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The effect of lairage time and transport density on live weight losses and meat quality in slaughter ostriches

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SUMMARY

Although transport and lairage of ostriches are accepted causes of production losses, these losses have not yet been quantified. Transport and lairage regulations focus on the wellbeing of the birds and, by default reduce some losses. This thesis investigated weight losses and meat quality of ostriches as a result of transport density, lairage duration and lairage feed availability (*ad libitum*). All birds were reared on the same farm and loaded and transported together. They were randomly divided into their respective groups on loading for the transport trial, and on offloading for the lairage trial. Bird grouping was according to density for the transport trial (4 groups, H1, H2: 0.56m^2/bird and L1, L2: 0.96m^2/bird) and according to time spent in lairage and feed availability for the lairage trial (n=30 birds/group; L0hr and L24hr; n=15 birds/group L48hr and L48hr *ad libitum* feed).

Behavioural observations of the ostriches showed a tendency of the ostriches to lean against objects and to orientate towards forces exerted on it to help keep its balance. Reactions to sound fluctuations were noted, with birds reacting towards changes in sound volume during transport and lairage. Timepoint numbers were allocated for each time the birds were weighed during the trial. Time points 1, 2, 3, 4 and 5 were allocated to loading, arrival, 19 hr in lairage, 31 hr in lairage and 39 hr in lairage respectively for Trial 2. Results showed no differences ($P > 0.05$) in live weights or meat quality parameters between Groups H1, H2, L1 and L2 during transport. Differences ($P < 0.05$) were found in cumulative weight losses between L0hr and the rest of the groups for time point 1. Differences between L48hr and L48hr *ad libitum* were found for time point 4 for cumulative weight loss. L48hr also differed significantly between the other lairage duration groups for dressing percentages as a function of loading weight. *Ad libitum* feed availability had a significant effect on live body weight changes but not the meat quality parameters for the groups held for 48hr in lairage. The number of birds having bruises (≈50% per group) was similar between groups and lairage had no influence on bruising. Results seem to indicate that the evaluated transport densities had no effect on the weight loss or meat quality of ostriches. However, the results indicate that the lairage period should be studied further with specific reference to weight losses during lairage. Meat quality was unaffected by the lairage parameters reported in this thesis.

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OPSOMMING

Hoewel vervoer en voorslag hou ("lairage") aanvaar word as die oorsake van produksieverliese, is hierdie aspekte nog nie gekwantifiseer nie. Regulasies in terme van vervoer en voorslag hou fokus op die welstand van die voëls en gevolglik verminder sommige verliese met hierdie faktore. Konflik tussen boere en sekondêre produsente (abattoirs en verwerkers) oor die kostes verbonde aan hierdie gewigsverliese, is in wese 'n oorsaak van oorbruggingsfases (vervoer). Daarom is die voorslag hou-parameters van tyd, voerbeskikbaarheid en laai digtheid ondersoek. Twee proewe is geloods; een vir vervoer en een vir voorslag aanhou. Voëls is voor hulle op vragmotors gelaai is, geweeg en op spesifieke tye daarna geweeg. Diere van die laaidigtheidsproef is ingedeel in vier groepe (H1, H2: 0.56m^2/voel en L1, L2: 0.96m^2/voel). Met aflaai is die eerste toets groep geslag terwyl die tweede toets groep ingedeel is volgens die tyd gespandeer en voer beskikbaarheid in voorslag aanhou (n=30 voëls/groep - Groepe L0u, L24u; n=15 voëls/groep - L48u en L48u *ad libitum* gevoer).

Gedrag van voëls is aangeteken tydens vervoer. Finale gewig was slagpale gewig en elke tyd periode waar die voëls geweeg is, is aangeteken as 'n tydpunt. Vleiskwaliteit analises is gedoen op die linker boud se fan filet. 'n Standaard rak leeftyd toets is ook gedoen om kwaliteit parameters te toets oor tyd. pH is gekatorigiseer volgens groepe om moontlike verskille te beklemtoon. Gedrag is opgeteken volgens die oriëntasie en steunings gewoontes van die volstruise gedurende vervoer. Voëls probeer hulle balans te hou deur op voorwerpe en op mekaar te leun, asook om hul liggaam oriëntasie te gebruik. Reaksies tot klank veranderinge is ook opgemerk tydens vervoer en voorslag aanhou. Resultate toon geen betekenisvolle verkille tussen digtheid groepe H1, H2, L1 en L2 nie. Geen gewigsverskille is gevind tussen vervoerdigtheid groepe nie. Vleiskwaliteit-parameters is ook ondersoek en geen verskille is gevind tussen groepe nie. Resultate toon wel betekenisvolle verskille in gewigsverlies-persentasie tussen groepe vir tyd in voorslag aanhou. Groepe L48u en L48u *ad libitum*, het onder andere betekenisvol verskille getoon vir tydpunt 4. Betekenisvolle verskille in uitslagpersentasie as funksie van begin-gewig tussen 48 uur en die res van die groepe is ook gevind. Verdere vleiskwaliteit-parameters (drupverlies, pH, ens.) tussen groepe het geen betekenisvolle verskille getoon nie. Resultate dui aan dat voer beskikbaarheid het 'n invloed op gewigsverskille in voorslag aanhou. Regulasies vir voorslag hou, vervoer en welstand van volstruise sal moontlik verder ondersoek moet word met spesiale klem op die nut van voorslag aanhou en die se invloed op gewigsverlies. Vleiskwaliteits-parameters is nie beïnvloed deur die aspekte ondersoek nie.

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LIST OF ABBREVIATIONS

Kg	kilogram
g	gram
kN	kilo Newton
Km	kilometre
SAOBC	South African Ostrich Business Chamber
°C	degrees Celsius
LWCC	Livestock Welfare Coordinating Committee
SD	standard deviation
SE	standard error
TP	total protein
LDH	lactate dehydrogenase
AP	alkaline phosphatase
AST	aspartate aminotransferase
ATP	adenosine triphosphate
ADP	adenosine diphosphate
GGT	Gamma-glutamyltransferase
NS	non-significant
GCs	glucocorticoids
L*	lightness
a*	red-green colour range
b*	blue-yellow colour range
H_{ab}	hue angle
C*	chroma
ANOVA	analysis of variance
WHC	water-holding capacity
PSE	pale, soft and exudative
DFD	dark, firm and dry
pH_u	ultimate pH
$pH_{(30\ min)}$	pH after 30 minutes *post-mortem*
$pH_{(1\ hour)}$	pH after 1 hour *post-mortem*
pH_u	ultimate pH *post-mortem*
pH_{24}	pH after 24 hours *post-mortem*
m^2	square meter
Proc GLM	procedure generalised linear model
N	Newton
vs	versus
Fisher LSD	Fisher least significant difference

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NOTES

This thesis represents a compilation of manuscripts; each chapter is an individual entity and some repetition between chapters, especially in the Materials and Methods sections, is therefore unavoidable.

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Chapter 1

Introduction

Traditionally, since its conception in the 1860's, the focus in the ostrich industry was production of feathers (Smit, 1963). The First World War and the economic depression that followed, combined with alternative materials, caused a shift in the production focus of the ostrich industry towards the production of leather and meat. After this gradual shift almost two thirds of the ostrich industry was focused on meat production (Brand, 2010). This has led to an increase in research evaluating the influence of various intrinsic and extrinsic factors on ostrich meat quality.

Ostrich meat has increased both in monetary value as well as in popularity amongst consumers due to a strong marketing drive focused on the healthy and safe meat demand due to the red meat scares in Europe and other first world countries (Lambrechts and Kruger, 2006; Claassen, 1991). This popularity is driven by the worldwide increase in nutritional awareness that emphasises healthy eating. Ostrich meat, being naturally low in cholesterol and having a beneficial fatty acid profile, has made it a favourite in the health conscious society. These factors both contribute to lowering the risk of coronary heart disease (Sales, 1996). The beneficial fatty acid profile of ostrich meat is due to the high amount of polyunsaturated fat it contains (Horbanczuk *et al*., 1998). This has additional benefits in ostrich meat labelling and marketing as it can be sold as promoting a variety of health benefits. Further research is being conducted to ensure this consumer perspective is supported and extended. The health conscious trend was one of the major drivers for growth in the ostrich meat industry, resulting in a substantial European export industry (Lambrechts and Kruger, 2006).

The ostrich production system can be classified as being horizontal because of sector autonomy (where each phase of animal production is owned by a different entity/company). Because of different sectors having different priorities and autonomy, there is an increasing debate regarding the responsibility of transport and lairage losses. Research is limited on how different factors in these phases affect meat quality. This, in combination with the bird's physical nature such as being bipedal, could lead to an increase in the incidence of bruising, injuries and mortalities that negatively influence

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meat quality during transport and lairage (Reiner *et al*., 1996; Wotten and Hewitt, 1999; Hoffman *et al*., 2010).

An intrinsic meat quality characteristic of ostrich is the consumer's perception that ostrich meat is darker than traditional red meat which exhibits a cherry red colour (Grunert *et al*., 2004; Mancini and Hunt 2005). The consumer perceives a cherry red colour in meat as an indicator of freshness. The reason for the dark colour of ostrich meat is attributed to the high level of myoglobin found in the meat coupled with a high ultimate pH (Leygonie *et al.,* 2011 a,b). This darker coloured meat could lead the consumer to perceive the meat as being of a lesser quality and having the negative effects related to Dry Firm Dark (DFD) meat. Although ostrich meat can be classified as slightly DFD, the quality loss connected to this is not comparable to full DFD meat (Wolmarans, 2011). Generally it is accepted that an increase in production in all ostrich production sectors resulted in a subsequent increase in both quality awareness and losses suffered during the production process. It is particularly the *ante-mortem* stress and its effects on these losses that is of concern to the different industry sectors. The concerns are linked to the live weight losses experienced during transport and lairage, which result in monetary losses. These bridging phases of transport and lairage are important in the ostrich meat production system as they influence the final product. This has resulted in research, albeit limited, studying the effect of transport and lairage on stress, haematology, product quality and live weight loss and how these affect animal production and products (Mitchell *et al*., 1996; Van Schalkwyk *et al*., 2005; Fasone *et al*., 2005).

Continued research, focussing on understanding and controlling of the factors affecting the quality of ostrich meat, will lead to a better understanding of the impact of both transport and lairage on the production sector, thereby leading to a better understanding of meat quality parameters as influenced by lairage, transport and husbandry in the final production phase.

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Chapter 2

Literature Review

This chapter encompasses information pertaining to the different farming, *ante-mortem* and *post-mortem* systems influencing meat quality, with an emphasis on ostrich meat quality. Other relevant theory, applicable to discussions in the chapters to follow, has also been incorporated in this chapter.

Bridging phases were developed to help link the different sectors (hatchery, juvenile rearing, slaughter bird production, abattoir) found in the horizontal ostrich production system. These bridging phases (transport and lairage) have become increasingly important as increased transport periods and lairage times have been attributed to an increase in live weight loss (Minka and Ayo, 2007a,b; 2008; 2009). The effects these phases have on the quality and quantity of animal products (and thus commercial value) have caused an increase in producer awareness on the crucial role of the bridging phases.

Ostriches are normally transported three times in their production cycle. The first period where ostriches are exposed to transport is in the movement of day old chicks from the hatching facility to the juvenile rearing facility (Verwoerd *et al*., 1998). The second occurrence of transport is the relocation of juveniles to a grow-out phase or pastoral system. Then finally, the transport of slaughter birds to the abattoir (Wotten and Hewitt, 1999). During each transport cycle, precautions should be taken to ensure the bird's safety and limit exposure to any stressful stimuli. The transport regulations pertaining to the transport of live ostriches are set in the World Ostrich Association Welfare Codes for Ostriches (2007). These regulations state that workers should be on the trucks with the ostriches. Their presence is a precautionary measure to ensure animal safety by decreasing the incidence of trampling and/or lying down, thereby decreasing bruising in the final transport phase (Wotten and Hewitt, 1999). These consequences caused by transporting live animals could be seen as being unavoidable as transport of animals between sectors is a necessity.

Lairage is defined as the time from the arrival of the animals at the abattoir until their slaughter. The inclusion of lairage as a bridging phase has resulted in some division between farmers and abattoirs on the responsibility of weight or animals losses during this period. The reason for lairage is that processing practices (abattoirs) and agricultural practices (farmers) do not share the

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same time lines and operating procedures. Lairage is therefore considered as a compromise to help bridge this time gap to ensure a smooth flow of birds through to the slaughter line. Lairage also helps the birds familiarize themselves with their new surroundings. This allows time for the animal to return to a relaxed state before slaughter, thereby improving meat quality (Hoffman and Lambrechts, 2011).

2.1 **Stress factors during transport**

Transportation of birds could have an influence on meat quality and quantity. The meat quality is affected by the endocrine response system reacting to physiological stress thereby influencing meat quality indirectly (Lawrie, 1998). The meat quantity is also affected by the bruising of the animal *ante-mortem* causing loss of meat during trimming of the carcasses (Hofmann *et al*. 2010), and the live weight losses during transport (Minka and Ayo, 2007a,b; 2008; 2009) and income loss to processing plants due to poor/ ineffective use/ realisation of resources.

During transport, the birds are exposed to various stressors, including nutrient deprivation, heat exposure, wind chill, social anxiety, equilibrium control and physical harm. All of these have varying degrees of influence on the quality and quantity of meat. The measure of influence depends on the bird's predisposition to the specific stressor and the amount of time it takes the bird to return to physiological homeostasis after reacting to stress. Therefore, information on the effect of transport is of concern to both primary and secondary producers as it affects live and carcass weight losses as well as meat quality (Minka and Ayo, 2007a,b; 2008; 2009).

2.1.1 Nutrient deprivation

It is generally known that nutrient deprivation is the shortage of nutritional products which can be caused by factors such as water and feed removal. This has various effects on the bird's physiological status, which takes time to correct. The limitation of energy caused by nutrient deprivation influences the para-sympathetic response as well as influencing the glucose levels in the tissue and organs of the animal. These changes have a direct influence on the quality and quantity of meat *ante-mortem* by influencing the glucose levels available in the muscle *post-mortem*. While the birds are being transported, their natural biological rhythm is disturbed (Eikelboon *et al*., 1991; Beattiea *et al*., 2002).

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Nutritional supplementation during transport, as a means of controlling this effect, is neither practical nor economical. However, the feeding of animals is a welfare requirement when they are being transported for more than a specific length of time or distance. European Union (EU) regulations have specific species recommendations for this but make no mention of regulations pertaining to ostriches (EFSA Panel on Animal Health and Welfare (AHAW) 2011). The length of time or distance transported is dependent on the welfare regulations, which are country and animal specific. Transport feeding also causes problems relating to which sector of the industry should carry the costs involved – not only of the feed but also of the removal of additional faecal waste. Understanding the different effects that nutrient deprivation have on the ostrich will probably lead to a better understanding on how to approach and solve these problems.

2.1.2 Energy deprivation

The amount of energy found in muscle *post-mortem* affects the quality characteristics of meat (Eikelboon *et al*., 1991; Beattie *et al*., 2001) as energy is a major component in the conversion of muscle to meat. This is discussed in more detail in section 2.3.1. The deprivation of energy lowers the available amount of glucose in the muscle *ante-mortem,* before any stress stimuli have been initiated or the animal enters the *post-mortem* state. The low available energy levels caused by energy deprivation in combination with a stress response, would then further decrease the energy levels of the meat and affect meat quality negatively (Lawrie, 1998).

The physiological energy requirement of specific organs and tissues increases with the introduction of a stressor or stressors. The endocrine non-specific response ensures increased blood glucose levels to compensate for this, but at the expense of the other physiological processes. The nutrient intake, limited by the act of transport places further strain on fulfilling this required increase in energy. The lowered energy intake with increased energy output requirement, combined with the non-specific endocrine response to stress, leads to changes in energy metabolism. In extreme cases, this can lead to protein denaturation of the muscle to provide more energy in an attempt to fulfil the energy requirements (Wotton and Hewitt, 1999). The availability of an energy rich feed can help the animal to recover energy deprivation as a result of stress during transport. This could possibly return the energy levels to the baseline requirements before the stress was introduced, and help in the conversion of muscle to meat. However considering this, the costs and time involved with returning the energy

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baseline levels to normal could be considered uneconomical, emphasising the importance of low stress bridging phases.

