THE EFFECT OF HACCP IMPLEMENTATION ON THE MICROBIAL PROFILE OF A POULTRY ABATTOIR

by

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This dissertation is dedicated to my family

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CHAPTER 1

GENERAL INTRODUCTION

Over the last few years the idea that red meat is the only rich source of protein has changed. There is now a greater demand for other sources of protein rich meat such as poultry. Chicken is one of the most widely accepted muscle foods in the world which has resulted in an increase in consumption and this is also true for South Africa (Macrae, Robinson & Sadler, 1993).

There is also a world wide increase in concern over foodborne disease. This together with the increased consumption of poultry meat has resulted in a need for increased hygienic practices at factories. Poultry and poultry products have repeatedly been implicated as a source or vehicle of foodborne infection in humans. Salmonellae are the predominant cause of foodborne disease (Bok, Holzapfel, Odendaal & van der Linde, 1986; Hafez, 1999).

Modern husbandry practices, high stocking densities, uniform age-distribution of birds and continuous feeding promote the spread of potential spoilage bacteria and human pathogens (Aho, 1992). As a result of this, colonization of chicks by human pathogens such as *Staphylococcus aureus* can take place soon after hatching, and thus colonize the bird before slaughtering (Gibbs, Patterson & Thompson, 1978; Mead & Dodd, 1990; Musgrove, Berrang, Byrd, Stern & Cox, 2001).

Many of the process steps in the factory, such as scalding, plucking, evisceration and immersion chilling, are often implicated as the sites of cross-contamination by pathogenic micro-organisms (Mulder & Veerkamp, 1974; Humphrey, Lanning & Beresford, 1981; Okrend, Johnston & Moran, 1986; Jones, Axtell, Rives, Scheideler, Tarver, Walker & Wineland, 1991). These process steps can contaminate other carcasses and the equipment. The bacteria become more resistant to sanitizers and other antimicrobial agents once they become attached to a surface of the equipment. This leads to resident bacteria in the factory which are difficult to remove.

It is also important to keep the carcasses clean of pathogens before packaging and storage as bacteria such as *Listeria monocytogenes* and yeasts and moulds can grow at refrigerated conditions where they will be able to multiply and spoil the product (Palumbo, 1986; Krysinski, Brown & Marchisello, 1992).

There was thus the need for a monitoring system in the poultry factory in this study that will enhance the quality of the product by reducing or eliminating the bacterial and pathogen load on the carcasses. It was found that the best means to achieve the desired results was by implementing the Hazard Analysis Critical Control Point system (HACCP) (Tompkin, 1990; Cross, 1996; Cates, Anderson, Karns & Brown, 2001; Gilling, Taylor, Kane & Taylor, 2001). The HACCP system is a step by step program that identifies possible hazards in the processing line and the desired control measures are implemented to try and eliminated these hazards.

1.1 OBJECTIVES

A good quality chicken product will enhance consumer confidence in the safety of the food supply. This means that the bacterial load on the product must be reduced in order to produce a product that is free of pathogens and spoilage bacteria.

The main objective of this study was, therefore, to first determine the extent of spoilage and presence of health risk bacteria in the poultry abattoir and to determine the effect of HACCP implementation on the extent and presence of these bacteria.

CHAPTER 2

LITERATURE REVIEW

2.1 INTRODUCTION

Chicken is a very common food source in the world and the demand has increased over the years. It is also a very favourable food source as it is high in protein (about 19.5%) and relative low in fat content (about 11%). Chicken contains all the essential amino acids, B vitamins and minerals such as iron and phosphorus (Macrae *et al.*, 1993).

The process to transform a live bird to a ready-to-cook form starts at the farm with the catching of the birds, crating, transporting and unloading at the factory. At the factory the process starts with hanging the birds on shackles, stunning, slaughtering and bleeding, scalding, defeathering, eviscerating, cutting, washing, chilling and packing (Macrae *et al.*, 1993).

There is a world-wide increase in concern over food borne diseases associated with poultry. This has forced the poultry industry to improve the monitoring and control over pathogens and spoilage bacteria. The best means to achieve this is by implementing the Hazard Analysis Critical Control Point program (HACCP).

The HACCP system identifies specific hazards in the processing system. Preventative measures are implemented for the control of these hazards to ensure the safety of the product. HACCP is thus a tool that enable one to assess hazards and establish control systems that prevents or minimize these hazards (Tompkin, 1990; Cross, 1996; Cates, Anderson, Karns & Brown, 2001; Gilling, Taylor, Kane & Taylor, 2001).

The aim of this literature study was to emphasize the use of a quality control program such as HACCP through the discussion of the extent of foodborne illnesses, factors contributing to foodborne illnesses, pathogens of concern in poultry, contamination of poultry and measures of microbial control.

2.2 THE EXTENT OF FOODBORNE ILLNESSES

Research have shown that beef consumption has decreased since 1992 in European countries, especially in Germany, Ireland and the United Kingdom, while pork consumption has only shown a small tendency towards reduction. In contrast to this tendency, chicken consumption has increased in each of the countries in the last five years (Tarrant, 1998).

Poultry is often the origin of foodborne disease due to the fact that large numbers of birds are kept in close proximity. It can thus lead to the fast spread of bacteria between the birds (Silliker, Baird-Parker, Bryan, Christian, Roberts & Tompkin, 1990). Poultry is also considered a major

vehicle for the spreading of foodborne disease, and also appears to be the major risk factor for sporadic cases (Bean & Griffin, 1990).

According to Notermans, Dufrenne & van Leeuwen (1982), data from six countries indicated that up to 22.9% of all outbreaks of foodborne disease are associated with poultry. Bean & Griffin (1990) illustrated the number of foodborne outbreaks associated with different foods from 1973 to 1987 in the United States (Table 2.1).

Table 2.1: Number of foodborne outbreaks by etiologic agent and food vehicle, 1973 to 1987 (Bean & Griffin, 1990).

Etiologic agent	Bakery products	Beef	Chicken	Chinese food	Dairy products	Eggs	Finfish	Fruits and Veg.	Ice cream
Campylobacter	1	0	2	1	25	1	0	1	0
Clostridium perfringens	0	51	9	0	0	0	3	1	0
Escherichia coli	0	3	9	0	0	0	3	1	0
Salmonella	12	77	30	2	22	16	5	9	28
S. aureus	26	22	14	0	5	9	3	4	1
Yersinia enterocolitica	0	0	0	0	2	0	0	2	0

Meat and poultry products were implicated in 54% of all the reported outbreaks of foodborne illnesses in the U.S. between 1968 and 1977 and a further 33% between 1977 and 1984 (Tompkin, 1990).

The outbreaks are mainly from a bacterial origin (± 66%), viruses account for about 5%, parasites for 4-5%, chemicals for about 25% and unknown etiological agents for the rest (Bean & Griffin, 1990; Bean, Griffin, Goulding & Ivey, 1990).

Millions of Americans are ill each year due to food they consumed, of which 9000 die each year. In Britain more people were poisoned by food in 1997 than ever before and it was estimated that one million cases occurred (Tarrant, 1998).

Foodborne disease costs countries billions due to illness, death and business lost. As was shown by Bean & Griffin (1990) and Todd (1989), the preliminary estimates for the United States were 12.6 million cases of foodborne disease which have a cost of $ 7.7 to $ 8.4 billion annually.

Bacterial and viral diseases represented 84% of the costs, with salmonellosis and staphylococcal intoxication the highest at $ 4.0 billion and $ 1.5 billion respectively. Listeriosis accounted for $ 313 million, *E. coli* for $ 223 million, campylobacteriosis for $ 156 million and *C. perfringens* enteritis for $ 123 million (Todd, 1989).

A ten year study of foodborne disease outbreaks from 1975 to 1984 in Canada, recorded an average of 5.6 deaths per year. *Salmonella, Clostridium botulinum* and *Listeria monocytogenes* were responsible for most of these deaths and the foods most frequently implicated were meat and poultry. Poultry was implied in 10% of the outbreaks and 20% of the cases (Todd, 1992). Since foodborne diseases are not reported regularly in South Africa, no data are available for South Africa. The reason for this is because there is no system in place where doctors report the occurrence of foodborne disease outbreaks.

2.3 FACTORS CONTRIBUTING TO FOODBORNE ILLNESSES

The greatest problem with meat products is the time and temperature factors involved. This is as a result of inadequate preparation of the food by means of inadequate cooking, improper reheating, preparation to far in advance, improper warm holding, storage at ambient temperatures and improper cooling. Other factors such as poor personal hygiene of the food handlers and contaminated equipment also play an important role. Food from an unsafe source can also lead to an outbreak. These factors do not cause foodborne illness, but due to the inadequate storage of the food, causing spores to survive or recontaminate the food, growth of pathogens may occur, which increase the chance of a foodborne illness outbreak (Bryan, 1980; Bean *et al.*, 1990; Bean & Griffin, 1990; Tompkin, 1990).

Another important factor is the occurrence of a summer peak due to higher temperatures which leads to faster growth of bacteria on contaminated food. Twenty-five percent of outbreaks due to bacteria occurred in the warmer months while *S. aureus* showed a seasonal shift that peaked sharply in late summer (Figure 2.1; Bean *et al.*, 1990; Bean & Griffin, 1990; Todd, 1992).

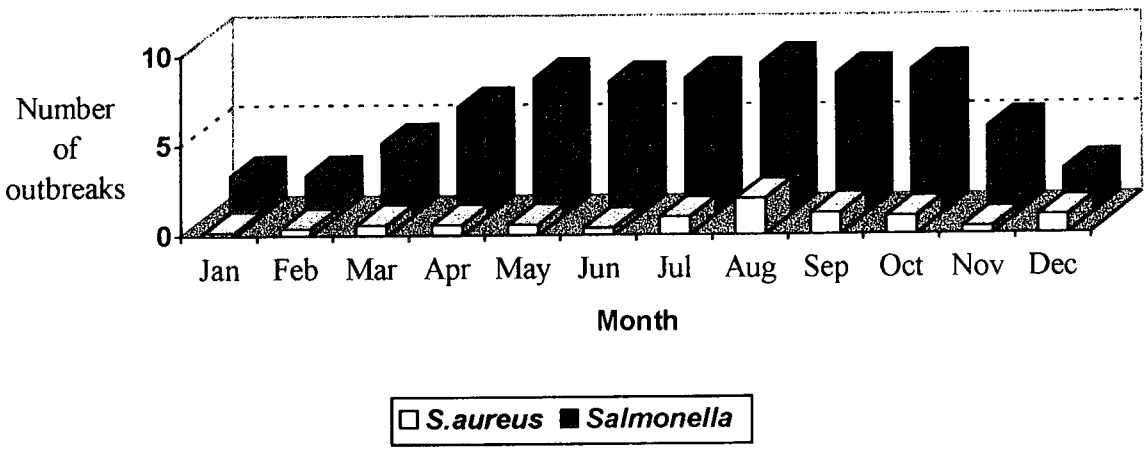

Figure 2.1: The average number of outbreaks in the United States due to *Salmonella* and *S. aureus* by month of occurrence – May to October are warmer months (Bean *et al.*, 1990).

2.4 PATHOGENS OF CONCERN FOUND ON POULTRY CARCASSES

Over the past few years there has been numerous bacteria emerging as pathogens. *Listeria monocytogenes*, *Escherichia coli* O157:H7 and *Campylobacter* species were not recognized as foodborne pathogens 20 years ago. *Salmonella enteritidis* and *Salmonella typhimurium* DT104 were only recognized in the UK in 1984 and in the US in 1996. This is not due to the fact that there are new species, but because of factors which interrelate with each other. This leads to conditions which favour some microorganisms and thus lead to new foodborne disease outbreaks. Organisms are also adapting to refrigeration, heat, pH and disinfection techniques (Cox, 1989; Tarrant, 1998).

Pathogens that may be found on poultry are *Escherichia coli*, *Salmonella* spp., *Yersinia enterocolitica*, *Listeria monocytogenes*, *Staphylococcus aureus*, *Campylobacter jejuni/coli*, *Clostridium perfringens* and *Aeromonas hydrophila* (Bok *et al.*, 1986; Macrae *et al.*, 1993; Kotula & Pandya, 1995).

2.4.1 *Escherichia coli*

Escherichia coli is generally harmless and part of the normal microflora of the gut of humans and other animals, but a few groups are pathogenic to humans. These organisms are divided into groups due to the different toxins they produce, their mechanism of disease production and symptoms (Wilson & Miles, 1961; Buchanan & Gibbons, 1974; Palumbo, 1986; Hitchins, Hartman & Todd, 1992; Starr & Taggart, 1992; Batt, 2000).

These groups are:

a) Enteropathogenic *E. coli* (EPEC)

b) Enteroinvasive *E. coli* (EIEC)

c) Enterotoxigenic *E. coli* (ETEC)

d) Enterohaemorrhagic *E. coli* (EHEC) or verocytotoxic (VTEC)

e) Enteroaggregative *E. coli* (EaggEC)

EPEC, EIEC and ETEC strains cause gastroenteritis in babies and children. EPEC can also cause gastroenteritis in adults and domestic animals. The origin of these *E. coli* groups is humans, either as symptomless carriers or infected people. The vehicle is mainly contaminated water, either through direct consumption, food washed or irrigated with contaminated water or direct faecal transfer (Garbutt, 1997; Batt, 2000).

The EHEC group are the cause of one of the most severe forms of disease which results in haemolytic-uraemic syndrome. They have the ability to produce adherence factors, enterohaemolysins and Shiga toxins (Batt, 2000). EHEC *E. coli* O157:H7 is often associated with ground meat as vehicle of infection, but it was found that this organism can readily colonize the caeca of chickens without clinical signs. It can be excreted in the feces for several months, making chickens a reservoir of this organism. This strain of *E. coli* causes diarrhoea and abdominal pain with bleeding due to inflammation of the colon. This leads to renal failure and internal bleeding which results in brain damage. It is also thought that the infective dose may be as low as 10 – 100 organisms (Doyle & Schoeni, 1987; Todd, 1992; Garbutt, 1997; Hafez, 1999; Batt, 2000). Between 1982 and 1984 there were 2 outbreaks and 22 cases of *E. coli* O157:H7 foodborne disease annually (Todd, 1992). As shown in Table 2.2 it is clear that *E. coli* is an important organism that causes many outbreaks and even death in some cases.

Table 2.2: Confirmed foodborne outbreaks, cases, and deaths, by *E. coli* in the United States (Bean *et al.*, 1990).

Year	Outbreaks		Cases		Deaths	
	No.	%	No.	%	No.	%
1983	3	1.6	157	2.0	0	0.0
1984	2	1.1	76	0.9	4	36.4
1985	1	0.5	370	1.6	0	0.0
1986	1	0.6	37	0.6	0	0.0

2.4.2 *Salmonella*

Salmonella is associated with domestic and wild animals, poultry and wild birds, insects and man. The usual habitat is the intestinal tract of the host, but are also found in blood, lymphatic nodes, the ovary, the eggs of fowls, water and sewage. It is commonly found in 36% of chicken carcasses. It is also found in beef, sausages, pork, cakes, milk, cheese, salads and sandwiches as vehicle of infection (Wilson & Miles, 1961; Todd, 1992).

Fowl, cattle and other food-source animals sometimes become infected or contaminated with *Salmonella* while on the farms, and when processed the animals contaminate the processing plants. This can lead to contamination of the workers who spread the contamination (Bryan, 1980). In the poultry industry there are five major sources of *Salmonella* contamination – feed, carrier birds, litter, nest boxes and the environment (Bains & MacKenzie, 1974).

Most of the *Salmonella* species cause acute gastro-enteritis of the food-poisoning type in children and adults, or acute enteritis in infants characterized by a short incubation period and the predominance of intestinal over septicaemic symptoms. The host that become infected may harbour the organism for a varying period of time without showing any signs of disease. Some *Salmonella* species such as *Salmonella gallinarum*, that are regarded as restricted to fowls, has been isolated from human patients (Wilson & Miles, 1961).

Salmonella accounted for 57% of the bacterial disease outbreaks during 1983 to 1987 in the United States, and was the most frequently reported pathogen during each year (Bean *et al.*, 1990). The factors which contribute to these outbreaks were improper holding temperatures, inadequate cooking, contaminated equipment, food from unsafe sources and poor personal hygiene (Bean *et al.*, 1990).

In the early 1980's a survey was carried out in Europe which found that *Salmonella* incidence figures were as high as 90% (Bok *et al.*, 1986). In the US alone *Salmonella* spp. account for between 8 – 50% of the pathogens found on poultry products (Waldroup, 1996). The incidence of *Salmonella* spp. on raw poultry internationally from 1969 to 1993 is given in Table 2.3.

2.4.3 *Yersinia enterocolitica*

Swine, rodents and some humans are carriers of *Yersinia* in their intestinal tract (Palumbo, 1986; Schiemann & Wauters, 1992; Waldroup, 1996; Sammarco, Ripabelli, Ruberto, Iannitto & Grasso, 1997). *Yersinia enterocolitica* are the cause of 1-2% of human cases of acute enteritis. Refrigerated foods are often the vehicle of contamination due to the fact that they are cold tolerant and can multiply at 4 °C. It contains a 70-75 kilo base pair plasmid causing its pathogenicity (Bean & Griffin, 1990; de Boer, 1995; Sammarco *et al.*, 1997; Bhaduri, 2000). Yersiniosis is caused by ingestion of the organism (Garbutt, 1997). They cause gastroenteritis, mesenteric lymphadenitis and pseudoappendicitis which predominate in children.

Table 2.3: Incidence of *Salmonella* spp. on raw poultry (Waldroup, 1996).

