Coefficients for response surface model	
Chlorine	$y = 1.03^a - 0.0258x_1 + 0.1263x_2 - 0.0006x_1^2 + 0.0009x_2^2 + 0.0001x_1x_2$
TSP	$y = 2.69^a + 0.0771x_1 + 0.1016x_2 + 0.0023x_1^2 - 0.0025x_2^2 - 0.0006x_1x_2$
Avgard™	$y = 7.13^a - 0.1202x_1 + 0.0308x_2 + 0.0079x_1^2 - 0.0020x_2^2 - 0.0002x_1x_2$
Lactic acid	$y = 10.68^a - 1.9625x_1 - 0.0434x_2 + 0.1270x_1^2 + 0.0000x_2^2 + 0.0004x_1x_2$

(ii) Significance (i.e., p values) of estimates of response surface models

Treatment	*y*-intercept[a]	Linear effect of		Quadratic effect of		Interactive
		Concentration	Pressure	Concentration	Pressure	effect
Chlorine	0.9021	0.6367	0.5065	0.5017	0.4233	0.9429
TSP	0.4055	0.8857	0.3057	0.7555	0.705	0.5000
Avgard™	0.8949	0.6852	0.7381	0.6223	0.6779	0.9536
Lactic acid	0.5384	0.0159	0.5045	0.0001	0.4698	0.8650

[a] *y*-intercepts are biased and are not unique estimators for the model

Table 3.4: The elimination of *Salmonella* spp. from turkey carcasses by carcass washing.

Treatment #[1]	*Salmonella* spp. testing using MSRV medium			
	Chlorine	TSP	Avgard	Lactic
1	+\+[2]	+\+	+\+	+\+
2	+\+	+\+	+\+	+\+
3	+\+	+\+	+\-	-\-
4	+\+	+\+	+\-	-\-
5	+\+	+\+	+\-	+\+
6	+\+	+\+	+\+	-\-
7	+\+	+\+	-\+	-\+
8	+\+	+\+	-\-	-\-
9	+\+	+\+	-\-	+\-
10	+\+	+\+	+\-	+\-
11	+\+	+\+	+\-	-\-
12	+\+	+\+	+\-	+\-
13	+\+	+\+	+\-	+\-

[1]Conditions of concentration and pressure are given in Table 3.2.
[2]+\+ Presumptive *Salmonella* spp. isolated from MSRV in both trials.
+\- Presumptive *Salmonella* spp. isolated from MSRV in first trial but not in second trial.
-/+Presumptive *Salmonella* spp. isolated from MSRV in second trial but not in first trial
-/-Presumptive *Salmonella* spp. not isolated from MSRV in either trial.

treated carcasses may increase the antimicrobial potential of chlorine. The relatively low reductions in microbial load in this study may be due to evaporation of the chlorine (Teotia and Miller, 1975), the short contact time and/or the low level of chlorine used during treatment. Emswiler *et al.* (1976) observed 1 to 2 $\log_{10}$ cfu/mL reduction in total microbial load at 100 to 400 ppm chlorine. Thiessen *et al.* (1984) observed greater reductions in total and coliform counts using chlorine treatments. However, it has been reported that chlorine can become inactivated in the presence of organic matter (Baran *et al.*,1973; Mead and Thomas, 1973b; Teotia and Miller, 1975). For example, Baran *et al.* (1973) reported that levels of active chlorine dropped to less than 1 to 2 mg/mL upon contact with turkey surfaces.

Presumptive testing indicated that chlorine did not eliminate *Salmonella* spp. from carcasses at any of the levels used (Table 3.4). Others have reported that higher chlorine concentrations are effective. Teotia and Miller (1975) reported that up to 3 400 ppm of chlorine was necessary to reduce *Salmonella* on turkey. Chlorine dioxide at 1.33 mg/L (Thiessen *et al.*,1984) and 200 ppm chlorine (Morrison and Fleet, 1985) were effective against *Salmonella* spp. on carcasses. However, Teotia and Miller (1975) reported that high chlorine concentration had caused adverse effects on the physical appearance and flavour of poultry meat. At levels of 60 ppm, chlorine still caused off-flavours in turkey meat (Wabeck *et al.*,1968).

Overall, indications are that the chlorine concentration and pressure combinations used in this study did not significantly reduce microbial load or

eliminate *Salmonella* spp. from carcasses. Reports of higher concentration of chlorine have shown to be effective (Wabeck, 1994). Unfortunately, regulatory limits restrict the level of chlorine between 20 to 50 ppm (Dye and Mead, 1972; Morrison and Fleet, 1985). Therefore, higher concentrations of chlorine cannot be used due to government regulations specifying allowable limits for food use.

3.3.2 Trisodium Phosphate

Trisodium phosphate caused a maximum 1.8 and 1.7 $\log_{10}$ cfu/mL reduction in total and coliform counts, respectively (Figures 3.2a and 3.2b). However, these reductions were not significant ($p>0.20$) for any of the pressures and concentrations used in this study (Tables 3.3 a and b). Comparable results were obtained by Kim *et al.* (1994a). The antimicrobial properties of TSP may be due to the detachment of contaminants from skin surfaces and its high alkalinity (Kim *et al.*,1994a). Other reports indicate that the antimicrobial effects of TSP will be more apparent during storage of treated carcasses (Thompson *et al.*,1979; Kim *et al.*,1994b).

Presumptive testing indicated that TSP did not affect *Salmonella* spp. at any concentration and pressure combinations (Table 3.4). The inadequacy of this treatment was also reported by Morrison and Fleet (1985). They observed that phosphates (3%) used for immersion of carcasses did not significantly reduce the total and *Salmonella* spp. counts compared to untreated controls.

The results obtained suggest that TSP is not an effective bactericide for

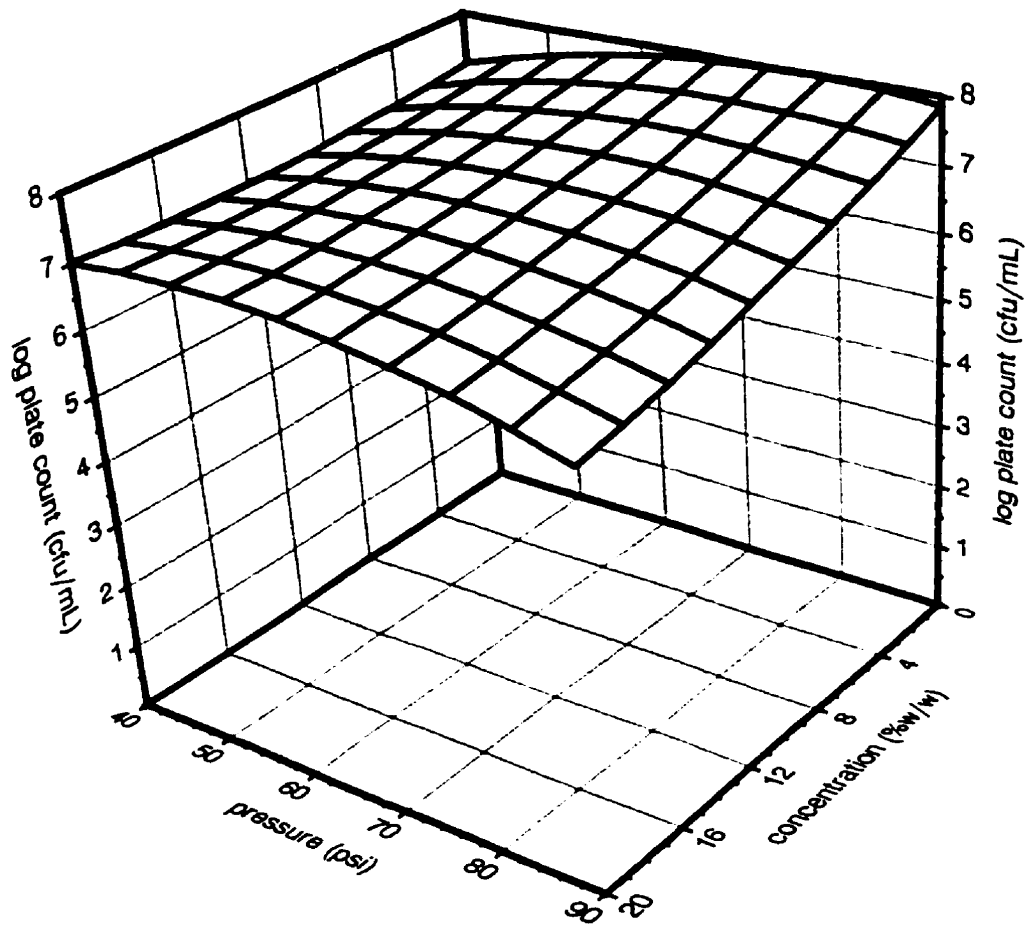


Figure 3.2a: Response surface diagram showing the effect of concentration of trisodium phosphate and spray pressure on the total aerobic count on turkey carcasses inoculated with intestinal contents

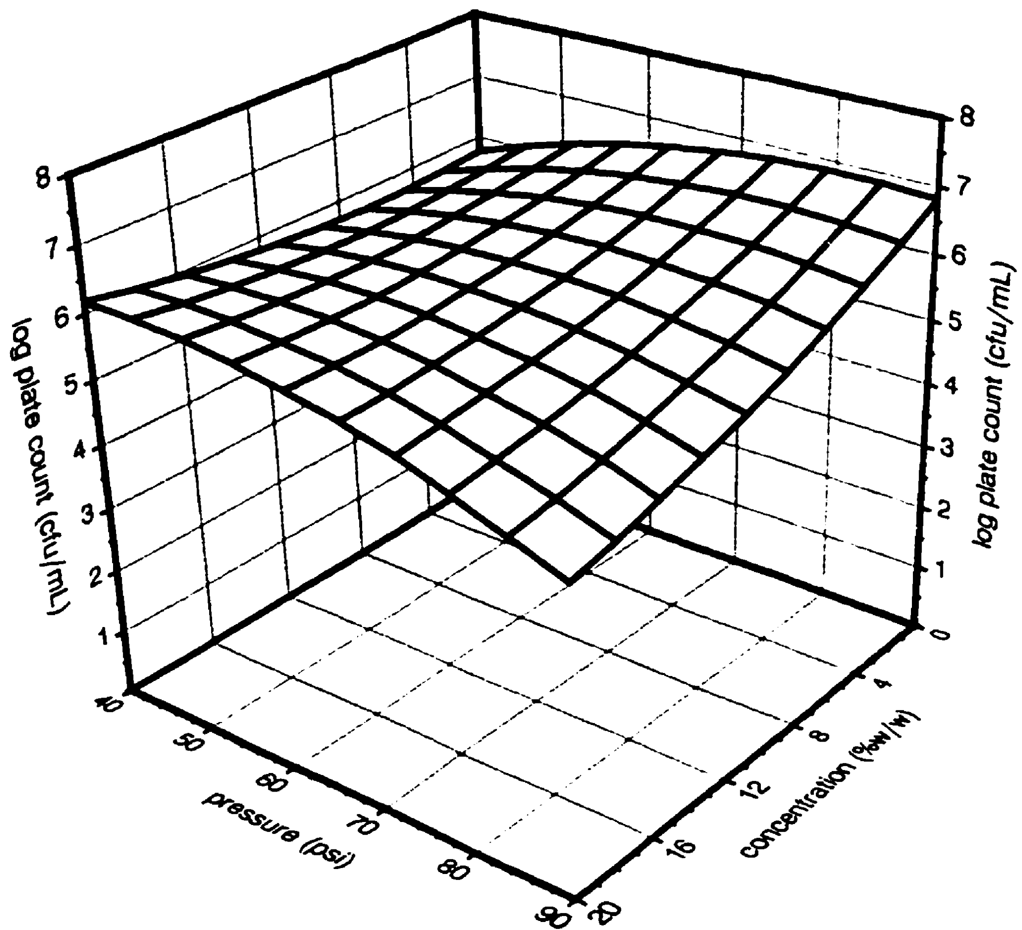


Figure 3.2b: Response surface diagram showing the effect of concentration of trisodium phosphate and spray pressure on the coliform count on turkey carcasses inoculated with intestinal contents

poultry processing at the concentrations and pressures used in this study. Even at concentrations of 20%, TSP was still ineffective, as well as difficult to solubilize.

3.3.3 Avgard™

Avgard™ resulted in a maximum 2.3 and 1.3 $\log_{10}$ cfu/mL reductions in total and coliform counts, respectively (Figures 3.3 a and 3.3b). However, the efficacy of this bactericide was not significant ($p>0.20$) at any of the pressure and concentration combinations (Tables 3.3 a and b). Ward (1992) reported that low concentrations of Avgard™ reduced bacterial levels by 2 $\log_{10}$ cfu/mL on chicken carcasses. They attributed the reduction to the sensitivity of coliforms.

Presumptive testing indicated that Avgard™ may affect the level *of Salmonella* spp. on carcasses (Table 3.4). Giesse (1993) reported that Avgard™ may be less effective at reducing the total microbial load than *Salmonella* spp. However, there was no distinct pattern of efficacy with increasing concentration of Avgard™ for both replicates in this study.

The results indicate that Avgard™ would not be highly effective in decontaminating poultry carcasses at the concentrations and pressures used in this study.

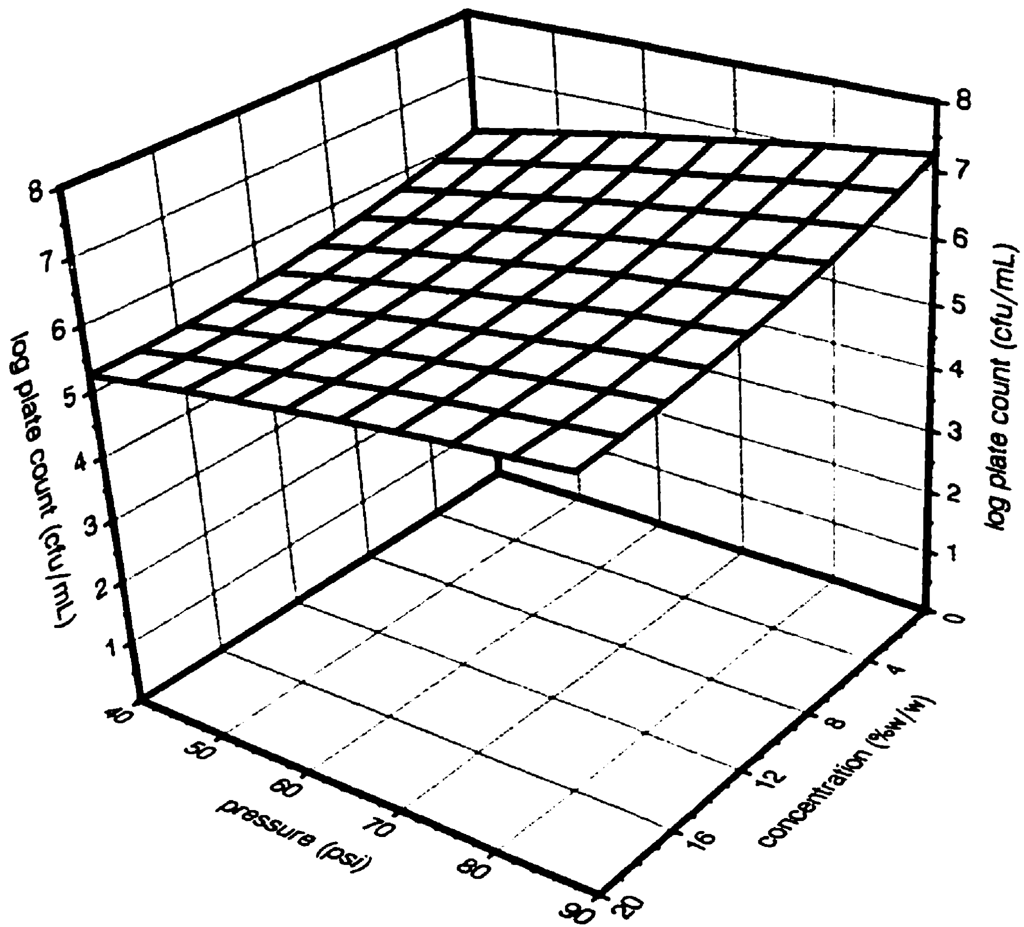


Figure 3.3a: Response surface diagram showing the effect of concentration of Avgard™ and spray pressure on the total aerobic count on turkey carcasses inoculated with intestinal contents

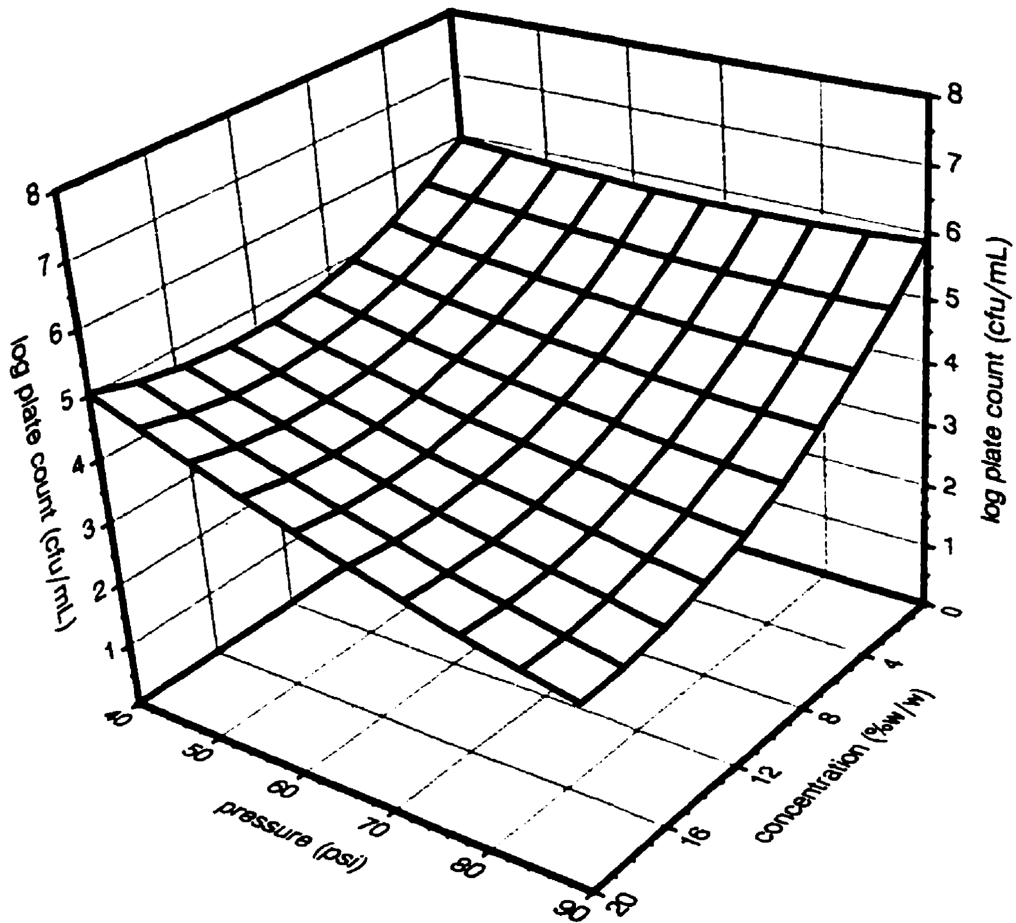


Figure 3.3b: Response surface diagram showing the effect of concentration of Avgard™ and spray pressure on the coliform count on turkey carcasses inoculated with intestinal contents

3.3.4 Lactic Acid

The concentration of lactic acid had a significant ($p<0.05$) impact on total and coliform counts (Tables 3.3 a and 3b). At a lactic acid concentration of 1.24% (w/w), total aerobic bacteria were estimated to be approximately 2.4 $\log_{10}$ cfu/mL lower than the initial inoculation level. Snidjers *et al.* (1985) and Anderson and Marshall (1990b) observed similar results for lactic acid. Microbial levels were even further reduced (4.4 $\log_{10}$ cfu/mL) at 4.25% (w/w) lactic acid. At the same lactic acid concentration, coliform counts were 5.5 $\log_{10}$ cfu/mL lower than the original inoculated controls. This is typical since various reports have indicated that Enterobacteriaceae are sensitive to higher lactic acid concentrations (Gill and Newton, 1982; Snijders, *et al.*,1985, Van der Marel *et al.*,1988; Zeitoun and Debevere, 1990). The effectiveness of lactic acid is based on the nature of initial contamination, concentration of the solution and the stage at which it is applied (Smulders, 1987). The high acid concentrations (1.24 to 8.5% w/w) used in the present study were more effective than the lower concentrations cited in the literature (Woolthius *et al.*,1984). Other researchers demonstrated delayed antimicrobial effects of lactic acid (Snijders *et al.*,1985; Shelef, 1994; Woolthius *et al.*,1984; Prasai *et al.*,1991). The delayed effect has been attributed to an increase in acidity on the carcass tissues. This would favour the growth of lactic acid bacteria and inhibit gram-negative bacteria (Anderson *et al.*,1992; Anderson and Marshallb, 1990b; Siragusa and Dickson 1993). Lactic acid concentrations of 1 to 2%

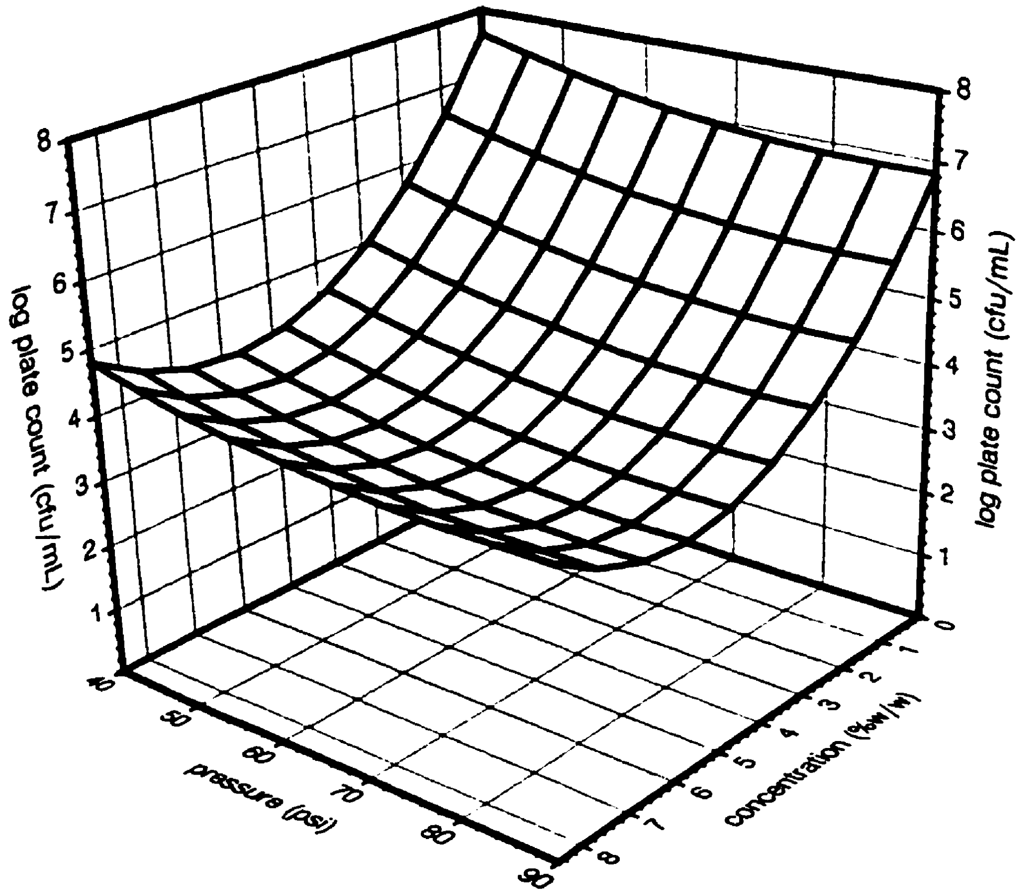


Figure 3.4a: Response surface diagram showing the effect of concentration of lactic acid and spray pressure on the total count on turkey carcasses inoculated with intestinal contents

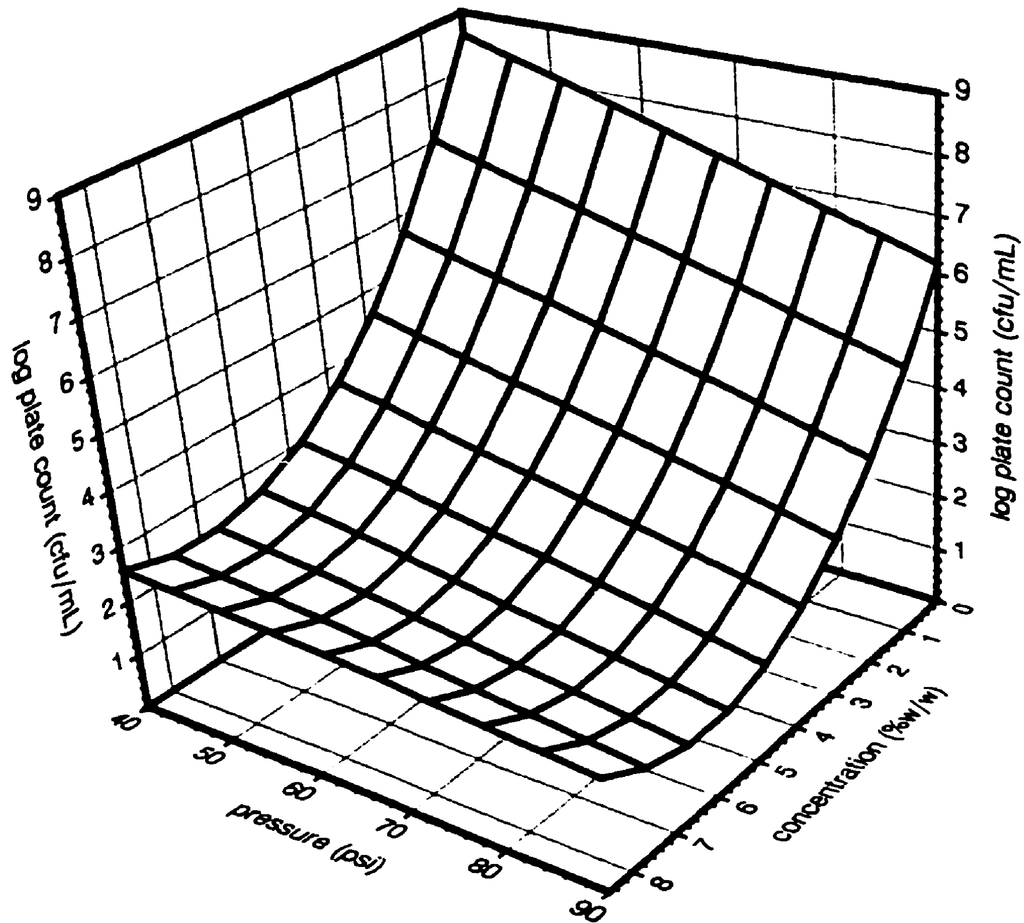


Figure 3.4b: Response surface diagram showing the effect of concentration of lactic acid and spray pressure on the coliform count on turkey carcasses inoculated with intestinal contents

have been shown not to produce immediate changes to the sensory quality of poultry (Smulders, 1987).

Presumptive testing for *Salmonella* spp. indicated that a lactic acid concentration as low as 4.25% (w/w) could be effective against *Salmonella* spp. (Table 3.4). Other work has also shown that lactic acid is effective against *Salmonella* spp. as well as *Campylobacter* spp. (Izat *et al.*,1990; Lillard, 1989; Prasai *et al.*,1991; Epling *et al.*,1993). In one report, *Salmonella* spp. were affected by a 120 second contact time, at 37°C in a pre-chill dip (Izat *et al.*,1990).

Van der Marel *et al.* (1988) had also observed a significant improvement of bacterial safety and shelf life of broiler carcasses. They observed 1.5 and 1.2 $\log_{10}$ cfu/mL reductions of mesophilic bacteria and *Enterobacteriaceae*, respectively, on chicken carcasses treated with 1 to 2% lactic acid prior to chilling. They used similar concentrations of lactic acid but as an immersion treatment for 15 sec.

