THE MICROBIAL QUALITY OF OSTRICH CARCASES PRODUCED AT AN EXPORT-APPROVED SOUTH AFRICAN ABATTOIR

By

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to my parents who have always believed

in the importance of education

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SUMMARY

THE MICROBIAL QUALITY OF OSTRICH CARCASES PRODUCED IN A EXPORT-APPROVED SOUTH AFRICAN ABATTOIR

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The aim of this study was to evaluate the microbial quality of ostrich carcases produced in a South African export-approved ostrich abattoir. Ninety surface samples were collected on 30 ostrich carcases at three processing points in the abattoir: post-flaying, post-evisceration and post-chilling. Carcase samples were evaluated for the Aerobic Plate Count (APC), *Pseudomonas* spp., Enterobacteriaceae, *Staphylococcus aureus* and for the presence of *Escherichia coli* and presumptive *Salmonella* spp. One hundred isolates obtained from the APC were identified.

The mean log CFU/cm^2 and standard deviations for surface counts at post-flaying, post-evisceration and post-chilling processing points respectively were: 4.32 ±0.62, 4.21 ±0.63 and 4.57 ±0.48 for the APC; 2.82 ±1.65, 2.86 ±1.53 and 3.75

±0.94 for *Pseudomonas* spp.; 2.89 ±0.78, 2.90 ±0.53 and 2.38 ±0.67 for *S. aureus* and 2.55 ±1.53, 2.78 ±1.31 and 2.73 ±1.46 for Enterobacteriaceae.

No significant differences were detected between the mean log counts of the post-flaying and post-evisceration processing points for the above-mentioned bacterial counts. However, statistically significant differences were detected between the mean log CFU/cm^2 counts for post-flaying and post-chilling and between the counts for the post-evisceration and the post-chilling processing points for the APC, *Pseudomonas* spp. and *S. aureus*. The trend was towards a marginal increase for the APC, and a negligible decrease for *S. aureus* counts obtained on samples collected post-chilling. However, there was an increase of practical significance for *Pseudomonas* spp. counts obtained post-chilling.

Seventeen out of 90 (18.8%) samples were positive for *E. coli* in terms of samples collected and 13 out of 30 (43%) in terms of carcases sampled. Log CFU/cm^2 counts for *E. coli* positive samples ranged from 1.0 to 3.79, with a mean log count of 2.15. Most of the samples, which were positive for *E. coli* were collected post-evisceration. The prevalence rate for presumptive *Salmonella* spp. on both Brilliant Green Agar and Xylose Lysine Desoxycolate Agar was 15.5% in terms of samples collected and 23.3% in terms of carcases sampled. Most of the positive samples were collected post-evisceration.

The proportional distribution of one hundred (100) bacterial isolates identified was Enterobacteriaceae: 57%, *Acinetobacter* spp.: 24 %, *Pseudomonas* spp.: 11%, *Aeromonas* spp.: 3%, *Micrococcus* spp.: 3%, St*aphylococcus* spp.: 1% and yeasts: 1%. Enterobacteriaceae were the predominant bacteria in terms of the total number of isolates identified per processing point and for the whole study.

CHAPTER 1

INTRODUCTION AND LITERATURE REVIEW

BACKGROUND

Ostrich farming in South Africa today plays a minor role in agriculture, but in earlier years it played a major part in the economy of certain regions of the country. At the peak of ostrich farming in South Africa (1913), there must have been at least one million birds being farmed. Ostrich feather was ranked fourth in value after gold, diamonds and wool, on the list of exports from the then Union of South Africa. The feather market collapsed at the onset of World War I (Smit 1963, Osterhoff 1979, Bertram 1992).

The natural home of the ostrich is Africa. Keeping ostriches has a long history dating back to the Egyptian, Babylonian, Greek and Roman empires. The Sahara desert contained many ostriches and was used as a hunting ground. Ostriches also inhabited Palestine, Persia and the Arabian Desert. Large numbers of ostriches were exported from Africa in the latter half of 19th century to Australia, New Zealand, Europe, North and South America (Osterhoff 1979, Bertram 1992).

The ostriches farmed in Southern Africa differ from wild ostriches. The differences result from selective breeding for 100 years, from the sub-species *Struthio camelus australis* Guerney and *Struthio camelus camelus* Linnaeus selected on the basis of their size, live weight gain, carcase weight and quality of feathers and leather. Farmed ostriches are also called *Struthio camelus var. domesticus*. Their body weight is 30 - 40 kg less than the weight of wild ostriches (which can weigh up to 150 kg at an adult age) and their legs are shorter, but the feather quality is much better. Ostriches have a life span of 30 - 70 years (Hallam 1992, Hildebrandt & Raucher 1999).

Wild ostriches are very unmanageable. The first ostriches were only tamed by about 1863. It seems that a few farmers in the Karoo and Eastern Cape started this branch of agriculture at approximately the same time (Osterhoff 1979).

The main products obtained from ostriches are plumes (feathers), ostrich skin and a variety of meat products, for example, the liver, the heart and fresh meat (steaks and roasts), processed meats (sausage, ham-type products, salami and biltong) and health care products (ostrich fat) (Jones *et al*. 1997).

Feathers are used in the household and motorcar industries as feather dusters. They are also used in the fashion industry as feather fans and capes, artificial flowers, feather-trimmed hats and frocks. Emptied, cleaned and carved, unhatched eggs are commercialised for the tourist industry.

The leather of ostriches is the most valuable product. Leather is imported by countries that are orientated towards the fashion industry. These countries buy tanned skins from South Africa and process them into handbags, purses, briefcases, footwear, belts, upholstery, and jackets. Approximately 1.3 m^2 of leather is produced by a 12 - 14 month old bird (Hastings 1991).

According to Odendaal (2000), ostriches are being explored for medical and medicinal purposes. The tendons of the ostrich leg are used to replace torn tendons in humans, as they are long and strong enough for the human leg. Recent research in ophthalmology points to the possible use of ostrich eyes in corneal transplants. Furthermore, the ostrich brain produces a substance that is being studied for the treatment of Alzheimer's disease and other types of dementia.

Although ostriches are poultry, the pH of their flesh is similar to that of beef. Therefore, some classify ostriches as "red meat". In ostriches, there is no breast meat (no white meat). The bulk of the meat is obtained from the leg and thigh (Anonymous 1996). With regard to the nutrient profile of cooked lean meat from

ostrich carcases, ostrich meat is low in fat (0.5%). The cholesterol content of raw ostrich meat is 62 mg/100 g, which compares favourably with that of chicken (with skin) at 98 mg/100 g. In view of the trend towards the consumption of lean meat, this should make ostrich meat suitable for the health-conscious consumer. The iron content of ostrich meat is closer to that found in beef rather than that found in cooked lean meat from chickens. This is one reason why ostrich meat is more red in appearance than conventional poultry meat (Kuhne 1977, Harris *et al*. 1993, Pollok *et al*. 1997).

In South Africa, ostriches are slaughtered mainly for the export market. In 1993, income generated from ostrich meat was 31.4 million rands. The total income from all ostrich products combined (leather, feathers and meat) was 189.9 million rands in the same year. In 1995, about 170 000 ostriches were slaughtered in South Africa at six European Union approved abattoirs. Calculations were that the rest of the world was slaughtering approximately 15 000 - 20 000 ostriches (Mellet 1995, Van Zyl 1996).

Ostrich meat, once only served locally in the production area in South Africa (fresh and biltong), has long been served in gourmet restaurants in Europe. Demand is growing in the Pacific Rim countries and in the United States (Anonymous 1997a).

MICROBIAL QUALITY OF MEAT

Post mortem meat inspection has been designed to ensure that meat and meat products entering the human food chain are safe, sound and wholesome. However, it is clear that post mortem meat inspection does not deal adequately with the problem of microbial contamination of meat during the slaughter process, and its consequences for human food-borne diseases (Hathaway & McKenzie 1991, Hudson *et al*. 1996).

Meat quality is dependent on the entire meat production chain from the farm where animals are conceived to the consumer. It covers sensory and microbiological properties (colour, tenderness, smell, taste, microbial load and shelf-life) (Monin & Ouali 1991).

Many of the procedures involved in stages of breeding and fattening meat animals to processing them into meat for the table, serve to spread the micro-organisms from animal to animal and from carcase to carcase. The spread of contamination can be divided into several stages: on the farm, during transport and holding prior to slaughter, during slaughter and post-slaughter (Roberts 1982).

The level of contamination of the carcase depends on the cleanliness of the animal before slaughter, the number of bacteria introduced during slaughter and processing, as well as the temperature, the time and the conditions of storage and distribution (Nortje *et al*. 1990b, Grau 1979).

PRE-HARVEST SOURCES OF MEAT CONTAMINATION

On the farm, heavy soil and poor drainage often result in animals arriving at the abattoir with muddy feet and abdomens, thus the state of the animal at slaughter is important. Dirty skins provide major sources of microbial contamination for the carcase. Soiling can be influenced by many factors including the prevalence of

diarrhoea in animals, climatic conditions on the farm and the length of time spent in the lairage. The design of transport trucks and abattoir lairages can also make a significant contribution to the level of soiling (Magraph & Patterson 1969, Nottingham 1982, Edwards *et al*. 1997, Hadley *et al*. 1997).

Concerning the design of ostrich lairages, it has been observed that ostriches penned on cement or tiles are restless and defecate readily when compared to those penned on sand. Cement or tiled flooring become wet and soiled and when ostriches lie down, expensive body feathers are soiled with faeces and urine. On the other hand, ostriches penned on sand are less restless and defecate less. Another advantage of sand is that the urine drains away in the sand, keeping the surface dry, so that when ostriches lie down their feathers are less soiled (Burger *et al*. 1995).

A study done by Burger *et al*. (1995), concerning the microbial assessment of two methods of ostrich lairage, on sand and cement, found that penning ostriches on clean river sand had to be well-managed by adhering to strict management procedures. The physical condition of the sand had to be efficiently monitored by keeping it well drained, raked at least once a day and kept dry at all times to prevent soilage of birds while lying down.

Animals from feedlots frequently carry variable amounts of manure, bedding and soil on their skins when they enter the abattoir. Mud, bedding and manure adheres to the skin of the animal and may contribute to microbiological contamination of carcases during skin removal. Microbial contamination from the skin normally includes staphylococci, micrococci, pseudomonads, yeasts and moulds. Skins may also carry as many as log 9 bacteria of soil or faecal origin per cm^2 of skin. Mud and faeces may contain food-borne pathogens like *E. coli*, *Clostridium perfringens* and *Salmonella* spp. (Ayres 1955, Reed 1996, Van Donkersgoed *et al*. 1997).

Stress before slaughter also contributes to meat contamination in the live animal. Transport stress may lead to increased frequency of defecation and discharge of caecal contents resulting in shedding of bacteria in the faeces, with increased risk of contamination of hides and subsequently of carcase meat (Mead 1982).

Meat from animals which have undergone prolonged muscular activity or stress before slaughter, with consequent depletion of glycogen reserves in muscles, undergoes spoilage at low cell (bacterial) densities (10^6/cm²). This meat contains little or no glycogen and, therefore, spoilage bacteria growing on such meat, immediately attack amino acids, so that spoilage odours and ammonia are detected (Nortje *et al*. 1985).

Pre-slaughter handling of animals influences to a large extent the rate of pH decline in the muscles after slaughter. According to Sales & Mellet (1996), the mean ultimate pH of ostrich muscles suggest that ostrich meat may be classified as an intermediate type between normal (pH <5,8) and extreme Dark Firm Dry meat (pH >6.2). Dark Firm Dry is a condition normally associated with pre-slaughter stress. It occurs mostly in beef, if muscle glycogen reserves are depleted before slaughter, with subsequent production of meat with a low shelf-life (Gill 1986, Lawrie 1990, Gracey & Collins 1992).

Symptomless carriers of pathogenic infections are also of particular significance in meat contamination. In symptomless carriers, the pathogens are generally found in the gastrointestinal tract, but they may also be confined to the mesenteric lymph nodes and the gallbladder (Brown & Baird-Parker 1982, Samuel *et al*. 1979). It has been recognised for decades that pigs and poultry are major reservoirs of *Salmonella* spp. (Roberts 1982).

HARVEST SOURCES OF MEAT CONTAMINATION

The slaughter process inevitably involves some degree of meat contamination, whether from the animals themselves, the abattoir environment or through contact with personnel and equipment as carcases move through the process (Hudson *et al*. 1996).

Before slaughter, meat and other edible organs without contact with the exterior of healthy and physiologically normal animals, may be regarded as sterile with the exception of the gastrointestinal tract and the tongue. Usually, meat contamination occurs during the slaughter process due to contact with the skin, hair, wool or feathers and the gastrointestinal tract contents. Contamination of carcases during the slaughter process depends on care taken during flaying and evisceration. The skin and viscera are both reservoirs of human pathogens and spoilage micro-organisms (Nottingham 1982, Roberts *et al*. 1984, Snijders *et al*. 1984, Grau 1986, Gracey & Collins 1992).

During the flaying process, when an incorrect technique is used, most of the carcase bacterial contamination is acquired on the first incision, when the knife being used for slaughter penetrates a heavily contaminated skin and comes into contact with the underlying tissue. Further contamination occurs, if the skin or workers' hands come into contact with the carcase (Grau 1986).

During the evisceration process, contamination occurs if there is puncture or spillage of intestinal or bile content on the carcase. The operations involved in the freeing of the anal sphincter and the rectal end of the intestine constitute an important source of contamination for the carcase. The perianal region of the carcases is often heavily contaminated with *E. coli* and *Salmonella* spp. The incision of the gallbladder, lymph nodes and bile ducts may contribute to contamination of the carcase with *Salmonella* spp. and *Campylobacter* spp. (Peel & Simmons 1978, Grau 1979, Samuel *et al*. 1979, Samuel *et al*. 1980).

Other sources of meat contamination during the slaughter process include clothing of workers, processing equipment such as saws, boning tables, conveyors and mincers, and the water used to wash carcases, hands and equipment. It has been demonstrated that there is a significant decrease in the degree of contamination of meat, if the hands and tools of operators are thoroughly cleaned. Although water at 82°C is provided for decontamination of equipment used during the slaughter process, the time of immersion is usually not enough (must be at least 10 seconds) to kill bacteria (Peel & Simmons 1978, Nottingham 1982, Nortje *et al.* 1990a, Samarco *et al*. 1997, Upmann *et al.* 2000).

Although contamination during the slaughter process is inevitable, the first aim of the abattoir is to harvest the edible tissue (meat) with as little contamination as possible, by ensuring that the contamination of dressed carcases and edible offal from sources within the abattoir itself is kept to a minimum. This can only be achieved by the use of good manufacturing practices. This entails specific measures to prevent meat contamination at all stages of meat production resulting in prevention of microbial contamination of meat during chilling, freezing, deboning and cutting, packaging and distribution to the consumer (Grau 1986, Hudson *et al*. 1996).

At the end of the slaughter process, beef carcasses are likely to have an aerobic count/cm^2 of 10^3 - 10^5 on the meat surface, mostly less than 10^2 psychotrophs/cm^2 and 10^1 -10^2 coliforms/cm^2 of meat surface. Sheep carcasses usually have a slightly higher level of contamination than beef with 10^3 - 10^6 aerobes/cm^2, about 20% of samples have up to 10^3 or more psychotrophs/cm^2 of meat surface (International Commission on Microbiological Specifications for Food (ICMSF) 1980).

A less documented source of meat contamination is airborne contamination. It

appears that airborne bacteria contribute to carcase contamination. Rahkio & Korkeala (1997), studied microbiological contamination of abattoirs. They found out that there was an association between microbiological contamination of air and carcase contamination, and the movement of personnel between the clean and dirty areas, appeared to be associated with higher carcase contamination level. Airborne contamination originates from skins of animals and lairages. Separation of the clean and unclean areas of the abattoir decreases the level of contamination.