Year	Country	Food	Incidence (% positive)	Numbers (cfu/unit)	No. samples evaluated
1969	USA	Processed chicken	20.5	<30/bird	?
1969	Ireland	Poultry carcasses	0	-	803
1973	Netherlands	Chicken parts	57	ND	?
		Poultry sausage	12	ND	?
1974	Brazil	Carcasses	23.3	ND	?
		Carcasses	16.7	ND	?
1975	India	Carcasses	2.8	ND	71
1975	Germany	Broiler	51	ND	465
1976	Brazil	Carcasses	18	ND	50
1977	Canada	Fresh chicken	34.8	ND	69
1978	USA	Retail poultry	14.8	ND	?
1979	Canada	Chicken parts	71	ND	7
1980	Germany	Chicken livers	50	ND	?
		Frozen broilers	14	ND	?
1980	Ontario	Poultry samples	2.4	ND	4240
		Retail carcasses	19.8	ND	96
1981	Israel	Frozen broilers	15-55	ND	444
1981	Netherlands	Fresh retail poultry	?	63/bird	42
1981	Sweden	Frozen chicken	1.2	ND	82
1981	Japan	Fresh chicken	25	<100/bird	674*
1982	USA	Broiler carcasses	36.9	ND	601
1983	Mexico	Chicken	80	ND	40
		Chicken liver	82.5	ND	40
1983	USA	Broiler carcasses	11.6	<1/bird	?
1983	Israel	Ground poultry meat	33	ND	172
1984	Czechoslovakia	Deboned poultry	0	-	
1985	Canada	Chicken liver	21	ND	165
1986	S. Africa	Broilers	49	ND	102
1986	Iraq	Retail chicken	25.9	ND	81
1987	Sweden	Fresh chicken	0	-	?
1987	Spain	Chilled chicken	22	ND	51
1988	UK	Fresh chicken	2.9	ND	69
1988	Japan	Chicken meat, minced	50-66.6	ND	?
1989	Portugal	Retail non-refrigerated and refrigerated chicken	31.7-60.5	ND	300
1990	India	Dressed broilers	4	ND	50
1990	Netherlands	Chicken cuts and liver	54	ND	81
1991	USA	Retail broilers	17-50	5-34/bird	36
1991	India	Fresh and frozen chicken	100	300/bird	?
1991	UK	Retail chicken	48	ND	292
1992	Bavaria	Fresh poultry	51.7	ND	238
1992	USA	Postchill broiler carcasses	40.8	1.8/bird	560
1993	USA	Prechill broiler carcasses	21.8	1.4/bird	500
1993	Germany	Poultry products	12.7	ND	?

cfu – colony-forming units
ND – no data
* 2% of positive carcasses had levels > 1000 cfu/bird

Acute abdominal disorders, diarrhea and arthritis primarily occur in adults and erythema nodosum in older persons (Palumbo, 1986; Garbutt, 1997). The incidence of *Yersinia enterocolitica* on raw poultry from 1975-1990 is given in Table 2.4.

Table 2.4: Incidence and numbers of *Yersinia enterocolitica* on raw poultry (Waldroup, 1996).

Year	Country	Food	Incidence (% positive)	Numbers (cfu/unit)	No. samples evaluated
1975	Germany	Poultry meat	29.9	ND	117
1981	The Netherlands	Raw poultry	68.0	ND	108
1981	Sweden	Frozen chicken	24.5	ND	82
1983	Italy	Raw chicken	<1.0	ND	150
1985	France	Raw poultry cutlets	57.1	ND	?
1986	S. Africa	Retail broilers	3.0	ND	102
1987	Spain	Broilers	2.0	ND	50
1987	Brazil	Chicken giblets	80.0	ND	25
1989	France	Raw turkey	18.4	ND	38
		Raw chicken	2.0	ND	50
		MDPM	25.0	ND	32
1990	USA	Retail broilers	26.7	ND	60
1990	France	Raw poultry	20.0	ND	35

cfu – colony-forming units
ND – no data
MDPM – mechanically deboned poultry meat

2.4.4 *Listeria monocytogenes*

Listeria monocytogenes is ubiquitous in nature, occurring in dust, soil, water, sewage, silage, decaying vegetation, damp earth, feces, wild and domestic animals and animal feeds (Wilson & Miles, 1961; Donnelly, Brackett, Doores, Lee & Lovett, 1992; Martin & Fisher, 2000).

In most cases infection is caused by ingestion of the organism which can cause various disorders: meningo-encephalitis, flu-like low grade septicemia *in gravida*, septicemia in the perinatal period, infectious mononucleosis-like syndrome, septicaemia in adults, pneumonia, endocarditis, urethritis and abortions (Palumbo, 1986; Donnelly *et al.*, 1992; Garbutt, 1997). The disease has a fatality rate of about 30%, and can be carried without effect for varying periods prior to onset of symptoms (Cox, 1989).

In the United States, listeriosis fatalities represent 13% of the total annual number of deaths (Todd, 1989). Listeriosis has increased by almost 150% since 1986 in England and Wales. This has been linked to the increased consumption of chilled food, specifically chicken (Cox, 1989). Since 1989 numerous studies have shown that *Listeria* spp. are regularly found on poultry products (Table 2.5). *Listeria monocytogenes* constitute 2 – 50% of the species found (Waldroup, 1996).

Listeria monocytogenes is of great concern in the meat and poultry industry since it has the ability to grow at refrigerated temperatures (Palumbo, 1986; Bean & Griffin, 1990). This means that once the abattoir environment is contaminated with *Listeria*, the bacteria may establish itself in the plant. The abattoir environment can then play a major role in the spreading of contamination to the carcasses (Cox, 1989; Sammarco *et al.*, 1997).

Table 2.5: Incidence and numbers of *Listeria* spp. on raw poultry (Waldroup, 1996).

Year	Country	Food	Incidence (% positive)	Numbers (cfu/unit)	No. samples evaluated	Comments
1975	UK	Processed chicken	53	ND	51	*L. monocytogenes*
		Retail chicken	50	ND	38	
		Frozen chicken	64	ND	64	
1978	Netherlands	Broiler intestines	7.9	ND	3090	
1989	USA	Chicken:				*L. monocytogenes* - high incidence on hands and gloves
		skin & drumsticks	36.7	ND	?	
		wings	70	ND	?	
		liver	33.3	ND	?	
1989	Canada	Chicken legs	56.3	ND	16	
1990	Taiwan	Raw chicken	50	ND	?	
1990	Australia	Fresh chicken	2.1	ND	48	
		Frozen chicken	15	ND	80	
1990	Canada	Raw poultry	29	ND	7	
1991	Italy	Raw chicken	37	ND	27	15% *L. monocytogenes*
1991	UK	Raw poultry	94	ND	32	
1991	China	Raw poultry	52	ND	21	one sample was *L. monocytogenes*
1991	Czechoslovakia	Raw poultry	10	ND	?	
1991	Italy	Raw poultry	>50	ND	?	
1992	USA	Postchill broiler carcasses	27.3	4.7/bird	480	
1992	Japan	Raw meat and poultry	36.2	<100/g	762	*L. monocytogenes* – incidence lower in poultry samples
1992	Italy	Poultry skin	36	ND	50	

cfu – colony-forming units
ND – no data

2.4.5 *Staphylococcus aureus*

Staphylococcus aureus are ubiquitous in nature and can be found in the air, water, milk and sewage, but its primary habitat is on the skin, in the nose, hair follicles and throat of man and animals. In humans up to 50% may be healthy carriers of *S. aureus* (Wilson & Miles, 1961; Buchanan & Gibbons, 1974; Shapton & Shapton,1991; Lancette & Tatini, 1992; Starr & Taggart, 1992; Harvey & Gilmour, 2000).

Despite the fact that they are not motile and susceptibile to bacteriocins, bacteriophages and simple bacterial products of competing bacteria, they are able to survive well outside their natural hosts (Harvey & Gilmour, 2000).

Intoxication is caused by ingestion of enterotoxins which are secreted into the food during growth. Only 1 μg toxin / 100 g of food can cause illness. This causes nausea, vomiting, diarrhoea and abdominal pain, 2-6 hours after consuming the contaminated food which can lead to dehydration. Recovery can take 1 to 3 days (Baird & Lee, 1995; Garbutt, 1997).

Staphylococcus aureus is often associated with pork, turkey, chicken, cheese, pasta, salads and sandwiches as vehicle of infection (Todd, 1992). Chicken becomes a vehicle of staphylococcal enterotoxins during processing. The carcasses go through a "kill" step and this can kill all the *S. aureus* and any other competitive organisms. When the carcasses are handled by the personnel during processing, the carcasses can be recontaminated with *S. aureus* through persons who are nose, mouth or skin carriers of *S. aureus*. There is then no competition for the *S. aureus* and they can then multiply and produce high concentrations of enterotoxins (Bryan, 1980; Lancette & Tatini, 1992). The incidence of *S. aureus* on raw poultry from 1978 to 1993 is given in Table 2.6.

Data from Canada between 1975 to 1984 showed that there was a constant number of outbreaks, 23 to 37, each year (Todd, 1992). In England and Wales, *S. aureus* intoxication attributed to 25% of poultry outbreaks (Notermans *et al.*, 1982). The factors contributing to these outbreaks are mainly improper holding temperatures and poor personal hygiene, but

contaminated equipment and inadequate cooking may also play a role (Bean *et al.*, 1990). It was found that mucoid growth facilitated the attachment of *S. aureus* to the defeathering equipment and this lead to the colonization of the equipment by the bacterium (Harvey & Gilmour, 2000).

Table 2.6: Incidence and numbers of *Staphylococcus aureus* on raw poultry (Waldroup, 1996).

Year	Country	Food	Incidence (% positive)	Numbers (cfu/unit)	No. samples evaluated
1978	Poland	Skin and meat of raw broilers	78.2	ND	?
1979	Germany	Broiler carcasses	35-47	ND	?
1980	Sweden	MDPM	80	>1 000/g	?
1981	Netherlands	Poultry skin	?	10-50 000/g	?
1983	Czechoslovakia	Poultry carcasses	?	2 400/cm^2	?
1987	Spain	Refrigerated chicken	43.1	ND	51
1991	India	Fresh/frozen chicken	most	15 000/cm^2	25
1991	Japan	Retail chicken	92.7	ND	110
1993	UK	Chicken/turkey carcasses	71	<1000/g	140*

cfu – colony-forming units
ND – no data
MDPM – mechanically deboned poultry meat
* before and after defeathering

2.4.6 *Campylobacter* spp.

They are found in sea- and untreated freshwater, insects, the intestinal tract, reproductive organs and oral cavity of warm-blooded animals such as rodents, wild birds, pets, farm animals and chickens. It is rarely found in poultry feed or hatcheries. Improperly handled and cooked foods are responsible for the majority of human infections. Especially undercooked poultry products are associated with sporadic cases of campylobacteriosis. *Campylobacter* are not found in the environment due to the fact that they have limited defences against oxygen, high minimum growth temperatures

and complex nutritional requirements (Shanker, Rosenfield, Davey & Sorrell, 1982; Silliker *et al.*, 1990; Shapton & Shapton, 1991; Stern, Patton, Doyle, Park & McCardell, 1992; Bailey, 1993; Kotula & Pandya, 1995; Garbutt, 1997; Hafez, 1999; Rowe & Madden, 2000).

Shanker *et al.* (1982) and Shapton & Shapton (1991) found that between 1.8 and 83% of broilers in the USA and between 14 and 91% of the broilers in the UK had *Campylobacter* present in their gastrointestinal tract. Although rarely found in hatcheries, Hafez (1999) found that two hatcheries had 17.6 and 42.9% infected broiler chicks on their farms.

Campylobacter spp. is isolated more frequently than *Salmonella* spp. in human gastroenteritis patients. The incubation period is usually 1 to 7 days with symptoms of fever with confusion or delirium and general malaise followed by severe abdominal cramping which is followed by profuse diarrhoea that lasts 2 to 7 days. The infective dose is as low as 5 to 800 organisms. Poultry is seen as the most important vehicle for the transmission of *Campylobacter* in the USA (Shapton & Shapton, 1991; Stern *et al.*, 1992; Rowe & Madden, 2000). *Campylobacter* have been found in up to 14% of patients with acute gastrointestinal symptoms (Shanker *et al.*, 1982). *Campylobacter* was also identified as the leading cause of bacterial diarrhea (Bean & Griffin, 1990). The incidence of *Campylobacter* spp. on raw poultry from 1974 to 1993 is given in Table 2.7.

Table 2.7: Incidence and numbers of *Campylobacter* spp. on raw poultry (Waldroup, 1996).

Year	Country	Food	Incidence (% positive)	Numbers (cfu/unit)	No. samples evaluated
1974	USA	Poultry	1.8	ND	165
1981	Sweden	Frozen chicken	22	ND	82
1981	Canada	Retail broilers in: Ontario	62	>100/bird	50
		Ohio	54	>100/bird	50
1982	Australia	Processed broilers	45	ND	40
		Cloacal swabs	41	ND	327
1982	USA	Frozen chicken gizzards	20	ND	5
1983	USA	Fresh turkey wings	64.1	740/wing	184
		Frozen turkey wings	55.6	890/wing	81
1983	USA	RTC broiler carcasses	68	ND	?
1984	Norway	Broiler carcasses	13.8	ND	Total = 691
		Turkey carcasses	56.7	ND	
		Hen carcasses	48.7	ND	
1984	USA	Chicken carcasses and livers	31.8	ND	405
1985	USA	Broilers	97	ND	50
1985	USA	Retail chicken	35	ND	?
1985	USA	Poultry	30	ND	360
1985	France	Guinea fowl: caecae	85.7	ND	224
		abdominal cavity	22.2	ND	224
1986	S. Africa	Broilers	4	ND	102
1987	Spain	Poultry	12	ND	51
1987	USA	Duck: liver	34	ND	?
		gizzard	20	ND	?
		heart	6	ND	?
		skin	6.7	ND	?
1988	UK	Broilers: fresh processed	48	1.5×10^6/bird	46
		uneviscerated	100	2.4×10^7/bird	12
		frozen carcasses	4.2	350/bird	24
1988	Finland	Live broilers:	1.7	100-10 000/bird	199
		caecae	24		
		deep frozen carcasses	7		
1988	USA	Poultry	> 95	$<10^1$/1 000cm^2	?
1989	Yugoslavia	Chicken carcasses: large plant	26.2	ND	?
		small plant	14.1	ND	?
1989	UK	Poultry	55.5	ND	?
1990	The Netherlands	Chicken cuts and livers	61	ND	279
1992	Mexico	Retail chicken	36	ND	92
1992	Portugal	Live chicken	60.2	ND	98
		Live ducks	40.5	ND	
1992	USA	Retail broilers	98	ND	50
1992	USA	Postchill broiler carcasses	90.8	3142/bird	480
1993	USA	Prechill broiler carcasses	86.4	9120/bird	500

RTC – ready to cook
cfu – colony-forming units
ND – no data

2.4.7 *Clostridium perfringens*

Clostridium perfringens is found in the intestine of animals (Porter, 1998). They are divided into five types on the basis of the production of four major heat-labile enterotoxins (Buchanan & Gibbons, 1974; Shapton & Shapton, 1991; Garbutt, 1997; Blaschek, 2000).

Type A can be found in soil, feces, marine sediments and dust and they are the main cause of illness in humans. Types B, C, D and E are obligate parasites of animals, and are only occasionally found in man (Bryan, 1980; Shapton & Shapton, 1991).

Sporulating cells produce a heat-labile enterotoxin that is released *in vivo* in the intestine that induces the major symptom of diarrhea and stomach cramps. The vehicle is mostly cooked meat or poultry (Table 2.8). The infective dose is 10^6-10^7 cells per gram of food and the incubation period is usually 8-24 hours (Shapton & Shapton, 1991; Labbe & Harmon, 1992; Garbutt, 1997; Blaschek, 2000). In addition to food poisoning they are also responsible for gas gangrene, necrotic enteritis, lamb dysentery and minor wound infections (Blaschek, 2000).

Clostridium perfringens are responsible for about 10 000 cases of food poisoning each year and approximately 10% of the food-borne disease outbreaks in the USA (Blaschek, 2000).

Table 2.8: Incidence and numbers of *Clostridium perfringens* on raw poultry (Waldroup, 1996).

Year	Country	Food	Incidence (% positive)	Numbers (cfu/unit)	No. samples evaluated
1963	USA	Meat, poultry, fish	16.4	ND	122
1971	USA	Raw turkey	20.0	ND	35
1971	USA	Processed carcasses	10.0	<10/cm^2	20
		Neck skin	38.5	<10/g	13
1973	USA	Fresh turkey skin and meat	62.0	?	85
1974	USA	Raw turkey	41.6	ND	24
1984	USA	Broiler skin	79	ND	48
		Broiler breast	23	ND	48
		Broiler thigh	30.5	ND	48
1987	Spain	Refrigerated chicken	19.6	ND	51
1989	USA	Raw fresh or frozen poultry	30-80	ND	?

cfu – colony-forming units
ND – no data

2.4.8 *Aeromonas hydrophila*

They are ubiquitous in nature, but found particularly in fresh water, brackish water and sewage (Buchanan & Gibbons, 1974; Palumbo, 1986; Palumbo, Abeyta & Stelma, 1992; Jeppesen, 1995; Garbutt, 1997; Blair, McMahon & McDowell, 2000).

Aeromonas hydrophila have been isolated from chlorinated drinking water in the USA. They are associated with wound infections, septicemia and meningitis, but this is usually in immunocompromised patients such as victims of leukemia or cirrhosis and also gastroenteritis which causes cholera-like illness or dysentery-like illness. They produce cytotoxic and cytotonic enterotoxins (Palumbo *et al.*, 1992).

Blair *et al.* (2000) showed that *A. hydrophila* was present in 38.7% of raw chicken. This is due to their psychrotrophic nature and their ability to express a range of virulence factors under refrigerated storage conditions (Waldroup, 1996). The incidence and numbers of *Aeromonas* spp. on raw poultry is given in Table 2.9.

Table 2.9: Incidence and numbers of *Aeromonas* spp. on raw poultry (Waldroup, 1996).

Year	Country	Food	Incidence (% positive)	Numbers (cfu/unit)	No. samples evaluated
1985	USA	Chicken	75-100	100-50 000/g	8
1986	S. Africa	Broilers	6.0	ND	102
1987	USA	Retail chicken	100.0	4-4 000/g	10
1987	Spain	Refrigerated chicken	29.4	ND	51
1987	Sweden	Chicken	24-33	occasional >100/cm^2	45
1989	USA	Broiler carcasses	98.0	<600/ml	25
1989	UK	Retail poultry	79.3	ND	?
1993	Finland	Retail chicken	62.0	ND	16

cfu – colony-forming units
ND – no data

2.5 CONTAMINATION OF POULTRY CARCASSES

The most important factors that determine the microbiological quality of meat are the condition of the animal during slaughtering, the spread of contamination and the temperature, time and other conditions during processing, storage and distribution.

Contamination of carcasses are either from intrinsic or extrinsic sources. Intrinsic sources are the nutrient content of the food, natural antimicrobial substances, pH of the food, buffering capacity of the food, oxidation reduction potential (Eh), water activity and mechanical barriers to microbial

invasion. Extrinsic sources are the storing temperature, gaseous atmosphere surrounding the food, relative humidity of the atmosphere and time (Nortjé, Nel, Jordaan, Badenhorst, Goedhart, Holzapfel & Grimbeek, 1990; Garbutt, 1997).

Contamination occurs mostly by means of the animal's exterior surface, the gastrointestinal tract and the introduction of pathogens onto the carcasses during processing either by the equipment or the workers in the plants. This can lead to further contamination among the carcasses (Macrae *et al.*, 1993; Sammarco *et al.*, 1997). The contamination level of products at retail premises are due to the combination of microbial quality of the carcasses and the sanitation program of the premises (Nortjé *et al.*, 1990). Pathogens can be introduced during the rearing, transport, processing, packaging, distribution or preparation of the poultry (Hafez, 1999).