In this study, the overall mechanical action of spraying with lactic acid was more effective than the immersion techniques described by Van der Marel *et al.* (1988) for reducing microbial levels (Figures 3.4 a and b). However, increasing the spray pressure did not significantly ($p>0.20$) improve the efficacy of the lactic acid treatment (Table 3.3 a and b). The enhanced bactericidal effect of lactic acid in this study may be due to the utilization of freshly processed, pre-chilled (post-eviscerated) carcass. It may also be possible that early application of the

bactericide to the carcasses may suppress bacterial attachment as well as growth (Snijders *et al.*,1985).

Lactic acid treatment at a concentration >1.24% used in this study caused discolouration of the skin tissues. This observation has been previously seen with concentrations greater than 1.25% (Mulder *et al.*,1987; Izat *et al.*,1990; Snijders *et al.*, 1985). Discolouration of the skin may be the result of oxidation reactions (Mendonca *et al.*,1989) and is more apparent with increasing concentration. However, the discolouration can be reversed with other treatments such as colour stabilizers or water immersion during the chilling process (Snijders *et al.*,1985). Although discolouration of the skin occurred in the present study, other sensory qualities may not have been affected since the skin acts as a protective layer preventing penetration of acid residues into the muscle tissues (Van der Marel *et al.*,1989).

Since a maximum reduction in total counts was obtained at 4.25% lactic acid in this study (calculated from Table 3.2a and 3.2b), it may be possible to use lower concentrations of the acid at higher temperatures to enhance the effects of the treatment. However, the reduction in the acid concentration and the use of an elevated temperature must also be evaluated for its effectiveness and economic feasibility and sensory effects.

3.4 Conclusions

The results of this research suggest that lactic acid is the most promising bactericide for use in decontaminating carcasses prior to chilling. Application of lactic acid could significantly improve the microbiological quality and safety of contaminated poultry carcasses. Furthermore, the low concentration of lactic acid required to reduce microorganisms on carcasses would have good economic and environmental implications for the poultry industry. The issues of discolouration can be minimized with shorter contact times, immediate rinsing with water and subsequent water chilling. Presumptive tests indicated that lactic acid and Avgard™ were effective against *Salmonella* spp. However, the smaller reductions in total and coliform count obtained by chlorine, TSP and Avgard™ may have been due to the presence of organic material which reduced the efficacy of these compounds. Furthermore, pressure did not significantly ($p>0.05$) influence the levels of total and coliform count on carcasses for any of the four bactericides.

4.0 Optimization of lactic acid solution used to treat contaminated poultry surfaces

4.1 Introduction

Due to the steady decline in the number of slaughtering plants and the rising demand for poultry meat, processors are under pressure to increase productivity and capacity of their facilities (Duewer *et al.,*1993; Van der Sluis, 1994; Austic and Nesheim, 1990). In consequence, the increased level of processing may intensify hygiene problems during meat production (Mead, 1989). One particular area of concern is contamination during poultry processing.

Contamination of poultry carcasses with fecal material can present problems for the meat industry. As a solution, the meat industry will either rework the carcass (e.g., hot deboning) to retrieve uncontaminated parts or completely remove it from the process (i.e., condemned). In either case, there is a loss of revenue. A report by Hundt (1995) estimated that fecal contamination constitutes a loss to the poultry industry of nearly $8 million annually. There have been attempts to improve the situation through better hygiene practices but they have not been entirely successful (Mossel, 1984).

Sanitizing carcasses has been suggested as a means of improving meat

quality and safety (Wabeck, 1994). Many types of sanitizers have been proposed for a variety of meat products (Siragusa, 1995) but the one that shows the most promise is lactic acid (Shelef, 1994; Bautista *et al.*,1995b). Lactic acid is a GRAS substance that has been widely used in other food applications. Some of the major uses of lactic acid are flavour enhancement, prevention of butyric fermentation in beer and suppression of crystallization in hard candies (Shelef. 1994).

Lactic acid has several antimicrobial properties. It can denature proteins along the bacterial membrane, destabilize its internal pH and disrupt cellular functions within the cell (Eklund, 1980; Tastayre and Holley, 1986; Mountney and O'Malley, 1965; Smulders and Woolthuis, 1985; Gould *et al.*,1983). To improve the efficacy of lactic acid treatments, research has been directed towards studying the effects of higher application temperatures (Prasai *et al.*,1991) and combination with sodium lactate to provide buffering capacity during storage (Zeitoun and Debevere, 1990; 1991).

In light of previous research, the objective of this study was to determine the optimum pH and application temperature for a lactic acid/sodium lactate buffered solution for the decontamination of poultry carcasses.

4.2 Materials and Methods

4.2.1 Microbial efficacy of buffered lactic acid

4.2.1.1 Sample preparation

During the experimental period,washed, post-eviscerated, pre-chilled broiler hen carcasses (n=15) were collected daily from a local commercial processing plant. Carcasses were pre-rinsed with water and inoculated with 2 mL of a fecal slurry (1:10 dilution with sterile water) originally collected from the intestines of two different carcasses on each day of the experiment. Anderson and Marshall (1990) observed that bacteria from fresh manure were more susceptible to acid treatments than organisms grown in broth. Therefore, the fecal slurry used in this study may more accurately reflect a poultry processing scenario.

Inoculation was carried out according to the procedures described in Section 3.2.1

4.2.1.2 Microbiological Analyses

The same microbiological analyses were used as in Section 3.2.2.

4.2.1.3 Experimental design for the evaluation of bactericidal efficacy of buffered lactic acid at various temperature and pH combinations

The experiment was conducted using a response surface central composite design (Cochran and Cox, 1957). The treatments were based on buffered lactic acid used at concentration of 4.25% w/w. A previous study by Bautista *et al.*,(1996b) indicated that this concentration of lactic acid was most effective against total aerobic bacteria and coliforms (see Section 3.0). Treatments were applied at nine pH and temperature combinations. Two individual replicates were performed at different occasions. A summary of pH levels and dispensing temperature for the lactic acid/sodium lactate treatment is presented in Table 4.1. There were 13 treatment combinations of pH and application temperatures, including five centre points that were used for experimentation with response surface designs (Table 4.2).

4.2.1.4 Statistical Analysis of Data

Data were analyzed using the General Linear Model of the Statistical Analysis System SAS® (SAS, 1988) after employing a $\log_{10}$ transformation. Estimates for the linear, quadratic and interaction effects of each parameter (e.g., pH and temperature) were developed to fit the following equation:

Equation 1:

$$y = \beta_0 + \beta_1 x_1 + \beta_2 x_2 + \beta_{11} x_1^2 + \beta_{22} x_2^2 + \beta_{12} x_1 x_2 + \varepsilon$$

y = bacterial level at a specific pH and application temperature combination
x_1 = pH of lactic acid (4.25% w/w)
x_2 = application temperature of lactic acid solution (at 4.25% w/w)
β_0 - estimate for the y - intercept
β_1 - estimate for the linear effect by pH of solution
β_2 - estimate for the linear effect by temperature of solution
β_{11} - estimate for the quadratic effect by pH of solution
β_{22} - estimate for the quadratic effect by temperture of solution
β_{12} - estimate for the interactive effect between pH and temperature of solution
ϵ - error term

Table 4.1: Levels of pH and temperature used to determine maximum efficacy of the buffered lactic acid/sodium lactate solution for treatment on contaminated carcasses

Level	pH	Temperature
1	2.5	10
2	2.9	20
3	3.5	35
4	4.1	50
5	4.5	60

Table 4.2: An experimental setup to determine optimal conditions based on pH and dispensing temperature by a response surface design

	Combination of levels based on response surface designs		Treatments used for experiment	
treatment number	pH	dispensing temperature (°C)	pH	dispensing temperature(°C)
1	2	2	2.9	20
2	2	4	2.9	50
3	4	2	4.1	20
4	4	4	4.1	50
5	1	3	2.5	35
6	5	3	4.5	35
7	3	1	3.5	10
8	3	5	3.5	60
9	3	3	3.5	35
10	3	3	3.5	35
11	3	3	3.5	35
12	3	3	3.5	35
13	3	3	3.5	35

4.3 Results and Discussion

The effect of pH, and temperature of lactic acid at 4.25% (w/w) on total aerobic bacterial and coliform count is illustrated in Figure 4.1a and 4.1b, respectively, and mathematically represented in Table 4.3.

The results indicate that organisms were significantly ($p<0.05$) affected by temperature during application and not significantly ($p<0.15$) affected by pH of the lactic acid/ sodium lactate solution. However, by removing the quadratic function of the model, the pH of the solution was shown to significantly ($p<0.05$) affect the aerobic plate counts. The interaction effect between pH and temperature did show a significant synergistic effect ($p < 0.10$). However, no significant change of the interaction was observed by removing the quadratic function from the model. Therefore, the results show that the efficacy of buffered lactic acid is dependant on the temperature during treatment and may be influenced by the pH of the solution. Depending on the treatment conditions, the reduction of total aerobic bacteria after treatment varied from 2 to 6 $\log_{10}$ cfu/mL in comparison with non-treated samples.

Coliforms were initially reduced by 2 to 3 $\log_{10}$ cfu/mL after carcasses were treated with lactic acid (4.25% w/w). This is in contrast to Zeitoun *et al.* (1994) who were only able to achieve a 1 $\log_{10}$ cfu/mL reduction of Enterobacteriaceae counts with buffered lactic acid (pH = 3.0) at a concentration of 10% (w/v). For this

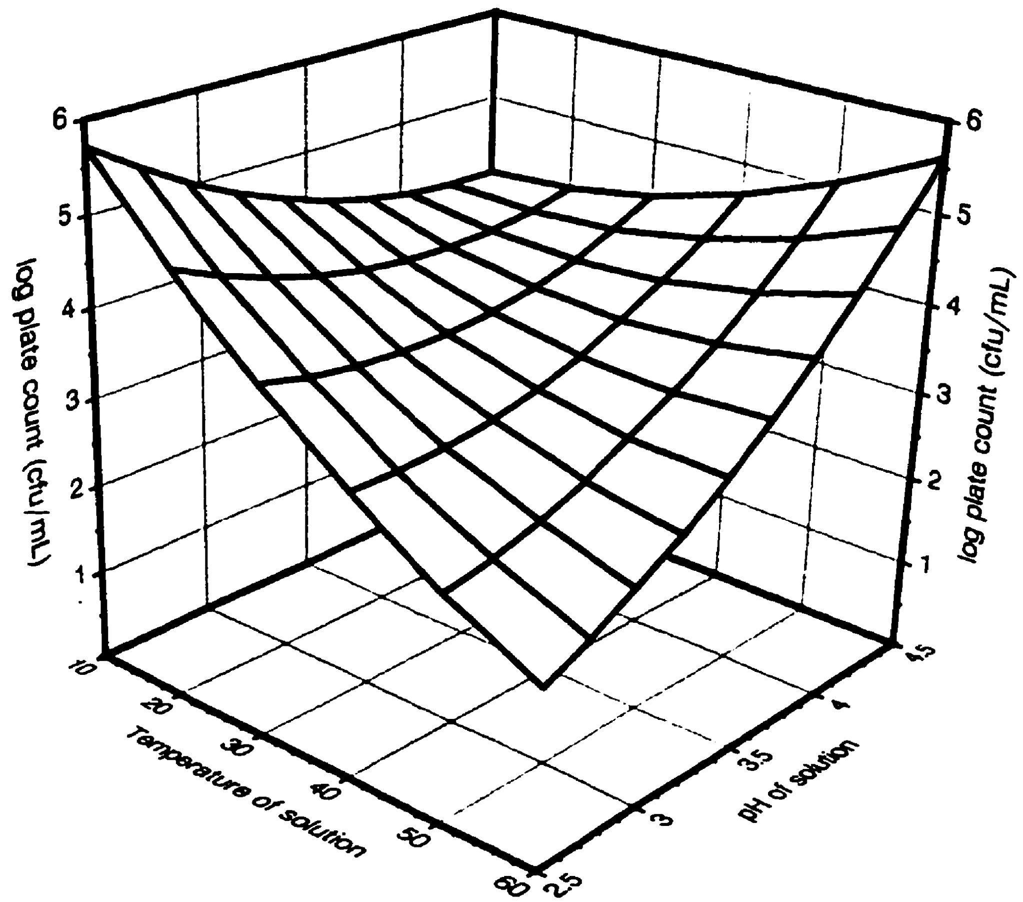


Figure 4.1a: Response surface diagram showing the effect of temperature and pH of lactic acid on the total aerobic count on chicken carcasses inoculated with intestinal contents

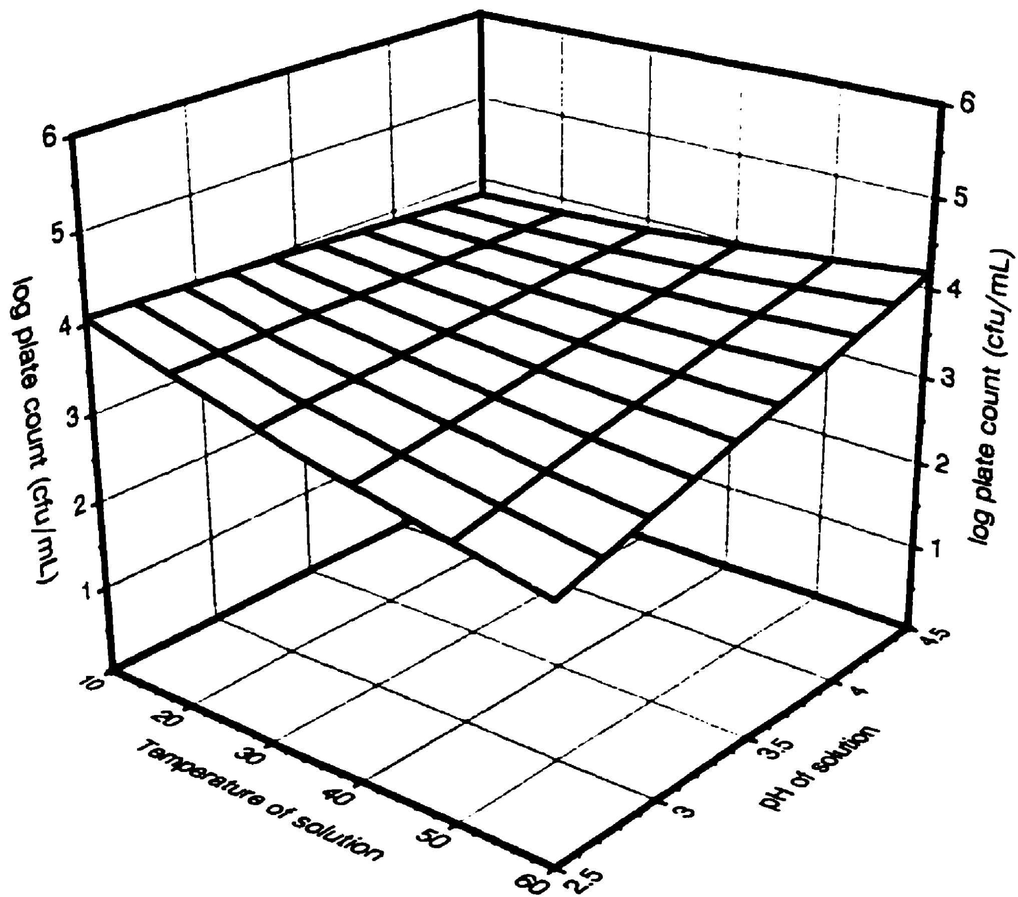


Figure 4.1b: Response surface diagram showing the effect of temperature and pH of lactic acid spray on the coliform count on chicken carcasses inoculated with intestinal contents

Table 4.3: Response surface models describing total aerobic count and coliform count at different temperature (x_1) and pH (x_2) for lactic acid treatments

Analysis	Model

(I) Coefficients for response surface model

Aerobic	$y = 15.3224^{a} - 0.247297x_1 - 4.5451x_2 - 0.0006x_1^2 + 0.4569x_2^2 + 0.05277x_1x_2$
Coliform	$y = 4.4379^{a} - 0.0740x_1 - 0.2938x_2 + 0.0001x_1^2 - 0.0001x_2^2 - 0.0161x_1x_2$

(ii) Significance (i.e., p values) of estimates of response surface models

Treatment	y-intercept[a]	Linear effect of		Quadratic effect of		Interactive
		Temperature	pH	Temperature	pH	effect
Aerobic	0.2882	0.0268	0.1143	0.3313	0.2254	0.0635
Coliform	0.5444	0.6265	0.9420	0.8798	0.9998	0.6832

[a] *y*-intercepts are biased and are not unique estimators for the model

study, the reduction in coliform counts were neither significantly ($p > 0.20$) affected by pH nor by temperature of the treatments (Figure 4.1b and Table 4.3).

Presumptive *Salmonella* spp. testing resulted in positive identification of the organism on carcasses for all of the pH and temperature combinations of the lactic acid/sodium lactate treatments. Therefore, the results suggest that the effect of lactic acid on *Salmonella* spp. organisms is based on the concentration of lactic acid and is not more effective with pH or temperature adjustments. Other studies have shown that the bactericidal effect is based on the concentration of the lactic acid treatment (Bautista *et al.*,1996b; Zeitoun and Debervere, 1991; 1994).

The findings suggest that lactic acid (4.25% w/w) should be dispensed at a temperature of 60°C and at pH 2.5 (i.e., 0% sodium lactate). Unfortunately, it was determined by some representatives of the poultry industry that the conditions prescribed for lactic acid/sodium lactate would not be economical. This premise was based on the corrosive nature of lactic acid on stainless steel equipment and the cost associated with operating at high temperatures (i.e. 60°C). These opinions were based on subjective reviews of previous reports on the use of lactic acid utilized during processing (Shelef,1994; Wabeck, 1994).

Upon further consultation with the poultry industry, it was decided to use the lactic acid/sodium lactate treatment with a dispensing temperature of 40°C and at pH 3.0.

4.4 Conclusions

Based on the encouraging results illustrated in this study, lactic acid may play a key role in controlling post-evisceration contamination. The results show that lactic acid/sodium lactate treatment can reduce microbial levels on poultry carcass tissues. Although coliform counts were initially reduced, pH and temperature of the lactic acid/sodium lactate solution did not significantly ($p > 0.20$) change the coliform population.

The only drawback seems to be the slight discolouration of the skin by lactic acid treatments. However, it should not pose a major problem because preliminary work has shown that chilling with water can be used to minimize discolouration. In addition, a qualitative assessment is required to ascertain whether or not lactic acid treatments can impart undesirable qualities onto the meat. This should help to determine the acceptability among consumers. Future work was conducted to investigate the reversal of discolouration effects on meat surfaces produced by lactic acid and an organoleptic assessment of poultry subjected to lactic acid treatments.

5.0 In-plant application of an optimized lactic acid treatment for the improvement of hygiene on fecal contaminated turkey carcasses

5.1 Introduction

Most bactericidal application studies on food products have been laboratory based and few investigations have been performed at food processing establishments. Recently, Avgard™ was used to treat poultry products at a processing plant. The results indicated that Avgard™ had an effect on *Salmonella* spp. on poultry products (Rhone-Poulenc, 1994; personal communication). However, the results that were generated were questionable. Also, the results were unable to demonstrate significant differences between test groups. Nevertheless, Avgard™ was approved by the USDA for the treating poultry carcasses (Giesse, 1993).

In light of this situation, more objective and comprehensive statistical methods should be stressed when new washing techniques are introduced into the food industry. There are many statistical designs available that could aid with large scale experiments at food processing establishments (Cochran and Cox, 1957). Poultry processors are encouraged to investigate this approach as a more accurate

way of determining of the efficacy of bactericidal treatments.

Due to the encouraging results with lactic acid treatments (Section 3.0 and 4.0), a "pilot-scale" study was designed to investigate the "in-plant" application of lactic acid/sodium lactate treatment to reduce microbial levels on condemned turkey carcasses.

5.2 Materials and Methods

5.2.1 On-site laboratory setup for treatment and microbial analysis

For treatment of carcasses, an area within a commercial poultry processing plant was chosen that was not heavily exposed to cross contamination from processing and totally removed from the slaughter area.

Microbiological testing was performed in an area that was free from positive ventilation, fully enclosed, and removed from the normal traffic areas used by workers.

5.2.2 Types of carcasses used for analysis of hygiene

At the plant, several types of carcasses were examined for microbial contamination to make an objective comparison to carcasses treated with lactic acid. The following categories were used to differentiate between carcasses. To

make sampling and the treatment manageable, the experiment was restricted to turkey hens.

I) Healthy carcasses - These are carcasses that have passed initial inspection. Sampling occurred at a point right after first inspection.

II) Condemned carcasses - These are carcasses that have been condemned by meat inspection due to heavy fecal contamination and removed from further processing. Sampling occurred at a point right after first inspection.

III) Regular carcasses - These are carcasses that were passed by initial inspection and final inspection. Sampling occurred at a point right after carcasses were treated with an inside/outside wash.

IV) Condemned turkey carcasses treated with lactic acid - These are carcasses that have been condemned by meat inspection due to heavy fecal contamination, removed from further processing and treated with a prescribed lactic acid treatment. Condemned carcasses were taken immediately after inspection and treated with a prescribed lactic acid solution at a concentration of 4.25 % (w/w), buffered to pH 3.0 with sodium lactate and dispensed at 40°C. Condemned carcasses were suspended on a rotating spool in a spray chamber and treated by spraying both inside and outside with the lactic acid treatment for 10 sec.

5.2.3 Sample preparation for microbial analysis

Immediately after sampling or right after the lactic acid treatment, carcasses were placed in a large plastic bag and rinsed with 500 mL sterile water in a mechanical shaker (McNab *et al.*,1993) The rinse water was retrieved for microbiological analysis.

5.2.4 Microbial Analysis

Serial dilutions (11:99 mL of rinse water in 0.1% Peptone Water; Difco Labs., Detroit, MI) were prepared. Undiluted and 10^{-2} dilutions were plated onto Standard Plate Count and Coliform agars using Petrifilm™ (3M, Inc.) for determining total and coliform counts, respectively. Petrifilms™were incubated at 37°C for 18 to 24 hrs before results were analysed.

5.2.5 Experimental Design and Statistical Analysis

The experiment was designed as a randomized incomplete block (Cochrane and Cox, 1957). For each day of production, 5 flocks of poultry were examined for each of the carcass types in Section 5.2.2. The experiment was replicated over 4 days giving a total of 80 observations (i.e., $5 \times 4 \times 4 = 80$). Data were analyzed using the General Linear Model of the Statistical Analysis System SAS® (SAS, 1988) after employing $\log_{10}$ transformation.

5.3 Results and Discussion

A student t-test analysis of microbial analyses ($n = 3$) performed on 2 different occasions ($3 \times 2 = 6$) from a single carcass rinse showed no significant ($p > 0.20$) difference, indicating that repeatability of microbial analysis was good for each carcass sample. The Univariate and General Linear Procedure of SAS® (1988) indicated that the data were normally distributed.

Microbial counts on each carcass type were significantly ($p < 0.001$) different from each other. The Least Square means of aerobic and coliform counts are illustrated in Figures 5.1 and 5.2, respectively. The average microbial counts of condemned birds were significantly higher ($p < 0.001$) than the other carcass types.

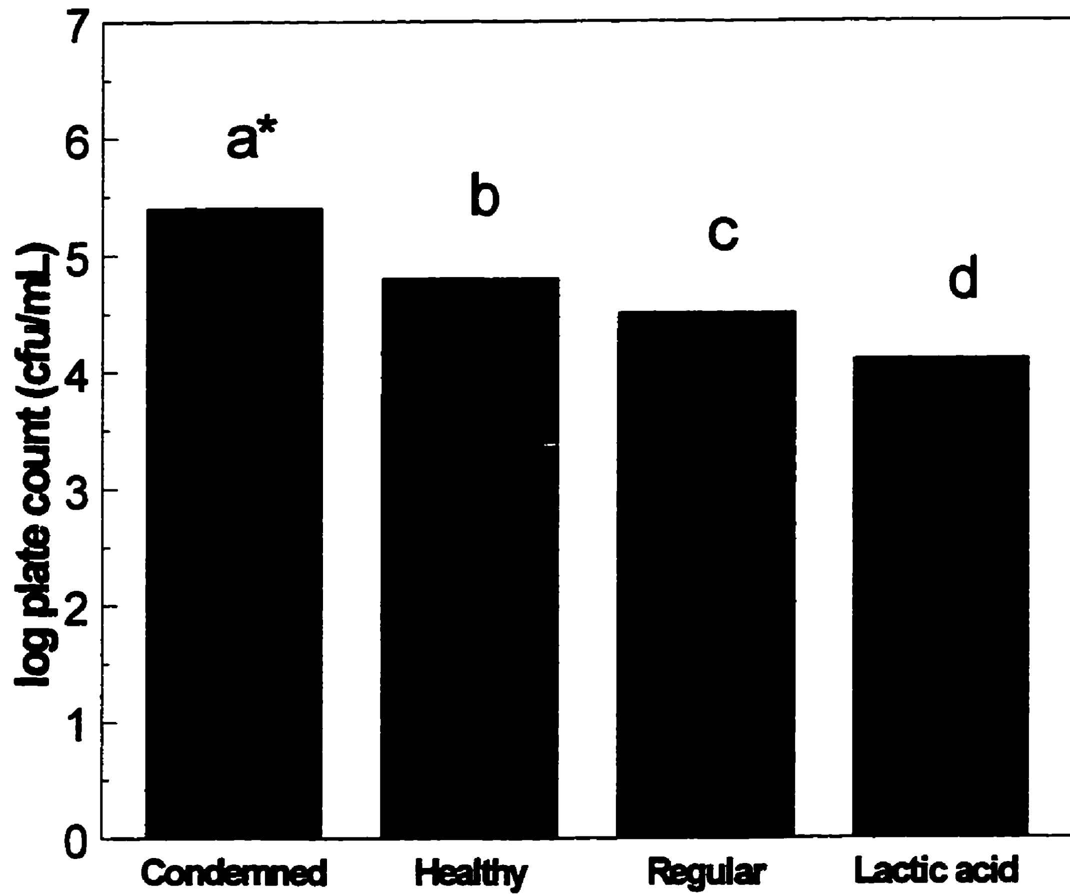


Figure 5.1: Least mean squares of aerobic plate count from several carcass categories under various treatment conditions

(* Means with different letters are significantly different; $p < 0.05$)

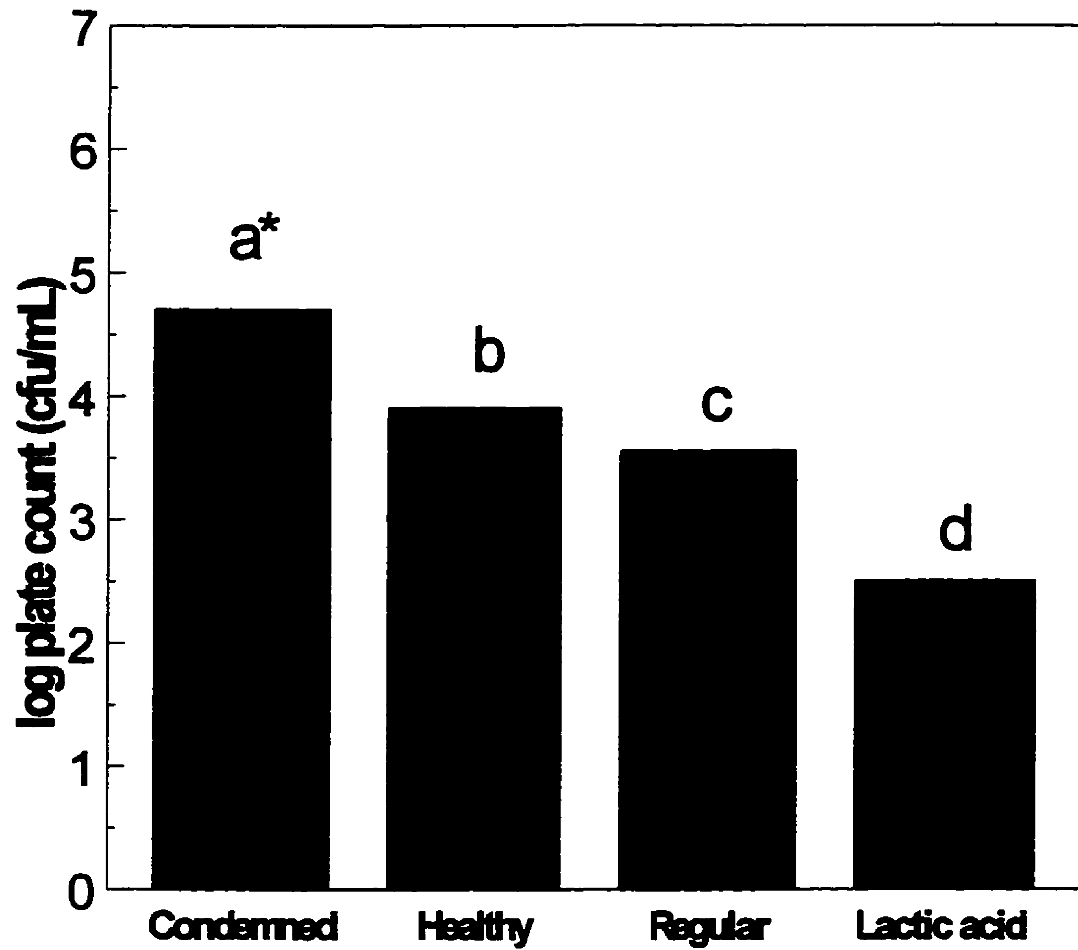


Figure 5.2: Least mean square of coliform count from several carcass categories under various treatment conditions

(* Means with different letters are significantly different; $p < 0.05$)

The order of contamination associated with each carcass type was condemned > healthy > regular > treated. The lowest microbial counts were found on birds treated with the optimized lactic acid treatment. The overall counts on these carcasses were at least 1 log_{10} cfu/mL lower than condemned birds and, on average, better than regular processed birds.

