THE MICROBIOLOGY OF OSTRICH MEAT WITH REFERENCE TO PREVALENT MICROBIAL GROWTH AND BRUISES ON CARCASSES

by

Demona Charlotte Schnetler

Thesis present in partial fulfilment of the requirements for the degree of Master of Science (Food Science)

at

Stellenbosch University

Department of Food Science

Faculty of AgriScience

Supervisor: Prof LC Hoffman
Co-Supervisor: Prof TJ Britz

Date: *March 2009*

Declaration

By submitting this thesis electronically, I declare that the entirety of the work contained therein is my own, original work and that I have not previously in its entirety or in part submitted it for obtaining any qualification.

Date:

Abstract

Fresh ostrich meat competes in well regulated and competitive international markets; therefore food quality and safety are of the utmost importance. At the same time the production process must be well controlled to be cost effective. Losses in meat yield through bruising and the trimming thereof as well as a high initial microbial load that causes a decrease in shelf-life is thus undesirable. The main objectives of this study were firstly to investigate the expected prevalent microbial growth on ostrich meat as well as possible environmental contaminants to establish which bears the greatest risk. Secondly to establish the best practice of removing bruised areas from carcasses from both a microbiological and meat yield perspective. Lastly to investigate bruises on carcasses to predict the possible causes thereof so as to minimize bruising during transport and handling. From this study it was concluded that the prevalent growth on carcasses was predominantly Gram-positive which increased ten fold from post-evisceration to post-chilling, this was also associated with a marked increase in Gram-negative organisms. The most dangerous vector for contamination was found to be standing water containing Gram-negative human pathogens including *Shigella, Salmonella* and *E. coli*. Bruises to the necks (52.58% of all bruises) were the most frequent, the high side railings on transport trucks the probable cause thereof. It was indicated that aerobic viable counts decreased after cold trimming, where the opposite occurred on warm trimmed surfaces, while the average loss in meat yield per bird due to bruising was smaller for cold trimming.

Opsomming

Vars volstruisvleis kompeteer in goed gereguleerde en kompeterende internasionale markte; dus is voedselkwaliteit en –veiligheid baie belangrik. Terselfdertyd moet die produksieproses goed beheer word en koste effektief wees. Verliese aan vleisopbrengs as gevolg van kneusings en die verwydering daarvan, sowel as ‘n hoë inisiële mikro-organisme lading wat ‘n verkorte rakleeftyd tot gevolg het, is dus ongewens. Die hoofdoelwitte van die studie was eerstens om die verwagte mikro-organisme groei op volstruisvleis en op moontlike omgewings kontaminasie bronne te ondersoek om vas te stel watter bronne die grootste risiko dra vir besmetting. Tweedens om die beste praktyd vir die verwydering van kneusings van die volstruiskarkasse te bepaal uit beide ‘n mikrobiologiese en vleisopbrengs oogpunt. Laastens om die omvang en verspreiding van karkaskneusings te ondersoek om die oorsaak daarvan te probeer aandui en sodoende kneusings tydens vervoer en hantering te verminder. Uit die studie was die volgende duidelik; die mikrobiese groei op karkasses was hoofsaaklik Gram-positief, tellings het tienvoudig toegeneem vanaf ontweiding tot na verkoeling, met ‘n gepaardgaande merkbare toename in Gram-negatiewe organismes. Die gevaarlikste oorsaak van omgewingskontaminasie was staande water wat Gram-negatiewe menslike patogene (insluitend; *Shigella, Salmonella* en *E. coli*) bevat het. Nekkneusings (52.58% van all kneusings) was die algemeenste; met die hoogte van die kantreëlings van die volstruistrokke die moontlike oorsaak daarvan. Dit is bewys dat die aerobe mesofiele plaattelings afgeneem het na koue verwydering, maar dat die teenoorgestelde gesien is op warm gesnyde areas; die gemiddelde verlies in vleisopbrengs per volstruis as gevolg van kneusingverwydering is kleiner tydens koue verwydering.

Dedicated to my son,

Donald McKinnon

Acknowledgements

My sincere gratitude to the following persons and institutions that formed an integral part of this research:

Prof. L.C. Hoffman of the Department of Animal Sciences, University of Stellenbosch, as Study Leader, for his technical support, mentoring throughout the completion of this study and for convincing me that I can achieve this;

Prof. T.J. Britz of the Department of Food Science, University of Stellenbosch, as Co-study Leader, for his technical support, expert guidance and for his enthusiasm for microbiology that has inspired me;

Klein Karoo International (Pty) Ltd, for financial support, access to their laboratory and abattoir information and facilities;

Drr. W.P. Burger, A.J. Olivier and the rest of the personnel of KKI Research, for their interest, participation and assistance with the completion of this study.

My family and friends and specifically my parents, who believed in me and supported me through out the duration of this study.

Johan and Donald for their love and support and for sacrificing our precious family time to facilitate the writing of the thesis.

The Lord for blessing me with opportunities to develop my talents and the health and means to complete this project.

Table of Contents

Language and style used in this dissertation are in accordance with the requirements of the *International Journal of Food Science and Technology*. This dissertation represents a compilation of manuscripts where each chapter is an individual entity and some repetition between chapters has, therefore, been unavoidable.

Chapter 1
INTRODUCTION

In the new millennium there is a worldwide shift towards the eating of healthier meats and this has led to increased popularity of ostrich meat, which has less cholesterol and total lipid content and a relatively higher content of polyunsaturated fatty acids than beef (Paleari *et al*., 1997). Within the ostrich industry itself the marketing focus also recently shifted from the leather that for many years has been the most important commodity from the ostrich, towards the meat. Ostriches have been farmed commercially in South Africa for more that a century, but recently the species was also introduced to other countries. While South Africa is still the leading exporter of the meat, it is now world widely available to the public (Lawrie & Ledward, 2006).

Research on ostrich meat, however, is still scanty and the bulk of the information available focuses on the physical eating quality and nutrition of the meat. Published research on the microbiology of ostrich meat and specifically on the expected shelf-life of the meat is not only limited, but in most instances the studies were performed either on meat obtained from the retail, or from previously frozen meat and the data from these studies vary widely (Alonso-Calleja *et al*., 2003; Otremba *et al*., 1999). There is still a need for comprehensive research on the microbiological quality of ostrich meat, the shelf-life, as well as processing technology thereof.

Up to 70% of the ostrich meat consumed internationally is slaughtered in South Africa (Hoffman, 2005), in ten abattoirs approved for the export of ostrich meat to the European Union and other markets. The birds are transported, often over very long distances to be slaughtered in these abattoirs. Despite stringent measures to prevent injuries, the transport and loading practices can often lead to serious injury to the animals, causing either lesions on the skins, still an expensive commodity responsible for a large percentage of the income from the birds, or in the meat, or both. Slaughter and de-boning procedures in these abattoirs were developed in co-operation with the Department of Agriculture (Veterinary services), who also oversee the actions in the abattoirs through on-line meat inspection services and certifying veterinarians.

Because of the fact that ostrich meat became more readily accessible in competitive and well regulated markets: competition arose between processors to be able to put their product on the shelves at the best possible price; while a greater emphasis is also placed upon meat quality and safety. Losses in meat yield from carcasses is one of the factors that can cause processing costs to increase, another is the loss of the meat due to premature bacterial spoilage.

Bruises to carcasses render pieces of meat unacceptable to consumers, either from a food safety perspective where the bruises became infectious, or from an aesthetic perspective. Furthermore injured or stressed ostriches have an abnormally high pH due to glycogen depletion and thus a lower production of lactic acid in the muscles. This in turn causes ostrich meat to better support microbiological growth, spoil more readily and have a shorter shelf-life (Chambers *et al*., 2004). Another contributing factor to loss of meat yield in the abattoir where these studies were completed was the trimming of excessive amounts of meat during the removal of the bruises sustained on ostrich carcasses during primary meat inspection. The standard practice in this and other abattoirs were for the meat inspectors to remove all bruises; large, infected lesions as well as the small (minor) ones from the warm carcasses.

Red meats and specifically ostrich meat, due to the relatively high pH thereof (Hoffman, 1998), is very susceptible to bacterial spoilage. While the ostrich carcasses are sterile when protected with skins (Karama, 2001), as soon as these are removed, carcass contamination sets in. Because ostrich meat is primarily exported and retailed as fresh meat cuts, there is no processing step to reverse the effect of the bacterial contamination in causing meat spoilage, loss of food safety and an adverse effect on the shelf-life of the product. Cold chain management of the meat to below 4ºC during de-boning, packaging and cold storage will only inhibit the growth of microorganisms (Scott & Stevenson, 2006) and not provide a low initial bacterial count and a subsequent acceptable shelf-life (McKinnon *et al*., 2005). The only way to ensure a low initial microbial load is to prevent contamination of the ostrich carcasses during slaughter and handling up to vacuum packing of the product. There can be many sources of this on-line contamination such as from the air supply to the work areas, from the water supply or standing water, from work surfaces or from personnel (Karama, 2001) and further research is warranted to identify the most hazardous of these sources and to be able to effectively manage them.

Therefore studies were initiated to investigate the causes and possible measures to prevent carcass bruising as well as to investigate the best practices in handling the carcasses post-slaughter to ensure better microbiological as well as meat yield results.

The objectives of these studies were firstly to investigate the microbiological organisms deposited on ostrich carcasses on the slaughter-line to give an indication of what types of growth is expected. The possible environmental sources of contamination will also be evaluated to determine which is most detrimental to carcass quality and subsequently meat hygiene. This information will be used to equip abattoir management with information on compiling a hygiene programme to keep the meat safe. Secondly to investigate ways to minimize the loss of utilizable meat due to excessive trimming of bruised meat. In this study the microbiological as well as meat yield advantages of the cold trimming of bruises on carcasses as opposed to the current practice of warm removal will be investigated. Lastly, the frequency and distribution of the bruises on ostrich carcasses will be scrutinized to try to determine what could lead to the incidence of bruising and of course how to prevent the bruising on the carcasses.

REFERENCES

Alonso-Calleja, C., Martinez-Fernandez, B., Prieto, M. & Capita, R. (2003). Microbiological quality of vacuum-packed retail ostrich meat in Spain. *Food Microbiology*, **21**, 241-246.

Chambers, P.G., Grandin, T., Heinz, G. & Srisuvan, T. (2004). Effects of stress and injury on meat and by-product quality. In: Guidelines for Humane Handling, Transport and Slaughter of Livestock. FAO Corporate document repository. [WWW document]. URL. http://www.fao.org/DOCREP/003/X6909E/x6909e04.htm. 28 August 2008.

Hoffman, K. (1988). pH – a quality criterion for meat. *Fleischwirtschaft*, **68**, 67-70.

Hoffman, L.C. (2005). A Review of the research conducted on ostrich meat. In: *Proceedings of the 3rd International Ratite Science Symposium of the WPSA*. Pp. 107-119. October 2005. Madrid, Spain.

Karama, M. (2001). The microbial quality of ostrich carcasses produced at an export-approved South African abattoir. MMedVet (Hyg) study at University of Pretoria. Pretoria, South Africa.

Lawrie, R.A, & Ledward, D.A. (2006). *Lawrie's Meat Science*, Seventh Edition. Woodhead Publishing Limited, Cambridge. Pp. 179-180.

McKinnon, D.C., Heyneke P.G., Olivier, A.J., Mulder, C., Britz, T.J. & Hoffman, L.C. (2005). Effect of ozone and UV on the microbial load of muscle surfaces of ostrich carcasses. *Proceedings of the 3rd International Ratite Science Symposium of the WPSA*. Pp. 276. October 2005. Madrid, Spain.

Otremba, M.M., Dikeman, M.E. & Boyle, E.A.E. (1999). Refrigerated shelf-life of vacuum-packaged, previously frozen ostrich meat. *Meat Science,* **52**, 279-283.

Paleari, M.A., Camisasca, S., Beretta, G., Renon, P., Corsico, P., Bertolo, G. & Crivelli, G. (1997). Ostrich meat: Physico-chemical characteristics and comparison with turkey and bovine meat. *Meat Science,* 48, 205-210.

Scott, V.N. & Stevenson, K.E. (2006). *HACCP A systematic approach to food safety*. 4th Ed. The Food Products Association, 1350 I Street, Washington D.C., 20005.

Chapter 2

LITERATURE REVIEW

A. INTRODUCTION

The exotic taste and the unique physico-chemical properties of ostrich meat has lead to a recent worldwide increase in interest in the bird itself as well as the consumption of the meat. In the story of the golden Camel bird, van Waart (1995) mentioned that in the fairy tale it is the goose that lays the golden eggs, while in the Little Karoo (South Africa's primary ostrich producing area) it is the ostrich. It is indeed true of these desert birds that almost all components are utilized post slaughter. In the past the ostrich feathers, and later the exotic ostrich leather, were the sought after commodities. However, since the turn of the century, more and more emphasis has been placed on the marketing of the ostrich meat (Hoffman, 2005).

Over the last decade commercial ostrich farming has spread too many other parts of the world and as noted by Hoffman (2005), currently, the ostrich meat represents about 45% of the income generated from an ostrich carcass. As the ostrich meat enters the competitive and well regulated international markets an increasingly greater emphasis is placed on the safety and quality of the meat. Ostrich meat is most often exported fresh, and therefore the shelf-life of the products is of great importance. McKinnon *et al.* (2005) reported that a low initial microbial load is the most effective way to ensure proper shelf-life and in order to achieve this, focus on all parameters that influence the microbial quality of ostrich meat, is necessary.

Another factor that can compromise the competitiveness of producers in the international market is losses due to a reduction in meat yield or utilizable meat due to aesthetically or microbiologically unacceptable meat caused by excessive bruising (or the removal thereof) on carcasses (Chambers *et al*., 2004).

The aim of this review is to assess the availability of information regarding the microbial quality of ostrich meat. In this chapter the focus is solely on research conducted in terms of ostrich microbiology and the articles discussed are all specifically about ostriches or as comparisons to other farmed game, exotic meats or red meat. No articles regarding the microbiology of poultry or red meat abattoirs in general will be included. Research on the eating quality, physical quality characteristics and nutritional traits of ostrich meat will not be taken into account (Hoffman, 2005; 2008; Sabbioni *et al*., 2003).

B. MICROBIOLOGY OF OSTRICH CARCASSES

Inherent microorganisms

Microbial information of ostrich meat is limited as only a few studies have been done on these commercially important birds. The quality of meat obtained from ostriches (as with other large game animals and birds) will depend upon the types of microorganisms carried by the ostriches (internally and externally), the conditions at slaughter, and the environment under which the carcass is dressed and butchered. Additionally, the microbial population that develops during storage will also be dependant on storage conditions and the intrinsic biochemical qualities of the meat (Gill, 2007). In a comparison of the meat from large game animals and birds, Gill (2007) concluded that the microbiological quality of farmed game meat is likely to be better than that of meat

obtained from hunted animals. The most important reasons for the difference in meat microbiological quality will lie in the differences in slaughter practices. Firstly wild game is harvested in the fields and poor placement of shots can expose the meat to bacteria both externally (ground and air) or internally (from damaged intestines). Secondly the wild game is often eviscerated in the field where hygiene and carcass chilling facilities are not always readily available or up to required abattoir standards. Farmed game, on the contrary are slaughtered and eviscerated under standardized abattoir conditions and the meat quality thereof can thus be compared with that of domesticated animals (Gill, 2007).

The question that thus arises is: what are the prevalent microorganism groups that can be expected on farmed ostriches and in what numbers are they likely to occur?

Harris *et al*. (1993) conducted a study in a processing plant in Texas, USA and found that the predominant types of microorganisms on the carcasses were environmental bacteria and those that are prevalent on animal and human skins. He reported that *Micrococcus* spp., a widely distributed group of environmental bacteria, were the dominant organism on all carcasses. Karama (2001) found the prevalent microbial groups on ostrich carcasses sampled at different sites down the slaughter-line to include: *Enterobacteriaceae* 57%; *Acinetobacter* spp. 24%; *Pseudomonas* spp. 11%; *Aeromonas* spp. 3%; *Micrococcus* spp. 3%; yeast 1% and *Staphylococcus aureus* 1%.

Once again limited published research is available on the prevalence of possible food borne pathogens (e.g. selected species of *Escherichia coli*, *Salmonella* and *Campylobacter*) on ostrich carcasses. In a study to determine the pathogens present in the ostrich carcass micro-population, Ley *et al*. (2001) reported that even though no *E. coli* O157:H7 were present on the ostrich carcasses sampled, 91% of dressed carcasses were positive for *E. coli*. *Salmonella* was isolated from one carcass and *Campylobacter* was present in 10% of the carcasses. Likewise, Harris *et al*. (1993) isolated pathogenic *Salmonella* from one carcass but found no *Campylobacter* even though this organism was suspected to be present due to its association with poultry carcasses (Scott & Stevenson, 2006). Non-pathogenic *Listeria* was isolated from three carcasses. *E. coli* was also detected by Bobbit (2002) while sampling ostrich carcasses for faecal indicators and food borne pathogens.

Salmonella is considered an important food safety hazard as it predominates in poultry and is one of the mayor hazards identified in HACCP systems in ostrich abattoirs. Whether or not hazards are included in a HACCP study is determined by the likelihood of the hazard occurring in the process and the severity of the outcome if the hazard is not controlled (Scott & Stevenson, 2006). The consequence of a salmonellosis outbreak being severe (Jay, 1992) and the likelihood of an occurrence of *Salmonella* spp. on meat and products in an ostrich abattoir were studied by Gopo & Banda (1997). They analyzed a large number of samples collected from various parts of the carcasses, raw water and wash water from carcasses and feathers, the results there of are shown in Table 1.

Table 1 Incidence of *Salmonella* at an ostrich abattoir, and the numbers and types of ostrich products that were found to be *Salmonella* positive (Gopo & Banda, 1997)

Sample type			Number tested	Number positive	Percent positive
1.	Water: (a) Raw water		51	17	33.3
	(b) Water before wash (treated water)	58	0	0	
2.	Water after wash (treated water) (a) Feathers		120	61	50.8
	(b) Carcasses		120	40	33.3
3.	Faeces		120	53	44.2
4.	Heart		70	0	0
5.	Liver		70	0	0
6.	Gizzards		100	5.0	5.0
7.	Fillet		120	0	0
8.	Skin		120	10	8.3
9.	Bloodmeal		120	5.0	4.2
10.	Meat and bone meal		120	0	0
11.	Small intestines	120	19	16.1	
12.	Large intestines	120	31	26.2	
		Total	**1429**	**241**	**16.9%**

They reported that of the samples tested, 16.9% were positive for the presence of *Salmonella* (Table 1) and that 50.8% of ostriches delivered to the abattoir and 33.3% of carcasses on the processing line also tested positive. This indicated that ostriches may already have been contaminated during rearing on the farm, during transport, or even at the slaughter-house. In this study they did not differentiate between Salmonella species to determine if pathogenic strains were present.

All *Salmonella* positive products were either by-products, intestines or faeces. Products which tested negative for *Salmonella* spp. included the liver, fillet steak and meat-and-bone-meal. The study concluded that most of the edible meat products from ostriches were free from *Salmonella.* This corresponds with the findings of Ley *et al*. (2001) and Harris *et al*. (1993).