POST-HARVEST SOURCES OF MEAT CONTAMINATION

The contamination of meat during storage in chillers has also been shown. Organisms like *Pseudomonas* spp. were found on structural surfaces in the chillers. It was demonstrated that contamination during chilling was also airborne. The presence of spoilage flora in chillers indicated that the disinfection and cleaning routines were inadequate with regard to removal of spoilage micro-organisms (Gutavson & Borch 1993, Nortje *et al*. 1990a).

MICRO-ORGANISMS CONTAMINATING MEAT

To get a reliable indication on the hygienic quality of meat and meat products, micro-organisms on the meat surfaces must be enumerated. One would want to know the identity and numbers of all the micro-organisms on the carcase, but this is impractical. The best way is to make separate estimates of a few organisms or groups of particular significance for hygiene (Ingram & Roberts 1976).

The microbes on carcases and primal cuts will usually be most numerous on the surfaces. Exceptions to this do occur from time to time as in the case of bone taint, but it is rare. Routine sampling of whole joints is usually confined to the surface of the meat (Kilsby 1982).

A number of infectious micro-organisms associated with food have been identified. These include *Aeromonas hydrophylia, Bacillus cereus, Campylobacter jejuni, Clostridium perfringens, E. coli* 0157:H7, *Klebsiella pneumonia, Listeria monocytogenes,* Norwalk virus, *Plesiomonas shigelloides, Serratia marcescens, Toxoplasma gondii, Vibrio vulnificus, Vibrio parahaemolyticus* and *Yersinia enterocolitica*. Of particular importance are *Salmonella* spp., such as *Salmonella enteriditis* PT4 in poultry (Ternstrom & Molin 1987, Geonaras *et al*. 1996, Mortimore & Wallace 1994).

Microbial contaminants that are associated with meat will also include some species of the following genera: *Bacillus* spp., *Aeromonas* spp., *Corynebacterium* spp., *Staphylococcus* spp., *Alcaligenes* spp., *Proteus* spp., *Alteromonas* spp., *Psychrobacter* spp., the *Moraxella/Acinetobacter* group, *Kingella* spp., Micrococcaceae and lactic acid bacteria. Dainty *et al*. (1985), studied the events taking place and their influence on meat quality when P*seudomonas* spp. and *Brochothrix* spp. contaminate meat.

INDICATOR ORGANISMS

The term "indicator organisms" can be applied to any taxonomic, physiological or ecological group of organisms whose presence or absence provides indirect evidence concerning a particular feature in the past (usually recent) history of the sample (Harrigan & McCance 1976). An indicator organism is a micro-organism or group of micro-organisms that indicate that a food has been exposed to conditions that pose an increased risk, that the food may have been contaminated with a pathogen or held under conditions conducive to pathogen growth (Buchanan 2000).

Assessment for various groups and individual indicator organisms has been used to obtain information about the microbiological quality and safety of meat. The

concept of testing for indicator bacteria rather than pathogenic bacteria dates back to 1892, when Shardingerm instituted the practice of testing water for *E. coli* as an indication of faecal contamination and the possible presence of *Salmonella typhi* (Banwart 1989, Jay 1992*)*.

It has traditionally been preferred to search for the more numerous and more readily determined indicator organisms. When one tries to recover pathogenic bacteria they can be so few that they often escape detection because of problems of sampling and recovery. However, indicator organisms only give an indication that the pathogen may be present and not necessarily that they are present (Ingram & Roberts 1976).

Jay (1992) elaborated on some criteria for the use of indicator organisms. An indicator bacterium should be detectable in all foods whose quality is to be evaluated. Growth and numbers of indicator bacteria should have a direct negative correlation with quality and the indicator bacteria should be easily and rapidly detected and counted. The indicator bacteria should be easily distinguishable from other bacteria. Other bacteria normally present in food should not inhibit the growth of the indicator bacteria.

According to Tompkin (1983), the choice of an indicator is product and process specific, when evaluating the microbiological quality of food. Indicator organisms have been used in meat and poultry products to assess three factors: microbiological safety, hygiene during slaughter and processing, and the keeping quality of the product. Indicators are used to monitor meat hygiene at various stages of processing and distribution to forewarn of potential microbiological problems. The economic incentive accompanying longer shelf life has led industry to also use indicators to try and assess the keeping quality of the meat.

In the present study, the APC, *Pseudomonas* spp., *S. aureus*, Enterobacteriaceae

and *E. coli* were used as slaughter hygiene indicators for ostrich meat.

A high APC on carcases usually indicates the degree of care taken during slaughter and unsuitable time or temperature conditions during the production and storage of meat. It can also indicate heavy post-slaughter or post-processing contamination. The presence of a high APC may also mean that the plant used has been poorly cleaned or is contaminated with raw product. In addition, high counts can predict the likelihood of product spoilage (ICMSF 1973, Tompkin 1983, Brown & Baird-Parker 1982, Buchanan 2000).

Because of differences in slaughter and dressing techniques used for different meat animal species, the significance of the APC will not be the same for all meats. For example, in the production of pig and poultry carcases, the skin is not removed so that the number of organisms on the skin is a reflection of the destruction of organisms by scalding (and singeing) and of recontamination in the abattoir. On ostriches, sheep and cattle, the number of APC is a consequence of contamination of a surface, which was sterile before removal of skin or viscera (Grau 1986).

Aerobic organisms as detected with APC on carcases varies with the incubation temperature used for their culture. The approach of the Meat Industry Centre laboratory of the Agricultural Research Council Animal Nutrition and Animal Products Institute (ARC-ANPI), and many other laboratories in the world, is to use an incubation temperature from 20°C - 30°C. The rationale behind the use of this incubation temperature (20°C - 30°C) is that many bacteria present on meat are unable to grow above 30°C.

Another reason is that, since the APC is done with the intention of enumerating bacteria which may spoil the product and to check the level of hygiene during slaughter, a temperature from 20°C to 30°C would be suitable for the recovery of

the combined flora on meat which is psychrotrophilic (spoilage) and mesophilic because they both grow in this range (ICMSF 1980, Tompkin 1983, Grau 1986, Kilsby 1982).

Counts of Enterobacteriaceae and *E. coli* have been used as indicators of direct contamination of carcases with organisms associated with faecal material. The detection of such organisms on carcases could also indicate indirect contamination from the intestinal tract during slaughter, since these organisms, along with *Salmonella* spp. are frequently found on the outside surface of animals. There is usually not a very large difference between counts of Enterobacteriaceae and *E. coli* obtained from intestinal tract contents. Enterobacteriaceae, on the outside surfaces of animals, are often 100 to 1 000-fold more numerous than *E. coli* (Grau 1986, Notermans *et al*. 1977).

The presence of *E. coli* on meat does not necessarily mean that a pathogen could be present, it only implies that there may be a risk of pathogens of faecal origin like *Salmonella* spp., *Campylobacter* spp. and *E. coli* 0157:H7 being present (ICMSF 1973, Simonsen 1989, Billy 1997, Calicioglu *et al.* 1999). *Salmonella* spp. have been isolated from samples taken from carcases in which the *E. coli* count ranged from 0.1 to 1 800 per cm^2 of meat surface (Grau 1979), and the count of Enterobacteriaceae ranged from less than 20 to more than 1 000 per cm^2 of meat surface (Gerats 1987). Nevertheless, with these observations, *E. coli a*nd Enterobacteriaceae can be useful in the definition of the stages of slaughtering and dressing responsible for contamination, and the sites on carcases most likely to be contaminated with *Salmonella* spp. (Grau 1986).

It has been suggested that generally *E. coli* comprises a greater proportion of the total aerobic flora of the intestine than that of the hide or fleece. The ratio of *E. coli* to total aerobic count can be used as an indicator of whether the major source of carcase contamination is the intestinal tract or skin (Nottingham 1982).

Some laboratories prefer to use coliform counts instead of Enterobacteriaceae. A European Economic Community (EEC) study carried out to compare the coliform count (on the Violet Red Bile Agar medium) and the Enterobacteriaceae count (on the Violet Red Bile Glucose Agar), demonstrated a high correlation between these two types of counts on samples of poultry carcases taken at different stages of processing. Enterobacteriaceae counts were generally higher, and the coliforms constituted 80 – 90% of the total Enterobacteriaceae count. From this correlation, it was established that either group of organisms could be used for hygiene control checks (Simonsen 1989).

Care must be exercised when interpreting Enterobacteriaceae count on carcases as indicators of intestinal tract content contamination. Mead *et al.* (1989) (cited by Grau 1986), found that most of the Enterobacteriaceae on poultry carcases were psychrotrophic and originated from the equipment used for slaughter. Because of the presence of psychrotrophic bacteria in the Enterobacteriaceae group, they were found to be less reliable as indicators of contamination with mesophilic organisms when used for chilled meat.

Enterobacteriaceae species able to grow at low temperatures include members of the genus *Kluyvera, Citrobacter feundii, Enterobacter cloacae, Erwinia herbicola, Serratia liquefaciens, Klebsiella aerogenes* and *Enterobacter hafniae* (Kleeburger *et al.* 1980). The mesophile Enterobacteriaceae are the pathogenic ones**:** *E. coli, Salmonella* spp., *Yersinia* spp., *Shigella* spp. and *Edwardsiella* spp. (Simonsen 1989).

SPOILAGE ORGANISMS

The keeping quality of meat and poultry products can be predicted by monitoring for spoilage organisms, e.g. pseudomonads (Gill 1983).

Pseudomonads have been identified as important organisms in the aerobic spoilage of meat (Nortje 1987, Dainty *et al*. 1985). Other important spoilage bacteria are Enterobacteriaceae, lactic acid bacteria (vacuum-packed meats) and *Alteromonas* spp. *Pseudomonas* spp. usually dominate on aerobically cold stored meat, while lactic acid bacteria usually dominate in vacuum and modified atmospheres packages having elevated carbon dioxide concentrations. The number of spoilage bacteria is usually low in cold storage meat, and spoilage bacteria therefore constitute a minor part of the total APC (Gustavson & Borch 1993, Nottingham 1982).

Spoilage is caused predominantly by organisms capable of growth at refrigeration temperatures (of <5°C). Such spoilage organisms are all psychrotrophs; i.e. they are capable of growth at temperatures close to zero and an optimum temperature of 20°C - 30°C (Gill 1986, Jay 1992).

The members of the genus *Pseudomonas* are common inhabitants of soil, fresh water and marine environments, where their activities are important in mineralisation of organic matter. Pseudomonads are unaffected by pH in the range that occurs in meat at chill temperatures and therefore grow faster than competing species (Gill 1983).

The *Moraxella/Acinetobacter* group is inhibited by the combined effect of a low pH and a low temperature in chilled meat. At slightly higher temperatures they can overcome the effects of a low pH and are able to compete more successfully with other organisms (Gill & Newton 1982).

Good control of the carcase chilling process will limit the growth of spoilage organisms, and low microbial numbers can be maintained during the cutting of carcases, if equipment and surfaces are properly cleaned to eliminate material that may harbour high populations of spoilage bacteria (Gerats 1987).

PATHOGENS

As previously shown, a number of different bacteria, viruses and other infectious agents can occur on meat. In South Africa, records for food-related disease have been kept since 1989 and when an outbreak of food poisoning occurs, records are kept only when at least three people are affected and they report to one doctor (Anonymous 1997b). Records are usually poor, and do not list the causative organism of food poisoning.

In many bacterial infections, toxins cause the characteristic pathology of disease. The toxins may exert their pathogenic effects directly on target cells, or may interact with cells of the immune system, resulting in the release of immunological mediators that cause pathophysiological effects.

There are two main types of toxin that have been described: endotoxin (a component of the outer membrane of Gram-negative bacteria) and exotoxins that are elaborated by both Gram-positive and Gram-negative organisms (Eley 1994).

In the present study, presumptive *Salmonella* spp. and *S. aureus* were the only pathogens evaluated.

1. *Salmonella spp*.

Salmonella spp. in meat is a world-wide problem. The incidence and numbers of *Salmonella* spp. in meat and poultry varies with the species of animal from which

it is derived, the geographical location, pre-slaughter holding conditions, processing conditions and other factors like stress and husbandry practices. Poultry and pig meat have the highest contamination followed by cattle and sheep, which have a lower contamination due to feeding practices and slaughter procedures (Roberts 1982, Silliker & Gabis 1986).

There are over 2 000 strains of *Salmonella* spp., many of which affect man, but some are specific to birds and certain animals. There is often a direct link between the occurrence of *Salmonella* spp. in living animals, on the meat derived from them, and human salmonellosis. Meat and poultry are the vehicles responsible for most outbreaks of salmonellosis.

Salmonella spp. infection in humans affects predominantly the very young, the elderly and immune compromised individuals. It is in most cases caused by contaminated food products. Studies reveal that a principle source of *Salmonella* spp. infection in humans is contaminated poultry products (Curtis *et al*. 1991).

Currently, it is difficult to assure the production of carcase meat and poultry that is free of *Salmonella* spp. Salmonellae are often associated with animals, and the introduction of these organisms into the food processing plant, the food service establishment, or the home, is almost inevitable.

Man induces salmonellosis through improper food handling practices. Most salmonellae are transmitted through the food chain by faecal contamination of carcases during dressing. *Salmonella* spp. contaminates food in many different ways: either directly at slaughter from animal excreta transferred to food by hands, utensils, equipment, flies, etc. Man perpetuates salmonellosis through recontamination of rendered animal by-products, which are incorporated into livestock feeds (Silliker & Gabis 1986, Bailey *et al*. 1987).

The optimum temperature for *Salmonella* spp. growth is 35ºC - 37°C. Environmental factors including the substrate, pH, water activity, and competing microflora affect the range.

In 1993, *Salmonella* spp. was isolated from the intestines of 94 ratites at the Oklahoma Animal Disease Diagnostic Laboratory. Forty-six of the 248 isolation attempts were positive in ostrich, 34 of 99 in emus and 16 of 60 in rheas. The total incidence was approximately 23% (96/407). *Salmonella* spp. was isolated from birds five days to four years of age. The affected birds were from flocks that had fence-to-fence contact with other animal species (Vanhooser & Welsh 1995).

Intensively reared ostrich chicks, which have failed to establish a normal intestinal flora, are susceptible to infections with *Salmonella* spp. and other enterobacteria. Older birds appear to be relatively resistant to these infections, although salmonellae have been isolated from faecal swabs of ostriches in quarantine; more than likely stress precipitated. Faecal contamination of meat during slaughter remains a possibility (Huchzermeyer 1997).

2. *Staphylococcus aureus*

Staphylococci are ubiquitous in nature and human carriers of these organisms are numerous and are often the source of food poisoning outbreaks. *S. aureus* occurs with considerable frequency on the skin and nasal membranes, in the intestinal tract, and as the causative agent of a variety of cutaneous infections in human populations, meat animals and poultry (Evans 1986, Gracey & Collins 1992).

The primary, and almost exclusive reason for concern about staphylococci on meat is the potential ability of many strains to produce heat-resistant enterotoxins that are a major cause of food poisoning. So far, six serologically distinct staphylococcal enterotoxins have been identified and are designated as SEA,

SEB, SEC_{1-3} SED, SEE and SEF. SEF is the most common cause of staphylococcal food poisoning (Ewald 1987, Isigidi *et al*. 1992, Banwart 1989).