2.1.3 Water deprivation

Dehydration is a major concern in the transport of animals, especially during daytime heat. Extreme heat exposure can lead to an increase in dead on arrival (DOA). The loss of water experienced by the animal during transport is caused by the bird's thermoregulation to counteract the increased core and skin temperatures and can lead to a significant decrease in bodyweight but without a major change in muscle weight (Van Schalkwyk *et al*., 2005). This loss in live weight in ostriches is speculated to be a result of water loss *pre-* and *post-mortem* (Minka and Ayo, 2007a,b, 2008, 2009). In birds, temperature is regulated mainly through movement of air in the airways (panting) (Richards, 1970), and excretion of metabolic waste products. Birds do not have sweat glands and thus cannot reduce body temperature by sweating through the skin. Panting and increased excretion cause loss of water which in some circumstances, can cause dehydration.

An increased breathing rate is induced when core temperatures increase as the body attempts to release excess heat. The evaporation of water in the airways is a key response to thermogenesis and the removal of the heat from the bird's core. This is an effective mechanism but causes increased moisture loss.

The increased moisture loss stimulates the hypothalamus to activate the water retention mechanism. This increases water retention by the kidneys and water absorption in die distal intestines. The extraction of water from tissue is governed according to organ function priority. As such, the retraction of water from muscle is found in the earlier stages of dehydration (Sanger, 1981). This in combination with over rehydration afterwards has been found to induce sweaty carcass syndrome *post-mortem*. This syndrome has undesirable effects on carcass quality characteristics such as light absorption, water binding capacity and texture. The losses in muscle proteins and water also directly convert into carcass weight losses. As an animal's muscle is made up of about 70% water, dehydration could possible lead to a decrease in carcass weight and the resulting monetary losses (Sanger, 1981).

Water economics plays a key role in the survival of animals, especially animals such as the ostrich, found naturally in arid conditions. The ostrich has a frugal water economy making it well

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adapted to arid conditions (Williams *et al*., 1993). They calculated the ratio of water influx to field metabolic rate, and the water economy index (WEI) of ostriches and found them similar to other desert birds. Ostriches'' WEI average is 0.17 mL/kJ, compared to other desert bird species averaging at 0.16 mL/kJ (Maloney, 2008). Transport and lairage regulations could therefore benefit from fully understanding ostrich water control mechanisms during both stages.

The regulations on possible water breaks (intake) during transportation are governed by transportation times and distance. They are also country and animal specific although all regulations are in agreement that the wellbeing of the animal is the highest priority. Therefore, the availability of water is of utmost importance when animals are transported long distances or for long time periods. European Union regulations state that animals may be transported for a maximum of 24 hours when they are afforded an hour's rest, water and food every eight hours, but these regulations do not mention ostriches. World Ostrich Association Welfare Codes for Ostrich (2007) state that when transporting ostriches for trips of approximately 12 hours, feed should be removed 10 hours before loading. This is to reduce the amount of wet droppings on the vehicle or pen floor and thus decreases the instances of ostriches slipping and falling. No provision has been made in the regulations for provision of water or feed while transporting ostriches for periods longer than 12 hours.

2.1.4 Heat exposure

The most prevalent stressor affecting the physiological homeostasis of ostriches in Sub-Saharan Africa is heat exposure. Excessive heat exposure causes a raised core temperature, which in turn causes a stress response, dehydration, heat shock and feed refusal (Guerrini, 1981). These negatively affect the bird's health status and affect the quantity and quality of meat by lowering glycogen reserves in the muscle and inducing muscle protein denaturation (Fuquay, 1981).

Heat stress has long been recognised as important in animal health care and animal production. Heat as a stressor induces a physiological need in the animal to reduce its core temperature to an acceptable level. The animal's physiological status and thermoregulatory system is responsible for maintaining core temperature. The intensity of the stress response stimulated by this increase in core temperature is dependent on the age, health status of the animal and the environment. The main objective of the animals, when exposed to increased ambient temperatures, is

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to maintain their core temperatures in the thermo neutral zone. The thermo neutral zone (TNZ) is defined as the range where heat loss and heat production are at basal levels (IUPS Thermal Commission, 2001). The lower thermal point (Ta) and higher thermal point (Tb) of ostriches indicates that the birds are adapted to live in a variety of climates (Table 2.1) (Maloney, 2008).

Table 2.1**:** Basic thermoregulatory information available for ostriches (adapted from Maloney, 2008)

Species		Mass (kg)	Ta (°C)	Tb (°C)	BMR (Watts)	TLC (°C)	TEWL (Watts)	CEWL (%)
Ostrich	*Struthio camelus*	100	25	39.3	152		160	
		80-100	20-33		57		31.2	42

T, ambient temperature; Tb, body core temperature; BMR, basal metabolic rate; TLC, lower critical temperature; TEWL, total evaporative water loss; a CEWL, cutaneous evaporative water loss;

The TNZ of the core temperature differs between young or old animals (thermo sensitive) when compared to animals in their prime. This means that the stress response stimulated by a change in core temperature is activated earlier in these thermo sensitive age groups (young and old). The response stimulated by this would also take longer to reverse if the TNZ is compromised, specifically when transporting young, health challenged or breeding stock animals (Blake *et al*., 1991).

The animal health status affects the ability of the animal to respond to, endure and reverse stress stimuli. If the animal's physiological equilibrium is challenged in any way, it affects the level of stress response induced and its reversal. The stress response could be physiologically exaggerated causing an increase in health related complications, such as increasing core temperatures to combat infection. The combined effect of a raised core temperature due to infection and heat stress could overtax the animal's physiological system. This results in the production of heat shock proteins to combat the increased effects caused by high core temperatures and an overacting thermoregulation system. In extreme cases, this can cause heat shock or sudden death syndrome. The core temperature in such a situation would pass the Tb range where protein denaturation and brain death can occur (Blake *et al*., 1991).

Thermoregulation also affects the brain by inducing feed refusal in an attempt to reduce core temperature levels. Feed refusal is a thermo-regulating behavioural response to an increase in core temperature above its normal range (TNZ). The actual process of digestion and absorption of food

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generates heat and increases the core temperature of the animal. By refusing certain feed components, the animals can minimize internal heat generating processes (Guerrini, 1981).

2.1.5 Social anxiety

Most production animals can be classified as herd animals and therefore certain social interactions can be expected when they are in transport and lairage. Animal herd dynamics are affected by hierarchy or social dominance within the herd as first postulated by Schelderup-Ebbe (1922), and as seen in Wood-Gush's (1955) "*The behaviour of the domestic chicken: A review of the literature*." The rearrangement of animal groups has also been documented to increase social anxiety (Archer, 1987). This social anxiety is a function of increased animal aggression as a result of a change in hierarchy. This increased anxiety leads to an increase in stress (Arey and Edwards, 1998). Bartos *et al.* (1993) showed that mixing and regrouping of pigs had detrimental effects on meat quality due to the stress factor, often resulting in dark firm and dry meat. Changes in the group structure also lowers feed intake of the animals in normal farming circumstances (Nakanishi *et al*., 1993; Stookey and Gonyou, 1994). An increase in anxiety could induce acute stress responses, which take a substantial amount of time for the animals' para-sympathetic system to correct (Hoffman and Lambrechts, 2011). Return to physiological homeostasis only occurs after the stress stimulus has become redundant or is removed. Stressors become redundant when the response is made innate, or cannot be surpassed or intensified by further exposure to it, the latter can also be seen as learned helplessness (McBride, 1984), which occurs when an animal fails to adapt, which causes it to stop reacting to the stress stimuli.

During the process of hierarchy determination, anxiety would continue until all the animals have determined their order in the hierarchical structure. The time it takes to determine the hierarchy is influenced by the herd/flock size and the animals' familiarity with one another (Bernstein, 1981). The mixing of animals from different groups during transport and lairage increases aggression due to low familiarity with the other animals, as well as the close proximity of humans. Again, these stresses would lower natural feed intake, further supporting the removal of feed during lairage (Nakanishi *et al*., 1993; Stookey and Gonyou, 1994). The number of animals per pen prolongs the time it takes to determine hierarchy within the group and increases the amount of anxiety experienced. This also

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increases the amount of time needed to load the animals for transport as well as increasing the potential harm to both animals and handlers (Grandin, 1980).

2.1.6 Equilibrium in transport

Equilibrium control is the measure of the animal's ability to keep its centre of gravity stable. Factors such as the location of the animal's centre of gravity and the nature of the external forces being exerted on the animal contribute to difficulty in equilibrium control during transport (Stephens and Perry, 1990). These horizontal and vertical forces exerted on the animals during transport can be simultaneous or separate. The animal then exerts a force equal to or greater than the forces acting on it, to counteract the exerted forces and ensure that the centre of gravity is kept as stationary as possible. External forces are continually changing the animal's centre of gravity and influencing their balance, whether the animal is moving or stationary. However, the intensity and number of forces exerted increases during transportation, which requires increased energy to counteract. Codazza *et al*. (1974) noted that horses transported for 300km showed the same effect on muscle function compared to those that had been vigorously exercised. Clark *et al*. (1993) also noted that horses did not need to use the sideboards of the transport pens to maintain balance when the transporter was travelling at a normal pace. However, horses are quadrupeds, and bipeds such as ostriches might differ in this regard. Animals in transport were also noted to align themselves either parallel to, or perpendicular to the direction of travel (Eldridge *et al*., 1988; Tarrant *et al*., 1988, 1992). Most likely, this is an attempt to lower the amount of external force exerted on the body, or to counteract these forces more effectively. The increased energy expenditure to maintain balance could lead to a secondary stressor, namely energy depletion. The low intensity, high frequency forces, such as vibrations that continually exert force on the animals' centre of gravity during transport, utilises a lot of energy, in some cases causing physiological syndromes such as muscle myopathy, which could lead to death in certain animals and/or negatively affect the meat quality in others (Wotton and Hewitt, 1999).

In some cases vibrations caused by transport have been linked to a condition named 'Capture Myopathy' (Wotton and Hewitt, 1999) which can be caused by the over-taxation of muscles. Jarrett *et al*. (1964) first described capture myopathy in hunted hartebeest. Herbert and Cowan (1971) also described it as a syndrome linked to white muscles. The white muscle described in some cases in

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capture myopathy is due to the denaturation of the myoglobin protein in an attempt to replenish energy reserves overtaxed by continuous use. This syndrome has been noted to be found in some avian species although it is not as common as with ruminants (Spraker *et al*., 1987).

There is a substantial increase of muscle usage in maintaining the animal's equilibrium. This results in a short-term increase in heart output, leading to an increase in oxygen and energy availability for the muscle. However, the continuous use of muscle without rest leads to energy deprivation that ultimately leads to a change from aerobic to anaerobic respiration. The switch from aerobic to anaerobic respiration causes a build-up of lactic acid in the muscle. The blood becomes slightly acidic as the lactic acid is removed from the muscle by the circulatory system. This acidification of blood causes the heart output to reduce further thereby decreasing the amount of oxygen circulated in the body. This further decrease in oxygen potential, coupled with energy shortages in the muscle, leads to a further increase in anaerobic respiration resulting in a further acidification of the blood. If this positive-return system is not stopped by resting the muscles and/or the supplementation of energy, muscle death can occur (Beringer *et al*., 1996; Harthoorn, 1983).

The death of muscle is connected to the onset of acidosis in the muscle. This includes the necrosis of the skeletal muscles responsible for maintaining balance, as well as the heart muscle itself. The necrosis of muscle causes the release of degraded myoglobin into the circulatory system of the animal, which puts substantial pressure on the kidney and liver function (Harthoorn, 1983). This physiological pressure can cause the animal's liver and kidney function to fail and cause the animal's death (Williams and Thorne 1996; Nicholson *et al*., 2000). The effects of capture myopathy can range from hyper-acute to chronic, and can induce death because of certain metabolic complications that can occur in the acid-base- and electrolyte balances (Fowler, 1989).

Animals of both domestic species and non-domestic species ("wild") to be transported after capture or mustering should be rested with adequate feed and water in order to regain homeostasis. These precautions decrease the number of DOA's. However, animal death might still occur up to 26 days later due to complications. Additionally, vitamin E injections have been found to aid prevention of extreme cases due to its anti-oxidant action in managing the acid-balance of the blood. Selenium injections have also been found to be effective; however, the frequency of the injections is higher (Businga *et al*., 2007). The animals could also be injected with sedatives or muscle relaxers to ensure no over-taxation of the muscles. An intravenous fluid drip will increase blood supply to the kidneys

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and dilute the lactic acid and myoglobin present in the blood. However, the ease of use and costs involved with these methods make them less viable for production systems where large numbers of animals are involved. Ostriches are farmed both intensively and extensively. The latter incorporates mustering and capturing of the birds for transport and it is anticipated that the above will apply to ostriches as well.

2.2 Stress factors in lairage

Lairage encompasses the period directly after transportation to the abattoir and before the animal is slaughtered. This period could range from 0 to 4 days because of working holidays and weekends (Van Schalkwyk *et al*., 2005); however, it is uncommon for the lairage period to be longer than 24 hours. Therefore, the understanding of lairage stress factors could be instrumental in the control and management of stress during lairage (Hoffman and Lambrechts, 2011). The main stress factors in lairage are social anxiety, lack of familiarization with the new environment, nutrient deprivation and lairage conditions. Transport and lairage stress interact making the identification and effect of each factor on meat quality difficult to ascertain (Fernandez and Tornberg, 1991; Geverink *et al*., 1996; Warriss *et al*., 1990, 1998).

As noted, social anxiety can be induced by the animals interacting with each other. Animals from different backgrounds and production systems are normally kept separate from each other during lairage. Separation of these animals in different pens (as standard in the ostrich industry) should help minimise anxiety but interaction still occurs between pens. Mixing of unfamiliar animals in pens post-transport has also been linked to an increase in social aggression and physical injuries (Barton-Gade *et al*., 1992), leading to an increase in acute and/or chronic stress. This in turn affects meat quality due to the possible formation of PSE (pale soft exudative), RSE (Red soft exudative) or DFD (dark dry firm) meat, depending on the physiological state of the animal (beef and pigs) (Kauffman *et al*., 1993).

During lairage, animals are frequently moved between pens and then to the killing point. This movement of animals in the lairage area could stimulate the herding instinct of the animals causing handling and safety problems. The herding instinct induced by moving the animals increases the frequency of animals tripping, falling and being trampled on, leading to a loss in product quality (leather) and quantity (bruising loss) (Hofmann *et al*., 2010).

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The exposure of unfamiliar noises and smells at different intensities during lairage also affects the animals. The noise generated by distressed animals, motorised mechanisms and humans could induce a fight or flight response from the animal (Grandin and Collins, 1996; Hoffman and Lambrechts, 2011). The smell of blood could also induce this fight or flight response, it is speculated that this response is caused by the fear hormones in the blood rather than the blood itself (Grandin and Collins, 1996). This needs some further investigation in the future.

Hormones released by the animals when sexually active are known to induce physiological and behavioural changes in the opposite sex. The recent reviews by Hagelin (2007), Hagelin and Jones (2007), and Balthazart and Taziaux (2009) made it apparent that certain avian species are capable of aromatic communication and new consideration should be given to the role of odours in avian communication. This could a problem when slaughtering breeding animals as the older birds might induce sexual behaviour in the younger birds or they themselves may be sexually stimulated. The breeding birds need not be in the same pen to stimulate this response. Ratite species such as emu and ostriches are dangerous and aggressive when sexually active. The sexual behaviour of male ostriches is marked by an increase in aggression towards intruders that enter their territory and this increases the possibility of physical injuries to the animal and the handler. In females, it can lead to egg production and sexual behaviour being induced. These behavioural changes such as cuing and displays then further increase the sexual activity of the males in lairage (Bertram, 1980).

2.2.1 Ostrich behaviour

Ostrich behaviour has been described as being on *par* in complexity and variability as animals belonging to the most developed and complex social orders (Cooper *et al*., 2010). This makes the identification of standard behaviour patterns and abnormal behaviour difficult to ascertain. Ostriches are driven by diurnal and annual patterns like most birds and are active during the daylight hours of the day, and sitting down and becoming inactive during the evening unless disturbed (Degen and Rosenstrauch, 1989). Sauer (1970) found that ostriches have been shown to be active during moonlit hours indicating sensitivity to light. They are also found in “nomadic groups” which vary in size and makeup. Groups are made up of an unspecific assortment of young, old, related and unrelated animals that change with the seasons. Inter-species interaction is minimal with only a low ranked hierarchy being exhibited at waterholes. No specific intra species interaction is noted between small

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family groupings of ostriches within the nomadic groups. Behaviour studies by Faki (2001) showed that ostriches spend 28.7% of their time walking or running, which is lower than what Deeming (1998) found in wild ostriches, but still indicates a high movement and activity level. The decrease of space associated sometimes with placing ostriches in lairage might incur abnormal behaviour and stress due to movement restriction as a result of over population. The increased density might also influence vigilance and lower stress as increased group numbers has been shown to lower the overall vigilance per bird allowing more time for feeding (Bertram, 1980). Behaviour such as panting, yawning and stretching in contrast help regulate the physiological balance.