Expected pH of the meat

One of the intrinsic parameters of foods that determine how effective certain bacteria can grow on these foods is the pH thereof. Most bacteria grow best at a neutral pH of around 7.0 (Jay, 1992). Therefore it is important to establish the pH of ostrich meat and its influence on microbial growth on the meat. Sales & Mellet (1996) found the pH of ostrich meat to be intermediate to high; between normal (pH <5.8) and extreme dark, firm and dry (DFD) (pH >6.2). The average values (24 h *post-mortem*) of the six muscle groups evaluated by the authors varied between 5.84 and 6.13. Most spoilage bacteria grow well at pH values higher than 5.8 (Alonso-Calleja *et al.,* 2003) and this inherent characteristic will thus cause ostrich meat to spoil easily. It can thus be assumed that

ostrich meat will have a limited shelf-life (Hoffman, 1988). It has also been reported (Scott & Stevenson, 2006) that most food borne pathogens prefer to grow at pH values above 6. Paleari *et al*. (1997) found the mean pH of ostrich meat to be 5.86 ± 0.35 which was slightly higher than that of beef (Blixt & Borch, 2002).

It can thus be expected, based on the higher pH values, that ostrich meat will support the growth of bacteria to a greater extent than other red meats and that the shelf-life might thus be shorter. It is thus important that care should be taken to prevent the cross contamination of ostrich meat in order to ensure a low initial microbial load.

Influence of transport and lairage practices on the microbial load of ostriches

Research was performed at a South African export approved abattoir by Burger *et al*. (1995) on the difference in microbiological quality of ostrich meat after lairage of the birds on two different types of surfaces. In this study two groups of ostriches were kept for approximately 24 h in lairage at the abattoir; one group in pens with clean river sand as flooring; the other in pens with cement flooring. The ostriches were slaughtered under identical conditions and meat was sampled after overnight chilling in cold rooms. No statistically significant differences were found between the aerobic plate counts on the meat from the ostriches penned on sand or cement.

Van Schalkwyk *et al*. (2005) reported on the effect of feed withdrawal lairage on meat quality characteristics in ostriches. After evisceration the mass of the full stomachs and the stomach contents of the stressed groups (feed deprived) were found to be lower than that found for the control group, but the mass of the full alimentary tract and the alimentary tract contents were slightly higher for the stressed group. It was thus suspected that feed withdrawal will reduce the risk of carcass contamination at evisceration due to decreased viscera volume that prevents the puncturing of the intestines. There was, however, a significant difference in intra-muscular pH between the control and the stressed groups in the study of van Schalkwyk *et al*. (2005). At one hour *post-mortem*, the readings of the stressed birds were 0.22 units higher and after 26.5 h in the cold room the readings were 0.25 units higher than the control. These high pH values (between 6.03 and 6.46) in the stressed group could make the meat of the stressed birds more susceptible to microbial growth and could be indicative of meat with a shorter shelf-life (Hoffman, 1988).

Ostriches defecate more readily during penning (Burger *et al*., 1995) and the subsequent soilage of the hides is one of the main contributors of bacteria of faecal origin on ostrich meat. At South African abattoirs, the birds also have unrestricted access to drinking water (Van Schalkwyk *et al*., 2005); too much water leads to an increase of alimentary tract volume which complicates evisceration and often leads to contamination of carcasses through rupturing of the full intestines.

Fasone & Priolo (2005) reported that ostriches which had been stressed from both transport and lairage practices had a significantly higher ultimate pH (6.95 vs 5.94) than the unstressed control group. This corresponds well with results found in practice for birds delivered stressed at the abattoir where the research for the rest of this study was conducted. The unstressed control group values reported by Fasone & Priolo (2005) correspond to those reported by Sales & Mellet (1996) and Paleari *et al*. (1997). Crowther *et al.* (2002) reported that ostriches are markedly less stressed when transported at night rather than during the day. On the basis of the above data it can be assumed that proper management of transport and lairage practices to minimize stress on the birds will result in a lower ultimate pH in the meat and better holding quality. Very little research was

found in the literature on the effect of transport practices on ostrich meat quality or microbiology and this field requires attention.

The causes of bruising and the influence on microbial load

Ostriches are often transported over long distances to slaughter-houses and the transporting on trucks, the on and off-loading from the trucks and lairaging at the abattoir has proven to be the most common causes for bruising on livestock carcasses (Grandin, 1990; 1991). In addition to the usual hazards for livestock transportation, ostriches have the added disadvantages that they are; bipedal, have two-toed feet and a high centre of gravity, which all contribute to ostriches having trouble in keeping their balance on the trucks (Wotton & Hewitt, 1999). Thus, the ostriches have a tendency to sit down during transport, this lead to severe injuries due to trampling in the confined truck partitions or in the pens.

Producers, transporters and abattoir management in South Africa adhere to strict animal welfare codes (SAOBC, 2001) regarding the treatment of ostriches during transport and pre-slaughter practices, to prevent unnecessary bruising or damage to the skins. The preventative measures during transport include: keeping the birds calm; keeping to prescribed numbers of birds per truck partition; having handlers travel with the birds on the trucks and designating experienced drivers for the trucks. Furthermore the trucks, loading areas and pens are constructed with rounded corners, no protruding elements and slip free flooring. Despite these measures, Wotton & Hewitt (1999) reported lacerations and bruises on the necks and lower legs were common on ostriches delivered to South African abattoirs.

Wotton & Sparrey (2002) reporting on these precautionary measures taken during transport and handling at a South African abattoir, highlighted the serious damage that can be inflicted to both skins and meat by kicking, bruising or fresh wounds. They reported that animal welfare was found to be of prime importance and that ostriches with fresh wounds would often be returned to the farms to heal.

In a FAO document (Chambers *et al*., 2004) with reference to all livestock species, including ostriches, on the effects of stress and injury on meat quality, it was indicated that because of glycogen depletion during transport and pre-slaughter stress, there is little lactic acid production in the muscles and this caused the meat pH to be higher than ideal. This higher pH would then better support microbial growth and the meat from animals that were stressed, injured or diseased before slaughter will have a shorter shelf-life. The FAO document indicated that bruised meat is wasted due to aesthetic unacceptability to consumers and the fact that it decomposes and spoils rapidly due to the bloody meat that is an ideal growth medium for bacteria. This is then the reason for the removal or trimming of these bruises during primary meat inspection, this practice, if not well controlled, can also lead to unforeseen losses in meat yield.

Very little published research was found evaluating the influence of these bruises on the microbiology of the ostrich meat or the aesthetic acceptability thereof and this warrants further investigation.

Influence of slaughter practices on microbial load

In the ostrich industry slaughter facilities often slaughter and dress animals of more than one species. Gill *et al*. (2000) found that in comparing the microbial load after the dressing of ostriches and that of other animals, that the dressing of each species should be regarded as a unique process. The specific method used for skinning

and eviscerating the carcasses of a certain type of animal can contain the bacterial contamination on those carcasses, but the same procedure will most probably not be effective in preventing bacterial contamination in the dressing of a different species such as an ostrich carcass. Processors should know how to control the hygienic quality of the process to slaughter each type of animal handled in their abattoirs to minimize interspecies as well as extra species contamination (Gill *et al*., 2000). Research regarding cross contamination between ostriches and other species handled in the same facility is lacking and warrants further investigation as there are a number of abattoirs practicing lairage of two or more species in close proximity to each other (Gill *et al*., 2000).

To be able to effectively control slaughter practices and ensure ostrich meat of low initial microbial load, consideration has to be taken of which steps in the slaughter process are most hazardous in increasing the bacterial load and which steps can control or minimize the load effectively. Research by Karama (2001) suggested that most of the indicator organisms were already deposited during the flaying step and will thus be derived directly or indirectly from the hides. This was concluded from the data indicating that there was no significant change in the log cfu.cm^{-2} values for aerobic plate counts (APC) (4.32, 4.21 and 4.57), *Stahylococcus aureus* (2.89, 2.90 and 2.38) and *Enterobacteriaceae* (2.55, 2.78 and 2.73) from post-flaying to post-evisceration and post-chilling. This confirms the results of Harris *et al*. (1993). The high percentage of samples found to be positive for *E. coli* (53% of the 17 out of 90 positive isolates) and *Salmonella* (±45% of the ±25 out of 90 positive isolates, there was a slight variation between results on different types of media) on post-evisceration samples indicated that this is the process step most likely to add faecal contamination if it is not controlled. Further more, overnight chilling of carcasses between 0 - 4°C did not significantly reduce or increase microbial counts, except for psychrophilic micro-organisms (*Pseudomonas* spp. (post-flaying = 2.82, post-evisceration = 2.86 and post-chilling = 3.75)) which increased. Severini *et al*. (2003) investigated the influence of different skinning and dressing procedures on the microbial load of ostrich carcasses. He found that the skinning method assisted by mechanical air inflation did not negatively affect microbial quality (Table 2) and that currently the practice is not considered or forbidden under European Union (EU) legislation.

The EU previously only permitted the rinsing of red meat and poultry carcasses with potable water. In January 2004 new hygiene laws were promulgated (Anon., 2004), providing a legal basis to permit the use of substances other than potable water to remove surface contamination from products of animal origin. The EU Commission is also considering lifting an 11 y ban on imports of USA poultry rinsed in chemicals (phosphate, acidified chlorite, chlorine dioxide or peroxyacid) stating that these chemicals do not pose a risk to human health (Rne, 2008). The South African legislation (Anon., 2007a) allows carcass wash with potable water, while the use of any anti- microbial agents is only permissible with the approval per individual case from the provincial executive officer. It would thus be worthwhile to investigate the different methods of washing of ostrich carcasses in pursuing a low post-evisceration microbial load. Severini *et al*. (2003) commented on final carcass wash (without addition of anti-microbial substances) after dressing and reported that it could have a positive effect in lowering carcass surface microbial load, but that more research on this practice is required. In the study performed by Gill *et al*. (2000) carcasses from all six species under investigation were washed with water at 50ºC from a spray nozzle. The final mean log cfu.cm^{-2} APC value of 2.15 on ostrich carcasses was lower than those reported above (Karama, 2001; Harris *et al*., 1993) and could indicate that the procedure is effective in

lowering microbiological counts. Maunsell & Bolton (2004) discussed different methods of carcass decontamination including: vacuum cleaning; hot water washing while vacuum cleaning; spraying with low concentration lactic acid and hot water or steam pasteurization. They reported that these practices were common in USA abattoirs, but not in the EU; this report however, focuses on the meat industry as a whole and not specifically on ostriches. Huffman (2002) discussed current and future carcass decontamination techniques of livestock carcasses and listed post-harvest techniques including hot water rinsing, steam pasteurization, chemical rinses, lactoferrin and combined treatments (hurdle technology). Under chemical treatments, he specifically listed the organic acids, such as acetic, lactic and citric acids approved by the USDA in concentrations of 1.5 to 2.5%. In New Zealand, rinsing of ostrich carcasses are common practice and their processing standards (Ostrich and Emu Standards Council, 2002) prescribed both a pre-evisceration and a post-evisceration (final) carcass wash with either potable water or a sanitizer solution. The use of low concentration organic acids is gaining popularity in red meat abattoirs in New Zealand, Australia and the USA, but unfortunately no published research on the success of these substances in ostrich carcass rinsing is available and this warrants further research.

Expected levels of microbial load on ostrich carcasses before packaging

Research by several groups has reported on the APC and levels of other indicator organisms on ostrich carcasses (Harris *et al*., 1993; Gill *et al*., 2000; Karama, 2001). A comparison of the APC values is a useful tool to evaluate microbiological quality and thus the level of hygiene attained in an ostrich abattoir. Further more it can be indicative of the expected shelf-life of the meat and facilitate evaluating whether the regulatory microbiological level which was specified for other red meats is also attainable for ostrich meat.

Physical values reported by Harris *et al*. (1993) were: APC averaging 4.0, 3.2 and 3.6 log cfu.cm^{-2} on three groups of carcasses, whilst those by Karama (2001) were slightly higher (4.32, 4.21 and 4.57 log cfu.cm^{-2} respectively, for carcasses post-flaying, post-evisceration and post-chilling) and those found by Gill *et al*. (2000) were markedly lower (2.98 log cfu.cm^{-2}).

The results of the study by Karama (2001) showed higher log mean values than for other studies under review on the indicator organisms (also commented on by Gill, 2007). Bobbit (2002) reported a shelf-life of four weeks for vacuum packed ostrich meat with an initial APC of <3 log cfu.g^{-1}. From the above data it can be concluded that ostrich carcasses slaughtered and dressed under proper process control is expected to carry a microbial load of between ±3.0 and 4.5 log cfu.cm^{-2}. South Africa contributes up to 70% of the ostrich meat produced internationally (Hoffman, 2005). The largest volume (more than 90%) of the ostrich meat produced in South Africa is exported (SAOBC, 2007) to the European Union (EU) and thus the expected initial microbial load on carcasses falls well with-in the limits specified by the EU regulation (Anon., 2007b) for red meats of APC 3.5-5.0 log cfu.cm^{-2}.

C. MICROBIOLOGY OF PACKAGED OSTRICH MEAT

The handling and packaging of ostrich meat cuts after de-boning will influence the microbiological population in the meat as well as the numbers in which the organisms are present; this will differ from microbiological results

reported for ostriches on the slaughter-line. Ostrich meat is most often vacuum packed and sold at refrigerated temperatures (Alonso-Calleja *et al*., 2003; Hoffman, 2008); both practices are intended to protect the meat against spoilage and thus increase its keeping quality (Capita *et al*., 2006). Research conducted on the effectiveness of vacuum packaging, levels of oxygen (O_2) exclusion and low temperature maintenance in controlling microbial growth on ostrich meat will be discussed further.

Microbial growth and the pH of vacuum packed ostrich meat

Ostrich meat is vacuum packed to suppress the growth of aerobic bacteria and subsequently to prevent spoilage due to primarily *Pseudomonas* spp. when stored at chilled temperatures. Alonso-Calleja *et al*. (2003) investigated microbial levels of retail refrigerated vacuum packed ostrich steaks in Spain as well as the influence of final pH on the bacterial levels of the meat. The data summarized in Table 2 show the microbial counts in both $\log_{10}$ cfu.g^{-1} and $\log_{10}$ cfu.cm^{-2}. They found that, contained in the total aerobic growth, there were significantly more mesophilic than phychrophilic bacteria. However, this did not correlate well with previous studies (Capita *et al.*, 2001) and the difference was ascribed to either possible temperature abuse of the retail products or possibly to the relatively short (2-7 d) vacuum packaged storage time. The APC of >7 $\log_{10}$ cfu.g^{-1} was high when compared to other studies (Otremba *et al*., 1999; Capita *et al*., 2006). This can once again be ascribed to a possible break in the cold chain.

Table 2 Microbial counts ($\log_{10}$) in retail ostrich meat fillet steaks (Alonso-Calleja *et al.*, 2003)

Variable	Log Mean Values	
Aerobic Plate Count 30°C	7.32[a]	6.69[b]
Aerobic Plate Count 37°C	7.09	6.45
Psychrotrophs	6.62	5.98
Pseudomonads	6.05	5.42
Fluorescent Psudomonads	3.29	2.66
Enterobacteriaceae	5.29	4.66
Enterococci	0.86	0.22
Lactic Acid Bacteria	6.86	6.23
Yeast and Moulds	4.90	4.27

[a] $\log_{10}$ cfu.g^{-1}

[b] $\log_{10}$ cfu.cm^{-2}

The significantly higher counts than that found in the studies of Harris *et al*. (1993) and Karama (2001) can be ascribed to the fact that the samples in these two studies were taken on whole carcasses and thus before de-boning, portioning, packing, storage and distribution. These actions all entail contact with personnel, work surfaces and packaging that could add to microbiological cross contamination, if the cold chain is not properly maintained during these periods, already deposited organisms could also grow and replicate. These actions could thus lead to an increase in APC.

In this study (Alonso-Calleja *et al*., 2003) samples were purchased from retailers on between 3-7 d after packaging and were immediately analyzed. Pseudomonads accounted for a fairly low percentage of the APC (±28%) at the time of sampling; this was expected, taking into account the decrease in pseudomonas throughout storage on vacuum-packed meat (Lawrie & Ledward, 2006). The lactic acid bacteria (LAB) were found to be the most abundant of all bacterial groups and comprised ±37% of the total counts on the meat. These findings supported the expected negative correlation between pseudomonads and LAB in vacuum-packed meat; where the respiratory pseudomonads will probably be inhibited and the microbial population's composition will shift to a more facultative anaerobic population including the LAB (Gram *et al*., 2002). The LAB's were the prevalent group of bacteria and this corresponds with literature on refrigerated vacuum-packed red meats where a dominance of this bacterial group was indicated (Jay, 1992; Blixt & Borch, 2002). All samples with LAB levels of more than 7 $\log_{10}$ cfu.g^{-1} were found to have off-odours, which also corresponds with results from the literature that state that 7 $\log_{10}$ lactobacilli.g^{-1} is the limit for perception of off-odours in vacuum-packed meat.

Alonso-Calleja *et al*. (2003) reported a positive correlation between high pH values and high microbial levels, with the lowest microbial loads found on meat with a pH ≤ 5.8. The influence of pH on microbial load of refrigerated vacuum-packed ostrich meat suggests a positive benefit of ensuring a low final pH in ostrich products so as to improve the quality of these products. The pH observed (6.00 ± 0.39) was similar to that reported in other ostrich studies (Sales & Mellet, 1996; Paleari *et al*., 1997).

The positive correlation between high pH values and high levels of microorganism growth was also reported by Gill & Gill (2005) who found that the storage life of vacuum packed, chilled meat depends on the extent of contamination with spoilage organisms at the time of packaging as well as the meat pH. They also found that bacteria with high spoilage potential can grow rapidly on muscle tissue at pH > 5.8 and thus can cause early spoilage of the vacuum packaged meat.

The influence of O_2 exclusion and temperature control on the microbial population

Capita *et al*. (2006) performed a study to compare the microbial levels of ostrich steaks packaged under vacuum or under aerobic atmosphere and then stored for 9 d at different temperatures. They showed that both the specific temperature and oxygen exclusion proved to be critical factors on most bacterial groups. The meat was divided into two groups, where one was packed in air, and the other packed under vacuum in bags with a low O_2 transmission rate. Half the packs of each group were stored at 4°C and the other half at 10°C. On days 0, 3, 6 and 9 packs from each group were analyzed for pH and microbial counts. Part of the data obtained is shown in Table 3.

From Table 3 where total viable counts are summarized, it can be seen that the storage temperature had a significant influence on microbial counts; this was also the main barrier to microbial growth up to day 6. This highlights the importance of maintaining the cold chain right from the packaging of the ostrich meat in order to inhibit microbial growth and thus assists in achieving a proper shelf-life for the meat. It was found that oxygen exclusion had a significant influence on the APC, psychrotrophics, *Pseudomonas* and, fluorescent *Pseudomonas* counts, and on the pH values, which had shown a favourable decrease in levels when the meat was vacuum packed (Table 4). It was also found that at the end of the storage period the samples packed under vacuum showed lower counts than those packaged in air at both temperatures (Capita *et al*., 2006).