S. aureus is the most enterotoxigenic staphylococcal species causing food-borne disease. In the food processing industry, it is usual to identify only this group to the level of coagulase-positive staphylococci (CPS), as most enterotoxigenic strains of *S. aureus* produce coagulase. However, there are exceptions. Other staphylococcal species, such as *S. hyicus* and *S. intermedius* also produce coagulase and may produce staphylococcal enterotoxins, and some enterotoxigenic *S. aureus* do not produce coagulase (Jablonski & Blohach 1997).

Even though the enumeration of CPS in foods is not highly specific, it has been proven to be an effective indicator of the degree of contamination with potentially pathogenic strains, particularly from human sources. Human isolates of CPS are reported to produce staphylococcal enterotoxins more frequently than meat isolates (Desmarchelier *et al*. 1999, Rosec *et al*. 1997, Isigidi *et al*. 1992).

Contamination of food with *S. aureus* may be traced to food handlers with minor septic hand infections, or severe nasal infections, with subsequent heavy growth on the food medium and production of sufficient enterotoxin to cause vomiting and diarrhoea. The nasal mucous membrane, particularly, is another likely source of staphylococci of human origin. Consequently, they constitute part of the flora on meat products although normally as a minor component (Evans 1986, Gracey & Collins 1992).

Notermans *et al*. (1982), found *S. aureus* population on the skin of broilers before processing to be less than 10/g, but after processing the counts had increased to more than 1 000/g. The plucking and eviscerating operations were the major source of the increased *S. aureus*.

Desmarchelier *et al*. (1999) tested 126 CPS isolates from beef carcases for staphylococcal enterotoxin production. Staphylococcal enterotoxin was detected from 70% of isolates examined.

Isigidi *et al*. (1992) examined human biotypes of *S. aureus* strains and found that 77% produced staphylococcal enterotoxins, so the human biotype may be considered the most dangerous biotype of public health significance.

Staphylococci grow vigorously under anaerobic conditions and even better under aerobic conditions. Strains have generally a minimum growth temperature a few degrees below 10°C and a maximum growth temperature only a few degrees above 45°C. Since staphylococci cannot grow under normal refrigeration conditions, they do not represent a spoilage problem for fresh meat and poultry (Evans 1986).

High counts of *S. aureus* in a foodstuff indicate a potential health hazard due to staphylococcal enterotoxins. *S. aureus* is also an indicator of questionable food sanitation, especially in processes of handling food by human handlers (Banwart 1989).

SAMPLING AND ENUMERATION OF MICRO-ORGANISMS ON MEAT

Many techniques have been developed for counting micro-organisms on surfaces of carcases. Various destructive and non-destructive methods may be used to estimate the number of microbes present on any surface and they provide different types of information about it. When choosing a method, it is important to understand what information it gives and to assess its sensitivity and reproducibility.

Some non-destructive methods, which have been used to enumerate bacteria on

carcase surfaces are: agar syringes, rodac plates, agar sausages and membrane filter blots. The main criticism against these methods is that they are of poor precision. Another non-destructive method is the use of adhesive tapes for removal of bacterial cells on a carcase. These methods do not yield representative microbial counts. The direct surface agar can give good counts, but only at low contamination levels (Sharpe *et al*. 1996).

Some non-destructive methods break-up colonies releasing large numbers of free bacteria into the eluting fluid (swabbing or rinsing), whilst others only replicate the surface colonies (contact plate) producing apparently lower counts which may be erroneously considered as indicating cleaner meat. In general, results indicate that destructive methods involving sample removal and maceration give higher and less variable counts than contact plates and swab methods (Brown & Baird-Parker 1982).

Dorsa *et al*. (1996) compared six sampling methods (excision, swabbing with cheesecloth, sponge, cotton-tipped wooden swabs, griddle screen and 3M mesh) for recovery of bacteria from beef carcase surfaces. They concluded that the excision method was the most effective for sampling carcases. When other methods were compared to excision, none of them yielded 100% recovery of the bacteria present on a carcase surface. The excision method requires a certain amount of both time and proficiency. The excision method is capable of recovering the more representative and less variable counts of the microbial flora present on beef carcases (Brown & Baird-Parker 1982, Ingram & Roberts 1976, Rivas *et al*.1993). When recovery of specific bacteria from a carcase surface is required, this method is considered the most effective and it is commonly used by researchers (Charlebois *et al*. 1991).

The main criticism against the excision method is the amount of time it requires and the tissue damage it causes with subsequent lowering of the value of the

carcase (Anderson *et al* 1987, Ware *et al*. 1999).

Another alternative for the excision method, which has been proposed, especially in sanitation checks, where rapid results are required is the swab technique (Ware *et al*. 1999). Gill & Jones (2000), suggested that there was no difference with regard to recovery of total APC between sampling by excision and swabbing with gauze or sponge. However, swabbing with cotton wool recovered fewer bacteria.

Recovery of bacteria from meat surfaces by swabbing with sterile cellulose sponges has been controversial. Cellulose sponges may contain inhibitory detergents, which are bactericidal for certain bacteria (*E. coli*). Ware *et al*. (1999) suggested that recovery of bacteria through both sponging and excision were similar following inoculation of beef carcase tissue samples, but sampling by excision after 24 hours of carcase storage (7°C) of inoculated beef samples resulted in higher bacterial recovery as compared to sponging. This again reinforces the fact that excision is better than swabbing.

Concerning the sampling sites on animal carcases during slaughter, the approach currently adopted for the evaluation of the level of hygiene during the slaughter process is to collect samples from likely heavily contaminated sites on carcases leaving a certain processing point. Furthermore, the selection of likely heavily contaminated sampling sites on carcases depends mostly on observations made during the slaughter process, which lead to the assessment of higher contamination risks at particular sites of the carcases (Mackey & Roberts 1993, Gill *et al*. 1996a, Nutsch *et al*. 1997, Untermann *et al*. 1997).

In two studies, one done in Canada and another one in the USA, surface contamination of ostrich carcases was evaluated. The sampling sites used on the carcase were the drum, the thigh, and the sides of the carcase's back, proximal to the thigh, and the vent area. In these two studies, ostrich carcases were evaluated

for different pathogens: *Salmonella* spp., *Listeria* spp., and *Campylobacter* spp. Indicators like the APC, total coliforms and *E. coli* were also evaluated. In the Canadian study, it was determined that the microbiological state of the ostrich carcase would be comparable to that of a beef carcase (Harris *et al*. 1993, Bryant 1998).

The choice of sampling at certain processing points along the line in the abattoir is motivated by the fact that the hygienic state of the abattoir and particular abattoir practices can have a large effect on the distribution of microbes on the carcase surface, or add to the microbes brought in on the animals or birds to be processed (Roberts 1980). These are processing points (flaying, evisceration and chilling) at which, if the abattoir operator loses control, meat will become contaminated. At these points or stages in the processing of a carcase, a significant shift in flora numbers or composition might be occurring (Gill & Bryant 1992).

The skin and viscera being the largest potential contributors to carcase contamination, any loss of control or mishandling of the flaying and evisceration processes (rolling of skin on carcase, accidental puncture of stomach and intestines), during slaughter might result in a shift in numbers or composition of the microbial flora on the carcase surface (Brown & Baird-Parker 1982).

The reason behind the sampling of carcases after chilling is to assess the effectiveness of the chilling or storage procedures in the abattoir. As mentioned before, microbial activity from psychrotrophs may occur at refrigeration temperatures, with ultimate spoilage, or in unusual cases, with possibility of food-borne disease (Kraft 1986).

As to the time of sampling during the year (summer), seasonal variations in psychrotrophic counts can be found and it is often suggested that cool, wet

weather favours their growth on the hide and within the abattoir. In wet weather, animals arrive at the abattoir in a very dirty condition. Increased counts have been correlated positively with rainfall and negatively with temperature (Brown & Baird-Parker 1982, Gracey & Collins 1992).

Concerning the number of samples needed to evaluate the distribution of bacteria on all forms of meat, it has been found that 25 samples are convenient in practice, for assessing the microbiological conditions of carcases after dressing or chilling. This stems from the fact that the distribution of bacteria on meat surfaces usually approximates the log normal, and for this reason, it has been suggested that the bacterial population on a group of meat items can be estimated with confidence, on at least 25 samples (Kilsby 1982, Brown & Baird-Parker 1982, Gill *et al*. 1997, Gill & Jones 2000).

Another way of reducing variability when sampling is to increase the size of the sample unit analysed. Microbiologists use between 10 and 25 g of food per analysis, combined with a thorough mixing of contents of each sample unit, especially when samples are pooled, during sample preparation. Stomaching or blending is believed to result in as complete a recovery of attached bacteria as is possible (Kilsby 1982).

JUSTIFICATION

In spite of developments in the ostrich industry around the world, there have been up to now few scientific publications concerning the microbial quality of ostrich carcases produced. Due to the financial implications in this highly competitive industry, the studies that have been undertaken in South Africa (and internationally) are mostly of a confidential nature.

The aim of this research project was to evaluate the microbial quality of ostrich

carcases produced in a South African export-approved abattoir.

The objectives of this study were to:

- Investigate the number and types of micro-organisms present on fresh ostrich carcases and
- Identify the predominant bacterial populations at potential critical control points along the slaughtering line.
- Compare the results with the available literature on meat, draw conclusions and make relevant recommendation if need be, concerning the improvement of the quality of ostrich meat produced in a South African export-approved abattoir.

OSTRICH SLAUGHTER PROCESS

PRE-SLAUGHTER HUSBANDRY PRACTICES

In South Africa, ostriches are mainly raised in feedlots. Their basic feed is mostly a ready mix of maize and lucerne, and according to legislation, without any additives or hormones, antibiotics or growth promoters. These requirements are monitored at three-month intervals by random sampling for chloramphenicol, nitrofurans and zeranol.

According to the National Directorate of Veterinary Services (Odendaal 2000), a veterinarian must certify that the ostriches being sent to an abattoir have been vaccinated with a registered vaccine against Newcastle disease, under the supervision of an authorised person at least 30 days, but not longer than six months prior to slaughter. The veterinarian must also certify that ostriches being sent to slaughter have been dipped with a registered acaricide for ostriches, at least 14 days but not longer than 30 days, prior to slaughter. To avoid contamination or cross-contamination by rodents, dipped birds are kept in a separate area.

On arrival at the abattoir, all consignments of ostriches are inspected for the presence of ticks before slaughter. Special attention must be given to the belly, the pygostyle and the periorbital areas. To verify the efficiency of the dipping procedure, a minimum of five percent of ostriches to be slaughtered must be sampled for the presence of ticks (Odendaal 2000).

OSTRICH SLAUGHTER PRACTICES

In South Africa, ostriches are slaughtered at 12 - 14 months, at the expected body weight of 75 - 95 kg, and a dressed carcase yield of 35 - 40 kg. The bulk of the meat is obtained from the leg and thigh, which represent approximately 38% of

the dressed carcase mass. When this meat (bulk) is deboned, it represents approximately 17 - 20 kg (Campodonico & Masson 1992, Tuckwell & Rice 1994, Anonymous 1996).

For identification purposes, from the carcase back to the live bird, ostriches are identified by microchip implants. Once approved after meat inspection, the carcases are marked on both legs with a carcase number and an official approval mark or stamp, that displays the abattoir approval number. This is an important measure from an epidemiological or animal health point of view, because it allows trace back of an animal to origin, if a disease of public heath importance is diagnosed during post mortem inspection.

Considerable variation in slaughter techniques of ostriches occurs. The following is a description of the slaughter process at the abattoir where the present study was conducted.

The ostriches were loaded on a vehicle on their farm of origin and sent to the abattoir where they were rested (Figure 1) in lairages for a period of not less than 12 hours but not more than 72 hours before slaughter. This makes them easier to handle, improves meat quality and allows for the emptying of the intestine overnight. Each pen in the lairage has a holding capacity of not more than 20 birds. Every pen contains a drinking trough, which must contain potable water at all times when ostriches are in the lairage.

Before slaughter, ostriches must undergo an ante-mortem inspection. According to South African regulations, animals for slaughter should be inspected on the day of arrival at the abattoir and the inspection should be repeated on the day of slaughter, if the animal has remained in the lairage for more than 24 hours (Government Notice, 1969). Ante-mortem inspection enables the veterinarian to

assess the health status and the welfare conditions in which the animal is placed and to have as few abnormal animals as possible slaughtered.

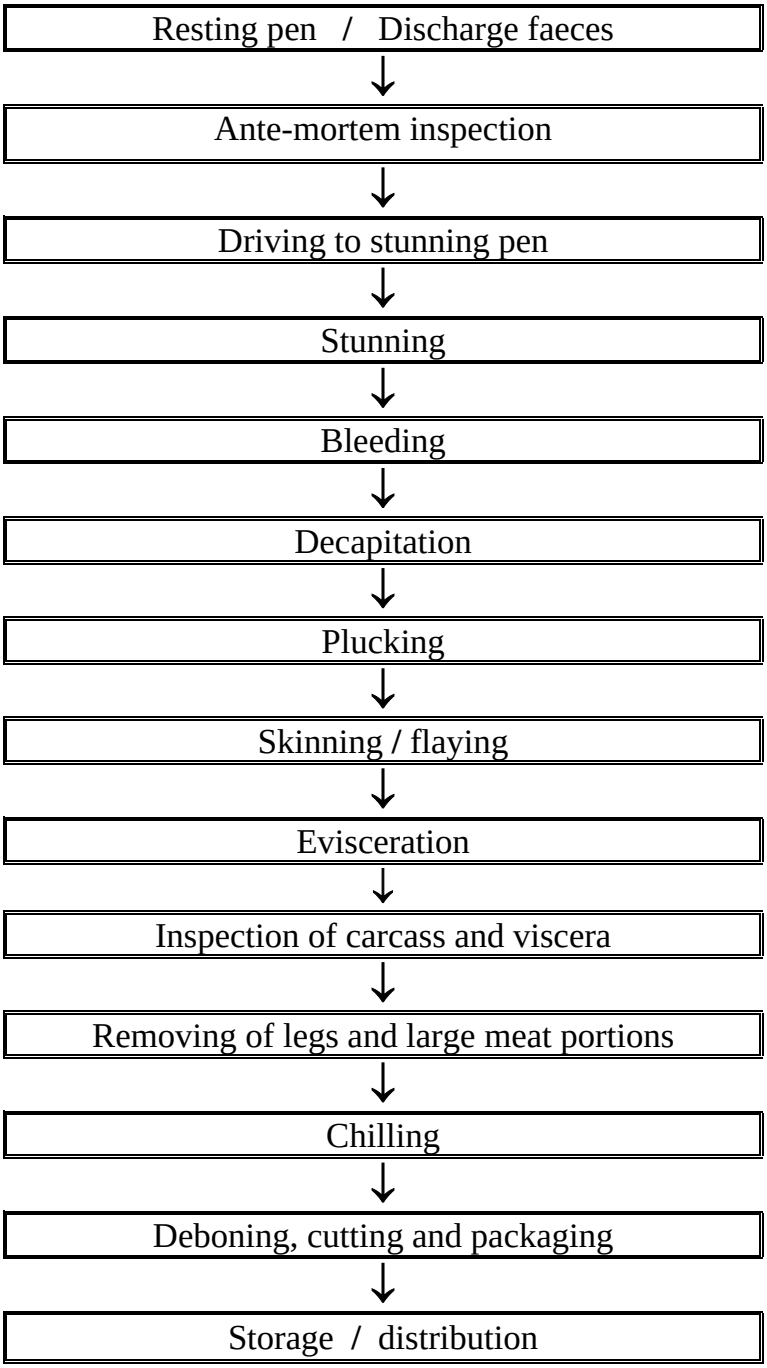

Figure 1: Ostrich slaughter flow-diagram.