Of the studies that used lactic acid at meat processing plants, results showed a similar reduction in the level of microorganisms on pork, beef and poultry products (Smulders, 1987; Van der Marel *et al.*,1988). A report by Van Netten *et al.*,(1995) demonstrated that 100 cfu/cm^2 of *S. typhimurium* were eliminated from pork carcasses when treated with 2% lactic acid at 55°C for 60 sec during production.

5.4 Conclusions

The results show that a lactic acid/sodium lactate treatment, (Section 4.0) can improve the microbiological quality of contaminated poultry carcasses. However, the reduction of bacterial counts found in this and other studies still needs to be investigated for practical significance. It may be useful to conduct a shelf-life study to determine overall improvement in hygiene and safety of poultry products treated with lactic acid.

Although this treatment was mainly intended for use with condemned carcasses, it may be possible to implement the treatment for all carcasses during processing. With proper engineering, lactic acid treatments could improve the quality of carcasses during production.

6.0 Organoleptic analysis of turkey carcasses treated with lactic acid

6.1 Introduction

The need for methods to improve carcass hygiene is of importance to the poultry industry. Presently, the Canadian poultry industry is investigating the use of bactericides for decontamination of carcasses. One bactericide that is of interest is lactic acid. It has been demonstrated, in this thesis and by other studies, that lactic acid can significantly ($p<0.05$) reduce the level of bacteria on meat samples (Bautista *et al.*,1996b; Smulder, 1987; Van Netten, 1995). Unfortunately, lactic acid treatments have been shown to produce adverse effects on the organoleptic quality of meat products (Reynolds and Carpenter, 1974; Ingham, 1989; Mendonca *et al.*,1989).

Lactic acid treatments at high concentrations (>3%) will cause a notable discolouration of meat surfaces (Van Netten *et al.*,1995). At lower concentrations (~1.24% w/w), lactic acid has been reported to cause odours on the skin and muscle tissues of meat samples.

Other reports show that lactic acid neither produced a strong odour nor produced off flavours in meat products at concentrations below 1%. Smulders

(1987) reported that a concentration of 1% lactic acid (pH 2.4) did not adversely affect the appearance of meat, but at a 2% concentration caused the development of off-flavours. Similar results have been reported for acid concentrations greater than 1.25% (Mulder *et al.*,1987; Izat *et al.*,1990; Snijders *et al.*,1985).

The purpose of this study was to evaluate the magnitude of discolouration produced by a range of lactic acid treatments and to determine if a lactic acid treatment (4.25% w/w, buffered to pH 3.0 and dispensed at 40°C; See Section 4.0) produces any undesirable organoleptic characteristics.

6.2 Materials and Methods

6.2.1 Evaluation of colour of skin tissues treated with buffered lactic acid

6.2.1.1 Sample preparation

Turkey breast skins were used as a medium for analyzing the effects of buffered lactic acid treatments. These skins were obtained from a local turkey processing plant.

Prior to experimentation, the skins were gently rinsed in lukewarm water to remove extraneous material and kept moist with sterile distilled water. The turkey skins were cut into 5 × 5 cm^2 squaresand fastened onto a plastic cylinder (diameter 4 cm) with elastic bands. The skins were kept moist with sterile distilled water until

ready for treatment. All samples were collected on the same day.

6.2.1.2 Experimental design for the evaluation of the discolouration effect of buffered lactic acid over time

Treatments over a range of temperature and pH values were examined. The experiment followed the same response surface design described previously for the optimization of lactic acid treatments (Section 4.0). The two main factors of interest for this design were the temperature and pH of the lactic acid solution (Table 6.1). The response surface design included a time factor that described the colour output of the turkey skins before treatment, immediately after treatment and at a series of subsequent time intervals after a post-treatment with water at 10°C (10, 20, 30, 40, 50, 60, 120 mins). The light reflectance was measured by a chromameter (Minolta, Japan. Model CR-200b) using the Hunter scale for L, a, b values. The design was performed in duplicate.

6.2.1.3 Statistical analysis of data

Data were analyzed using the General Linear Model of the Statistical Analysis System SAS© (SAS, 1988). Estimates for the linear, quadratic and interaction effects of each parameter (i.e., temperature and pH) were developed to

fit an equation that included the influence of time. The equation is described below.

Equation 1:

$$y = time + \beta_0 + \beta_1 x_1 + \beta_2 x_2 + \beta_{11} x_1^2 + \beta_{22} x_2^2 + \beta_{12} x_1 x_2 + \varepsilon$$

y = result of colour determination (i.e., Hunter L, a or b)
time = measurement at time interval
x_1= pH of lactic acid (at 4.25% w/w)
x_2 = application temperature of lactic acid solution (at 4.25% w/w)
β_0 - estimate for the y - intercept
β_1 - estimate for the linear effect by pH of solution
β_2 - estimate for the linear effect by temperature of solution
β_{11} - estimate for the quadratic effect by pH of solution
β_{22} - estimate for the quadratic effect by temperature
β_{12} - estimate for the interactive effect between pH and temperature of solution
ϵ - error term

6.2.2 A consumer taste panel survey of turkey carcasses treated with lactic acid and regular washing methods

6.2.2.1 Sample preparation and cooking methods for turkey carcasses

From a commercial turkey processing plant, two fully intact turkey hen carcasses were retrieved prior to the chill immersion area of the processing line for each day of experimentation. Turkey carcasses were refrigerated (4° C) upon arrival at the laboratory. Carcasses were gently rinsed with water to remove any extraneous material.

Each of the two carcasses was subjected to one of two treatments. Using the spray chamber described earlier, one of the carcasses was subjected to be prescribed lactic acid treatment (Section 4.0). The parameters for this treatment were defined as a concentration of 4.25% (w/w), buffered with sodium lactate to a pH of 3.0 and dispensed at a temperature of 40°C with an application pressure of 40 psi. Afterwards, the treated carcass was rinsed with water at a temperature of 15°C. The other carcass was subjected to rinse treatment with water only at a temperature of 15°C.

Following treatment, both carcasses were placed into cooking pans (Pyrex, Corning, Inc.) and placed into an oven (Dacor Manufacturing, Model W305C) at a cooking temperature of 375°C. Both carcasses were cooked for a period of 4 hrs.

6.2.2.2 Taste panel survey comparing control and treated carcasses

A comparison between control and treated carcasses was performed using a triangle test (Poste, 1991). The survey was conducted as a consumer taste panel on 3 separate days with 3 different test groups for each day. The entire survey group consisted of 54 individual judges. Both white and dark meat were presented to each judge who were asked to consume both samples entirely.

6.2.2.3 Statistical analysis of the triangle test

Data were analyzed using the General Linear Model procedure of SAS© (SAS, 1988) to determine if the organoleptic quality between carcasses treated with lactic acid/sodium lactate and no treatment were significantly different.

6.3 Results and Discussion

6.3.1 Colour evaluation of turkey skins

The Univariate procedure of SAS® (SAS, 1988) indicated that the data were normally distributed.

The discolouration produced by the lactic acid treatments is described in Figures 6.1 to 6.3. The graphs illustrate that lactic acid, at various pH and temperature combinations, had a significant ($p < 0.05$) effect on the L (i.e., lightness scale) and the a (green-red scale) values immediately after treatment and after 10 mins immersion chilling (Figures 6.1 and 6.2, respectively). However, the discolouration was reduced to initial, untreated levels after 20 mins for both Hunter L and a values.

With Hunter b (yellow-blue) values (Figure 6.3), a significant ($p < 0.05$) change in colour did occur 10 mins after treatment and remained throughout the test period. The results indicated that the tissue changed from a yellow to a blue colour. Furthermore, the effect remained significant even after 120 min following treatment. It should be noted that the overall change in b value is less that 4 units and does fluctuate within the range of -2 to 2. Upon visual inspection of the tissues, an initial discolouration was observed but the effects were reduced after 10 mins of immersion chilling. The colour of the tissues after 50 to 120 mins of immersion chilling were comparable to tissues not treated with lactic acid when determined by

eye.

Lactic acid has been shown to affect the colour of meat surfaces and this effect may be dependent on the concentration of the acid (Cudjoe, 1988; Smulders *et al.*,1986). Generally, low concentrations (< 2.0 %) of lactic acid are unable to severely discolour meat surfaces (Snijders *et al.*,1985; Woolthuis and Smulders, 1985). Lactic acid concentrations greater than 2.0% can cause noticeable discolouration of poultry (Labots *et al.*,1983; Van der Marel *et al.*,1989).

Most of the reports that described the discolouration effects involved the lactic acid treatments in which the acid remained on the surface of food samples (Snijder *et al.*,1985; Smulders and Woolthuis, 1985). In previous studies, researchers commented that the discolouration effect may have resulted from oxidation reactions (Mendonca *et al.*,1989). Another reason could be related to the dehydration effects of proteins in skin tissues (Hedrick *et al.*, 1989). Acidic conditions are known to decrease water binding capacity of proteins. Several reports have indicated that concentration and temperature of lactic acid can promote further discolouration of skin tissues (Izat *et al.*,1990; Kotula and Thelappurate, 1994). However, it has been suggested that the problem can be remedied with stabilizers such as ascorbic acid and nicotinic acid (i.e., niacin).

For this study, lactic acid was to be used as a short-term treatment to lower bacterial levels and was not intended to be used as a preservative. Immediate

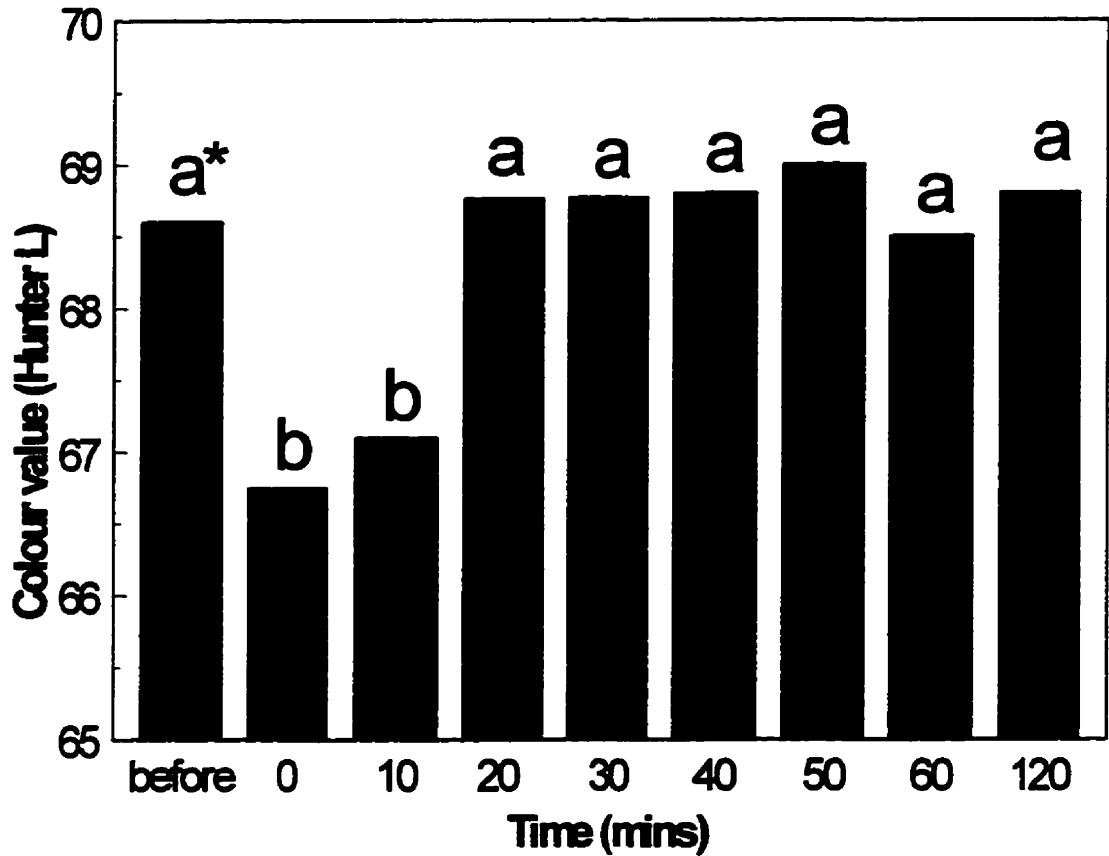


Figure 6.1: The overall effect of buffered lactic acid treatments (4.25% w/w) on Hunter L values (i.e., lightness) on turkey skins.

(* Means with different letters are significantly different; $p < 0.05$)

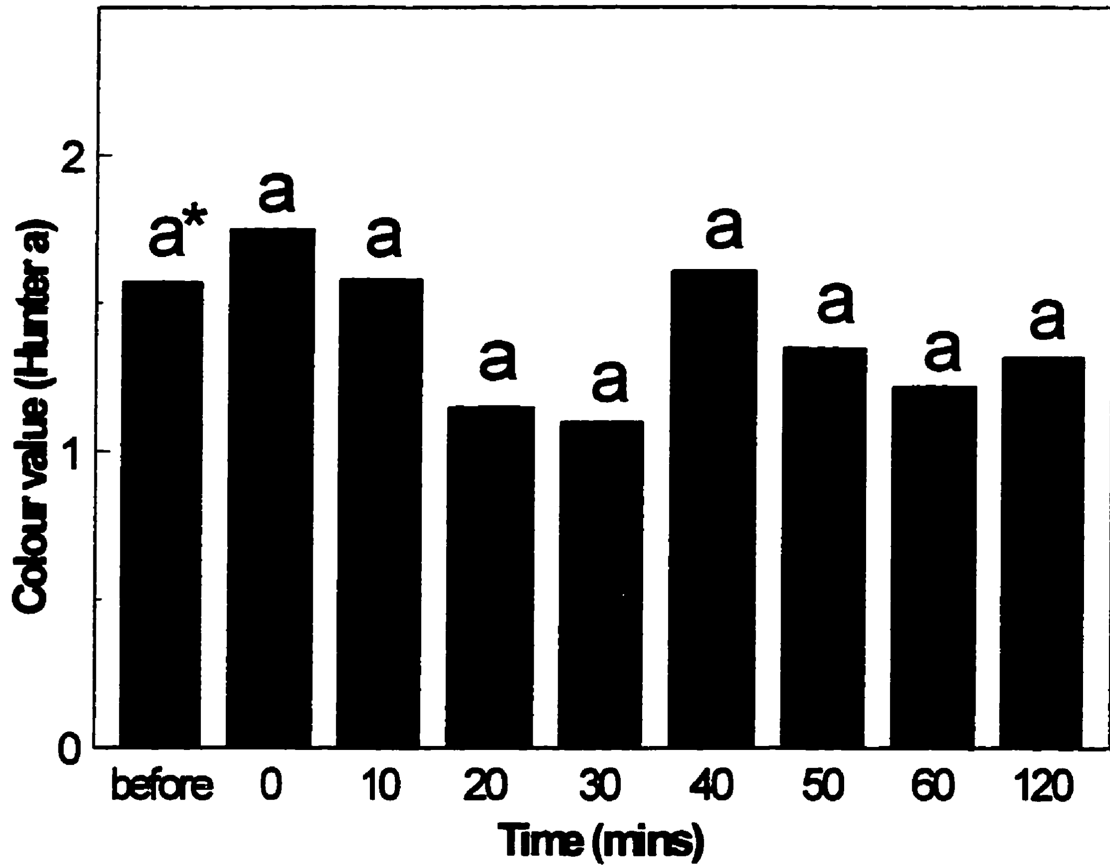


Figure 6.2: The overall effect of buffered lactic acid treatment (4.25% w/w) on Hunter a values (i.e, green-red) on turkey skins

(* Means with different letters are significantly different; $p < 0.05$)

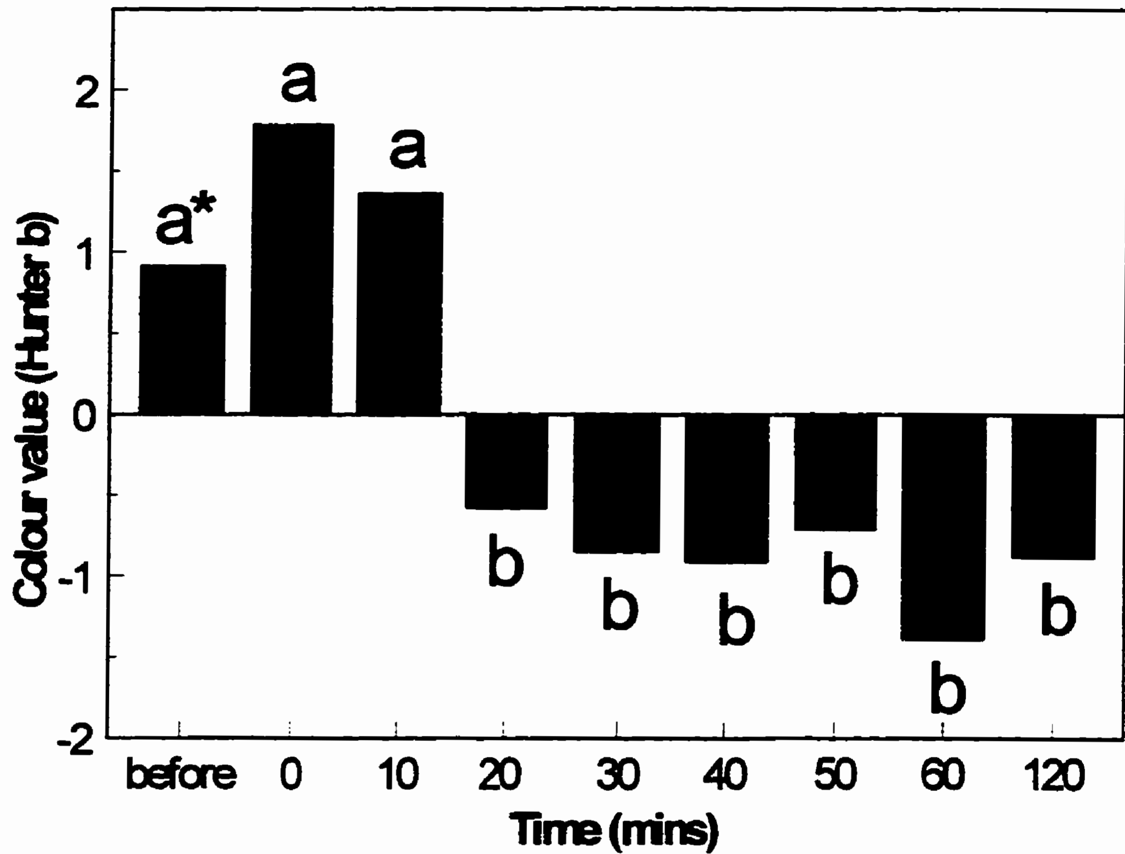


Figure 6.3: The overall effect of buffered lactic acid treatment (4.25%w/w) on Hunter b values (i.e., yellow - blue) on turkey skins.

(* Means with different letters are significantly different; $p < 0.05$)

rinsing with water after the lactic acid treatment was effective in minimizing the changes in L and a Hunter values. Although the Hunter b values had significantly and permanently changed over time, the overall change was small. Water immersion minimizes the discoloration effect of lactic acid by diluting the tissues, increasing the pH and allowing proteins to increase water binding capacity (Hedrick *et al.,* 1989). Snijders *et al.* (1985) also reported that water treatment following lactic acid treatments was able to minimize discolouration effects.

6.3.2 A consumer taste panel survey of the lactic acid treatment on turkey carcasses

Using the triangle test for the sensory evaluation, the results from the statistical analysis indicated that judges were unable to determine the difference between treated and control carcasses ($p > 0.30$). There were no adverse comments from any of the judges for any of the samples. General comments from the judges for treated carcasses ranged from "dry and not as tasty" to "a juicier and fuller taste". There was no associated acidic taste (e.g., vinegar taste) or other objectionable flavours identified by any of the judges.

Gill and Penney (1985) found that panelists could not identify differences in flavour of vacuum packaged lamb treated with 5% lactic acid. Van der Marel *et al.* (1989) also found no deleterious effect of lactic acid (1.0 %) on the taste of grilled

chicken legs. However, other reports have indicated that lactic acid can impart characteristic flavours and off odours on meat samples (Smulders *et al.*,1986; Kotula and Thelappurate, 1994). In most cases, lactic acid was used as a preservative throughout storage of food samples.

6.4 Conclusions

The findings of this study suggest that lactic acid could be used to treat carcasses without imposing undesirable flavour attributes. Based on the taste panel survey, the optimized lactic acid treatment for poultry products does not adversely affect the organoleptic quality of skin or muscle tissues after cooking. Although there was a permanent colour change after lactic acid treatments, it was not noticeable or unpleasing. This was especially true after cooking.

7.0 The construction and stability testing of an auto-bioluminescent wild-type *Salmonella* spp. for the purpose of monitoring metabolic activity

7.1 Introduction

Conventional microbiological techniques can be inadequate for determining the viability and extent of injury of microorganisms when exposed to certain stimuli (e.g., bactericidal treatments). This is because the technique relies on the ability of microorganisms to be completely recoverable under defined culture conditions. There is now substantial evidence indicating that sub-lethally injured cells can be non-culturable (Leriche and Carpentier, 1995). As a result, conclusions drawn from antimicrobial, challenge, storage and other studies that rely on cultural methods for quantification may not be entirely valid.

A novel approach that could prove to be very useful for monitoring viability of organisms is bacterial bioluminescence. The method involves the incorporation of a genetic sequence that encodes for enzymes required for the production of light by naturally luminescent bacteria into bacteria of interest (Baker *et al.,*1992; Chen and Griffiths, 1996; Meighen and Dunlap, 1993; Stewart and Williams, 1992). The luminescence genes can be easily incorporated into a target organism by either transformation (e.g., electroporation techniques) with plasmids or transduction with genetically modified, host-specific bacteriophages.

The major advantage of bacterial bioluminescence is the ability to monitor the metabolic activity of the modified bacteria directly in samples and in "real-time". For example, the light emission decreases with time when the organism is exposed to a negative stimulus. The opposite is true for favourable conditions. Since the samples are continuously used and relatively undisturbed during analysis, bacterial bioluminescence may be more useful for challenge and storage experiments than cultural techniques.

The purpose of this study was twofold. First, a study was performed to determine if *Salmonella* spp. can be successfully engineered to express the entire *lux* gene cassette (i.e., *lux* CDABE), thus becoming luminescent. Secondly, based on light output, a series of experiments was performed that evaluated the metabolic status of the modified organism upon exposure to different conditions.

7.2 Materials and Methods

7.2.1 Isolation, enrichment and identification of wildtype *Salmonella* spp. from carcasses at a poultry abattoir

At a poultry abattoir, condemned carcasses (n = 15) were examined for signs of fecal contamination. If fecal contamination was present, a 10x10 cm^2 area of the contaminated skin was excised aseptically from the carcass. The excised samples were immediately placed into 100 mL of 1% peptone (Difco) and incubated for 18 to 24 hrs at 37° C.

To check for the presence of *Salmonella* spp., an aliquot (0.1 mL) from each sample was inoculated on MSRV (Modified Semisolid Rapport Vasilidas, Difco) agar. MSRV has been shown to be an excellent medium for the isolation and detection of *Salmonella* spp. (Dusch and Altwegg, 1995). Only samples producing a distinctive migration of growth and a change in colour were further examined.

Presumptive *Salmonella* spp. on MSRV were streaked onto Salmonella/Shigella agar (BBL Media Products). Only colonies that were able to reduce iron (indicated by black dots in the center of the colony) were further investigated to confirm the presence of *Salmonella* spp.

The isolates were serologically identified by Dr. C. Poppe, Health Canada. All confirmed isolates were saved on BHI (Brain Heart Infusion) agar slants and stored at 4° C.

7.2.2 Transformation of the *lux* (CDABE) genes into target organisms

7.2.2.1 Preparation of *E.coli* JM109 for classical transformation with calcium chloride ($CaCl_2$)

Escherichia coli JM109 was inoculated into 3 mL of Luria-Bertani (LB) broth and incubated overnight at 37°C. An aliquot (0.5 mL) of the overnight culture was inoculated onto 19.5 mL of fresh LB broth and incubated at 37° C for an additional 75 to 90 mins.

The subculture was cooled on ice for 75 to 90 mins and centrifuged at 5000 rpm for 5 mins at 4°C. The supernatant was discarded and the pellet resuspended in cold (0°C), 50 mM $CaCl_2$ solution and placed on ice for 30 to 60 mins. The suspension was centrifuged again at 5000 rpm for 5 mins and resuspended in 2 mL of cold 50 mM $CaCl_2$ solution. The final preparation was dispensed (0.2 mL) into sterile eppendorf tubes and stored at 4°C. Cells remain competent for up to 6 months under these conditions (Sambrook *et al.*, 1989).

The competent *E. coli* JM109 were transformed with *lux* (CDABE). The full *lux* (CDABE) cassette and the expression vector were the generous donation of Edward A. Meighen, McGill University, Canada. Meighen and Szittner (1992) had successfully isolated the *lux* cassette from *Xenorhabdus luminescens* and inserted the DNA fragment into the plasmid pT7 at the EcoRI site. To 0.2 mL of the refrigerated (4°C) and competent *E. coli* JM109 in a sterile eppendorf tube, 10 µL

of plasmid were added. The mixture was cooled on ice for 30 to 60 mins. Afterwards, the mixture was heat shocked by placing the tube into a waterbath at 42°C and allowed to incubate for 90 sec. Then, the mixture was immediately cooled on ice for an additional 120 sec. To resuscitate the cells, LB broth (0.2 mL) was added to the transformed cells and the suspension incubated at 37°C. At 0.5, 1, 5 and 24 hrs, an aliquot (100 µL) was withdrawn from the transformed sample and plated onto LB agar plates containing 50 µg/mL ampicillin. Plates were incubated for 24 hrs.

Plates were analysed for light output using a photon counting CCD image analyser (BIQ Cambridge Imaging). Any colonies exhibiting light output were subcultured and stored (-20° C) in LB broth containing 15% glycerol.