Table 3 Total of aerobic viable counts (log_{10} cfu.g^{-1}) obtained over a storage period of 9 d on ostrich meat steaks packed and stored under different conditions (Capita *et al.*, 2006)

Temperature & O_2 exclusion	Storage (days)			
	0	3	6	9
Air – 4°C	5.4 ±0.7$^{a}_{a}$	5.9 ±1.5$^{a}_{a}$	8.5 ±1.2$^{b}_{a}$	10.2 ±0.6$^{c}_{a}$
Air - 10°C	5.4 ±0.7$^{a}_{a}$	9.3 ±0.6$^{b}_{b}$	10.1 ±0.6$^{c}_{b}$	9.8 ±0.5$^{bc}_{ab}$
Vacuum - 4°C	4.9 ±0.2$^{a}_{a}$	6.4 ±0.9$^{b}_{ac}$	7.1 ±1.5$^{bc}_{c}$	8.0 ±1.3$^{c}_{c}$
Vacuum - 10°C	4.9 ±0.2$^{a}_{a}$	7.7 ±1.9$^{b}_{c}$	9.0 ±0.4$^{b}_{ab}$	8.7 ±1.6$^{b}_{bc}$

Results are reported as log means ± standard deviation (n=6). Means in the same row (same processing) that are not followed by the same letter (superscript) are significantly different ($P < 0.05$). Means in the same column (same sampling time) that are not followed by the same letter (subscript) are significantly different ($P < 0.05$).

The data also showed that the influence of oxygen exclusion is only seen after day 3 of storage. From the above data it is evident that a combination of both temperature control and oxygen exclusion is required to inhibit microbial growth in packaged ostrich meat.

Table 4 pH values throughout storage on ostrich meat steaks packed and stored under different conditions (adapted from Capita *et al*., 2006)

Temperature & O_2 exclusion	Storage (days)			
	0	3	6	9
Air - 4°C	6.7 ±0.3$^{a}_{a}$	6.8 ±0.2$^{ab}_{a}$	6.9 ±0.2$^{b}_{ab}$	6.8 ±0.2$^{ab}_{a}$
Air - 10°C	6.7 ±0.3$^{a}_{a}$	6.9 ±0.1$^{bc}_{a}$	7.0 ±0.2$^{c}_{a}$	6.7 ±0.1$^{a}_{ab}$
Vacuum - 4°C	6.7 ±0.2$^{ab}_{a}$	6.8 ±0.2$^{ab}_{a}$	6.8 ±0.1$^{a}_{b}$	6.6 ±0.2$^{b}_{ab}$
Vacuum - 10°C	6.7 ±0.2$^{ab}_{a}$	6.9 ±0.1$^{a}_{a}$	6.4 ±0.3$^{c}_{c}$	6.6 ±0.4$^{bc}_{b}$

Results are reported as log means ± standard deviation (n=6). Means in the same row (same processing) that are not followed by the same letter (superscript) are significantly different ($P < 0.05$). Means in the same column (same sampling time) that are not followed by the same letter (subscript) are significantly different ($P < 0.05$).

D. SHELF-LIFE OF OSTRICH MEAT

Expected shelf-life

Retail packs of fresh refrigerated vacuum-packed portioned ostrich meat in South Africa are labelled with a shelf-life of between 21 and 40 d. For minced ostrich meat the period is between 10 and 21 d (R Dempsey, Klein Karoo International, Oudtshoorn, South Africa, personal communication). Bacterial spoilage, and thus, the end of the shelf-life are defined as the time in days after packaging, when spoilage organisms (specifically *Pseudomonas* spp. in aerobically stored meat and *Lactobacillus* spp. in vacuum-packed meats) reach levels of ≥10^7 cfu.cm^{-2} or cfu.g^{-1} (Bobbit, 2002).

Bobbit (2002) while performing a validation study for the Australia ostrich industry on vacuum-packed ostrich primal cuts allotted a four week shelf-life (28 d) at 4°C to meat of good initial microbial quality where the levels

were of 3 log cfu.g^{-1} APC. These results confirmed what McKinnon *et al*. (2005) reported regarding the low initial microbiological counts being a pre-requisite for attaining a shelf-life that would be acceptable to the industry (more than four weeks).

Otremba *et al*. (1999) studied the shelf-life of vacuum packed, previously frozen ostrich meat steaks and mince in the USA. They observed that there was no significant increase in APC counts from day 0 to day 7, but a significant increase was found from day 7 to day 28 on both the intact and the minced meat. The initial APC counts for intact steaks was low (2 log cfu.cm^{-2}) and stayed below 7 log cfu.cm^{-2} for up to 21 d and reached 7.2 log cfu.cm^{-2} by day 28, corresponding with the 28 d shelf-life of Bobbit (2002). The APC for minced meat peaked at 6.1 log cfu.cm^{-2} at day 21, thus the minced meat attained a longer shelf-life period than the intact muscles. From literature (Jay, 1992) it was expected that the minced meat would have higher APC levels and thus, a shorter shelf-life than intact muscles, primarily because of excessive handling and a greater surface area of minced meat compared with that of intact muscles. The resulting pH of each product may be the reason for the lower final APC of the minced meat (Fig. 1). There was a greater decrease in pH of the minced meat after day 14 (from pH 6.25 to 5.7) than for the intact meat (increased from pH 6.35 to pH 6.4). The minced meat also had slightly higher counts of LAB and a resulting drop in pH that could inhibit the growth of the aerobic bacteria – this drop was larger than expected (from pH 6.4 at 3 d to pH 5.7 at 28 d).

Taking all other parameters into account, Otremba *et al.* (1999) concluded that refrigerated, previously frozen, vacuum-packed ostrich meat should be used within 10 d. In contrast, Seydim *et al*. (2006a) found that ground ostrich meat was below saleable quality in less than 6 d. In their study oxidation seemed to be the limiting factor for shelf-life of ground ostrich meat and a shelf-life of <3 days was therefore suggested.

There is a large variation between results from different studies and also between these results and those commonly found in the ostrich industry. Thus, further research is warranted to give a more realistic guideline on the expected shelf-life of vacuum-packed ostrich meat and other meat products.

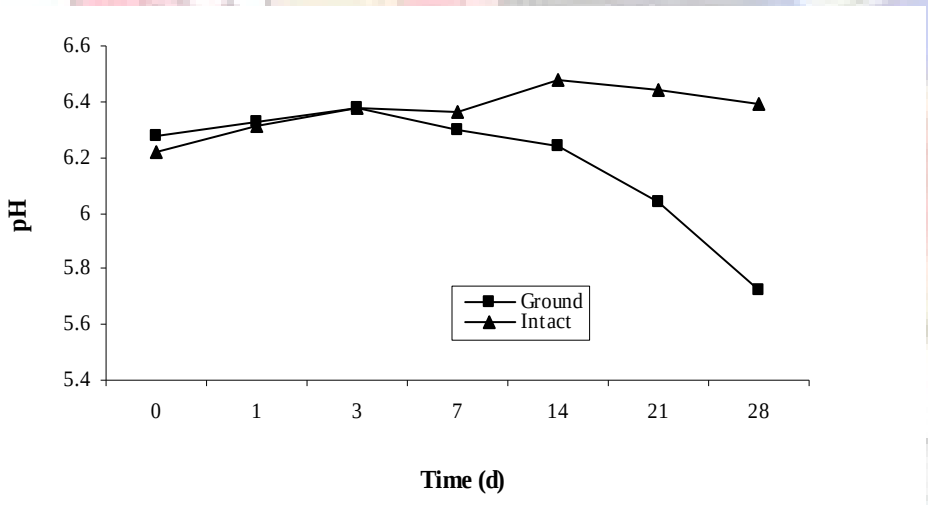

Figure 1 pH of intact vacuum-packaged ostrich steaks and ground ostrich meat during storage at 0°C (Otremba *et al*., 1999)

Interventions on ostrich meat shelf-life

Research on the impact of packaging atmosphere on the shelf-life of minced ostrich meat was reported by Seydim *et al*. (2006a) who stated that modern meat packaging techniques are employed to maintain meat quality. They also found that product shelf-life can be extended by inhibiting microbial growth through micro-environment manipulation. In the food industry this is attained through vacuum and modified atmosphere packaging (MAP). MAP is generally divided into two categories: low oxygen modified atmosphere (including vacuum packing, CO_2 gas flushing, N_2 gas flushing); and high oxygen modified atmosphere.

In the study of Seydim *et al*. (2006a), the shelf-life of the minced meat packed under different atmospheres was evaluated in terms of pH changes, microbial quality, oxidative changes and other shelf-life parameters. They packaged the samples under four different conditions namely, high oxygen (O_2), high nitrogen (N_2), vacuum (VAC) and ambient air (AIR). The initial average pH of the meat was 6.15 and did not change significantly over time of storage for the meat under different atmospheres. The pH values correspond to data obtained by Capita *et al*. (2006) and Seydim *et al*. (2006b). Seydim *et al*. (2006a) however, did note a slight decrease in pH which was slightly larger for vacuum and N_2 samples (the difference in pH values between those for O_2 and air and that for N_2 and VAC was significant with $P \leq 0.05$) which can possibly be ascribed to LAB increases and thus a lower pH. Microbial counts in all four the groups (O_2, N_2, VAC & AIR) increased with storage time where it was found that the initial APC values were 4 $\log_{10}$ cfu.g^{-1} with an increase to 7.8 $\log_{10}$ cfu.g^{-1} by day 6. It was also reported that all packaging atmospheres showed generally similar effects on microbial growth, but the differences found did not support the practical selection of one system over the other.

Seydim *et al.* (2006b) found that sodium lactate, alone or in combination with rosemary extracts, was effective in inhibiting bacterial growth on ostrich minced meat and provided a 2-log reduction in microbial counts over the storage period of 9 days. Rosemary extract on its own did not display a significant impact on bacterial growth.

The use of ultra violet rays (UV) and ozone (O_3) in chillers for overnight chilling of ostrich carcasses were found to reduce the aerobic viable counts and *Enterobacteriaceae* counts by more than 90% and could be an effective method to enhance the shelf-life of ostrich meat (McKinnon *et al*., 2005). However, the practical application and cost effectiveness of these techniques still require evaluation and confirmation.

E. QUALITY OF OSTRICH MEAT

Microbiological quality under different conditions – hot or cold deboned

In the literature (Botha *et al*., 2007; Hoffman *et al*., 2006) articles on meat quality under different de-boning conditions do not directly deal with the microbial quality of the meat, but all gave the pH and indications of the expected microbial population. In normal ostriches the muscle pH varies between individual carcasses. This is a phenomenon that might be due to the intrinsic variation between ostriches and the different levels of *anti-mortem* stress and consequent levels of *post-mortem* glycogen in muscles (Botha *et al*., 2007). In Botha's study it was also found that 24 h *post-mortem* there was not a significant difference in the pH of hot (5.8±0.1) and cold (5.86±0.08) deboned and vacuum packed ostrich meat. During cold storage for 21 d the pH for both groups of meat followed the same trend and was found to be just above pH 5.7 by the end of the period. This could

indicate that deboning under different conditions will not influence microbial quality of ostrich meat on the basis of pH variation. However, as Botha reported the difference in the rate of *post-mortem* temperature decline and the resulting difference in the pH after 24 h between hot and cold deboned ostrich meat warrants further research.

Hoffman *et al.* (2006) studied the muscle pH changes in hot and cold deboned meat and reported that hot deboned muscles take longer to reach the point of minimum pH than the intact muscles, but there is not a significant difference in the final pH reached (5.91 ± 0.26). After an initial decrease to a minimum, the pH increased again to on average >6.0 after 22 h. The reason for this increase warrants further investigation because if this can be avoided, it could lead to better keeping quality because of the lower pH.

F. DISCUSSION

From the research reviewed on the microbiological quality of ostrich meat, the following can be concluded: The microbial population on ostrich carcasses is influenced by pre-slaughter handling, skinning and evisceration practices. Most of the bacteria are deposited initially during flaying and secondly, during evisceration. Based on the literature these processes should be specifically adapted for the dressing of ostrich carcasses to prevent unnecessary contamination. The effectiveness and legality of rinsing carcasses with anti-microbial substances to reduce the microbial load post-evisceration needs to be investigated. The impact of transport and pre-slaughter handling and subsequent bruising as well as the trimming of the affected meat on the microbial quality of the meat is not given in the literature and it is essential that this be evaluated. Whether the slaughter and lairaging of ostriches and other species in the same abattoir would lead to cross contamination between the species also needs to be evaluated.

In the literature it was shown that overnight chilling of the carcasses post-slaughter only inhibits growth of bacteria but it does not reduce the number of organisms present. The expected dominant growth is of bacteria found on the skins of the birds and humans and these consist mostly of *Micrococcus* spp, *Enterobacteriaceae* and *Acinetobacter* spp. The only commonly isolated foodborne pathogen on ostrich carcasses so far reported was *E. coli*. Comprehensive research on the expected microbial population and the effect of slaughter practices is, however, minimal and the prevalent growth on ostrich carcasses as well as the influence of environmental contaminants must be further investigated.

The levels of the aerobic organisms present on carcasses as reported in several studies were found to vary from 2.5 to 4.5 $\log_{10}$ cfu.cm^{-2}. These values are higher than the average counts from carcasses found on routine tests in commercial export abattoirs in South Africa (between 2 and 3 $\log_{10}$ cfu.cm^{-2} on average). Thus, a more focused study on expected levels of organisms is warranted so as to make predictions on shelf-life and conformance to specifications.

In all research on ostrich meat the pH of the meat was found to be on average between 5.8 and 6.0 and often even higher. This pH might be more conducive to microbial growth and indicates that the meat may thus in turn be more susceptible than other red meats to microbial growth and subsequent spoilage. Thus, hygiene practices in the abattoirs and cutting plants to prevent cross contamination is of the utmost importance to ensure a good final shelf-life. Much of the research on the shelf-life of ostrich meat was done on meat from retail outlets

or on previously frozen meat, but more information on the expected shelf-life of fresh ostrich meat is warranted. Research on the microbiology and shelf-life of ostrich meat products is also lacking.

G. REFERENCES

Alonso-Calleja, C., Martinez-Fernandez, B., Prieto, M. & Capita, R. (2003). Microbiological quality of vacuum-packed retail ostrich meat in Spain. *Food Microbiology*, **21**, 241-246.

Anonymous (2004). Laying down specific hygiene rules for the hygiene of foodstuffs. Regulation (EC) No 853/2004 of the European Parliament and of the Council. URL http://www.eur-lex.europa.eu/. 4 April 2008.

Anonymous (2007a). Ostrich Regulations under the Meat Safety Act. R54, G.N.R. 29559/2007 02 02. Johannesburg, South Africa: Lex Patria Publishers.

Anonymous (2007b). Microbiological criteria for foodstuffs. Commission Regulation (EC) 1441/2007 amending Regulation (EC) No 2073/2005. URL http://www.eur-lex.europa.eu/. 4 April 2008.

Blixt, Y. & Borch, E. (2002). Comparison of shelf life of vacuum-packed pork and beef. *Meat Science,* **60**, 371-378.

Bobbit, J. (2002). Shelf life and microbiological safety of selected new and emerging meats – destined for export markets. A report for the Rural Industries Research and Development Corporation, Level 1, AMA House, 42 Macquarie Street, Barton ACT, 2600.

Botha, S.St.C., Hoffman, L.C. & Britz, T.J. (2007). Physical meat quality characteristics of hot-deboned ostrich (*Struthio camelus var. domesticus*) *Muscularis gastrocnemius, pars interna* during post-mortem aging. *Meat Science,* **75**, 709-718.

Burger, W.P., Peyrot, B., Bekker, A., Swart, B., Theron, L.P., De Jesus, A.E. & Van Zyl, E. (1995). Microbiological assessment of two methods of ostrich lairage – on sand and cement flooring at an ostrich abattoir. Directorate of Veterinary Public Health, Private Bag X138, Pretoria, 0001, South Africa.

Capita, R., Alonso-Calleja, C., García-Fernández, M.C., Moreno, B., 2001. Microbiological Quality of retail poultry carcasses in Spain. *Journal of Food Protection*, **64**, 1961-1966.

Capita, R., Diaz-Rodriguez, N. Prieto, M. & Alonso-Calleja, C. (2006). Effects of temperature, oxygen exclusion, and storage on the microbial loads and pH of packed ostrich steaks. *Meat Science*, **73**, 498-502.

Chambers, P.G., Grandin, T., Heinz, G. & Srisuvan, T. (2004). Effects of stress and injury on meat and by-product quality. In: Guidelines for Humane Handling, Transport and Slaughter of Livestock. FAO Corporate document repository. [WWW document]. URL. http://www.fao.org/DOCREP/003/X6909E/x6909e04.htm. 28 August 2008.

Crowther, C., Davies, R. & Glass, W. (2002). The effect of night transportation on the heart rate and skin temperature of ostriches during real transportation. *Meat Science*, **64**, 365-370.

Dempsey, R. (2008). Klein Karoo International (KKI) Meat Marketing, Oudtshoorn, South Africa. Personal communication.

Fasone, V. & Priolo, A. (2005). Effect of stress on ostrich meat quality. Proceedings of the 3rd International Ratite Science Symposium of the WPSA, Madrid, October 2005.

Gill, C.O. (2007). Microbiological conditions of meats from large game animals and birds. *Meat Science,* **77**, 149-160.

Gill, A.O. & Gill, C.O. (2005). Preservative packaging for fresh meats, poultry and fin fish. In: J.H. Han, *Innovations in Food Packaging*. Pp.204-226. Amsterdam: Elsevier.

Gill, C.O., Jones, T., Bryant, J. & Brereton, D.A. (2000). The microbiological conditions of the carcasses of six species after dressing at a small abattoir. *Food Microbiology*, **17**, 233-239.

Gopo, J.M. & Banda, G.N. (1997). Occurrence of Salmonella on meat and products in an ostrich abattoir determined with a DNA probe. *South African Journal of Animal Science*, **27**, 1-6.

Gram, L., Ravn, L., Rasch, M., Bruhn, J.B., Christensen, A.B., Givskov, M., (2002). Food spoilage-interactions between food spoilage bacteria. *International Journal of Food Microbiology,* **78**, 79-97.

Grandin, T. (1990). Design of loading and holding pens. *Applied Animal Behavior Science,* **28,** 187-201.

Grandin, T. (1991). Recommended Animal Handling Guidelines for Meat Packer. American Meat Institute, Washington, D.C.

Harris, S.D., Morris, C.A., Jackson, T.C., May S.G., Lucia, L.M., Hale, S.D., Miller, R.K., Keeton, J.T., Savell, J.W., & Acuff, G.R. (1993). Ostrich meat industry development, Final report to: American Ostrich Association. Texas Agricultural Extension Service 348, Kleberg, 14.

Hoffman, K. (1988). pH – a quality criterion for meat. *Fleischwirtschaft*, **68**, 67-70.

Hoffman, L.C. (2005). A Review of the research conducted on ostrich meat. In: *Proceedings of the 3rd International Ratite Science Symposium of the WPSA*. Pp. 107-119. October 2005. Madrid, Spain.

Hoffman, L.C. (2008). Value adding and processing of Ratite meat: a review. *Australian Journal of Experimental Agriculture,* **48,** 1-6.

Hoffman, L.C., Botha, S. St.C. & Britz, T.J. (2006). Sensory properties of hot-deboned ostrich. *Meat Science,* **72**, 734-740.