1. Stunning and bleeding

The slaughter process of ostriches involves bringing the ostriches into the slaughter facility and their subsequent electrical stunning. After being adequately rested, the ostriches are driven into a restraining pen, after which they are led through a passage which opens on the stunning area inside the abattoir. Ostriches waiting to be slaughtered must not be able to view stunning and slaughtering of other ostriches.

Stunning results from the passing of an alternating electric current through the brain. It is done by employing low voltage (90 volts), through a manually operated stunning device. Effective electroplectic shock in ostriches is characterised by the presence of muscle spasms, especially through lifting of the wings and caudal flexion of the neck, as well as the absence of the corneal reflex.

After stunning, the unconscious bird is suspended by both legs with chains hanging from the ends of an upturned T-hook (a horizontal bar), its vertical and load-bearing axis being directly connected to the trolley on an overhead monorail. The bird is pushed manually to the bleeding area and then bled. The stunning to bleeding time must never exceed 60 seconds.

The bleeding method is a combination of an incision across the throat region under the head and sticking. Sticking consists of inserting a knife in the midline of the neck, at the depression in front of the breastbone. A knife is pushed upward to sever the anterior vena cava at the entrance to the chest. Bleeding is continued in the hanging position for at least six minutes.

2. Plucking

After bleeding, the body feathers are removed by dry manual plucking. Five minutes plucking time is suggested as a general guideline. Contamination of the slaughter hall with dust from the plucking area is prevented by total separation of

the slaughter hall from the feather plucking area with a hatch and a sliding door for carcase movement. A self-closing, lockable door for the sole movement of supervisors is allowed. An effective air extraction facility is also provided. Feather plucking is followed by decapitation, by severing the occipito-atlantal joint. Flaying is done after plucking.

3. Flaying

Flaying starts by severing the wingtips, whereafter the appropriate spear cut incisions are made to loosen the skin on the wings and the breast (Figure 2). The callus on the breast is cut away and the skin of the wings and the forequarters are flayed. The bird is then inverted and hung by its wingtips. The feet are removed by severing the tibio-tarsal joint. Spear cut mid ventral incisions are made to loosen the skin from the neck.

A vertical spear cut mid ventral incision is made from the breast to the cloaca, whereafter a horizontal spear cut incision is made across the belly to the tip of the tibio-tarsal joint. The bird is then flayed by pulling or peeling off the hide manually, and using spear cut incisions to loosen the skin where necessary, while taking care not to damage it.

4. Evisceration

After the hide is removed, the sternum is split and the oesophagus is exposed by a transverse cut into the neck, separated from the trachea and tied to prevent microbial contamination from the gastrointestinal tract. The anal tissue and cloaca are also loosened by knife and tied to prevent faecal material contamination during evisceration. The abdomen is opened through a cut along the linea alba. The heart, lungs and liver are removed, followed by evisceration of the abdominal cavity. Care must be taken not to damage the air sacs when the heart and liver are removed. The kidneys are not removed.

Figure 2: Ostrich flaying process. Solid lines indicate the cutting pattern during the flaying process.

After evisceration, the ostrich carcase and the internal organs are presented for inspection to the meat inspector. Any part found unsuitable for human consumption is condemned and the rest passed.

After meat inspection, the carcase is quartered by cutting loose all the muscle attachments to the pelvic girdle and detaching the femur from the acetabulum. The internal obturator muscle is cut loose from its attachment to the ischium and pubis, as well as from its tendon insertion on the acetabulum. The legs and the internal obturator muscles are removed, and then immediately chilled at 0°C to reach an internal temperature of 4°C in 24 hours before further processing (cutting and deboning). The rest of the carcase and neck are loaded in a refrigerated truck for the local market.

MINIMUM OR BASIC HYGIENE STANDARDS FOR EXPORT ABATTOIRS

1. Building

According to the National Directorate of Veterinary Services (Odendaal 2000), abattoirs and cutting plants that work according to standards of the EEC must comply with specified basic hygiene requirements. The abattoir is divided into separate working areas according to the hygiene level of the work performed in specific areas:

- Restraining, stunning and bleeding (one room is permissible).
- Feather plucking area.
- Slaughter hall divided in two sections with a floor to ceiling separation from wall to wall with a hatch for carcase passage between the point of skin removal and evisceration. Quartering of the carcase is done at the end of the slaughter line before the chilling area.
- Chiller room must function at 0 – 2°C to reduce the temperature of meat to an internal (deep muscle) temperature of ±4°C within 24 hours, and

maintaining meat at this temperature after 24 hours. This room must have both dial thermometers and thermograph recorders.

- Dispatch area: must be chilled to 12°C and both dial thermometers and thermograph recorders must be provided.
- Equipment cleaning rooms.
- Deboning plant with a deboning room which shall be chilled to 12°C.

Apart from the above-mentioned areas or rooms, there are many other areas and rooms which are not relevant for the sake of this review. Two separate air circulation systems for the slaughter line ("unclean") and the meat cutting section ("clean") must be provided. In principle, airflow is received from outside through a filter system onto the processing floor in such a way that air moves from clean to dirty areas to prevent dust, steam and vacuum contamination.

2. Personnel

Once a year, the abattoir workers undergo health checks for tuberculosis (X-ray and intradermal tests), for *Salmonella* spp. (faecal samples), and on voluntary basis, for HIV infection.

MICROBIOLOGICAL MONITORING PROGRAMME

According to the National Directorate of Veterinary Services (Anonymous 2000), routine microbiological monitoring of the slaughter process must be carried out weekly at a government laboratory. Sampling of carcases on the slaughterline is done if carcases are the final product of the abattoir, or in the case of a follow-up of a breakdown in hygienic practices, indicated by sampling in the deboning plant.

Aseptic samples are collected using a core borer from the following sites on an ostrich carcase: medial side of leg between knee and joint, lateral side of leg

above knee joint and on the *M. obturatorius medialis* (long fillet). Samples from these sites are pooled to make up at least five bags of composite samples of ±60 g each and transported to the laboratory for evaluation. Samples are evaluated for the Aerobic plate count (APC), coliforms, *Escherichia coli (E. coli), Salmonella* spp., *Staphylococcus aureus (S. aureus)* and *Campylobacter* spp. (optional).

Laboratory results of microbial evaluation are sent to the State Veterinarian in charge of the Food Safety and Veterinary Public Health, who reviews all test results and makes decisions on the effectiveness of the Hygiene Management System at the abattoir and on the suitability of meat for export. It is the duty of the State Veterinarian to inform the management of the abattoir and the Director of Food Safety and Veterinary Public Health for any negative trends, so that appropriate corrective measures can be implemented. Results are compared against South African standards for chilled export or frozen export depending on the product exported by the abattoir (Table 1).

Table 1*:* **Limits of Colony Forming Units / gram (CFU/g) for export meat according to South African standards.**

Micro-organism	CFU/g for chilled export	CFU/g for frozen export
Aerobic plate count	$<10^4$/g	$<10^5$/g
Salmonella spp.	-	-
Staphylococcus aureus	$<10^2$/g	$<10^2$/g
Coliforms	$< 10^3$/g	$< 10^3$/g
Escherichia coli	$< 10^1$/g	$< 10^2$/g
Campylobacter spp. (optional)	$< 10^3$/g	-

(Anonymous 2000).

PUBLIC HEALTH RISKS FROM OSTRICH MEAT

Although ostriches do not have species-specific diseases, they are nevertheless susceptible to common avian and some mammalian infectious diseases (Table 2). Some of these diseases may pose a public health threat. Some of them have been found to cause disease in zoo ostriches, e.g. *Erysipelothrix rhusiopathiae*, *Mycobacterium avium* and *Chlamydia psittaci*, due to contact with other zoo animals. No cases of human disease have been reported in connection with the eating of slaughtered ostriches, and, on the other hand, the likelihood of infected birds coming to slaughter, although extremely slim, cannot be excluded (Huchzermeyer 1997, Post *et al*. 1992, Vanhooser & Welsh 1995, Welsh *et al*. 1997).

Table 2*:* **Diseases transmissible from ostriches and ostrich products to man.**

Bacterial agents	Viral agents
Bacillus anthracis	Crimean-Congo haemorrhagic fever
Salmonellae	Spongiform encephalopathy
Pasteurella multocida	Newcastle disease
Chlamydia psittaci	
Mycobacterium avium	
Erysipelothrix rhusiopathiae	
Escherichia coli	
Campylobacter spp.	
Clostridium spp.	

CHAPTER 2

MATERIALS AND METHODS

PILOT STUDY

1. Design

A pilot study was conducted in order to make an informed decision on which sites should be used as sampling sites for pooled samples in the survey. The sites chosen for the pilot sampling were selected after a careful observation of the slaughter practices along the slaughter line.

The pre-selected sites were the neck, both thighs (inside), both thighs outside, sides of the carcase's back proximal to the thighs, the vent area and the drum. These sites were chosen because they were more likely to be heavily contaminated during slaughter.

The pilot study was conducted on 10 carcases by assessing the aerobic plate count of each of the six pre-selected sites individually (non-pooled), on an ostrich carcase after evisceration (n = 60). From these results, the three sites with a high aerobic plate count were selected for the survey.

Excision surface samples (5 cm^2 x 0.5 cm) for the aerobic plate count were taken aseptically from the above-mentioned sites on the carcase, by using a specially designed core borer (Nortje *et al*. 1982, Dorsa *et al*. 1996, Sharpe *et al*. 1996, Dorsa *et al*. 1997, Gill & Jones 2000, Anderson *et al*. 1987). The samples were then placed in sterile stomacher bags and stored in a cooler box at 4°C. They were transported to the microbiology laboratory, at the ARC-ANPI, Meat

Industry Centre, at Irene, where they were processed within three hours after sample collection.

2. Results

The highest mean log counts were for the thigh (inside), followed by the drum, neck, thigh (outside), vent area and the back area proximal to the thighs respectively (Table 3).

Table 3: Mean APC (log CFU/cm^2) from six different sites per carcase on ten ostrich carcases sampled in the pilot study.

Sites	Mean log counts/cm^2	Standard deviations
Thigh inside	4.0	0.16
Thigh outside	2.82	1.13
Neck	2.86	1.33
Drum	3.07	1.19
Vent	2.25	1.11
Back	1.68	1.09

The thigh (inside) and the thigh (outside) and the drum were chosen as sampling sites for the main study, as these items were exported. Although the counts on the neck were high, the neck was not included as a sampling site in the present study because it is not exported.

SAMPLING MATERIALS AND METHODS FOR THE MAIN STUDY

1. Sampling sites on the carcase

Surface excision samples (5 cm^2 x 0.5 cm) for bacteriological assessment were taken aseptically from both thighs (inside), both thighs (outside) and the drum, since these sites had revealed higher APC readings in the pilot study. Samples

from different sites were pooled and evaluated as a single sample for each carcase (Desmachelier *et al*. 1999, Untermann *et al*. 1997).

2. Processing points in the abattoir

Six carcases were sampled per visit at each of the three processing points in the abattoir (n = 3 samples per carcase): post-flaying, post-evisceration and after an average of 20 hours post-chilling. This amounted to 18 samples per visit. These processing points were chosen to identify the effects of flaying, evisceration and chilling on meat contamination. A significant shift in numbers of organisms might be occurring at these processing points in the abattoir (Ingram & Roberts 1976, Grau 1986, Desmarchelier *et al*. 1999).

The activities associated with skinning and evisceration are the principal ways through which contamination of previously sterile surfaces on meat carcases occurs (Biss & Hathaway 1996).

The sampling procedure (Table 4) was repeated on five different occasions during summer. This was done to assess the reproducibility of data and permit valid statistical analysis. In total, 30 carcases were sampled during the survey, amounting to 90 samples for the whole survey.

3. Sample collection

At the abattoir, samples were taken in the morning (zero hour 7:30 - 9:30). A sterile core borer (5 cm^2 x 0.5 cm) was used to delimit an area of 5 cm^2, with a tissue depth of approximately 5 mm excise portion as surface sample. Delimited patches of tissue were then excised, using a sterile scalpel and tweezers.

Pooled samples from each site on the carcase were placed in sterile plastic bags (stomacher bags), transported on ice (in a cooler box at 4°C) to the microbiology laboratory, where they were processed within three hours after sample collection.

4. Sample preparation

At the laboratory, the samples in stomacher bags were weighed and the exact mass noted. To be able to express microbial counts as log CFU/cm^2 of meat surface or log CFU/g of meat, all samples were weighed (more or less 25 g). Sterile Buffered Peptone Water (Oxoid CM 509), which had been prepared according to the manufacturer's instructions, was added to the samples in a 1 + 9 mass/volume ratio based on the exact mass of the sample. Thus, for example, for 25 g sampled +225 ml diluent were used. Each sample was macerated in a Colworth Stomacher^{400} for two minutes.

After maceration, appropriate serial dilutions were made. Serial dilutions were prepared in the usual way to a dilution of 10^7 using 9 ml aliquots of Buffered Peptone Water. These dilutions were done with a Scorex automatic pipette (200 – 1 000 µl) or a Brand Transferpette (200 – 1 000 µl).

Table 4: Summary of sampling sites and micro-organisms evaluated.

Visits	Carcases per visit	Processing points in the abattoir	Number of samples	Micro-organisms Evaluated
5	6*	After: - flaying, - evisceration - chilling	18 samples per visit	Aerobic Plate Count *Pseudomonas* spp. Enterobacteriaceae *Escherichia coli* Presumptive *Salmonella* spp. *Staphylococcus aureus*
TOTAL NUMBER OF SAMPLES = 18 x 5 replicates = 90				

*One sample per carcase consisting of pooled material from three sites on the carcase.

CULTURE, ISOLATION AND EVALUATION OF MICRO-ORGANISMS

1. Aerobic Plate Count:

The aerobic plate count was determined by spread plating 0.015 ml aliquots of the appropriate dilutions (10^{-1} – 10^{-7}) onto quarter plates of Tryptone Soy (TS) Agar (Oxoid CM 131) containing 3% yeast extract (Oxoid L 21) and incubated aerobically at 20°C for 48 – 72 hours.

2. *Pseudomonas* spp.

The prevalence of *Pseudomonas* spp. was determined by spread plating 0.015 ml aliquots of the appropriate dilutions (10^{-1} – 10^{-7}) onto quarter plates of Pseudomonas Agar (Oxoid CM 559) with Cemetridine Fucidine Cephaloridine (CFC) (Oxoid SR 103) as a supplement and incubated at 20°C for 48 hours. All colonies on the Agar were considered to be *Pseudomonas* spp.

3. *Staphylococcus aureus*

The prevalence of *S. aureus* was determined by spreading 1 ml aliquots of the appropriate dilutions (dilutions usually 10^{-1} – 10^{-4}) on Baird Parker Agar (Oxoid CM 275) containing Egg Yolk Tellurite (Oxoid SR 54) as a supplement and incubated at 37°C for 48 hours. Grey-black, shiny convex colonies with 1 - 1.5 mm up to 3 mm in diameter, with a narrow white entire margin surrounded by a clear zone were considered to be *S. aureus*. *S. aureus* colonies were tested for the ability to produce coagulase with the Staphytect test (Oxoid DR 850).

4. Enterobacteriaceae

The prevalence of Enterobacteriaceae was determined by spreading 1 ml aliquots on of the appropriate dilutions (dilutions usually 10^{-1}–10^{-4}) on Violet Red Bile Glucose Agar (Oxoid CM 485) and incubated at 37°C for 24 hours. Round purple colonies, surrounded by a purple halo, were considered to be Enterobacteriaceae.