7.2.2.2 Amplification of the *lux* (CDABE) genes in *E. coli* JM109

The transformant, *E. coli* JM109 + *lux* (CDABE), was grown overnight on LB agar containing 50 µg/mL ampicillin at 37°C. An isolate from the LB plate was inoculated into 10 mL of LB broth containing 50 µg/mL ampicillin and incubated at 37°C for an additional 8 hrs. The culture was centrifuged at 4000 rpm for 10 mins. The sample was decanted and the pellet initially resuspended in 200 µL of an alkali solution plus detergent (Solution I; Sambrook *et al.*,1989) at 4°C. Then, 400 µL of a secondary solution (Solution II; Sambrook *et al.*,1989) was used to completely

lyse the cells. Lastly, 300 μL of neutralizing agent (Solution III; Sambrook *et al.*,1989) was added to the mixture.

The suspension was centrifuged at 14 000 rpm for 5 min at 4°C. An aliquot (600 μL) of the supernatant was added to an equal volume of phenol:chloroform solution (600 μL) in a sterile eppendorf tube. This suspension was centrifuged, again, at 14 000 rpm at 4°C for 2 mins. From the supernatant, 600 μL was retrieved, added to a new eppendorf tube with 600 μL of ethanol (70%) and left to stand for 2 mins. Then, this mixture was centrifuged, again, at 14 000 rpm for 10 mins at 4°C. The supernatant was decanted and the pellet allowed to air dry. The pellet was resuspended in 1 mL of cold (4°C) 70% ethanol, decanted and allowed to air dry for 10 mins. The pellet was redissolved in 50 μL of TE buffer (10 mM Tris*Cl, 1 mM EDTA; pH 8.0) containing 20 μg/mL RNase (Boehringermannheim, Inc.). Purified plasmid was stored at -20° C.

7.2.2.3 Electroporation of wildtype *Salmonella* spp. with the *lux* (CDABE) cassette

Confirmed *Salmonella* spp. (Section 7.2.1) isolates were revived and grown overnight in 3 mL LB broth at 37°C. To prepare the cells for competency, the same protocol for the preparation of *E. coli* JM109 was used (Section 7.2.2.1). However, sterile distilled water was used to resuspend the cells prior to electroporation instead of 50 mM $CaCl_2$.

To 200 µL of chilled (4°C) competent cells, 2 µL of plasmid pT7 + *lux* CDABE were combined in a prechilled electroporation tube (Biorad,Inc. Cat# 165-2086). The electroporation apparatus (BioRad gene pulser, Product # 1652076) was set to 2.5 kV, 25 µF and the pulse controller set to 400 ohms. The cuvette was installed into the electroporation apparatus and a pulse was applied to the mixture. SOC (see appendix A) medium (800 µL) was immediately added and the culture allowed to incubate up to 24 hrs at 37° C. After 0.5, 1, 2, 3 and 24 hrs, 100 µL of the transformed culture were inoculated onto LB agar plates containing ampicillin (50 µg/mL).

7.2.3 Morphological, biochemical and serotypical characterization of *Salmonella* spp. before and after transformation

Transformants (lux^+) were subjected to a series of tests to confirm that the modified bacteria had not substantially deviated from their original characteristics.

7.2.3.1 Colonial morphology

The parent strain and the *lux*$^+$ transformants were inoculated onto BHI (Difco) agar, MSRV (Difco) agar and SSA (BBL). The colonial morphology and behaviour on each of the agars was noted.

7.2.3.2 Cellular morphology

The parent strain and the *lux*$^+$ tranformants were studied by phase-contrast microscopy, gram-stain and scanning electron microscopy.

7.2.3.2 Biochemical testing

The biochemical profiles of the parent strain and *lux*$^+$ transformants were studied using the Vitek Jr. (Biomerieux Vitek, Inc.) using the GNI (Gram negative identification; Product # V 1306) card. Both the parent strain and the transformant were analysed in duplicate.

7.2.3.3 Serological testing

The *lux*$^+$ transformants were tested against Group B and C antisera (Factor 4 and 6,8 respectively, Difco, Inc.) to verify that the indicator antigens for the *Salmonella* spp. were present after transformation.

7.2.4 Stability studies of bioluminescence output of the successfully transformed *Salmonella* spp.

The following tests were conducted to determine the behaviour of *lux*$^+$ transformants under various conditions.

7.2.4.1 Correlation of light output and plate count of *lux*$^+$ transformants

Transformants were grown overnight in 10 mL of SOB (See appendix A) + 50 µg/mL ampicillin at 37°C. The light output from serial dilutions (10^0, 10^{-2}, 10^{-4} and 10^{-6}) of the overnight cultures was monitored using a Multilite luminometer (Biotrace, Inc., Bridgend, U.K.). Correlations were performed on light output with corresponding plate counts for each dilution.

7.2.4.2 Growth determination based on conductance and light measurements

Growth of the original *Salmonella* spp. and the corresponding *lux*$^+$ transformants was measured by an impedimetric method (Firstenberg-Eden, 1983). Both isolates were inoculated in General Purpose Medium (Biomerieux, Vitek) and analysed by conductance measurements (Bactometer; Biomerieux, Vitek) over a 24 hrs period at 37°C. The analysis was performed in duplicate.

Growth of the *lux*$^+$ transformants was also determined by light output. Serial dilutions (10^0, 10^{-2}, 10^{-3} and 10^{-4}) of the *lux*$^+$ transformants were inoculated (10 µL) into a microtitre plate containing 100 µL of LB broth with and without ampicillin (50 µg/mL). Light output was repeatedly measured using a Galaxy BioOrbit 1258 Plate Reader (Mandel Scientific, Inc., Guelph, Ontario) every 10 mins over a period of 24 hrs at 37°C. The experiment was performed in duplicate.

7.2.4.3 The response of bioluminescent output to lactic acid at different levels of pH

A culture of *S. hadar* (*lux*$^+$) was grown in 10 mL of SOB + 50 µg/mL ampicillin overnight at 37°C and subcultured into fresh medium (i.e., SOB + 50 µg/mL ampicillin) for an additional 90 mins at 37°C. To concentrate bacterial cells, a filter unit was used. It consisted of a 5 mL syringe attached to a Swinnex filter unit (Model Swinnex SX0001300 ; Millipore, Inc) containing a removable glass fibre filter (Biotrace, Inc. MMK/100).

A sample (2mL) of culture was filtered and the membrane was subsequently rinsed with 5 mL of one of the buffered lactic acid solutions (pH 2.83, 3.01, 4.02, 4.35, 4.40) for a period of 10 sec. To reduce the effects of lactic acid, the filters were rinsed with 5 mL of phosphate buffered saline (PBS). The filter was removed from the filter unit and placed into a cuvette containing 5 mL of BHI broth. Filters were observed for bioluminescence output at 0, 10, 20 and 60 mins by the Multilite luminometer (Biotrace, Inc.). The experiment was repeated twice. To serve as a control, a non-treated sample was filtered prior to each treatment without any lactic acid, the membrane placed into 5 mL of BHI broth and the bioluminescence output measured by the Multilite luminometer (Biotrace, Inc.).

After 24 hrs, total aerobic plate counts were performed on all samples in duplicate using a Spiral Plater Model D (Spiral Biotech, Inc., Bethesda, MD) to inoculate Standard plate count agar (SPCA alpha grade: Oxoid CM463)

7.2.4.4 Temperature stability of transformant at 40°C

A culture of *S. hadar* (*lux*$^{+}$) was grown in 10 mL of SOB + 50 µg/mL ampicillin overnight at 37°C and subcultured into fresh media (i.e., SOB + 50 µg/mL ampicillin) for an additional 90 mins at 37°C. Samples of the subculture (100 µL)

Table 7.1: Summary of physical characteristics of original and transformed (*lux*$^+$) *Salmonella hadar*

	original bacteria	transformed bacteria
cellular morphology	Gram negative rod	Gram negative rod
motility	yes	yes
colonial morphology on BHI agar	pale white round convex	pale white round convex
colonial morphology on SSA	characteristic grey colonies with black center	characteristic grey colonies with black center
growth on MSRV with 2% novobiocin	Yes	Yes

Table 7.2: Serological testing of unmodified and transformed (*lux*$^{+}$) *Salmonella hadar*

Type of *Salmonella* spp. testing	original bacteria	transformed bacteria
polyantisera	+	+
Group B antisera	-	-
Group C antisera	+	+

Table 7.3: Results of biochemical testing of the original and transformed *Salmonella hadar* by Vitek Jr. (Biomerieux Vitek) in duplicate

	original	transformant
GLUCOSE FERMENTATION (DP 300)	-/-	-/-
UREA	-/-	-/-
MALTOSE	+/+	+/+
INOSITOL	-/-	-/-
L-ARABINOSE	+/+	+/+
GLUCOSE OXIDATION	+/+	+/+
CITRATE	+/+	+/+
MANNITOL	+/+	+/+
ADONITOL	-/-	-/-
GLUCOSE	+/+	+/+
GROWTH CONTROL	+/+	+/+
MALONATE	-/-	-/-
XYLOSE	+/+	+/+
p-COUMARIC	+/+	+/+
ARGININE	-/-	-/-
ACETAMIDE	-/-	-/-
TRYPTOPHAN	-/-	-/-
RAFFINOSE	-/-	-/-
HYDROGEN SULFIDE	+/+	+/+
LYSINE	+/+	+/+
ESCULIN	-/-	-/-
POLYMYXIN B	-/-	-/-

Table 7.3 : Results of biochemical testing of the original and transformed *Salmonella hadar* by Vitek Jr. (Biomerieux Vitek) in duplicate (continued)

	original	transformant
SORBITOL	+/+	+/+
ONPG[a] FERMENTATION	-/-	-/-
ORNITHINE	+/+	+/+
PLANT INDICAN	-/-	-/-
LACTOSE	-/-	-/-
SUCROSE	-/-	-/-
RHAMNOSE	+/+	+/+

[a] ONPG : O-Nitrophenyl-β-D-galactopyranoside

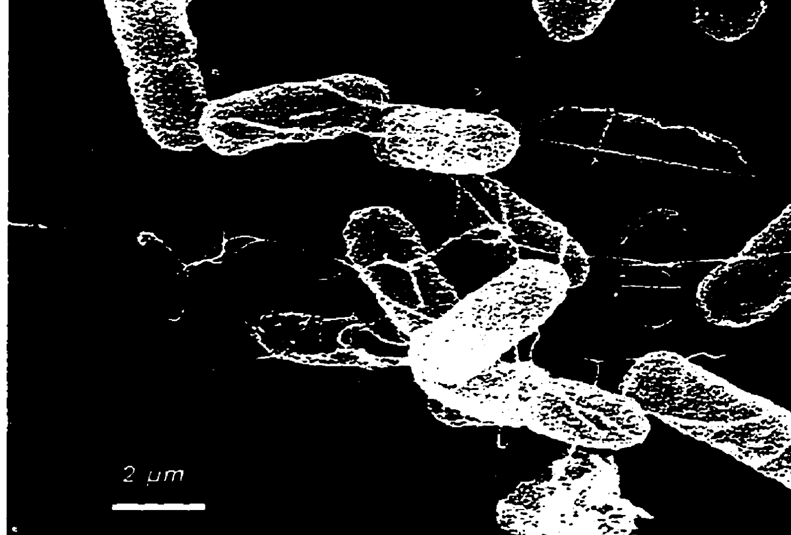


Figure 7.1a: Scanning electron microscopy of original *Salmonella hadar* isolated from a poultry abattoir

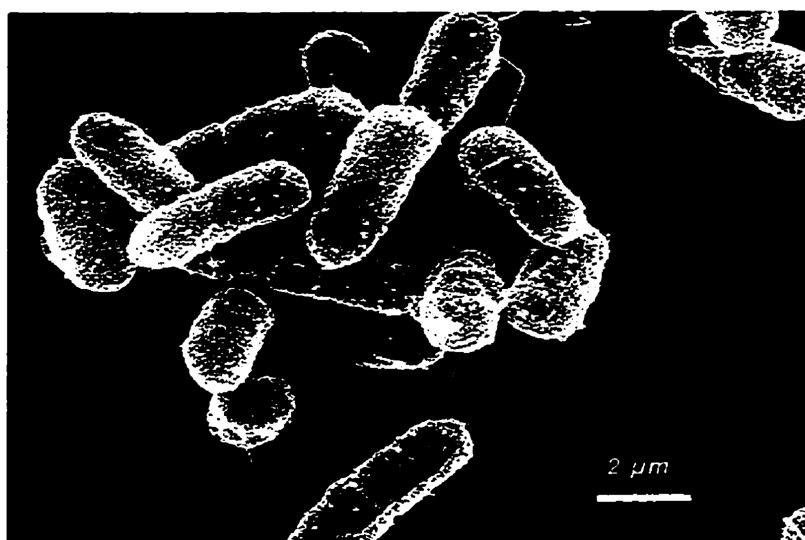


Figure 7.1b: Scanning electron microscopy of the transformed *Salmonella hadar* (*lux*$^+$).

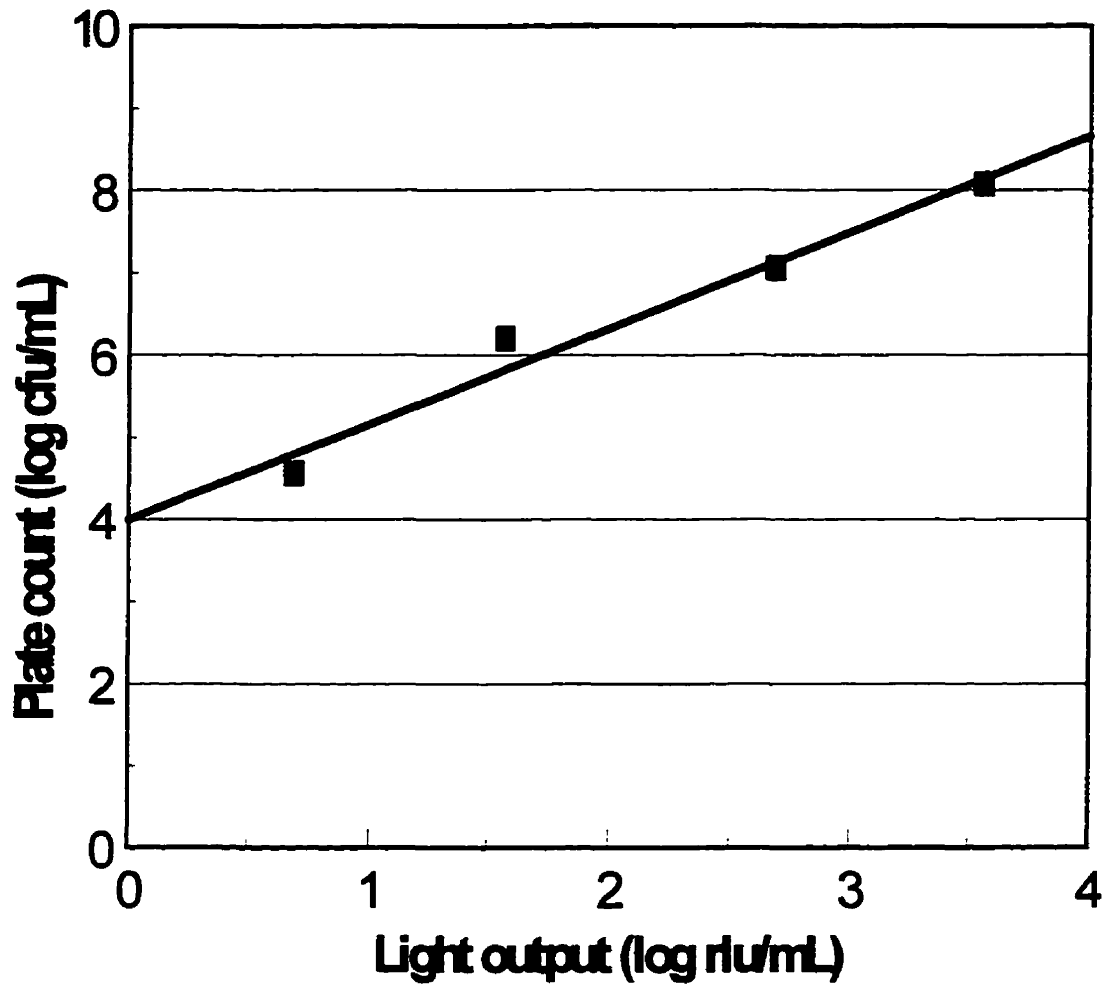


Figure 7.2: Relation between plate count and bioluminescence readings of *Salmonella hadar* (lux^+)

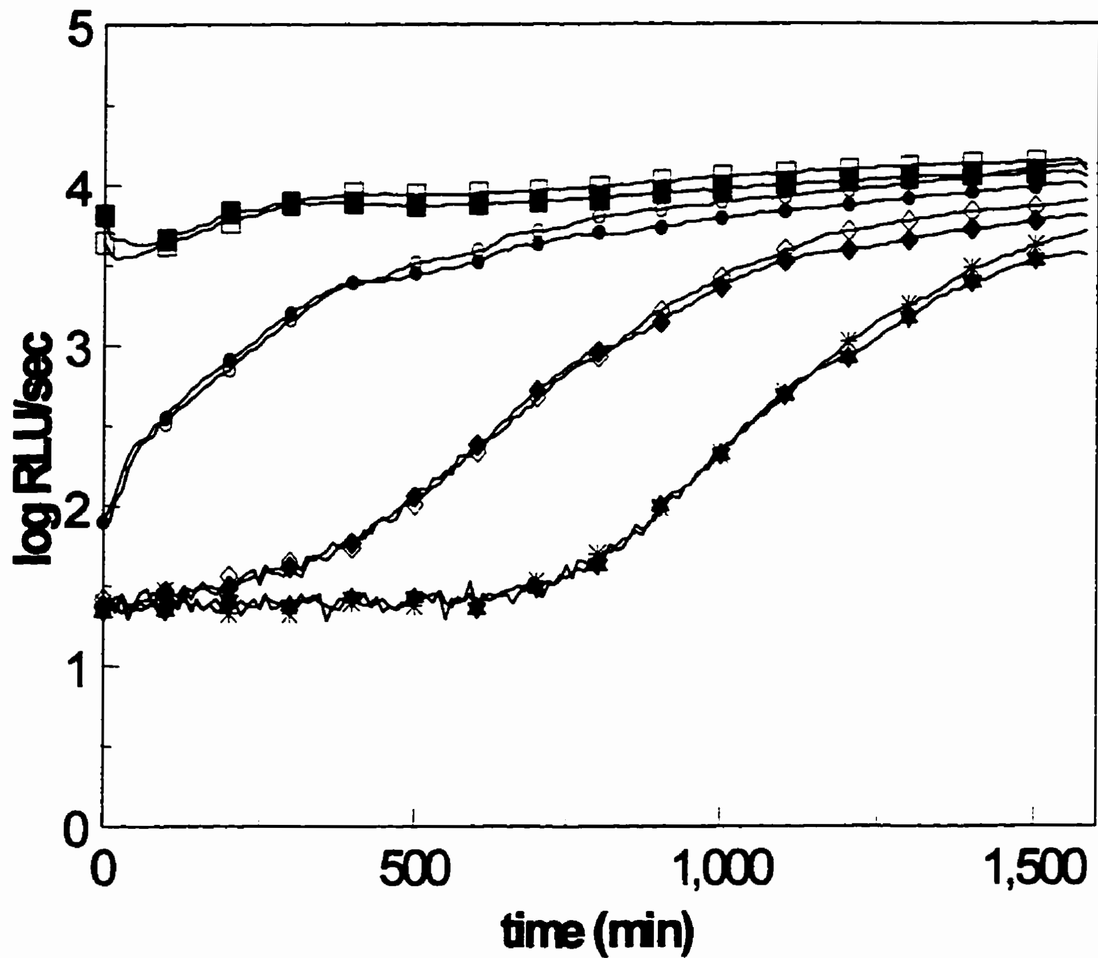


Figure 7.3: The effect of dilution (10^0 ■,□; 10^{-2} ●,○; 10^{-3} ◆,◇; 10^{-4} ✱,✳) on light output of the *Salmonella hadar* (*lux*$^+$) over time with (closed symbols) and without (open symbols) ampicillin in LB broth at 37°C.

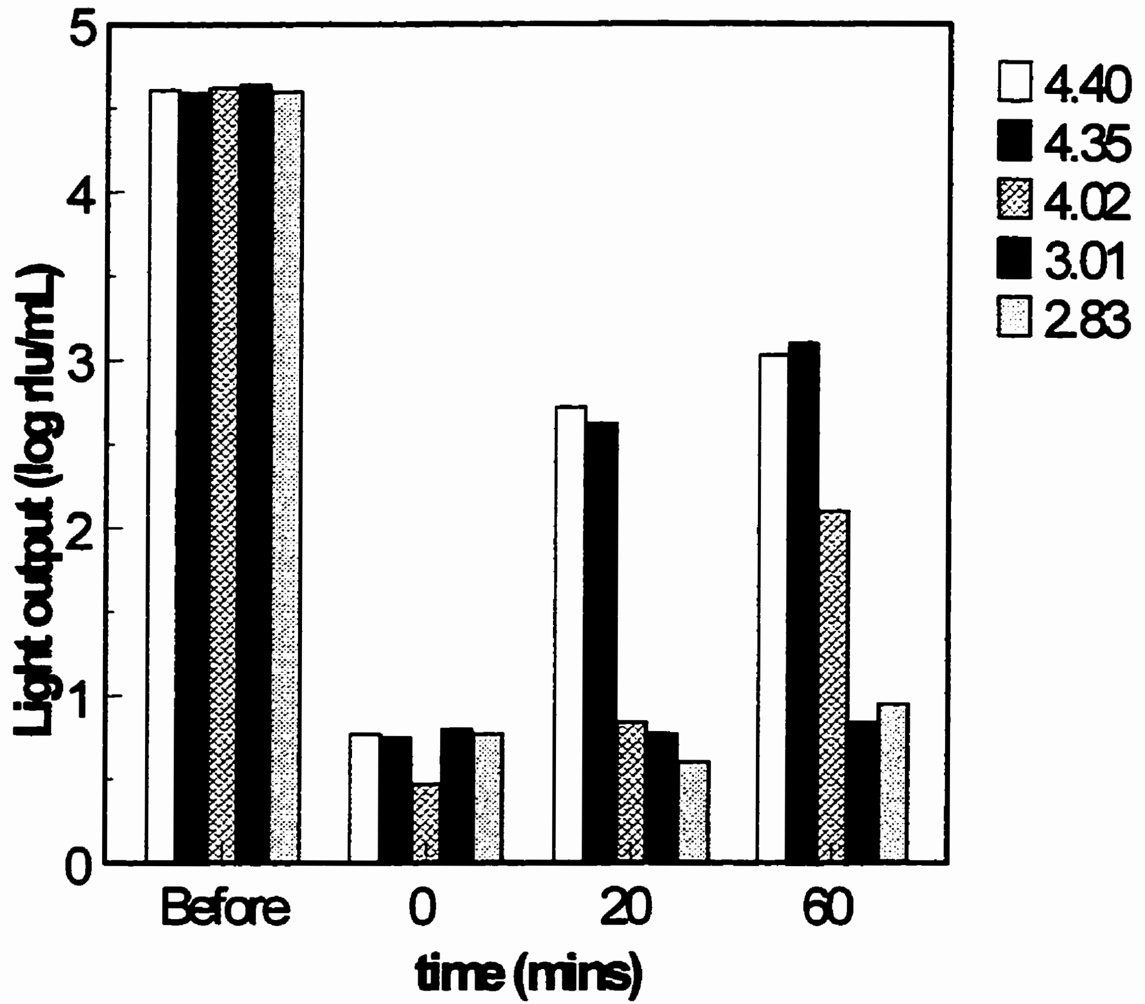


Figure 7.4: Bioluminescence output of *Salmonella hadar* (*lux*$^+$) before treatment and during recovery after exposure to different pH levels.

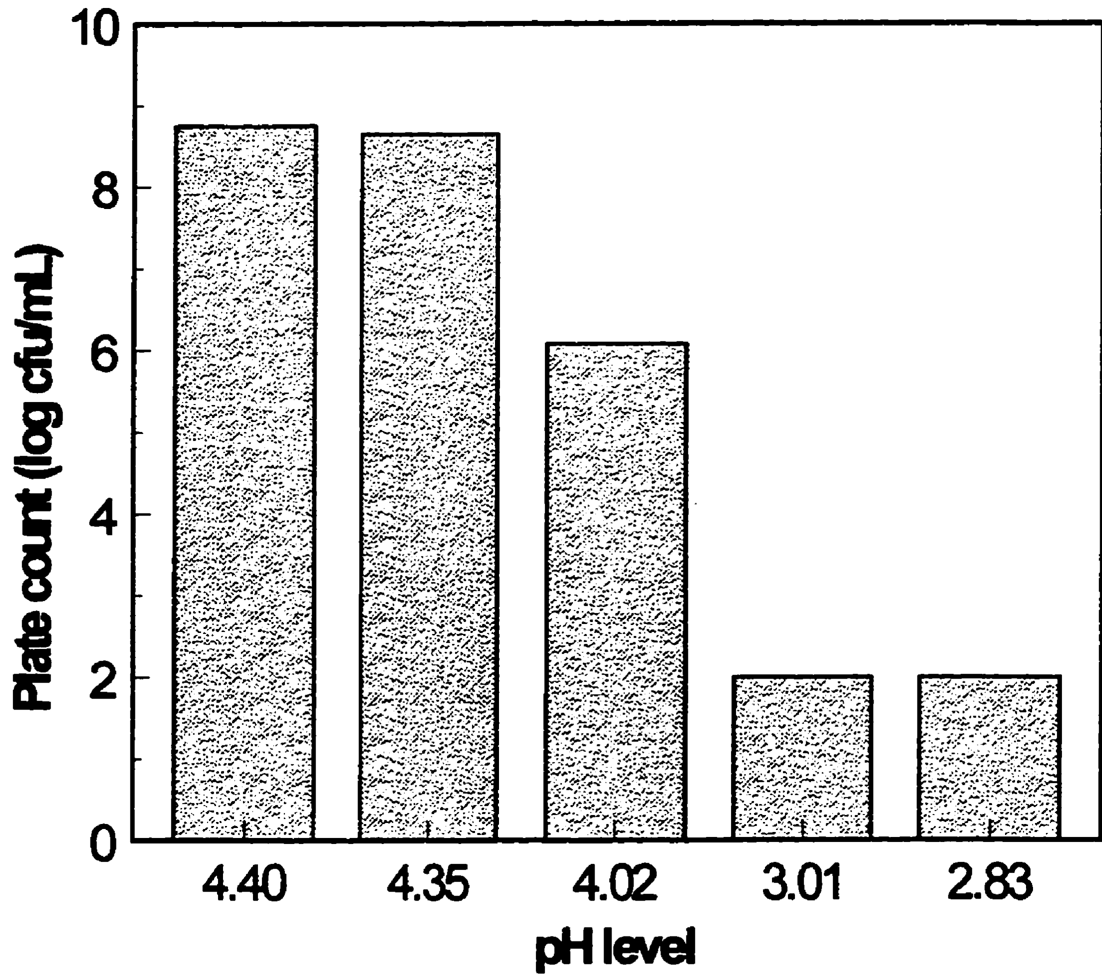


Figure 7.5: Plate count of *Salmonella hadar* (*lux*$^{+}$) 24 hrs after exposure to different pH levels

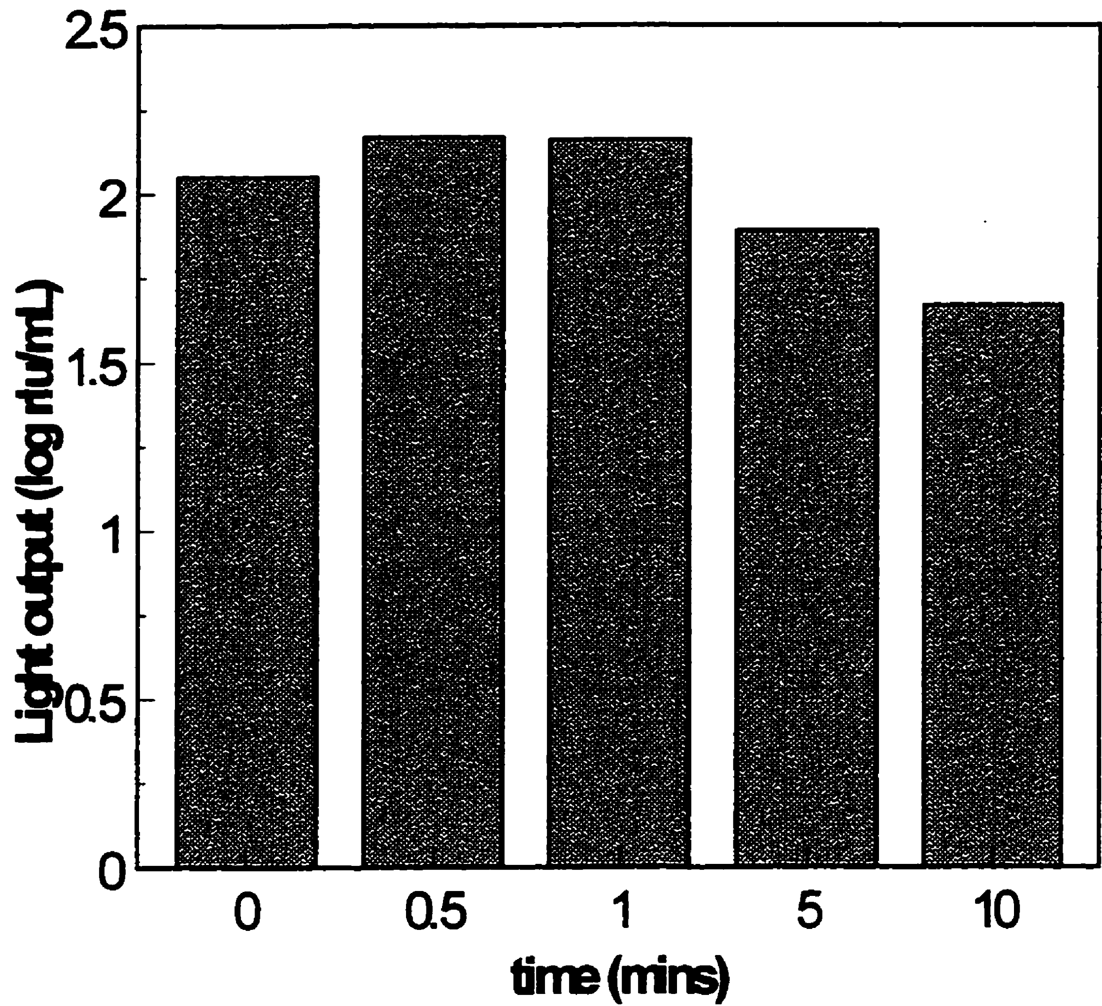


Figure 7.6: Bioluminescence output of *Salmonella hadar* (*lux*$^+$) over time at 40°C

were dispensed into five sterile eppendorf tubes and treated at 40°C in a waterbath. Bioluminescence was measured before treatment and at 0, 0.5, 1, 5 and 10 mins using a Multilite luminometer (Biotrace, Inc.).