Huffman, R.D. (2002). Current and future technologies for the decontamination of carcasses and fresh meat. *Meat Science*, **62**, 285-294.

Jay, J. (1992). *Modern Food Microbiology*. 4th Ed., Chapman and Hall, New York. Pp. 38-44, 64-65, 71-73.

Karama, M, (2001). The microbial quality of ostrich carcasses produced at an export-approved South African abattoir. MMedVet (Hyg) study at University of Pretoria. Pretoria, South Africa.

Lawrie, R.A, & Ledward, D.A. (2006). *Lawrie's Meat Science*, Seventh Edition. Woodhead Publishing Limited, Cambridge. Pp. 179-180.

Ley, E.C., Morishita, T.Y., Brisker, T. & Harr, B.S. (2001). Prevalence of *Salmonella, Campylobacter*, and *Escherichia coli* on ostrich carcasses and the susceptibility of ostrich-origin *E.coli* isolates to various antibiotics. *Avian Diseases,* **45**, 696-700.

Maunsell, B. & Bolton, D.J. (2004). Critical Control Points from Farm to Fork. In: Conference report of the EU-RAIN conference on Farm to Fork Food Safety: A Call for Common Sense, Athens, Greece.

McKinnon, D.C., Heyneke P.G., Olivier, A.J., Mulder, C., Britz, T.J. & Hoffman, L.C. (2005). Effect of ozone and UV on the microbial load of muscle surfaces of ostrich carcasses. *Proceedings of the 3rd International Ratite Science Symposium of the WPSA*. Pp. 276. October 2005. Madrid, Spain.

Ostrich and Emu Standards Council of New Zealand. (2002). Ostrich *and Emu Processing Standard 5* (OEPS5).

Otremba, M.M., Dikeman, M.E. & Boyle, E.A.E. (1999). Refrigerated shelf-life of vacuum-packaged, previously frozen ostrich meat. *Meat Science,* **52**, 279-283.

Paleari, M.A., Camisasca, S., Beretta, G., Renon, P., Corsico, P., Bertolo, G. & Crivelli, G. (1997). Ostrich meat: Physico-chemical characteristics and comparison with turkey and bovine meat. *Meat Science,* 48, 205-210.

Rne, J. (2008). EU proposal to allow chicken imports faces opposition. Food productiondaily.com. (WWW document). URL http://foodproductiondaily.com. 14 August 2008.

Sabbioni, A., Superchi, P., Sussi, C., Quarantelli, A., Bracchi, P.G., Pizza, A., Barbieri, G., Beretti, V., Zanon, A., Zambini, E.M. & Renzi, M. (2003). Factors affecting Ostrich meat composition and quality. *Annali Facoltà di Medicina Veterinaria di Parma*, **23**, 243-252.

Sales, J. & Mellet, F.D. (1996). Post-mortem pH decline in different ostrich muscles. *Meat Science,* **42**, 235-238.

Scott, V.N. & Stevenson, K.E. (2006). *HACCP A systematic approach to food safety*. 4th Ed. The Food Products Association, 1350 I Street, Washington D.C., 20005.

Severini, M., Ranucci, D., Miraglia, D. & Branciari, R. (2003). Preliminary study on the microbiological quality of ostrich (*Struthio camelus*) carcasses dressed in small Italian abattoirs. *Italian Journal of Food Science,* **15**, 295-300.

Seydim, A.C., Acton, J.C., Hall, M.A. & Dawson, P.L. (2006a). Effects of packaging atmospheres on shelf-life quality of ground ostrich meat. *Meat Science,* **73**, 503-510.

Seydim, A.C., Guzel-Seydim, Z.B., Acton, J.C. & Dawson, P.L. (2006b). Effects of rosemary extract and sodium lactate on quality of vacuum-packaged ostrich meat. *Journal of Food Science*, **71**, 71-76.

South African Ostrich Business Chamber (SAOBC) in conjunction with the National Council of Societies for the Prevention of Cruelty to Animals (SPCA) and the ARC – Animal Nutrition and Animal Products Institute (2001). *Code of Practice for the Transport, Handling and Slaughter of Ostriches.*

South African Ostrich Business Chamber (SAOBC) (2007). *General information on the ostrich industry.*

Van Schalkwyk, S.J., Hoffman, L.C., Cloete, S.W.P. & Mellet, F.D. (2005). The effect of feed withdrawal during lairage on meat quality characteristics in ostriches. *Meat Science*, **69**, 647-651.

Van Waart, S. (1995). *The Golden Camel Bird and the Story of Klein Karoo*. Klein Karoo Koöperasie, Koöperasie street, Oudtshoorn, 6625.

Wotton, S.B. & Hewitt, L. (1999). Transportation of ostriches – a review. *The Veterinary Record*, **145**, 725-731.

Wotton, S. & Sparrey, J. (2002). Stunning and slaughter of ostriches. *Meat Science*, **60**, 389-394.

Chapter 3

PREVALENT ORGANISMS ON OSTRICH CARCASSES AND THOSE FOUND IN A COMMERCIAL ABATTOIR

Abstract

The prevalent microbial growth on carcasses before and after overnight cooling in an ostrich abattoir and de-boning plant was investigated. The effect of warm or cold trimming of the carcasses was examined together with possible causes of contamination along the processing line. An attempt was made to link the prevalent micro-organisms that were identified from carcasses to that from specific external contamination sources. Samples of carcasses and possible contaminants were collected in the plant, plated out and selected organisms were typed using a commercial rapid identification system. It was indicated that the cold trim (mainly of bruises) of carcasses were advantageous in terms of microbiological meat quality. Results indicated pooled water in the abattoir as the most hazardous vector for carcass contamination and that contamination from this source is mostly Gram-negative pathogens. *Pseudomonas* and *Shigella* were frequently isolated from surface and air samples and indicate that the control of total plant hygiene is a requirement for producing ostrich meat that is safe to consume and has an acceptable shelf-life.

INTRODUCTION

The meat of animal carcasses is still sterile while protected by the skin or by the hide (Karama, 2001). In most instances, immediately after slaughter, the skins / hides are removed and the meat is exposed. From the moment of the first incision in the skin during the flaying process, bacterial contamination may occur (Grau, 1986). Microbial contamination can come from a wide range of external sources, including workers' hands and clothing, water supply, air supply and the slaughter equipment. These contaminating organisms can in the end cause bacterial spoilage of the meat, loss of shelf-life in the end product or in the worst case, food infection or poisoning for the consumers. Right through the abattoir or processing plant these vectors of microbiological contamination should be identified and monitored to minimize the damage done to the meat products.

This study focuses on ostrich meat and as reported (Hoffman, 2005; 2008), the main export product of the ostrich industry is fresh meat and it is therefore important to have as long a shelf-life as possible. As noted by McKinnon (2005), one effective way to ensure a good shelf-life is to ensure a low initial microbial load. Karama (2001) reported that there was no significant increase in microbial count on carcasses from post-flaying to post-evisceration; but a significant increase was detected from post-evisceration to post-chilling. Bearing this in mind and in relation to the rest of this study where a chapter is designated to the differences in warm and cold removal of bruises, prevalent organisms were isolated from bruised areas on the carcasses directly post-evisceration and from hot trimmed exposed meat after 24 h chilling in the de-boning area. This was done to get an indication of microbial populations found on the ostrich carcasses at different processing points. To monitor

for possible environmental contamination, samples from work surfaces, different water sources and personnel were taken in the same production areas.

Selected micro-organisms from the meat samples as well as the environmental samples were identified. It was attempted to establish the mayor cause of contamination and in this way equip abattoir management with a strategy to prevent meat contamination and increase shelf-life.

MATERIAL AND METHODS

Abattoir

In a modern European Union (EU) export approved ostrich abattoir in South Africa (Klein Karoo International Abattoir 1, ZA92), commercially reared ostriches were slaughtered, their feathers plucked, the skins removed (flaying) and the intestines removed (eviscerating) (Hoffman & Fischer, 2001). In this abattoir, the thighs are removed from the carcasses post-evisceration and the ribcages are discarded (hot de-boning), as only meat from the thighs is utilized commercially. The thighs are moved into an overnight chiller operating at 0-4°C, where they are cooled for approximately 24 h.

Trials and sampling

This study comprised of five trials, each of these trials was performed over a two day period. The bacteria from only two of the five trials were characterized and identified because of the time and financial implications. One of the two trials was performed in summer, the other during late autumn. Ostriches with bruises on their thighs or back muscles were randomly selected from the birds slaughtered on the selected day. Suitable experimental carcasses were selected at the primary meat inspection point, just after the evisceration process.

For each of the five studies performed, at least six carcasses were identified, each with a visible bruise. The warm trimmed carcasses were sampled in the following manner: for the first set of samples, a piece of bruised meat was removed from each of at least three carcasses during primary meat inspection (on day one) these carcasses were then warm trimmed (the bruises cut away); for the second set (on day two in the de-boning department) of samples (carcasses warm trimmed on day one) a piece of meat was removed from the exposed area where the bruise was trimmed away on day one. For these samples n = 5 x 3 = 15.

Of the initial six carcasses with identified bruising, three bruises were not removed on day one, but had been left intact on the carcasses during overnight cooling to be cold trimmed on day two. The carcasses for cold trimming were sampled in the following manner: for the first set of samples, a piece of meat was taken on each of the remaining intact carcasses adjacent to the bruised area (on day one during primary meat inspection); for the second set of samples (on day two in the de-boning department) a piece of meat was taken from the intact bruised area; for the third set of samples (on day two in the de-boning department) the bruises were cold trimmed by the de-boning personnel and the newly cold trimmed areas on the carcasses were sampled. Thus, for the second set of samples also, n = 5 x 3 = 15. The sampling procedure is summarized in Table 1. These samples were all taken in a destructive manner, i.e. pieces of meat were aseptically removed from the bruised area on the carcasses.

Table 1 Procedure for the sampling of bruises where carcasses are either warm or cold trimmed

Warm trimmed		
Set 1 Bruises	Day 1 at Primary meat inspection Bruises removed by meat inspectors	3 samples in bruises
Set 2	Day 2 at De-boning	3 samples on the same carcasses on exposed trimmed areas
Cold trimmed		
Set 1	Day 1 at Primary meat inspection Bruises left intact overnight	3 samples next to bruises
Set 2 Bruises removed by de-boning staff after sampling	Day 2 at De-boning	3 samples in bruised area of the same carcasses after cooling
Set 3	Day 2 at De-boning	3 samples on the newly exposed trimmed area of the same carcasses

At evisceration and all along the line, up to the de-boning department, environmental samples were also taken. The air samples in each area were taken by placing open agar plates in the area for 10 min. The water from taps, hoses and water brooms were also collected in sterile specimen jars, whilst water from the drains and platforms for personnel in the evisceration and primary meat inspection areas were collected in injection syringes. The swabs on work surfaces, workers' hands and knives were taken with the Rodac plate method (Lemmen *et al*., 2001).

Handling of samples

The meat samples were placed in sterile stomacher bags, identified with a permanent marker, rolled up to prevent opening and stored on ice in cooler bags. The specimen jars with water samples as well as the air and Rodac plates were also stored on ice in cooler bags. All the samples were transported in the coolers to the Klein Karoo International Research Laboratory, where they were analyzed on the same day in accordance to South African National Standards (SANS) these standards are based on the international ISO methods (SANS ISO 6887-1, 1999 & SANS 11133, 2004).

Microbial analysis

At the lab a dilution series was prepared for each sample, all the samples were plated out or drawn at the abattoir (Rodac plates) on three selected media: Plate Count Agar (PCA, Biolab code C6), Violet Red Bile Glucose Agar (VRBG Oxoid code CM485) and MRS (MRS, Biolab code C86). The PCA plates were incubated for 48 h at 30 ± 1°C, the VRBG at 37 ± 1°C for 24 h and the MRS at 37 ± 1°C for 48 h. On the PCA plates the

aerobic viable counts were evaluated to provide an indication of both Gram- positive and negative organisms and a general indication of hygiene on the carcasses and in the plant (SANS ISO, 1998). From the VRBG plates the *Enterobacteriaceae* counts (SANS, 2005) were obtained and only Gram-negative rods were expected to grow. The MRS media was not acidified or incubated under anaerobic or micro-aerophilic conditions as it was decided to rather use the media's enrichment properties than its selective properties for lactobacilli. The expected growth on these plates included *Lactobacillus, Streptococcus, Pediococcus* and *Leuconostoc* which are all Gram-positive, catalase and oxidase negative organisms.

Isolation of colonies

Colonies from the above plates were selected for identification. On plates with less than five colonies, all colonies that were morphologically unique were isolated. On plates with heavier growth, the Harrison's disc method (Harrigan & McCance, 1976) was used to select colonies. In this procedure the plates were superimposed on the disc proposed by Harrison and the colonies that fell inside the demarcated vectors were typed. With this method it is possible to calculate the percentage distribution of various organisms on the plates when it is not possible to type all of the organisms. Selected colonies were aseptically picked off the plates by making use of a sterile inoculation loop.

All selected colonies were plated out for single colonies on blood agar plates (Columbia agar, Oxoid code CM331 with addition of five % ostrich blood). These plates were incubated at 37 ± 2°C for 48 h.

Characterization

Isolated pure colonies were removed from these plates for Gram staining according to Preston & Morrel's (1962) method. During microscopic inspection of the stained slides, Gram-positive organisms, Gram-negative organisms and yeasts were distinguished. The colony morphology was also recorded, i.e. cocci, rods, etc. The Gram-positive colonies were tested for catalase and the Gram-negatives were tested for oxidase activity.

Identification

On the basis of the above information, the colonies were identified using the Remel RapID systems (Remel, 2005a). Each of the RapID systems has vials with either 1 or 2 ml of inoculation fluid. The fluids were adjusted by the addition of pure colonies to a density specified for each system, based on the McFarland Equivalence Turbidity Standards. The turbid inoculation fluid was pipetted with a glass pipet into the appropriate RapID identification panel with dehydrated reagents for biochemical identification reactions in the wells. The panels with the wells were tilted, firstly to the side to distribute the fluid evenly and eliminate possible air bubbles and then forward to fill up the wells. The reagents dissolved and during incubation reacted with the organisms present in the fluid to produce easy to interpret colour reactions from the degradation of both chromogenic and conventional substrates. The panels were incubated according to prescribed procedures, in most instances for 4h at 37 ± 2°C (Remel, 2000). After incubation the RapID Color guides were used to read and score each well on the grounds of a positive or negative reaction. The scores for the panels were electronically captured into the ERIC (Electronic RapID Compendium) (Remel. (2005b), a windows based program, developed to process micro

codes for all the RapID identification systems (Remel, 2000). ERIC processed the micro codes and gave the microorganism options, including probability percentages, on the grounds of the reactions in the wells.

The RapID identification systems were designed as biochemical diagnostic tools in human medicine. It was decided to use these systems on the basis of time, cost and lack of available laboratories in South Africa that were prepared to attempt the typing of such a large number of organisms. Out of the eight individual RapID systems, the following six systems were used: RapID ONE for the identification of Enterobacteriaceae and other oxidase negative bacteria; RapID CB Plus for the identification of *Corynebacterium, Actinomycis* spp. and other irregular, Gram-positive coryneform bacilli; RapID NH system for the identification of *Neisseriaceae, Haemophilus* and other related bacteria; RapID NF Plus for the identification of glucose fermenting and non-fermenting Gram- negative bacteria not belonging to the family *Enterobacteriaceae*; RapID Yeast Plus for the identification of medically important yeasts; RapID STR for the identification of streptococci and other similar Gram-positive bacteria. All Gram-positive, catalase positive coccoid morfological colonies were tested on the Oxoid Staphylases test kit (Oxoid code DR595A) to identify *Staphylococcus aureus* colonies (Oxoid, 2003).

RESULTS AND DISCUSSION

Enumeration of colonies

The aerobic plate count (APC) found on carcasses on the slaughter-line was 219.47 cfu.g^{-1} on carcasses where the bruises were already trimmed and 99.73 cfu.g^{-1} on carcasses where the bruises were left intact. These values are very low when compared to those found by Harris *et al*. (1993) and Karama (2001) (APC values averaging 3.2 to 4.21 log cfu.cm^{-2} on groups of carcasses post-evisceration). It was even lower than those reported by Gill *et al*. (2000) (2.98 log cfu.cm^{-2}).

The values increased considerably from where the samples were initially taken on day one, immediately post-evisceration, to where it was sampled again on day two in the de-boning hall: for the warm trimmed carcasses, counts increased from 219.47 cfu.g^{-1} to an average value on day two of 3 494.07 cfu.g^{-1}; the cold trimmed carcasses surface values increased from 99.73 cfu.g^{-1} to 2 142.00 cfu.g^{-1} on day two, after overnight cooling, and then decreased to 887.93 cfu.g^{-1} after cold trimming. All three post-chill average values were still significantly lower than the 4.57 log cfu.cm^{-2} reported by Karama (2001) for post-chilling samples. From the data in Fig. 1 and Fig. 2 portraying the overnight changes on carcasses warm vs. cold trimmed, it is evident from the decrease in microorganisms after the cold trim, that, as indicated elsewhere in these studies in more detail, it is beneficial from a microbiological point of view to rather cold trim than warm trim carcasses.

From the data obtained in these studies it was evident that Karama (2001) was correct in stating that the biggest increase in bacterial growth is from post-evisceration to post-chilling (Fig. 1).