5. *Escherichia coli*

The prevalence of *E. coli* was determined by spread plating 1 ml aliquots of the appropriate dilutions on Violet Red Bile Agar (Oxoid CMI 71) with MUG (4-methyllumbelliferryl-b-D-glucuronide) Agar Supplement (Oxoid BR 71) and incubated at 37°C for 24 hours. The plates were observed under ultra-violet light (366 nm). All colonies showing blue/green fluorescence in the surrounding medium were considered to be *E. coli*.

6. Presumptive *Salmonella* spp.

The prevalence of presumptive *Salmonella* spp. was determined after incubating stomacher bags containing homogenised 25 g of sample + 225 ml of Buffered Peptone Water, at 37°C for 20 hours. Subsequently, 0.1 ml of the pre-enriched Buffered Peptone Water culture was added to 10 ml of enrichment Rappaport Vassiliadis Soya (RVS) Peptone Broth (Oxoid CM 886), and incubated at 42°C for 24 hours. The enriched broth was subcultured by streaking onto plates of Brilliant Green Agar (BGA) (modified) (Oxoid CM 469) with Sulphamandelate as a supplement (Oxoid SR 87) and Xylose Lysine Desoxycolate Agar (XLD) (Oxoid CM 469).

The BGA plates were incubated at 42°C for 24 hours, while the XLD Agar plates were incubated at 37°C for 24 hours. On XLD Agar red colonies and red colonies with black centres were considered to be presumptive *Salmonella* spp. On BGA red colonies surrounded by bright red medium were considered to be presumptive *Salmonella* spp.

Table 5: Summary of culture, analysis and enumeration methods used for evaluation of micro-organisms.

Organism	Media	Cultivation conditions
Aerobic Plate Count	Tryptone Soy Agar (Oxoid CM 131) + 0.3% yeast extract	20ºC for 48–72 hours
***Pseudomonas* spp.**	Pseudomonas Agar (Oxoid CM 559) CFC Supplement (Oxoid SR 103)	20ºC for 48 hours
Enterobacteriaceae	Violet Red Bile Glucose Agar (Oxoid CM485)	37ºC for 24 hours
Escherichia. coli	Violet Red Bile Agar (Oxoid CMI 71) + MUG Supplement (Oxoid BR 71)	37ºC for 24 hours
Presumptive *Salmonella* spp.	Rappaport Vasiliadis Soya (RVS) Peptone Broth (Oxoid CM 886) Brilliant Green Agar (modified) (Oxoid CM 329) + Sulphamandelate Supplement (Oxoid SR 87) XLD Agar (CM469)	37ºC for 20 hours 42ºC for 24 hours 42ºC for 24 hours 37ºC for 24 hours
Staphylococcus. aureus	Baird-Parker Medium (Oxoid CM275) with egg yolk tellurite emulsion + Oxoid Staphytect (DR850)	37ºC for 48 hours

(Harris *et al*. 1993, Rivas *et al*. 1993, Jericho *et al*. 1994, Dorsa *et al*. 1996, Gill *et al*.1996b, Bryant 1998, De Boer 1998).

BACTERIAL IDENTIFICATION

For identification purposes, three to four colonies per site, for each carcase were selected from the highest APC dilution. The identification of bacterial isolates was done as follows: 10 colonies were selected from viable APC where colonies were separate, by means of a Harrison's disk (Harrigan & McCance 1976). This amounted to 10 isolates per carcase and 300 isolates in total for the whole trial. These isolates were stored and preserved in a freezing mixture (Table 6). Due to financial constraints, only 100 (from the initial 300) were identified for the purpose of this study.

The identification procedure consisted of thawing frozen isolates at room temperature, and then resuscitating them by inoculation in TS Broth (Oxoid CM 129) and incubation at 20°C for 72 hours. After incubation, the isolates were purified by plating them on TS Agar (Oxoid CM 131) + 0.3% yeast extract and incubated at 20°C for 72 hours. The purification process was repeated until a pure culture was obtained. Once a pure culture was obtained, a 24-hour pure culture was used for identification.

The following tests were used for identification purposes: Gram stain, catalase test, oxidase test, motility test, oxidation fermentation test and Triple Sugar Iron Agar test (Harrigan & McCance 1976). In addition the above-mentioned tests, the morphology of the isolates was also determined under a microscope. The Gram-positive and Gram-negative bacteria were then identified according to the dichotomous keys of Dainty *et al.* (1979), based on morphological and biochemical features.

STATISTICAL ANALYSIS OF DATA

For the purpose of statistical analysis, counts were converted into log values of colony forming units per cm^2 (CFU/cm^2). Data in tables were arranged per processing point in the abattoir. There were three sets (three processing points in the abattoir) of 30 counts per table. Mean log and the standard deviation (SD) values were computed for each set of data per processing point, for the APC, *Pseudomonas* spp., Enterobacteriaceae and *S. aureus* counts. A Wilk-Shapiro test for normal distribution of data was done for these sets of data. An analysis of variance (ANOVA) was determined for the data using the SAS software, version 6.09 (SAS Institute, Inc. Cary, NC, USA).

A p-value was computed by comparing the processing points with each other, to detect if there were significant differences between processing points. Significance was defined at 95% confidence level ($p \leq 0.05$).

For presumptive *Salmonella* spp. and *E. coli*, the data from all samples, which yielded positive results, were arranged according to processing points in the abattoir and percentages were computed to detect increase or decrease in microbial counts.

Results obtained after identification of isolates were also arranged per bacteria detected and per processing point, and then percentages were computed.

The computer spreadsheet Microsoft Excel 97 (1985 – 1997 Microsoft Corporation) was used to make graphs.

Table 6: Freezing mixture for – 70°C (Modified Trypticase Soy Broth)

Peptone from Casein	20.0g
Peptone from Soy Meal	3.0g
Glucose	2.5g
Di-potassium H-phosphate	2.5g
Beef Extract	2.8g
Glycerol	60.0g
NaCl	5.0g

Add water to make one litre. Inoculate and incubate at 25 °C for 48 hour. Maintain at – 70°C

CHAPTER 3

RESULTS

In the present study, the microbiological status of ostrich carcases produced in a South African export-approved abattoir was evaluated. This study was undertaken for a period of six weeks, in summer. These results present the counts for the APC, *Pseudomonas* spp., *S. aureus*, Enterobacteriaceae and *E. coli* in log CFU/cm^2 at three selected processing points during the slaughter process in the abattoir. Prevalence rates for presumptive *Salmonella* spp. and the results obtained after identification of different bacteria colonising ostrich carcases are also presented.

BACTERIAL COUNTS

Aerobic plate count

The log means for the APC (Table 8 and Figure 3), post-flaying, post-evisceration and post-chilling were 4.32, 4.21 and 4.57 log CFU/cm^2 respectively. The Standard Deviations (SD) were ±0.62, ±0.63 and ±0.68 respectively.

No significant differences were detected ($p = 0.2490$) between the log means for counts of samples collected post-flaying and post-evisceration. However, a significant difference was detected between the means of samples collected post-evisceration and post-chilling ($p = 0.0022$), and between the means of samples collected post-flaying and post-chilling ($p = 0.0190$). For both of these last two comparisons, the trend was towards a statistical increase in the log mean CFU/cm^2 for samples collected post-chilling.

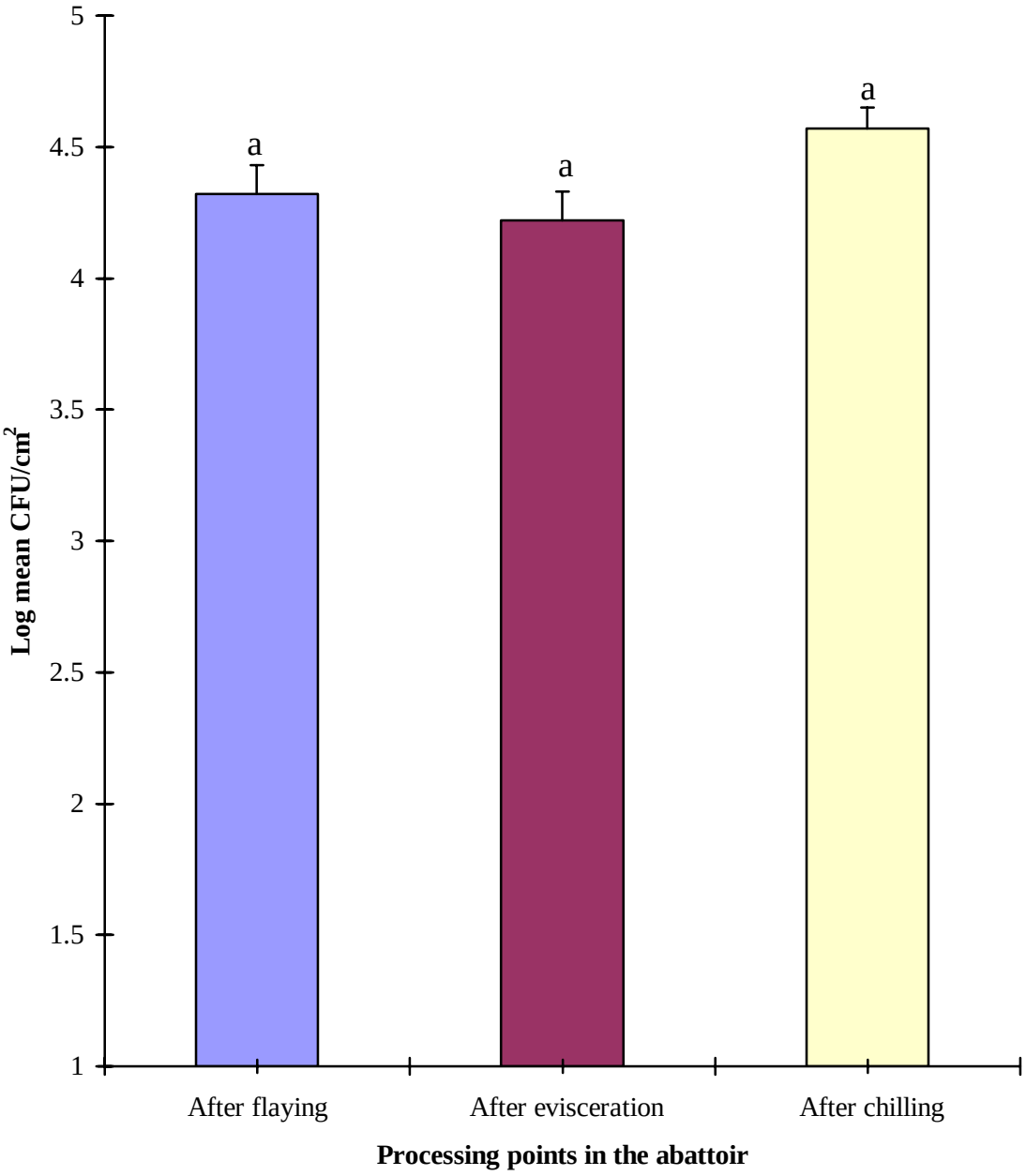

Figure 3: **Mean Aerobic Plate count on ostrich carcases at three processing points in a South African export-approved abattoir.**

Pseudomonas spp.

The log means of *Pseudomonas* spp. CFU/cm^2 counts (Table 9 and Figure 4), were 2.82, 2.86 and 3.75 for the processing points post-flaying, post-evisceration, and post-chilling respectively. The SD were ±1.65, ±1.53 and ±1.94 respectively.

No significant difference was detected between the log mean CFU/cm^2 for the post-flaying and post-evisceration processing points ($p = 0.8845$). However, there was a significant difference ($p = 0.0072$) between the log mean CFU/cm^2 of samples collected post-evisceration and post-chilling, and between post-flaying and post-chilling processing points ($p = 0.0063$). The trend was towards an increase in the counts after chilling.

Staphylococcus aureus

Staphylococcus aureus log mean counts along the slaughterline (Table 10 and Figure 5), were 2.89, 2.90 and 2.38 for the post-flaying, post-evisceration and post-chilling processing points respectively. The SD were ±0.78, ±0.53 and ±0.67 respectively.

No significant differences were detected between the post-flaying and post-evisceration processing points ($p = 0.9736$). However, there was a significant difference ($p = 0$) between the post-evisceration and post-chilling processing points and between the post-flaying and post-chilling processing points ($p = 0$) log mean CFU/cm^2 counts. For these last two comparisons, the trend was towards a decrease in bacterial counts.

Enterobacteriaceae

Log mean counts of Enterobacteriaceae (Table 11 and Figure 6), were 2.55, 2.78 and 2.73 for post-flaying, post-evisceration and post-chilling respectively. The standard deviations were ±1.53, ±1.31 and ±1.46 respectively.

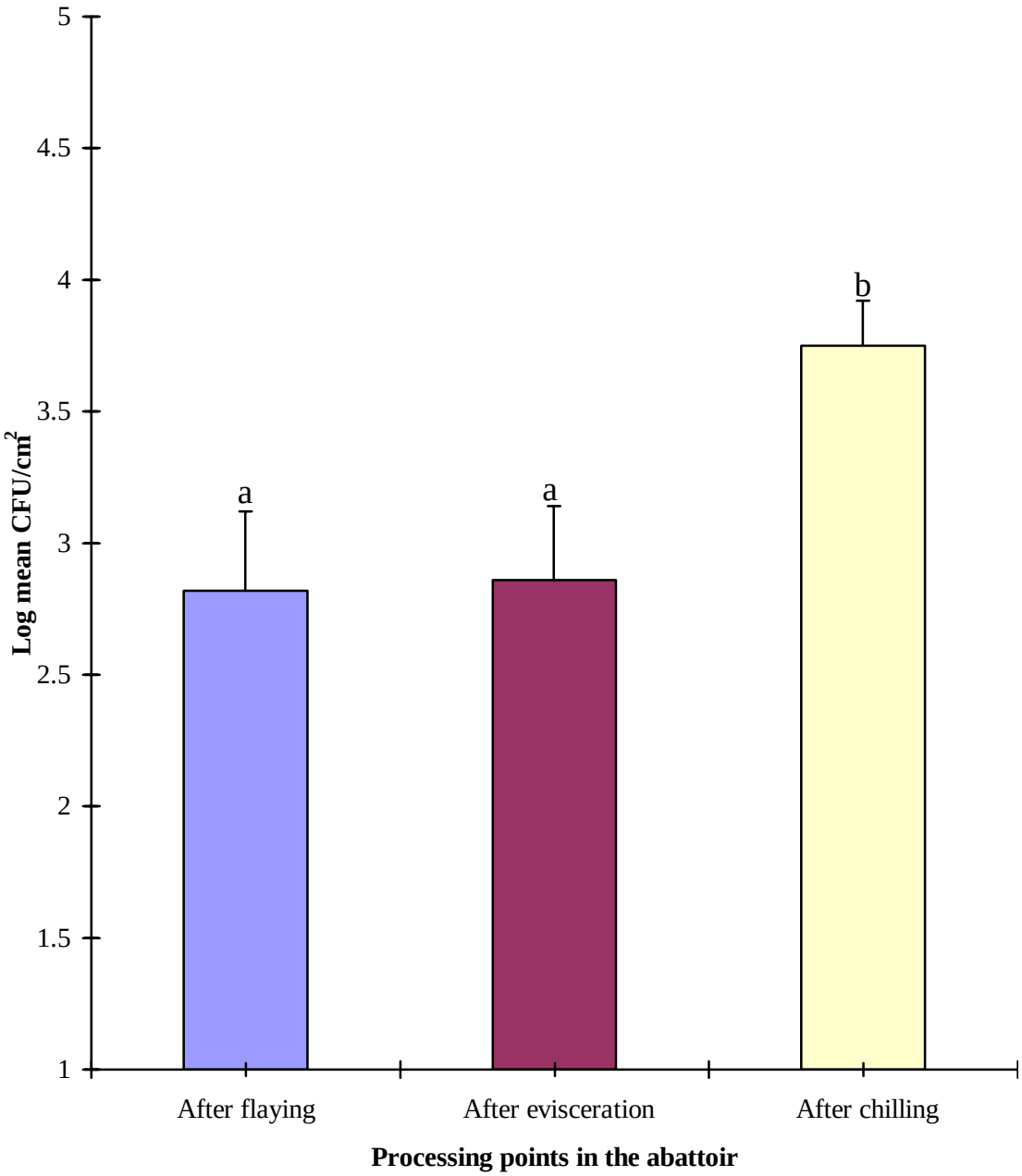

Figure 4: **Mean *Pseudomonas* spp. count on ostrich carcases at three processing points in a South African export-approved abattoir.**