Courtship behaviour is dependent on the sex of the ostrich. Male mating behaviour is key, marked by kantling, wing swinging and pacing. Hen behaviour in comparison is marked by eliciting behaviour such as clucking and responding to the kantling behaviour. Common mating behaviour markers are pacing, approaching mates and feeding. Current literature into the behaviour of ostriches shows complexity in social dynamics but possible resistance to behavioural stressors are more prevalent in other domesticated animals such as pigs, sheep and cattle, where a more defined hierarchy and social interaction exists. This makes identification of stressors and abnormal behaviour difficult to ascertain.

2.2.2 Human-animal interaction

Animal behaviour during lairage is influenced by the animal's familiarity with humans and the handlers' level of experience and their understanding and application of animal welfare regulations (Grandin and Collins, 1996). An increase in the above mentioned will probably decrease the amount of stress induced by human-animal interactions during lairage and also decrease both handler and animal injuries. It is generally accepted that the animal's reaction to the stressors prior to slaughter will influence its meat quality (Grandin and Collins, 1996).

Animal familiarity to humans could be attributed to the level of agitation and comfort animals experience when exposed to humans. This is largely a consequence of the production method used in the rearing of the animals. Intensively raised animals are more familiar with humans than extensively raised birds (Neindre *et al*., 1996). This is because of increased exposure to humans in intensive production systems. A decrease in familiarity or increase in anxiety is connected to negative experiences by the animal when exposed to humans. This may occur during capture, mustering,

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transport of animals or during their stay in lairage. The extensively raised animals' lower familiarity with humans, in conjunction with a genetic predisposition to exaggerate the stress response, may induce increased levels of DOA's and lairage deaths. This phenomenon can also be found in halothane sensitive pigs and their response to transportation and human interaction (Gregory, 1998). The inappropriate and incorrect use of herding tools by handlers such as prodders or whips increases discomfort for the animals inducing a stress response and causing increased bruising (not limited to handling) *ante-mortem*. This is of particular interest to the ostrich industry as the ostrich skin is used in the manufacture of leather products. Bleeding under the skin will decrease the value of the leather (Engelbrecht *et al*., 2009). The bruising will also lower the carcass yield due to trimming (Hofmann *et al*., 2010).

The design of the lairage facility is therefore important in helping the handlers effectively move animals without extensive use of herding tools at abattoirs. The effective design of the lairage area would lead to improved management of the animals during lairage, thereby increasing production output. Nominal or incorrectly designed lairage and slaughter plants are one of the main causes of welfare concerns in animal abattoirs. Handler training is also important as it ensures that there is a full understanding of how to use the herding tools effectively, thereby lowering related problems. Improved training also increases handler and animal safety as certain animal species can cause fatal injuries to themselves as well as the handlers if not correctly handled (Grandin and Collins, 1996).

2.2.3 Feed removal

It is generally known that feeding of animals before slaughter has negative effects on both the slaughter line health and the production speed of the abattoir. Withholding feed during lairage is seen as a common practice in abattoirs as a management tool to increase productivity and reduce lairage costs. However, feeding animals during lairage could possibly decrease the stress experienced by the animal. The allocation of responsibility for feeding the animals during lairage, especially concerning the costs, can also be a contributing factor to feed removal.

Feeding of the animals before slaughter could cause the digestive track to become extended. This extension impedes the process of keeping the slaughter line hygienic, as the extended digestive

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tract is more prone to puncturing during evisceration. The digestive track contains bacteria, so the puncturing thereof will increase the possibility of carcass and line contamination.

The removal of feed during lairage ensures a non-extended digestive track during processing. This increases the ease of evisceration, thereby increasing production efficiency while limiting contamination. Some countries use animal blood in the production of feed products, thus the sanitation of blood collected during slaughter becomes increasingly important. Animals fed before slaughter could still have food present in the higher digestive track when stunned, which during exsanguinations may lead to feed contaminating the blood collection. To this end, the overall exclusion of feed is found to be a compromise that supports all the various parties involved as well as being financially beneficial (Eikelboon *et al*., 1991).

2.2.4 Lairage conditions

Lairage contains a multitude of factors that have an impact on the animal welfare and product quality, of which those that have a major influence have been discussed. All lairage conditions share a common fact in that they are influenced to some degree by lairage design. The main functions of a lairage area are the containment and protection of the animal before slaughter. The efficiency of these functions is determined according to the materials and construction of the lairage area. It is typical for ostrich pens to have an octagonal design so that no sharp corners exist for the birds to flock together in and thus cause bruising and/or damage to themselves. Drinking troughs are placed outside the pens at an acceptable height to allow the birds to drink comfortably. The lairage floors are either sand or cement with grooves to stop birds slipping – metal grids may also be placed on top of the cement floors to fulfil the same function, or of an expanded metal grid. However, the effects of these different flooring types on the birds' behaviour and on skin and meat quality have not been quantified (Grandin and Collins, 1996).

The reason animals are in containment prior to slaughter is to ease the management of the abattoir and the production line, as well as ensuring the traceability of the animals. For these reasons the lairage area should be kept separate from the slaughter line (Grandin and Collins, 1996). This is to ensure that the animals are not distressed by the sounds emanating from the slaughter line and also ensures that hygienic principles are adhered to.

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The movement of animals during containment is also important. Distances between offloading of animals to lairage pens and the pens at the abattoir should maintain the minimal distance possible so as to decrease physical exertion which decreases the glycogen reserves prior to slaughter. Therefore, the construction of the lairage area should have a pro-animal relocation design (Grandin and Collins, 1996). Accordingly, the animal pens should also be sizeable as to accommodate a number of animals without making it unmanageable within regulation guidelines. This eases lairage management as well as ensuring that the groups of animals transported together can be kept together, thereby lowering social anxiety. Adequate water should always be available to the animal at all times. This is done in accordance with the standard animal welfare regulations and codes of conduct (World Ostrich Association Welfare Code, 2010). These welfare requirements also include adequate protection against the weather elements. Exposure to the elements is generally considered by industry to be a prevalent stressor and should be minimized by lairage construction and layout.

Considering the function of lairage, rest and replenishment of energy of animals is currently seen secondary to other possible contributors to animal welfare in lairage and is not well researched and documented.

2.3 Physical characteristics of meat

2.3.1 Conversion of muscle to meat

The slaughter process has distinct effects on an animal's biochemical chemistry and physiological processes. When the animal is killed, the heart ceases to function and the blood circulation throughout the body stops. This results in oxygen and energy deprivation to all parts of the physiological system including the muscle. However, the normal metabolic processes of the body continue after brain death until an inflection point is reached where the material available for metabolism is not enough to maintain normal homeostasis of the body (Lawrie, 1998). When this shortage in first metabolites occurs, the metabolic processes either cease or switch to a more energy efficient process. Alternative processes may also be utilized to fulfil the metabolic requirements left wanting by the inactive processes. The fall in oxygen potential causes the muscle metabolism to switch from aerobic respiration to anaerobic respiration as a response to this restriction of metabolites and to prevent the permanent binding of actomyosin cross-bridges (Scheffler and Gerard, 2007).

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The metabolite ATP is required by the muscle to return muscle fibres to a pre-contractive state. The *post-mortem* ATP levels are initially high at *mortem* but, due to the high turnover rate required to return the muscle to a pre-contractive state, those levels are quickly exhausted (Bate-Smith and Bendall, 1949 as cited by Lawrie, 1998). This high turnover rate is supplied by aerobic respiration metabolites when the oxygen potential is still high *ante mortem*. The cytochrome enzyme system within the muscle is used to resynthesize ATP from ADP (Lawrie, 1998). However, when the oxygen potential falls, glycogen is metabolized through a process of anaerobic respiration to supply the ATP required at a slower turnover rate, resulting in a deficiency of ATP.

The switch from an aerobic to anaerobic state is essential to provide the necessary energy for the re-phosphorylation of ADP to ATP in order for the latter to prevent permanent binding of actomyosin cross-bridges (Scheffler and Gerard, 2007). However, as glycogen levels are finite *post mortem*, a point is reached where the muscle will be unable to return to its pre-contractive state due to a lack of ATP. This heralds the entering of muscle into *rigor mortis* (Greaser, 2001; Lawrie, 1998). This change in muscle state can be measured by the build-up of metabolic waste products such as hydrogen molecules inside the muscle.

This build-up of metabolic waste products in the physiological systems has a number of consequences. Respiration waste products include hydrogen (H^+) molecules and lactic acid. The impact of these waste products on the acid base balance of the body *ante mortem* is maintained in balance by the circulatory system. However, with the inability of the muscle to remove these waste products *post mortem*, the pH in the tissues decreases. The rate and extent of the pH decline in these tissues is influenced by the metabolic processes still present in the muscles and the inherent tissue structure (Warris, 2000)

The decrease in pH is time limited depending on the amount of glycogen available in the muscle at time of death. However, the process of glycolysis is not primarily metabolite dependant as this process has been noted to cease before all glycogen has been utilized (Greaser, 2001). This event has been attributed to factors such as enzyme pH sensitivity, or because of metabolic derivatives (adenine nucleotides to ionise derivatives), that may halt the glycolytic flux (Greaser, 2001).

The decrease in pH is still time oriented and glycogen reserves are finite, therefore, the pH decline can be seen to follow a certain trend. However, not all species and subspecies share the

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exact same pH trend or pH decrease. Rather this is attributed to different intrinsic and extrinsic factors. The initial meat/muscle pH of most animals is the physiological pH, which is close to neutral (pH 7) and by the end of *post mortem* glycolysis, the final pH is in the region of 5.5. The final pH is considered the point at which glycogen reserves are exhausted and the muscle is thus unable to return to its pre-contractive state (Warriss, 2000).

As pH decreases, muscle shows a reduction of extensibility stated as a slow degeneration of the pre-contractive state of muscle. pH decrease coincides with ATP depletion, as stated earlier, and when the ultimate pH is reached, the muscle is deemed to have entered *rigor mortis* (Greaser, 2001; Lawrie, 1998) This is because of troponin's inability to prevent binding of actin and myosin, the thin and thick filaments found in muscle, because of ATP depletion, causing the muscle to become inflexible (Swatland, 1994). *Rigor mortis* is also affected by factors that influence both glycogen and creatine phosphate, both of which are needed in the reversion of muscle to a pre-contractive state and which affect pH decline. This extensibility reduction also proceeds accordingly to phases. The first phase is considered the slow phase which is followed by a rapid phase and then finally, a slowing down phase. The last phase is completed when all ATP has been exhausted (Lawrie, 1998). This signals the complete conversion of muscle into meat (Swatland, 1994).

2.3.2 Colour

Humans make decisions based on information available. Our senses are used to interact with the world and retrieve this information. Then, with these sensory inputs and by using our intellect, we decide whether to buy a product or not (Lawrie, 1998).

The sense of sight is thought to be the most instrumental in the purchasing of meat where colour is the main purchasing criteria (Warriss, 2000). This is coupled with the pre-conceived opinions on what is considered healthy and not healthy. The colour changes normally seen in meat can be connected to quality deterioration or contamination of meat but can also be a symptomatic of other factors not connected to the deterioration or contamination of meat. The understanding of an acceptable meat colour is seen with consumer discrimination against brown meat, even though this is not always an indication of lower meat quality (Ouali *et al.*, 2006). Consumers are known to prefer beef of a cherry red colour above that of purple or brown (Carpenter *et al*., 2001). The colour change

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exhibited by meat is influenced by a variety of factors ranging from pH decrease *post-mortem* to muscle-fibre type (Lawrie, 1998). The muscle fibre type influences the amount of myoglobin found in the muscle, which in turn is linked to the metabolism of the muscle fibre. The concentration of these molecules in the muscle affects the colour intensity and the rate of colour deterioration (Honikel, 1998; Lawrie, 1998).

Hemo-myoglobin was considered for a time to be the reason behind the red colour exhibited by meat, but this opinion was changed as early as 1932 (Lawrie, 1998). This is because hemo-myoglobin was found to be limited to the fine vascular supply web found in the muscle (Lawrie, 1998), and not the muscle itself. It was later shown to influence only meat colouring if incorrect exsanguination techniques were used. Therefore, there has to be an intermediate molecule between the haemoglobin and cytochromes of mitochondria for the transfer of oxygen needed for respiration, and which affects the final colour of the meat. Myoglobin was found to be the molecule enabling this transfer. This protein is structurally and chemically very similar to hemo-myoglobin which consists of a globular protein of approximately 153 amino acids surrounding a porphyrin ring structure held in a pocket of the protein. The ionic-state of the iron within the porphyrin ring is responsible for the colouring exhibited by the protein (Greaser, 2001). The different colour spectra of red exhibited by meat are influenced by a variety of factors, ranging from atmospheric content, myoglobin content, microbial activity and genetic predisposition (Lawrie, 1998).

The intensity of meat colour can be attributed to the amount of myoglobin present in the meat (Sales, 1996). The greater the amount of myoglobin, the darker the meat will be (Lawrie, 1998). Myoglobin content in the meat is influenced by the oxygen consumption or need of the tissue (Meyer, 2004). Therefore, muscles that are more active will have higher levels of myoglobin than less active muscles (Greaser, 2001). As game is generally more active than domestic animals, game meat will contain more myoglobin, and therefore has a darker shade of red compared to domesticated animals (Hoffman, 2000).

The chemical state of myoglobin reflects the meat colour seen in the visible light spectrum. As previously noted, the porphyrin ring's reduction by an oxygen molecule causes the noted cherry red colour found favoured by consumers (Ouali *et al*., 2006). The most acceptable red colour by consumer standards is attained by the exposure of myoglobin to oxygen and through the process of oxygenation, oxy-myoglobin is formed which is responsible for the surface red colour shade most

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preferred by consumers (Mancini and Hunt, 2005). The conversion of myoglobin is not only limited to oxygen molecules alone, and if exposed for any extended period to the atmosphere, the more phylic-molecules found in the atmosphere could interact with the myoglobin molecule inducing a colour pallet not acceptable to consumers (Ouali *et al*., 2006). The most notable example is the oxidation of the iron in the porphorin ring from a ferric to ferrous state (Mancini and Hunt, 2005; Ouali *et al.*, 2006). This process is influenced by different factors such as oxygen partial pressure, low pH, high temperatures and meat's reducing activity (Mancini and Hunt, 2005).

Consumer perspective of meat is influenced by sight and it is therefore difficult to standardise an acceptable meat colour. A standardised test to measure colour and thus make impartial recommendations was therefore needed. This was achieved by using a spectrophotometer and the CIELab colour scale standard (Honikel, 1998).

The CIELab standard has three measurements *L*, a** and *b*.* In the CIELab scale, the *L** value indicates the lightness of the meat, the *a** value indicates the positioning of the meat colour on the green-red range and the *b** value the position on the blue-yellow range. By using these measurements, calculations can be made to determine both the hue angle and chroma (Hue angle: $hab = \tan^{-1}(b^*/a^*)$; Chroma value $C^* = [(a^*)^2 + (b^*)^2]1/2$). Using the hue and chroma standard, a more impartial decision can be made on the freshness of meat.

Ostrich meat colour is characterised by a L* value lower than 40 and a* values that are relatively higher than the b* values (Volpelli *et al*., 2003). This can be indicated by the values gathered in a study by Hoffman and Fisher (2001), where ostriches were used ranging from ages 14 months to 8 years, receiving values of 29.42 ± 0.041 and 24.84 ± 0.574 for L*, 5.48 ± 0.383 and 9.45 ± 0.541 for a* and 3.51 ± 0.27 and 4.68 ± 0.382 for b*,* respectively. The relative dark colour of ostrich meat can probably affect the consumer's perspective.