The attachment of bacteria to the meat surface can be considered as the first step in the microbial contamination of meat. When organisms attach themselves to meat surfaces in low numbers, their continued presence will depend on their ability to remain attached to the meat surfaces. Bacterial attachment is divided into two stages. The first stage is the reversible attachment of bacteria to the surface when they are trapped in a water film on the surface. This allows a portion of the population to bond with the surface. The second stage is the irreversible attachment of bacteria to the surface which is influenced by cell surface charge, hydrophobicity and the presence of flagella, fimbriae and extracellular polysaccharides (Benito, Pin, Marin, Garcia, Selgas & Casas, 1997; Selgas, Marin, Pin & Casas, 1992).

The nutrient content of poultry meat is ideal for bacterial growth, as 3.5% by weight of meat muscle is made up of water soluble materials. The most significant of these for bacterial growth is the low level of glucose (0.01%), amino acids (0.35%), nucleotides, vitamins, inorganic salts and trace elements. The water activity of meat is also high (0.99) and ideal for bacterial growth (Garbutt, 1997).

The sources of microbial contamination of poultry carcasses will now be discussed in more detail.

2.5.1 Flock Contamination

Harrigan (1998) and Silliker *et al.* (1990) described how microbial contamination can start at the egg during its development and later through the shell by *Salmonella* species. It is thus very important to have an effective HACCP program on the farm to exclude *Salmonella* from the flocks. *Staphylococcus aureus* can also colonize the skin of the chicks as soon as they hatch. The population will increase until the seventh week, the point at which many poultry are slaughtered (Mead & Dodd, 1990).

The chickens can acquire salmonellae from animate environmental contact, such as rodents, wild birds, insects and workers (Bryan, 1980; Silliker *et al.*, 1990; Bailey, 1993; Garbutt, 1997; Hafez, 1999). Both rats and mice suffer naturally from infection with *Salmonella typhimurium* and *Salmonella enteritidis* (Wilson & Miles, 1961). Bains & MacKenzie (1974) found that a plague of rodents in the grain growing area lead to heavy contamination of

the grain with *Salmonella typhimurium*, and the contamination spread to the flocks and to the factory. This meant that the carcasses was heavily contaminated while the rodent plague was present.

They can also acquire salmonellae from inanimate environmental contact, such as from feed, feed ingredients, water and equipment (Bryan, 1980; Silliker *et al.*, 1990; Bailey, 1993; Garbutt, 1997; Hafez, 1999). Dougherty (1976) found that *Salmonella* species that were present in the feed of the chicks were *S. derby* and *S. drypool* from meat and bone meal and *S. senftenberg* from fish meal.

Dougherty (1976) also found that 37.5% of the chicks placed in the poultry house were already positive for *Salmonella*, which were acquired from the breeder flock or the hatchery. If the flock is contaminated with *Salmonella*, the contamination will be spread to the processing plant (Bryan, 1980). The main sources of *Salmonella* contamination of the flock is, therefore, either species entering the poultry house or species residing in the house. Bailey (1993) found that day-old chicks could be colonized with less than five cells of *Salmonella*, but later colonization was irregular and required higher doses. Two-week-old chicks have mature gut microflora and are thus more resistant to intestinal colonization. It also takes only one *Salmonella* per gram of feed to colonize 1 to 7-day-old chicks. Bailey (1993) also found that the highest levels of intestinal colonization of salmonellae occurs during the second and third weeks of growout, unless disease or temperature stress occurred, and from there it is typically a gradual decline in frequency until the time of processing.

Escherichia coli O157:H7 can colonize the caeca of chickens, and the chickens can then be a reservoir for the organism. The *E. coli* can be excreted in the feces for several months implying that an infected bird can spread the bacteria to the entire flock (Notermans, van Leusden & Schothorst, 1977; Doyle & Schoeni, 1987).

Staphylococcus aureus is considered to be part of the normal flora of live poultry. Chickens become contaminated with *S. aureus* during the first few days of life, but the initial colonization is low during the first weeks. Low numbers are present in the intestinal tract (Notermans *et al.*, 1982).

2.5.2 Transport

During the transportation of the flock to the factory, there is also a high degree of contamination. This is mainly due to crowded conditions and transport over long distances. Contamination of the skin and feathers with faecal organisms then occur (Silliker *et al.*, 1990; Garbutt, 1997).

Kotula & Pandya (1995) tested the broilers from four different farms as they arrived at the factory. They found that *E. coli* was 100% present on all broilers from all the farms, *Salmonella* spp. was present on 100% of all the broilers of one farm, 90% on two farms and 60% of the broilers from the other farm and *C. jejuni/coli* was present on all the broilers from three farms and on 80% of the fourth farm.

2.5.3 Equipment and Environment Contamination

Due to the high throughputs in factories, this increases the spread of contamination between the carcasses (Garbutt, 1997). When the flock is contaminated with *Salmonella*, it is usually in fecal material on the feet, skin and feathers of the animals. This means that equipment such as the defeathering machines and eviscerating equipment can be contaminated by these birds, and the equipment will then contaminate other carcasses with *Salmonella* (Bryan, 1980).

Dodd, Mead & Waites (1988) found that the counts on the defeathering machinery yielded counts of *ca.* 10^3/swab at the entry of the first plucker and increased to *ca.* 10^7 at the exit of the first plucker. There was a slight decrease through the second plucker and the rest.

The floors of the slaughtering area, cold room floors and worktables are important sites in abattoirs that may harbor pathogens like *Salmonella* spp. and *Listeria monocytogenes* (Sammarco *et al.*, 1997). Pathogens like *Listeria* that are able to grow at refrigerated temperatures can also establish itself in the factory and lead to contamination from the environment (Bean & Griffin, 1990; Sammarco *et al.*, 1997).

There is also the practice of allowing sanitizers and cleaners to flow off of the equipment and walls onto the floor after they have been cleaned. This does not eliminate the pathogenic microorganisms from the environment (Sammarco *et al.*, 1997).

The air in the slaughtering, scalding, defeathering and evisceration areas may have high numbers of bacteria, particularly aerobic and coliform bacteria. This is due to the high humidity in these areas that could stimulate or increase the microbial loads in the air (Abu-Ruwaida, Sawaya, Dashti, Murad and Al-Othman, 1994).

2.5.4 Personnel Contamination

The workers can become carriers of salmonellae, either through contact with contaminated birds during processing or after processing when contaminated meat is consumed. This means that the workers can then contaminate other carcasses and equipment. After the "killing" steps, this can lead to recontamination of the carcasses (Bryan, 1980; Sammarco *et al.*, 1997). Man is also the natural habitat of *Staphylococcus aureus*, and 40 – 44% of the population can be nasal carriers and 14 – 40% can be hand carriers. This means that *S. aureus* is always a problem as the workers can contaminate the meat (Wilson & Miles, 1961; Shapton & Shapton, 1991; Harvey & Gilmour, 2000).

2.5.5 Storage Contamination

Temperature abuse of food can generate a hazard because some pathogens are capable of competitive growth at 5 °C, e.g. enterotoxigenic *Escherichia coli* and *Listeria monocytogenes*. Other pathogens, e.g. *Salmonella* and *Staphylococcus aureus*, are able to grow at temperatures slightly above 5 °C

up to 12 °C. Yeasts and moulds may also be found on poultry held at 5 °C (Palumbo, 1986).

2.5.6 Processing steps

Abu-Ruwaida *et al.* (1994) found that during processing most of the gram-positive bacteria that are already present on the birds when they arrive at the slaughter house, are removed. It is replaced by a heterogeneous population largely composed of gram-negative bacteria such as pseudomonads, flavobacteria, *Acinetobacter/Moraxella* and Enterobacteriaceae.

Their presence indicates unsatisfactory processing, improper sanitation and hygienic practices in the factory. The finished product may contain spoilage-causing organisms, such as *Pseudomonas* spp. or pathogens, such as *Salmonella*, *Campylobacter*, *Clostridium perfringens* and *Staphylococcus aureus* (Abu-Ruwaida *et al.*, 1994).

Slaughtering

During processing, the lines move at such a speed (125 chickens/minute) that it is impossible to have any hygienic separation between the carcasses. This means that contamination of one carcass can spread to many other carcasses through many pieces of equipment that come in contact with each of the carcasses (Silliker *et al.*, 1990).

Scalding

Scalding is used to facilitate feather removal. Because all the carcasses are immersed in the same water, this facilitates the spread of organisms. Contamination can occur from the feathers, skin, intestinal track and respiratory track (Silliker *et al.*, 1990). Humphrey *et al.* (1981) and Abu-Ruwaida *et al.* (1994) showed that high counts were recovered after scalding, which indicates that cross-contamination occurred in the scalding tank, possibly from the scalding water and other carcasses. The high counts for *Salmonella* and *S. aureus* on the carcasses showed that the microflora on the carcasses survived scalding at 51 to 53.5 °C for 3 minutes. This was due to the pH of 5.9 – 6.0 in the scalding tanks which is close to the optimum for heat resistance of salmonellas.

When the chicken muscle fascia and muscle perimysium are immersed in the water, this causes the collagen associated with the connective tissue to expand and form a dense network of fibers on the surface. *Salmonella* spp. are able to attach to the collagen fibers if the muscle is immersed for extended times in the water. The skin also absorbs water which causes capillary-size channels trapping bacteria. More water is retained as a surface film which can also trap more bacteria (Selgas *et al.*, 1992).

Plucking

Abu-Ruwaida *et al.* (1994) showed that the microbial loads on carcass skin can be increased by the defeathering machine or other carcasses. If there is not an adequate sanitation step for the plucking machine, and especially the

rubber fingers, this could become a significant source of contamination as bacteria can be present on these rubber fingers. During the plucking step bacteria can be transferred from the rubber fingers to the carcasses and become firmly attached to the skin surface or enter the feather follicles and thus become difficult to remove (Silliker *et al.*, 1990).

Dodd *et al.* (1988) found that the numbers of *S. aureus* on the carcasses increased after defeathering. The strains isolated from the pluckers were of different biotypes or phage types from those found on the freshly slaughtered carcasses. These endemic strains often showed a particular clumping phenotype which enables them to adhere to surfaces and increases their resistance to hypochlorite. They also found that the problem was more severe when serial pluckers are used.

Gibbs *et al.* (1978) and Mead & Dodd (1990) found that the atmosphere inside the machines is moist and warm, up to 30 °C for the first two units. This, together with the residual carcass-blood and other organic materials, is a good place for bacteria to grow. There is also difficulty in effectively cleaning and sanitizing the machines, especially the rubber fingers. These fingers become worn and cracked during use, which means that bacteria such as *S. aureus* can penetrate below the surface of the rubber and is thus protected from the sanitizing agents. Many of the strains isolated from the plucker fingers has chlorine resistance making it more difficult to reduce the numbers.

Washing

Washing removes the contamination from the carcasses, especially fecal contamination which is a primary avenue of contamination (Mulder & Veerkamp, 1974; Cross, 1996). Abu-Ruwaida *et al.* (1994) found that washing of the carcasses had less of an effect on reduction of Enterobacteriaceae, *Escherichia coli* and coliform counts, probably due to the strong attachment of these organisms to the carcass skin.

Eviscerating

During evisceration there is also a degree of contamination, particularly with intestinal organisms, during the removal of the intestines. This can lead to the contamination of the inside and outside of the carcass. There is also the chance of contamination from the workers of the manual evisceration line. Mechanical evisceration reduces the contamination from workers, but if the equipment is not functioning correctly this can lead to contamination from the intestines if they are damaged (Silliker *et al.*, 1990).

Chilling

Chilling is an important step because it is a major source of organisms. The factory in this study made use of spin-chilling, but spray-chilling and air-chilling is also available. Cross contamination can occur during chilling in water. The immersion washer water, immersion chiller water and ice used to cool immersion chiller water can be major sources of contamination (Mulder

& Veerkamp, 1974; Thomas & McMeekin, 1980; Silliker *et al.*, 1990). As shown by Abu-Ruwaida *et al.* (1994), air-chilling did not reduce or increase the bacterial counts on carcasses while properly controlled immersion chillers can lower the bacterial load of carcasses.

2.6 HAZARD ANALYSIS CRITICAL CONTROL POINT SYSTEM

The Hazard Analysis Critical Control Point (HACCP) system is a quality control management technique. Its purpose is to identify and eliminate potential problems which could occur during or after the operation (Tompkin, 1990; Scarlett, 1991). Each potential hazard (unacceptable contamination, unacceptable growth or unacceptable survival by microorganisms of concern to safety and spoilage) must be considered and means must be established to minimize or prevent its occurrence. Critical control points defines the limits of what should be achieved when a HACCP program is established to prevent contamination which could lead to unacceptable growth. There should also be corrective action procedures which can be taken when monitoring results which indicate that part of the operation is not under control and can lead to a hazard (Tompkin, 1990).

Table 2.10 describes the principles and related stages of a HACCP system.

Table 2.10 : Principles and stages of HACCP (Bekker, 2001).

Principles		Stages	
1.	Conduct a hazard analysis.	1.	Total management commitment
		2.	Select HACCP team
		3.	Define terms of reference
		4.	Describe the product
		5.	Identify intended use
		6.	Construct a flow diagram
		7.	On-site verification of flow diagram
		8.	Identify hazards and describe preventative measures
2.	Identify critical control points (CCPs) in the process.	9.	Apply HACCP decision tree to each step in order to identify CCPs
3.	Establish critical limits for preventative measures.	10.	Establish target level(s) and tolerance for each CCP
4.	Establish CCP monitoring requirements.	11.	Establish a monitoring system for each CCP
5.	Establish corrective actions when monitoring indicates a deviation.	12.	Establish corrective plan of action
6.	Establish record keeping procedures.	13.	Establish record keeping
7.	Establish procedures for verification.	14.	Verification
		15.	Review

2.6.1 Conduct a hazard analysis

2.6.1.1 Total management commitment

Management of the factory should be involved in the implementation of the HACCP system in order for them to understand the HACCP system and

understand the benefits it can offer the factory. This will enable management to understand what resources is needed in the implementation of the system (Bekker, 2001).

2.6.1.2 Select HACCP team

The HACCP team should be multi-disciplinary, and the team should be members of the factory as they will have the knowledge of the conditions that occur in the factory. The personnel should also receive training such as meat inspection and HACCP (Bekker, 2001).

2.6.1.3 Define terms of reference

It is necessary to define terms of reference in order to decide which types of hazards can occur and whether the HACCP system will cover the whole factory or only a certain division. In order to decide where to implement the HACCP system, one first have to answer the five basic HACCP questions (Bekker, 2001):

1.) Is the product microbiologically sensitive?
2.) Can the product become contaminated with foreign materials and chemical residues?
3.) Can the raw material, process or product become adulterated by pests?
4.) Can the raw material, process or product support the growth of pathogenic microbes ?
5.) Are there points in the system which, if they went out of control, might alter the product in such a way as to render it below standard, unprofitable or unfit for human consumption?

2.6.1.4 Describe the product

It is important that the HACCP team have a description of their product in order to better understand the hazards that could occur on their product. There are seven questions that can be asked to describe the product (Bekker, 2001):

1.) Composition
2.) Structure
3.) Processing
4.) Packaging system
5.) Storage and distribution system
6.) Required shelf life
7.) Instructions for use

2.6.1.5 Identify intended use

It is important to know the products intended use and the consumer target groups (Bekker, 2001).

2.6.1.6 Construct a flow diagram

The HACCP team should draw a flow diagram of the process from the receiving of the live birds to the final packaging and transport of the final product. This will help the team in deciding where CCPs are and where the process can be improved (Bekker, 2001).

2.6.1.7 On-site verification of flow diagram

It is important that the team verify that the flow diagram is correct be going through the whole process and verify each step. This will also help the team to observe important steps in the process (Bekker, 2001).

2.6.1.8 Identify hazards and describe preventative measures

All the hazards that could occur on the product are described, together with preventative measures that can be used to control the hazards. No CCPs are determined at this step, only the hazards are determined. This includes hazards that are not present at the moment but can occur in the future (Bekker, 2001).

2.6.2 Identify critical control points (CCPs) in the process

2.6.2.1 Apply HACCP decision tree to each step in order to identify CCPs

Potential critical control points where hazards could be controlled were then identified by means of the CCP decision tree (Figure 2.2).

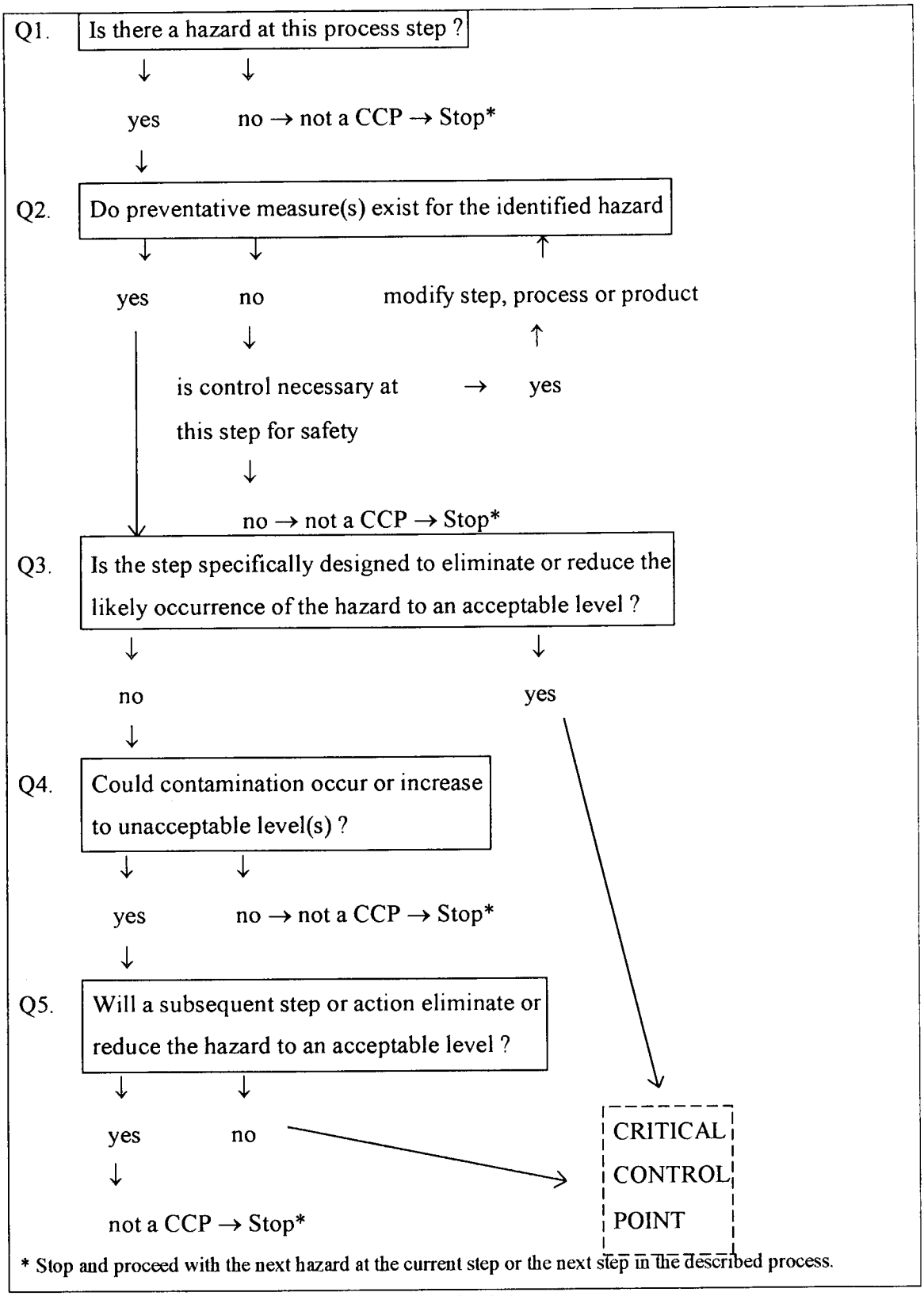

Figure 2.2 : The critical control point decision tree.