7.3 Results and Discussion

Several presumptive *Salmonella* spp. (n=14) were isolated from the poultry processing plant. However, serotyping by Dr. C. Poppe, Health Canada revealed that only 7 isolates were positive for *Salmonella* spp. (See Appendix B). The isolates identified were *S. hadar* (n=2; Antigens 6,8:z10:x) and *S. heidelberg* (n=5; Antigens 4:r:2). An attempt was made to transform all these species/strains, however, only one of the isolates, *S. hadar* 6,8:z10:x, was successfully transformed with the *lux* (CDABE) cassette. For *Salmonella* spp. that were unable to be transformed, all were resistant to ampicillin. The successful transformant did not possess this characteristic.

The transformed organism was not significantly different in its biochemical, serotypical and phenotypical characteristics from the parent strain (Tables 7.1 to 7.3). The only substantial difference was apparent under scanning electron microscopy. In Figures 7.1 a and b, it is evident that the long thin strands are in more numbers on the parent strain of *S. hadar*.

The light output correlated well with plate counts ($r = 0.98$ $p<0.01$; Figure 7.2). Growth of the parent strain and the transformant were comparable when measured by conductance (Bactometer; Mean Detection times = 6.2 and 6.7 hrs, respectively). Growth curves by bioluminescence output of the *S. hadar* (*lux*$^+$) strain resembled typical growth curves by cultural methods at different cell concentrations (Figure 7.3). Furthermore, results of growth curves were similar with and without ampicillin. Therefore, ampicillin was not required to induce bioluminescence in the organism.

Immediately after exposure to different pH levels, there was an immediate decrease in light output for all treatments (Figure 7.4). However, after 20 mins of recovery, the *S. hadar* (*lux*$^+$) was able to produce bioluminescence after exposure to pH levels, 4.40 and 4.35. There was also recovery of the transformant after 30 mins from exposure to a pH level of 4.02. Recoveries at the lower pH levels (i.e., 3.01 and 2.30) were nominal. Plate count of *S. hadar* after a recovery time of 24 hrs for all treatments showed that decreasing pH levels affected the population (Figure 7.5).

Bacterial bioluminescence has been used to predict growth and behaviour of modified organisms. However, certain limitations have been recognized (Duffy *et al.*,1995; Ellison *et al.*, 1991). The major problems are the effect of extreme pH conditions and/or temperatures on the bioluminescence reaction. Both pH and

temperature play a vital role in the conformation of proteins. Therefore, luciferase enzyme and the bioluminescence reaction of modified organisms could be greatly affected under constant acidic and/or extreme temperature conditions. However, the treatment described in this study was used for a short period of time (10 sec) and recovery occurred under favourable conditions. Furthermore, colonies from plate counts (i.e., after a 24 hrs recovery) were still able to produce bioluminescence. Therefore, the bioluminescence reaction of *S. hadar* (*lux*$^{+}$) was not permanently damaged by the treatment. The results indicated that the light output of *S.hadar* at 40°C was maintained for up to 10 mins (Figure 7.6).

Previous studies have indicated that the incorporation of the full *lux* (CDABE) gene cassette might be very energy consuming for target organisms (Baker *et al.*, 1992). This could result in a transformed organism that may exhibit odd behaviour. Fortunately, the transformed *S. hadar* did not exhibit any serious differences from the parent strain in terms of phenotypical characteristics. The only concern is the absence of the long thin strands of the transformant which might hamper the adhesion of the organism to surfaces.

Most important, the transformed organism was able to produce a light signal that was responsive to its environment. This is clearly indicated throughout Figures 7.2 to 7.6. Note, that in all of the studies, when conditions were less than optimal the "real time" response was a decrease in light output.

7.4 Conclusions

Bacterial bioluminescence can be a valuable tool for studying the metabolic activity of organisms. This technique could possibly lead to other uses in research. For example, microaerophilic organisms, such as *Campylobacter* spp., can be analyzed for metabolic activity without disturbing their environment. Bacterial bioluminescence could also be used to obtain a better picture of responses to antibiotics. Other researchers should be encouraged to find more uses for this technology.

8.0 The determination of the long-term efficacy of an optimized lactic acid treatment under various storage conditions using a *Salmonella hadar* (*lux*$^+$)

8.1 Introduction

Treatment of meat carcasses with bactericides is a means by which processors can improve the quality and safety of their products. There are a number of food-grade chemicals that have anti-microbial effects (See Section 2.0). However, many studies that investigated the efficacy of these materials were unable to verify their performance in an industrial setting. The primary method of investigation in these studies was based on the conventional aerobic plate count.

Generally, conventional microbiology relies on the ability of microorganisms to grow from a single cell into a visible colony under aerobic conditions. In the case of sublethal injury or in periods of stress, the growth can be hampered and this inhibition results in longer recovery or lag times (Leriche and Carpentier, 1995). Therefore, there is a strong possibility that the true physiological status of the microbial population is not determined in these studies.

An alternative to conventional microbiology could be bacterial bioluminescence. There are many organisms that are naturally luminescent (Baker

et al.,1992; Meighen and Szittner, 1992). The genes that encode the enzymes responsible for bioluminescence can be cloned into microorganisms of interest by molecular techniques (Baker *et al.*,1992). The result is a genetically modified organism that can be easily identified and detected (Duffy *et al.*,1995; Ellison *et al.*, 1991).

Another major advantage of bacterial bioluminescence is the capability to monitor the metabolic activity of the microorganism under any given conditions and in "real-time". Metabolic activity may be a better indication of the organism's ability to recover from an injured state or stress situations than conventional cultural techniques. Furthermore, this method can be used nondestructively during storage or challenge studies. As a result, more accurate interpretations of the organism's response to bacterial treatments can be obtained on a single sample during the entire course of study.

The purpose of this study was to determine the effectiveness of a lactic acid treatment against a bioluminescent *Salmonella hadar* strain that had been genetically modified to carry the *lux* gene (CDABE) cassette. The light output was used as an indicator for viability and recovery of the organism.

8.2 Materials and Methods

8.2.1 Bacterial strain used for study

A strain of *Salmonella hadar* was retrieved from a poultry abattoir and transformed to become bioluminescent with a constitutive *lux* (CDABE) expression carried on plasmid pT7 (Meighen and Szittner, 1992). The plasmid was a generous donation by Dr. E. Meighen, McGill University.

8.2.2 Preparation of the bioluminescent *Salmonella hadar* (*lux*$^+$) for artificial inoculation onto turkey carcasses

For each experimental run, a loopful of frozen culture was inoculated into a test tube containing 1.5 mL SOB + ampicillin (50 µg/mL). The culture was grown in a shaking incubator for 10 to 12 hrs. at 37°C. After incubation, 1 mL of the culture was spread onto petri plates containing SOB agar + ampicillin (50 µg/mL). The plates were incubated at 37°C for about 5 hrs so that there was a complete lawn of growth.

8.2.3 Preparation of turkey samples for artificial inoculation

On each day of the experiment, one whole, government inspected, grade A turkey tom was used for each replicate. Turkey carcasses were obtained from a poultry processing plant and transported to the laboratory on ice. Carcasses were rinsed in tap water to remove any extraneous material and allowed to drain.

The breast meat, with the skin intact, was removed from the bones and cut up into 16 individual squares (approximately 5×5 cm^2) with a thickness of about 2 cm. Following treatment, each breast piece was placed into resealable aluminum foil pans ($10 \times 5 \times 2$ cm^3; Alcan, Inc.) lined with black absorbent tissue. To prevent the sample from drying out, each absorbent tissue in the sample tray was wetted with sterile distilled water (about 5 mL) between readings.

8.2.4 Inoculation of turkey samples with *S. hadar* (*lux*$^+$) using a sponge imprint technique

Cleaning sponges (WashRack Supply, Inc. Toronto, Ont.) were cut into dimensions of $2 \times 2 \times 5$ cm^3 pieces. Using the lawn of growth of *S. hadar* (*lux* CDABE) generated earlier (Section 8.2.2), the square end (i.e., 2×2 cm^2) of the sponge was pressed firmly into the prepared culture of the bioluminescent *S. hadar* and immediately transferred onto turkey breast samples (Section 8.2.3). This method was used for all treatment samples except uninoculated controls.

8.2.5 An investigation of the viability of *S. hadar* (*lux*$^+$) exposed to different treatments and storage conditions

The following categories were used to compare the viability of *S. hadar* (*lux*$^+$) on turkey breast samples after treatment. Bioluminescence output was used as an indicator of viability per sample.

I) Non-inoculated turkey samples (negative control) - Samples of turkey breasts, not inoculated with the modified organism, were placed into the resealable containers and monitored for background readings over time.

II) Inoculated turkey samples with no treatment (i.e., positive control) - Samples of turkey breast, inoculated with the modified organism, were placed into the resealable containers and monitored for light output over time.

III) Inoculated turkey samples treated with water only (treatment 1) - Samples of turkey breast, inoculated with the modified organism, were rinsed off with water sprays described below. Samples were placed into resealable containers and monitored for light output over time.

IV) Inoculated turkey samples treated with lactic acid (treatment 2) - Samples of turkey breast, inoculated with the modified organism, and rinsed off with lactic acid spray treatment. The lactic acid solution was applied at a concentration of 4.25%, pH 3.0 and heated to a temperature of 40°C. Afterwards, samples were lightly rinsed with sterile distilled water at a temperature of 15°C. The samples

were monitored for light output over time.

For conditions III) and IV), a lawn and garden sprayer (Canadian Tire; model no. 59-3950-2) was used to spray the carcasses. The equipment was filled to a volume of 2 L and was primed by pumping up the device 20 full strokes. The spray was directed evenly over the samples by a back and forth motion and for a duration of 10 sec.

Following all treatments, samples were stored at either -12, 0, 5 or 10°C according to the statistical design (Section 8.2.8). For samples stored at 10°C, light output was monitored by the BIQ Bioview (Cambridge Imaging, Ltd.) daily for a period of 10 days. For samples stored at -12, 0, and 5°C, light output was monitored by the BIQ Bioview (Cambridge Imaging, Ltd.) at 7, 14 and 21 days.

8.2.6 Conditions to investigate the recovery of *S. hadar* (*lux*$^+$)

After each of the storage periods, samples were tempered in an incubator at 22°C to initiate recovery of the bacterium. Bioluminescence was monitored and recorded at 0, 1, 5 and 10 hrs with the BIQ Bioview (Cambridge Imaging, Ltd.)

8.2.7 Quantification of light output using a CCD image analyzer (BIQ Bioview, Cambridge Image, Ltd.)

The f-stop on the CCD camera lens of the BIQ Bioview was set to an aperture of 2.0. The "Blots and Gels" software package, included with the BIQ Bioview, was used to locate and quantify bioluminescence output. The dimension of the area for quantification of light output was restricted to 2×2 cm^2 and a template was created to fix the sample between readings. Light emission measurements were integrated over 10 sec.

8.2.8 Statistical design

The study was set up as a 4×4 factorial design (4 treatments $\times$ 4 storage temperatures = 16 combinations) and replicated over 4 day period. The total number of observations was 64 ($4 \times 4 \times 4 = 64$).

8.2.8.1 Analysis of data from the viability study of turkey breast samples inoculated with *S. hadar* (*lux*$^+$)

Initial analysis of the results indicated that an inverse transformation of the bioluminescence output over time was required to improve the interpretation of the viability study. An example of the viability curve and the subsequent transformation are illustrated in Figure 8.1.

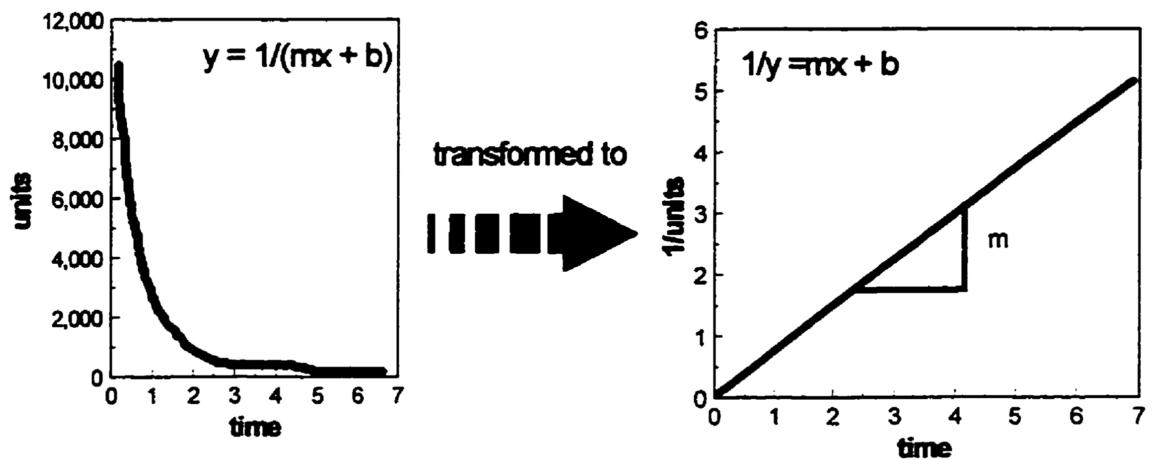


Figure 8.1: An example of raw data from the viability study and the subsequent transformation of bioluminescent output

Using the regression procedure of SAS® (1988), positive slopes (i.e., m_S) were calculated from the inverse transformations. A statistical evaluation was performed to determine if the treatment combinations had an effect on the reduction of metabolic activity of the organisms over time. The following model was used for interpretation of the results.

Model 1:

$$SLOPE(m_S)_{ijk}=\beta_0+trial_i+treat_j+temp_k+(treat\times temp)_{ij}+\epsilon$$

SLOPE (m_S) - rate of the bioluminescent output over time for viability
β_0 - intercept value
trial - effect of experimental run
treat - effect of treatment of either negative, positive, water or lactic acid
temp - effect of storage temperature of either -12, 0, 5,or 10°C
treat × temp - effect of the combination of treatment and temperature
ϵ - error term

These data were analysed using the General Linear Model of SAS® (SAS Institute, 1988).

8.2.8.2 Analysis of data from the recovery study of turkey samples inoculated with *S. hadar* (*lux*$^+$)

A $\log_{10}$ transformation of the bioluminescence output over time was performed to facilitate analysis of the data. An example of the initial recovery curve and the subsequent transformation is illustrated in Figure 8.2.

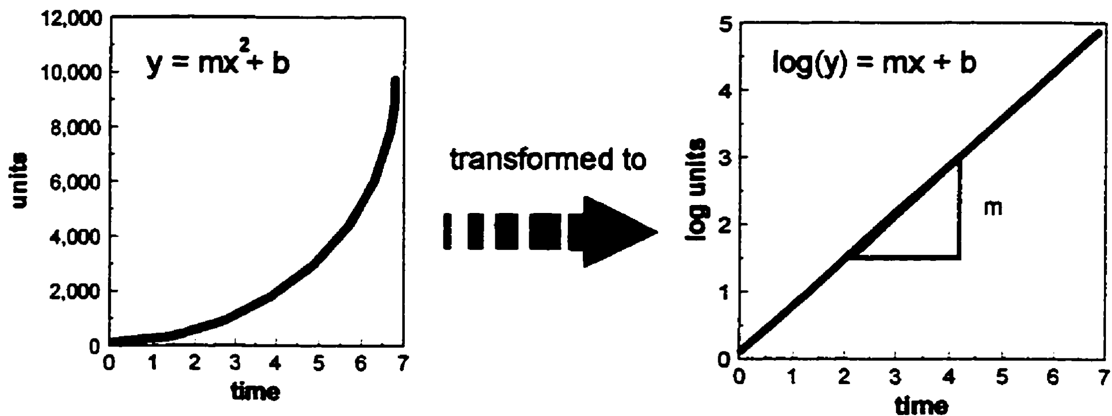


Figure 8.2: An example of raw data from the recovery study and the subsequent transformation of bioluminescent output

Using the regression procedure of SAS® (1988), positive slopes (i.e., m_R) were calculated from the $\log_{10}$ transformation of data. A statistical evaluation was performed to determine if the treatment combinations had an effect on the recovery of organisms over time. The following model was used for interpretation of the results.

Model 2:

$$SLOPE(m_R)_{ijk}=\beta_0+trial_i+treat_j+temp_k+(treat\times temp)_{ij}$$

SLOPE (m_R) - rate of the bioluminescent output over time for recovery
β_0 - intercept value
trial - effect of experimental run
treat - effect of treatment of either negative, positive, water or lactic acid
temp - effect of storage temperature of either -12, 0, 5, or 10°C
treat × temp - effect of the combination of treatment and temperature
ϵ - error term

All data were analysed using the General Linear Model of SAS® (SAS Institute, 1988).

8.3 Results and Discussion

To remove drift associated with the BIQ Bioview (Cambridge Imaging, Ltd.), readings obtained from negative controls were subtracted from all test bioluminescent readings. A student t-test on two groups (i.e., each group consisted of 3 samples) of breast samples inoculated by the sponge imprint technique showed no significant ($p > 0.20$) difference in light output. Therefore, repeatability of the inoculation method on turkey breast samples was good. Both the Univariate and General Linear Model procedures indicated that the data were of a normal distribution and there was no significant ($p<0.15$) difference between trials.

Initially, an attempt was made to fit a linear expression to the bioluminescence data over time for both the viability and recovery studies. However, the linear model analysis by the Regression analysis procedure of SAS® revealed that most of the data did not follow a significant ($p > 0.20$) linear trend. Therefore, it was necessary to convert the bioluminescence data to fit a nonlinear expression to improve the interpretation of the results. Transformation of the data greatly improved the significance values ($p < 0.05$) when the inverse and $\log_{10}$ cfu/mL interpretation of the bioluminescence output were used for the viability and recovery studies over time, respectively.

Differences in light output from *S. hadar* (*lux*$^{+}$) immediately after treatments,

as indicated by y-intercepts values (Figures 8.3 a to d), were significantly ($p < 0.05$) different. Therefore, the *S. hadar* population was affected by each treatment, initially.

The viability study indicated that there was a significant ($p < 0.01$) negative curvilinear relation between the bioluminescence output and time. Illustrations of the viability studies are shown in Figures 8.3 a to d. Based on the results in Figure 8.4, viability of *S. hadar* (i.e., m_s) significantly ($p<0.05$) decreased with treatment type; Control (+) < water< Lactic acid. Therefore, lactic acid was the most effective treatment for eliminating *S. hadar* (*lux*$^+$) from turkey carcasses.

Further analysis determined that storage temperatures were also important. The rate of metabolic activity was significantly ($p<0.05$) higher at 10°C than at -12, 0 and 5°C. There were no significant ($p>0.15$) differences observed between the three lower storage temperatures. There was also no significant ($p > 0.20$) interactive effect between temperature and treatment during the viability study. Bactericidal treatments and low storage temperatures have been effective in reducing microbial contamination. Therefore, it is no surprise that the bioluminescence output confirms these findings.

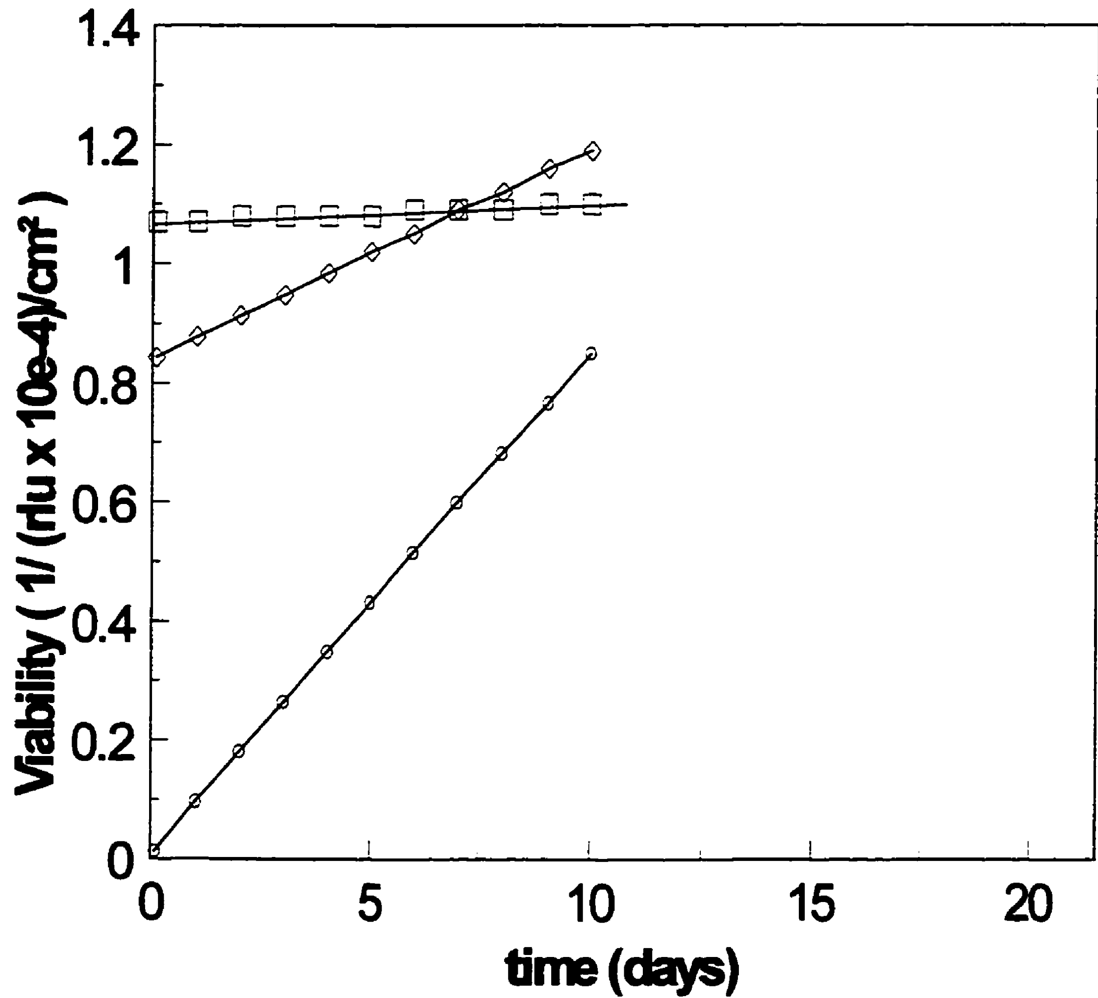


Figure 8.3a: Viability of *Salmonella hadar* (*lux*$^+$) over time (i.e., based on bioluminescence output) after exposure to three different treatments (control ○; water ◇; lactic acid □) and storage at 10°C.