2.3.3 Water-holding capacity

The main saturating chemical agent in most organic products is water. Depending on the organic compound, water can be found in a variety of states and forms. The water holding capacity is defined as the meat's ability to retain and/or absorb water in the absence or presence of external forces such as external pressure or heat exposure (Honikel, 2004; Swatland, 1994). Meat is considered to contain approximately 75% water (Offer and Trinick, 1983; Honikel, 2004). The amount of water

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found in the muscle is dependent on the leanness and composition of the muscle (Honikel, 2004). Water is localized in different areas and structures within meat. These are the filament, inter-filament and extracellular spaces (Offer and Trinick, 1983).

This water is also characterized by the ionized state of the water found in these different spaces. The water state in meat can be classified into three classes in descending order of their levels found in the meat: “free”, “bound” and “immobilised” water. Free water is kept in place by the structural and textural composition of meat, and is the highest contributor to drip-loss experienced by meat. Bound water is mostly associated with functions of the hydrogen molecule found in water and it’s binding with myofibrillar proteins. Protein makes up a small part (≈22%) of meat. Bound water only amounts to a tenth of the total amount of water found in meat (Huff-Lonergan and Lonergan, 2005).

Immobilised water is contained in the structure of meat through different steric effects and phylic/phobic attractions. These steric effects and particle interactions are influenced by space and volume changes of the meat structure, and therefore immobilised water is most affected by the conversion of muscle to meat (Huff-Lonergan and Lonergan, 2005; Lawrie, 1998).

Muscle conversion to meat influences the structural format and integrity of the tissue and will therefore change the amount and ratio of “bound”, “immobilised”, and “free” water. The factor that contributes the most in the determination of water holding capacity is inter-filament spacing (Lawrie, 1998). Lateral changes of these filaments cause both the volume and space between filaments to increase and decrease. These changes influence the expulsion and absorption of water. Long-range electrostatic forces that also affect myosin-actin interactions (Warriss, 2000) cause the expansion and contraction of these filaments. This could affect the expulsion of water. Electrostatic forces are influenced by the number of positive and negative charges in the proteins of meat that also change with the acidification of meat – it is at its lowest when the meat proteins are at their iso-electric pH of ≈5.4 (Lawrie, 1998).

Muscle respiration changes *post mortem*, which causes a shift in the loaded charges within the meat because of the build up of lactic acid and hydrogen molecules. As these molecules build up without being removed, the pH drops and passes the isoelectric point of major muscle proteins. The isoelectric point indicates when the positive charges within the muscle are almost exactly the same as the negative charges. This causes an increase in phylic attraction between the opposite charges

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within the protein resulting in a reduction in protein size. The repulsion forces exhibited by two similarly charged molecules are reduced, further decreasing the space between molecules within the protein (Warriss, 2000; Huff-Lonergan and Lonergan, 2005).

This reduction in space causes the expulsion of “immobilised” water to the extra-cellular space of the meat superstructure (Warriss, 2000). This water later manifests itself outside the meat and is then referred to as exudate or “drip”. The decrease of pH *post mortem* is a non-changeable factor in the conversion of muscle to meat. Therefore, there will always be an expulsion of water from meat *post mortem* in the form of drip (Honikel, 2004). It is also conceivable that pH and the water-holding capacity have a high correlation with one another (Bouton *et al*., 1971).

The water holding capacity changes when meat is cooked, because of the structural change induced in meat by exposure to high temperatures. The act of exposing meat to high temperatures has a distinct effect on the structural integrity of meat (Lawrie, 1998). When cooking meat at temperatures ranging from 36°C to 75°C, the proteins in the meat are denatured and certain cellular structures disrupted. Muscle structure is influenced by the denaturation of cell walls and the shrinkage of connective tissue (Honikel, 2004). The most notable are the changes exhibited by collagen of the perimysium and endomysium, both of which shrink and cause expulsion of free-water from the superstructure (Sims and Bailey, 1981). The amount of change experienced by the structure and the water loss induced is correlated with the amount of energy available (heat) for the reaction to take place and also the time it takes for the reaction to complete. In the case of meat cooking, the loss is influenced by temperature, duration of cooking and the tissues’ integrity (Lawrie, 1998). Meat structural integrity or tissue integrity is negatively correlated to a decrease in pH found in meat *post mortem,* compared to the pH which was found to be linearly correlated to cooking loss (Marsh *et al*., 1987). The pH of meat can therefore be used as a relative indicator of the water holding capacity of meat.

2.3.4 Tenderness

Food enters the mouth where mastication takes place. Mastication is the reduction of a material into a smaller state through application of force by the teeth and mouth of an animal or human (Cooper and Horbanczuk, 2002). The ease of mastication of meat could be an indication of the tenderness of

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meat. It makes sense then that a lower amount of energy required in the act of mastication would be indicative of an increase of the tenderness of the meat (Cooper and Horbanczuk, 2002).

The tenderness of meat is a main contributing factor in consumer preference of meat (Risvik, 1994; Cooper and Horbanczuk, 2002). This is supported by the consumers' preference of tenderloin (*M. psoas major*) over other juicier and more flavourable cuts such as the rump and loin (Savell and Shackelford, 1992). Consumers perceive game meat as being less tender than conventional livestock. This perception has been shown to be incorrect by Von La Chevallerie (1972) who showed that seven different game species did not follow this trend.

Ostrich meat tenderness has been rated by some consumer panels as being on par with that of beef loin steak (Harris *et al*., 1994). However, the variation found in ostrich meat in relation to tenderness has also led Sales and Oliver-Lyons (1996) to confirm the work of Von La Chevallerie (1972) that no fixed trend in the shear force of mastication or tenderness can be made.

Toughness in meat is the function of three different factors: background toughness, toughening phase, and a tenderizing phase. Of these factors, only background toughness can take place *ante mortem* and as such be influenced while the animals are still alive.

Background toughness is the combination of intrinsic meat characteristics of the animals with those of extrinsic living factors accumulated throughout the animal's life (Koohmaraie and Geesink, 2006). These factors ultimately contribute to the overall tenderness of the meat. The intrinsic factors focus primarily on the genetics of the animals such as the animal's predisposition towards connective tissue deposition and the ratio of collagen to muscle fibres. The ratio of permanent banded collagen to non-permanent banded collagen is also important in the determination of background toughness (Lawrie, 1998). These intrinsic characteristics are related to the animal's level of maturity and the quantity of connective tissue present in the muscle (Olsson *et al*., 1994).

Extrinsic factors, such as nutritional level, focus on the living conditions that contribute to meat toughness. A higher nutritional level during the animal's life is connected to a higher level of adipose tissue saturation and the formation of intra-muscular fat that lowers toughness (Lawrie, 1998).

The main types of connective tissue found in muscle are collagen and elastin. The latter tissue type is found in such small amounts that its contribution to toughness is deemed minimal.

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Elastin becomes inflexible when exposed to high temperatures of cooking but because of its minimal presence in meat; its effect on toughness is much lower in comparison to collagen (Lawrie, 1998).

Collagen amounts to almost 30% of all protein found in the muscle structure and it is a major contributor to the toughness of meat. Collagen tissue is straight and inextensible in muscle even prior to cooking. The characteristic of collagen to extend itself through meat in a three dimensional matrix contributes to the toughening effect by increasing the amount of connective tissue needed to be sheared through per square cm (Lawrie, 1998; Young and Gregory, 2001). The formation of non-reducible cross-links between collagen molecules also increases its tensile strength and meat toughness (Davis *et al*., 1979). This formation of cross-links also increases with the age of the animal and is subject to other intrinsic factors, such as animal genetics. The non-reducible cross-links are also heat resistant and can therefore endure heat that would induce structural change in other proteins (Young and Gregory, 2001).

The toughening phase takes place *post mortem* and is characterized by the shortening of the sarcomeres through ATP depletion and the development of *rigor mortis* (Koohmariae, 1996). This phase takes place after the animal's death, and thus after cessation of blood circulation, causing lowered ATP levels. It is completed in approximately 12-24 hours *post mortem*. However in ostriches *rigor mortis* can be achieved within a few hours *post mortem* as noted by Botha *et al*. (2004). The muscle enters rigor when the ATP is depleted and causes the overlapping of myofibril contracting proteins (actin and myosin). This shortens the A band and sarcomeres increasing the amount of tissue per square cm. This indicates an inverse relationship between the sarcomere length and the toughness of meat (Bouton *et al.*, 1978; Herring *et al.*, 1967). Sarcomere length shortening can also be influenced by low or high temperature ranges; high temperatures induced by cooking or low temperatures that cause cold shortening (Hopkins and Thompson, 2002).

As the animal muscle is converted into meat and just before *rigor* is completed, another effect helps increase the tenderness of meat and marks the tenderisation phase of meat and takes place after *rigor*. This effect is the proteolysis of a range of muscle proteins, including to a lesser degree the connective tissue, which lowers toughness (Hopkins and Thompson, 2002). The proteins responsible for this phase of tenderisation are related to the calpain system. This process of proteolyzation is an effect dependent on pH levels (below 6) and Ca^{2+} availability, which both only reach acceptable levels *post mortem*. Calpain enzymes are affected by the level of calcium (Ca^{2+})

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available in the muscle, as they are dependent on Ca^{2+} for the proteolyzation of the protein calpastatin that in turn controls calpain activity.

The calpain system consists of m-calpain, μ-calpain, and skeletal muscle specific calpain, all of which are inhibited by calpastatin (Lawrie, 1998). Calpastatin therefore, determines the range of proteolytic activity (Koohmaraie and Geesink, 2006). The main substrate of the calpain system does not include major muscle proteins such as actin or myosin but influences the substructure through use of troponin I, troponin T, connecting, desmin, m- line proteins and tropomyosin as substrates for their activity (Lawrie, 1998). Calpain activity is also influenced by temperature where 35°C was found to contain more active forms of the enzyme than at 5°C. Calpain activity is also influenced by the level of calcium available to the system, as a decrease of calcium availability would decrease calpain enzyme activity by increasing calpastatin activity, indicating that the inhibitor calpastatin will be affected by the onset of *rigor mortis*.

Differences in muscle tenderness through proteolytic activity have been attributed to a combination of intrinsic and extrinsic factors that range from calcium availability to level of exercise and growth rate. These factors affect the toughness of the meat through their interaction with the different toughening phases or change in muscle structure. Although differences are to be expected between animals because of these factors, some studies have shown no significant differences in some ostrich sub-species when considering these factors (Hoffman *et al*., 2008).

2.3.5 The relationship between pH and characteristics of meat

The pH decline *post mortem* is associated with meat characteristics as the major effect caused by the death of the animal. The acidification of meat is the indicator of the change in respiration activity in the muscle in its conversion to meat. The change to anaerobic respiration in the muscle as its oxygen potential falls leads to a hydrogen ion build up in the muscles and therefore acidification of meat. The number of hydrogen ions is measured in the meat by means of pH (Warriss, 2000).

The number of hydrogen ions in meat is maintained at its physiological level by the reduction thereof in the process of aerobic respiration. Aerobic respiration uses a higher percentage of hydrogen ions in the production of ATP than anaerobic respiration. The rate of change and final pH of meat is therefore, affected by the level of anaerobic respiration found in the muscle. This type of respiration is determined by the first and second limiting metabolites, which are the oxygen and/or

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energy available in the muscle *post mortem*. The complete depletion of energy marking the stop in anaerobic respiration would be the cause in deviation from the expected linear fall in pH to an exponential fall in pH (Bruce *et al*., 2001).

As the pH decreases, the precipitation of meat proteins increases. The myo-filament lattice shrinks, increasing the amount of water found in the superstructure of the muscle. This increases the myofibril refractive index that lowers the amount of light absorbed by myoglobin. This ultimately leads to the pale colour which meat exhibits as time increases and pH decreases (Swatland, 1994). The pH level and iso-electric point of the meat are also factors influencing this colour reflection. The iso-electric point indicates an inflection point where the colour readings, water holding capacity (WHC) and protein denaturation are all affected. Higher ultimate pH increases redness and light absorbency of meat, whereas a lower pH would have the opposite effect.

As discussed, the water holding capacity (WHC) is influenced by the pH level involved in the precipitation of proteins in meat decreasing the available area for binding and storage of water. Furthermore, when the pH levels that are optimal for the function of proteolytic enzymes are reached, denaturation of hydro-philic proteins occurs, further lowering the WHC. Offer and Knight (1988) postulated that a combination of low pH and exposure to high temperatures would increase denaturation of water-soluble proteins, causing a lowered WHC.

Ostrich meat has a dark colour and a high WHC which are both related to meat pH levels. Ostrich meat is also known for its high ultimate pH. These high pH levels correlate positively to changes exhibited in the superstructure in meat reducing the water mobility. This is achieved by limiting the space between filament fibres causing them to be packed more closely together (Hoffman, 2005) and limiting water movement thereby increasing WHC. The water holding capacity was also found to be influenced by the amount of intramuscular fat. The extent and level of adipose saturation found in the meat effectively loosens the micro-structure thereby increasing the water movement and philic/phobic reactions and ultimately affecting tenderness (Harris *et al*., 1994).

Tenderness is influenced by a range of factors of which the most prevalent are the proteolytic enzymes' concentrations and activity. The activity of these enzymes is again influenced by a variety of factors such as temperature, substrate and pH (Cheftel, 1992; Kunugi, 1992). The pH level of the environment is of importance as enzymes have an optimal activity pH range. Purchas (1990) postulated that there is a curvilinear relationship between pH and tenderness where the inflection

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point is reached in the pH range of 5.5 - 6 after which it becomes increasingly tender again. This could be attributed to the pH range of activity for the main proteolytic enzymes that function beneath par at the ranges from 5.8 - 6.2 (Yu and Lee, 1986).

2.4 Effect of stress on meat quality

2.4.1 Defining stress

Stress could be defined as the reaction of a biological mass to a change in its internal or external environment by stressors that can be classified as “irritant” or “intermittent” depending on its origin (Crowther *et al*., 2003). The reaction to a stressor could be physiologically non-specific but this then limits the ability to effectively respond to the stressor. Other factors, such as changes in an animal’s behaviour, are also involved in the control of stressors.

The animal’s ability to react behaviourally, specific to the stressors, in combination with non-specific physiological changes, is how the animal effectively controls its body homeostasis in the presence of stressors. The level of these reactions from the animal in the presence of a stressor is determined by the intensity of the stressor and the animal’s ability to react to it (Dantzer and Mormède, 1983).

2.4.2 Stress, related to transport

Transport stress is a combination of all the external stimuli that influence the animal’s biological homeostasis because of being transported. These stressors can be classified as “irritant” and/or “intermittent” (Crowther *et al*., 2003).

Irritant stressors are associated with long-term stress effects due to the low frequency and extended length of exposure to the stressor. These stressors include vibration, noise, light, confinement and exposure to the elements, which may also be classified as intermittent stressors if experienced for short periods. Intermittent stressors are associated with high frequency and intermittent periods, which induces the animal’s natural fight or flight response at different times. The length of exposure to the stressors is shorter, therefore, the responses associated with intermittent stressors are considered less invasive and easily reversed (Crowther *et al.,* 2003).

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Vibration is considered an irritant stressor because of its continuous nature in combination with its high frequency level during transport. Sensitivity of the animal to this stressor is associated with the bio-kinetic build of the animal and the animals' centre of gravity. An ostrich's sensitivity to transport vibrations is high because of its bio-kinetic build and high centre of gravity. This can contribute to an increase in the transport mortality rates and muscle necrosis (myopathy) of ostriches when they are exposed to long periods (more than about 8 hours) of transport (Foggin, 1992; Wotton and Hewitt, 1999).

The average ostrich slaughter weight is around 120-150 kg (also sometimes quoted as 95 to 105 kg) with a height of approximately 2.75 meters. This, in combination with it being a bipedal animal, and having a high centre of gravity, increases the amount of energy required to keep its balance (Foggin, 1992; Wotton and Hewitt, 1999). This, when combined with other stressors such as elemental exposure, increases the physiological strain on the animal's body and increases the possibility of death (Beringer *et al*., 1996; Harthoorn, 1983).

Of the different forms of elemental exposure that animals can experience, the most discussed in research is that of heat exposure. The exposure of animals to high temperatures without adequate ventilation or water causes heat stress and can also lead to dehydration which makes it a major factor to consider during transport (Crowther *et al*., 2003). Statutory regulations are quite explicit on the levels of temperature exposure the animals may experience and the steps to be taken to prevent heat stress and dehydration during transport. Temperature levels which are deemed acceptable to welfare regulation are determined according to the animal's physiological needs and therefore vary accordingly (South African Oostrich Transport Codes and Conduct, 2011).