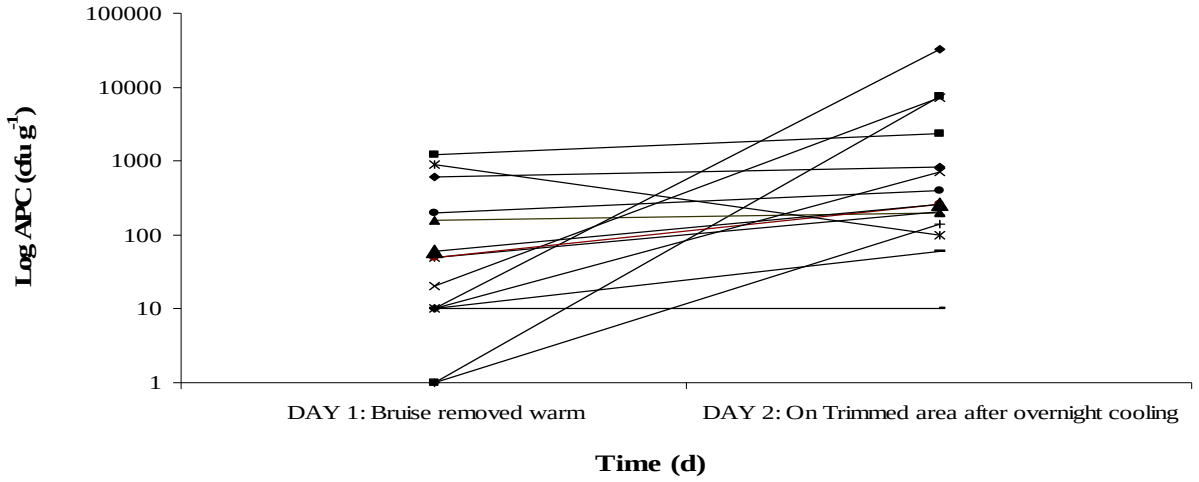

Figure 1 Aerobic plate counts on ostrich carcasses on the areas where bruises were removed post-evisceration; before vs. after overnight chilling

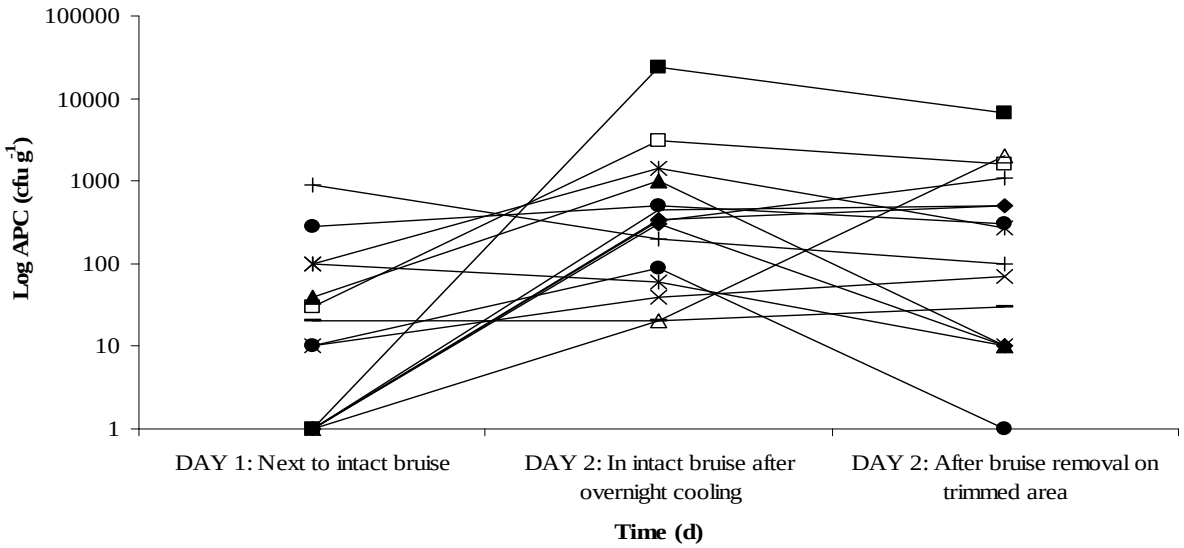

Figure 2 Aerobic plate counts on ostrich carcasses where bruises were removed cold on day 2; sampled before and after over night chilling as well as post trimming on day 2

Identification

A total of 198 organisms were selected on the basis of Gram stain. These organisms were collected from both carcass and environmental samples. Of these, 91 organisms were found to be Gram- positive, 82 Gram-negative and 25 were identified as yeasts. Only one mould was found. Out of the 198 organisms that were gram stained, 161 were further evaluated for oxidase or catalase activity to determine on which RapID systems

they should be inoculated. Of the 91 Gram-positive organisms, 74 were catalase positive and 9 were catalase negative. Of the 82 Gram-negative bacteria, 25 were found to be oxidase positive and 53 oxidase negative.

For the identification of the micro-organisms, the organisms isolated from carcasses were divided into two groups, post-evisceration (day one on the slaughter-line) (Table 2) and post-chilling (day two in the de-boning hall) (Table 3). It can be seen from the summary in Table 4 that the prevalent growth on the bruised carcasses directly post-evisceration was predominantly Gram-positive organisms.

After overnight cooling to 4ºC, the bacterial counts were on average 10 times higher than on the post-evisceration samples and more isolates were selected for identification. While there were still many Gram-positive isolates, there was also now a pronounced presence of Gram-negative bacilli. This indicates that the contaminants were both Gram-positive and Gram-negative, but that some or all of the Gram-negative organisms were probably more psychrophilic and grew better under refrigerated conditions. From Table 4 it can be seen that out of the 41 organisms isolated from carcasses post chilling, nine were identified as yeasts. All of the yeasts were isolated during the trial that was conducted late in the production season (autumn) and all eight were collected from samples in the de-boning hall on day two after chilling. Jay (1992) indicated that yeasts can grow over a wide range of temperatures (comfortable at chiller temperature) and relative humidity, thus, conditions inside the chillers would also support the growth of yeasts. Many of the yeasts could not be effectively identified (Tables 1, 2 & 3) because the RapID Yeast Plus system were only obtained later during the study and it only covers options for yeasts associated with human medicine.

The isolates identified from the air sample plates were more evenly distributed between Gram-positive (15) and Gram-negative (20) organisms. On the contact plates of the first trial (summer), the growth was predominantly Gram-negative (66.67% of isolates), while on that of the second trial (autumn), apart from the *Enterobacteriaceae* (VRBD) plates, the growth was markedly more Gram-positive (63.89% of isolates from PCA and MRS plates). Also in the second trial a very high number of yeast colonies (69.23% of isolates, n = 9) were isolated from the MRS media. If the colonies on the contact plates are split into contamination from workers and that from surfaces for both trials, the prevalent growth is again evenly distributed between Gram-positive and negatives, with a significant yeast population as well. The growth on water samples tended to be predominantly Gram-negative (81.82% of isolates, n = 11).

Table 2 The identification of organisms recovered from ostrich carcasses post evisceration in the abattoir

Sample	Gram stain	Morphology	Catalase activity	Oxidase activity	Identified As	% Certain
1	+	Cocci	+		*Gemella morbillorum*	>99.9
2	-	Rods		-	*Shigella* sp.	>99.9
3	+	Cocci	+		*Pediococcus pentosaceus*	75.97
					Gemella morbillorum	16.18
					Pediococcus acidilactici	7.85
4	+	Cocci	+		*Gemella morbillorum*	>99.9
5	+	Cocci	+		*Gemella morbillorum*	>99.9
6	NA	Yeast like	NA	NA	No choices	

Table 2 (continued) The identification of organisms recovered from ostrich carcasses post evisceration in the abattoir

Sample	Gram stain	Morphology	Catalase activity	Oxidase activity	Identified As	% Certain
7	-	Rods		-	*Serratia marcescens*	>99.9
8	-	Rods		+	*Aeromonas hydrophila*	>99.9
9	+	Cocci	+		No choices	
10	+	Cocci	+		*Pediococcus pentosaceus*	75.97
					Gemella morbillorum	16.18
					Pediococcus acidilactici	7.85
11	+	Cocci	+		*Gemella morbillorum*	99.83
					Streptococcus mitis	0.17
12	+	Rods	+		*Brevibacterium* (Group B)	66.68
					Brevibacterium casei	33.32
13	+	Cocci	+		*Pediococcus pentosaceus*	75.97
					Gemella morbillorum	16.18
					Pediococcus acidilactici	7.85
14	NA	Yeast like	NA	NA	No choices	

From the typing of the carcass samples before overnight chilling (Table 2) it can be seen that the prevalent organisms isolated from ostrich carcasses were the Gram-positive cocci (64.29% of isolates, n = 14), *Gemella morbillorum* (4 isolates), closely resembling the *Streptococcus* spp. and *Pediococcus pentosaceus* (3 isolates), a member of the lactic acid bacteria group (Sneath *et al*., 1986). The samples of carcasses post-chilling still had a high percentage of the above two organisms present in the growth, with Gram-positive organisms accounting for 50.00% of the organisms identified, but now there was also a wide variety of Gram-negative organisms (26.19% of organisms identified) (Table 3).

Table 3 The identification of organisms recovered from ostrich carcasses post chilling in the De-boning hall

Sample	Gram stain	Morphology	Catalase activity	Oxidase activity	Identified As	% Certain
1	+	Coccoid	+		*Gemella morbillorum*	>99.9
2	+	Coccoid	+		*Pediococcus pentosaceus*	75.97
					Gemella morbillorum	16.18
					Pediococcus acidilactici	7.85
3	+	Coccoid	+		*Gemella morbillorum*	>99.9
4	+	Coccoid	+		*Pediococcus pentosaceus*	75.97
					Gemella morbillorum	16.18
					Pediococcus acidilactici	7.85
5	+	Coccoid	+		*Gemella morbillorum*	72.20
					Pediococcus acidilactici	27.01
					Pediococcus pentosaceus	0.79
6	NA	Mould like	NA	NA	Mould	
7	-	Coccoid	+		*Gardnerella vaginalis*	>99.9
8	NA	Yeast like	NA	NA	*Rhodoturula minuta*	95.29
					Cryptococcus uniguttulatus	4.21
					Rhodoturula rubra	0.35
					Trichosporon beigelii	0.14
9	+	Coccoid	+		*Pediococcus pentosaceus*	75.97
					Gemella morbillorum	16.18
					Pediococcus acidilactici	7.85
10	+	Coccoid	+		*Pediococcus pentosaceus*	75.97
					Gemella morbillorum	16.18
					Pediococcus acidilactici	7.85
11	NA	Yeast like	NA	NA	No choices	
12	+	Coccoid	+		*Gemella morbillorum*	>99.9
13	-	Rod shaped		-	*Acinetobacter calcoaceticus*	>99.9

Table 3 (continued) The identification of organisms recovered from ostrich carcasses post chilling in the De-boning hall

Sample	Gram stain	Morphology	Catalase activity	Oxidase activity	Identified As	% Certain
14	+	Coccoid	+		*Pediococcus pentosaceus*	75.97
					Gemella morbillorum	16.18
					Pediococcus acidilactici	7.85
15	-	Rod shaped		-	*Shigella* sp.	>99.9
16	-	Rod shaped		-	*Serratia plymuthica*	>99.9
17	+	Coccoid	-		*Aerococcus* sp.	91.28
					Gemella morbillorum	8.72
18	+	Coccoid	+		*Streptococcus salivarius*	>99.9
19	+	Coccoid	+		*Pediococcus pentosaceus*	75.97
					Gemella morbillorum	16.18
					Pediococcus acidilactici	7.85
20	+	Coccoid	+		*Gemella morbillorum*	72.20
					Pediococcus acidilactici	27.01
					Pediococcus pentosaceus	0.79
21	+	Coccoid	+		*Pediococcus pentosaceus*	75.97
					Gemella morbillorum	16.18
					Pediococcus acidilactici	7.85
22	-	Rod shaped		-	*Yersinia pseudotuberculosis*	65.43
					Shigella sp.	34.57
23	-	Bent rods		+	*Neisseria weaveri / elongata*	>99.9
24	+	Coccoid	+		*Pediococcus pentosaceus*	75.97
					Gemella morbillorum	16.18
					Pediococcus acidilactici	7.85
25	NA	Yeast like	NA	NA	No choices	
26	+	Coccoid	+		*Gemella morbillorum*	>99.9
27	NA	Yeast like	NA	NA	No choices	
28	+	Coccoid	+		*Gemella morbillorum*	>99.9

Table 3 (continued) The identification of organisms recovered from ostrich carcasses post chilling in the De-boning hall

Sample	Gram stain	Morphology	Catalase activity	Oxidase activity	Identified As	% Certain
29	+	Coccoid	+		*Gemella morbillorum*	>99.9
30	+	Coccoid	+		*Gemella morbillorum*	>99.9
31	-	Rod shaped		-	*Stenotrophomonas maltophila*	>99.9
32	NA	Yeast like	NA	NA	*Cryptococcus uniguttulatus*	99.29
					Trichosporon beigelii	0.54
					Rhodoturula rubra	0.16
33	-	Rod shaped		+	*Flavobacterium* IIb	99.33
					Sphingomonas paucimobilis	0.67
34	-	Rod shaped	-		*Proteus vulgaris* Group II	>99.9
35	+	Coccoid	-		*Gemella morbillorum*	>99.9
36	-	Rod shaped		-	*Providencia rettgeri*	>99.9
37	NA	Yeast like	NA	NA	No choices	
38	-	Rod shaped		-	*Pantoea agglomerans*	>99.9
39	NA	Yeast like	NA	NA	No choices	
40	NA	Yeast like	NA	NA	No choices	
41	NA	Yeast like	NA	NA	No choices	
42	+	Coccoid	+		No choices	

According to Jay (1992), all the above microorganisms have the environment, animals and/or humans as important sources. These results confirm what Harris *et al.* (1993) reported regarding the growth on ostrich carcasses, that it is mostly associated with those organisms native to the environment and the skin of animals and humans. No *Salmonella* colonies were isolated from the carcasses, which correspond with Gopo & Banda (1997) who found no *Salmonella* on any ostrich meat intended for human consumption.

The Gram-positive isolates from the air sample plates that could be positively identified were primarily *Gemella morbillorum.* Some of the organisms selected died off during isolation or there were not enough options under the Remel RapID systems to facilitate their successful identification. As the Remel RapID systems were developed specifically for human clinical specimens it focuses only on microorganisms associated with human diseases and infections. During the use of these systems for the identification of organisms in the abattoir and on the meat it was found, that while there were enough options on the Gram-negative side to ensure the successful typing of all organisms, there were limited options with only the RapID STR and RapID CB Plus systems to identify the Gram-positive organisms. The RapID systems did not cover all the options for the Gram-positive environmental organisms, including *Bacillus* spp. and *Staphylococcus* spp.

The Gram-negative organisms on the air plates consisted primarily of *Shigella* spp., most probably from the Evisceration hall, because these organisms are associated with the gastrointestinal tract of animals and not with the environment (Jay, 1992). *Proteus* spp. was also isolated from the air plates and the air supply in the abattoir could possibly contribute to the Gram-negative contamination of the carcasses. From the cold rooms and De-boning area *Stenotrophomonas maltophilia* were isolated, these organisms are associated with the Pseudomonads (Pearson, 2005) and they are thus able to grow and contaminate the meat in these temperature controlled areas.

The prevalent Gram-positive organisms isolated from both surfaces and workers were *Gemella* (11 out of 44) and *Pediococcus* (13 out of 44). The Gram-negative organisms consisted of predominantly *Shigella* and *Serratia* spp., but also included *Pseudomonas aeruginosa, Burkholderia cepacia*, *Stenotrophomonas maltophilia* and other organisms. The latter two species are also closely related to the Pseudomonads (Pearson, 2005). These organisms have not been recovered from the carcasses in these trials, which indicate that these vectors of contamination are well controlled. Care should however always be taken to prevent these spoilage organisms from contaminating the meat.

Yeast colonies were isolated from almost all the environmental samples, with a high incidence on the surface swabs (only from the knives and the workers' hands). From the carcasses pre-chilling, only two yeast colonies were isolated, but post-chilling in the De-boning area, nine yeast isolates were found. This indicated that the carcasses were contaminated with these organisms; most probably from the knives and workers. Not all yeast isolates were identified, but from the successfully identified species, more than 90% were typed as a *Cryptococcus* sp., a type of yeast that is known to occur on fresh refrigerated meat (Jay, 1992).

Table 4 Summary of the prevalent organisms recovered from ostrich carcasses and the environmental contaminants identified inside the abattoir and de-boning hall

Sample	Gram stain	Most common organism isolated	Number of isolates
Carcasses post-evisceration	2 yeast isolates	Not identified or no choices	
	3 Gram negative	*Shigella, Serratia marcescens, Aeromonas hydrophilia*	1 each
	9 Gram positive	*Gemella morbillorum*	4
		Pedicococcus pentasosaceus	3
		Brevibacterium (Group B)	1
		No choices within acceptable limits	1

Table 4 (continued) Summary of the prevalent organisms recovered from ostrich carcasses and the environmental contaminants identified inside the abattoir and de-boning hall

Sample	Gram stain	Most common organism isolated	Number of isolates
Carcasses post-chilling	9 yeast isolates	*Rhodoturula minuta Cryptococcus uniguttulatus*	1
		Not identified or no choices	1
			7
	11 Gram negative	*Gardnerella vaginalis*	1
		Acinetobacter calcoaceticus	1
		Shigella sp.	1
		Serratia plymuthica	1
		Yersinia pseudotuberculosis	1
		Neisseria weaveri / elongate	1
		Stenotrophomonas maltophila	1
		Flavobacterium IIb	1
		Proteus vulgaris Group II	1
		Providencia rettgeri	1
		Pantoea agglomerans	1
	21 Gram positive	*Gemella morbillorum*	10
		Pedicococcus pentasosaceus	8
		Aerococcus sp.	1
		Streptococcus salivarius	1
		No choices within acceptable limits	1
Air plates	3 yeast isolates	Not identified or no choices	
	20 Gram negative	*Shigella* spp.	4
		Proteus spp.	2
		Stenotrophomonas maltophilia	3
		Pantoea agglomerans	2
		Alcaligenes faecalis	2
		Tatumella ptyseos	1
		Acinetobacter calcoaceticus	1
		Serratia marcescens	1
		No choices within acceptable limits	4
	15 Gram positive	*Gemella morbillorum*	6
		Pedicococcus pentasosaceus	2
		Brevibacterium casei	1
		Enterococcus asburiae	1
		No choices within acceptable limits	5
Contact plates: workers	5 yeast isolates	*Rhodoturla rubra*	1
		Cryptococcus neoformans	3
		Saccharomyces cerevisiae	1
	22 Gram negative	*Alcaligenes faecalis*	3
		Shigella spp.	3
		Serratia spp.	4
		Pseudomonas aeruginosa	2
		Stenotrophomonas maltophilia	2
		Neisseria gonorrhoeae	1
		Alcaligenes faecalis	2
		Moraxella osloensis	1
		Klebsiella rhinosclromatis	1
		No choices within acceptable limits	3
	24 Gram positive	*Pediococcus spp*	7
		Gemella morbillorum	7
		Tatumella ptyseos	1
		Enterococcus faecalis	1
		Staphylococcus aureus	1
		No choices within acceptable limits	7

Table 4 (continued) Summary of the prevalent organisms recovered from ostrich carcasses and the environmental contaminants identified inside the abattoir and de-boning hall

Sample	Gram stain	Most common organism isolated	Number of isolates
Contact plates: surfaces	6 yeast isolates	*Cryptococcus spp*	3
		Rhodoturula glutinis	1
		Not identified or no choices	2
	17 Gram negative	*Serratia spp*	4
		Shigella spp.	4
		Burkholderia cepacia	2
		Proteus penneri	1
		Leminorella grimontii	1
		Alcaligenes faecalis	1
		Shewanella putrefaciens	1
		No choices within acceptable limits	3
	20 Gram positive	*Pedicococcus Pentasosaceus*	6
		Gemella morbillorum	4
		Streptococcus equines	1
		Enterococcus avium	1
		No choices within acceptable limits	8
Water	0 yeast isolates		
	9 Gram negative	*Shigella* spp.	3
		Escherichia coli	2
		Salmonella typhi	1
		Alcaligenes faecalis	1
		Burkholdria cepacia	1
		No choices within acceptable limits	1
	2 Gram positive	*Gemella morbillorum*	2

In this abattoir, the water from taps, hoses and water brooms are all from the water reticulation system that is treated with chlorine dioxide and no organisms were found in these water samples. The water collected from drains and platforms showed very high counts (>2 x 10^3 cfu.ml^{-1} on all three media), the growth was predominantly Gram-negative (81.82% of isolates, n = 11) (Table 5).

Many of the organisms were human pathogens (*Shigella*, *E. coli* and *Salmonella*) and even though *E. coli* and *Salmonella* were not recovered from the carcasses in these trials, the standing water in the drains and on the platforms pose a very real risk to food safety and it should be prevented as far as possible.