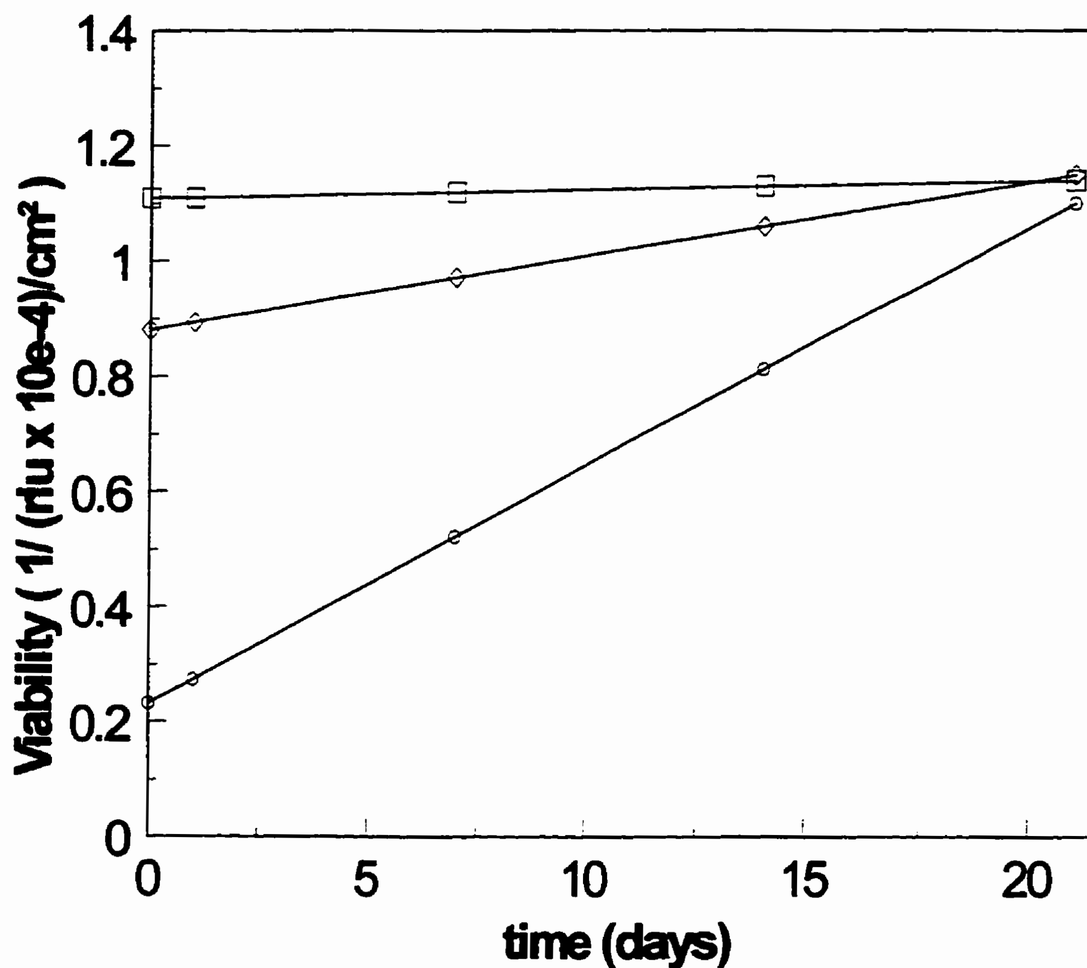


Figure 8.3b: Viability of *Salmonella hadar* (*lux*$^+$) over time (i.e., based on bioluminescence output) after exposure to three different treatments (control ○; water ◇; lactic acid □) and storage at 5°C

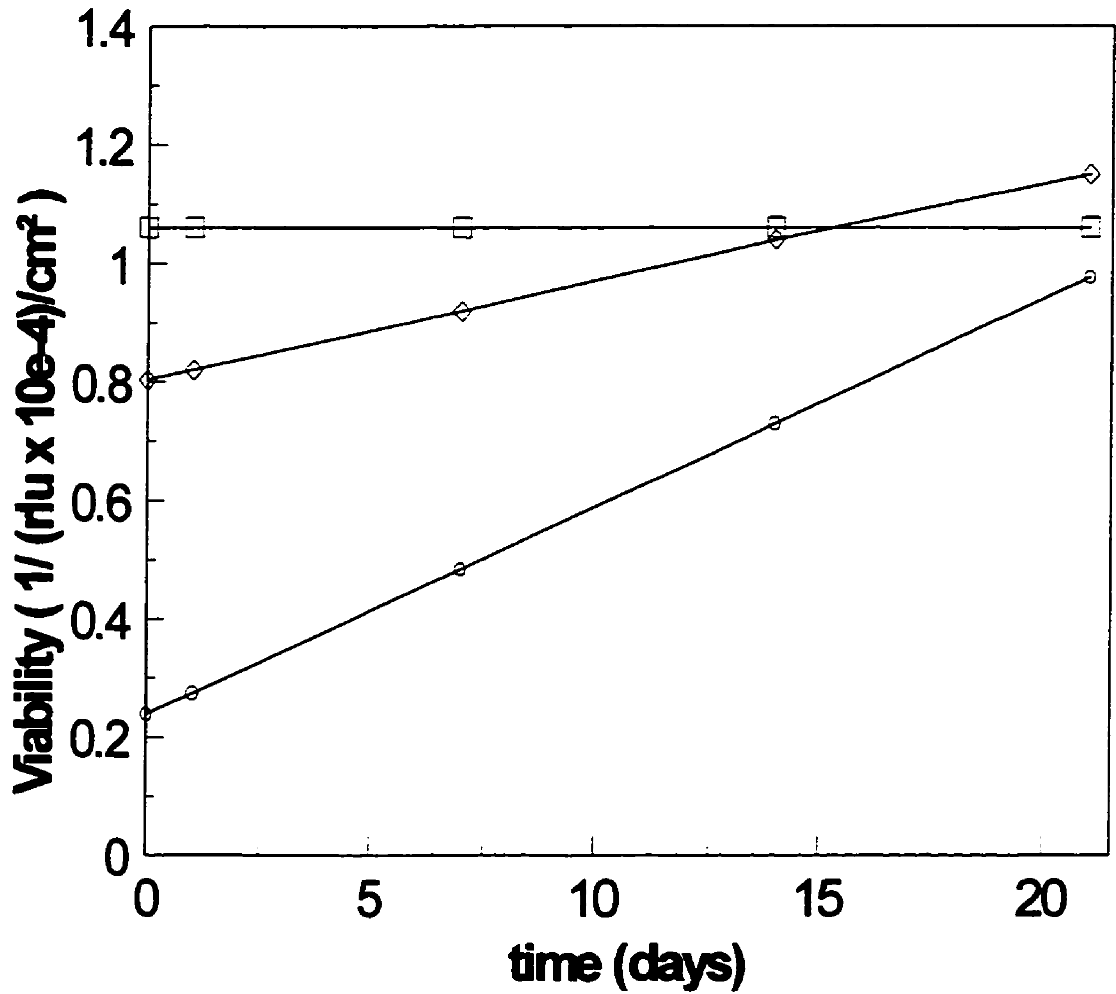


Figure 8.3c: Viability of *Salmonella hadar* (*lux*⁺) over time (i.e., based on bioluminescence output) after exposure to three different treatments (control ○; water ◇; lactic acid □) and storage at 0°C

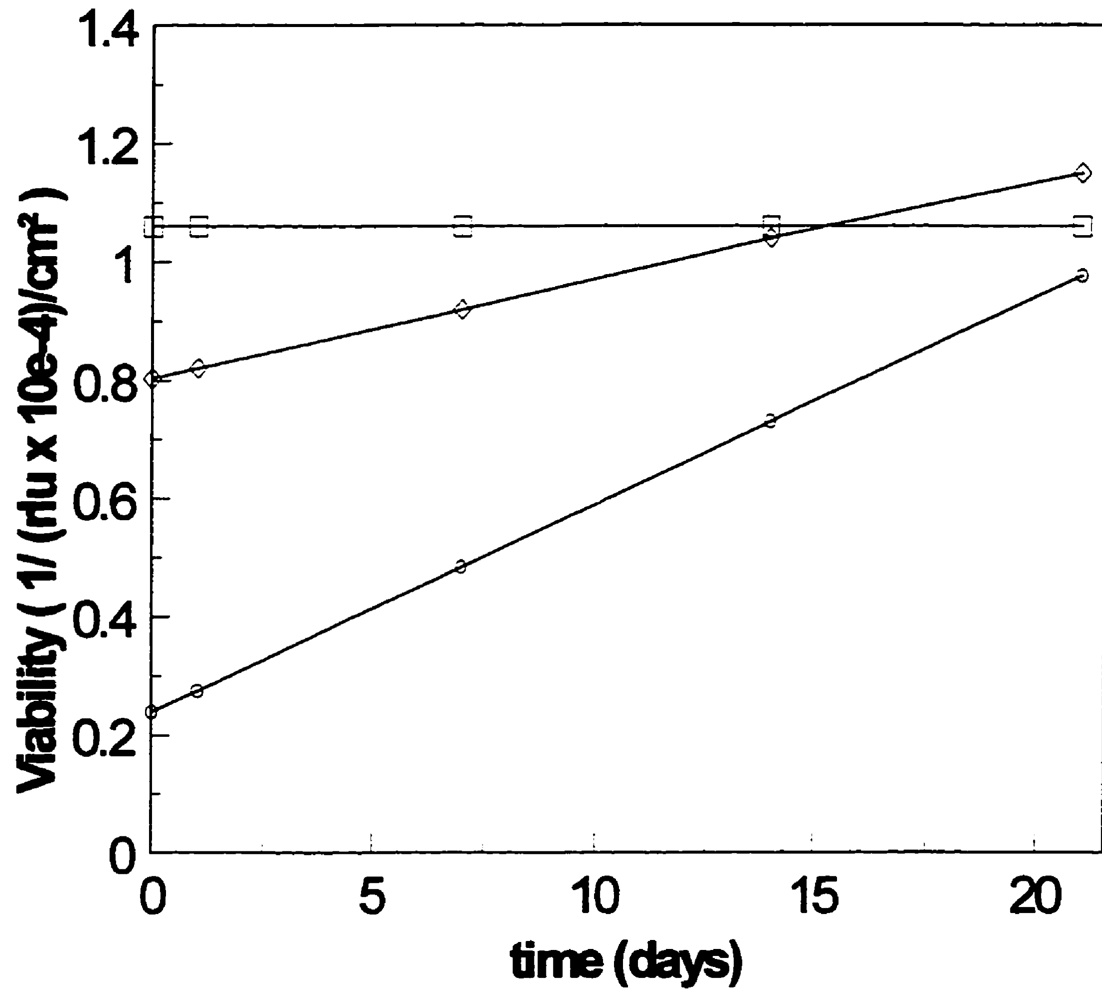


Figure 8.3d: Viability of *Salmonella hadar* (*lux*$^{+}$) over time (i.e., based on bioluminescence output) after exposure to three different treatments (control O; water ◇; lactic acid □) and storage at - 12°C.

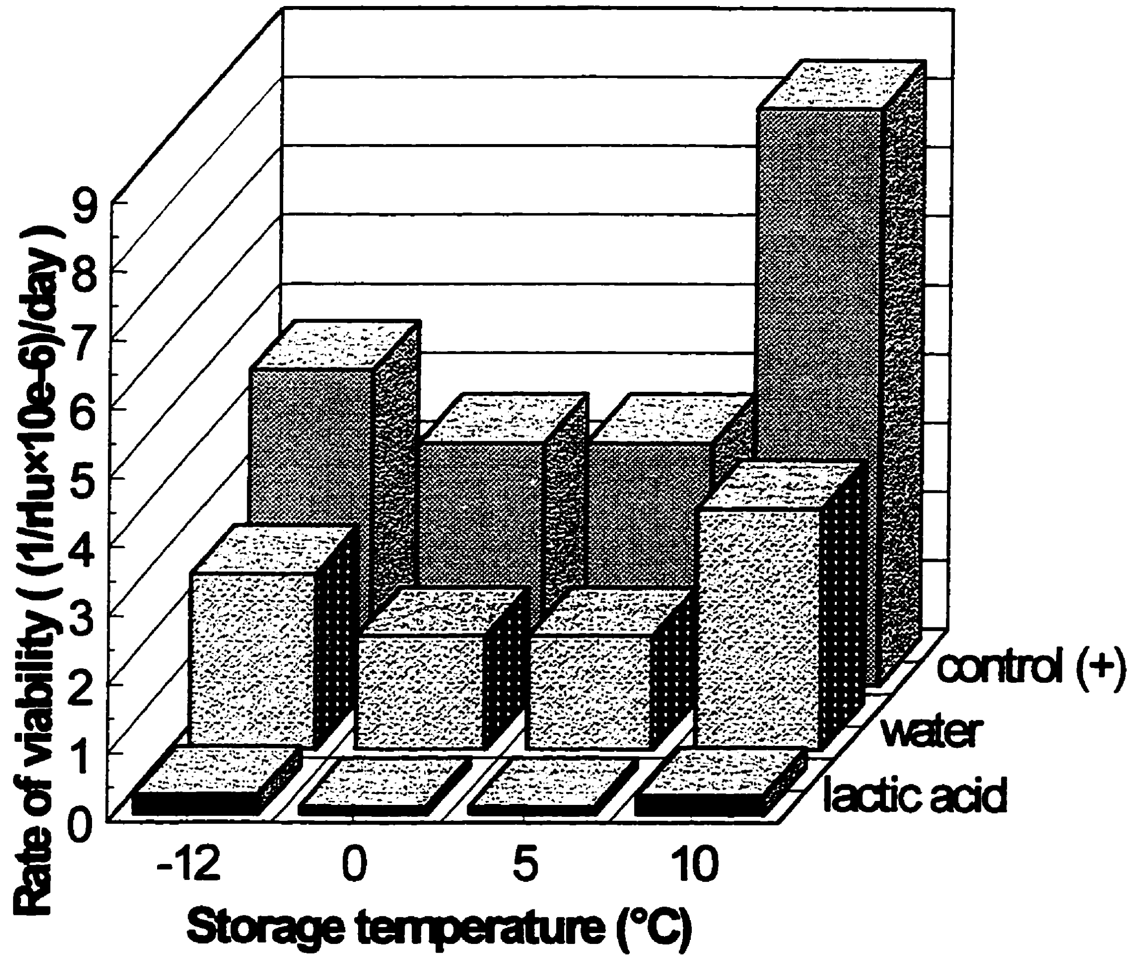
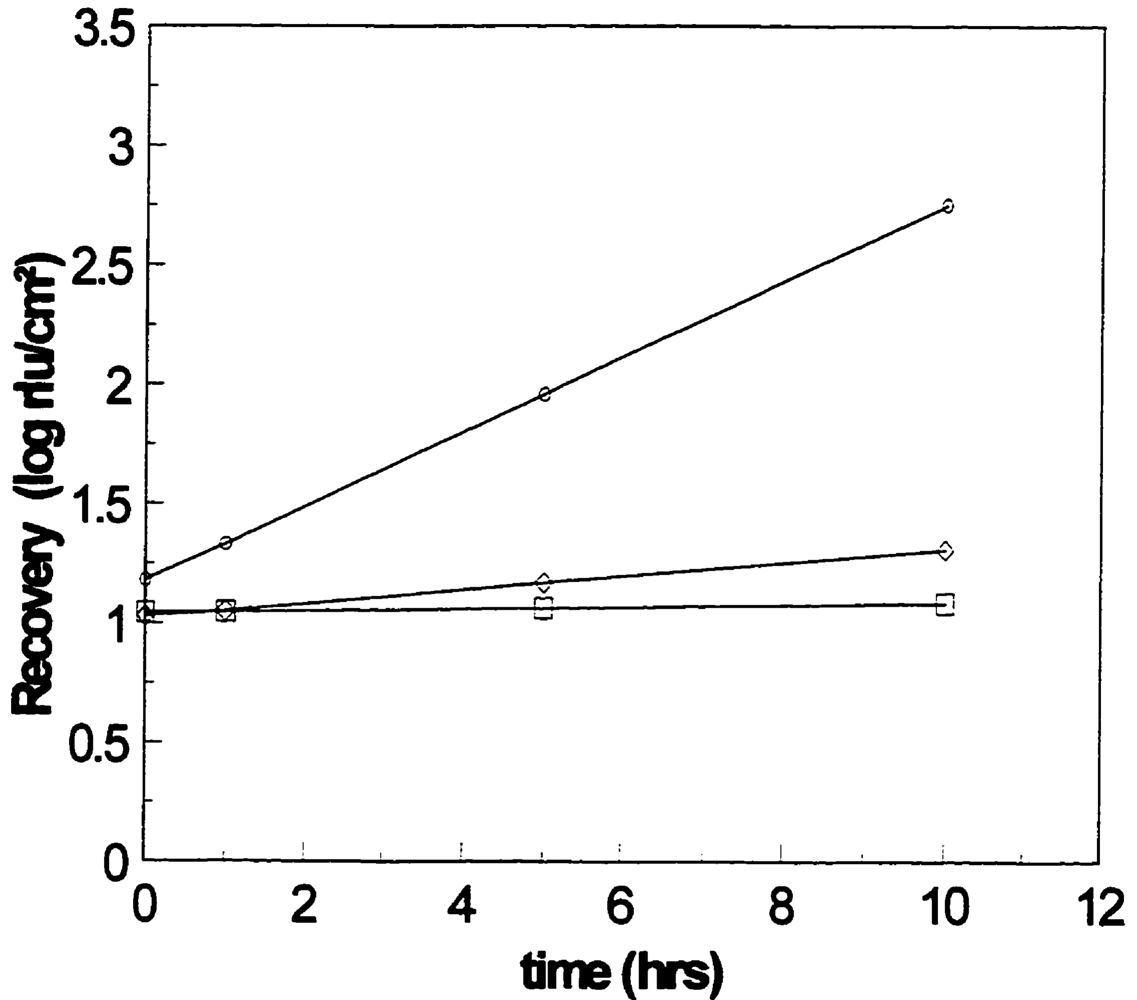


Figure 8.4: Least Square means on the rates of viability from *Salmonella hadar* (*lux*$^+$) (i.e., based on rates of bioluminescence output) after exposure to different treatments and storage at various temperatures (Std. error = 0.90 (1/rlu× 10^{-6}/day))

The recovery study indicated that there was a significant ($p < 0.05$) positive curvilinear response of the bioluminescence output over time during resuscitation of the samples. Illustrations of the recovery curves are shown in Figures 8.5 a to d. Based on the statistical analysis, the results indicated that the rate of recovery (i.e.,m_R) of the S. *hadar* significantly ($p<0.05$) increased with treatment type; Control(+) > water > lactic acid. Recovery was slowest for the lactic acid treatments. There was a significant ($p < 0.05$) interactive effect between treatment and temperature on the rate of recovery of the *S. hadar* (*lux*$^+$). Furthermore, it was determined that temperature had a significant ($p < 0.05$) effect on the rates of recovery of the *S. hadar* (*lux*$^+$). Recovery was greatest following storage at -12°C. There was no significant ($p>0.20$) difference between rates of recovery following storage at 10 and 0°C, but both temperatures produced faster recovery rates than storage at 5°C. Ingram (1951) has also described an adverse effect of cold environments on microorganisms, but at - 2°C.

An explanation of the odd behaviour of the organism during recovery could partly be attributed to the behaviour of water under cold conditions. Water increases in density as it approaches 4°C (Gillespie *et al.*,1986). Therefore, it may be possible that an organism at a storage temperature of 5°C would have to



operate in a more viscous cytoplasm than at higher temperatures. In turn, the

Figure 8.5a: Recovery of *Salmonella hadar* (*lux*$^+$) over time (i.e., based on bioluminescence output) from exposure to three different treatments (control ○; water ◇; lactic acid □) and from storage at 10°C.

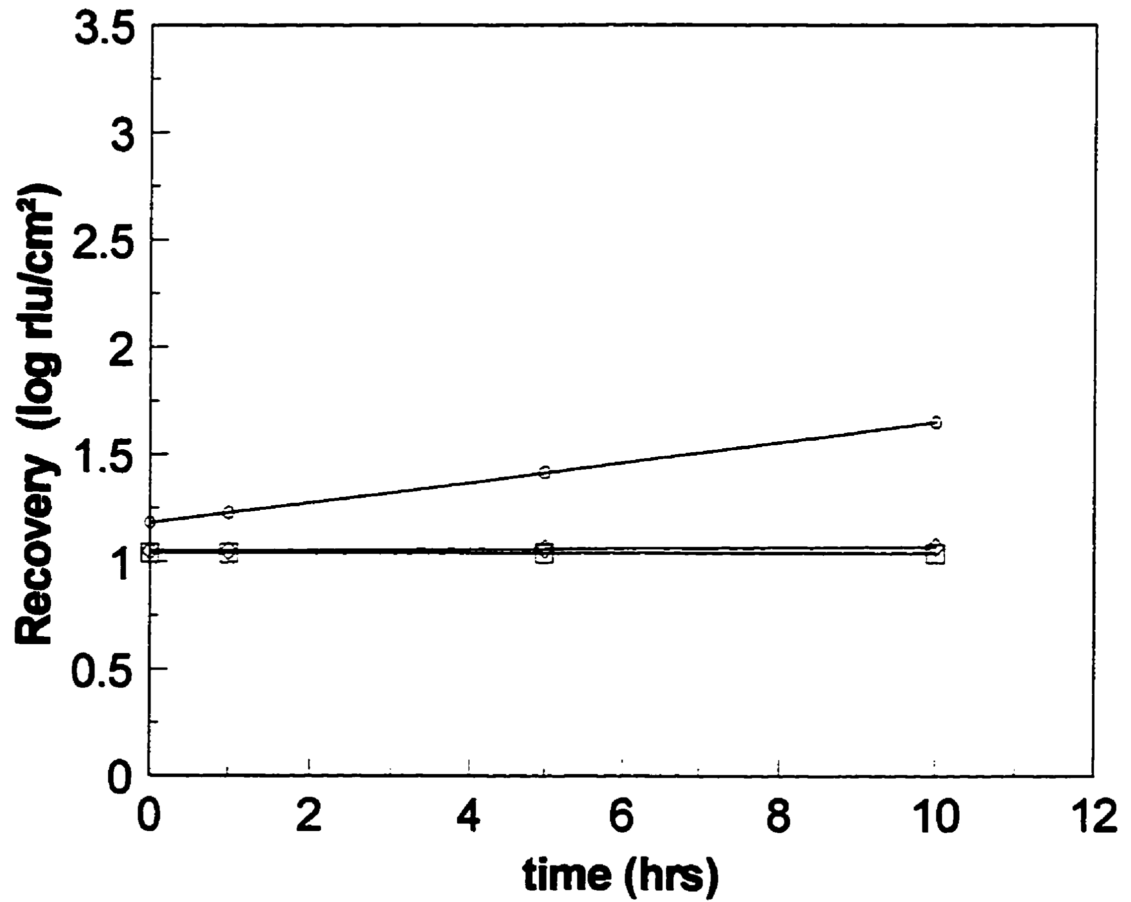


Figure 8.5b: Recovery of *Salmonella hadar* (*lux*$^+$) over time (i.e., based on bioluminescence output) from exposure to three different treatments (control ○; water ◇; lactic acid □) and from storage at 5°C

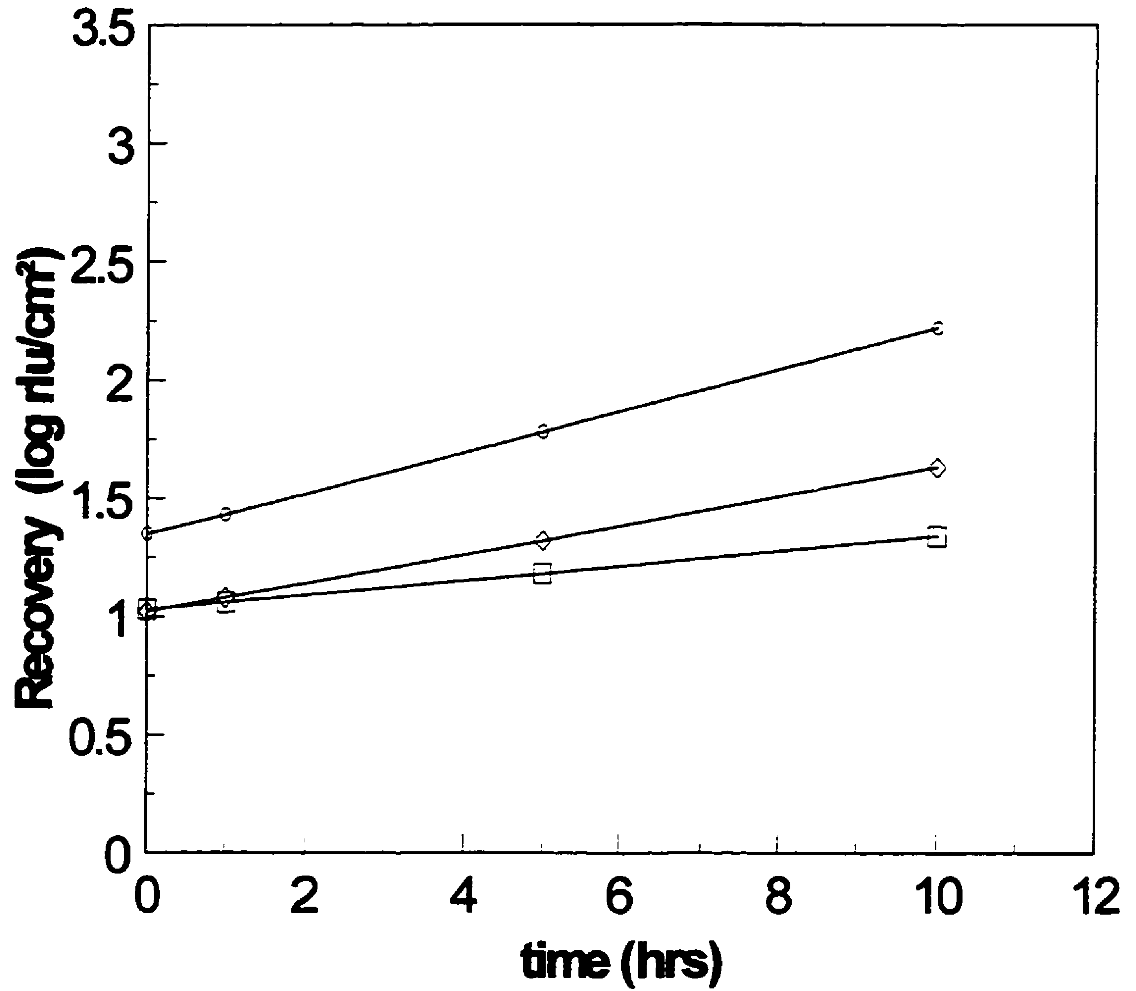


Figure 8.5c: Recovery of *Salmonella hadar* (*lux*$^{+}$) over time (i.e., based on bioluminescence output) from exposure to three different treatments (control ○; water ◇; lactic acid □) and from storage at 0°C

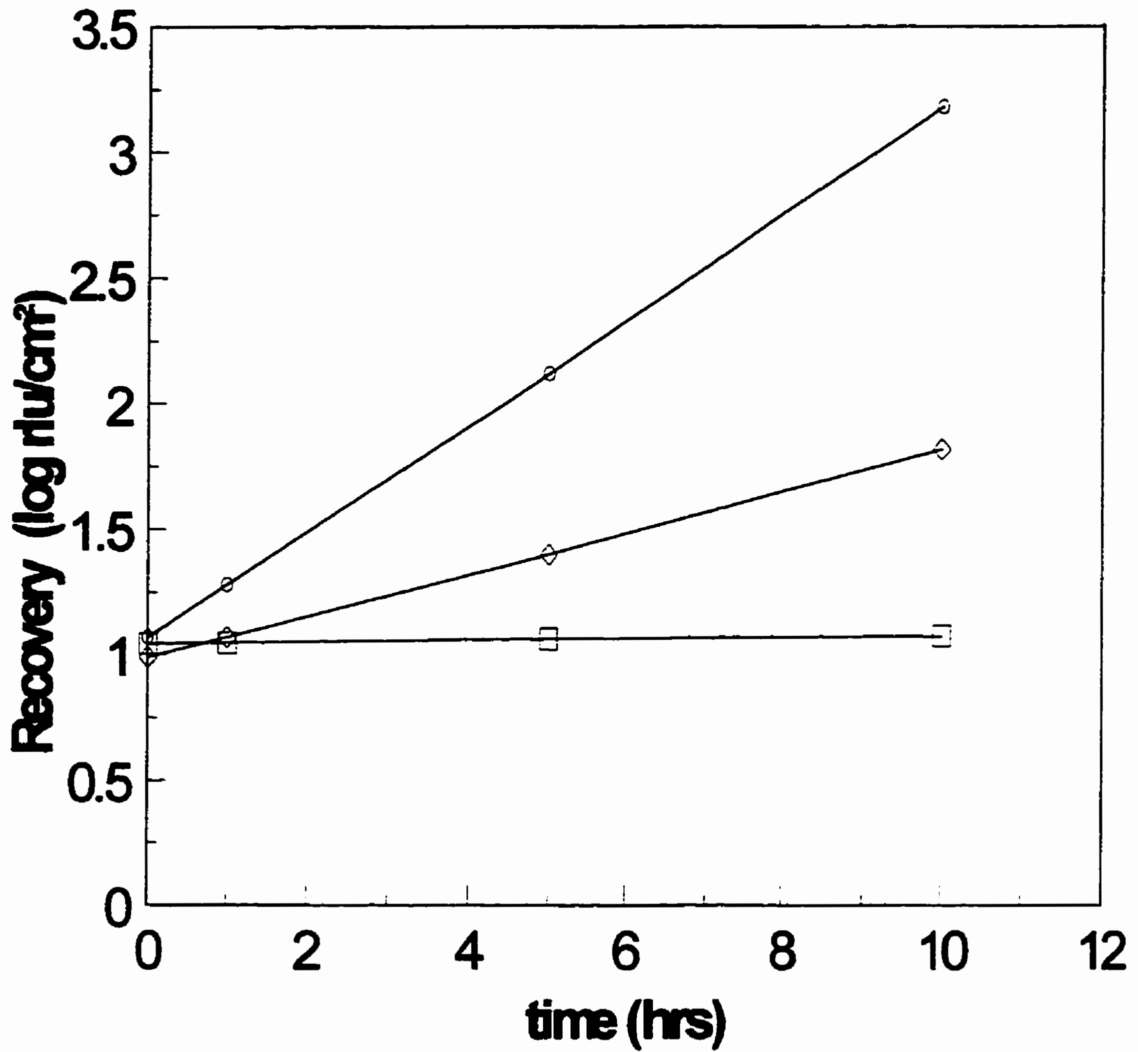


Figure 8.5d: Recovery of *Salmonella hadar* (*lux*⁺) over time (i.e., based on bioluminescence output) from exposure to three different treatments (control ○; water ◇; lactic acid □) and from storage at -12°C

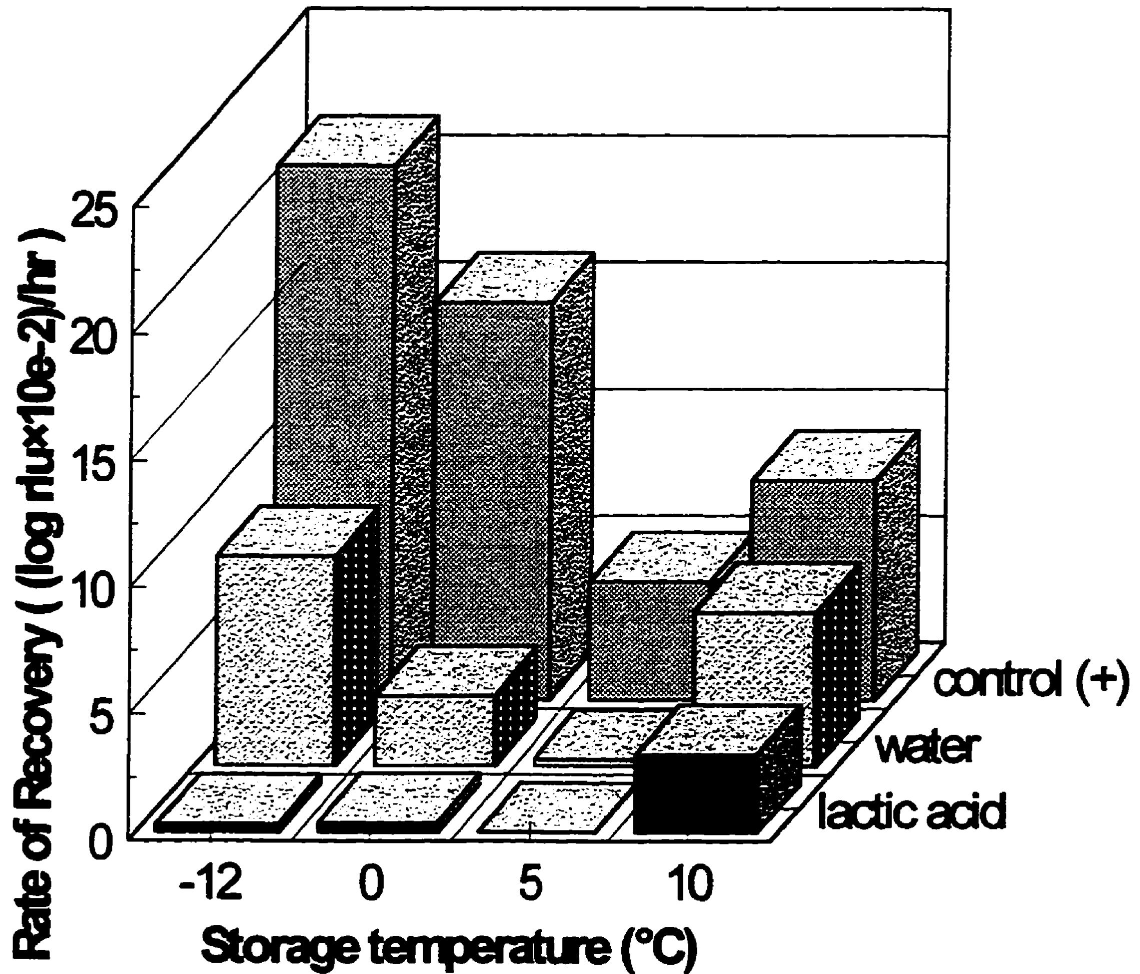


Figure 8.6: Least Square means on the rates of recovery from *Salmonella hadar* (*lux*$^+$) (i.e., based on rates of bioluminescence output) from exposure to different treatments and from various storage temperatures (Std. error = 0.02 (log rlu ×10^{-2})/hr).

cellular functions may be adversely affected. Another explanation could be the proliferation of psychrotrophic bacteria at 5°C that could have a negative effect on *S. hadar* (*lux*$^+$) during storage and subsequently, during resuscitation.