Finch (1976) stated that the relationship between the animal and its thermal environment determines the degree to which an animal keeps itself in thermal equilibrium. These factors (radiation, conductivity, etc.) combined with high levels of humidity which limits water evaporation from the skin, could increase instances of heat stress and shock in transport (Mitchell and Kettlewell, 1998). The cooling effect of evaporation of water is possible through human intervention by spraying ostriches with water as they do not contain any sweat glands. However, this practise has two major drawbacks. Firstly it lowers the value of the feathers and secondly, it requires water to be carried and be readily available on the transport vehicle.

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The construction of the open roofed transportation truck and climate associated with the area are also factors that should be taken into account during transport. The manner in which ostriches control thermal equilibrium is affected by physiological and behavioural changes. Ostriches exercise a fugal water economy that stems from the dry arid and semi-arid regions with regular high climatic temperatures where they evolved from. This behavioural technique, in combination with the ostrich's ability to raise their body temperatures when exposed to high ambient temperatures, makes them more heat tolerant than other species (Cooper, 2007; Paleari *et al*., 1995). This can also explain the observations by Crowther *et al*. (2003) that ostriches did not consume water during transport.

Heat conduction and radiation from other ostriches lead to increased body temperatures when space is limited. This heat exchange is limited by the adequate control of pen stocking density and increasing the animal space allowance. Closed roof trucks with adequate ventilation, in conjunction with a space allowance of 0.75m^2 per bird (Mitchell, 1999), should minimise heat related damages in transport. The transport of ostriches during cooler periods of the day may also be an effective management tool.

The time of day when animals are transported has a significant effect on stressors such as elemental exposure, light and noise. When transporting animals in the dark, Crowther *et al*. (2003) observed a lowering of heart rate possibly indicating lowered stress levels. Night transport lowers the radiation from the sun experienced by animals during normal daytime transport. Mitchell (1999) and Andersen (2000) also noted that ostriches transport better during the night than in daytime. However, ostriches tend to sit down when exposed to low light intensities, which again increases skin damage percentages. The decrease in light intensity during night transport induces the ostriches to sit down and sleep, which lowers energy expenditure in maintaining balance, lowering the possibility of external rhabdomyolyis and stress (Wotton and Hewitt, 1999). However, this would also increase the incidences of skin irritation, skin bleaching, skin damage and bruising, lowering the price of the leather. The reduction in traffic during night transport could also contribute to the reduction of accompanying noise levels, which lowers the animal's anxiety which is directly related to its stress levels.

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2.4.3 Physiological response to stress

2.4.3.1 Endocrine response system

The endocrine system is the control mechanism of the autonomic nervous system, which is uncontrolled and divided into two components, e.g. the parasympathetic and sympathetic nervous systems (Warriss, 2000). Both are responsible for continuation of homeostasis in the body.

The sympathetic nervous system is responsible for the release of the hormones that combat stress, not accommodate it. The pathways, which are responsible for stress hormones are used to define the stress response. These are related to the length and intensity of the stress response (Siegel, 1980).

The best known response is that of the fight or flight syndrome, which is characterised by a fast change in energy stores, and the short time span associated with the response. The pathway first described by Cannon (1935) involves the stimulation of two organs of the sympathetic nervous system. These are the hypothalamus and the adrenal medulla which secrete catecholamine hormones, epinephrine and norepinephrine. These hormones and the adrenalin secretion influence the metabolism in the organs and the glucose levels in the blood, which in turn prepares the body for a strenuous event (Cannon, 1935).

The other response pathway is induced by long term exposure to stress which activates the secretion of corticotrophin-releasing hormone (CRH),which stimulate the anterior pituitary to secrete adrenocorticotropic hormone (ACTH) (Selye, 1956). This activates the adrenal cortex and adrenalin secretion. This pathway is marked by the duration of the response and the amount of time needed by the para-sympathetic system to reverse its effects. This response is also known as the hypothalamic-pituitary-adrenocorticoid system (HPA axis) as first described by Selye (1956).

The endocrine system is marked by the production and release of hormones involved in the combat and control of stressors. The response and intensity of the endocrine system response is dependent on the origins and characteristics of the stressor and the length of exposure to the stressor. The fight or flight response, is a short term stress response, while the general adaptive syndrome takes place after this, and is considerably longer in duration (Selye, 1956).

When exposed to a stressor, the sympathetic nervous system, which is connected to the adrenal glands, stimulates the release of nor-epinephrine. Nor-epinephrine acts as a

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neurotransmitter stimulating the secretion of epinephrine and catecholamines. Both epinephrine and norepinephrine inhibit the function of insulin in the body increasing the blood glucose levels, while simultaneously stimulating glucagon secretion in the pancreas (Hill *et al*., 2004). Catecholamine's on the other hand, affects a range of organs and biochemical processes which include the increase of respiration rate, heart rate, arousal, and dilation of airways. Biochemically, glycogen and adipose tissue breakdown increases to increase blood energy levels (Hill *et al*., 2004). All of these take place to ensure the body's preparation for a strenuous event.

In understanding the process of hormonal responses to stress, researchers are now able to determine to a certain degree, the animal stress levels through hormone levels. However, the lack of detailed information available on blood hematologic and biochemical values of ostriches makes effective use of this knowledge problematic (Van Heerden *et al*., 1985; Levy *et al*., 1989; Verstappen *et al*., 2002). This information is still useful to some degree, even though incomplete, as some of the hormones involved in combating stress have a detrimental effect on meat quality. An example of this could be the increase in catecholamines (Hill *et al*., 2004). The increase in catecholamines would lead to an increase in *post mortem* glycolysis by increasing the process associated with phosphorylase. Therefore, measurement of catecholamines could help with the identification of long-term stress. However, results should be interpreted with reference to the diurnal cycle of the animal as this influences catecholamine levels during the day (Shaw and Tume, 1992).

Long-term stress or chronic stress is associated with the HPA axis and the subsequent secretion of glucocorticoids, which mainly have a catabolic function. This increase in glucocorticoids reduces the mass of major muscles through increased proteolytic activity. This catabolic effect in turn during stress increases the anabolic activity in the liver. These processes include gluconeogenesis and protein synthesis which attempts to reverse the catabolic effect. This is done to reverse the effects of the HPA axis (Mormède *et al*., 2007).

The release of glucocorticoids will also suppress the release of other hormones. These include growth hormone (GH), thyroid stimulating hormone, and some gonadotropins. The postulation for this event is that by the suppression of these hormones, the subsequent processes involved will be stopped, making extra energy available for the body's stress response (Sherwood *et al.*, 2005).

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Both the short-term and long-term pathways described have a negative feedback loop which helps maintain the system's homeostasis. The fight and flight response is regulated by the secretion of epinephrine, which at certain levels suppresses the release of CRH. Glucocorticoids help the regulation of the HPA axis through suppression of CRH by the hypothalamus. In addition, the sensitivity to CRH is lowered by glucocorticoids causing a fall in effectiveness of the ACTH cells located in the anterior pituitary. This in turn causes decrease in ACTH secretion and the lowered stimulus for inducing glucocorticoid release at the adrenal cortex (Hill *et al*., 2004).

2.4.4 Effect of stress on post-mortem pH and implications thereof on meat quality

The acidification of meat has already been stated as a contributor in the conversion of muscle to meat and thereby as having an effect on meat quality. Of the factors involved in the acidification of meat, the most important is the glycogen available in the muscle *post mortem.* The amount of glycogen available determines the duration of *post mortem* glycolysis, which is responsible for the decrease in the pH of the meat. The amount of glycogen available in the animal's muscle *post mortem* is determined by various factors such as the muscle type, species of animal and the animal's nutritional level as well as its *ante mortem* stress levels (Lawrie, 1998).

Stress responses therefore influence the amount of glycogen available for utilization. Acute stress involves the release of catecholamines, which increase the amount of glycogen available for utilization in the muscle. However, the death of the animal causes this large amount of glycogen to be utilized in the process of anaerobic respiration because of the low oxygen potential in the muscle *post mortem* (Lawrie, 1998).

Chronic stress is associated with the release of glucocorticoids. This, coupled with the increased period of stress, causes a state of near depletion of glycogen reserves in the muscle *ante mortem*, lowering glycolysis period *post-mortem*. This results is a decrease in the drop in pH *post mortem* and a higher than optimum final pH. The variation in rate of pH decline leads to effects influencing the meat quality (Warriss, 2000).

The first effect connected to acute stress *ante mortem* is a lower than optimum final pH. This is caused by a rapid fall in pH at high temperatures, causing protein denaturation which leads to PSE meat. However, no cases of this phenomenon have been reported in ostrich muscle. Chronic stress

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by comparison causes DFD (dark, firm and dry) meat and is marked by higher than optimum final pH (Lawrie, 1998).

As both these effects are connected to the rate of acidification in meat, they can be plotted relative to the pH fall as seen in Figure 2.1. In birds, long-term stress has been found to be a significant factor in the determination of *post mortem* glycolysis and ultimate pH (Fasone *et al*., 2005). The decline in pH of ostrich meat stabilises quickly *post mortem* at around 2-6 hours *post mortem*, reaching an average ultimate pH of ≈5.8. This susceptibility to high ultimate pH levels makes ostrich meat, in some cases, to be seen as DFD which effect can only be seen at pH levels >6.2 (Sales and Mellett, 1996). This ultimate pH range level of 5.8 might be of concern to processors because of the low secondary production (mince, sausages, etc.) ability of high pH meat and the consumer preference and perspective (Warriss, 2000).

Dark firm and dry meat is correlated to low glycogen levels in the muscle *post mortem*. Lower glycogen levels *post mortem* mean direct decreases in the rate of glycolysis lowering the lactic acid build-up in the muscle. This ultimately leads to a higher than optimum ultimate pH. This influences the amount of proteolytic activity in the meat (Guignot *et al*., 1993) as well as possibly decreasing the sarcomere length and increasing WHC (Hoffman, 2005).

This high pH level leads to a change in the ratio of biochemically bound, hydrostatically bound and free water within the meat. The high amount of water bound proteins (because of lower proteolytic activity), in combination with the alkaline environmental charge, helps to increase the amount of bound water and WHC. The high WHC in DFD is connected to the dry taste experienced by consumers during mastication because of the strength of the bond between water and the proteins, resulting in less water being released during mastication of the meat, making it less juicy (Hoffman, 2005).

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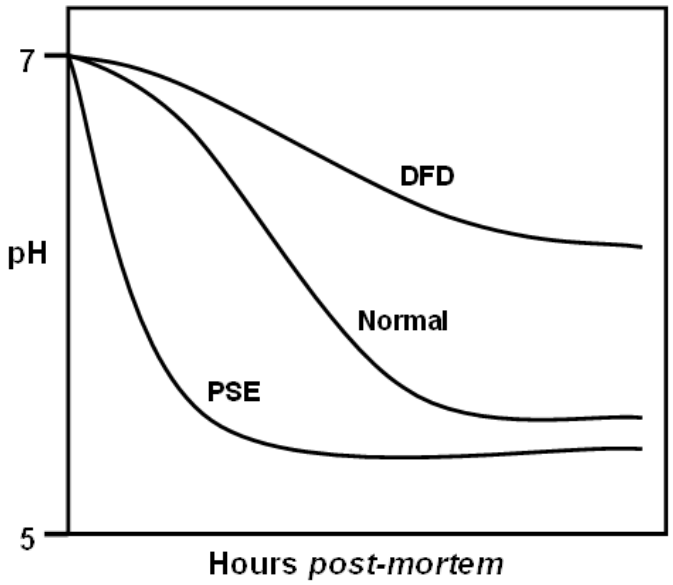

Figure 2.1: The pattern of acidification in pale soft and exudative, dark firm and dry and normal meat illustrated schematically, x-axis is hours post-mortem,y-axis is pH of meat measured. (Warriss, 2000).

This high WHC also leads to a higher light absorption value of the meat and lower reflection index. This makes the meat appear darker than normal (Balog and Almeida Paz, 2007). The closely packed structure of the meat (sarcomere length), also leads to the decrease of oxygen permeability into the internal structure of the meat. This leads to the surface of the meat being oxygenated and forming red myoglobin. However, with an increase in meat depth level, the amount of non-oxygenated purple myoglobin increases. This non-oxygenated myoglobin shines through the surface of the meat giving the meat its dark colour (Warriss, 2000). The high pH environment connected with DFD meat also promotes bacterial growth and bacterial proteolysis within the meat, and although glucose levels in DFD are limited, other meat proteins and molecules serve as substrates (Lawrie, 1998). This is the reason why DFD meat is also associated with low shelf life expectancy. This is especially true when meat is vacuum packed. These factors are documented as a common occurrence in ostrich products (Balog and Almeida Paz, 2007).

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2.6 **Summary**

Currently information pertaining to the behaviour of domestically reared ostriches is limited. This is especially true for the reactions of these birds to external stimuli which are caused by processes that are part of the production system. Transport and lairage are unavoidable production phases and are contributory factors to losses incurred during production.

Behavioural changes have been used in studies to identify stressors and subsequently how to best approach counteracting their effects. The limited nature of information concerning ostrich behaviour during transport and lairage was therefore important and a contributing factor for the initiation of this thesis.

The lack of accepting responsibility by various stakeholders for transport and lairage losses causes concern about the specific identification of the causes of the losses, and where they occur. Transport loading requirements are standard according to animal biomass and are noted to affect the end weight of meat produced. However little or no information exists on the effect of transport density on meat quality. Considering that transport is classified as a stressful process, this needed to be investigated.

Lairage as a bridge between agricultural and industrial norms is unexplored when considering weight losses incurred and the effects on meat quality. As ostriches are left without food and water for a set period, in an unfamiliar environment, these stressors were investigated.

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Chapter 3

Transport and lairage behavioural observations in ostriches causing bruising altercations

ABSTRACT

Observed ostrich behaviour from two trials was correlated with meat quality. Behavioural patterns were observed which were of interest and notable for both trials. Transport density was changed in one trial but kept uniform for the other. Animals were transported with a single truck containing four compartments. For the trial of different loading densities, the compartments (single tests) were allocated numbers H1, H2, L1 and L2, representing two high density compartments and two low density compartments, respectively. The space allocated for each bird was 0.56m^2 for both high density (H1 and H2) compartments, and 0.65m^2 and 0.91m^2 for the low density (L1 and L2) compartments, respectively. The transport density trial birds were slaughtered on arrival and the second trial had groups being slaughtered on arrival, after 24 hours, and after 48 hours in lairage. Feed was also supplied *ad libitum* to one group in lairage. Bruising weight percentages of chest, drum and neck were taken for possible correlation with transport density changes. During transport, ostriches were found to lean against objects and other ostriches if possible. They were also noted to orientate themselves to better resist forces influencing their balance. Bruising trimmed from the drumstick and chest did not differ between transport densities, but did in the weights trimmed from the neck being higher in the H treatments. This is possibly due to more animals being located at the sides of the compartments where ostriches tend to stretch and rest their necks. Bruising total percentages for the lairage trial indicated no significant differences for the different groups. Ostriches were also noted to react to sound emanating from traffic as well as trains near the lairage area. The effect of sound on ostrich behaviour should be examined in more detail as it might be a possible stressor which could be controlled. Ostrich behaviour is complex and differs from other production animals. It is therefore advisable that further research on ostrich behaviour during lairage, production, and transport, be done to quantify and explain the noted observations.

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Introduction

Ostriches are a major contributor to the alternative red meat market and are a relatively recent addition to the group of domesticated animals. The increased animal density caused by intensive ostrich production systems has possibly induced some changes in standard ostrich behaviour such as bird-bird and human-bird interaction. These differences between domesticated and wild ostrich behaviour, results in increased difficulty in identifying animal specific behaviour patterns. As explained in section 2.2.1, little information exists on the behaviour of ostriches during transport and lairage.

It is important to understand the stressors in the animal production system and the effect thereof on the meat quality. This is especially true for stressors in the bridging phases of the final production phase from grow out camp to slaughter (i.e. transport and lairage). The physiological response of any bird to stress is the non-specific endocrine response in combination with specific behavioural changes (Dantzer and Mormède, 1983). Behavioural changes attempt to minimize the impact of the stressors, while the endocrine response combats it indirectly through preparing the body's internal environment to cope with the stress. The interaction between different factors within transport systems however, also makes the identification of specific effects and behavioural changes difficult to ascertain (Fernandez and Tornberg, 1991; Geverink *et al.*, 1996; Warriss *et al*., 1990; Warriss *et al*., 1998).