Table 5 The identification of organisms recovered from water pooling on platforms and drains in the Evisceration area of the abattoir

Sample	Gram stain	Morphology	Catalase activity	Oxidase activity	Identified As	% Certain
1	+	Coccoid	+		*Gemella morbillorum*	72.20
					Pediococcus acidilactici	27.01
					Pediococcus pentosaceus	0.79
2	-	Rod shaped		-	*Shigella* sp.	>99.9
3	-	Rod shaped		+	*Alcaligenes faecalis*	>99.9

Table 5 (continued) The identification of organisms recovered from water pooling on platforms and drains in the Evisceration area of the abattoir

Sample	Gram stain	Morphology	Catalase activity	Oxidase activity	Identified As	% Certain
4	-	Rod shaped		-	*Escherichia coli*	>99.9
5	-	Rod shaped		+	*Burkholdria cepacia*	>99.9
6	-	Rod shaped		-	*Shigella* sp.	95.39
					Acinetobacter calcoaceticus	4.21
					Tatumella ptyseos	0.34
7	-	Rod shaped		-	*Shigella sonnei*	90.83
					Escherichia coli	8.31
					Shigella sp.	0.87
8	-	Rod shaped		-	*Salmonella typhi*	94.22
					Salmonella paratyphi A	2.73
					Salmonella 1 Most	1.87
					Shigella sp.	1.18
9	-	Rod shaped		-	*Escherichia coli*	>99.9
10	-	Rod shaped		-	no choices	
11	+	Coccoid	+		*Gemella morbillorum*	>99.9

CONCLUSION

The initial results from this study would seem to indicate microbiological benefits for removing minor bruises cold rather than warm (immediately post-evisceration). The adoption of this practice could lead to an increase in shelf-life because of the reduced initial microbial load directly prior to vacuum packing. This practice warrants further research.

From the typing of environmental organisms, by far the most dangerous vector of contamination is the water standing in the drainage and on platforms as these water sources contained dangerous food pathogens associated with feacal contamination. These included *E. coli*, *Salmonella* and *Shigella* spp. and could be the reason for the Gram-negative contamination of the meat. Pathogens from these water sources could contaminate carcasses from high pressure hoses or brooms spraying the standing water, or from workers' boots stepping in the pools, or from any equipment (knives, saws or crates) dropped on the floors or platforms and not re-sterilized before use on the carcasses. Therefore, water containment on the slaughter floor is of the utmost importance.

The workers' hands, surfaces and air borne bacteria can not be excluded or indicated as the sole contributor to the microbial load of the carcasses and further research in this area is warranted. The incidence of *Shigella* spp. (possibly human pathogenic organisms) and *Pseudomonas* (strongly associated with spoilage of

refrigerated meat; Jay, 1992) and related species on surfaces and in air, however, indicates that these sources of contamination also warrant strict hygiene control in ostrich meat handling establishments.

The relatively high incidence of yeast growth and specifically *Cryptococcus* spp. growth on the ostrich meat is an interesting finding and the significance / spoilage potential of yeast species in an ostrich abattoir warrants further investigation.

In conclusion and based on the data collected in this trial it can be recommended that proper environmental management in the abattoir can be used to control the microbiological load on ostrich meat before packaging to a large extent. Primarily by employing the unique slaughter practices developed specifically for ostriches, which focus on the prevention of contamination during flaying and evisceration, by for example, tying the cloacae, inverting the bird, making incisions from the inside to the outside of the skin and not damaging intestines during the evisceration step. Secondly by removing bruised meat cold during de-boning and lastly by controlling the external sources of microbiological contamination inside the slaughter-house. With regard to the management of contamination: a programme for water containment is of high importance, this should include the prevention of pooling of water or blockage of drains and the control over the usage of high pressure hoses; subsequently a programme for the general hygiene of the abattoir can not be neglected. This will focus on good manufacturing practices and have to include the implementation of filtered air supply and regular cleaning of air ducting, the cleaning and sanitizing of work surfaces and equipment and personnel hygiene (including proper medical screening, clean protective wear and the frequent cleaning and sanitizing of workers' hands).

REFERENCES

Gill, C.O., Jones, T., Bryant, J. & Brereton, D.A. (2000). The microbiological conditions of the carcasses of six species after dressing at a small abattoir. *Food Microbiology*, **17**, 233-239.

Gopo, J.M. & Banda, G.N. (1997). Occurrence of Salmonella on meat and products in an ostrich abattoir as determined with a DNA probe. *South African Journal of Animal Science*, **27**, 1-6.

Grau, F.H. (1986). Microbial ecology of meat and poultry. In: Pearson A. M. & Dutson T.R. (Eds), *Advances in Meat Research*, Volume 2, Meat And Poultry Microbiology, Department of Food Science and Nutrition, Michigan State University, East Lansing, Michigan. Pp. 1-36.

Harris, S.D., Morris, C.A., Jackson, T.C., May S.G., Lucia, L.M., Hale, S.D., Miller, R.K., Keeton, J.T., Savell, J.W. & Acuff, G.R. (1993). Ostrich meat industry development, Final report to: American Ostrich Association. Texas Agricultural Extension Service 348, Kleberg. Pp. 14.

Harrigan, W.F. & McCance, M.E. (1976). Preface to "Laboratory methods in Microbiology". In: *Laboratory Methods in Food and Dairy Microbiology*. Revised edition. London, New York & San Francisco: Academic Press. Pp. 47-49.

Hoffman, L.C. (2005). A Review of the research conducted on ostrich meat. In: *Proceedings of the 3rd International Ratite Science Symposium of the WPSA*. Pp. 107-119. October 2005. Madrid, Spain.

Hoffman, L.C. (2008). Value adding and processing of Ratite meat: a review. *Australian Journal of Experimental Agriculture,* **48,** 1-6.

Hoffman, L.C. & Fisher, P. (2001). Comparison of meat quality characteristics between young and old ostriches. *Meat Science*, **59**, 335-337.

Jay, J. 1992. *Modern Food Microbiology*. 4th Ed. Chapman and Hall, New York. Pp.13-86, 201-203.

Karama, M. (2001). The microbial Quality of Ostrich Carcasses produced at an export-approved South African Abattoir. MMedVet (Hyg) study at University of Pretoria. Pretoria, South Africa.

Lemmen, S.W., Häfner, H., Zolldann, D., Amedick, G. & Lutticken, R. (2001). Comparison of two sampling methods for the detection of Gram-positive and Gram-negative bacteria in the environment: moistened swabs versus Rodac plates. *International Journal of Hygiene and Environmental Health*, **203**, 245-248.

McKinnon, D.C., Heyneke P.G., Olivier, A.J., Mulder, C., Britz, T.J. & Hoffman, L.C. (2005). Effect of ozone and UV on the microbial load of muscle surfaces of ostrich carcasses. *Proceedings of the 3rd International Ratite Science Symposium of the WPSA*. Pp. 276. October 2005. Madrid, Spain.

Oxoid. (2003). *Diagnostic Reagents: Staphylase test*. Oxoid, Basinstoke, Hampshire, England.

Pearson, A. 2005. *Pseudomonas* spp and *Stenotrophomonas malthophilia* bacteraemia: England, Wales, and Northern Ireland: 2004. CDR Weekly, Volume 15, No 39. Pp. 1-6.

Preston, N.W. & Morrell, A. (1962). Reproducible results with the Gram stain. *Journal of pathology and bacteriology*, **84**, 241-243.

Remel. (2000). *IDS RapID Systems*. Lenexa K.S., United States of America.

Remel. (2005a). *RapID Systems*. Lenexa K.S., United States of America.

Remel. (2005b). *RapID with ERIC means results*. Lenexa K.S., United States of America.

SANS ISO 15214. (1998). *Horizontal method for the enumeration of mesophilic lactic acid bacteria – Colony count technique at 30ºC.*

SANS ISO 6887-1. (1999*). Preparation of test samples, initial suspension and decimal dilutions for microbiological examination – general rules.*

SANS 11133. (2004). *Microbiology of food and feeding stuffs – Guidelines on preparation and production of culture media.*

SANS 218528. (2005). *Microbiology of food and feeding stuffs – Horizontal method for the detection and enumeration of Enterobacteriaceae*

Sneath, P.H.A., Mair, N.S., Sharpe, M.E. & Holt, J.G. (1986). *Bergey's manual of Systematic Bacteriology*, Vol. 2. Pp. 1075, 1081.

Chapter 4

BRUISING ON OSTRICH CARCASSES AND THE IMPLICATIONS ON THE MICROBIOLOGY AND LOSSES IN UTILIZABLE MEAT WHEN REMOVING THEM POST-EVISCERATION OR POST-CHILLING

Abstract

The ostrich meat industry is continuously searching for means to increase the meat yield from carcasses. One reason for the loss of utilizable meat is sustained bruises. In ostrich abattoirs these bruised areas are removed as part of the primary meat inspection, performed directly after evisceration. To investigate the implications of removing the bruises at primary meat inspection or after overnight cooling of the carcasses (0-4°C), three separate studies were conducted to determine the advantages and disadvantages of both practices. The bruises on the carcasses were also investigated to determine their frequency and distribution to try to establish the most obvious causes of bruising and subsequently possible preventative measures. The bruises on the necks represented 52.58% of all bruises found; with the high side railings of the transport vehicles the most probable cause of the injuries. Large and multiple bruising seen on the carcasses were probably from ostriches trampling on birds sitting down. In the warm vs. cold trimmed studies it was established that when the bruises were trimmed on the warm carcasses the total aerobic viable counts on the trimmed surfaces increased significantly during overnight chilling. However, when the bruises were left on the carcasses during overnight chilling, counts decreased after cold trimming. The cold trimming of minor bruises together with better management of trimming practices also led to a decrease in meat yield losses. From both a microbiological and an utilizable meat yield point of view, it is advantageous to remove the bruises after overnight cooling.

INTRODUCTION

South African ostrich meat is utilized primarily for the export market; ostriches from across the country are therefore slaughtered in one of the only 10 export approved abattoirs (Anonymous, 2005). Due to the extended demographics of South Africa, birds are often transported per truck over long distances. Compared to other farms where the ostriches are just herded to the abattoirs (this practice has been suspended in the mean time due to animal welfare concerns). In both instances and despite transport measures, as well as receiving and keeping facilities at the abattoirs that adhere to stringent animal welfare codes (SAOBC, 2001); the birds still often sustain bruises to their bodies. Bruises could also be inflicted due to handling or inter ostrich contact during rearing on the farms. Unless there are visible lesions on the external surface of the thighs of the ostriches, these bruises will only become evident after slaughtering and de-hiding.

Bruises or contusions are described as superficial discoloration due to haemorrhage into the tissue from ruptured blood vessels beneath the skin surface, without the skin being broken. In the contusions the blood accumulates in surrounding tissues, producing pain, swelling and tenderness (Blood & Studdert, 1988). The bruising can be caused by a physical blow from a stick or stone, a metal projection or an animal fall (Chambers *et al.*, 2004). These bruises vary widely in location, severity and appearance. Factors such as the age, size, depth and sterility of the bruise would influence both severity and appearance. The distribution of the locations of

the bruises as well as the frequency in which the bruising is present are indicative of primarily the transport, loading and lairage practices as these steps are the most likely to cause harm to the animals (Jago *et al*., 1996; Grandin, 1990; 1991). Thus, information from the bruising patterns can indicate what practices should be better managed to minimize bruising on carcasses.

From a consumer point of view the welfare of the animals before slaughter is a concern, as is a bruised area in meat is not desirable (Chambers *et al*., 2004) and it could possibly also pose a health risk if the bruise is infected. Bruised areas in the meat would also decompose and spoil more rapidly if the bruises are not removed, because the bloody areas would be conducive to bacterial growth. Furthermore, it was indicated that stressed or injured (bruised) ostriches would have an abnormally high pH because of glycogen depletion and the subsequent lower production of lactic acid in the muscles of stressed animals. This higher pH would then better support microbial growth and the meat from animals that were stressed, injured or diseased before slaughter will have a shorter shelf-life. (Chambers *et al*., 2004)

Ostriches are slaughtered and the carcasses de-feathered, skinned and eviscerated (Hoffman *et al*., 2006), where after the carcasses are inspected. This process is known as the primary meat inspection and is performed in the export abattoirs by Department of Agriculture meat inspectors, appointed on authority of the Regulations under Act 40 (Anon., 2004). While inspecting the carcasses for bruises and injuries the inspectors must trim away visible bruises according to the appropriate Veterinary Procedural Notice (Anon., 2007b). All these actions take place on day one, within one hour *post-mortem*. This action of warm trimming of bruises has in the past been known to contribute to significant losses in meat yield per carcass in the abattoir where the studies were conducted.

In the export approved ostrich abattoir chosen for the studies (Klein Karoo International Abattoir 1, ZA92), the thighs are removed from the carcasses directly after the primary meat inspection. Only the meat from the ostrich thighs is utilized for export. The thighs are then moved into large cold rooms at 0-4°C, for overnight chilling to below a 4°C meat core temperature and are then cold de-boned on day two.

Petersen (1978) reported that in a study of bruised lamb carcasses in New Zealand, most of the bruises found by meat inspectors are classified as minor and these bruises are a company responsibility and removed later in the process. The same may be true for ostriches and requires further investigation.

The aim of the study is therefore to evaluate the advantages and disadvantages of removing bruised tissue during primary meat inspection (day one) or during cold de-boning (day two) as well as to evaluate the distribution of the bruises to identify the risks during transport and lairage for the birds. It is studied on the basis of microbiological analysis of the cut muscle surface, visual evaluation of the bruised areas and meat yield from the ostrich carcasses.

MATERIAL AND METHODS

Ostrich transport

Commercially reared slaughter ostriches of an age between 12 and 14 months were transported or moved to the abattoir for slaughter by their owners or contracted transporters. The birds were either transported by truck or pick-up, or shepherded (this practice has in the mean time been suspended) to the overnight pens of the abattoir

from nearby farms. The trucks in which the ostriches were transported are open-topped with railed sides (Wotton & Sparrey, 2002). The group of ostriches transported together was separated into compartments on the trucks with a maximum of 12 ostriches per section. The ostriches were loaded onto the trucks and transported to the abattoir, keeping them as calm as possible to prevent them from unnecessary injury. The handlers traveling with the birds on the back of the trucks take all possible measures to prevent the ostriches from loosing their footage or sitting down during transport, to prevent the birds from being trampled by their peers (SAOBC, 2001). Ostriches have difficulty to maintain their balance during transport, because they are large animals with only two legs, they have two-toed feet and a high centre of gravity (Wotton & Hewitt, 1999).

Off-loading and lairaging

The birds were off-loaded from the vehicles at a horizontal loading bay and moved into the covered, but open-sided pens. At the abattoir great care was taken to prevent injury during off-loading, standing in the pens or during movement to the slaughter area. Grandin (1990, 1991) reported that these first phase processing steps are the most likely to cause bruises to the animals. Furthermore, the lairages and walkways have sand floors to prevent the birds from slipping. The corners of the pens and water troughs are rounded to prevent injury. Care was also taken to keep the birds as calm as possible during off-loading and pen allocation (SAOBC, 2001).

The day after arrival at the abattoir the birds were moved to the slaughter pens via a sanded path with rounded wooden constraints. The slaughter pens have gridded cement flooring to make it slip free and once again the pens and troughs have rounded corners. During transport, off-loading and standing in the pens guidelines on maximum numbers of ostriches per truck compartment or lairage (Anon., 1993; 2001) were adhered to, to further try and prevent the ostriches from injuring or bruising each other.

Slaughtering studies for microbiological analysis

Three individual microbiological studies, each lasting two days were performed over a three month period. In all three studies the ostriches were slaughtered in the export approved ostrich abattoir in Oudtshoorn, South Africa (Klein Karoo International Abattoir 1, ZA92). Once inside the abattoir, the ostriches were slaughtered, bled, plucked, de-skinned and eviscerated according to the prescribed standard operating procedures (Anonymous, 2002; 2007a).

Ostriches with bruises on their thighs or back muscles were selected at the primary meat inspection point, just after the evisceration process, from the birds slaughtered on the specific day. Bruised areas of larger than 2.5 cm and up to 10 cm in diameter that did not show green discoloration (i.e. an old injury) or any sign of infection or abscess were selected for the study. The size of the bruises was selected on the basis of experience on the slaughter floor that suggested that smaller bruises (< 2.5 cm in diameter) are mostly negligible and larger bruises (> 10 cm in diameter) are usually so severe that they have to be removed by the inspectors.

Warm trimmed

For each of the three studies 10 carcasses were identified with visible bruises as discussed above. Five bruises (one bruise per carcass from five carcasses) were removed (warm trimmed) during the primary meat inspection by the meat inspectors (on day one, 30-45 minutes *post-mortem*) and five bruises (one identified per carcass)

were left on the carcass. The first set of microbiological samples was taken right after primary meat inspection. Next to the bruised areas that were sampled, a set of control samples were also taken at each sampling point.

Cold trimmed

The latter five bruises were cold trimmed by personnel in the de-boning department the next morning (on day two, ±24 h *post-mortem,* temperature = 0-4°C). In this abattoir the carcasses are warm quartered, i.e. before overnight chilling the thighs are removed from the ribcages and only the meat from the thighs is utilized commercially. The quartered thighs were hung on a stainless steel frame and moved into a refrigeration room operating at 0-4°C. The next morning the thighs were moved out onto an automatic overhead conveyor into the de-boning area where the muscle groups were separated onto de-boning tables. On these tables the remaining five bruises where removed (cold trimmed) and the second set of microbiological samples was taken (meat temperature ≤4ºC). The sampling sites on the carcasses were identified with sterilized steel pins that were stuck in the physical sample area to ensure that as near as possible to the same area was tested on day two.

Microbial sampling

Microbial samples of the bruised carcass muscle surfaces were taken in a destructive manner, i.e. pieces of meat were removed from the surface muscles, using aseptic techniques. All groups of five samples per sampling point were pooled together, i.e. the five pieces of meat from the individual carcasses were placed together in one bag to give one value for the five carcasses.

Day one

At the first sampling point (day one) the following samples were taken:

For the five bruised carcasses that were warm trimmed on day one;

A1. One sample per carcass cut from the newly exposed trimmed area where the bruises were removed, pooled into one sterile bag.

A2. One sample per carcass from the undamaged area right next to the trimmed area, pooled into one sterile bag (control sample).

For the five bruised carcasses that were not trimmed on day one (bruises on these carcasses will be cold trimmed on day two);

B1. One sample per carcass cut from the bruised area, pooled into one sterile bag.

B2. One sample per carcass from the undamaged area right next to the trimmed area, pooled into one sterile bag (control sample).

Day two

At the second sampling point (day two) the following samples were taken:

For the five bruised carcasses that were warm trimmed on day one;

A3. One sample per carcass cut from the exposed trimmed area where the bruises were removed, pooled into one sterile bag.

A4. One sample per carcass from the undamaged area right next to the trimmed area, pooled into one sterile bag (control sample).

For the five bruised carcasses that were cold trimmed on day two;

B3. One per carcass cut from the newly exposed trimmed area after the bruises were removed, pooled into one sterile bag.