2.6.3 Establish critical limits for preventative measures

2.6.3.1 Establish target level(s) and tolerance for each CCP

The target levels for each CCP must be defined. Critical limits define the boundaries between a safe and unsafe product. The critical limits enable the monitoring of the CCPs (Bekker, 2001).

2.6.4 Establish CCP monitoring requirements

2.6.4.1 Establish a monitoring system for each CCP

Monitoring is very important in the HACCP system, as this ensures that the product is manufactured safely. Monitoring can either be an on-line system, where the factors are measured during the production, or an off-line system, where samples are taken and measured (Bekker, 2001).

2.6.5 Establish corrective actions when monitoring indicates a deviation

2.6.5.1 Establish corrective plan of action

Corrective action follows to prevent deviation and when there is a deviation in the monitoring results of a CCP, action must be taken to correct the deviation and bring it under control (Figure 2.3).

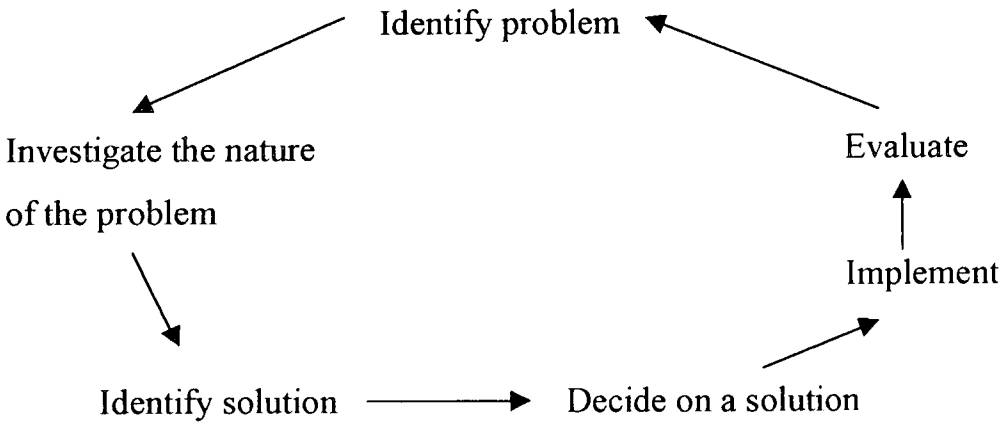

Figure 2.3 : Corrective action loop (Bekker, 2001).

2.6.6 Establish record keeping procedures

2.6.6.1 Establish record keeping

Records should be kept of everything that is done by the HACCP team in order to verify that the HACCP system is working correctly (Bekker, 2001).

2.6.7 Establish procedures for verification

2.6.7.1 Verification

There should be verification that the HACCP system is working correctly and whether the system is still appropriate for the product and its hazards (Bekker, 2001).

2.6.7.2 Review

There should also be a review of the HACCP plan at least every 6 months (Bekker, 2001).

2.7 MEASURES OF MICROBIAL CONTROL

A typical HACCP flow chart with causes of contamination of raw poultry meat is given in Figure 2.4. The control measures for each of the contamination sources will be discussed in more detail.

2.7.1 Flock Contamination

It is important that the factory receive high quality flock in order to produce high quality products (Silliker *et al.*, 1990; Shapton & Shapton, 1991). This means that it is critical that bacteria such as *Salmonella* must be controlled during the growout stage of the chickens in order to produce *Salmonella*-free chickens that can be delivered to the factory (Bailey, 1993).

The flock is received from poultry houses which should be kept locked and visitors prevented from entering. The workers on the farm should undergo regular bacteriological examinations. This will identify carriers of pathogens. Protective clothing and disinfectant footbaths are also essential. Another important practice is an all-inn, all-out rearing system, because this will prevent cross-contamination from one flock to another (Hafez, 1999).

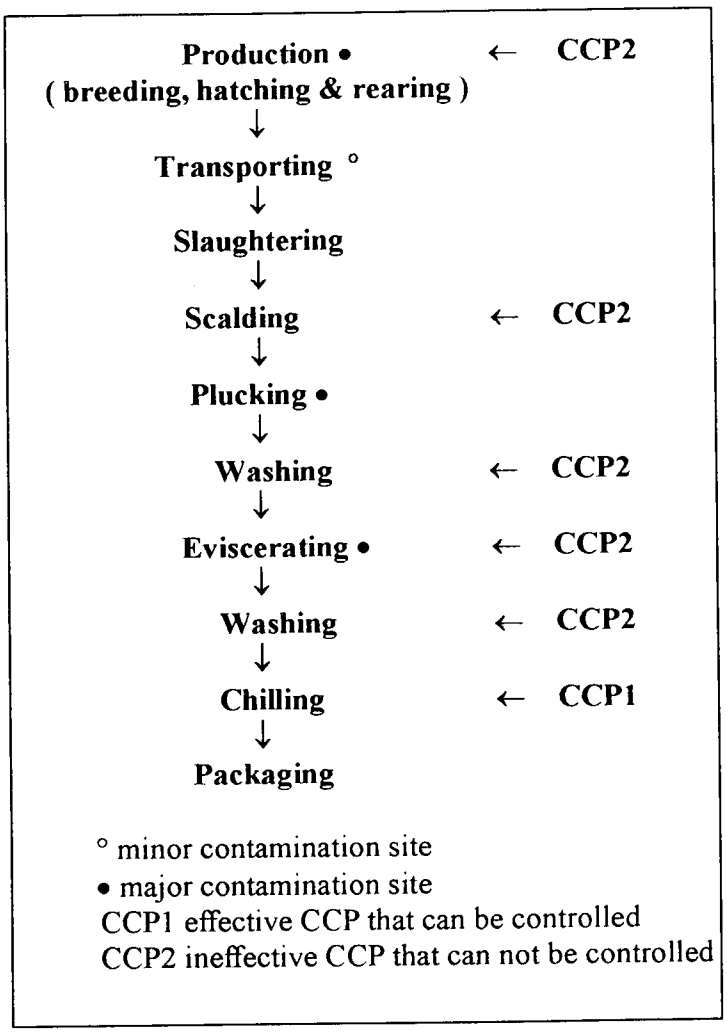

Figure 2.4: Causes of contamination during production of raw poultry meat (Silliker *et al.*, 1990).

Microbial contamination can start at the egg, either during its development or later through the shell (Silliker *et al.*, 1990). This means that breeder flock should be fed *Salmonella*-free feed and kept away from other animals such as birds and rodents. Contaminated feed is seen as the most common source of infection in poultry flocks (Hafez, 1999). It is thus very important to have good cleaning and sanitation programs that are systematically monitored to

prevent the build up of pathogens. A good pest control program should also be followed to prevent pests from coming into contact with the carcasses, packaging material and the environment of the food (Garbutt, 1997).

Feed additives such as short chain organic acids (formic and propionic acids) can be used for decontamination and prevention of recontamination of feed. Carbohydrates (lactose, mannose, galactose and sucrose) can influence the caecal environment by increasing the acid production of bacterial fermentation, and thus decreasing the pH. This will then reduce the amount of *Salmonella* in the caeca (Hafez, 1999).

Competitive exclusion can also be used as this involves the oral introduction of intestinal microflora from *Salmonella*-free adult chickens into newly hatched chicks. The introduction of intestinal microflora speeds the maturation process of the gut microflora and this will increase the resistance of most chicks to *Salmonella* colonization (Bailey, 1993). *Salmonella* infection of poultry can be decreased by use of probiotics such as lactobacilli, streptococci, bifidobacteria, bacilli and yeasts for competitive exclusion and introduction of adult microflorae as early as possible. The probiotics are able to inhibit the growth of pathogens by decreasing the pH through the production of lactate, lactic acid and volatile fatty acids (Hafez, 1999). Competitive exclusion together with good farm hygiene will prevent *Salmonella* infection throughout the flock. In Finland more than 70% of the farms introduced competitive exclusion with the result that less than 5% of the flocks were *Salmonella* positive (Harrigan, 1998; Hafez, 1999).

Live and inactivated vaccines are available especially for the treatment of *S. typhimurium* and *S. enteritidis* infections, and the vaccines also reduced the number of carriers if a flock is infected with a field strain of *Salmonella* (Hafez, 1999).

Animals can be tested for specific pathogens, and antibiotic therapy can be used to treat infected animals to thus remove the food poisoning bacteria from their intestines. Inspection of the live birds and carcasses at the farm and factory is very important to remove sick or diseased animals from the food chain (Garbutt, 1997).

2.7.2 Transport

The catching and transporting of the birds should be done by trained personnel to ensure that the birds are handled carefully and do not cause them distress. All equipment used for the collecting of the birds must be cleaned and disinfected (Hafez, 1999). The removal of feeders and waterers prior to the catching of the birds will minimize the bruising of the chickens (Macrae *et al.*, 1993).

During transport there is cross-contamination between birds, due to faecal organisms on the feathers and skin. Trucks should, therefore, be cleaned after use (Silliker *et al.*, 1990; Kotula & Pandya, 1995). Contamination during transport can be reduced by taking the birds off feed 8 to 12 hours before the time of transportation to reduce the chance of faecal contamination during transport and during the processing of the carcasses

(May, Lott & Deaton, 1990; Lyon, Papa & Wilson, 1991; Macrae *et al.*, 1993).

2.7.3 Equipment and Environment Contamination

The use of an 'in-line' processing system is also a means of reducing the contamination of carcasses. This is because it is not necessary for the personnel to handle the carcasses from the time they are hung on the line before stunning until the carcasses arrive at the packaging department (Hafez, 1999). The practice of high throughputs in the poultry plant can increase the spread of organisms from one carcass to another. It is important to have properly designed equipment in combination with efficient cleaning and sanitation programs as this will prevent contamination (Garbutt, 1997).

It is necessary to apply stronger sanitizers to the walls and floors to eliminate the pathogens which end up here from the cleaning process of the equipment and to eliminate bacteria that are harbored in the factory environment. It is also important to have effective cleaning procedures for refrigerated areas (Sammarco *et al.*, 1997).

Measurements for evaluating whether equipment is hygienically acceptable are as follows (Tompkin, 1990):

a.) Monitoring measurements

1. Visual
2. Aroma

3. Touch

b.) Verification measurements

1. Microbiological assays

- samples from equipment
- flow sheet samples
- analysis of finished product
- shelf life tests

2. Records and reports

2.7.4 Personnel Contamination

By implementing the correct personal hygiene practices, the risk of product contamination can be reduced (Tompkin, 1990). Training of the personnel to make use of good personal hygiene practices also plays an important part in the reduction of contamination.

2.7.5 Storage Contamination

It is very important to have a good monitoring system of all the refrigerated areas, as temperature abuse of the product can generate a hazard (Palumbo, 1986).

2.7.6 Processing steps

Slaughtering

The process line move at high speeds (125 chickens/minute) and can not be slowed down. It is thus important to minimize the spread of bacteria at points of high contamination between carcasses by giving special attention to these points which could lead to problems (Silliker *et al.*, 1990).

Scalding

Many bacteria are washed from the carcasses and contaminate the scald water. The contamination during scalding can be prevented by an overflow system, replacement of the scald water or through the use of high temperatures.

A minimum overflow of 1 liter per bird and the use of a scalding temperature of 60 °C or higher is adequate (Silliker *et al.*, 1990; Hafez, 1999). When carcasses are mildly scalded (55.5 °C, soft scalding) there is only a slight decrease in the Enterobacteriaceae, *E. coli* and total counts. When carcasses are scalded at 60 °C (hard scalding), nearly all Enterobacteriaceae and *E. coli* are destroyed. Psychrophiles are decreased to undetectable levels on carcasses during scalding (Notermans *et al.*, 1977). Dodd *et al.* (1988) found that scalding decreased the initial counts on the carcasses by 100-fold.

The change in the pH of the scalding tanks can also lead to the reduction of bacteria on the carcasses and prevent the growth of the bacteria in the scalding tanks. Humphrey *et al.* (1981) found that *Salmonella typhimurium* can survive the scalding process at pH 6.1 as it has a *D*52 °C value of 34.5 minutes which is the optimum for heat resistance. At pH 4.0 or 9.0 the *D*52 °C values were 0.75 and 1.25 minutes. This means that at pH 4.0 or 8.5 or above there will be a reduction in salmonellas that will reduce cross-contamination. At pH 9.0 the total bacterial count reduced by 71.7% and coliform counts dropped by 87.9%. There was also a reduction in the amount of faecal and other organic matter on the feathers. A pH of 8.5 were regarded as optimum for plucking rather than at pH 4.0. It was even more effective than raised water temperature. At 56 °C or above the cuticle becomes damaged and is removed during plucking rendering the flesh less tender.

The use of counter-current flow rather that parallel-flow systems in the scalding tanks will also reduce the spread of bacteria and other organic material. The use of larger amounts of water will dilute the level of contamination on the carcasses (Kotula & Pandya, 1995). The use of simultaneous scalding and plucking with the use of steam at 65 °C will lead to lower contamination of carcasses (Mulder & Veerkamp, 1974).

In a study by Kaufman, Klose, Bayne, Pool & Lineweaver (1972), steam was used to loosen the feathers at 51 °C for 2 minutes and compared to birds that were scalded in a tank at 53 °C for 2 minutes. There were little or no

abrasions on the outside of the steam scalded carcasses, and the air sacs contained 1000-fold less bacteria than those of tank scalded birds.

Plucking

Plucking allows for the spread of organisms. With automatic machinery, there is a considerable amount of scattering of bacteria which leads to cross-contamination between the carcasses (Hafez, 1999). If the rubber fingers are not cleaned effectively, they can harbour bacteria and this facilitates the spread of organisms (Silliker *et al.*, 1990). The fingers must be replaced frequently due to the fact that bacteria can be protected from sanitizing agents between the cracks in the rubber (Mead & Dodd, 1990). Although plucking is the major site of cross-contamination, the degree at which it occurs is related to the effect of the scalding process to lower the bacterial load on the carcasses (Humphrey *et al.*, 1981).

Washing

It is very important to remove faecal contamination on the carcasses because this can prevent contamination of the plant and the spread of pathogens which are common in faecal contamination (Cross, 1996). Spray washing can remove loosely bound organisms and other material and can be an important process to lower contamination (Silliker *et al.*, 1990). When efficient cleaning and sanitation programs are in place and systematically monitored, there is an even better chance of lowering the amount of bacteria on the carcasses (Garbutt, 1997).

Eviscerating

During evisceration there can be contamination from the intestines if they are damaged. This can lead to cross-contamination as a result of the feces. To prevent the spread of contamination during evisceration, there is a need for operator skill, the use of correct techniques and a mechanical line in a good working order. The equipment should be properly adjusted and monitored regularly (Silliker *et al.*, 1990; Hafez, 1999).

Chilling

Spoilage occur only before freezing and after thawing. Poultry stored at or near −18 °C will not spoil and there is a drastic reduction in the number of viable organisms. Aerobic plate counts of poultry skin were found to decrease by 10 to 95% (Shapton & Shapton, 1991). Chilling is, therefore, an important step to reduce contamination, because it delays the growth of the bacteria. During chilling the carcasses are immersed into water which should be chlorinated up to 50 p.p.m. total residual to decrease the amount of bacteria and to prevent cross contamination (Silliker *et al.*, 1990).

Packaging

With hygiene precautions there should not be any contamination during the cut-up and packaging of the carcasses. With manual cut-up there is a risk of contamination from the workers, but this risk is reduced when the carcasses are cut-up mechanically. It is very important to have a good sanitation step to clean the cutting equipment and machinery or they can harbour bacteria.

Packaging also protects the meat from further contamination (Silliker *et al.*, 1990; Abu-Ruwaida *et al.*, 1994).

2.7.7 Sampling Methods

There are various methods that can be used to sample poultry meat for their microbial content. The four main methods are (Avens & Miller, 1970b):

1) Direct contact plating which results in the clumping of bacterial cells. Colonies are therefore, not distinguishable from one another and bacteria are not completely removed. This method can however be used where low contamination is expected.

2) Swab sampling which leads to incomplete and inconsistent bacterial removal as only weakly attached bacteria are removed.

3) Rinse sampling which leads to incomplete and inconsistent bacterial removal as there is not enough friction to remove the bacterial cells. It is difficult to perform and all the carcasses have to be the same size to estimate the amount of bacteria on the carcass, as it cannot be related to a unit of skin.

4) Skin tissue removal delivers the best results as all the bacteria on the skin become dislodged.

The skin tissue removal method is seen as the best method for accurately and precisely determining the amount of bacteria on the skin. This involves the

removal of a known area of skin by using sterile cutting tools and then the sample is blended with the appropriate fluid. The blending of the skin tissue removes the bacteria from the skin and uniformly distributes the bacteria in the fluid (Avens & Miller, 1970a; Avens & Miller, 1970b; Simonsen, 1971; Notermans, Kampelmacher & van Schothorst, 1975; Notermans *et al.*, 1977).

The swabbing method may be used on packaging material and the hands of personnel. This should be done with the use of a template and a moistened cotton swab (Avens & Miller, 1970b).