As external stimuli can also induce stress, a better understanding of the bird's senses could be used to better explain some behavioural changes and anomalies. Through the understanding of the binocular sight range of the ostrich (Martin *et al*., 2000), it was possible for the industry to implement methods that increased feed location and improved feed positioning. These, in combination with the colour preferences exhibited by ostriches, have helped to increase the average daily gain of growing birds (van Vuuren *et al*., 2009). Sight is therefore considered by most researchers (Zeigler and Bischof, 1993; Davies and Green, 1994) to be the most important sense for triggering a behavioural response. Sight is also used for the confirmation of a threat and is highly correlated to inducing a fight or flight response, and vigilance behaviour (Graham, 2007). The fight or flight response can also be induced by other senses stimulated by methods such as whipping or

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noise. However, it is possibly due to a lack of visible confirmation that the other senses can initiate a priority stimulus and cause a behavioural response.

Sound is the other major sense used in the detection of threats by the bird species. Bird hearing is ranged at a lower frequency spectrum making higher frequencies above 20 kHz inaudible (Schwartzkopff, 1973). The frequency range indicated by an ostrich's audiogram could therefore become an important managing tool in animal behaviour and welfare. Ostriches are able to handle noise levels of 90 dB and less which, according to animal welfare standards of acceptable sound levels in production systems should not be exceeded (Code of Conduct for the Commercial Production of Ostriches 2009). However, this noise level requirement is rarely considered in abattoir construction and logistics.

The hearing ability of animals is also important in the maintenance of balance. The bipedal nature of ostriches, combined with a higher centre of gravity and non-accommodating upper limbs, makes balance control difficult, and can possibly influence behaviour and cause distress (Foggin, 1992; Wotton and Hewitt, 1999). Touch is not a very strong trigger in initiating a behavioural response. However, it is possible that in combination with the other senses being initiated, it may induce behavioural changes. Taste faculties and a small range of selective smell faculties are indicative of the bird genus. This is sometimes utilized in the formulation of poultry feeds that are less palatable by mammalian standards than avian standards (Mason and Clark, 1992). However, these senses are only relevant if water and feed are supplied to the birds during transport and lairage. In South Africa, it is not common to supply birds with feed during transport and/or lairage due to limited time spent in lairage (less than 24 hours). Drinking water is supplied *ad libitum* during lairage, but not during transportation.

The olfactory senses of birds are generally perceived to be minimal and not a main contributor to animal behaviour. However, recent studies by Hagelin (2007), Hagelin and Jones (2007) and Balthazart and Taziaux (2009) have shown that birds are capable of olfactory communication. Therefore, ostrich behaviour might be influenced by smell and this should be considered.

The focus of this study was the identification of behavioural anomalies in ostriches during transport, including behavioural changes caused by different loading densities during transport, and lairage. It is accepted that transport and lairage induce stress in ostriches. Density levels during

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transport are usually standardized in accordance with the relevant regulations in use in that country. However, higher densities during transport could be a possible co-factor for the bruising found on carcasses. This study will therefore also focus on loading density levels and its effect on bruising percentage.

Materials and Methods

Trial 1 (transport density, bruising and environmental stimuli)

Ethical clearance (ref. 11LV_HOF01) for this investigation was obtained from the University's animal ethics committee.

Transport

The ostriches used in this investigation were intensively reared. However, the birds were all exposed to the same management practises and were all hatched within a two week period. The farming systems and management practises applied to these birds is typical of any used for grow out system of ostriches in South Africa. In the first trial (which evaluated the effect of transport density), 30 ostriches were transported from Elsenburg to Swellendam (200 km). External climatic conditions during transportation were a clear day with an ambient temperature range of 24-27 °C. Heat stress could become a contributing factor if ostriches are exposed to this heat level for too long (Watton and Hewitt, 1999). Time spent in transport was approximately two and a half hours at a speed of 110 km/hr. A single bed truck with four compartments was used. Each compartment was loaded with different densities, two high density compartments of 0.56 m^2 per bird, and two lower density compartments of 0.65 m^2 and 0.95 m^2 per bird, respectively.

Prior to loading, the birds were individually weighed. Birds were restrained into the weighing box, although as is this a research farm by nature, the birds were used to being handled and weighed. Human handling could thus be considered a non-attributing stressor. After being weighed, their necks were painted either red, blue, yellow or yellow-blue – the allocation of the specific colour being done according to treatment (birds were randomly allocated to treatments). These colours would indicate the different groups to be separated after transport.

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There was little difficulty in completing the administration tasks such as weighing, so handling stress was minimal. This could be as a result of the birds being used to humans. After weighing, the birds were herded using a funnel passage with coloured (to ensure visibility) sides to an inclined ramp with rubber matting, used for loading. The passageway sides consisted of normal wired fencing which was a height of 1.25 m, acceptable for ostriches (World Ostrich Association Welfare Codes for Ostrich, 2007). The top of the ramp was level with the transport truck and trailers. A bridging board covered with a rubber mat was placed down to cover the gap between the truck and ramp. The rubber mat was to insure that the animals did not trip and fall, thereby injuring themselves. Animals were then loaded one at a time to prevent trampling, injuries and stress. Each trailer was divided into four compartments 2.54 m x 1.82 m containing a metal grid floor (15 cm^2, rod diameter of 10 mm) to minimise slipping. Railings were 2.5 m to ensure birds could not jump out, although their heads were able to look out over the railings. There were no metal protrusions or loose wires present anywhere on the truck which could cause damage to the birds. The truck and compartments were all cleaned prior to loading. Each compartment was separated by a removable steel plate door. After the first compartment was filled the door was placed in such a manner to separate it from the rest of the truck. This process continued until the last compartment in the trailer was filled; the latter is frequently filled with any sick and/or compromised birds. One bird was injured during loading and was loaded last.

Defecation by some of the birds was noted during the trial but was not measured for the purposes of this trial. Faeces were in some cases watery but the faeces still retained its white colour. Although some birds exhibited excited behaviour just after being loaded, such as continuous bobbing of the head and pacing, they calmed down within five minutes after being loaded. The birds were all calm before transport to the abattoir commenced. Every four compartments had a handler to ensure that the animals did not lie down to limit bruising (see section 2.1.6). Stockmen on board were all experienced and handled animals with a calm demeanour so as not to agitate or stress the birds. At the start of transport all the birds within a compartment grouped together, seemingly for balance support.

Birds from each loading density were marked prior to loading with coloured neck tags for ease of identification. The lowest density was comparable to Trial 2's density levels of 1 m^2. This was done to determine any relationship between density and bruising percentage. The floor of the compartment was a metal grid, with holes 15 cm^2 in size and with rods having a 10 mm diameter. Trucks were

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clean and complied to all safety and health regulations. The same truck company used for Trial 1 was used for Trial 2. All loading procedures for Trial 1 were followed in Trial 2 to ensure comparability of observations. Additionally, decibel readings were taken during Trial 2 in an attempt to ascertain average decibel ranges during transport and lairage. The decibel meter was used at a frequency range of 75 Hz. Three readings were taken for one minute every ten minutes during transport.

Offloading

After arrival at the abattoir the truck stopped and positioned itself horizontal to an offloading ramp. Birds were then offloaded one compartment at a time. A rubber mat was placed over the ramp to ensure secure footing. Birds were then funnelled to their respective pens according to neck colouring codes. Abattoir handlers were responsible for the movement of the birds after offloading. Upon arrival and offloading at the abattoir, all birds were evaluated by a veterinarian to determine if emergency slaughter was needed; this is normally applicable where sick and/or compromised birds are encountered. At this abattoir, each holding pen has a maximum capacity of 60 birds, each at 1 m^2 per bird (World Ostrich Association Welfare Code, 2007). Thus density in lairage was not a limiting factor considering the groups' sizes did not exceed 30 birds. Although the pens were open, exposure to the natural elements was minimised with a roof and netting cover. All the pens had earth flooring. Birds had *ad libitum* access to water.

Trial 2 (lairage duration and feeding, bruising and environmental stimuli)

Ethical clearance (ref. 1oLV_HOF01) for this investigation was obtained from the University's animal ethics committee.

Transport

The rearing and management practises of the birds from this trial were similar to that of Trial 1. Ninety ostriches were transported from Elsenburg to Swellendam, a distance of 200 km during a rainy, overcast day with a maximum temperature of 25^{o}C reported by the weather bureau. Prior to transport, the birds were grouped together for a period exceeding 6 weeks and all fed the same diet. Animal interaction and social behaviour could be considered to have stabilized after this period. The same

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handling procedures described for Trial 1 were followed for Trial 2. However, the bird size and weight varied as noted in Table 3.1. Stocking density was 1 m^2 for each bird with no more than 10 birds to a compartment. This gave adequate space according to South African guidelines.

Birds were transported from Elsenburg to Swellendam, a distance of 200 km on a Monday during a rainy overcast day. Heat stress could be described in such circumstances as a non-contributable factor. Each truck and trailer had an observer to note any behavioural anomalies or changes in the birds. Observations were made every 5 minutes and the behaviour written down. Behavioural activities noted were bird orientation, bird interests and stressful behaviour such as pecking and panting. The birds were transported at an average speed of 110 km/hr to the target destination. Time spent in transport was two and a half hours. The road travelled exhibited free flowing traffic and the route taken went through a tunnel and a series of passes. Notations were made pertaining to the birds reactions during these specific route markers. The birds started to spread out from grouping approximately 5 min after transport commenced.

Lairage

After offloading, birds were herded to the lairage pens and separated according to the painted group markings on the neck made earlier that morning before loading. The number of birds per lairage group varied with group L0hr having 29 birds, group L24hr, 31 birds, group L48hr, 15 birds and group L48hr *ad lib.*, 16 birds). Adjacent pens were also filled with ostriches from other producers, thereby increasing possible cross-interaction activities. Group L0hr spent an hour in lairage before being moved to the abattoir for processing. All birds had access to water and any specific behavioural problems were noted. Groups L24hr, L48hr and L48hr *ad lib* were weighed between 17:00-19:00 that same day and any abnormal behaviour noted. The next day all groups were weighed between 06:00-08:00. Groups L48hr and L48hr *ad lib* were weighed again between 17:00-19:00. The next day Group L48hr and L48hr *ad lib* were weighed at 06:00-07:00. Bird groups were slaughtered according to their time spent in lairage (i.e., L24hr after 24 hrs and L48hr after 48 hrs of lairage, respectively). All behaviours were noted during this trial period. Birds from treatment L48hr *ad lib* received the same feed that they had been fed whilst growing *ad libitum* in the same feed troughs used during their growth period. The feed was available after the first weighing period. Feed added and refusals were weighed each day to ascertain feed intake (no water intake was measured).

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Table 3.1: Maximum and minimum weights in groups with difference shown.

Group*	Min. Weight (kg)	Max Weight (kg)	Difference (kg)
L0hr	59.5	120.5	61.0
L24hr	61.5	119.5	58.0
L48hr	87.0	116.5	29.5
L48hr *ad lib*	97.5	117.5	20.0

*grouping according to time spent in lairage and whether feed was available or not.

Slaughter

The same slaughter and dressing procedures were followed for both trials. The birds were also slaughtered at the same abattoir. Birds were herded into a passageway that leads to the slaughter area. There were no corners in the passageway to prevent crowding (Grandin, 1980). The passageway was covered to provide shade and ensure that the birds walked without hesitation (Grandin, 1980; Van Putten and Elshoff, 1978). In the first trial, the birds were slaughtered in a random order. In Trial 2, group L0hr was slaughtered at 12:30 on the day of arrival. Group L24hr was slaughtered the day after at 12:00, and the last two groups were slaughtered on the third day at 10:45. The variation in exact time of slaughter was due to abattoir time table changes because of other slaughter birds being late or absent. The birds were electrically stunned while restrained and encased in a box that rotates the animal whilst being stunned (Hoffman, 2012). This procedure ensures animal welfare and helps increase production speed, eases the shackling and ensures handler safety. The animal was then exsanguinated, shackled, hoisted and bled for an adequate amount of time to ensure an effective bleed. Thereafter, the carcasses were weighed before entering the processing line where they were plucked of their feathers. The animal was then skinned and tagged for data traceability before reaching the next slaughter station.

After the carcass had been eviscerated, the organs were taken to a separate area for further processing according to the abattoir regulations. After evisceration the carcass was inspected for disease, the bird's sex was noted and visible bruising trimmed (and weighed). The carcasses fat pads (abdominal belly flaps) were then removed and carcass weight (warm) was recorded.

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All bird handling and slaughter was done in accordance to the abattoir's quality assurance manual, guidelines and regulations and under direct inspection of a government veterinary official.

Results and Discussion

Transport

It was noted that the animals displayed an interest in sound fluctuations while being transported on the truck and when in lairage. This reaction could probably be attributed to the lack of traffic sounds from their farm origin until before being transported. The animals became less sensitive to the different transport noises with an increase in transport time. It was noted that the avoidance behaviour of ducking their heads in reaction to the noise decreased over the trip, and stopped just before reaching the destination. Sound measurements (every 10 min) were collected in order to compare sound levels in lairage to those of transport, as can be seen in Figure 3.1.

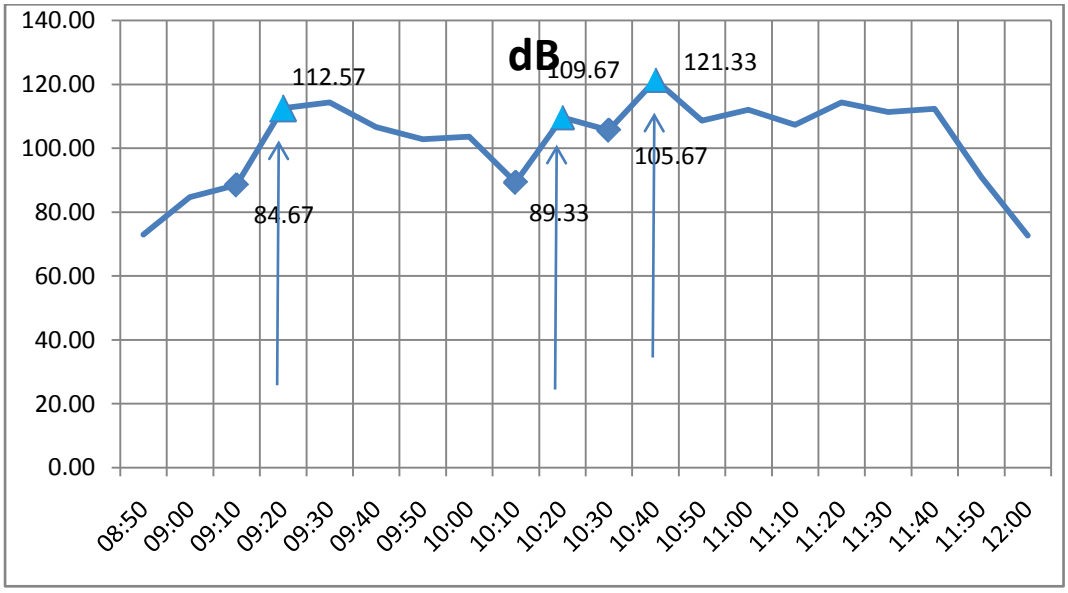

Figure 3.1: Volumel changes measured (dB) during transport of ostriches at commencement of density trials, with noted peaks. (Avoidance behaviour noted at 9:20 ; 10:20 ; 10:40)

It was noted that the animals reacted to fluctuations in sound caused by approaching cars and bridges as mentioned earlier (figure 3.1). The average decibel level on the back of the truck was 112 ± 5.5 dB at a speed of 110 km/hr. With the truck speed constant, and passing vehicle speed

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limited, the decibel fluctuation was limited to approximately ± 5.5 dB. It was noted that fluctuations of 10-15 dB and higher (figure 3.1) triggered the behavioural response noted from the animals

When in lairage the animal's environmental sound level was measured and was found to be around (average) 65 dB. This is deemed acceptable according to human welfare standards for a working environment. However, this increased to 125 dB (Table 3.2) when a train passed nearby eliciting a fleeing response from all the birds in the lairage. No train lines are located near the primary production facility, indicating that the sound of the train passing might be considered as a new stressor and unfamiliar stimulus. This is similar to their response noted in the second trial.

Table 3.2: Decibel range at specific points during density trial, with an accompanying description of noted occurrences.