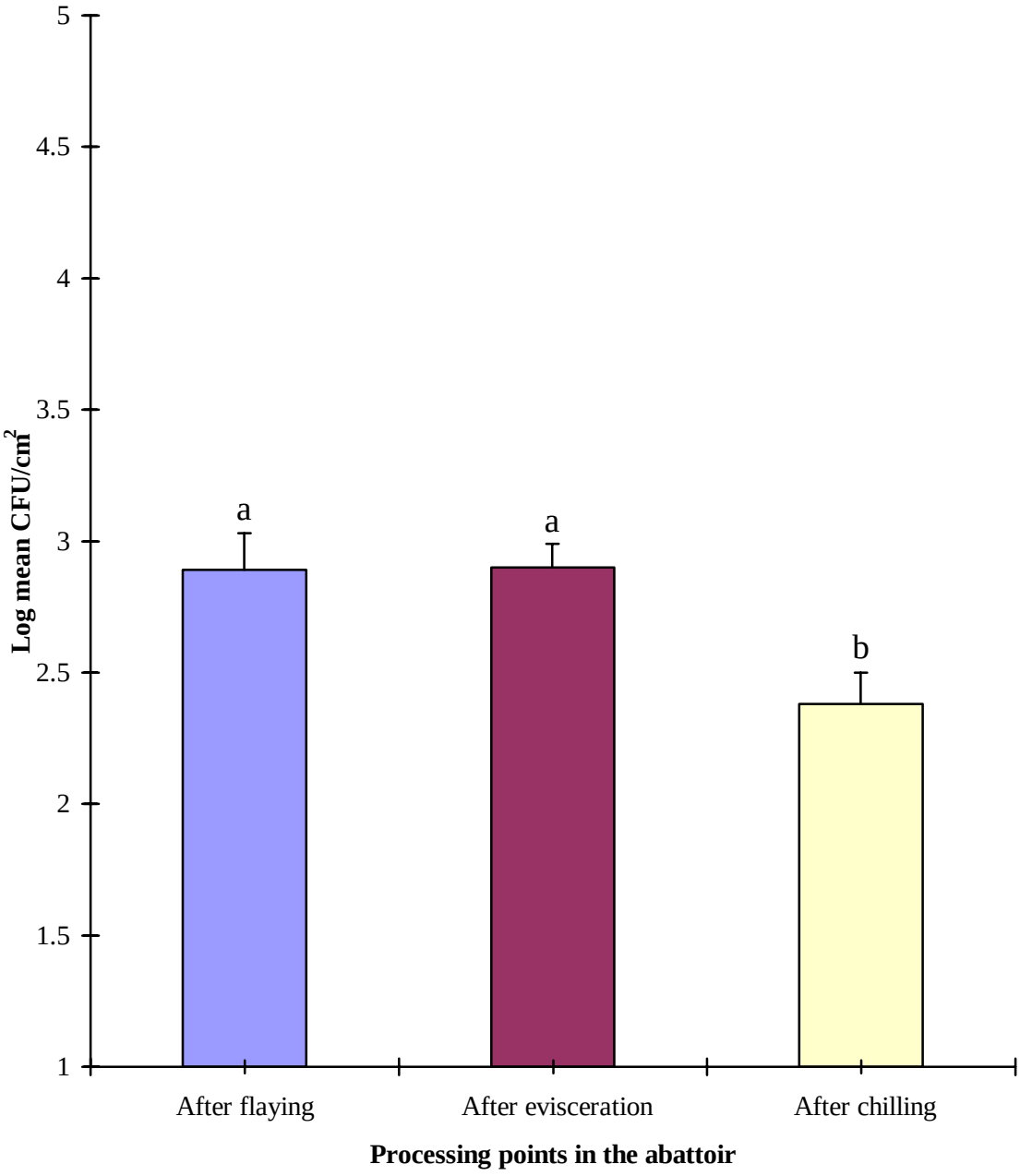

Figure 5: **Mean *Staphylococcus aureus* count on ostrich carcasses at three processing points in a South African export-approved abattoir.**

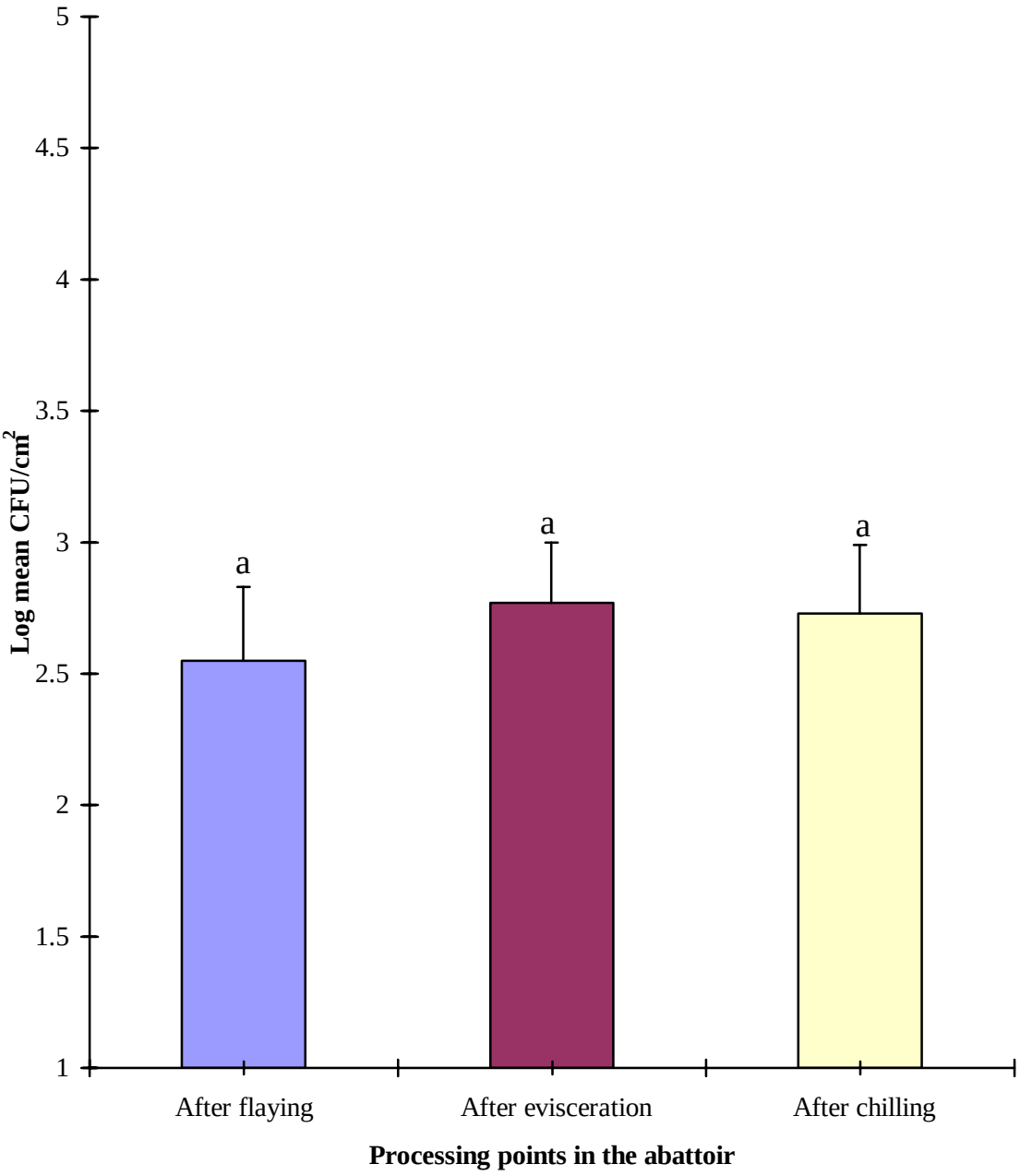

Figure 6: **Mean Enterobacteriaceae count on ostrich carcasses at three processing points in a South African export-approved abattoir.**

There was no significant difference for the log mean CFU/cm^2 counts between the post-flaying post-evisceration processing points (p = 0.2723). No significant differences were detected between the log mean CFU/cm^2 counts of samples collected post-evisceration (p = 0.8407) and post-chilling and between post-flaying and post-chilling processing points (p = 0.4453).

Escherichia coli

Of the 90 samples collected in this study, only 17 were positive for *E. coli*. This accounted for 18.8% or 17 samples out of 90 (n = 90). Log CFU/cm^2 counts for *E. coli* positive samples ranged from 1.0 to 3.79, with a log mean count of 2.15 and a SD of ±0.94. Of the 17/90 positive samples, 35% (six) were collected post-flaying, 53% (nine) post-evisceration and 12 % (two) post-chilling. In terms of all carcases sampled, 13 carcases out of 30 were positive for *E. coli*, this accounted for 43% of all carcasses sampled.

Presumptive *Salmonella* spp.

Presumptive *Salmonella* spp. were cultured on XLD and BGA media. These two media yielded different results for presumptive *Salmonella* spp. In terms of carcases sampled, 10/30 (30%) carcases were positive on XLD medium and 12 or (40%) carcases were positive on BGA media at one or more processing points.

In terms of the total number of samples analysed (n = 90), the XLD media yielded 22 (24%) positive samples and the BGA medium yielded 28 (31.1%) positive samples for presumptive *Salmonella* spp.

In terms of the different processing points in the abattoir, of the 22 positive samples on XLD medium, six or 27.2% were collected post-flaying, 10 or 45.6 % post-evisceration and six or 27.2% post-chilling. Of the 27 positive samples on BGA medium, there were eight positive samples or 29.6%, post-flaying, 12 or 44.5% post-evisceration and seven or 26% post-chilling.

Concerning the agreement between the two media in terms detecting presumptive *Salmonella* spp (XLD and BGA), there was agreement for only 14 samples out of 90. This means that the 14 or 15.5% of samples were collected from the same ostrich carcases. This converts to 23.3% or seven out of 30 carcases, which were positive for presumptive *Salmonella* spp.

Due to financial constraints, it was not possible to type the samples that yielded presumptive *Salmonella* spp., in this study.

BACTERIAL IDENTIFICATION

One hundred bacterial isolates picked from aerobic plate counts were characterised. The proportion of the isolates identified per processing point was 30 for post-flaying, 40 post-evisceration and 30 post-chilling processing points respectively (Table 7).

The predominant flora was Enterobacteriaceae 57%, followed by *Acinetobacter* spp. 24%, *Pseudomonas* spp. 11%, *Aeromonas* spp. 3%, *Micrococcus* spp. 3%, yeast 1% and S. *aureus* 1% (Figure 7).

Of the 30 isolates recovered post-flaying, 43% (13/30) were identified as Enterobacteriaceae, 40% (12/30) as *Acinetobacter* spp., 10% (3/30) as *Pseudomonas* spp., 7% (2/30) as *Micrococcus* spp. No *Aeromonas* spp., yeast or *S. aureus* was recovered post-flaying.

As for the 40 isolates recovered post-evisceration, 65% (26/40) were Enterobacteriaceae, 15% (16/40) *Acinetobacter* spp., 10% (4/40) *Pseudomonas* spp., 5% (2/40) *Aeromonas* spp., 2.5% (1/40) for *Staphylococcus* spp. and 2.5% 1/40) for yeasts. No *Micrococcus* spp. were recovered post-evisceration.

The proportional distribution for the 30 isolates recovered post-chilling was 60% (18/30) for Enterobacteriaceae, 20% (6/30) for *Acinetobacter* spp., 13.3% (4/30) for *Pseudomonas* spp., 3.3% (1/30) for *Aeromonas* spp. and *Micrococcus* spp. No *Staphylococcus* spp. were recovered post-chilling.

Table 7: Distribution bacterial isolates identified on ostrich carcases, at three processing points in a South African export-approved abattoir.

Bacterial isolates	Post-flaying	Post-evisceration	Post-chilling	Percentage
Enterobactericeae	43% (13)	65% (26)	60% (18)	57%
*Acinetobacte*r spp.	40% (12)	15% (6)	20% (6)	24%
Pseudomonas spp.	10% (3)	10% (4)	13.3% (4)	11%
Aeromonas spp.	-	5% (2)	3.3% (1)	3%
Micrococcus spp.	7% (2)	-	3.3% (1)	3%
Staphylococcus spp.	-	2.5% (1)	-	1%
Yeast	-	2.5% (1)	-	1%
Total number of isolates identified per processing point	**30**	**40**	**30**	**100**
Log mean APC per processing point	**4.32**	**4.21**	**4.57**	

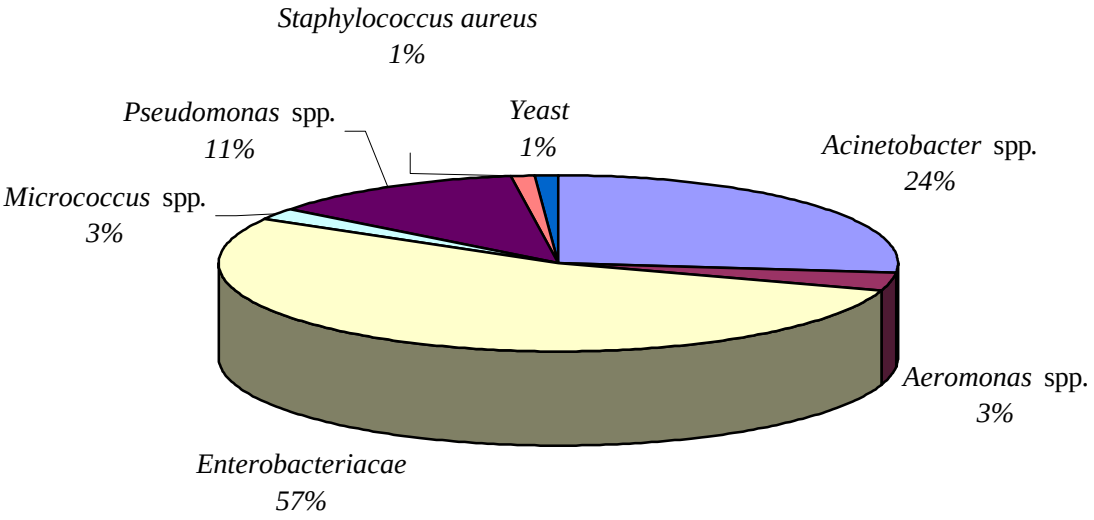

Figure 7: Distribution of bacterial isolates identified on ostrich carcases in a South African export-approved abattoir.

Table 8: Aerobic plate counts expressed as log CFU/cm^2 for 30 ostrich carcases at three processing points in a South African export-approved abattoir.