At the lower temperatures (i.e., - 12 and 0 ° C), recovery is dependant on the rate of freezing. Based on the amount of unbound water and the absence of ice crystallization, lower temperatures could promote the preservation of the bacterium and facilitate a better recovery. This may be especially true at a storage temperature of -12°C (Georgala and Hurst, 1963). The functionality of water at lower temperatures in food products has been reviewed by Goff (1992).

8.4 Conclusions

In conclusion, the lactic acid treatment was able to improve both hygiene and safety of meat products during storage and thawing. This was clearly shown by the reduced metabolic rate of *S. hadar* (*lux*$^+$) during the viability study and the reduced metabolic rate during the recovery study. The interpretation of the temperature effect from the recovery study is of paramount value. It presents further evidence of the danger of thawing poultry or meat products that may habour well preserved pathogenic bacteria at ambient temperatures (i.e., 22°C).

9.0 Quality assessment of a poultry processing facility using ATP bioluminescence and conventional microbiological techniques

9.1 Introduction

Programs have been developed for the food industry to help improve food safety and quality. One program of interest to the food industry is HACCP. An important part of a HACCP program is the verification procedures that indicate the system is functioning properly (The National Advisory Committee on the Microbiological Criteria for Foods, 1992). This is especially important for microbial hazards associated with the process (Simonsen *et al.,* 1987). Verification of microbial hazards may be achieved by routine analysis of carcasses using conventional microbiology (i.e., plate counts). However, an appropriate and meaningful sampling method has yet to be developed.

Furthermore, conventional methodology only provides information of a historical nature whereas it may be desirable to obtain results on a near "real-time" basis. A better approach may be the use of ATP bioluminescence assays. ATP bioluminescence assays can provide data on the microbiological quality of carcasses in minutes rather than days (Bautista *et al.,*1994; 1996a; Griffiths 1996;

Stanley, 1989). Using a simple biochemical/enzymatic reaction, the contamination of a surface can be determined (Stanley, 1989). In the food industry, hygiene monitoring tests based on ATP are widely used to determine the level of cleanliness of equipment. Some insight has been directed towards the application of this assay for HACCP verification and/or monitoring protocols.

The objective of this study was to develop a system of sampling that could effectively determine the level of hygiene on carcasses during poultry production. Both the ATP bioluminescence assay and conventional methodology were used to evaluate microbial loads on poultry carcasses during processing.

9.2 Materials and Methods

9.2.1 Sampling Protocol

At a poultry processing plant, 12 carcasses were examined for each of 4 flocks processed on each day. The experiment was repeated on 4 separate days. A total of 192 poultry carcasses was examined ($12 \times 4 \times 4 = 192$) during the entire study.

Sampling of chicken carcasses for bacterial content was carried out using a simultaneous dual swab technique on the chicken samples. The method involved swabbing the entire breast area with two cotton tip swabs, simultaneously. To

ensure adequate sampling, the breast area was swabbed in a horizontal pattern and secondly, swabbed once again in a vertical pattern. In addition, the swabs were rotated between the index finger and the thumb in a back and forth motion. This allowed for complete use of the entire swab surface. After sampling, one of the swabs was placed into 10 mL of 0.1% peptone (Difco, Inc.), the other swab was replaced into its container. Both swabs were brought back to the laboratory for further analysis by ATP bioluminescence and conventional microbiology.

9.2.2 Assessment of the microbiological quality of processed carcasses

9.2.2.1 Conventional microbiology

Serial dilutions (2 × 1:9 mL in 0.1% peptone) of the swab sample were prepared and 1 mL of the dilution was dispensed onto Petrifilm™ (3M, Inc.) for total aerobic counts. The Petrifilms™ were incubated for 24 hrs at 37°C and pink colonies counted.

9.2.2.2 ATP bioluminescence

The swab (designated for ATP analysis) was immersed in 1 mL of lysing solution (Bactex solution, MMK/100, Biotrace Inc.) for 1 min. An aliquot (200 µL) of the lysed solution was placed into a fresh cuvette (Biotrace, Inc.) and combined with

100 µL of prepared luciferin/luciferase solution (MMK/100 Biotrace, Inc.) ; diluted 1:10 with sterile distilled water. The cuvette was placed into a Multilite luminometer (Biotrace, Inc.) and the light output measured.

9.2.3 Layout of statistical design and analysis

An illustration of the poultry processing line is depicted in Figure 9.1. To determine baseline levels of contamination, carcasses at several key areas, identified as possible CCPs, were sampled to assess microbiological quality. A list of the 12 designated areas is provided in Table 9.1 and shown schematically in Figure 9.1. The statistical design was set up as a partially balanced incomplete latin square, where 4 flocks of poultry were analysed on each experimental day and each flock was analysed for all stations listed above. The order of station sampling was completely randomized within each flock processed. The experiment was replicated on 4 separate days ($12 \times 4 \times 4 = 192$).

The results were analyzed using the MIXED procedure of Statistical Analysis System SAS® (SAS, 1988).

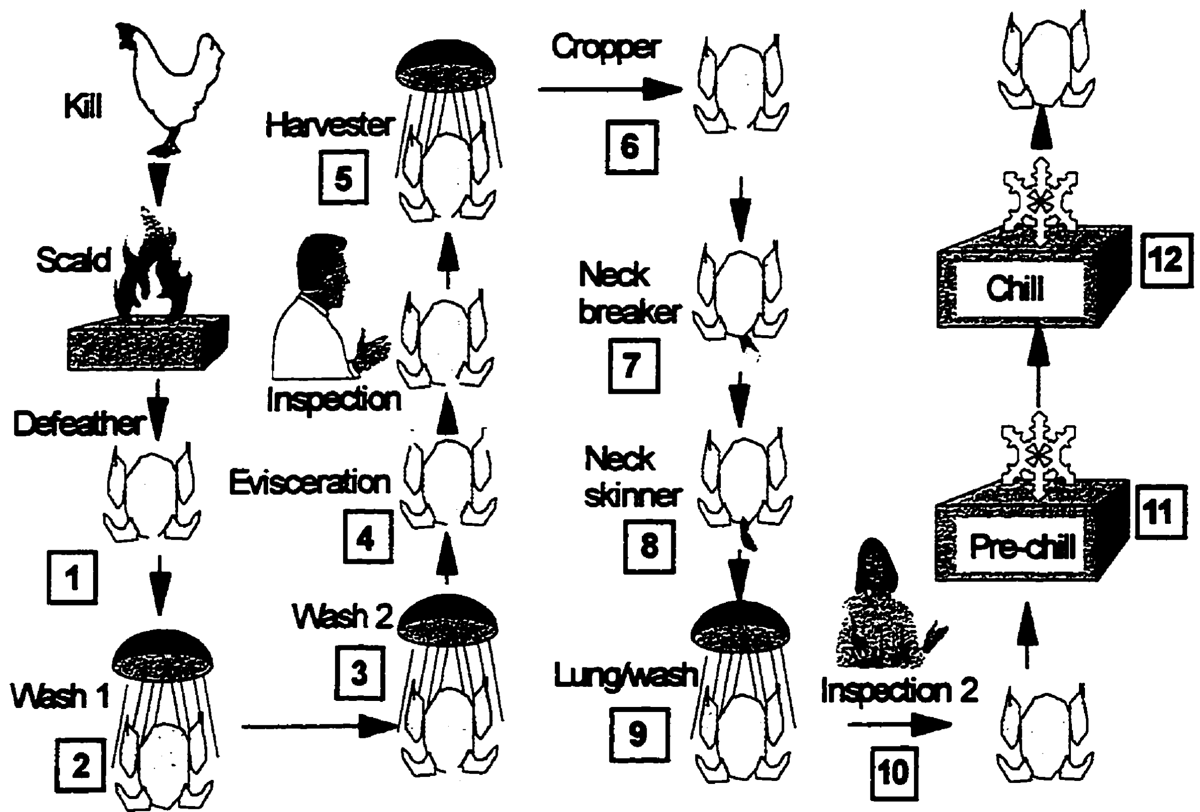


Figure 9.1: Illustration of the processing steps at a poultry abattoir and stations identified for poultry hygiene assessment during processing

Table 9.1: Summary of stations evaluated at a poultry processing plant

1) Before shower 1	7) After\before neck removal
2) After shower 1\before shower 2	8) After neck removal\before vacuum
3) After shower 2\before evisceration	9) After vacuum\before inside-outside wash
4) After evisceration\before inspection	10) After inside-outside wash\before prechilling
5) After inspection\before cropper	11) After prechilling\before chilling
6) After cropper\before neck breaker	12) After chilling

9.3 Results and Discussion

To test repeatability of the swabbing procedure within a sample, chicken carcasses (n=3) were obtained, the breasts separated from the carcass, and analysed for quality by the plate count method in duplicate. Results of a student t-test showed that there was no significant ($p>0.20$) difference in count between duplicates. The same result was true for the ATP bioluminescence assay. Therefore, the repeatability of the sampling procedure, within a sample, was sufficient for both the plate count procedure and the ATP bioluminescence assay.

9.3.1 The analysis of quality of carcasses at stations along the processing line by conventional microbiology and ATP bioluminescence

The Least Square means of microbial load on carcasses at each of the stations for the entire study are illustrated in Figure 9.2. As indicated by aerobic plate counts, the level of contamination on poultry carcasses was constant throughout stations 1 to 10. At stations 11 and 12, there was a significant ($p<0.001$) decrease in microbial levels on poultry carcasses. This was probably due to the chilling of carcasses in the prechill and chill tanks. The Least Square means of the microbial load by conventional methodology on carcasses at each of the stations for individual days are reported in Table 9.2. The results show similar trends of

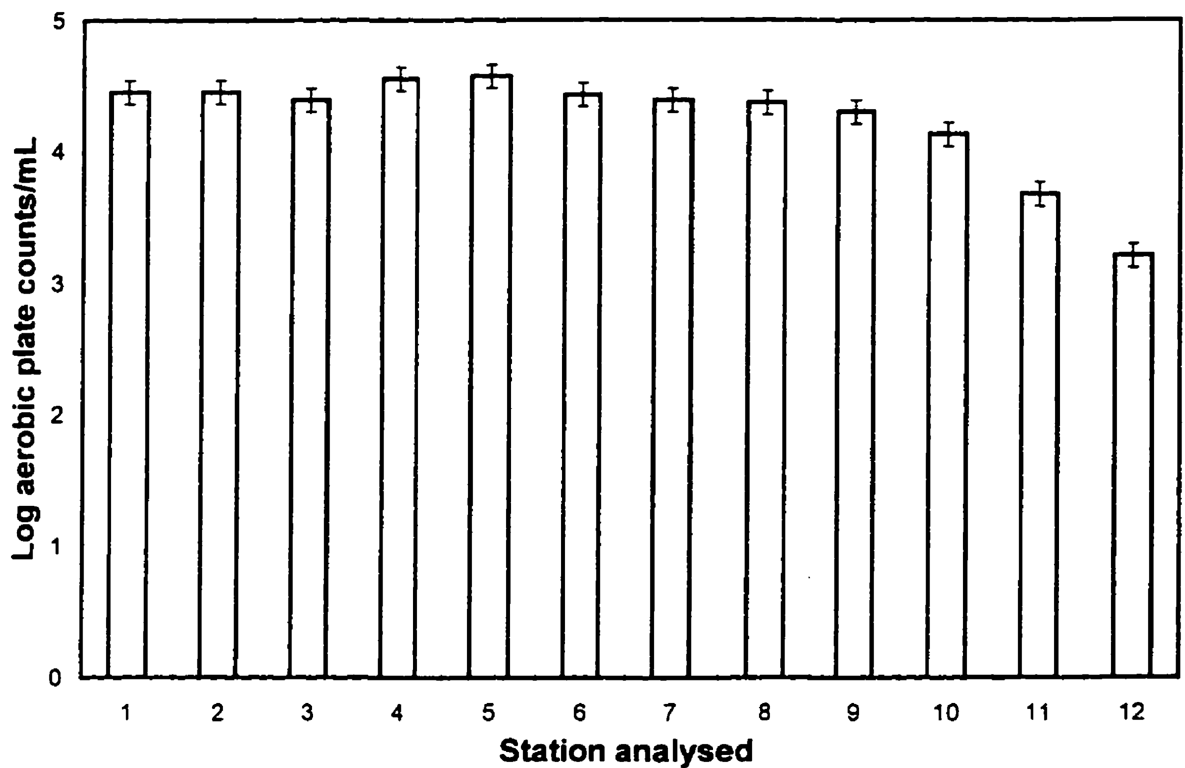


Figure 9.2: Least Square means of the microbiological quality (i.e., as determined by conventional methods) of carcasses sampled at different stations at a poultry processing plant for the entire study (n = 192).

Table 9.2: Least Square means of the microbial contamination (i.e., as determined by conventional methods; cfu/mL) of carcasses sampled at different stations at a poultry processing plant for individual days (Std. error = 0.10 $\log_{10}$ cfu/mL)

Station	Day 1	Day 2	Day 3	Day 4
1	4.66	4.58	4.36	4.20
2	4.52	4.42	4.27	4.58
3	4.24	4.38	4.42	4.51
4	4.60	4.39	4.68	4.55
5	4.74	4.55	4.27	4.72
6	4.57	4.58	4.31	4.25
7	4.69	4.31	4.10	4.48
8	4.41	4.44	4.17	4.45
9	4.54	4.26	3.97	4.42
10	4.33	4.15	3.70	4.33
11	3.86	4.02	3.41	3.44
12	3.45	3.39	2.88	3.15

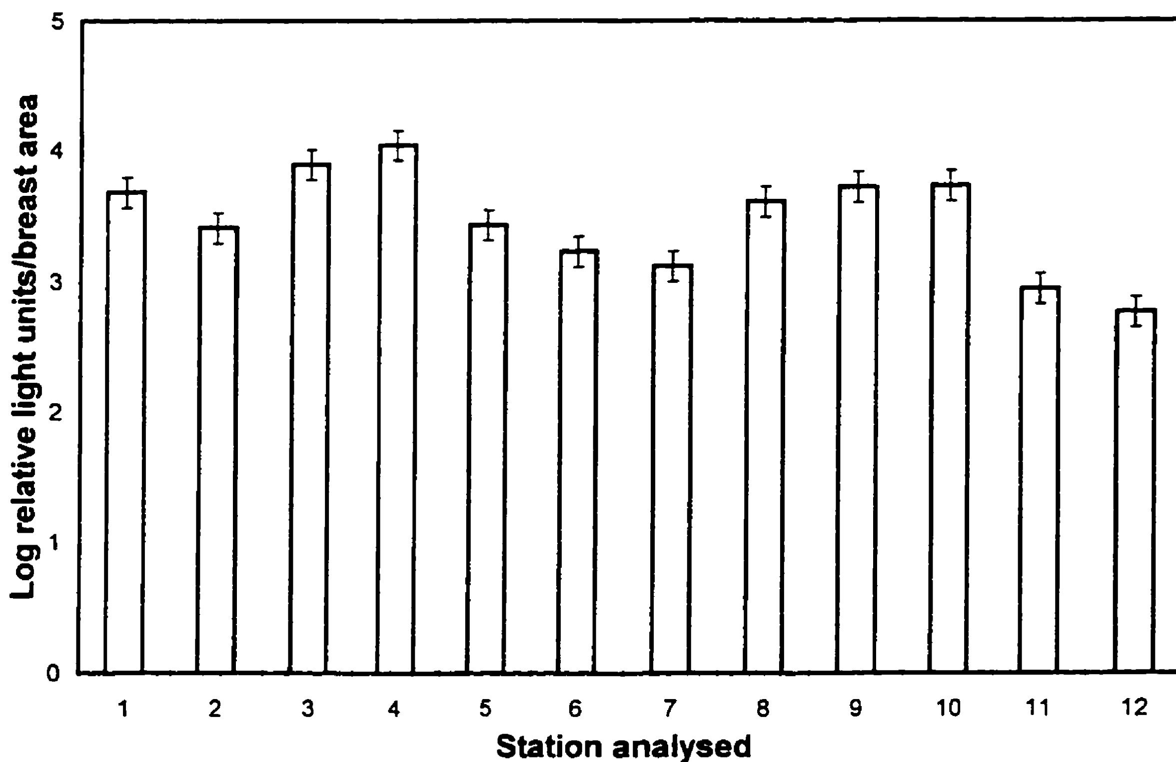


Figure 9.3: Least Square means of the overall quality (i.e., as determined by the ATP bioluminescence assay) of carcasses sampled at different stations at a poultry processing plant for the entire study (n = 192).

Table 9.3: Least Square means of the microbial contamination (i.e., as determined by the ATP bioluminescence assay; rlu/breast area) of carcasses sampled at different stations at a poultry processing plant for individual days

(n = 192; Std. error = 0.11 log rlu/breast area).

Station	Day 1	Day 2	Day 3	Day 4
1	4.03	3.78	3.34	3.57
2	3.96	2.95	3.36	3.38
3	3.97	3.85	3.89	3.85
4	4.39	3.29	4.07	4.41
5	3.37	2.99	3.87	3.50
6	3.50	2.75	3.55	3.12
7	2.90	3.20	3.15	3.24
8	3.68	3.55	3.70	3.52
9	3.71	3.40	3.73	4.05
10	3.78	3.77	3.66	3.72
11	3.03	2.73	2.92	3.14
12	2.64	2.79	2.89	2.79

microbial contamination on carcasses.

Similar significant reductions ($p<0.001$) are also illustrated at stations 11 and 12 for poultry carcasses analysed by the ATP bioluminescence method (Figure 9.3). However, the assay showed some variation in the Least Square means between stations 1 to 10 that was not observed when samples were assayed by conventional methodology. ATP levels were higher on carcasses sampled at points 3 and 4 and also at points 8, 9 and 10. The higher levels of contamination at station 3 and 4 were probably due to contamination associated with evisceration of the poultry carcass. The higher contamination levels at 8, 9 and 10 are not so easy to explain. Since carcasses are inverted during processing (i.e., head down), a possible reason could be due to some contents from the crop that may have worked its way onto the neck area. As carcasses are passed through the neck breaker, contamination can be transferred onto the carcass and surrounding carcasses.

The Least Square means of the microbial load determined by the ATP bioluminescence assay on carcasses at each of the stations for individual days are reported in Table 9.3. The results show similar trends of contamination levels at all stations throughout the entire study.

9.3.2 Analysis of overall quality of poultry slaughtered on individual days by conventional methods and the ATP bioluminescence assay

Results of analysis of microbiological quality and overall quality by conventional methods and the ATP bioluminescence assay, respectively, for individual days are illustrated in Figures 9.4 and 9.5. There were significant ($p<0.05$) differences of contamination levels on individual days by both conventional methodology and the ATP bioluminescence assay. However, the differences were within 1 $\log_{10}$ (i.e., between 3 and 4 $\log_{10}$ cfu/mL) and could be regarded as small.

9.3.3 Analysis of quality of chicken flocks on individual days by conventional methodology and the ATP bioluminescence assay

Least Square means of the quality of flocks analysed on a daily basis by conventional methods and the ATP bioluminescence assay are reported in Tables 9.4 and 9.5, respectively. For all days, flocks showed significant ($p<0.05$) differences in levels of quality during processing by plate count and the ATP bioluminescence assay.

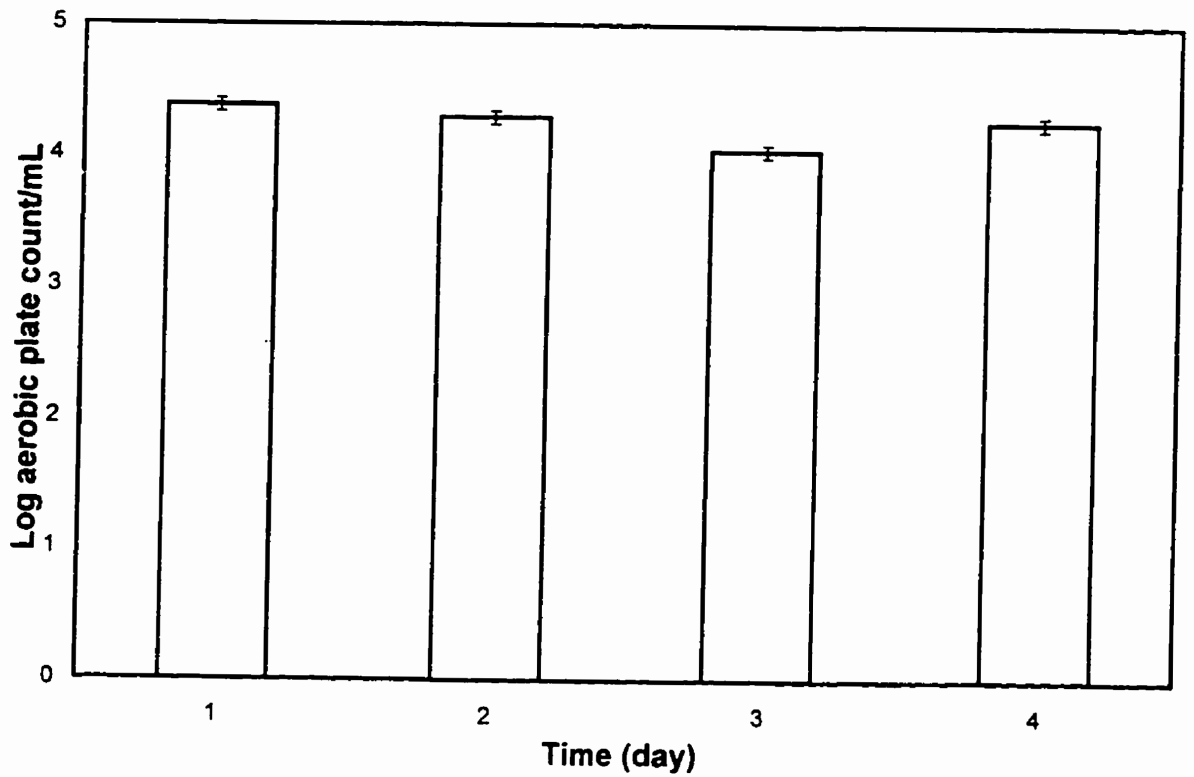


Figure 9.4: Least Square means of the microbiological quality (i.e., as determined by conventional methods) of carcasses sampled on individual days (n = 192).

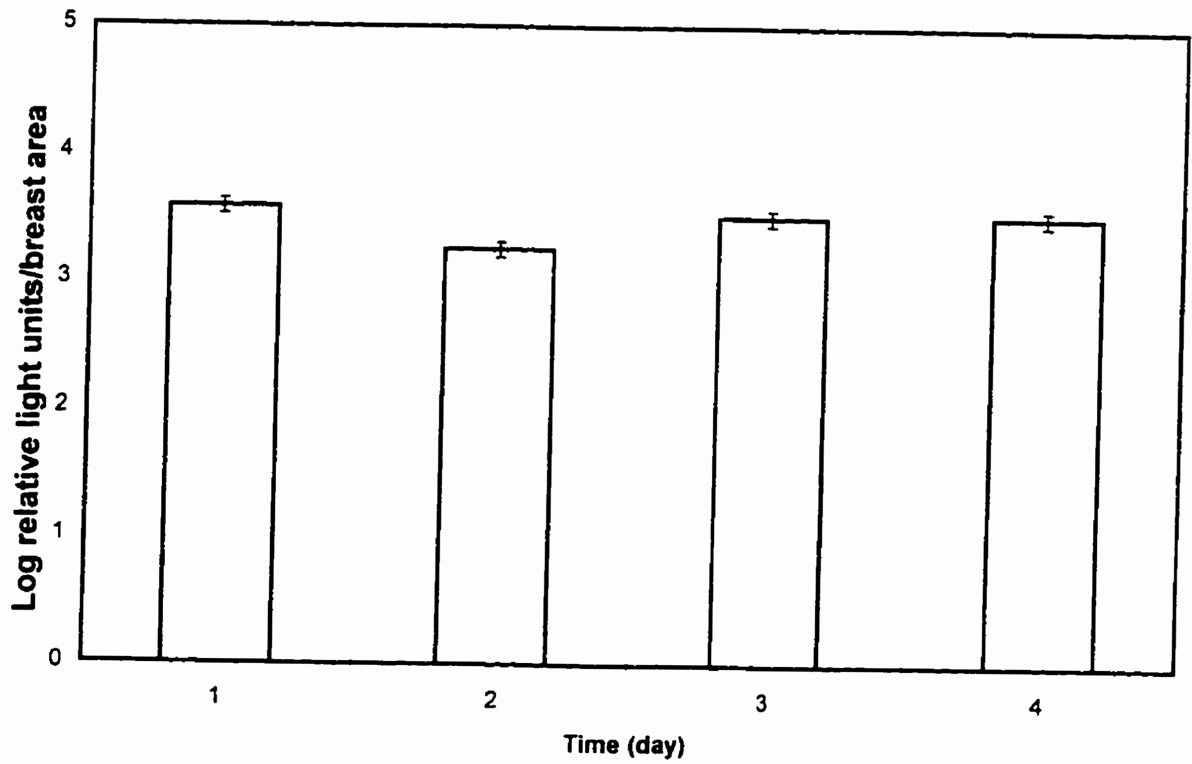


Figure 9.5: Least Square means of the overall quality (i.e., as determined by the ATP bioluminescence assay) of carcasses sampled on individual days (n = 192)

Table 9.4: Least Square means of the microbiological quality (i.e., as determined by conventional methods; cfu/mL) of flocks of poultry processed on individual days (n = 192; Std. error = 0.10 cfu/mL)

Flock	Day 1	Day 2	Day 3	Day 4
1	4.36	4.33	4.06	4.31
2	4.57	4.13	3.85	4.12
3	4.40	4.42	3.93	4.26
4	4.20	4.27	4.33	4.33

Table 9.5: Least Square means of the overall quality (i.e., as determined by the ATP bioluminescence assay; rlu/breast area) of flocks of poultry processed on individual days

(n = 192; Std. error = 0.13 rlu/breast area)

Flock	Day 1	Day 2	Day 3	Day 4
1	3.73	3.37	3.31	3.31
2	3.74	3.00	3.59	3.45
3	3.59	3.38	3.60	3.79
4	3.26	3.27	3.54	3.55

However, again these differences were within 1 $\log_{10}$ (i.e., between 3 and 4 log10 cfu/mL). Therefore, the differences in contamination levels were regarded as small.