2.8 SUMMARY

There is an ever increasing occurrence of foodborne disease outbreaks. Many pathogens and spoilage bacteria are regularly found on poultry as natural microflora. As the demand for poultry increases, so does the chance for a foodborne disease outbreak with poultry as vehicle, increase. Even with better hygienic practices, pathogens can never be completely eliminated from the abattoir. The only step that can be taken to eliminate or reduce the number of pathogens during the processing stages to acceptable levels and at all cost prevent the recontamination or increase of pathogens which have their origin from the processing plant and personnel. Hazard analysis critical control point (HACCP) is a management control system that may be used to control contamination of the poultry abattoir with spoilage bacteria and pathogens.

CHAPTER 3

MATERIALS AND METHODS

3.1 ABATTOIR PROCESS

The abattoir sampled in this study is a Grade A poultry abattoir, with a capacity of ca. 65 - 67 000 birds per day. The birds that was used in the factory came from the factories own farms or from contract breeders. The birds are hanged on shackles when they arrive at the factory and stunned at a voltage of 50 to 70 volts, and scalded at a temperature of 52 to 54 °C before they go through three defeathering machines which are arranged in series. The defeathered carcasses are divided into two lines, one line is eviscerated manually and the other mechanically. The manually eviscerated carcasses are spray washed after evisceration and then both lines enter the spin chiller. The carcasses are immersed chilled in the spin chiller, which operate in a counterflow system at 3 to 4 °C with a chlorine level of 30 p.p.m.. After 30 minutes the carcasses are packed as whole birds, portions or individually quick frozen portions (IQF). A diagrammatic drawing of the abattoir is given in Figure 3.1.

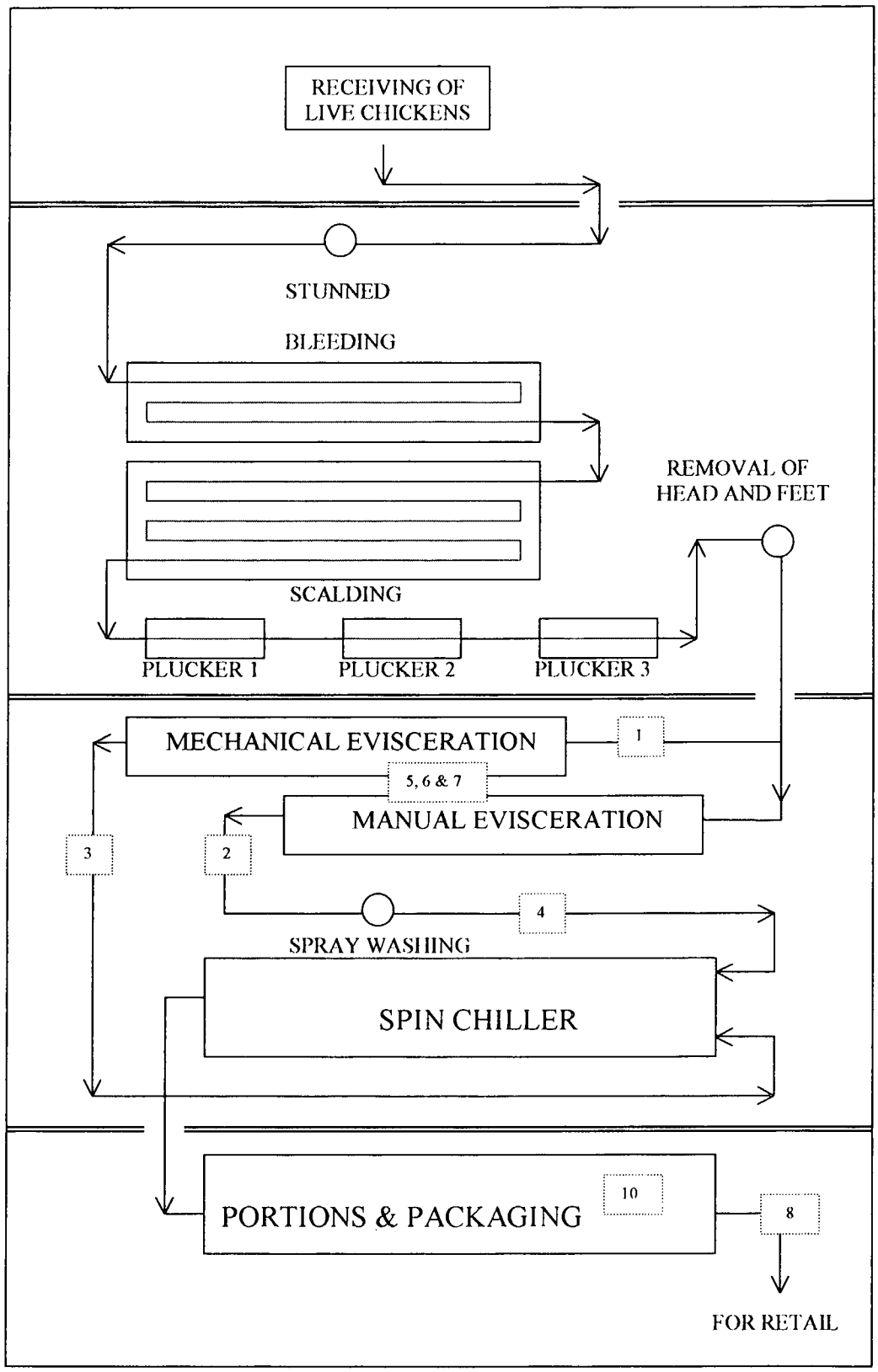

Figure 3.1: Scheme of the poultry abattoir (- Sample position; positions 9 & 11 were taken over the whole factory).

3.2 HACCP IMPLEMENTATION

The following critical points were addressed during HACCP implementation: the manual and mechanical evisceration lines which were sites of major contamination; after the spin chilling of the carcasses which was a site of effective microbiological control; cutting of the carcasses, packaging and packaging material were also critical control points that needed attention in order to prevent contamination. One of the most important critical control points was the improvement of the hygiene and training of the workers as this would improve the microbial contamination all over the factory.

3.3 MICROBIAL ANALYSIS

3.3.1 Collection and treatment of samples

Samples were taken at various points in the processing line (Figure 3.1). Swab samples were taken from the hands of workers in the factory and from the inside of the packaging material of the final product. Contact plates were used to sample the hearts, gizzards and livers conveyer belt, the whole chicken conveyer belt, the quick frozen portions conveyer belt and from each of the two portions conveyer belts. The degree of contamination was determined and corrective action was taken to improve these points. HACCP was implemented and the same points were sampled again to determine if there was an improvement at these points.

In order to perform statistical analysis on the results, two samples were taken at each point of interest on three visits to the plant. A total of 126 samples were taken from the various points in the factory during each visit. Out of this total, 48 samples were skin tissue removal samples, 60 samples were contact plates and 18 samples were swabs. The factory was sampled before HACCP implementation and again there after.

Skin tissue removal samples were taken from the neck skin of carcasses as it was shown by Simonsen (1971), Patterson (1972) and Notermans *et al.* (1977) that the neck skin of the carcasses were the most heavily contaminated. Six samples were taken at each point of interest in order to get enough tissue for all the analysis. The samples were cut with sterile scissors, placed in a sterile Whirl-pak bag (Nasco) and placed in a container with ice. All the samples were processed in 24 hours time from the time of sampling.

The samples were transported to the laboratory where they were aseptically weighed and divided into three sterile Whirl-pak bags containing 10 g each. To one bag 90 ml 0.1% buffered peptone water (Oxoid CM 509) was added for the determination of total aerobic mesophilic bacteria, coliforms, yeasts and moulds, *E. coli* and *S. aureus*. Buffered Peptone water (0.1%; Oxoid CM 509) was used as diluent since it did not cause any appreciable destruction of bacteria for 1 hour and was most frequently used as a diluent for determining bacteria counts on poultry meat (Avens & Miller, 1970a). To the second bag 90 ml selenite cystine broth (Oxoid CM 699) was added for the determination of *Salmonella*. To the third bag 90 ml UVM modified Listeria enrichment broth (Difco 0223-17) was added for the determination

of *L. monocytogenes*. Blending took place for one minute in a stomacher (Lab-blender 400).

Swab sampling was done by using a sterile metal template to outline a 6.25 cm^2 area which was swabbed using a moist sterile cotton swab. The swab was then broken off into the dilution fluid or enrichment broth. Six swabs were put in 10 ml 0.1% buffered peptone water (Oxoid CM 509) for determination of *E. coli*, *S. aureus*, yeasts and moulds and total aerobic mesophilic bacteria counts. Six swabs were put in 10 ml selenite cystine broth (Oxoid CM 699) for *Salmonella* detection and six swabs were put in 10 ml University of Vermont (UVM) modified Listeria enrichment broth (Difco 0223-17) for *L. monocytogenes* detection. The diluents containing the swabs were vortexed for 30 seconds. Tenfold serial dilutions of the neck skin and swab samples were made in 9ml 0.1% buffered peptone water and plated onto the specific media.

Contact plates (Rodac, Nunc Brand Products 240541) were taken from the five conveyor belts and used for determination of total aerobic mesophilic bacteria, coliforms and *E. coli*. Due to the fact that the conveyor belts had to be sampled while moving in order to determine their contribution to the microbial load of the carcasses, contact plates were the only means to do this.

3.3.2 Determination of bacterial groups

3.3.2.1 Total aerobic mesophilic count

The total aerobic mesophilic count was determined using standard plate count agar (Oxoid CM 463). Incubation was at 30 °C for 3 days (Notermans *et al.*, 1975; Notermans *et al.*, 1977).

3.3.2.2 Coliforms and *Escherichia coli*

Coliforms and *E. coli* were determined by using violet red bile agar with MUG (Oxoid CM 978). The plates were incubated at 37 °C for 18 to 24 hours. After incubation the plates were viewed under a long-wave UV light (366 nm) for fluorescence by *E. coli*. Total coliforms were regarded as forming pink to red colonies and included the *E. coli* colonies (Notermans *et al.*, 1975; Hitchins *et al.*, 1992; Blood & Curtis, 1995).

3.3.2.3 Yeasts and moulds count

Yeasts and moulds were determined by using rose-bengal chloramphenicol agar base (Oxoid CM 549) containing chloramphenicol selective supplement (Oxoid SR 78 E). The plates were incubated at 25 °C for 5 days (Mislivec, Beuchat & Cousin, 1992; Beuchat; 1995).

3.3.3 Determination of pathogenic bacteria

Although a number of pathogens may be present on poultry (see Chapter 2), only the three most frequently occurring pathogens associated with food borne outbreaks from poultry (Bean & Griffin, 1990) were investigated.

3.3.3.1 *Listeria monocytogenes*

The neck skin samples and swab samples were incubated at 30 °C for 24 hours in UVM modified listeria enrichment broth (Difco 0223-17). After incubation, 0.1 ml of the pre-enrichment broth were transferred to tubes containing 10 ml Fraser broth base (Oxoid CM 895) supplemented with Fraser selective supplement (Oxoid SR 156 E) and incubated at 37 °C for 40 hours. Black coloured test tubes indicated possible *L. monocytogenes* presence. A loopfull was streaked out on Listeria selective agar base (Oxoid CM 856) containing Listeria selective supplement (Oxoid SR 140 E). Typical *Listeria monocytogenes* colonies were round with black zones around them. The colonies were then gram stained and tested for oxidase activity. All the gram-positive coccoid rods that were oxidase-negative were regarded as *L. monocytogenes* (Donnelly *et al.*, 1992; Corry, Curtis & Baird, 1995; Curtis & Lee; 1995).

3.3.3.2 *Salmonella* spp.

The 10 g neck skin samples were mixed (Stomacher lab-blender 400) with 90 ml selenite cystine broth base (Oxoid CM 699) containing sodium

biselenite (Oxoid LP 121 A). The neck skin samples and the swabs in the 10 ml selenite cystine broth were incubated at 37 °C for 24 hours. After incubation, a loopful of the enrichment broth was streaked onto modified brilliant green agar (Oxoid CM 329) and incubated at 37 °C for 18 to 24 hours (Corry *et al.*, 1995).

Red, pink or white opaque colonies with a brilliant red zone were streaked onto Bacto urea agar base (Difco 0283-17-9) slants. It was incubated at 37 °C and examined after 24 and 48 hours. *Salmonella* gives a negative result on urease agar. All test tubes that did not discolour were streaked on xylose lysine desoxycholate agar (XLD; Oxoid CM 469). The plates were incubated at 37 °C for 24 hours. Typical *Salmonella* strains produce pink colonies with or without black centers. Some colonies may have large, glossy black centers or appear as completely black colonies due to a strong H_2S-production. Atypical lactose-positive and/or sucrose-positive *Salmonella* strains produce yellow colonies with or without black centers. The morphology, gram stain and oxidase reaction of the typical and atypical *Salmonella* colonies were determined. All the gram-negative rods that were oxidase-negative were regarded as *Salmonella* (Flowers, D'Aoust, Andrews & Bailey, 1992).

3.3.3.3 *Staphylococcus aureus*

From each dilution, 1 ml was added to 10 ml trypticase soy broth (Oxoid CM 129) containing 10% sodium chloride (NT Laboratory Supplies R 3060) and 1% sodium pyruvate (BDH 440943 N) in a sterile test tube. The test

tubes were incubated at 37 °C for 48 hours. The broth was used for the recovery of low numbers of *S. aureus* in food where there is a large population of competing bacteria (Corry *et al.*, 1995).

After 48 hours, 0.1 ml of each dilution was spread on Baird-Parker Medium (Oxoid CM 275) containing egg yolk-tellurite emulsion (Oxoid SR 54 C) using a sterile glass rod. The plates were incubated at 37 °C for 48 hours (Baird & Lee, 1995).

Colonies that produced black, shiny colonies with a white margin were then gram stained and their morphology observed. The gram-positive colonies that formed grape-like clusters were tested with the Staphylase Diagnostic Reagents test kit (Oxoid DR 595 A). All the colonies that resulted in clumping of the test cells were regarded as *S. aureus* (Buchanan & Gibbons, 1974; Lancette & Tatini, 1992).

3.4 STATISTICAL ANALYSIS

Statistical analysis was performed on the microbial parameters. Differences in parameters between different groups of samples were determined using an analysis of variance (ANOVA) procedure. The Tukey-Kramer multiple comparison test ($\alpha = 0.05$) was used to identify differences between treatment means (NCSS, 2001). Statistical analysis - 0.5 log cfu/g was entered for samples in which no counts were detected (Gill & Jones, 1998 ; Gill, Badoni & McGinnis, 1999).

CHAPTER 4

RESULTS AND DISCUSSION

4.1 HACCP IMPLEMENTATION

All the critical control points where a hazard could occur were determined and the preventative measures to improve the level of contamination or eliminate it was then determined and applied and are depicted in Table 4.1.

4.2 MICROBIAL ANALYSIS

All the data from the microbial tests were collected and were used to build a microbial profile of the poultry abattoir in order to assess the effectiveness of the implementation of a HACCP program on the microbial load on the carcasses. The results of each of the 11 sampling points will now be discussed.

4.2.1 Mechanical line, after defeathering

Before HACCP was implemented, the mean total aerobic mesophilic counts were log 7.65 cfu/g. After implementation the counts were significantly ($P<0.05$) reduced to log 6.42 cfu/g (Figure 4.1).

Table 4.1 : Hazards at various stages in the abattoir under investigation and their preventive measures.

PROCESS STEP	HAZARDS	PREVENTIVE MEASURES
Slaughtering	Incomplete bleeding	Extension of the bleeding time
Evisceration (manual line)	Microbial contamination Inadequate evisceration	Inspection at the manual evisceration line Extension of the shackle line before evisceration
Evisceration (mechanical line)	Microbial contamination Inadequate evisceration	Second inspection at the mechanical evisceration line Better equipment for evisceration at mechanical line
Inadequate evisceration	Microbial contamination	Installation of a recovery section for carcasses that are held back due to contamination
After spin chilling	Microbial contamination	Final inspection after spin chilling of both lines
Packaging (IQF)	Microbial contamination	Improved IQF conveyor belts
Packaging (mechanical)	Microbial contamination	Installation of mechanical cutting of the carcasses equipment
Packaging	Microbial contamination Inadequate packaging	Change of packaging materials from plastic to bags – less contact from personnel and less chance for contamination Wash points for the personnel's hands
Packaging and Transport	Microbial contamination	Installation of sterilization equipment for gloves and equipment
Personnel hands	Microbial contamination	Better hand and shoe washing points for workers before they enter the factory
Personnel hands	Microbial contamination	Installation of washing points for workers hands in the factory
Water supply	Microbial contamination	Better control over the chlorine content
Factory floor	Microbial contamination	Better drainage system
Personnel	Microbial contamination	Training of the personnel - Poultry meat examiners course - Poultry meat inspectors course - HACCP course for supervisors

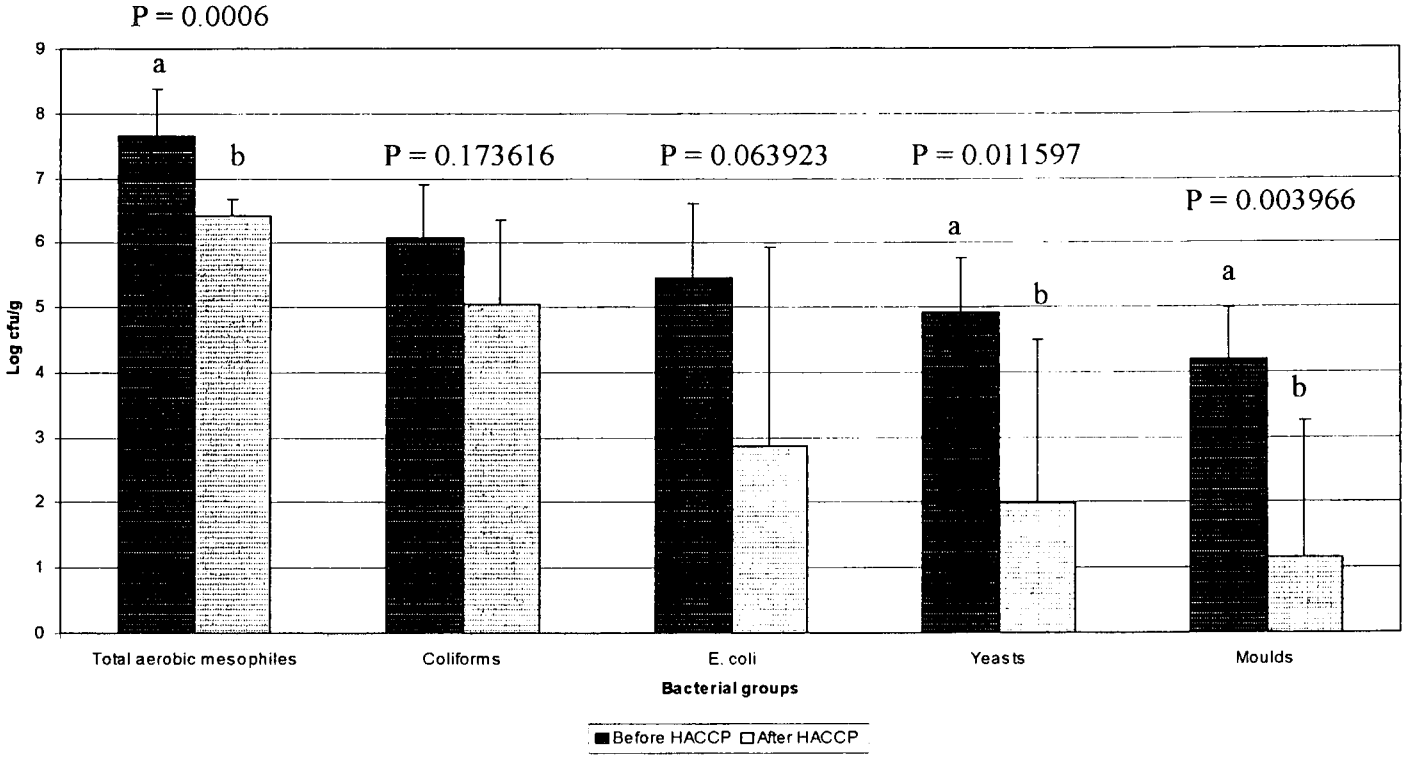

Figure 4.1 : Mean microbiological counts (log cfu/g) of samples, before and after HACCP implementation, from the mechanical line, after defeathering. Bars within a bacterial group with different superscripts are significantly different ($P<0.05$).