Carcase no	Post-flaying	Post-evisceration	Post-chilling
1	4.44	4.7	4.73
2	4.23	3.85	4.07
3	4.65	4.65	4.02
4	4.49	4.34	3.93
5	4.4	4.43	4.93
6	4.74	4.74	4.9
7	5.01	4.32	4.76
8	4.88	4.59	4.56
9	4.01	4.55	5.34
10	4.52	4.37	4.76
11	4.83	4.45	5.17
12	4.61	4.52	5.43
13	5.09	4.25	5.23
14	4.86	5.44	4.9
15	4.82	5.02	4.7
16	3.57	4.61	4.5
17	3.29	3.29	4.45
18	3.81	2.81	4.65
19	3.58	4.37	4.77
20	2.27	2.32	3.08
21	4.26	4.23	4.01
22	3.95	4.29	4.13
23	4.38	3.89	4.14
24	3.29	3.29	4.63
25	4.52	3.77	4.51
26	4.84	4.59	4.55
27	4.44	4.53	4.75
28	4.76	3.96	5.01
29	4.49	4.07	4.32
30	4.62	4.33	4.4
Log mean CFU/cm^2 per processing point	**4.32**[a]	**4.21** [a]	**4.57** [b]
SD	**±0.62**	**±0.63**	**±0.48**

[a] and [b] means no significant differences were detected for the means with the same letters.

Table 9: ***Pseudomonas*** **spp. counts expressed as log CFU/cm^2 for 30 ostrich carcases at three processing points in a South African export-approved abattoir.**

Carcase no.	Post-flaying	Post-evisceration	Post-chilling
1	ND	2.8	4.47
2	3.29	3.13	3.85
3	2.81	3.12	3.15
4	3.15	3.12	3.66
5	1.6	3.47	2.86
6	3.2	3.5	2.86
7	2.9	ND	2.94
8	3.3	3.25	3
9	ND	ND	4.95
10	ND	2.85	4.29
11	4.68	4.13	4.95
12	3.12	3.28	4.53
13	5.32	5.35	5.44
14	4.72	4.7	4.28
15	4.83	3.82	5.03
16	4.78	5.33	2.69
17	1.3	1.3	2.93
18	1	1.47	2.69
19	1	1	3.69
20	1	2.17	3.25
21	2.54	3.27	1.77
22	2.84	2.73	2.04
23	3	2.56	3.69
24	3.29	ND	4.94
25	4.33	4.76	4.86
26	4.38	4.7	4.5
27	3.11	3.59	3.92
28	ND	ND	3.68
29	4.92	3.12	3.29
30	4.37	3.29	4.31
Log mean CFU/cm^2 per processing point	**2.82^a**	**2.86^a**	**3.75^b**
SD	**±1.65**	**±1.53**	**±0.94**

^{a} and ^{b} means no significant differences were detected for the means with the same letters.
ND means not detected (≥ 10 CFU/cm^2).

Table 10: ***Staphylococcus aureus*** **counts expressed as log CFU/cm^2, for 30 ostrich carcases at three processing points in South African export-approved abattoir.**

Carcase no	Post-flaying	Post-evisceration	Post-chilling
1	2.81	2.47	1.81
2	3.46	3.14	2.77
3	ND	2.07	ND
4	2.69	2.34	2.3
5	3.63	3.24	3.05
6	2.6	3.06	2.07
7	3.09	2.99	2.82
8	2.6	3.06	2.07
9	2.58	3.24	2.7
10	3.75	3.64	3.09
11	2.87	3.25	2.81
12	3.16	4.05	3.29
13	4.11	3.46	2.92
14	3.67	3.68	3.25
15	2.47	2.84	3.34
16	3.04	3	2.38
17	2.96	2.95	2.38
18	3.63	3.05	2.48
19	3.41	3.15	2.32
20	2.89	2.78	2.72
21	3.82	3.59	2.31
22	2.6	3.06	2.2
23	3.89	1.6	2.07
24	1.77	2.6	1.2
25	3.06	2.59	2.57
26	2.8	2.58	2.56
27	2.61	2.71	2.31
28	2.81	2.23	1.6
29	2.2	2.14	2.14
30	2	2.55	2.14
Log mean CFU/cm^2 per processing point	**2.89^a**	**2.90^a**	**2.38^b**
SD	**± 0.78**	**± 0.53**	**± 0.67**

^{a} and ^{b} means no significant differences were detected for the means with the same letters.
ND means not detected (≥ 10 CFU/cm^2).

Table 11: Enterobacteriaceae counts expressed as log CFU/cm^2, for 30 ostrich carcases at three processing points in a South African export-approved abattoir.

Carcase no	Post-flaying	Post-evisceration	Post-chilling
1	2.79	1.6	3.51
2	2.3	3	3.06
3	2.3	2	ND
4	2.84	2.47	ND
5	ND	3.55	ND
6	3.42	3.45	3.29
7	2.36	2.47	3.58
8	2	3.56	3.86
9	1.84	2.93	3.2
10	3.58	2.47	3.64
11	3.43	4.04	4.81
12	4.59	4.96	4.26
13	3.85	4.9	2.46
14	4.34	4.81	3.96
15	4.78	4.6	4.52
16	4.78	3.85	3.85
17	1	1	2.11
18	1.9	1.84	2.46
19	1.84	1.3	2.11
20	ND	ND	ND
21	ND	2.11	1
22	ND	1.69	1
23	ND	ND	2.88
24	2.61	2.07	4.15
25	2.42	3.27	3.24
26	3.76	3.63	1.47
27	1.6	3.07	2.07
28	4.41	2	3.98
29	4.17	3.47	3.56
30	3.74	3.54	3.98
Log mean CFU/cm^2 per processing point	**2.55[a]**	**2.78[a]**	**2.73[a]**
SD	**± 1.53**	**± 1.31**	**± 1.46**

[a] and [b] means no significant differences were detected for the means with the same letters.
ND means not detected (≥ 10 CFU/cm^2).

CHAPTER 4

DISCUSSION AND CONCLUSIONS

BACTERIAL COUNTS

Aerobic Plate Count, *Pseudomonas* spp., Enterobacteriaceae and *Staphylococcus aureus*

The objectives of this study were to investigate the number and types of micro-organisms present on fresh ostrich carcases, and to identify the predominant bacterial populations at potential critical control points along the slaughter line.

Although the flesh of healthy slaughtered animals can be expected to be sterile, contamination of carcases and meat derived from carcases is difficult to avoid during the slaughter process. Slaughter techniques determine the extent of carcase contamination. In this study, the microbiological status of ostrich carcases was assessed in order to determine how ostrich carcases compare in terms of surface bacterial counts post-flaying, post-evisceration and post-chilling along the slaughter line.

The sets of log mean values obtained in this study for different bacterial counts, at different processing points in an ostrich abattoir, indicate that statistically significant differences were detected between the post-flaying and post-chilling processing points and between the post-evisceration and post-chilling processing points. There was a trend towards a statistical increase in bacterial counts for samples obtained post-chilling specifically as the mean differences suggest for the APC, *Pseudomonas* spp. and Enterobacteriaceae, whereas a trend towards the statistical reduction in bacterial counts was noted for *S. aureus* (Table 8 - 11).

However, it is important to state that, when a trend towards an increase or decrease in the log mean values of certain bacterial groups is observed, and in some instances where statistically significant differences are detected, when assessing replicated data of microbial counts for relative hygienic performances, differences between log mean values are only likely to be of practical importance, if they approach or exceed one log. Furthermore, values within ±0.5 log of the group mean can usually be regarded as of no practical significance or substantially not different (Gill *et al*. 1997, Gill & McGinnis 1999).

The results in this study suggest that there was no change of practical significance in the log CFU/cm^2 of APC, *S. aureus* and Enterobacteriaceae deposited on ostrich carcases at different processing points during the slaughter process. In other words, the initial numbers of bacteria present after skin removal or flaying, for practical purposes remained constant along the slaughter line without increasing or decreasing post-evisceration or during chilling in spite of statistically significant differences detected.

On the other hand, the counts for *Pseudomonas* spp.(refer to Table 8) (log mean 2.82 and 2.86 CFU/cm^2 for samples collected post-flaying and post-evisceration respectively), indicate that there was no modification of practical significance in the log numbers of *Pseudomonas* spp. bacterial counts deposited on ostrich carcases during the flaying and the evisceration operations, but an increase of practical significance (almost 1 log unit) in *Pseudomonas* spp. numbers occurred during the chilling process (log mean 3.75 CFU/cm^2).

The data also indicates that large numbers of aerobic bacteria as detected on APC (log mean CFU/cm^2 4.32 ±0.62; 4.21 ±0.63 and 4.57 ±0.48 for samples collected post-flaying, post-evisceration and post-chilling respectively), were deposited on carcases during the initial flaying operations and remained constant during evisceration and chilling, as suggested by the log mean count at different

processing points. More than 75% of APC counts were higher than log 4.0 CFU/cm^2 at all the three processing points.

As mentioned before, it will be difficult to compare this study with other studies, since research on the microbiological quality of ostrich carcases in South Africa and internationally is very scant. In addition, it is difficult to compare different studies on the microbiological contamination of carcases because of differences in objectives, sampling protocols and laboratory methods for the various studies of this nature which are found in literature.

According to Bryant (1998), the microbiological quality of ostrich carcases could be comparable to beef since the dressing process of both animals is more or less comparable except for defeathering during ostrich slaughter. In a recent study carried out by Gill *et al*. (2000), it was found that the estimated log mean APC numbers of ostriches and emus were greater than the corresponding values estimated for beef carcases.

In surveys of seven European beef abattoirs, carried out by Roberts *et al*. (1984), the mean APC of beef carcases ranged between 2.29 and 3.85 log CFU/cm^2. Other studies carried out in the United States of America (USA) (Sofos *et al*. 1999a, Cook *et al*. 1997) found that beef carcases had an APC ranging from 2.68 to 7 log CFU/cm^2. Similar findings have been reported for studies in Australia and New Zealand (Vanderlinde *et al*. 1998). In Germany Ingram & Roberts (1976), observed that beef carcase samples after slaughter had an average APC count of 4.58 log CFU/cm^2, and in the United Kingdom (UK) the mean APC ranged from 1.98 – 4.14 for the different abattoirs surveyed (Hudson *et al*. 1996).

When one compares results from the present study with results from the above-mentioned surveys, it would appear that the APC of ostrich carcases produced in this South African export-approved abattoir, is in the range of the APC for beef

carcases produced in other countries. However, the log mean APC obtained for the different processing points in the present survey were higher when compared to those obtained by Gill *et al*. (2000) in a study conducted on ostrich and emu carcases in Canada. The log mean APC was 2.15 for ostriches, and 2.85 for emus.

Although it is difficult to set categories of acceptance and rejection of carcases based on their APC (Hudson *et al*. 1996), the UK has attempted to set a scale of judgement for beef carcases, in order to facilitate the recognition of good hygiene procedures during slaughter. The following scale has been proposed for the logarithmic mean total viable count/cm^2 or APC:

- excellent: <2.0
- good: 2.0 - 2.9
- fair : 3.0 - 3.4
- poor: 3.5 - 4.5
- bad: >4.5

Since more than 75% of APC counts were higher than log 4.0 CFU/cm^2 at all the three processing points in this study, it appears that more than 75% of carcases for this survey would have been in the bad to poor category according to the UK scoring method, because of a high APC. A high APC usually relates to poorer quality and reduced shelf life (Eisel *et al*. 1997).

As most of the bacteria on flayed carcases are derived directly or indirectly from the hide and most types of bacteria are deposited on meat during dressing (Grau 1987, Gill *et al*. 2000), the above-mentioned findings indicate that the flaying operations during ostrich slaughter resulted in contamination of meat by the four groups of bacteria under discussion. In addition, the final microbiological condition of an ostrich carcase was largely determined by the state of the carcase before evisceration at the abattoir involved in this study.

The way in which contamination could have occurred during the flaying process, according to our visual observations, is by rolling of the outer surface of freed skin on the carcase (especially on the sites which were sampled) as well as the contact of unwashed hands or gloves of abattoir workers with the meat surface, after contacting the outer surface of the skin. These observations require corroboration. It is clear that measures need to be taken to reduce contamination during flaying. This should lead to lower overall contamination.

This study also suggests that the evisceration process was not an operation which contributed to an increase in the APC, Enterobacteriaceae, *Pseudomonas* spp. and *S. aureus*. This is in agreement with research done by Grau (1979), in which the evisceration process did not contribute significantly to a high aerobic count. The results in this study are not in agreement with Grau (1986) and Notermans *et al.* (1982), in which it was observed that a not properly performed evisceration process (in poultry abattoirs) contributed to an increase in the contamination of the carcase with Enterobacteriaceae and *S. aureus*.

The explanation behind the increase in Enterobacteriaceae of intestinal origin in poultry as compared to ostrich during evisceration could be the fact that, in poultry, evisceration through a small vent is technically more difficult than in ostriches, where the evisceration process is more or less comparable to the one of bovines.

Concerning the occurrence of *S. aureus* which is not usually detected in the intestinal tract, Notermans *et al.* (1982) in the above-mentioned study, suggested that *S. aureus* could have originated from sources other than the bird, since the strains involved appeared to be indigenous to the surveyed plant.

The lack of increase in Enterobacteriaceae counts after evisceration could falsely suggest that the evisceration process was well performed in the abattoir studied,

when one compares the results obtained after enumeration of Enterobacteriaceae with those obtained after identification (Table 11 and Table 7). Of all (57) Enterobacteriaceae identified, 46% (27/57) were obtained from samples collected after evisceration, 23 % (13/57) after flaying and 31% (18/57) after chilling. On the other hand, when one analyses the proportional distribution of bacteria bacterial isolates identified per processing point (Table 7), Enterobacteriaceae increased from 43% after flaying to 65% after evisceration with a slight decrease to 60% after ±20 hours of chilling (Table 7). These results when analysed in conjunction with *E. coli* counts and *Salmonella* spp. prevalence suggest a not well performed evisceration process (see later on).

There seems to be a contradiction between Enterobacteriaceae results obtained after bacterial counts and those obtained after identification. The means of Enterobacteriaceae counts were amongst the lowest of all bacterial groups enumerated (Table 8 – 11). However, Enterobacteriaceae constituted the highest proportion of all bacteria identified (Table 7). This apparent contradiction could stem from the fact that the methods used to culture bacteria during the enumeration of Enterobacteriaceae counts were different from those used for the identification of isolates (Table 5).

A strongly selective medium and a selective incubation temperature were used for Enterobacteriaceae enumeration. This was done by culturing Enterobacteriaceae VRBG Agar at an incubation temperature of 37°C for 24 hours (Table 5).

According to Frazier (1967), Enterobacteriaceae have the ability to grow over a wide range of temperature, from below 10°C to about 46°C. Originally, the culture of Enterobacteriaceae on VRBG Agar at 37°C for 24 hours was proposed by Mossel (1962) to favour the growth of pathogenic Enterobacteriaceae like *Salmonella, Shigella* and *Yersinia*. The temperature of 37°C favours the growth of those Enterobacteriaceae which only grow at an optimum temperature above

30°C to the detriment of those which have an optimum growth temperature of below 30°C. According to Mossel (Cited by The Oxoid Manual, 1990), media that contain bile salts like VRBG Agar have an intrinsic toxicity, even for cells that have not been under stress. If the Enterobacteriaceae are in any way stressed, they will not grow on this strongly selective medium at 37°C.

On the other hand, colonies for identification were obtained after culture on a specially designed non-selective Tryptone Soya Agar supplemented with Yeast extract usually used for the APC at an incubation temperature of 20°C (Table 5). The use of a non-selective medium and an incubation temperature of 20°C during identification was more conducive for the growth of a wide variety and a higher number of Enterobacteriaceae including those which could have been stressed.

The relationship between Enterobacteriaceae and APC counts in this study is in agreement with a study done by Reuter (1994) in beef and pork, in which it was observed that the difference between Enterobacteriaceae and APC counts ranged within two log cycles approximately at the end of the slaughter line just before chilling. This relationship remains nearly the same during and after chilling.