Both Renwick *et al.* (1993) and McNab *et al.* (1993) also confirmed that significant ($p<0.05$) levels of contamination were associated with flocks. They used an automated hydrophobic grid membrane filtration apparatus/ interpreter system to determine their results.

According to this limited study, both the ATP bioluminescence assay and the plate count method were able to evaluate the hygienic levels of a poultry processing system. Therefore, if the industry is considering using either technique, it will be necessary to implement some statistical design and analysis. The information obtained in this report cannot be achieved with occasional random sampling as proposed by the "Final Rules" guidelines or by the Canadian Generic HACCP model for poultry slaughter (Anon, 1997a; Anon, 1997b). The sample scheme must be planned such that the maximum information can be retrieved for interpretation of the system performance.

Also, one must consider the major advantage of using the ATP bioluminescence assay. The ATP bioluminescence assay has proved to be useful for the determination of hygiene of meat products (Bautista *et al.*,1994;1996a; Siragusa and Cutter, 1995; Siragusa *et al.*,1995) and equipment surfaces (Bautista

et al.,1994;Seeger and Griffiths, 1994). The test, as described in this study, can be performed in less that 5 mins and provides an assessment of carcass quality on a near "real-time" basis. This definitely would be a great help for HACCP programs.

Regression analysis of the Least Square means of conventional methodology and the ATP bioluminescence assay for the quality of stations for the entire study was satisfactory ($n = 12$ $r=0.69$ $p<0.05$). However, it should be noted that both assay systems measured two different parameters (i.e., microbial vs. overall cleanliness). Therefore, an exact relationship between the two assessments should not be expected and the assay systems should be interpreted individually.

The data indicated that a good statistical design and analysis can provide meaningful interpretation of complex systems. From the information gathered in this experiment, a CCP should be established in the areas of prechill and chill immersion. It has been recognized that adequate chilling and agitation in the prechill and chill tanks are able to reduce the levels of contamination significantly (ICMSF, 1988; Brock, 1991). Upon adopting the necessary specifications for this CCP, the poultry industry will control and likely reduce the overall microbial level on poultry carcasses. There is some indication that other areas can be effective CPPs (e.g., evisceration area) within a poultry processing system. However, more studies are required.

9.4 Conclusions

With the aid of statistical designs, the quality of a poultry process was effectively determined by both conventional methodology and the ATP bioluminescence assay. Although the results were meaningful, it is not an exhaustive study. More information can be obtained from an extended study at a poultry processing plant. The statistical method helped scale down this observational study into a manageable form.

The plate count method was able to provide similar information as the ATP bioluminescence assay. However, plate count results were obtained after 24 to 48 hrs of incubation time as opposed to minutes by the ATP bioluminescence assay. In a situation where a problem may require immediate attention, the plate count method fails. Furthermore, the importance of "real time" evaluation of microbial hazards must be recognized. It is only by adopting this philosophy that the food industry can assess and correct a microbial problem effectively.

10.0 General Discussion

The meat inspection service and food quality assurance programs have provided a level of safety to the consumer. Unfortunately, outbreaks of foodborne illness continue in spite of their efforts. Therefore, present methods of ensuring safety and quality are not always reliable and other technologies and philosophies should be introduced to provide an increased level of safety to the consumer. These philosophies and techniques can easily complement the meat inspection service.

As presented in this dissertation, bactericidal treatments and verification of HACCP programs can certainly improve the level of safety and quality of meat products. Based on overall performance, lactic acid treatments should be implemented to decontaminate poultry surfaces. However, a high concentration of lactic acid (> 4.0 % w/w) is necessary if food establishments want to achieve a significant improvement of meat surface hygiene. The research presented here and previous research activities have shown the poor efficacy of lactic acid treatments lower than 3% w/w (Bautista *et al.*, 1996b; Greer and Jones, 1991; van der Marel *et al.*, 1988). Although the present study focuses on the application of bactericidal treatment onto carcasses contaminated with feces, lactic acid may be used as a routine treatment to improve the hygiene of all carcasses during production. However, further research is required.

In addition, lactic acid can be regarded as a naturally occurring substance with low toxicity. Lactic acid is a fundamental component of carbohydrate and amino acid metabolism and is readily found in tissues, liquids, secreta and excreta of the body (Wagner, 1981). The metabolism of lactic acid is regulated by hormones (adrenalin, insulin, glucagon, etc.) and it has been reported that humans produce 117 to 144g lactate/24h/70kg (Connor *et al.*,1982). Furthermore, lactates have low acute toxicity in animals (Elias, 1987). The oral lethal dose (LD_{50}) in rodents is 2 to 5 g/kg body weight and dogs can ingest up to 1 600 mg/kg body weight over 2.5 months without ill effects. Thus, it has GRAS status.

Consumer acceptance of poultry products treated with lactic acid may or may not be a problem. The only concern is the discolouration the acid produces on meat surfaces. Although a post treatment with water significantly improved the appearance of poultry carcass surfaces, there was a significant ($p < 0.05$) irreversible change in Hunter b values (i.e., yellow - blue). However, a close visual inspection is required before the discoloration becomes apparent. In comparison to discoloured carcasses from an air-chilling process, lactic acid treatments may not pose an issue with consumers.

For in-plant use, spraying technology should be used to administer the lactic acid treatment. Spraying is a better approach than other techniques (i.e., dipping of carcasses in solution) because there is less opportunity for cross contamination.

Furthermore, the frequent use of a holding tank may harbour the possibility of introducing a lactic acid resistant microorganism onto food products. The problem could be the opportunistic colonization of the lactic acid resistant organism on the product. It is of utmost importance if the resistant organisms are pathogens. If a food processor still wishes to use immersion techniques, they should frequently change the bactericidal solution to maximize performance and reduce the prevalence of resistant bacteria.

As for storage and recovery studies, the use of bacterial bioluminescence as a "real-time" indicator of metabolic status of *S. hadar* (*lux*$^+$) provides an innovative approach for evaluating bactericidal efficacy. This especially came to light with the results from breast samples treated with lactic acid. Note, if a similar experiment was performed with conventional microbiological techniques, certain problems may arise that could affect the accuracy of the study. For example, due to the destructive nature of conventional microbial techniques, several samples would have to be used to accommodate the sampling interval for the shelf-life study. Furthermore, an assumption would have to be made that the level of inoculum on the sample would be the same throughout. Unless certain precautions are taken, this is difficult to do on a practical basis. Studies using bioluminescent organisms can be performed non-destructively and only one sample would be necessary per experimental run. Bacteria modified with bioluminescence genes have been used to detect

Salmonella spp. in food samples (Chen and Griffiths, 1996; Hudson *et al.*,1996; Tomicka *et al.*, 1996) and to evaluate metabolic activity under different environmental conditions (Walker *et al.*,1992; Ellison *et al.*,1991; Duffy *et al.*,1995).

Unfortunately, there were limitations of the instrument (i.e., BIQ; Cambridge Imaging, Ltd.) used to determine the bioluminescence output from poultry samples in this study. The minimum light output from a population of bacteria that could be detected corresponded to about 10 000 cfu/mL. Therefore, individual assessment of a single bioluminescent organism was not feasible. A microscopic assessment of individual bioluminescent microorganisms could lead to a clearer picture of microbial reduction, metabolic activity and response to the environment. The present study could only detect light output per unit of sample. However, there is a microscopic imaging system available from a company (Hamamatsu, Inc.) and this instrument will expand the usefulness of bioluminescence to food microbiologists.

Recently, the FSIS and CFIA produced several generic HACCP programs for meat processing plants. Two HACCP programs of key interest are the American and Canadian Generic HACCP Model for Poultry Slaughter (Anon 1994; Anon 1997b) (Section 2.5.5.3).

An attempt at regulation of microbial hazards is proposed by FSIS with the publication of the "Final Rules" for meat processing establishments (Anon., 1997a). However, the frequency rate for microbial analysis for post-harvest of animals is not

comprehensive. For example, a broiler is to be sampled for every 22 000 birds during processing on a weekly basis for *E. coli* (Anon, 1997a). For smaller volume plants, evaluation can be as infrequent as once a year.

Obviously, this type of hygiene assessment for quality and safety is unacceptable. Furthermore, several variables (e.g., seasonality, flocks of poultry, staff, etc.) can influence the outcome of the result that cannot be covered by the "Final Rules" protocol (Renwick *et al.*,1991; McNab *et al.*,1993). Therefore, there is a need for statistical designs and analysis that can determine hygiene at meat processing establishments on a routine basis.

As demonstrated in Section 9.0, the statistical design proved to be useful in determining the baseline values of contamination during processing. This was clearly seen using both the ATP bioluminescence assay and conventional microbial techniques to assess contamination. Although conventional microbiological techniques were able to determine contamination levels of the process, food processors are encouraged to see the value of immediate results from the ATP bioluminescence assay. The greatest advantage is the "real time" evaluation of CCPs that allow for immediate adjustments during processing. Furthermore, with the development of surface samplers (Sharpe *et al.*, 1996), more convenient and practical sampling methods can be developed to help food processors more easily determine hygiene during processing.

The results from this study provide an interesting look at novel approaches for the determination and control of the safety and quality of poultry products. However, it is not exhaustive and additional research is required before any of these techniques are implemented in meat processing establishments.

For lactic acid treatments, future work can be directed towards routine application of the acid for all poultry carcasses. However, further studies must be performed to find the right conditions for regular processed birds. The advantage of the treatment could not only improve safety, but extend shelf-life of poultry products, as well. In addition, studies should be performed to determine if bacteria, particularly pathogens, can become resistant to the treatment.

In regards to the *S. hadar* (*lux*$^+$), future work could involve an investigation of patterns of contamination during evisceration of carcasses. This can be easily achieved by administering the organism into feed or by oral-gavage. Then, patterns of light emissions can be mapped on the carcass. Knowledge of the patterns of contamination on carcasses could help with future designs of equipment for poultry processing.

11.0 Conclusions

1) Of the four bactericides studied, lactic acid was the most effective treatment that improved the hygiene of contaminated carcasses.

2) In an industrial setting, the lactic acid treatment outlined in Section 4.0 significantly ($p < 0.05$) improved the hygiene of contaminated carcasses to levels equal to or better than regular processed birds.

3) A prescribed lactic acid treatment did not impart any significant ($p > 0.20$) or undesirable taste qualities onto poultry products following water immersion treatments. Water immersion can also be used to minimize the discolouration effects from the treatment.

4) Based on bioluminescence studies with a modified *S. hadar* (*lux* CDABE), lactic acid treatments were able to reduce initial levels of contamination, reduce metabolic activity and suppress recovery of the organism.

5) Statistical planning and analysis could prove to be useful tools for verification of HACCP programs and, when combined with rapid microbiological tests, such as, ATP, bioluminescence, a "real time" evaluation of the program can be achieved.

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13.0 Appendices

SOB Medium

Per liter:

To 950 ml of deionized H_2O, add:

bacto-tryptone	20 g
bacto-yeast extract	5 g
NaCl	0.5 g

Shake until the solutes have dissolved. Add 10 ml of a 250 mM solution of KCl. (This solution is made by dissolving 1.86 g of KCl in 100 ml of deionized H_2O.) Adjust the pH to 7.0 with 5 N NaOH (~0.2 ml). Adjust the volume of the solution to 1 liter with deionized H_2O. Sterilize by autoclaving for 20 minutes at 15 lb/sq. in. on liquid cycle.

Just before use, add 5 ml of a sterile solution of 2 M $MgCl_2$. (This solution is made by dissolving 19 g of $MgCl_2$ in 90 ml of deionized H_2O. Adjust the volume of the solution to 100 ml with deionized H_2O and sterilize by autoclaving for 20 minutes at 15 lb/sq. in. on liquid cycle.)

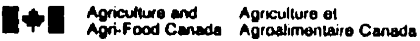
SOC Medium

SOC medium is identical to SOB medium, except that it contains 20 mM glucose. After the SOB medium has been autoclaved, allow it to cool to 60°C or less and then add 20 ml of a sterile 1 M solution of glucose. (This solution is made by dissolving 18 g of glucose in 90 ml of deionized H_2O. After the sugar has dissolved, adjust the volume of the solution to 100 ml with deionized H_2O and sterilize by filtration through a 0.22-micron filter.)

(Sambrook et al., 1989)

Appendix B

96·11·13 & (14)



Agriculture and Agri-Food Canada — Agriculture et Agroalimentaire Canada

SALMONELLA SEROTYPING REPORT — RAPPORT DE SÉROTYPAGE DES SALMONELLES

616

Submitted to:	Présenté à:	Report to (Name and Address) / Remettre à (Nom et adresse):
Agriculture and Agri-Food Canada Health of Animals Laboratory 110 Stone Rd. W. Guelph, Ontario N1G 3W4	Agriculture et Agroalimentaire Canada Laboratoire d'hygiène vétérinaire 110, Chemin Stone ouest Guelph (Ontario) N1G 3W4	D. Bautista c/o Food Sci University of Guelph Guelph, Ont. N1G 2W1 (519) 767-6226
		Signature- Submitter / Demandeur: [signature] Date: Nov 12/96

SPECIMEN INFORMATION / INFORMATION CONCERNANT LE PRÉLÈVEMENT

IDENTIFICATION	1	2	3	4
Submitting laboratory / Laboratoire d'origine	Food Sci - U Guelph	Food Sci - U Guelph	Food Sci - U Guelph	Food Sci - U Guelph
Specimen no / N° du prélèvement	1B	1D1	1D2	5E
Date of collection / Date de prélèvement	Oct 16/96	Oct 18/96	Oct 18/96	Oct 21/96
Province or country / Province ou pays	ONT	ONT	ONT	ONT
Establishment (Type and no) / Établissement (Type et numéro)				
County or municipality / Comté ou municipalité	NOM 2MO	NOM 2MO	NOM 2MO	NOM 2MO
Source: species or product / Source: espèce ou produit	Turkey POULTRY	Turkey POULTRY	Turkey POULTRY	Turkey POULTRY
Type of sample / Type d'échantillon	Rinse water	Rinse water	Rinse water	Rinse water
Program / Programme	Ph D	Ph. D	Ph. D	Ph. D
Priority / Priorité	Normal	Normal	Normal	Normal

LAB SEROTYPING REPORT / RAPPORT DE SÉROTYPAGE DE LABORATOIRE

616   616

REPORT / RAPPORT	1	2	3	4
Laboratoire no / N° de laboratoire	SA961954	SA961955	SA961956	SA961957
Date received / Date de réception du prélèvement	13.11.96	13.11.96	13.11.96	13.11.96
Date reported / Date d'envoi du rapport	20.11.96	20.11.96	20.11.96	21.11.96
Serotype / Sérotype	NOT SALMONELLA	NOT SALMONELLA	NOT SALMONELLA	HEIDELBERG
Antigens identified / Antigènes identifiés	-------	-------	-------	4:r:2
	Cit.freundii	E.coli	Unidentified	PHAGE TYPE 8

Signature: Dr. C. Poppe [signature]

AAFC / AAC3188 (89/96)

Agriculture and Agri-Food Canada / Agriculture et Agroalimentaire Canada

96.

SALMONELLA SEROTYPING REPORT

RAPPORT DE SÉROTYPAGE DES SALMONELLES

617

Submitted to:	Présenté à:	Report to (Name and Address) / Remettre à (Nom et adresse):
Agriculture and Agri-Food Canada Health of Animals Laboratory 110 Stone Rd. W. Guelph, Ontario N1G 3W4	Agriculture et Agroalimentaire Canada Laboratoire d'hygiène vétérinaire 110, Chemin Stone ouest Guelph (Ontario) N1G 3W4	D. Bautista c/o Food Sci (519) 767-6226 University of Guelph Guelph, Ont N1G 2W1 Signature- Submitter / Demandeur: [signature] Date: Nov 12/96

SPECIMEN INFORMATION / INFORMATION CONCERNANT LE PRÉLÈVEMENT

IDENTIFICATION	1	2	3	4
Submitting laboratory / Laboratoire d'origine	Food Sci. - U Guelph	Food Sci - U Guelph	Food Sci - U Guelph	Food Sc. - U Guelph
Specimen no. / Nº du prélèvement	3E	1E	2E1	2E2
Date of collection / Date de prélèvement	Oct 21/96	Oct 21/96	Oct 21/96	Oct 21/96
Province or country / Province ou pays	ONT	ONT	ONT	ONT
Establishment (Type and no.) / Établissement (Type et numéro)				
County or municipality / Comté ou municipalité	N0M 2M0	N0M 2M0	N0M 2M0	N0M 2M0
Source: species or product / Source: espèce ou produit	Turkey POULTRY	Turkey POULTRY	Turkey POULTRY	Turkey POULTRY
Type of sample / Type d'échantillon	Sp: Rinse water	Rinse water	Rinse water	Rinse water
Program / Programme	Ph. D.	Ph. D	Ph. D	Ph. D.
Priority / Priorité	Normal	Normal	Normal	Normal

LAB SEROTYPING REPORT / RAPPORT DE SÉROTYPAGE DE LABORATOIRE

617 617

REPORT / RAPPORT	1	2	3	4
Laboratory no. / Nº de laboratoire	SA961958	SA961959	SA961960	SA961961
Date received / Date de réception du prélèvement	13.11.96	13.11.96	13.11.96	13.11.96
Date reported / Date d'envoi du rapport	21.11.96	21.11.96	20.11.96	20.11.96
Serotype / Sérotype	HEIDELBERG	HEIDELBERG	NOT SALMONELLA	NOT SALMONELLA
Antigens identified / Antigènes identifiés	4:r:2	4:r:2	-------	-------
	PHAGE TYPE 8	PHAGE TYPE 8	Kleb.pneumoniae	Kleb.pneumoniae

96.11.28

Signature: Dr. C. Poppe [signature]

AFC / AAC3188 (89/06)

Agriculture and Agri-Food Canada / Agriculture et Agroalimentaire Canada

SALMONELLA SEROTYPING REPORT / RAPPORT DE SÉROTYPAGE DES SALMONELLES

618

Submitted to:	Présenté à:	Report to (Name and Address) / Remettre à (Nom et adresse):
Agriculture and Agri-Food Canada Health of Animals Laboratory 110 Stone Rd. W. Guelph, Ontario N1G 3W4	Agriculture et Agroalimentaire Canada Laboratoire d'hygiène vétérinaire 110, Chemin Stone ouest Guelph (Ontario) N1G 3W4	D. Bautista c/o Food Sci (519) 767-6226 University of Guelph Guelph, Ont N1G 2W1 Signature- Submitter / Demandeur: [signature] Date Nov 12/96

SPECIMEN INFORMATION / INFORMATION CONCERNANT LE PRÉLÈVEMENT	1	2	3	4
Submitting laboratory / Laboratoire d'origine	Food Sci - U Guelph	Food Sci - U Guelph	Food Sci - U Guelph	Food Sci - U Guelph
Specimen no / Nº du prélèvement	4C	1E	4E	5D
Date of collection / Date de prélèvement	Oct 17/96	Oct 21/96	Oct 21/96	Oct 18/96
Province or country / Province ou pays	ONT	ONT	ONT	ONT
Establishment (Type and no) / Établissement (Type et numéro)				
County or municipality / Comté ou municipalité	N0M 2M0	N0M 2M0	N0M 2M0	N0M 2M0
Source: species or product / Source: espèce ou produit	POULTRY Turkey	POULTRY Turkey	POULTRY Turkey	POULTRY Turkey
Type of sample / Type d'échantillon	Sk. Rinse water	Rinse water	Rinse water	Rinse water
Program / Programme	Ph.D.	Ph.D.	Ph.D	Ph.D.
Priority / Priorité	Normal	Normal	Normal	Normal

618 618

LAB SEROTYPING REPORT / RAPPORT DE SÉROTYPAGE DE LABORATOIRE	1	2	3	4
Laboratoire no. / Nº de laboratoire	SA961962	SA961963	SA961964	SA961965
Date received / Date de réception du prélèvement	13.11.96	13.11.96	13.11.96	13.11.96
Date reported / Date d'envoi du rapport	21.11.96	21.11.96	21.11.96	21.11.96
Serotype / Sérotype	HADAR	HEIDELBERG	HEIDELBERG	HADAR
Antigens identified / Antigènes identifiés	6,8:z10:x	4:r:2	4:r:2	6,8:z10:x
	PHAGE TYPE 14	PHAGE TYPE 8	PHAGE TYPE 8	PHAGE TYPE 56

Signature: Dr. C. Poppe [signature]

AAFC / AAC3198 (89/96)

Agriculture and Agri-Food Canada / Agriculture et Agroalimentaire Canada

SALMONELLA SEROTYPING REPORT

RAPPORT DE SÉROTYPAGE DES SALMONELLES

619

Submitted to:	Présenté à:	Report to (Name and Address) / Remettre à (Nom et adresse):
Agriculture and Agri-Food Canada Health of Animals Laboratory 110 Stone Rd. W. Guelph, Ontario N1G 3W4	Agriculture et Agroalimentaire Canada Laboratoire d'hygiène vétérinaire 110, Chemin Stone ouest Guelph (Ontario) N1G 3W4	D Bantister c/o Food Sci (514) 767-6226 University of Guelph Guelph, Ont N1G 2W1 Signature- Submittor / Demandeur: [signature] Date: Nov 12/96

SPECIMEN INFORMATION / INFORMATION CONCERNANT LE PRÉLÈVEMENT

IDENTIFICATION	1	2	3	4
Submitting laboratory / Laboratoire d'origine	Food Sci - U Guelph	Food Sci - U Guelph		
Specimen no / Nº du prélèvement	4D2	4D1		
Date of collection / Date de prélèvement	Oct 18/96	Oct 18/96		
Province or country / Province ou pays	ONT	ONT		
Establishment (Type and no) / Établissement (Type et numéro)				
County or municipality / Comté ou municipalité	~~POULTRY~~	~~POULTRY~~		
Source: species or product / Source: espèce ou produit	Turkey POULTRY	Turkey POULTRY		
Type of sample / Type d'échantillon	[illegible] Rinse water	Rinse water		
Program / Programme	Ph.D.	Ph.D.		
Priority / Priorité	Normal	Normal		

LAB SEROTYPING REPORT / RAPPORT DE SÉROTYPAGE DE LABORATOIRE 619 [initials] 619 [initials]

REPORT / RAPPORT	1	2	3	4
Laboratoire no. / Nº de laboratoire	SA961966	SA961967		
Date received / Date de réception du prélèvement	13.11.96	13.11.96		
Date reported / Date d'envoi du rapport	20.11.96	20.11.96		
Serotype / Sérotype	NOT SALMONELLA	NOT SALMONELLA		
Antigens identified / Antigènes identifiés	-------	-------		

Cit.freundii Cit.freundii

Signature: Dr. C. Poppe [signature]

AAFC / AAC3198 (95/06)

229

IMAGE EVALUATION
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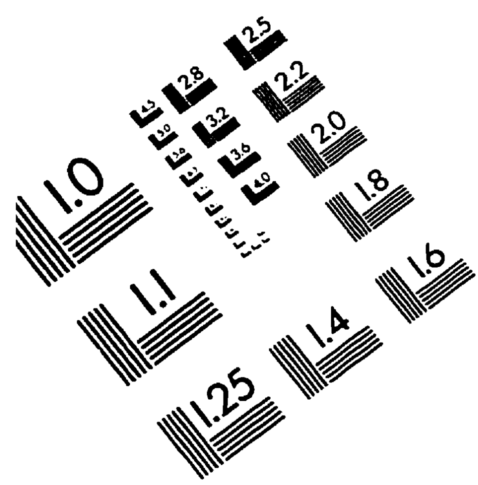
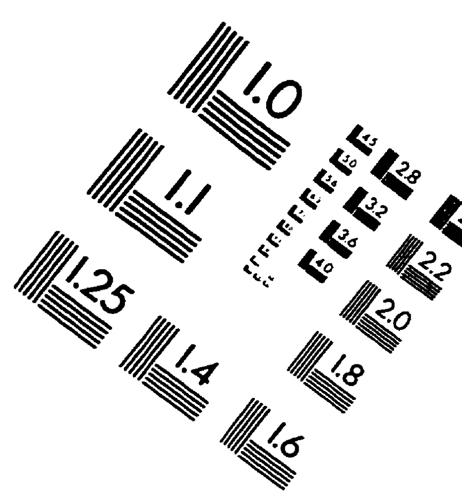
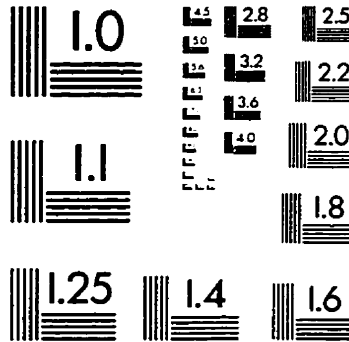
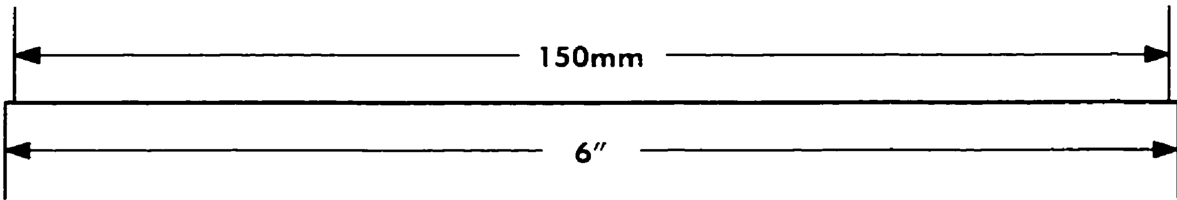

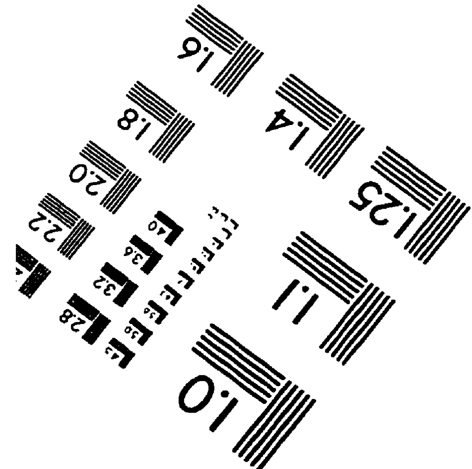

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