Patterson (1972) found that the total aerobic mesophilic counts after defeathering, before evisceration were log 2.91 cfu/g. Mulder & Veerkamp (1974) and Notermans *et al.* (1977) found counts of log 4.93 cfu/g and log 5.57 cfu/g respectively, while Abu-Ruwaida *et al.* (1994) found mean counts of log 7.1 cfu/g at this sampling point. Although the aerobic mesophilic counts in this study did not reach these low levels, there were a significant decrease in the counts. The differences in the counts by the various authors is due to the fact that no two abattoirs are the same.

The mean coliform count before HACCP implementation was log 6.08 cfu/g, which was not significantly reduced to log 5.05 cfu/g after HACCP implementation (Figure 4.1). The coliform counts after HACCP

implementation was higher than that found by Berrang, Dickens & Musgrove (2000), Berrang, Ladely & Buhr (2001), Notermans *et al.* (1977) and Mulder & Veerkamp (1974). Berrang *et al.* (2000; 2001) found the coliform counts to be as low as log 2.4 cfu/ml and log 3.2 cfu/ml respectively, while Notermans *et al.* (1977) and Mulder & Veerkamp (1974) found counts to be up to log 4.07 cfu/g and log 4.3 cfu/g. Abu-Ruwaida *et al.* (1994) found mean coliform counts of log 5.5 cfu/g at this sampling point which were higher than that found in the study.

The *E. coli* counts were also not significantly reduced after HACCP implementation with a mean count of log 2.87 cfu/g (Figure 1). Berrang *et al.* (2000; 2001) found *E. coli* counts to be log 2.1 cfu/ml and log 2.9 cfu/ml, which was lower than that found in the study, while Notermans *et al.* (1977) found counts to be log 4.07 cfu/g and Abu-Ruwaida *et al.* (1994) recorded mean *E. coli* counts of log 4.85 cfu/g which was higher than that found in the study after HACCP implementation.

The yeast and mould counts were log 4.91 cfu/g and log 4.19 cfu/g respectively before HACCP implementation and was significantly ($P<0.05$) reduced to log 1.98 cfu/g and log 1.16 cfu/g respectively after HACCP implementation (Figure 4.1).

Listeria monocytogenes was present on 33.33% of the carcasses before HACCP implementation at this point of sampling and were totally eliminated after the implementation (Table 4.2). There were no *Salmonella* species found on any of the carcasses on the mechanical line after

defeathering before and after HACCP implementation (Table 4.2).

Table 4.2 : The occurrence of pathogenic bacteria in samples, before and after HACCP implementation, from the mechanical line, after defeathering.

Pathogen	Baseline	HACCP	Reduction
Listeria monocytogenes	33.33% of carcasses	0% of carcasses	33.33%
Salmonella spp.	0% of carcasses	0% of carcasses	0%
Staphylococcus aureus	16.67% of carcasses	0% of carcasses	16.67%

Dougherty (1974) found *Salmonella* species in 80% of the carcasses tested at this point of sampling while Abu-Ruwaida *et al.* (1994) recorded a 100% presence.

Staphylococcus aureus was present on 16.67% of the carcasses with the baseline study which was eliminated after HACCP implementation (Table 4.2). *Staphylococcus aureus* counts of as low as log 2.73 cfu/g (Notermans *et al.*, 1982) and log 3.55 cfu/g (Abu-Ruwaida *et al.*, 1994) and as high as log 5.70 cfu/16cm^2 may be present on carcasses at this sampling point (Gibbs *et al.*, 1978).

The reason that the bacterial counts were so high at this sampling point before HACCP implementation was due to the fact that the scalding tank and defeathering machines lead to cross-contamination between the carcasses. The temperature in the defeathering machines can be as high as 30 °C, and together with the blood of the carcasses, this is a perfect place for growth of bacteria such as *E. coli*, *S. aureus* and *L. monocytogenes*. The

extension of the bleeding time reduced the chance of bacterial growth in the defeathering machines and improvement of worker hygiene. This is monitored through visual observation from trained workers.

The reduction in the bacterial counts and pathogens on the carcasses after HACCP implementation was mainly due to the improvement of the hygiene of the workers as they were required to re-hang the carcasses after plucking, before evisceration. This was achieved through the installation of better hand and shoe washing points before the workers entered the factory and hand washing points in the factory. This is monitored through trained workers that make use of visual observation (Table 4.1).

4.2.2 Manual line, after evisceration

The mean total aerobic mesophilic counts were log 7.54 cfu/g before HACCP, and were not significantly reduced (log 6.81 cfu/g) after the implementation (Figure 4.2). Counts after evisceration was found to be as low as log 3.95 cfu/cm^2 (Thomas & McMeekin, 1980) and as high as log 5.47 cfu/g (Notermans *et al.*, 1977). The total aerobic mesophilic counts were not as low as that of the literature after HACCP implementation.

There was a significant ($P<0.05$) decrease in the coliform and *E. coli* counts on the manual line after evisceration (Figure 4.2). Before HACCP implementation the mean coliform counts were log 6.61 cfu/g which was significantly ($P<0.05$) reduced to log 3.76 cfu/g after HACCP implementation. Mulder & Veerkamp (1974) found coliform counts to be log 3.24 cfu/g which is lower than that found in the study after HACCP

implementation, while Notermans *et al.* (1977) found counts up to log 4.2 cfu/g which is higher than the counts after HACCP implementation.

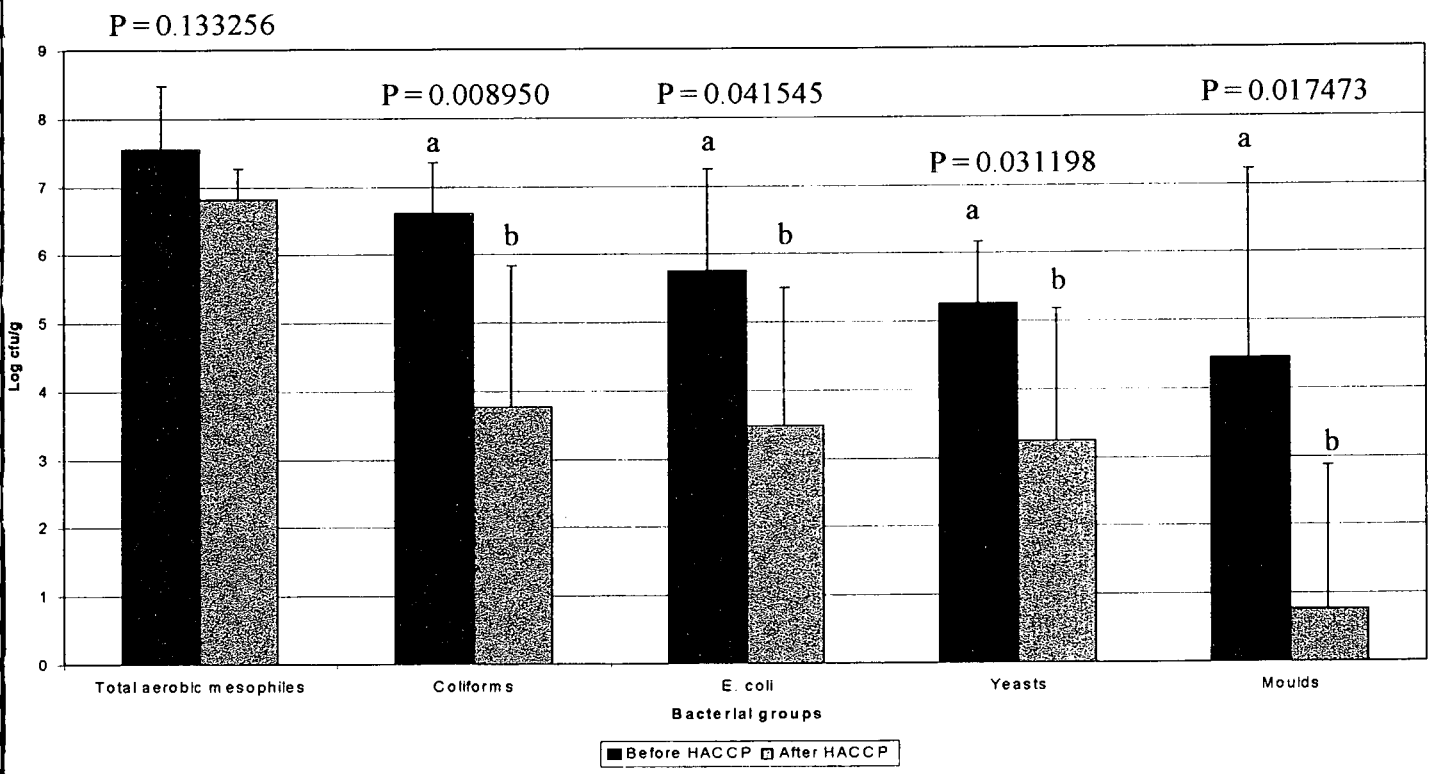

Figure 4.2 : Mean microbiological counts (log cfu/g) of samples, before and after HACCP implementation, from the manual line, after evisceration. Bars within a bacterial group with different superscripts are significantly different ($P<0.05$).

Notermans *et al.* (1977) found the mean *E. coli* count to be log 2.9 cfu/g. The mean *E. coli* count at this sampling point in this study was log 5.75 cfu/g, and was significantly ($P<0.05$) reduced to log 3.48 cfu/g after HACCP implementation (Figure 4.2) which is higher than the counts found by Notermans *et al.* (1977).

The mean yeast count were log 5.25 cfu/g before HACCP implementation which was significantly ($P<0.05$) reduced to log 3.23 cfu/g after HACCP implementation, while mould counts were also significantly ($P<0.05$) reduced from log 4.45 cfu/g to log 0.77 cfu/g after HACCP implementation (Figure 4.2).

Table 4.3 : The occurrence of pathogenic bacteria in samples, before and after HACCP implementation, from the manual line, after evisceration.

Pathogen	Baseline	HACCP	Reduction
Listeria monocytogenes	66.67% of carcasses	0% of carcasses	66.67%
Salmonella spp.	0% of carcasses	0% of carcasses	0%
Staphylococcus aureus	0% of carcasses	0% of carcasses	0%

At this point on the manual line, *Listeria monocytogenes* (66.67%) was present before HACCP, but there was a total reduction after HACCP implementation (Table 4.3). No *Salmonella* species or *Staphylococcus aureus* were present before and after HACCP implementation (Table 4.3). According to Notermans *et al.* (1982), *S. aureus* counts after evisceration can be as high as log 3.88 cfu/g (Table 4.3).

The improvement of all the bacterial and pathogen counts at this point was due to the extension of the line before evisceration (Table 4.1). This allowed the workers more time to eviscerate the carcasses better. Poor evisceration will lead to contamination of the carcass with intestinal bacteria such as *E. coli*. The workers also received training in meat inspection and the inspection of the carcasses ensured that inadequate carcasses were removed

from the line. The hygiene of the workers' hands was also improved due to more washing points in the factory. This is monitored through visual observation from trained workers.

4.2.3 Mechanical line, after evisceration

The mean total aerobic mesophilic count was significantly ($P<0.05$) reduced after HACCP implementation with counts that decreased from log 7.30 cfu/g to log 6.06 cfu/g (Figure 4.3). Counts after evisceration was found to be as low as log 3.66 cfu/cm^2 (Patterson, 1972) and log 4.28 cfu/g (Mulder & Veerkamp, 1974) and up to log 4.97 cfu/g (Notermans *et al.*, 1975). The counts before and after HACCP implementation was higher than that of the literature.

Before HACCP implementation the mean coliform counts were log 6.13 cfu/g and was significantly ($P<0.05$) reduced to log 4.11 cfu/g after HACCP implementation (Figure 4.3). According to Notermans *et al.* (1975; 1977) the coliform count were found to be log 3.65 cfu/g and up to log 4.2 cfu/g. The coliform counts after HACCP implementation were in the range of the results in the literature.

The mean *E. coli* count before HACCP was log 5.45 cfu/g, which was significantly ($P<0.05$) reduced to log 2.01 cfu/g after implementation (Figure 4.3). Notermans *et al.* (1977) found the mean *E. coli* count to be log 2.9 cfu/g at this sampling point which is higher than the counts found in this study after HACCP implementation.

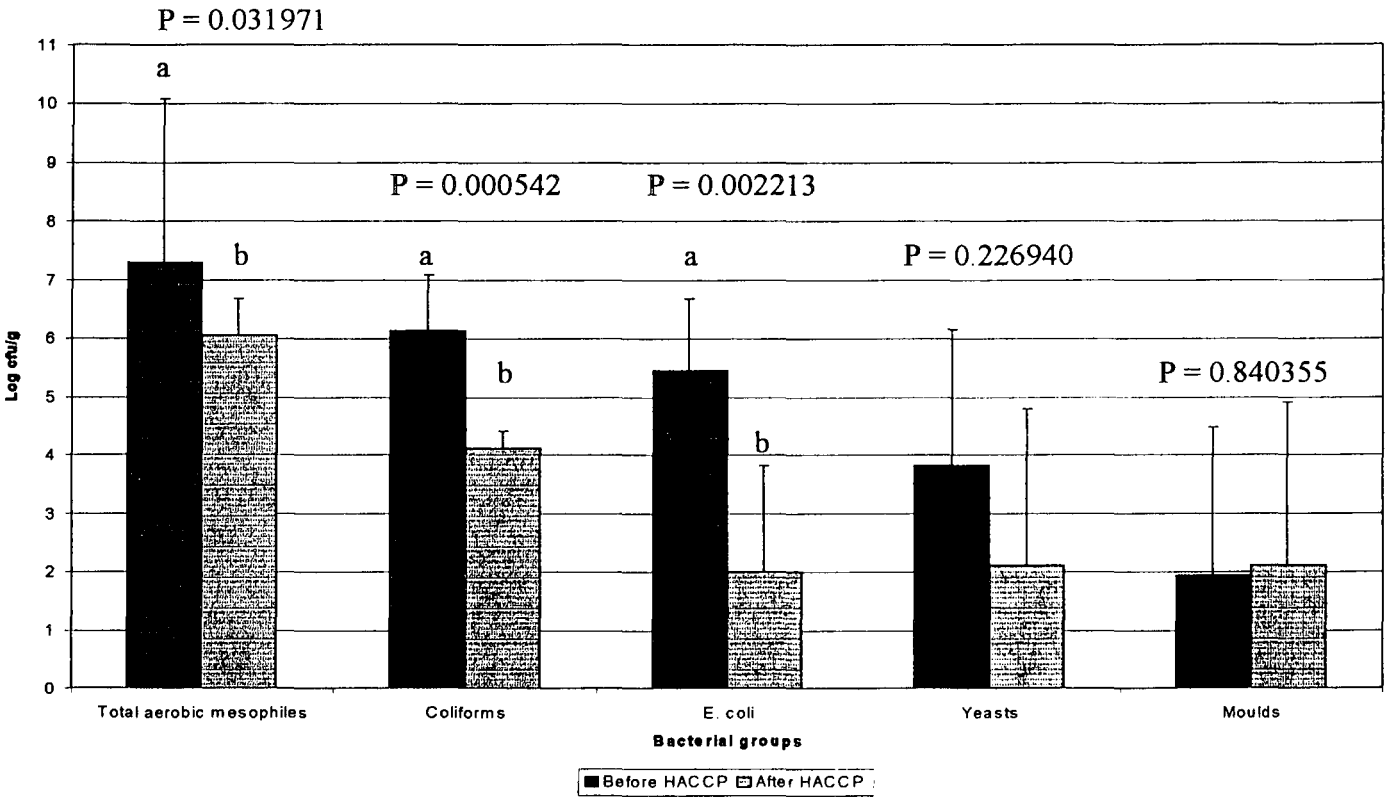

Figure 4.3 : Mean microbiological counts (log cfu/g) of samples, before and after HACCP implementation, from the mechanical line, after evisceration. Bars within a bacterial group with different superscripts are significantly different ($P<0.05$).

The mean yeast count before HACCP was log 3.82 cfu/g. It was, however, not significantly reduced to log 2.11 cfu/g after implementation. The mould counts were log 1.93 cfu/g before HACCP, but was slightly higher after the implementation, log 2.11 cfu/g (Figure 4.3). The increase in the mould count may be due to moulds from the air in the factory and contaminated birds that become resident on the equipment and start growing in and on the

equipment. The carcasses could then be contaminated with spores from the moulds as they passed through the equipment.

Listeria monocytogenes was present on 50% of the carcasses before HACCP implementation at this point, but there was a total reduction after HACCP implementation (Table 4.4). No *Salmonella* species and *Staphylococcus aureus* were present before and after HACCP implementation (Table 4.4). Gibbs *et al.* (1978), however, found that the *S. aureus* counts can be as high as log 5.70 cfu/16cm^2 at this point.

Table 4.4 : The occurrence of pathogenic bacteria in samples, before and after HACCP implementation, from the mechanical line, after evisceration.