With regard to bacterial counts on samples collected post-chilling, it is commonly believed in the meat trade, that chilling reduces the number of viable bacteria on the carcase, especially organisms like *S. aureus* which cause food poisoning, although contradictory results have been reported (Evans 1986, Nortje *et al.* 1990a). Chilling involves the exposure of carcases to a rapid flow of cold air as heat is extracted. In such a process, evaporation of water from the carcase tends to dry the carcase surface, thereby inhibiting and even reducing the number of bacteria on the carcase (Gill & Bryant 1997).

In general, this survey suggests that bacterial numbers were maintained at the initial level without any increase or decrease of practical importance in the level

of contamination during the chilling process, except for psychrophilic micro-organisms like *Pseudomonas* spp., which increased. Gill (1982), stated that if the chiller conditions allow carcase surfaces to remain moist and relatively warm for extended periods, then the psychrotrophic fraction of a flora will have the opportunity for substantial proliferation. Nottingham (1982), observed that adjustments of temperature, humidity and speed of the air to which cooling carcases are exposed, could result in increases, decreases and even no change in the total numbers of aerobic bacteria recoverable from the carcases.

This study is also in agreement with the findings of Jericho *et al.* (1997), which suggest that counts on chilled carcases appear to be comparable to counts on carcases at the end of the slaughter line, especially for the APC, although it is assumed that the ratio of psychrotrophic to mesophilic bacteria changes when meat is chilled.

2. *Escherichia coli*

The majority of carcases or samples surveyed were not positive for *E. coli*. According to Vanderlinde (1998), when the majority of samples are negative for *E. coli*, it is considered inadequate to use the mean of bacterial counts of all carcases sampled, in order to describe the level of contamination of carcases with *E. coli*.

Vanderlinde (1998), went on to suggest, that it was better to use the distribution of *E. coli* on carcases, since it had proved to be a better indicator of the degree of contamination than the mean. To preclude the limitation of using the mean, McNamara (1998) (cited by Vanderlinde 1998), suggested that one could use the mean of *E. coli* counts for only those carcases testing positive. In the present study, we used a combination of the two methods, since they give a better understanding concerning the level of contamination of carcases with *E. coli*.

The *E. coli* counts on the surface of ostrich carcases ranged from log 1 to log 3.79 CFU/cm^2, with a log mean of 2.15 CFU/cm^{2}of meat surface. These counts are comparable to those found on beef in New Zealand, Australia and the USA (Cook *et al.* 1997). While *E. coli* was detected to a maximum of log 2.11 in New Zealand, the maximum for carcases in the USA was log 6.0 (Sofos *et al.* 1999b).

Gill *et al* (2000), concluded that the contamination of ostriches (log mean 1.54) and emus (log mean 1.31) with *E. coli* was lower than *E. coli* contamination on beef (log mean 3.20) because beef hides were more likely to carry faecal material, and therefore, higher numbers of faecal micro-organisms like coliforms. The reason being that cattle are continually crowded during intensive rearing in feedlots, whereas adult ostriches are farmed under free-ranging conditions. On the other hand, ostrich farming aims at less faecal contamination before slaughter, because ostrich feathers must be kept as clean as possible, since they constitute a prized product which cannot be cleaned once contaminated with faeces.

In terms of *E. coli* distribution, in the present study, it was found that 43% (13/30) of all ostrich carcases sampled were positive for *E. coli* at some stage during processing. It was also observed that most of the samples 53% (9/17) which tested positive for *E. coli*, were collected post-evisceration. This suggests a relatively poor control of the evisceration process, which resulted in some avoidable contamination (Gill *et al.* 1996b, Sofos *et al.* 1999b, Ingham & Schmidt 2000).

It was also observed that a small number of samples 12% (2/17) tested positive for *E. coli* post-chilling. This confirms the studies by Calicioglu *et al.* (1999), who reported that *E. coli* was less cold-tolerant, and therefore, decreased during chilling. Gill & Bryant (1997), reported that chilling resulted in about a 2 log unit reduction in numbers of *E. coli*. In this study, the two (2) samples which were

positive post-chilling, decreased to one log CFU/cm2. A larger number of samples would be needed to warrant such a conclusion.

Certain strains of *E. coli* (e.g. *E. coli* O157: H7) have been recognized as being responsible for haemorrhagic colitis and haemolytic uremic syndrome in humans (Charlebois *et al.* 1991, Buncic & Avery 1997). Reducing *E. coli* in meat contributes to minimising the risk of human infection with *E. coli*. Indications are that the ostrich slaughter process in the abattoir, where this study was conducted, contributed to *E. coli* contamination. Therefore, it is important to exert control measures during the dressing procedures in order to minimise ostrich carcase contamination.

3. Presumptive *Salmonella* spp.

All the 90 samples from 30 carcases were evaluated for presumptive *Salmonella* spp. Presumptive *Salmonella* spp. evaluation was carried out on two different media which yielded slightly different prevalence rates. The agreement for the two media was only 14 samples collected from seven carcases. This converted to 15.3% in terms of samples evaluated, and 23.3% in terms of carcases evaluated.

The overall rate (23.3%) of contamination of ostrich carcases falls in the range of 20 to 25% reported in the UK (22.8%), and Korea (25.9%) on chicken (Plummer *et al*. 1995, Chang, 2000). This prevalence rate was slightly higher than the one found on US chicken carcases (19.4%). Beef showed lower prevalence rates of *Salmonella* spp: 1.0% for steer-heifers and 2.7% for cow-bull carcases (Sofos *et al*. 1999c).

When one compares this study to other studies done by Aoust (1989) and Bensink (1991), (cited by Rickard *et al*. 1995) the prevalence of presumptive *Salmonella* spp. on ostrich carcases were lower or higher when compared to those found in various meat industries in Australia: kangaroo 11.1% (with a range of 7.8 - 15%),

pigs 16% (with a range of 0.4 - 76%), poultry 33.4% (with a range of 5.0 - 79%) and feral pigs 34.4% (with a range of 5.6 - 68%). The major source of *Salmonella* spp. is farm animals, which may frequently be intestinal carriers of the organism. Pigs and poultry are particularly incriminated in this regard, and to a lesser extent cattle and sheep (Oosterom 1991).

The positive results at different processing points showed an increase in numbers of presumptive *Salmonella* spp. in samples collected post-evisceration for both media from 27.2% (six) post-flaying to 45% (10/22) positive samples for XLD medium, and from 29.6% (eight) post-flaying to 44.4% (12/27) positive samples for the BGA medium. These results point again towards a lapse of hygiene during the evisceration process, especially if one observes them in conjunction with *E. coli* positive samples, which were obtained post-evisceration from 35% (six) post-flaying to 53% (9 out of 17). A higher number of *E. coli* positive samples implies that enteric pathogens, such as *Salmonella*e will most likely be present on the carcase surface (Vanderlinde *et al*. 1999).

BACTERIAL IDENTIFICATION

The dominant microbial flora identified in this survey (Table 7) are comparable to those identified by previous researchers (Lehellec & Colin 1979, Gill & Newton 1982, Nortje *et al*. 1990a, Fries 1996, Kawadza 1997, Geonaras *et al*. 1998, Olivier 1998, Buys 2000), who characterised the microbial flora on beef and poultry carcases.

In this survey, most of the micro-organisms identified, were the same that usually occur on meat (ICMSF 1980). The major groups of micro-organisms isolated from ostrich carcases consisted of Gram-negative saprophytic species, and the micrococci group (mainly *Micrococcus* spp. and *Staphylococcus* spp.).

In the above-mentioned studies on bacterial isolates, found on fresh meat, the dominant bacterial group alternated between *Pseudomonas* spp. and Enterobacteriaceae, depending on the processing stage at which the samples were taken.

According to Gill & Newton (1979) and Gill (1983), the predominance of *Pseudomonas* spp. or Enterobacteriaceae depends on how these bacterial groups are affected by meat pH and temperature. *Pseudomonas* spp. occurring on meat, are unaffected by pH over the range found in meat. The other group which is least affected by pH changes, is the Enterobacteriaceae group, and they also tend to grow very much slower than *Pseudomonas* spp. at chill temperatures.

In this study, it was observed that Enterobacteriaceae were predominant along the different processing points, followed by the Acinetobacter group and *Pseudomonas* spp. respectively. The reason behind this compositional distribution lies in the fact that most of the samples were taken before chilling. For those samples collected after chilling (±20 hours); as shown by the bacterial counts, the chilling process had not been long enough to favour the predominance of psychrophiles like *Pseudomonas* spp., which become dominant during prolonged chiller storage (Grau 1981).

In addition to being psychrophiles, and not being affected by the drop in meat pH during prolonged chilling, *Pseudomonas* spp. inhibit the growth of other species by competitive exclusion (Gill & Newton 1978, Gill 1983).

According to Gill & Newton (1980), at elevated temperatures, there is predominance of the Acinetobacter group and Enterobacteriaceae, which include psychrophiles and mesophiles instead of the pseudomonads. The decisive factor in the dominance of the *Pseudomonas* spp. on aerobically stored chilled meats is their advantage to grow at chill temperatures (Gill & Newton 1982).

On the other hand, Lehellac & Colin (1979), found that *Pseudomonas* spp. were predominant in abattoirs where much water was used on carcases. This also depended of course, on the water quality. *Pseudomonas* spp. can also grow in hoses and taps, etc., where water is in contact with air. This would be another explanation, why *Pseudomonas* spp. were found to be in low proportion as compared to the Enterobacteriaceae and the Acinetobacter group, since during slaughter of ostriches, no water is used on carcases. In a water environment, *Pseudomonas* spp. are more resistant to chlorine, and therefore, may survive the normal water treatment, but they can be eliminated by super chlorination (Mead 1989).

Compared to the Enterobacteriaceae and *Pseudomonas* spp., the Acinetobacter group is inhibited by the low pH of normal meat, but flourish at an elevated temperature because the effects of pH are less pronounced (Gill 1991). This bacterium could be a major constituent of meat flora in circumstances where meat of high ultimate pH contains a relatively high proportion of this bacteria in the initial flora (Gill & Newton 1978, Nottingham 1982). Since the ultimate pH of ostrich meat is relatively high (Sales & Mellet 1996) and on the other hand, most of the samples were collected while the temperature was still high, this combination of factors would probably explain the high proportion of the Acinetobacter group among the variety of bacteria isolated from ostrich meat.

Concerning the proportion of different bacterial groups at particular processing points, the only noteworthy observation was that a high number of Enterobacteriaceae were isolated from samples taken post-evisceration 65% (26/40), as compared to the post-flaying process 44% (13/30). According to Brown & Baird-Parker (1982), in fresh meats, most of the Enterobacteriaceae originate from faecal contamination, and consequently, their occurrence in high numbers may indicate unhygienic processing or storage. This might again point

to a lapse of hygiene during the evisceration process, although the data are too few to make definite conclusions.

Apart from hides and faecal material contaminating ostrich carcases during the slaughter process, another possible source of contamination could be contaminated air (Rahkio & Korkeala 1997). In a study done in a poultry abattoir, it was found that Enterobacteriaceae, *Acinetobacter* spp. *Micrococcus* spp. *S. aureus* and yeasts were some of the most prevalent micro-organisms in the air at different processing points (Ellerbroek 1997).

The dominant bacterial groups isolated on ostrich carcases play a major role in meat aerobic spoilage (Nortje & Naude 1981, Gill & Newton 1982). Spoilage flora mostly originate from the hides of animals (Newton *et al*. 1978, Nottingham 1982). If there is effective effort during the dressing of cattle, sheep and ostrich, to avoid both direct and indirect contact between hide and flayed surfaces, then few spoilage organisms will be deposited on the meat from the source. Spoilage organisms may also originate from water, and especially from taps and hose pipes (Gill 1987).

Gill (1982) described how spoilage due to *Pseudomonas* spp. occurs in meat. Pseudomonads preferentially utilise glucose. While using this substrate they do not produce malodorous compounds. However, when glucose is exhausted they attack amino acids with malodorous sulfides, esters, acids, etc., being formed as by-products. The odours of such by-products are usually the first symptom of chill temperature spoilage.

The Enterobacteriaceae group plays a minor role in the aerobic spoilage of meat. Like pseudomonads, they preferentially utilise glucose, and when glucose is finished, they attack amino acids which produce organic sulfides. Many strains of Enterobacteriaceae can also release H_2S, which with decarboxylated amino-

acids are responsible for malodours. This shows that the spoilage potential of Enterobacteriaceae can be high if conditions are favourable (Gill & Newton 1978, Gill 1986).

Other bacteria with spoilage potential are the Acinetobacter group. They preferentially utilise amino acids while growing on meat, but do not seem to produce malodours by degrading amino acids, and therefore, have a low spoilage potential. However, when they are a major component of the spoilage flora, they enhance the spoilage activities of pseudomonads by restricting oxygen available to pseudomonads (Gill & Newton 1978; Gill 1986).

Aeromonas spp. and *Micrococcus* spp. were among bacteria isolated on ostrich meat, but to a lesser extent. *Aeromonas* spp. have been isolated from a number of meats (beef, pork and poultry) and other animal products, such as seafood and dairy products, as part of the spoilage flora (Stelma 1989, Wang 1999). Usually their main sources is water, animal faeces or food handlers (Kirov 1993).

The most probable source of *Aeromonas* spp. in this survey, may have been the faeces (5% post-evisceration and 3.3% post-chilling). Water could not have been a source, since water is used in an ostrich abattoir only for cleaning purposes. *Aeromonas* spp. are considered the cause of emerging food-borne diseases causing septiceamia, gastro-enteritis, enterocolitis and wound infections in humans (Wang 1999).

Micrococcus spp. are also constituents of the microflora of meat. They are normally found on meat carcases and meat products. In general *Micrococcus* spp are widely distributed in the environment. The main sources are the air, the skin and hides, as well as in dust, soil and water (Jay 1992, Geornaras *et al*. 1996, Ellerbroek 1997).

CONCLUDING REMARKS

This study provides baseline data on the microbiological quality of ostrich carcases produced in a South African export-approved abattoir. Although not alarmingly high and comparable to other studies done on bovines and very scant studies on ostrich carcases, the results in this study indicate that the slaughter procedures in the abattoir studied contributed to the contamination of carcases during the flaying process and evisceration process.

The initial bacterial load deposited on the carcase during the flaying process was maintained at a controllable level in terms of the APC, *Pseudomonas* spp. and *S. aureus*. The implication being that, if the flaying process could be performed with care, this could contribute to a lower number of bacteria being deposited on meat, thereby improving the microbial quality of ostrich meat produced in the studied plant to a large extent.

The evisceration process was also found to be a contributing factor towards the contamination of ostrich carcases, especially when one analyses the results for *E. coli*, presumptive *Salmonella* spp. and the results obtained after identification, where Enterobacteriaceae were the predominant bacteria. This also would imply that there is a need for improving the evisceration process, in order to eliminate microbial contamination as this would contribute towards not only improving the quality but also the safety of ostrich meat produced in the abattoir studied.

It would be in the interest of the abattoir to implement more efficient quality assurance systems in order to ensure the production of ostrich meat of a better quality for the consumer and better economic returns for the abattoir. In order to achieve maximal success with these programmes, adequate and regular training of the slaughterers in the basic hygiene procedures is also needed.

There is also a need to compile more data on the microbiology of ostrich meat, by collecting samples from different abattoirs and comparing the results obtained after microbial evaluation. This would help the scientific community and regulatory authorities to get a larger picture on the microbiological quality of ostrich meat produced in South Africa, as this data is scanty. Another avenue for research would be a detailed study of pathogenic bacteria contaminating ostrich carcases in order to evaluate objectively the safety of meat and meat products produced from ostriches

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