B4. One sample per carcass from the undamaged area right next to the trimmed area, pooled into one sterile bag, this represents the control sample.

Transport and handling of samples

Thus, from each study there were eight pooled samples, four on day one and four on day two. This procedure was repeated over three periods. The sterile sampling bags were closed and transported on ice to the Klein Karoo International Research Laboratory where they were analyzed according to standard ISO (International Organization for Standardization) and SANS (South African National Standard) codes (SANS ISO, 1999; 2007a; 2007b; SANS, 2004)

Microbiological analysis

From each sample bag with the five pooled pieces of meat (minimum of 10 g) a 1:10 dilution series was prepared in buffered peptone water (code LP0034, Oxoid) and the samples were analyzed to determine the aerobic plate count (APC) (Plate count agar, Biolab code C6, Merck) as well as the total coliform and *E. coli* count (VRBA with MUG, code CM0978, Oxoid). The APC plates were incubated for 48 h at 30°C and those for coliforms and *E. coli* for 24 h at 37°C. The relevant colonies on the plates were counted and results were expressed as colony forming units (cfu) per 1 g of meat sample.

Abattoir analysis

At the sampling points in both the primary meat inspection area and the de-boning hall, the bruises or bruised areas were visually inspected and generally commented on in terms of size and acceptability.

It is a standard procedure in this abattoir that the meat trimmed off carcasses by the meat inspectors because of bruising, is collected in the “detained for inspection room (DFI)” and weighed at the end of each shift in order to calculate the amount of meat trimmed per carcass per day. The losses are expressed in grams per ostrich and are calculated by the weight of trimmed bruised meat divided by the number of approved ostrich carcasses for the specific day. These losses were calculated for each production day on the slaughter line and an average is reported for 12 months.

At the Klein Karoo International Research Laboratory routine weekly samples from the abattoir de-boning hall (meat just before packaging) is analyzed for APC counts (Plate count agar (Biolab code C6, supplied by Merck). The weekly APC results were used to calculate the geometric mean value for the week; and these values were then plotted on a geometric mean graph for a year period (Anon., 2002).

Inspection of bruise distribution

At the primary meat inspection point of the slaughter-line all the carcasses slaughtered over eight slaughter days were visually inspected for the presence of bruises. These bruises were identified to one of four areas on the carcasses: either on the neck; the back, the front of the thighs; or the back of the thighs. As Wotton & Hewitt (1999) reported ostriches are particularly prone to lacerations and bruising to the necks and legs. The number of bruises were recorded and commented on in terms of size. An A, B and C classification were awarded to the bruises on the basis of size, where A = <2 cm in diameter, B = 2–5 cm in diameter and C = >5 cm in diameter.

RESULTS AND DISCUSSION

Microbiological data

The results of the microbiological data, indicated that with the exception of one pooled sample with low coliform counts (<100 cfu.g^{-1}), all the carcasses sampled were free of coliforms and *E. coli*. The aerobic plate counts (APC) for the three studies are reported in Tables 1 and 2. In both Tables the sample numbers 1 to 3 represents the data from the three individual studies.

The data in Table 1 reflects the colony forming units per gram of meat (cfu.g^{-1}) of the warm trimmed samples, taken directly after primary meat inspection (on day one) on the trimmed surfaces and again on the exposed trimmed surface on day two after overnight cooling (A1 tot A4).

The data in Table 2 represents the cfu.g^{-1} values of the cold trimmed carcasses. These samples were taken on day one in the untrimmed bruised areas and again after overnight cooling and cold trimming of the areas at de-boning (on day two). The number of carcasses sampled for the three pooled trials was n = 3 x 5 x 2 = 30 and the same number (n = 30) was sampled as control.

Table 1 Aerobic plate counts (cfu.g^{-1}) on ostrich meat from bruised areas trimmed warm (at primary meat inspection point) - performed in triplicate

Sample no	**Bruised area**		**Undamaged next to bruise (control)**	
	On Day 1 (A1)	On Day 2 (A2)	On Day 1 (A3)	On Day 2 (A4)
1	<10	1073	10	110
2	50	509	170	130
3	40	236	218	355

Table 2 Aerobic plate counts (cfu.g^{-1}) on ostrich meat from bruised areas trimmed cold (at de-boning) - performed in triplicate

Sample no	**Bruised area**		**Undamaged next to bruise (control)**	
	On Day 1 (B1)	On Day 2 (B2)	On Day 1 (B3)	On Day 2 (B4)
1	120	50	30	630
2	6782	1009	6855	1127
3	20	<10	173	155

As can be seen in the data from Table 1 and Fig. 1 representing the bruised areas, aerobic plate counts (APC) for carcasses that were warm trimmed on day one all increased, in most instances with more than a log phase (<10 to 1073, 50 to 509 and 40 to 236). From the data in Table 2 and Fig. 1 it can be seen that the APC in the bruised areas for carcasses that were cold trimmed all presented a small decrease. Thus the final microbial load on the primal meat cuts in the de-boning area was lower when bruises were cold trimmed rather than warm trimmed and as McKinnon *et al.* (2005) reported this should lead to an increase in shelf-life.

The reason for this increase in microbial growth on the trimmed areas could be the fact that the muscle area is exposed during trimming; the meat is thus more susceptible to aerobic bacterial contamination and growth. As indicated elsewhere in these studies, sources of bacterial contamination are present all along the slaughter-line, in the cooling rooms and the de-boning facilities. The sources identified include the air in the areas, the hygiene of workers and surfaces and most importantly, the water pooling on platforms and drainage areas. Microorganisms from the indicated sources can thus readily cross contaminate the meat on the exposed trimmed areas.

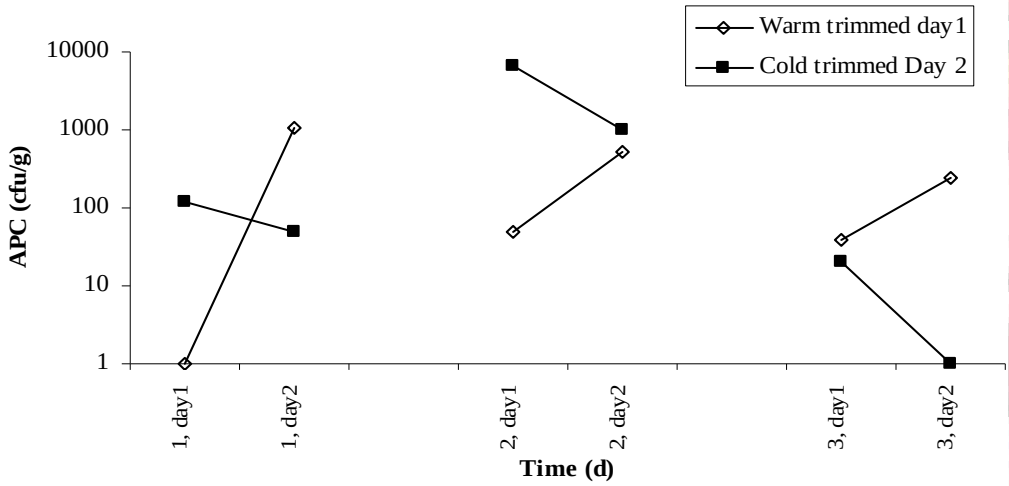

Figure 1 Changes over 2 days in aerobic plate counts on bruised areas for the three trials for both cold and warm trimmed carcasses

For the control samples (normal, not bruised and untrimmed carcasses) there is no obvious trend in the microbial growth data from day one to day two (Fig. 2). However, in most instances there is a slight increase which corresponds well with what Karama (2001) reported; "for APC counts in an South African ostrich abattoir there tends to be an increase in counts from post-evisceration to post-chilling, this increase however, is not significant."

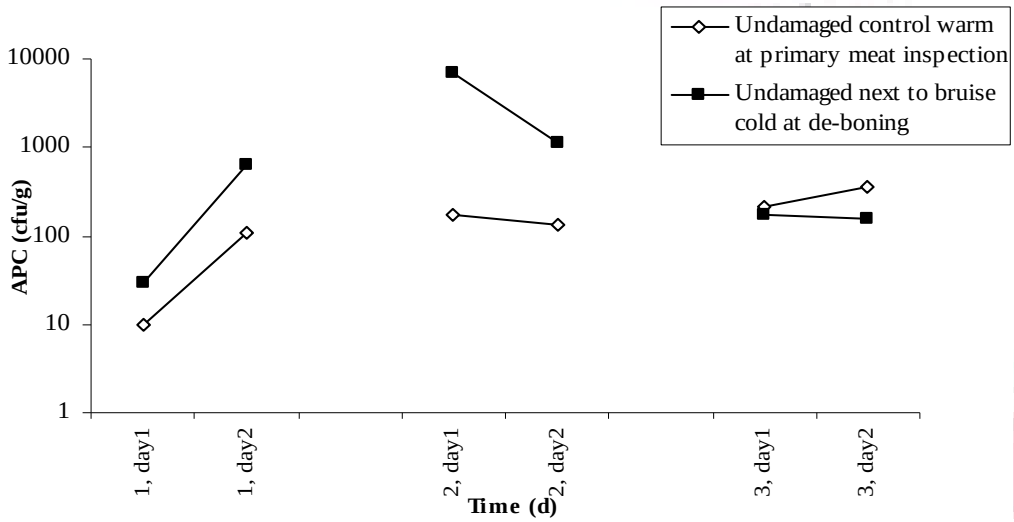

Figure 2 Changes over 2 days in aerobic plate counts on undamaged control areas for the three trials for both cold and warm trimmed carcasses

Practical implications

Results from this study have led to the permanent acceptance of the practice of cold removal of minor bruises in this abattoir, where minor bruises are classified as for this study; between 2.5 and 10 cm in diameter, non-infectious and not showing any green discolouration. From the results presented in Fig. 3, as expected, it can be seen that average aerobic meat counts in the de-boning hall was lower for the second period of the year, when this practice was implemented and these bruises were removed from chilled carcasses (cold trimmed). The cold trimming of bruised carcasses aided in better microbiological control of the meat at this abattoir and the subsequent lower initial microbial load on the ostrich meat will lead to an increased shelf-life.

The bruises that were not removed at primary meat inspection were visually inspected on day one before overnight chilling and again on day two directly prior to trimming of the muscles in the de-boning hall. In all instances the visibly bruised areas had diminished. *Rigor mortis* sets in within a few hours after the animal is killed (Hoffman *et al*., 2006) and this condition is associated with a contraction of muscle fibers (Potter, 1986). The diminishing of the visible bruised area can probably be explained in terms of *rigor mortis*, where the capillary veins that cause the bleeding associated with bruises, contracts with the muscle fibers to miniaturize the appearance of the bruises.

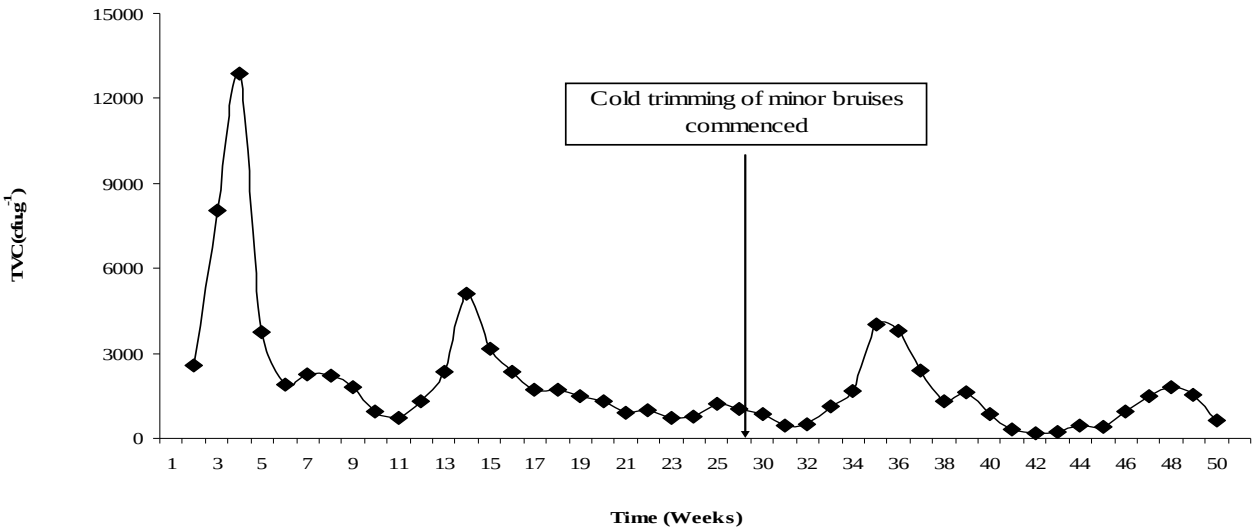

Figure 3 Geometric mean results of aerobic plate counts on primary ostrich meat cuts in the de-boning area where cold trimming of minor bruises commenced on week 30

Physical measurements were not taken and further investigation of the extent of this decrease is advised. Due to this crimping effect the area that had to be trimmed away to remove the bruise was smaller than on day one and this led to an increased meat yield.

Since the practice of cold trimming of the meat in the de-boning area commenced, the trained personnel in the area would assess the condition of the bruised meat that is to be removed from the back or thigh muscles. Any piece of bruised meat that is deemed to pose a health risk due to infection or that is visually unacceptable due to excessive bloodiness is trimmed off and condemned for human consumption. Slight bruises are removed and the trimmed meat is then added to other off-cuts for use in minced and processed products. This practice is acceptable as Rogers (1993) reported that up to 30% bruised beef can be incorporated into fresh sausages with no detrimental effect on product quality as assessed by a sensory panel. This also leads to an increase in utilizable meat. The practice of adding the trimmed bruises to the rest of the meat trimmings destined for use in minced meat preparations did not have a marked negative effect on the microbiological quality of the trimmings. The trimmings were routinely monitored at the facility and conform to the specifications under the Red Meat Act (Anon., 2004).

The third parameter that was monitored in this study was the meat losses associated with the removal of bruised areas during primary meat inspection. As can be seen from the data in Fig. 4, the average meat loss per carcass due to the warm trimming of bruises at this particular abattoir for the year period preceding the change in procedure to cold trimming was on average 264 g per bird.

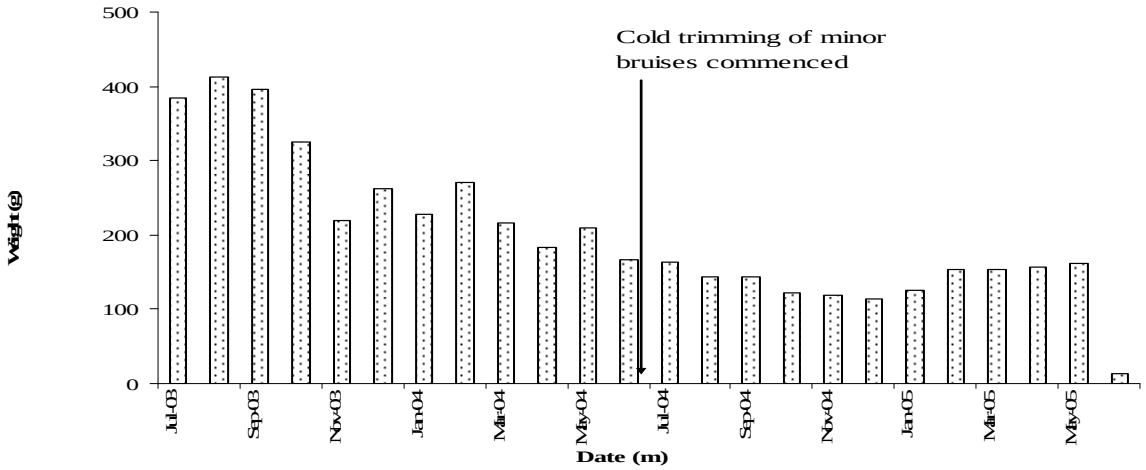

Figure 4 The average monthly meat losses per carcass in gram (g) due to the removal (trimming) of bruises at primary meat inspection, indicating the commencing of cold trimming

The average loss per carcass for July to October of 2003 was above 300g, this figure was higher than expected and higher than for other abattoirs in the ostrich industry (W P Burger, Klein Karoo International, Oudtshoorn, South Africa, personal communication). Discussions were held with the meat inspectors and the supervising official state veterinarian to try to curb the losses by responsible trimming of bruises, this already aided in lowering meat losses as can be seen from the data in Fig. 4 from November 2003 to June 2004. The study was compiled from May to June 2004 and from July 2004 all minor bruises were removed in the de-boning area with the approval of veterinary services (W P Burger, Klein Karoo International, Oudtshoorn, South Africa, personal communication). The decrease in meat losses since July 2004 is evident. The average meat loss due to the trimming of bruised meat decreased from an average of > 250 g per bird for warm trimmed carcasses to an average of just over 130 g per bird for cold trimmed carcasses.

Distribution of bruises

The data from the investigation into the distribution of bruises over the ostrich carcass as well as the frequencies of bruising is summarized in Table 3. A total of 3153 birds were evaluated and on them 789 bruises were found. From the data it was seen that the necks (52.58%) were clearly the most pronounced area for bruising and this will obviously lead to significant losses in the use of the meat from the necks. As mentioned before the reasons for the bruises were expected to lie in the transport and lairage practices and thus, this high incidence of injuries on the ostrich necks can most probably be traced back to the construction of the trucks transporting them to the abattoir. These trucks are constructed with high side panels, according to the guidelines of the Code of Practice for Transport of Ostriches (SAOBC, 2001), which states under point 1.4.8 that “the sides of the vehicles must be at least as high as the top of the neck, just below the head, to prevent ostriches from jumping out of the vehicle.” A practice that the ostriches do attempt, despite the high panels (personal observation) and this point in the standard might need to be revised.

From the data in Table 3 it is seen that the other area on the carcass which showed clear indication of bruising was the front of the thighs (36.98%). The bruising in this area was most likely caused during loading and off-loading practices, when the ostriches jump on or off the trucks and often into either the sides of the

trucks or the walkways. To a lesser degree these bruises might be caused by injuries in the pens and during herding between pens. Protecting the sides of the vehicles and the ramps with a softer barrier might aid in minimizing this area of bruising. Current practices of keeping the ostriches calm during handling and ensuring that all the passages, water troughs and pens have rounded corners should also be maintained.