Function	Measured Decibels (dB)
Truck movement at 110 km/hr	112 dB (± 5.5)
Approaching vehicles and bridges	122-127
Lairage	65
Train passing lairage	125

Different loading densities were explored to investigate the speculation by farmers and transport co-ordinators that an increase in loading density during transport leads to a decrease in bruising and animal losses, since the space in which the animals can fall or trip is limited. The increase in density would also lower the operational cost of transport. If sufficient evidence could be gathered, it would provide credibility to this speculation. This would, however, be contradictory to the transport regulations for ostriches that specify a density of 0.5 m^2 per 80 kg bird (Code of Conduct for the commercial production of ostriches, 2009). For this reason, the densities were increased to see if a decrease in animal space would lead to a decrease in bruising.

Visual observations indicated that with an increase in density from 0.91 m^2 to 0.56 m^2 per bird, a decrease of falling and tripping was observed, probably due to the increase in animal balance support provided by other birds as described. However, an increase in trampling and harrying such as pecking of heads was observed as the birds had less space to avoid one another, which could be related to the large variation in their sizes.

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The herding dynamics of ostriches are not well defined in literature and the birds' social dynamics are described as being complex. This according to description is on par with those of more socially dynamic animals. In some animals, herding is known to influence the density observations with some animals grouping together. Exceptions, such as birds separated from the flock, could indicate that there is something wrong with the specified bird (Cooper *et al*., 2010). In this study, there appears to be an increase in grouping in corners with a decrease in density amongst the ostriches (can also be due to balance support). The corner groupings indicated no specific orientation overall, but birds not situated in the compartment corners were noted to orientate themselves, parallel to the forces exerted on them. This could possibly be attributed to the limited amount of balance support caused by the decrease in density, although ostriches were observed to lean on one another and group in Trial 2.

Force exertion relative to orientation in order to maintain balance was noted. Further indicators were thought to exist therefore continued observations were made during both trials. Animals were again seen orientating their bodies parallel in relation to the movement of the truck. This would support the previous observations of animals orientating themselves to counter the forces exerted on them by using forward and backward body movements.

No panting or defecation was noted during loading in Trial 2, which could have indicated possible stress. Birds were loaded onto the truck one at a time with the help of two stockmen. Limited resistance was noted but expected. Birds were loaded until the first compartment was full and closed. Birds grouped initially when moved into a compartment but spread out after approximately 5 min. This could possibly be due to adaptation to their environment. The level and speed of adaption to their environment could also be affected by their level of habitation and domestication. These birds were fairly well habituated to humans because of the research nature of the farm, as stated previously. Birds were able to look over the railings. This led to the birds head movements being erratic as they would move their heads to investigate sense stimuli. The birds did not react verbally or erratically when transport commenced but braced themselves against the movement of the vehicle. Birds that were not orientated parallel with the truck did so as movement commenced. The first 2 km travelled were on a gravel road until the freeway was reached. On the freeway the most notable reactions of the birds were to the noise generated from the cars passing the truck and the truck passing underneath bridges. This noise reaction might be caused by an acoustic phenomenon called

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convolution from the sound coming from the truck and traffic (Reijnen *et al*., 1996). This noise fluctuation seemed to cause distress to the birds and they would duck their heads in an avoidance manner. This was noted as an anomaly as the birds, when not exposed to this noise fluctuation, showed no specific head positioning or orientation. No visual confirmation by the bird of the object that generated the noise was needed to initiate the avoidance response. This makes noise the most probable stimuli of this reaction. It could, therefore, be stated that this behaviour was possibly triggered by both the sound generated by the movement of the cars in relation to the truck, and the truck in relation to the bridges. The noise output of cars is restricted by law, however the combination or convolution of the noise generated from free flowing traffic can surpass the noise restriction imposed by law (Johnson and Saunders, 1968). Although this noise is also audible in the human hearing spectrum, it is possible that human rationalisation ability can discard the sound as not indicative of a threat. Ostriches possibly do not have such high levels of rationalising faculties and might consider the noise as indicative of a threat, explaining the avoidance behaviour observed. The ostrich's reaction to sound also seemed to decrease with an increase in exposure to this sound fluctuation (McBride, 1984). Future research may determine whether this noise causes stress in the birds or merely increases its curiosity. Whether it is the frequency or the volume (dB) level that is causing this reaction should also be examined further.

The loose material of the stockman's hammock on the truck was caught by the wind, which caused it to flap and make a noise that attracted the attention of the ostriches. Each compartment has a hammock wherein the handler frequently rests although the main function of the hammock is to "hang" birds that are unable to stand into this hammock, thereby ensuring that they are not trampled on. It was initially speculated that the colours of the hammock material was responsible for the increased interest of the animals. The material ranged in colour from chromatic red to khaki. Only the latter colour is situated near the green colour spectrum preferred by ostriches (Burbier *et al.,* 1996). Therefore, it is possible that curiosity was initiated by the sound or movement of the materials. Ostriches, when placed under stressful situations, are noted to peck at objects although the reasons for this behaviour are not yet fully understood (Lambert *et al.,* 1995). Pecking behaviour was noted in this trial but only to a limited number of animals in different compartments. No common denominator between animals exhibiting the behaviour could be found.

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The majority of birds were seen orientating themselves parallel to the external forces being exerted on them. When the truck was moving in a straight line, without turns, with intermediate slowing down or even when stopping, the ostriches' bodies, legs and heads were facing forward in the direction of travel. As the truck moved around long bends in the road the animals were seen turning themselves horizontally in relation to the truck in an attempt to resist the forces, either by moving forward or backwards rather than sideways. Birds when orientating themselves had no preference in facing a specific direction, however, their body's always needed to be parallel with the forces being exerted on them. This behaviour has been noted concerning quadruped animals (Eldridge *et al.*, 1988; Tarrant *et al.*, 1988, 1992). This behaviour may also be due to the bipedal nature of the birds' anatomy limiting the ability to move sideways such as sidestepping and crossing over. Their bipedal nature makes them prone to use forward movement, stopping and initiating central body mass changes to orientate themselves during movement (Jindrich *et al.,* 2007). This behaviour can possibly be attributed to the animal's physical need to conserve energy. Therefore, the behavioural changes observed could be an attempt to minimize energy use in maintaining balance. Preliminarily, this behaviour indicates that it would be advisable to keep space open for the animals to orientate themselves. However, it was also observed that with an increase of space, there was also an increase in incidences of loss of balance, in some cases causing falling and tripping. These incidences are mostly observed with trucks going around corners and sudden changes in speed. Ostriches were also noted to be leaning against the sides of the compartments and in some cases exhibited a preference for leaning against each other. This leaning behaviour was constantly noted and no specific external factor could be ascribed to it. Speculatively, this behaviour might have been an attempt to reduce balancing problems. In addition, when the ostriches were seen leaning against objects it was also primarily on the parts of their bodies which they appear least capable of exerting force on. This seemed to be the sides of their main mass including the sides of their drum-sticks. This might cause increased bruising in these areas, although this could not be substantiated by increasing bruising percentages and the bruising location.

The falling and tripping of the animals that was observed, was in some instances caused by the metal grid mesh on the floors of the trucks. This may be cause for concern as the primary function of the grids is to ensure sure footing. The nail of the ostrich was noted to be a prominent reason for the animal to get stuck in the grid and tripping. Therefore, a lack of nails or change in grid size could

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possibly decrease instances of animals tripping or falling. However, the effect of nail removal/amputation in ostriches does have an effect on their maintenance of their balance and movement, and in some studies fewer splayed legs were noted after the removal of nails. Although nail removal is not allowed in South Africa, it is still practiced in some countries, although there are welfare considerations and a sometimes high chick mortality rate is associated with this method. (Alexander *et al*., 1979; Schaller *et al*., 2005). Grids such as these are used to stop birds from slipping and falling down whilst at the same time they improve feather and skin quality by limiting contact with wet faeces. However, these metal grids can also increase the overall skin damage and bruising if the animal does indeed sit or fall down for any reason. A number of transport trucks are now moving to the use of rubber matting to try and combat the described situation – this flooring warrants further research.

Lairage

The ostriches were calm in lairage with no agitation noted in comparison with their normal behaviour. The birds had adequate water and all regulations were adhered to in accordance with Animal Welfare regulations (Code of Conduct for the Commercial Production of Ostriches, 2009). Bird behaviour was observed to be flighty when exposed to the sound emanating from the railway track when a train passed. As stated earlier, the increase in volume range seemed to be the inducing agent for this reaction. Male ostriches did, however, exhibited increased aggression with an increase in time spent in lairage (particularly in Trial 2 for the two 48hr groups) and it could be speculated that mating behaviour was initiated (observed from increased neck and leg colouring) by this exposure to other ostriches which leads to territorial behaviour. This behaviour was not limited to any specific size of the male animals. Mating behaviour increased considerably with the time spent in lairage. When the birds were loaded into the truck it was almost impossible to distinguish between sexes without doing a cloacae inspection. This was due to the legs and beaks not showing any intense colouring as are observed with males in mating season. The feather colouring was also not distinctive due to the relative young age of the birds (averaging 300 days) and the dust covering the feathers. After being loaded and placed in pens, the birds immediately started to exhibit some sexual behaviour, with females cuing and males starting to show colouring (Bubier *et al*., 1996). This behaviour was not exclusive to the trial group and adjacent pens also showed signs of increased mating (colouring)

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behaviour. Some researchers have published similar results for other species, where the effect of transport stress can stimulate the onset of oestrus; for example in pigs (Hughes, 1982).

It is possible that the effect of new surroundings and foreign animals could be responsible for the stimulation of sexual behaviour rather than exposure to humans as postulated by Bubier *et al.* (1998). Animals were handled each day for administrative needs and data collection while in lairage (morning and afternoon for each group until slaughter). The handling difficulty of males increased each day with an observed increase in the number of males displaying colouring as well as the colouring intensity. When the days were compared it was found that on day one there was no visual difference between males and females, but by day three, all males were coloured and aggressive. Injuries (bumps and grazing) to handlers increased with time in lairage. No serious injuries occurred to the birds, but ankle and leg injuries occurred with increased resistance to handling – it is speculated that this phenomenon is due to these temperament changes. Reasons could include the stress of transport or the stimulation of external environmental stimuli such as new (female) birds already in the lairage area. The introduction of foreign birds from another farm may also have induced this behaviour as some breeding birds from the other farms may have been present in the lairage. Human imprinting has also been noted to trigger sexual behaviour but excludes cuing behaviour which was also observed during this trial (Bubier *et al.,* 1996).

Bruising

The weight of trimmed meat due to bruising as well as the location of bruising on the carcass was noted. All differences in bruising weights are considered to originate from the loading, transport, unloading and moving of the birds to be slaughtered, and not from lairage as the birds in Trial 1 had spent no time in lairage and were slaughtered on arrival. The weights of the bruised trimmings were not significant different between the loading density groups for the thighs or the chest regions, although they were significant for neck region: L1 differed significantly from H1 and H2 but did not differ significantly from L2. The latter (L2) did not differ from H1 and H2 (Table 3.3).

Table 3.3: Losses (kg) ± standard deviation associated with bruising of ostrich carcasses from birds transported at different densities

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Group	Loading densities			
	H1	H2	L1	L2
Drum bruising weight (kg)	0.230[a] ± 0.727	0.012[a] ± 0.693	1.959[a] ± 0.932	0.032[a] ± 0.977
Chest bruising weight (kg)	0.001[a] ± 0.011	0.011[a] ± 0.01	0.023[a] ± 0.014	0.049[a] ± 0.014
Neck bruising weight (kg)	0.028[b] ± 0.012	0.018 [b]± 0.011	0.041[ab] ± 0.015	0.086[a] ± 0.016

[a,b]means with the same superscript do not differ P>0.05

In Trial 2, the total bruising percentage was used to calculate if time spent in lairage affected the bruising percentage. Birds were randomly allocated when transported and the trucks used were of the same company, travelled the same distance and route. Transport bruising could be concluded to have been standardised between groups. The number of birds per lairage period varied with group L0hr (29), group L24hr (31), group L48hr (15) and group L48hr *ad lib.* (16). It is speculated that the group size variation contributed to no significant differences being found in bruising weight trimmed. The inability to being able to identify the origin of the bruises also contributed to this lack of significant difference.

Table 3.4: Total bruising weight trimmed off and averages trimmed of ostriches kept under different lairage periods (L0hr, L24hr, L48hr and L48hr *ad libitum*).

Groups	Total bruised weight trimmed (kg)	Average bruised weight trimmed per bird (g)
L0hr in lairage: immediate slaughter	2.76	9.52
L24hr in lairage	6.46	20.84
L48hr in lairage	2.15	14.33
L48hr (*ad libitum* feed) in lairage	0.52	3.27

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Conclusion

The complex nature of ostrich behaviour, adapted behavioural changes from domestication, and changes to their environmental, such as transport and lairage, make identification or clarification of stressors difficult. However, certain behavioural changes were noted that indicate more research is needed on factors which aren't usually quantified in stress studies. Balance and noise have been identified as inducing behaviour responses in this investigation but whether these cause significant levels of stress is not known. Some behavioural changes seemed to be caused by changes to the transport density. The most notable behavioural trend was the birds' tendency to group if allowed too much room for movement and to position them-selves for leaning purposes. This was speculated to have possible ramifications for bruising percentages, but the Trial 1 data showed that the only differences seen were on bruising weights removed from the neck. This could be because of the larger number of birds located at the sides of the compartments allowing more birds to look out over the sides of the truck compartment. Financially important carcass regions, such as the chest and drum/leg, were found not to have significantly different bruising across the loading densities evaluated. Trial 2 indicated that time in lairage might have an influence on bruising percentage but because of large within group variations and multiple possible causes of bruising, conclusions could not be substantiated.

Sound intensity (Decibel) readings were taken to quantify the sound environment of transport and lairage. Sound intensity changes were noted to affect behaviour and cause a possible fight or flight reaction that may result in increased bruising and possible skin and meat quality defects. The readings taken indicate that more focus should be given to noise and sound levels as possible stressors in abattoir and transport schemes. Birds were noted to react more to sound fluctuations than intensity or level. More research on the subject is needed to identify further application. Road traffic noise has been shown to affect breeding populations of birds in the wild (Reinjen *et al.,* 1995b, 1996: Reinjin and Foppen, 1995). The limited coverage of the welfare regulations concerning noise is also of concern. Further research should be conducted on this facet of production.

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Chapter 4

Effects of transport density and time in lairage on ostrich meat quality and carcass weights

Abstract

Transport density and lairage time were changed to determine their effect on ostrich live weight, carcass weights and meat quality parameters: toughness (shear force), pH_u, colour parameters, drip loss and cooking loss. From the previous section it was seen that transport densities had no significant effect on live weights and carcass weights or muscle quality. The lairage trial was divided into four groups relative to their time spent in lairage; one group had feed available (*ad libitum*). Groups were 0hr, 24hr, 48hr and 48hr *ad libitum*. Weight loss percentages and dressing percentage as function of loading weight differed significantly between lairage times, but not dead weight (abattoir weight). However, no differences between cumulative loss percentages were found between L48hr and L48hr with feed *ad libitum*. These results indicate that lairage time (>24hr) had an effect on the loading weight loss and dressing percentages of ostriches, but not dead weight loss and dressing percentage. No significant differences were found in any meat quality parameters for different transport densities or lairage periods. When ostriches were kept in lairage >24hr, a loss in weight and dressing percentages were found although feed availability was noted to help negate some of these losses. However, lairage time had no effect on the ostrich meat quality.

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Introduction

Progress in production systems can possibly be measured by the reduction of both monetary and product losses while increasing production output. It is difficult to allocate responsibility to the losses that are experienced in the horizontal production system of ostriches. The bridging phases of transport and lairage are responsible for most of these losses. However, they fall into a grey area of sector responsibility.

The lairage phase is used to bridge the differences between agriculture and industrial norms (Eikelboon *et al.,* 1991), rather than reducing stressors inherent to the process. Therefore, adequate investigation into lairage effects (duration) on product parameters is needed.

The time spent in lairage is important as it indicates the time the animals are exposed to possible stressors. Abattoirs are non-productive during the weekend as they are constrained by their work force requirements. Therefore, the maximum time an animal spends in lairage can be considered to be up to three days. During this time the animals are supplied only with water and shelter which has been shown to affect live weight percentages and possibly carcass dressing percentage in ostriches and other animals such as cattle (van Schalkwyk *et al*., 2005; Jones and Schaefer, 1992). If of sufficient duration, time in lairage can also be beneficial in the reversal of stress responses (Hoffman and Lambrechts, 2011). The further addition of feed to the animals during lairage could possibly lower stress levels but can also increase process costs by increasing evisceration difficulty and duration. Additionally, the responsible entity for the costs of feed and removal of additional faeces is never resolved satisfactorily between the livestock owner and the abattoir facility.