Pathogen	Baseline	HACCP	Reduction
Listeria monocytogenes	50% of carcasses	0% of carcasses	50%
Salmonella spp.	0% of carcasses	0% of carcasses	0%
Staphylococcus aureus	0% of carcasses	0% of carcasses	0%

The improvement of all the bacterial and pathogen counts except the mould count was due to the upgrading of the equipment. It eliminated the contamination from the intestines and thus reduced the amount of inadequate eviscerated carcasses. The workers also received training in meat inspection and the increase in inspection sites of the carcasses ensured that inadequate carcasses were removed from the line.

Comparison between the manual and mechanical lines

When the microbial results of the mechanical line after evisceration were compared to the microbial results of the manual line, it was clear that the mechanical line in general had lower counts than the manual line. The main reason for this was due to the fact that only a limited number of workers had contact with the carcasses on the mechanical line. The whole evisceration process of the manual line was done by workers who handled the carcasses which will increase the contamination of the carcasses and lead to increased cross contamination.

4.2.4 Manual line, before spin chilling after spray-washing

Before HACCP implementation the mean count was log 7.21 cfu/g which was significantly ($P<0.05$) reduced to log 5.75 cfu/g after HACCP implementation (Figure 4.4). The total aerobic mesophilic counts were found to range from log 3.46 cfu/g (Mulder & Veerkamp, 1974), log 4.03 cfu/g (Notermans *et al.*, 1975), log 4.2 cfu/ml (Berrang *et al.*, 2000) to log 4.33 cfu/ml (Berrang *et al.*, 2001). Abu-Ruwaida *et al.* (1994) found mean total aerobic mesophilic counts of log 6.15 cfu/g which is higher than the counts found in this study after HACCP implementation.

Mean coliform counts were log 5.94 cfu/g at baseline and was significantly ($P<0.05$) reduced to log 3.68 cfu/g after HACCP implementation (Figure 4.4). Notermans *et al.* (1975) found coliform counts of log 2.09 cfu/g, while Berrang *et al.* (2000) found counts of log 2.9 cfu/ml. Mulder & Veerkamp (1974) found coliform counts of log 2.37 cfu/g and Berrang *et al.* (2001)

found counts of log 2.97 cfu/ml at this sampling point, which is lower than that of this study, but Abu-Ruwaida *et al.* (1994), however, found mean coliform counts as high as log 5.15 cfu/g.

Mean *E. coli* counts were log 4.66 cfu/g before HACCP implementation, but was not significantly reduced to log 2.63 cfu/g after HACCP implementation (Figure 4.4). Berrang *et al.* (2000; 2001) found *E. coli* counts of log 2.2 cfu/ml and 2.57 cfu/ml which is close to the counts found in this study after HACCP implementation, while Abu-Ruwaida *et al.* (1994) found mean counts of log 4.5 cfu/g at this sampling point.

Mean yeast counts were log 4.52 cfu/g before HACCP and significantly ($P<0.05$) reduced to log 1.55 cfu/g after HACCP implementation. Mean mould counts were log 3.95 cfu/g before HACCP implementation, but was eliminated after HACCP implementation (Figure 4.4).

Listeria monocytogenes was present on 66.67% of the carcasses before HACCP, but was totally eliminated after implementation (Table 4.5). No *Salmonella* species were present before and after HACCP implementation (Table 4.5). Abu-Ruwaida *et al.* (1994) found a 100% presence of *Salmonella* at this sampling point. *Staphylococcus aureus* (16.67%) was present before HACCP, but there was a total reduction after HACCP implementation (Table 4.5). *Staphylococcus aureus* can be present at mean counts of log 3.55 cfu/g (Abu-Ruwaida *et al.*, 1994).

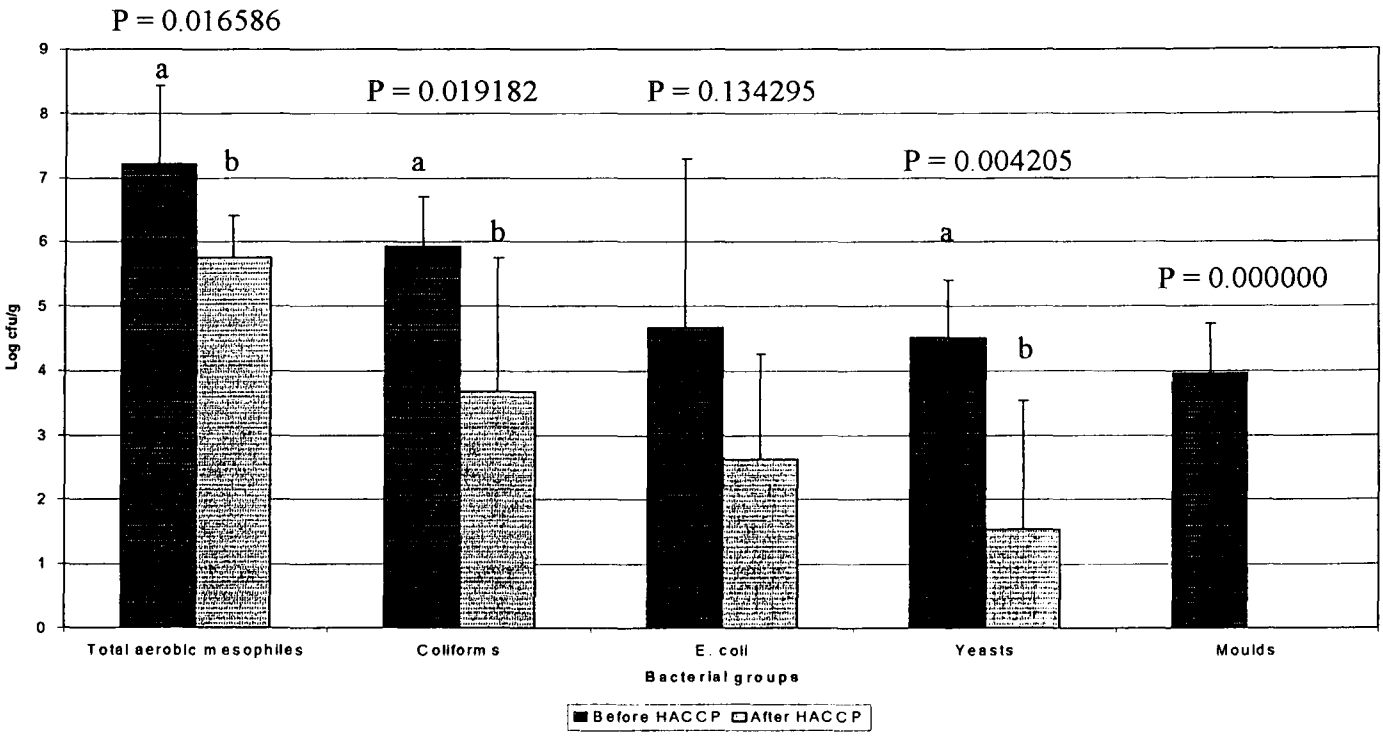

Figure 4.4 : Mean microbiological counts (log cfu/g) of samples, before and after HACCP implementation, from the manual line, before spin chilling after spray-washing. Bars within a bacterial group with different superscripts are significantly different ($P<0.05$).

Table 4.5 : The occurrence of pathogenic bacteria in samples, before and after HACCP implementation, from the manual line, after spray-washing, before spin chilling.

Pathogen	Baseline	HACCP	Reduction
Listeria monocytogenes	66.67% of carcasses	0% of carcasses	66.67%
Salmonella spp.	0% of carcasses	0% of carcasses	0%
Staphylococcus aureus	16.67% of carcasses	0% of carcasses	16.67%

At this point the carcasses had an overall reduction in contamination due to the improvement in the previous steps (Table 4.1). The spray-washing helped to reduce the microbial load on the carcasses which was also improved with better chlorine content control. Abu-Ruwaida *et al.* (1994) found that spray-washing reduced the coliform and *E. coli* counts and the total aerobic mesophilic counts by at least 1 log cycle.

4.2.5 Gizzards

The mean total aerobic mesophilic count before HACCP was log 5.72 cfu/g, which was significantly ($P<0.05$) reduced to log 0.99 cfu/g after HACCP implementation (Table 4.6). The mean coliform count before the implementation of HACCP was log 4.09 cfu/g and after HACCP implementation there was a significant ($P<0.05$) decrease to log 2.25 cfu/g (Table 4.6). The mean *E. coli* count was log 3.06 cfu/g before HACCP, and there was a total elimination after the implementation (Table 4.6).

The mean yeast count was log 2.06 cfu/g, while the mean mould count was log 0.55 cfu/g before HACCP implementation. After HACCP the mean yeast count was not significantly reduced to log 0.58 cfu/g, while the moulds were completely eliminated (Table 4.6).

Table 4.6 : Mean microbiological counts (log cfu/g) of the gizzard samples, before and after HACCP implementation. Means within rows with different superscripts are significantly different ($P < 0.05$).

Micro-organism	Baseline (log cfu/g)	HACCP (log cfu/g)	Reduction[1] (log cfu/g)	P – value
Total aerobic mesophiles	5.72 ±3.29[a]	0.99 ±2.63[b]	4.73	0.009693
Coliforms	4.09 ±1.21[a]	2.25 ±1.40[b]	1.84	0.026562
Escherichia coli	3.06 ±2.10	ND	3.06	
Yeasts	2.06 ±2.58	0.58 ±1.62	1.48	0.210550
Moulds	0.55 ±1.55	ND	0.55	

[1]Reduction = Log cfu/g Baseline – Log cfu/g after HACCP
ND = Not detected
Rows within a bacterial group with different superscripts are significantly different ($P<0.05$)

Listeria monocytogenes was found on 33.33% of the gizzard samples before HACCP, but was eliminated after the implementation (Table 4.7). Waldroup (1996) found that *L. monocytogenes* may be present on 7.9% of the intestines of poultry carcasses. *Salmonella* was found on 16.67% of the gizzard samples, but on none after the implementation of HACCP (Table 4.7). There were no *Staphylococcus aureus* present in any of the samples before and after HACCP implementation (Table 4.7).

Table 4.7 : The occurrence of pathogenic bacteria in gizzard samples, before and after HACCP implementation.

Pathogen	Baseline	HACCP	Reduction
Listeria monocytogenes	33.33% of carcasses	0% of carcasses	33.33%
Salmonella spp.	16.67% of carcasses	0% of carcasses	16.67%
Staphylococcus aureus	0% of carcasses	0% of carcasses	0%

The workers handled the gizzards as they were removed and packed. The reduction in the bacterial counts and pathogens on the gizzards was mainly due to the improvement of the hygiene of the workers. This was achieved through the installation of better hand and shoe washing points before the workers entered the factory and hand washing points in the factory. Workers were educated in the need for good hygiene in order to improve the effectiveness of the hand and shoe washing points.

4.2.6 Hearts

The mean total aerobic mesophilic count before HACCP implementation was log 6.20 cfu/g, which was not significantly reduced to log 4.34 cfu/g after implementation of HACCP (Table 4.8).

The mean coliform count before HACCP was log 5.60 cfu/g and was not significantly reduced after HACCP (Table 4.8). The mean *E. coli* count was log 2.86 cfu/g before HACCP, and also not significantly reduced to log 1.75 cfu/g after the implementation (Table 4.8).

The mean yeast count was log 4.13 cfu/g, while the mean mould count was log 3.15 cfu/g before HACCP implementation (Table 4.8). After HACCP the mean yeast and mould counts were significantly ($P<0.05$) reduced to log 0.69 cfu/g and log 0.58 cfu/g respectively.

Table 4.8 : Mean microbiological counts (log cfu/g) of the heart samples, before and after HACCP implementation. Means within rows with different superscripts are significantly different ($P < 0.05$).

Micro-organism	Baseline (log cfu/g)	HACCP (log cfu/g)	Reduction[1] (log cfu/g)	P – value
Total aerobic mesophiles	6.20 ±3.43	4.34 ±3.64	1.86	0.299292
Coliforms	5.60 ±1.67	4.47 ±0.45	1.13	0.141127
Escherichia coli	2.86 ±2.91	1.75 ±2.26	1.11	0.462008
Yeasts	4.13 ±2.41 [a]	0.69 ±1.91 [b]	3.44	0.012360
Moulds	3.15 ±1.82 [a]	0.58 ±1.62 [b]	2.57	0.014757

[1]Reduction = Log cfu/g Baseline – Log cfu/g after HACCP
Rows within a bacterial group with different superscripts are significantly different ($P<0.05$)

In this study there were no *L. monocytogenes*, *Salmonella* or *S. aureus* present in any of the samples before and after HACCP implementation (Table 4.9). *Listeria monocytogenes* can be present on 7.9% of the intestines of poultry carcasses (Waldroup, 1996).

Table 4.9 : The occurrence of pathogenic bacteria in heart samples, before and after HACCP implementation.

Pathogen	Baseline	HACCP	Reduction
Listeria monocytogenes	0% of carcasses	0% of carcasses	0%
Salmonella spp.	0% of carcasses	0% of carcasses	0%
Staphylococcus aureus	0% of carcasses	0% of carcasses	0%

The reduction in the bacterial counts and pathogens on the heart samples was also due to the improvement of the hygiene of the workers. The hearts were handled by the workers as they were removed and packed. The improvement in hygiene was achieved through the installation of better hand

and shoe washing points before the workers entered the factory and hand washing points in the factory.

4.2.7 Livers

The mean total aerobic mesophilic count before HACCP implementation was log 6.91 cfu/g, which was significantly (P<0.05) reduced to log 5.89 cfu/g after HACCP implementation (Table 4.10).

Table 4.10 : Mean microbiological counts (log cfu/g) of the liver samples, before and after HACCP implementation. Means within rows with different superscripts are significantly different (P < 0.05).

Micro-organism	Baseline (log cfu/g)	HACCP (log cfu/g)	Reduction[1] (log cfu/g)	P – value
Total aerobic mesophiles	6.91 ±1.07 [a]	5.89 ±0.66 [b]	1.02	0.007184
Coliforms	6.00 ±0.97 [a]	4.33 ±0.58 [b]	1.67	0.004907
Escherichia coli	5.40 ±1.31 [a]	0.62 ±1.71 [b]	4.78	0.000137
Yeasts	4.77 ±1.08 [a]	0.50 ±1.43 [b]	4.27	0.000074
Moulds	3.69 ±2.20	ND	3.69	

[1]Reduction = Log cfu/g Baseline – Log cfu/g after HACCP
ND = Not detected
Rows within a bacterial group with different superscripts are significantly different (P<0.05)

The mean coliform count before HACCP implementation was log 6.00 cfu/g and was significantly (P<0.05) reduced to log 4.33 cfu/g after HACCP implementation (Table 4.10). The mean *E. coli* count before HACCP implementation was log 5.40 cfu/g, and after the implementation it was significantly (P<0.05) reduced to log 0.62 cfu/g (Table 4.10).

The mean yeast count was log 4.77 cfu/g, while the mean mould count was log 3.69 cfu/g. After HACCP the mean yeast count was significantly ($P<0.05$) reduced to log 0.50 cfu/g, while the moulds were eliminated (Table 4.10).

There were no *L. monocytogenes* present in any of the samples before and after HACCP implementation (Table 4.11). Waldroup (1996) found that *L. monocytogenes* can be present on 33.3% of the livers of carcasses. There were no *Salmonella* or *S. aureus* present in any of the samples before and after HACCP implementation (Table 4.11). Waldroup (1996) found that *Salmonella* can be present on 21 to 82.5% of the liver samples of poultry carcasses.

Table 4.11 : The occurrence of pathogenic bacteria in livers samples, before and after HACCP implementation.

Pathogen	Baseline	HACCP	Reduction
Listeria monocytogenes	0% of carcasses	0% of carcasses	0%
Salmonella spp.	0% of carcasses	0% of carcasses	0%
Staphylococcus aureus	0% of carcasses	0% of carcasses	0%

The reduction in the bacterial counts and absence of pathogens on the liver samples was as a result of the improvement of the hygiene of the workers. When the livers were removed and packed, they were handled by the workers. By improving the hygiene of the workers through the installation of better hand and shoe washing points before the workers entered the factory and hand washing points in the factory, microbial quality of the livers have improved.

4.2.8 Portions

The mean total aerobic mesophilic count before HACCP implementation was log 5.61 cfu/g, which was significantly ($P<0.05$) reduced to log 0.83 cfu/g after HACCP implementation (Table 4.12). Berrang *et al.* (2001) found the mean total aerobic mesophilic count to be log 5.07 cfu/ml while Abu-Ruwaida *et al.* (1994) found mean counts as high as log 6.45 cfu/g which is much higher than the counts found in this study after HACCP implementation.

Table 4.12 : Mean microbiological counts (log cfu/g) of the portion samples, before and after HACCP implementation. Means within rows with different superscripts are significantly different ($P < 0.05$).

Micro-organism	Baseline (log cfu/g)	HACCP (log cfu/g)	Reduction[1] (log cfu/g)	P – value
Total aerobic mesophiles	5.61 ±3.22 [a]	0.83 ±2.25 [b]	4.78	0.005588
Coliforms	5.25 ±0.97 [a]	3.80 ±0.58 [b]	1.45	0.012732
Escherichia coli	3.77 ±1.27	2.64 ±1.70	1.13	0.215852
Yeasts	4.08 ±2.51	2.43 ±2.24	1.65	0.224819
Moulds	2.85 ±2.51	0.55 ±1.55	2.30	0.058802

[1]Reduction = Log cfu/g Baseline – Log cfu/g after HACCP
Rows within a bacterial group with different superscripts are significantly different ($P<0.05$)

The mean coliform count before HACCP was log 5.25 cfu/g and after HACCP it was significantly ($P<0.05$) reduced to log 3.80 cfu/g (Table 4.12). Berrang *et al.* (2001) found the mean coliform count to be log 2.43 cfu/ml, but Abu-Ruwaida *et al.* (1994) found mean coliform counts of log 4.9 cfu/g. The coliform counts after HACCP implementation was in the range of that found in the literature.

The mean *E. coli* count was log 3.77 cfu/g before HACCP, and there was a non-significant reduction to log 2.64 cfu/g after the implementation (Table 4.12). Berrang *et al.* (2001) found the mean *E. coli* count was log 2 cfu/ml, while Abu-Ruwaida *et al.* (1994) found mean counts of log 3.95 cfu/g at this sampling point. The *E. coli* counts recorded in this study after HACCP implementation fell between that of the literature. Doyle & Schoeni (1987) found *E. coli* on 1.5% of the samples and Bok *et al.* (1986) found *E. coli* on 6.85% of the samples.

The mean yeast count was log 4.08 cfu/g, while the mean mould count was log 2.85 cfu/g. After HACCP implementation there was a non-significant reduction of the mean yeast count to log 2.43 cfu/g, and the mould count to log 0.55 cfu/g (Table 4.12).