Table 3 Distribution of bruises on ostrich carcasses

Number of			**Number of bruises on**				**Percentage (%) of bruises on**				
Day	Birds	Bruise	Neck	Back	Thigh front	Thigh back	Total	Neck	Back	Thigh front	Thigh back
1	209	14	4	0	10	0	6.70	28.57	0.00	71.43	0.00
	91	5	0	0	2	3	5.49	0.00	0.00	40.00	60.00
2	27	4	3	0	1	0	14.81	75.00	0.00	25.00	0.00
	70	30	24	0	6	0	42.86	80.00	0.00	20.00	0.00
	55	17	10	0	7	0	30.91	58.82	0.00	41.18	0.00
	36	10	9	0	1	0	27.78	90.00	0.00	10.00	0.00
	61	12	4	1	4	3	19.67	33.33	8.33	33.33	25.00
	62	26	8	1	9	8	41.94	30.77	3.85	34.62	30.77
	42	27	14	0	10	3	64.29	51.85	0.00	37.04	11.11
	144	42	18	0	19	5	29.17	42.86	0.00	45.24	11.90
	48	11	7	0	4	0	22.92	63.64	0.00	36.36	0.00
	49	11	4	0	5	2	22.45	36.36	0.00	45.45	18.18
3	65	53	33	1	14	5	81.54	62.26	1.89	26.42	9.43
	41	9	5	4	0	0	21.95	55.56	44.44	0.00	0.00
	100	34	18	1	12	3	34.00	52.94	2.94	35.29	8.82
	100	38	19	0	13	6	38.00	50.00	0.00	34.21	15.79
	41	34	17	0	14	3	82.93	50.00	0.00	41.18	8.82
	176	69	31	0	30	8	39.20	44.93	0.00	43.48	11.59
	35	0	0	0	0	0	0.00	0.00	0.00	0.00	0.00
4	49	4	1	0	3	0	8.16	25.00	0.00	75.00	0.00
	38	3	1	1	1	0	7.89	33.33	33.33	33.33	0.00
	248	65	43	0	21	1	26.21	66.15	0.00	32.31	1.54
5	74	24	9	0	12	3	32.43	37.50	0.00	50.00	12.50
	76	22	6	0	10	6	28.95	27.27	0.00	45.45	27.27
	50	12	6	0	6	0	24.00	50.00	0.00	50.00	0.00
6	250	33	20	0	12	1	13.20	60.61	0.00	36.36	3.03
	85	11	4	0	6	1	12.94	36.36	0.00	54.55	9.09
	36	7	3	0	3	1	19.44	42.86	0.00	42.86	14.29
7	97	43	30	0	11	2	44.33	69.77	0.00	25.58	4.65
	56	6	5	0	1	0	10.71	83.33	0.00	16.67	0.00
	62	10	5	0	4	1	16.13	50.00	0.00	40.00	10.00
	33	1	0	0	1	0	3.03	0.00	0.00	100.0	0.00

Table 3 (continued) Distribution of bruises on ostrich carcasses

Number of			Number of bruises on				Percentage (%) of bruises on				
Day	Birds	Bruise	Neck	Back	Thigh front	Thigh back	Total	Neck	Back	Thigh front	Thigh back
8	226	28	17	0	10	1	12.39	60.71	0.00	35.71	3.57
	120	14	6	0	5	3	11.67	42.86	0.00	35.71	21.43
	14	9	3	0	5	1	64.29	33.33	0.00	55.56	11.11
	7	4	2	0	2	0	57.14	50.00	0.00	50.00	0.00
	180	53	29	0	20	4	29.44	54.72	0.00	37.74	7.55
Total	**3153**	**789**	**418**	**9**	**294**	**74**	**25.21**	**52.58**	**1.13**	**36.98**	**9.31**

Size of the bruises

On 168 carcasses (5.33% of the ostriches evaluated), more than one bruise were found (up to 4 large bruises on a single carcass) and the bruises evaluated from these carcasses with multiple bruising also tended to be larger in size (from 2 cm to > 5 cm). This has led to the assumption that these birds were injured through trampling by the other ostriches in the trucks or pens. These birds either accidentally loose their footage or sit down during transport or in the pens (they are referred to as downers) and the other ostriches step on them in the confined areas; this can lead to severe injuries. The ostriches should never be left unsupervised and downer birds should be helped up or moved to a separate pen as soon as possible to prevent bruising of the meat and damage to the skins.

Out of the 789 bruises evaluated, 47.06% of the bruises were indicated as >5 cm in diameter, thus, representing fairly large areas of meat and causing significant losses. Care should always be taken to adhere to all measures protecting the ostriches from bruising because of not only the loss of utilizable meat, but also damage to the skins that can cause a downgrade in skin classification and a loss in income for the producers. Furthermore these measures have as basis the humane treatment of the animals, which are under written by the various societies for the protection of animals and thus serve not only to benefit producers and processors, but also to look after the welfare of the birds.

CONCLUSION

From the data obtained in this study it is clear that the cold trimming of ostrich meat is advantageous to abattoir management in terms of meat yield. Thus, the better management of trimming practices has halved the losses due to bruising of carcasses. The cold trim practice also caused a reduction in meat microbial load and should result in a subsequent desired gain in shelf-life. Cold trimming of bruises is preferable to the process of warm trimming in so far minor, non-infectious bruises are concerned. As a result of these studies letters of motivation were sent to the Department of Agriculture in the Western Cape (Veterinary Services) and permission was granted to ostrich abattoirs to take responsibility for the handling of minor bruises (as at the discretion of meat inspectors). This practice contributed to both a gain in meat yield and increased hygiene control in this abattoir.

From the data regarding the distribution of the bruises it was evident that the ostrich necks and thighs were most often bruised during transport and handling practices. Keeping the ostriches calm during these initial processing steps, preventing rough edges or openings in lairages and walkways, covering the sides of trucks and off-loading areas with a softer barrier (such as rubber or conveyor belting) and a further investigation into raising the prescribed height of the truck sides should all aid to minimize bruising on ostrich carcasses and at the same time curb the subsequent financial losses.

REFERENCES

Anonymous (1993). Council Directive 93/119/EC on the Protection of Animals at the time of Slaughter or Killing, www.eur-lex.europa.eu. 2006.

Anonymous (2001). Commission Decision of 30 March 2001, Regarding protection of animals during transport, www.eur-lex.europa.eu. 2006.

Anonymous (2002). RSA export of ostrich meat to the EC – Policy, published as Veterinary Public Notices, www.nda.agric.za/vetweb/. 2002.

Anonymous (2004). The Meat Safety Act and Regulations. Act no. 40 of 2000, G.N.R. 8056. Johannesburg, South Africa: Lex Patria Publishers.

Anonymous (2005). Modification of Annex to Commission Decision 2001/96/EC from 16/03/2005, www.eur-lex.europa.eu. 2005.

Anonymous (2007a). Ostrich Regulations under the Meat Safety Act. R54, G.N.R. 29559/2007 02 02. Johannesburg, South Africa: Lex Patria Publishers.

Anonymous (2007b). VPN/13/2007-01 Standards for *ante-mortem* and *post-mortem* meat inspection and hygiene control at ostrich meat establishments, www.nda.agric.za/vetweb/. Augustus 2008.

Blood, D.C. & Studdert, V.P. (1988). *Baillière's Comprehensive Veterinary Dictionary*. Baillière Tindall, 24-28 Oval Road, London, NW1 &DX, UK.

Burger, W.P. (2004). Klein Karoo International, Oudtshoorn, South Africa. Personal communication.

Chambers, P.G., Grandin, T., Heinz, G. & Srisuvan, T. (2004). Effects of stress and injury on meat and by-product quality. In: Guidelines for Humane Handling, Transport and Slaughter of Livestock. FAO Corporate document repository. [WWW document]. URL. http://www.fao.org/DOCREP/003/X6909E/x6909e04.htm. 28 August 2008.

Grandin, T. (1990). Design of loading and holding pens. *Applied Animal Behavior Science,* **28,** 187-201.

Grandin, T. (1991). Recommended Animal Handling Guidelines for Meat Packer. American Meat Institute, Washington, D.C.

Hoffman, L.C., Botha, S. St.C. & Britz, T.J. (2006). Sensory properties of hot-deboned ostrich. *Meat Science*, **72**, 734-740.

Jago, J.G., *et al*. (1996). Risk factors Associated with Bruising in Red Deer at a Commercial Slaughter Plant. *Meat Science*, **40**, 181-191.

Karama, M. (2001). The microbial quality of ostrich carcasses produced at an export-approved South African abattoir. MMedVet (Hyg) study at University of Pretoria. Pretoria, South Africa.

McKinnon, D.C., Heyneke P.G., Olivier, A.J., Mulder, C., Britz, T.J. & Hoffman, L.C. (2005). Effect of ozone and UV on the microbial load of muscle surfaces of ostrich carcasses. *Proceedings of the 3rd International Ratite Science Symposium of the WPSA*. Pp. 276. October 2005. Madrid, Spain.

Petersen, G.V. (1978). Bacteriological evaluation of bruised tissue in lamb carcasses. *New Zealand Veterinary Journal*, **26,** 9-10.

Potter, N.N. (1986). *Food Science*, Fourth Edition. Van Nostrand Reinhold Company Inc. 115 Fifth Avenue, New York. Pp.390-401.

Rogers, S.A. (1993). The effect of bruised beef addition on the quality of processed meat products. *Meat Science*, **33**, 51-59.

SANS ISO 6887-1. (1999*). Preparation of test samples, initial suspension and decimal dilutions for microbiological examination – general rules.*

SANS 11133. (2004). *Microbiology of food and feeding stuffs – Guidelines on preparation and production of culture media.*

SANS ISO 4832. (2007a). *Horizontal method for the detection and enumeration of Coliforms – colony count technique.*

SANS ISO 4833. (2007b). *General guidance for the enumeration of micro-organisms – Colony Count Technique at 30ºC.*

South African Ostrich Business Chamber (SAOBC) in conjunction with the National Council of Societies for the Prevention of Cruelty to Animals (SPCA) and the ARC – Animal Nutrition and Animal Products Institute. (2001). *Code of Practice for the Transport, Handling and Slaughter of Ostriches.*

Wotton, S.B. & Hewitt, L. (1999). Transportation of ostriches – a review. *The Veterinary Record*, **145**, 725-731.

Wotton, S. & Sparrey, J. (2002). Stunning and slaughter of ostriches. *Meat Science*, **60**, 389-394.

Chapter 5

CONCLUSION AND RECOMMENDATIONS

South African ostrich meat is primarily vacuum packed and exported fresh (0-4ºC) to the European Union and other overseas markets. In order to be competitive in these markets the meat needs to have a shelf-life of at least four weeks to allow for distribution and retailing. Ostrich meat has an expected pH of between 5.8 and 6.2 (Sales & Mellet, 1996) which is intermediate to high compared to other red meats; it will thus render the meat more susceptible to microbial growth and subsequent spoilage. McKinnon *et al*. (2005) reported that the most effective way to ensure an acceptable shelf-life in ostrich meat is to keep the initial microbial load at vacuum packing low by minimizing carcass contamination. In this regard Karama (2001) reported that most of the contaminating organisms on the carcasses were already deposited during skin removal and other processes on the rest of the slaughter-line. He further established that the over-night chilling of the carcasses post-evisceration only inhibited the growth of these organisms and did not reduce the numbers thereof. According to literature the dominant growth found on ostrich carcasses on the slaughter-line is of bacteria found in the environment and on the skins of the birds and humans (Harris *et al*., 1993) and these consist mostly of *Micrococcus* spp, *Enterobacteriaceae* and *Acinetobacter* spp. (Karama, 2001). The only reported commonly isolated foodborne pathogen on ostrich carcasses was *E. coli* (Ley *et al*., 2001). Comprehensive research on the expected prevalent microorganism growth on ostrich carcasses as well as on the influence of different sources of environmental contaminants is minimal and warrants investigation.

Another parameter that influences the success of an export meat facility is its ability to operate cost effectively and supply the meat to clients at a competitive price. In this regard the management of meat yield from carcasses is an important factor. Meat yield is jeopardized by unnecessary bruising on carcasses and the excessive trimming of the carcasses when removing these bruises. The distribution of the bruises on the carcasses as well as the frequency of their presence would be indicative of the transport, loading and lairage practices as these handling steps are the most likely to cause injury to animals (Grandin, 1990; 1991). Published research on bruises on carcasses and the effect that the trimming of the affected meat has on the microbial quality of the ostrich meat is once again limited and it is essential that this be evaluated.

The aim of the first part of this study was to establish what the prevalent microorganism growth on ostrich carcasses would be; the meat was sampled from undamaged areas on the carcasses, from bruised areas as well as from the cut surfaces where bruised areas were removed. It was seen that the number of organisms increased from post-evisceration to post-chilling, corresponding to results of other studies, but that the numbers of bacteria found were lower than those reported by other authors (Harris et al., 1993; Karama, 2001). The organisms evaluated from post-evisceration samples were predominantly Gram-positive (64.29% of 14 samples). After overnight cooling there was still a large number of Gram-positive isolates (51.22%) but there was also an increased number of Gram-negative organisms present (26.83% of 41 samples). This indicated that the contaminating organisms were of both Gram types, but that some or all of the Gram-negatives were probably more psychrophilic and grew better under refrigerated conditions. The organisms identified from carcasses were mostly Gram-positive coccoid organisms including *Gemella morbillorum* (closely resembling

Streptococcus spp.) and *Pediococcus* spp.; these organisms are of environmental origin. The Gram-negative organisms identified very widely varied with *Shigella* and *Serratia* spp. the only organisms that were isolated more than once, no *Salmonella* or *E. coli* organisms were isolated. Of interest were the high incidence of yeast growth and specifically *Cryptococcus* spp. on the ostrich meat and the significance and spoilage potential of yeast species in an ostrich abattoir warrants further investigation.

The second aim of this trial was to determine which sources of environmental bacterial contamination were most hazardous to the ostrich meat quality and thus aid abattoir management in preventing carcass contamination. The air supply to the facility, the water sources including pooling water, the workers' hands and work surfaces and knives were tested and colonies for identification were selected from the media. From the data obtained the workers, surfaces and air borne organisms could not be excluded or indicated as the main contributor to microbial contamination on carcasses. However, from these sources *Shigella* spp. (possible human pathogenic organisms) and *Pseudomonas* (strongly associated with spoilage of refrigerated meat; Jay, 1992) were isolated, indicating that these vectors of possible contamination needs to be controlled. The water supplied through the reticulation system is treated with chlorine dioxide and no organisms were isolated from the taps. The water pooling in drains and on platforms, on the other hand, had a predominantly Gram-negative growth (81.82% of 11 organisms) and many of these were human pathogens (*Shigella, E. coli* and *Salmonella*), posing a threat to the food safety of the ostrich meat.

In conclusion and based on the data collected in this trial it can be recommended that water must be well contained on the slaughter-line through: the prevention of pooling of water on platforms; the prevention of drain blockages; the control over the usage of high pressure hoses and water brooms; training of personnel not to step in standing water. This should be supported by a well developed programme for the hygiene of workers, the areas, surfaces and the air supply. These good manufacturing practices (GMP) will have to include the implementation of filtered air supply and regular cleaning of air ducting, the cleaning and sanitizing of work surfaces and equipment and personnel hygiene (including proper medical screening, clean protective wear and the frequent cleaning and sanitizing of workers' hands).

In an attempt to curb meat yield losses at the abattoir where this study was performed, the practice of removing sustained bruises warm on the slaughter-line or cold, post-chilling, was investigated on grounds of both microbiological and meat yield parameters. Large, infectious bruises were not considered, since these need to be removed during primary meat inspection from a food safety perspective; in the study only minor bruises, non-infectious and <10 cm in diameter were considered. It was found that the cut made in the muscle to remove the bruises post-evisceration had negative implications from a microbiological perspective. The aerobic plate counts increased on this surface, despite over-night chilling to below 4ºC. This was probably due to the exposure of the meat on the still warm carcass (more or less 30ºC) to both oxygen and environmental contaminants. The opposite was true when these bruised meat surfaces were trimmed cold in the de-boning area; the exposed outer membrane and bruised meat is cut away, leaving a freshly cut, largely uncontaminated area on the muscle just before packaging, thus aiding a low initial microbial load and projected better shelf-life.

Furthermore the surface area of the bruises physically diminished during the cooling of the carcasses due to crimping of the muscles and the pieces that needed to be trimmed was smaller. When the bruises were cold removed the losses in meat yield were considerably smaller than when it was warm trimmed. On the basis of

the results from the study indicating microbiological as well as meat yield advantages of cold trimming, it was recommended to abattoir management and the regulatory authorities to adopt the practice of cold trimming of minor bruises in this abattoir.

As part of this second study the distribution and frequency of the bruises on 789 ostrich carcasses were evaluated on the slaughter-line. From the 3153 bruises seen, 52.58% were on the necks of the ostriches and 36.98% where on the front of the thighs, corresponding with Wotton & Hewitt (1999) that ostriches are particularly prone to injuries to the necks and legs. The pronounced bruising on the necks can most possibly be linked to the height of the side railings on the transport trucks and the tendency of the ostriches to try to jump out of the trucks. The height of the side panels are prescribed under point 1.4.8 in the Code of Practice for Transport of Ostriches (SAOBC, 2001) and it is recommended that this paragraph be revised to prevent the excessive bruising to the necks of the ostriches. The bruises to the front of the legs can be associated with loading and off-loading practices and the birds jumping or running into the sides of the trucks or the walls of the loading ramps. Here it is indicated that current practices of constructing facilities with rounded corners and no protruding elements as well as keeping the birds calm during handling is of the utmost importance. Furthermore it is recommended that the sides of trucks and the walls of the off-loading area be covered with a barrier to prevent these injuries. Large and multiple bruises were seen on 5.33 % of the ostrich carcasses evaluated, this could possibly be linked to downer birds that were trampled on the trucks or in the pens. Once again it is recommended that current practice of having handlers travel with the birds to prevent the birds from sitting down in the confined space of the truck compartments must be maintained.

REFERENCES

Grandin, T. (1990). Design of loading and holding pens. *Applied Animal Behavior Science,* **28,** 187-201.

Grandin, T. (1991). Recommended Animal Handling Guidelines for Meat Packer. American Meat Institute, Washington, D.C.

Harris, S.D., Morris, C.A., Jackson, T.C., May S.G., Lucia, L.M., Hale, S.D., Miller, R.K., Keeton, J.T., Savell, J.W., & Acuff, G.R. (1993). Ostrich meat industry development, Final report to: American Ostrich Association. Texas Agricultural Extension Service 348, Kleberg, 14.

Jay, J. (1992). *Modern Food Microbiology*. 4th Ed., Chapman and Hall, New York. Pp. 38-44, 64-65, 71-73.

Karama, M, (2001). The microbial quality of ostrich carcasses produced at an export-approved South African abattoir. MMedVet (Hyg) study at University of Pretoria. Pretoria, South Africa.

Ley, E.C., Morishita, T.Y., Brisker, T. & Harr, B.S. (2001). Prevalence of *Salmonella, Campylobacter*, and *Escherichia coli* on ostrich carcasses and the susceptibility of ostrich-origin *E.coli* isolates to various antibiotics. *Avian Diseases,* **45**, 696-700.

McKinnon, D.C., Heyneke P.G., Olivier, A.J., Mulder, C., Britz, T.J. & Hoffman, L.C. (2005). Effect of ozone and UV on the microbial load of muscle surfaces of ostrich carcasses. *Proceedings of the 3rd International Ratite Science Symposium of the WPSA*. Pp. 276. October 2005. Madrid, Spain.

Sales, J. & Mellet, F.D. (1996). Post-mortem pH decline in different ostrich muscles. *Meat Science,* **42**, 235-238.

South African Ostrich Business Chamber (SAOBC) in conjunction with the National Council of Societies for the Prevention of Cruelty to Animals (SPCA) and the ARC – Animal Nutrition and Animal Products Institute (2001). *Code of Practice for the Transport, Handling and Slaughter of Ostriches.*

Wotton, S.B. & Hewitt, L. (1999). Transportation of ostriches – a review. *The Veterinary Record*, **145**, 725-731.