Listeria monocytogenes was present on 50% of the samples before HACCP implementation, but there was a total reduction after the implementation (Table 4.13). *Listeria monocytogenes* can be present on 2.1 to 94% of the carcasses after packaging (Waldroup, 1996).

Table 4.13 : The occurrence of pathogenic bacteria in portion samples, before and after HACCP implementation.

Pathogen	Baseline	HACCP	Reduction
Listeria monocytogenes	50% of carcasses	0% of carcasses	50%
Salmonella spp.	0% of carcasses	0% of carcasses	0%
Staphylococcus aureus	16.67% of carcasses	0% of carcasses	16.67%

In this study, there were no *Salmonella* present in any of the samples before and after HACCP implementation (Table 4.13). Bailey (1993) found that *Salmonella* counts can be as low as <100 cfu/g. Bok *et al.* (1986) found *Salmonella* on 21.18% of the carcasses tested, while Jones *et al.* (1991) found *Salmonella* on 21.4% of the carcasses. Dougherty (1974) found *Salmonella* on 47 to 90% of the carcasses tested while Abu-Ruwaida *et al.* (1994) found *Salmonella* on 100% of the carcasses tested.

Staphylococcus aureus was found on 16.67% of the samples before HACCP implementation, but it was eliminated after the implementation (Table 4.13). Mead & Dodd (1990) found *S. aureus* to be present in 88% of the carcasses tested, but 84.2% of the carcasses had counts of log <3 cfu/16 cm^2, while 0.5% had counts of log >4 cfu/16 cm^2. Gibbs *et al.* (1978) found counts to be from log 3.11 cfu/16 cm^2 to log 5.04 cfu/16 cm^2 while Abu-Ruwaida *et al.* (1994) found mean counts of log 3.4 cfu/g.

The reduction of bacterial counts and elimination of pathogens was achieved by improving the hygiene of workers and improvement of packaging material. The replacement of plastic wrapping with plastic bags reduced the contact between the workers and the product, therefore reducing the contamination. The installation of a sterilizer for the equipment and gloves of the workers also improved the microbial quality of the product.

4.2.9 Hands

The mean total aerobic mesophilic count before HACCP implementation was log 4.90 cfu/cm^2, which was eliminated after HACCP implementation (Table 4.14).

Table 4.14 : Mean microbiological counts (log cfu/cm^2) of the hand swab samples, before and after HACCP implementation. Means within rows with different superscripts are significantly different ($P < 0.05$).

Micro-organism	Baseline (log cfu/cm^2)	HACCP (log cfu/cm^2)	Reduction[1] (log cfu/cm^2)	P – value
Total aerobic mesophiles	4.90 ±2.62	ND	4.90	
Coliforms	2.80 ±2.48	1.58 ±2.11	1.22	0.348245
Escherichia coli	1.78 ±2.28	1.01 ±1.96	0.77	0.529306
Yeasts	2.64 ±2.43	0.50 ±1.43	2.14	0.059328
Moulds	2.05 ±1.85	ND	2.05	

[1]Reduction = Log cfu/g Baseline – Log cfu/g after HACCP
ND = Not detected

The mean coliform count before HACCP implementation was log 2.80 cfu/cm^2 but was not significantly reduced after HACCP implementation (Table 4.14). The mean *E. coli* count before HACCP implementation was log 1.78 cfu/cm^2, and was also not significantly reduced after the implementation (Table 4.14).

The mean yeast count was log 2.64 cfu/cm^2, while the mean mould count was log 2.05 cfu/cm^2 before HACCP implementation. After HACCP the

mean yeast count was not significantly reduced to log 0.50 cfu/cm^2, while the moulds were eliminated (Table 4.14).

There were no *L. monocytogenes*, *Salmonella* or *S. aureus* present in any of the samples before and after HACCP implementation (Table 4.15). Gibbs *et al.* (1978), however, found that the *S. aureus* counts on hands can range from log <2 cfu/16 cm^2 to log 6.48 cfu/16 cm^2.

Table 4.15 : The occurrence of pathogenic bacteria on hand swab samples, before and after HACCP implementation.

Pathogen	Baseline	HACCP	Reduction
Listeria monocytogenes	0% of carcasses	0% of carcasses	0%
Salmonella spp.	0% of carcasses	0% of carcasses	0%
Staphylococcus aureus	0% of carcasses	0% of carcasses	0%

The reduction of bacteria and absence of pathogens on the hands of workers was due to the installation of improved hand and shoe washing points for the workers before they entered the factory as well as hand washing points in the factory. The improvement of the drainage of water also improved the hygiene of the factory floor and thus the hygiene of the workers.

4.2.10 Packaging material

Before HACCP was implemented, the mean total aerobic mesophilic counts were log 1.92 cfu/cm^2, and after implementation there was a total

elimination (Table 4.16). Abu-Ruwaida *et al.* (1994) found mean total aerobic mesophilic counts of log 0.6 cfu/cm^2 at this sampling point.

Table 4.16 : Mean microbiological counts (log cfu/cm^2) of the packaging material swab samples, before and after HACCP implementation. Means within rows with different superscripts are significantly different ($P < 0.05$).

Micro-organism	Baseline (log cfu/cm^2)	HACCP (log cfu/cm^2)	Reduction[1] (log cfu/cm^2)	P – value
Total aerobic mesophiles	1.92 ±3.33	ND	1.92	
Coliforms	0.91 ±1.80	0.90 ±1.65	0.01	0.963676
Escherichia coli	0.33 ±1.02	0.48 ±1.39	-0.15	0.834949
Yeasts	ND	ND	0	0
Moulds	ND	ND	0	0

[1]Reduction = Log cfu/g Baseline – Log cfu/g after HACCP
ND = Not detected

The mean coliform count before HACCP implementation was log 0.91 cfu/cm^2, which was not significantly reduced to log 0.90 cfu/cm^2 after HACCP implementation (Table 4.16). The baseline counts for *E. coli* were log 0.33 cfu/cm^2, and after HACCP implementation there was a slight non-significant increase to log 0.48 cfu/cm^2 (Table 4.16). The main cause may be due to contamination of the packaging material from workers. This happens when there is inadequate handling of the packaging material by the workers and inadequate storage.

There were no yeasts and moulds found before or after HACCP implementation (Table 4.16).

There were no *Listeria monocytogenes* or *Staphylococcus aureus* present before or after HACCP implementation (Table 4.17). *Salmonella* species were found on 16.67% of the samples before HACCP, but not on any samples after the implementation (Table 4.17).

Table 4.17 : The occurrence of pathogenic bacteria on packaging material swab samples, before and after HACCP implementation.

Pathogen	Baseline	HACCP	Reduction
Listeria monocytogenes	0% of carcasses	0% of carcasses	0%
Salmonella spp.	16.67% of carcasses	0% of carcasses	16.67%
Staphylococcus aureus	0% of carcasses	0% of carcasses	0%

As a result of improved hygiene of the workers and improved packaging material from plastic wrapping to plastic bags, there was a slight improvement in most of the bacterial counts and elimination of pathogenic organisms. Care should, however, be taken in contamination of the packaging material by workers and inadequate storage.

4.2.11 Conveyor belts

4.2.11.1 Hearts , gizzards and livers conveyer belt

Total aerobic mesophilic and coliform counts taken with contact plates before HACCP was implemented, was completely covered in bacterial growth. After HACCP implementation, the coliform plates were still completely covered in growth. Two of the six total aerobic mesophilic plates

had counts of log 0.95 cfu/cm^2 and 1.08 cfu/cm^2, but the rest were completely covered in growth.

4.2.11.2 Whole chicken conveyer belt

All the total aerobic mesophilic and coliform plates were completely covered in growth before and after HACCP implementation.

4.2.11.3 Quick frozen portions conveyer belt

Before HACCP, two coliform contact plates had counts of 0 and one had counts of log 1 cfu/cm^2. The rest of the coliform contact plates and total aerobic mesophilic contact plates were completely covered in growth. After HACCP implementation, all the plates were covered in bacterial growth.

4.2.11.4 Portions conveyer belts

Before HACCP was implemented, three of the coliform contact plates had counts of log 0.30 cfu/cm^2, log 0.48 cfu/cm^2 and log 0.85 cfu/cm^2, but the rest and all the total aerobic mesophilic plates were completely covered in growth. After HACCP implementation, four of the coliform contact plates had counts of 0, log 1 cfu/cm^2, log 0.3 cfu/cm^2 and 0.48 cfu/cm^2. Five of the total aerobic mesophilic contact plates had counts of 0, log 0.90 cfu/cm^2, log 1.04 cfu/cm^2, log 1.11 cfu/cm^2 and log 1.18 cfu/cm^2.

The reason that there was no reduction in the bacterial counts on the conveyor belts was due to the fact that the current conveyor belts were made

from plastic, they were old and not in a good condition. Some had cracks and torn edges that may harbour bacterial growth. There was no system in place that cleaned the conveyor belts, but future plans are to replace the plastic conveyor belts with interlink metal conveyor belts. The metal belts will then last longer and will be easier to clean. This will reduce the bacterial counts on the conveyor belts.

Alternative method that can be used in the future is the agar sausage in order to achieve better counts.

CHAPTER 5

CONCLUSIONS

The processing of poultry plays an important role in the quality of the final product. During processing, there are various factors that have an influence on the microbial profile of the product. It became clear in this study that the most important factors in maintaining a good quality product are the equipment, the hygiene and the training of the personnel. Training was measured by means of courses that the workers had to pass.

Contamination of the broilers with pathogens start at the hatching of the chicks. As a result of this it is virtually impossible to maintain a breeder flock free of pathogens. This means that there will always be some broilers which are contaminated before they enter the factory. Every step should, therefore, be taken to try and reduce or eliminate any bacteria on the carcasses as they go through the different processing steps in order to prevent cross-contamination between carcasses and contamination of the equipment.

This is where the HACCP system plays an important role. The HACCP system was designed to identify all the points in the factory where a hazard could occur. The HACCP system then helps to identify possible preventative measures that will reduce or eliminate the bacterial problem at that specific stage in the processing line.

The effect of HACCP implementation in the poultry factory in this study had the following effects on the microbial and pathogen load:

1. At the mechanical line, after defeathering there was an overall reduction in the microbiological counts. There was also a total elimination of pathogens found at this point.

2. At the manual line after evisceration, all the microbiological counts were lowered after HACCP implementation. All the pathogens that were found at this point were eliminated.

3. The microbiological counts at the mechanical line after evisceration were lowered except for a slight increase in the mould counts. There was a total elimination of all the pathogens.

4. The microbiological counts at the manual line, after spray-washing before spin chilling were lowered except for the mould counts that were totally eliminated. All the pathogens were eliminated after HACCP implementation.

5. The microbiological counts of the gizzard samples were lowered except for the mould counts that were totally eliminated. All the pathogens were eliminated at this sampling point.

6. There was an overall reduction in the microbiological counts of the heart samples and no pathogens were found.

7. There was an overall reduction of all the microbiological counts and elimination of mould counts of the liver samples and no pathogens were found.

8. All the microbiological counts of the portion samples were reduced and all the pathogens were eliminated.

9. There was an overall reduction of all the microbiological counts and elimination of total aerobic mesophilic and mould counts of the hand swab samples and no pathogens were found.

10. With the packaging swab samples there was a reduction in the coliform counts and elimination in the total aerobic mesophilic, yeast and mould counts. There was a slight increase in the *E. coli* counts but a total reduction of all the pathogens.

11. With the conveyor belts most of the rodac plates were overgrown with bacterial growth. This lead to inconsistent counts where possible and this shows the need for more attention at these points and the improvement of the equipment and cleaning techniques.

These findings could be ascribed to the following changes brought about by HACCP implementation in the poultry factory:

1. Better bleeding of the carcasses before scalding and defeathering by extending the bleeding time.

2. More inspection points by trained personnel and extension of the shackle line at the manual evisceration line to give workers more time to better eviscerate the carcasses.
3. Second inspection point by trained personnel and upgraded equipment that work better at the mechanical evisceration line.
4. Installation of a recovery section for carcasses that were inadequately eviscerated or became contaminated by other means.
5. Visual inspection by trained personnel of both manual and mechanical carcasses after spin chilling.
6. Installation of a mechanical carcass cutting machine before packaging that reduces contamination from workers.
7. Replacement of plastic wrapping with plastic bags to reduce worker contact with product.
8. Installation of sterilization equipment for workers' gloves and equipment.
9. Improvement of control over chlorine content in water supply.
10. Training of workers and installation of more and better washing points in and around the factory.
11. Improvement of factory drainage to remove excess water from the factory floor.

In conclusion it can be said that there was thus a definite improvement of the microbial profile in the poultry factory after HACCP was implemented. The fact that there are still bacteria present on the carcasses is not a sign that the HACCP system is not working as it is impossible to eliminate all the bacteria from the processing line and product.

The most important change that has to be made in the factory is the replacement of the current plastic conveyor belts with metal interlink conveyor belts and the installation of a cleaning system that will continuously clean the conveyor belt as it is in use. This will improve the counts on the conveyor belts as the metal belts will be easier to clean and will not crack and torn and thus harbour bacteria. Another improvement that can be made is the installation of more spray-washing points along the processing line. This will ensure an even higher reduction in microbial load on the carcasses and equipment. It will also reduce the intestinal contamination of the carcasses before they enter the spin chiller where cross-contamination could occur. These recommendations are made to the abattoir in this study and may differ to that of other abattoirs as no two abattoirs are the same.

Future research may include analyzing the presence of other pathogens such as *Campylobacter*, *Yersinia enterocolitica*, *Clostridium perfringens*, *Aeromonas hydrophila* and *Shigella* spp.

Since *E. coli* was still present after HACCP implementation, and the medium employed in this study could not detect *E. coli* O157:H7 presence, research could be aimed at these new emerging pathogens and the groups they belong to.

CHAPTER 6

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CHAPTER 7

SUMMARY

As the demand for poultry meat increases, so does the awareness of its potential to contain pathogens and other spoilage bacteria. Due to the nature of the product and the processing involved in the transformation of live birds into carcasses and portions, it is easy for the product to become contaminated.

The aim of the study was to determine the microbial profile of the factory before and after Hazard Analysis Critical Control Point (HACCP) implementation. Microbial analysis (total aerobic mesophilic count, coliform and *Escherichia coli* count, yeast and mould counts, *Listeria monocytogenes* presence, *Salmonella* presence and *Staphylococcus aureus* presence) were performed on the following places and products in the factory: mechanical line after defeathering, manual line after evisceration, mechanical line after evisceration, manual line after spray-washing but before spin chilling, gizzards, hearts, livers, portions, hand swabs, packaging material and conveyor belts.

Before HACCP implementation, most of the bacterial counts found in the study was higher than that of the literature. There was, however, a marked reduction in the microbial profile of the carcasses after HACCP implementation. All of the bacterial counts were lowered except for the following: mould counts from the mechanical line after evisceration, *E. coli*

count from the packaging material, total aerobic mesophilic and coliform counts from the conveyor belts. All the pathogens that were tested for were eliminated from the carcasses and other places and products. There was thus an improvement in the microbiological quality of the product. The only section that needed improvement was the conveyor belts as the HACCP system did not reduce or eliminate bacterial or pathogen counts on them.

Even though the amount of contamination in the factory was reduced, microbial loads will only be further improved if the broilers that arrive at the processing plant have low microbial contamination levels. The breeder farms should establish a HACCP system to reduce the initial contamination of the flock, in order to decrease the degree of contamination at the factory.

The conclusion was drawn that HACCP implementation at this poultry factory was very effective in reducing the microbial load and eliminated the presence of *Salmonella*, *S. aureus* and *L. monocytogenes*.

Key words: poultry, pathogens, spoilage bacteria, microbial profile, Hazard Analysis Critical Control Point (HACCP), quality, contamination.

OPSOMMING

Soos die aanvraag na pluimvee vleis toeneem, so ook is daar 'n toename in die bewuswording dat dit potensiële patogene en ander bederf bakteriëe kan bevat. As gevolg van die aard van die produk en prossesering betrokke in die transformasie vanaf lewendige hoender na karkasse en porsies, kan die produk maklik gekontamineer te word.

Die doel van hierdie studie was om die mikrobiese profiel van die fabriek voor en na "Hazard Analysis Critical Control Point (HACCP)" implementering te bepaal. Die mikrobiese analiese (totale aerobiese mesofiliese telling, kolivorm en *Escherichia coli* telling, giste en skimmel tellings, *Listeria monocytogenes* teenwoordigheid, *Salmonella* teenwoordigheid en *Staphylococcus aureus* teenwoordigheid) is uitgevoer op die volgende plekke en produkte in die fabriek: meganiese lyn na ontvering, hand-lyn na ontweiding, meganiese lyn na ontweiding, hand lyn na sproei-was, maar voor spin verkoeling, magies, harte, lewers, porsies, hande van personeel, verpakkingsmateriaal en vervoerbande.

Voor die implementering van "HACCP", was die meeste van die bakteriese tellings van die studie hoër as die waardes gevind in die literatuur. Hierdie studie het egter aangetoon dat daar 'n merkbare afname in die mikrobiese profiel van die karkasse was na "HACCP" implementering. Al die bakteriese tellings het gedaal behalwe vir die volgende: skimmel tellings van die meganiese lyn na ontweiding, *E. coli* telling vanaf die verpakkingsmateriaal, totale aerobies mesofiliese en kolivorme tellings vanaf die vervoerbande.

Die patogene waarvoor daar getoets is was uitgeskakel vanaf die karkasse en ander plekke en produkte. Daar was dus 'n definitiewe verbetering in die kwaliteit van die produk. Die enigste afdeling wat verbetering benodig is die vervoerbande, aangesien die huidige sisteem nie 'n vermindering of eliminering van bakteriese of patogeen tellings tot gevolg gehad het nie.

Alhoewel die vlak van kontaminasie in die fabriek verminder is, sal die mikrobiese las slegs verder verbeter as die hoenders wat by die prosseserings aanleg arriveer lae mikrobiese kontaminasie vlakke besit. Die broeiplase moet 'n "HACCP" sisteem vestig om die aanvanklike kontaminasie van die pluimvee te verminder om sodoende die graad van kontaminasie by die fabriek te verminder.

Die gevolgtrekking was dat "HACCP" implementasie by hierdie pluimvee fabriek baie effektief was in die vermindering van die mikrobiese lading en dat dit die teenwoordigheid van *Salmonella*, *S. aureus* en *L. monocytogenes* uitgeskakel het.

Sleutelwoorde: pluimvee, patogene, bederf-bakteriële, mikrobiologiese profiel, "Hazard Analysis Critical Control Point (HACCP)", kwaliteit, kontaminasie.