Transport has also been proven to induce stress in ostriches and influence meat quality (Reiner *et al.,* 1996; Wotten and Hewitt, 1999; Hoffman *et al.,* 2010). The different transport parameters are difficult to quantify because of cross interaction between behaviour and stressors and an undefined standard for ostrich behaviour (Fernandez and Tornberg, 1991; Geverink *et al.,* 1996; Warriss *et al.,* 1990*,* 1998). A popular belief by farmers and transporters is that increased transport density would lower instances of bruising, product losses and production costs. The opposite would then also be applicable and a decrease in density would increase bruising percentages and cause meat quality problems. Meat quality parameters are all affected by the rate of pH decline and final pH. Glycogen reserves at slaughter are a key aspect that influences these two parameters. Therefore, the

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manipulation of glycogen reserves through exposure to stressors, exercise and feed availability could in theory be used to control meat quality (Lawrie, 1998).

This chapter focuses on the transport parameters of loading density and lairage period and access to feed *ad libitum* whilst in lairage, and the effect thereof on meat quality.

Material and Methods

Ostrich origin

The ostriches used during the trials originated from the Kromme Rhee research farm located near Stellenbosch, Western Cape, South Africa. The animals were all raised in a grow-out system environment. Initially the animals were visually evaluated and deemed to be healthy and of adequate age for slaughter and trial evaluation. All selected animals were then examined by a veterinarian prior to transport and slaughter as pertaining to European Union Health, Slaughter and Safety Regulations. The birds numbered 90 African Black Ostriches (*Struthio camelus*) for the lairage trial and 30 for the transport density trial. All groups contained a random assortment of males and females. All birds used in the trials were 10 months ± 2 weeks of age with ± 80 kg live weight. Animal familiarization with humans was also high due to the research orientation of the farm, with live weights being taken weekly.

Transport and Lairage

The full experimental procedures for th transport densities and lairage duration trials are discussed in detail in the previous Chapter 3. Therefore only pertinent information to this chapter will be discussed /repeated.

The ostriches of Trial 1 were all slaughtered on arrival at the abattoir and therefore spent no time in lairage. The main variable that was inspected here were the effect of transport density on meat quality. Immediate slaughter of the animals ensured the removal of lairage as an additional factor in determining meat quality. The birds from Trial 2 were kept in lairage for different periods.

Live weights and carcass weights

Birds were weighed before departure for both trials. Birds were restrained in a weigh pen where the live weight was taken, the identification number confirmed and the neck marked randomly with paint

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for group identification (according to pen allocation for lairage time – Trial 2). When the birds of Trial 2 reached the abattoir they were allocated to their pens as previously described. Group L0hr was slaughtered on arrival at the plant. Their final weight was taken as dead weight prior to exsanguination (Table 4.1). Group L24hr, L48hr and L48hr *ad lib.* weights were also recorded each morning (± 07:00) and afternoon (± 17:00). Birds were handled gently but firmly to ensure minimal stress. Habituation of birds to humans possibly lowered stress caused by handling, as these birds had formed part of the experimental flock on the research farm. Group L24hr was then slaughtered and final weight was recorded as dead weight. On the second day, groups L48hr and L48hr *ad libitum* were again weighed and then slaughtered. Final weight was also recorded as dead weight. Differences between first and final weights were used to calculate if there were any significant differences between the groups.

Carcass weights were taken before trimming after evisceration for both trials. Dressout percentage as function of initial and dead weight was calculated as well as differences between final live weights.

Table 4.1: Time schedule for determination of ostrich weights during different lairage times and accompanying data.

Cumulative Percentages time points	Time weighed (Time points)	Treatment mean weights (kg)			
		L0hr	L24hr	L48hr	L48hr *ad lib*
Time point 1	Loading (X)	104.9	103.7	101.9	107.4
Time point 2	Arrival (Y)	104.5	99.9	97.6	102.0
Time point 3	Y + 19 hr		98.5	95.2	101.5
Time point 4	Y + 31 hr			94.0	103.4
Time point 5	Y + 39 hr			94.2	101.7

Slaughter

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Birds were removed from lairage *en masse* and herded into a passageway that leads to the slaughter area. There were no corners in the passageway to prevent crowding (Grandin, 1980). The passageway was covered to provide shade and to ensure that the birds walked without hesitation (Grandin, 1980; Van Putten and Elshoff, 1978). Group L0hr was slaughtered at 12:30 am on the day of arrival. Group L24hr was slaughtered the day after at 12:00 am, and the last two groups were slaughtered on the third day at 10:45 am. The variation in exact time of slaughter was due to abattoir time table changes because of other slaughter birds being late or absent. The birds were electrically stunned while restrained, encased in a box that rotates the animal whilst being stunned (Hoffman, 2012). This procedure ensures optimal bird welfare and helps increase production speed and eases the shackling of the birds. The birds were then exsanguinated and left to hang until bleeding was completed. Thereafter the birds were weighed (dead weight) before entering the rest of the processing line where they were plucked. All processes mentioned so far were constrained to the dirty area of the abattoir. The birds were then skinned in the clean area and tagged before reaching the next slaughter station. The tagging was done for data referencing purposes and ensures full traceability.

The birds were then eviscerated. The organs were taken to a separate area for further processing according to the abattoir regulations (SOP) and health code. The bird's gender was noted after evisceration through sex organ identification in addition to any possible oddities which could be connected to the animal's health. The carcasses were inspected for any indication of disease and any visual bruising removed. The bruising location and weight was recorded for both trials. The carcasses fat belly flaps were then removed and carcass weight (warm) was recorded, where after the pH 1 hour *post mortem* was measured in the left fan fillet (*M. Iliofibularis*). Prior to measuring the pH, the Testo 205 pH meter (Testo AG, Germany) was calibrated using standard buffers as per the manufactures instructions.

Carcasses were then refrigerated (4°C) prior to transport to the deboning plant in Oudtshoorn the next day.

Deboning

All further carcass processing took place on site at Oudtshoorn. All trial groups followed the same procedure of slaughter and processing. Before transport, the ultimate carcass pH was taken and

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recorded as pH_u. The carcasses were all transported in a cooler truck (4°C) which adhered to the transport regulation pertaining to meat products, thereby ensuring that the cold chain of the product was maintained. Carcasses were stored in a refrigerator at the deboning plant at a temperature of 4°C until further processing.

The left fan fillet (*M. iliofibularis*) was removed for further analysis. The fillets were weighed and labelled before being vacuum packed. These samples were then transported at 4°C on the next day to Stellenbosch University for analysis. The fan fillets were received in the meat laboratory at 17:00 (day 4 *post mortem*). Upon arrival at the Meat Science laboratory, standard meat quality tests were conducted. This included a standard shelf life test with data being taken for time points 4, 6, 8 and 10 days after slaughter. The samples were taken out of their packaging and a reverse drip calculation was performed on each fillet. A muscle pH reading was also taken before being prepared for analysis. Trial 2 samples were also received on the day after deboning (day 4 *post mortem*) in Oudtshoorn using the same procedures (deboning, packaging, transport). A standard shelf life test was done for time points 4, 6 and 8 days.

Preparation for shelf life test

Each sample fillet was cut into four steaks from the centre of the muscle moving outwards, each steak was ±1cm thick and was weighed. The steaks were then repackaged into a standard foam holder and overwrapped with 10 micron oxygen permeable Versafilm (Crown National, Montague Gardens, Cape Town, South Africa). The repackaged steaks were then stored in a cooler room at a temperature of 4ºC. Each individual fillet thus had four (or three for Trial 2) repackaged steaks at the end of preparation with each one representing an individual time point.

Physical analysis

Samples were opened and analysed on their respective time points (days). The steak was taken out of its packaging and the pH and its colour was then recorded. The steak was then gently patted dry with an absorbent paper towel and weighed for drip loss analysis. After this, a piece of muscle was cut off for water binding capacity analysis. The sample remaining was then weighed again and cooked to determine the cooking loss (Honikel, 1998).

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Reverse drip loss

Drip loss was calculated by use of the reverse drip loss method. This was done on all samples received on arrival. The meat analysis could only commence 4 days after slaughter (3 days after deboning) therefore, the reverse drip calculated was rendered as total drip loss for 3 days (Honikel, 1998).

Shelf life drip loss

Shelf life drip loss was measured by calculating the difference in fillet weight before packaging and after being patted dry on the designated sampling day. This provides a more accurate picture of the water lost during storage and shelf life (Honikel, 1998).

Water binding capacity (WBC)

Water binding capacity (WBC) was measured as an indicator of the rate at which the meat proteins were being denatured. This capacity also correlates with the drip loss and the juiciness of the meat. Water binding determination was done on each fillet sample at each time point allocated. A sample of 0.5 g was removed from the fillet and placed on a water absorbent paper. The sample and paper were then placed between two plastic lenses that fit into an area that is resistant to horizontal pressure, thereby ensuring that no side movements occurred between the plates. A clamp mechanism was then engaged to supply constant vertical pressure on the plastic lenses and sample. This causes the expulsion of free and suspended water out of the sample which is subsequently absorbed by the paper. After a set time period of one minute the pressure was released and the plastic lenses and sample removed. The absorbent paper was then placed on a flat surface and a photo taken at a 90 degree angle. The photos were then analysed using the ImageJ1.41 computer package to measure the different areas (Trout 1988). Values used in the calculation of the WBC were those taken from the complete exuded surface area and central meat surface area. The WBC value was given as the central meat surface area expressed as a percentage of the total surface area. Thus the smaller the percentage; the weaker the WBC.

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pH

The pH readings were taken in the fan fillet one hour and 24 hours *post mortem* by making a ±5 cm incision into the left fan fillet. Additional pH readings were taken when the fillets were removed prior to cutting and packing for the shelf life test. A pH measurement was also taken each time the sample was removed for further analysis. All measurements were done using the same Testo 205 pH meter (Testo AG, Germany) which was recalibrated for each time point to ensure accurate readings (Honikel, 1998).

Colour

Colour was measured at each time point for a comparison between groups. This was to note the colour stability of the meat or to which degree its colour changes. The fillets were left to bloom for 30 minutes (Leygonie *et al*., 2011) after which colour measurements were taken to determine the L*, a* and b* values prior to packaging. These values indicate the lightness (L*), red-green range (a*) and blue-yellow range (b*), respectively. Three measurements at random points on the surface were taken at each time point to ensure complete colour schematic representation (Stevenson, Seman, Weatherall and Littlejohn, 1989). Hue angles and Chroma values were also calculated using equations 1 and 2. These helped define the colour (Hue) and intensity (Chroma) of the measurements.

Hue angle: $hab = \tan^{-1}(b^*/a^*)$ (4.1)

Chroma value: $C^* = [(a^*)^2 + (b^*)^2]1/2$ (4.2)

Cooking loss

Cooking loss indicates the amount of water lost by the meat after it has been exposed to high temperatures for a specific period of time. Weighed samples were placed individually into a plastic bag, immersed in water at 80°C until the sample reached an internal temperature of 75°C (Honikel, 1998). Correct internal temperature was achieved by periodically removing the smallest sample and checking with a temperature probe. If the correct temperature was reached, the sample was then removed from the water bath and the second smallest sample taken and checked. This procedure is followed until all samples are removed at 75°C. Samples removed were then taken to a basin and all

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expelled water decanted, after which the sample was placed on ice in a cooler room. When the sample had cooled down to < 5°C, it was dried and weighed (Honikel, 1998).

Shear force

Shear force is measured by the amount of force needed to shear or separate muscle fibres. The Warner-Bratzler device, with a load of 2.000 kN, attached to a test model 4444 Instron Testing Instrument (Apollo Scientific cc, South Africa), was used in the determination of the maximum shear force of the individual samples. A cylindrical cutting tool (1.27mm in diameter) was used to produce test cores taken parallel to the grain of the meat. Special care was taken to ensure cores did not contain visible connective tissue which would influence readings. The cylindrical test cores were then placed perpendicular to the longitudinal and as centrally orientated as possible to ensure that the cutting surface was optimum. The test core was then sheared by the tooth of the Warner Brazler device and the maximum shear force needed to do this was recorded by the Instron. The Instron used had a V shaped tooth to hold the sample which was then moved up against a shearing blade, with the meat sample being sheared by upwards pressure from the bottom tooth. A minimum of three test cores per muscle sample were used to ensure an average shear force measurement (Honikel, 1998).

Statistical analysis

Data was analysed using SAS (SAS for windows Ver 9.1.3) and included an analysis of variance (ANOVA) with Fishers LSD (least significant differences) and *post hoc* tests. Analysis was done with mixed models with bird ID and Group as random variables. Weight loss data was calculated using the cumulative value for the number of time points. As the birds were of approximately the same age, the main effects considered were weight data at the different production time points. Weights for production time points were tested by means of a null hypothesis of u1=u2=u3=u4 and alternative u1≠u2≠u3≠u4, where appropriate. Data results are presented as means and standard errors. A p-value of < 0.05 was accepted as statistically significant using Fishers LSD.

Meat quality when analysed with ANOVA and Fishers LSD showed differences but had an extremely low R square. The data was further analysed by means of a Principle Component analysis (PCA), using XLSTAT (Software Package Windows Excel 2010), to identify possible associations and to explain these differences. A further Bonforonni test (Hommel, 1988) was compiled to ensure data

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correspondence with results retrieved from Fisher LSD Anova and associations of PCA. All the pH values of all the birds were divided into categorical data according to the pH value of the birds' muscles. The pH categories were pH > 6.2, 6 - 6.2, 5.8 - 5.99 and < 5.8. The percentage of birds that fell into each category was noted. This was done because individual birds differ in their response to stress and for abattoirs to determine the proportion of birds that has high (extreme) pH values that could result in DFD meat.

Results and discussion

Effect of transport loading density on meat quality

Weight loss results

The transport loading density trial showed no significant difference between groups when analysed with Fisher least mean square on the cumulative weight losses (Table 4.2). The lack of significant differences is attributed to the large variation measured within the groups. This is most probably due to the small size of the groups as well as the inherent genetic variation of ostriches. Even so, losses were experienced during transport, which could be attributed to stress (Wolmarans, 2011).

Table 4.2: Mean transport loading density group weights (± se) at loading, abattoir and carcass weights with accompanying weight differences between loading and abattoir weights.

Group	Loading Weight (LW)	Abattoir Weight (AW)	Carcass weight (cold)	Difference (LW – AW)
H1	102.6 ± 4.60	96.2 ± 4.73	44.37 ± 2.89	6.4 ± 0.90
H2	103.4 ± 2.57	98.5 ± 2.89	45.9 ± 1.40	4.9 ± 0.90
L1	126.3 ± 2.18	117.6 ± 2.24	54.9 ± 2.62	8.7 ± 0.82
L2	124.0 ± 7.85	115.4 ± 6.93	53.9 ± 4.55	8.6 ± 1.40

pH

Transport loading density groups showed no specific trend on initial (30 minutes *post mortem*) or pH_u. The pH of the majority (±80%) of the birds ranged between 5.8 and 6.2. The exception was H1 with 25% of the birds' pH_u ranging below 5.8. This might indicate that higher loading density might cause

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lower stress levels, although H2 was the same loading density and tended toward the higher pH ranges (Table 4.3). In contrast ≈50% of H2 and L1 can be classified as DFD. This further substantiates a postulation that density has no effect on the stress experienced by birds during transport. It can also be argued that if the experimental groups were larger, these differences may have been more significant or less – a phenomenon that warrants further research

Table 4.3: Summary of the pH categories of the pH (30 min post *mortem*) and pH_u of each of the four groups of ostriches that travelled at different loading densities prior to slaughter.

Time post mortem	pH category	H1 %	H2 %	L1 %	L2 %
Slaughter line (30 min)	> 6.2	9.0	6.7	4.3	7.0
	6 - 6.2	15.0	16.7	18.6	16.0
	5.8 - 5.99	59.0	36.7	40.0	50.0
	< 5.8	17.0	40.0	37.1	27.0
Chiller (24h) pH_u	> 6.2	11.0	23.3	11.4	13.0
	6 - 6.2	17.0	23.3	38.5	25.5
	5.8 - 5.99	47.0	53.3	47.1	48.0
	< 5.8	25.0	0	2.9	13.5

Meat quality parameters

No differences (P>0.05) were found between trial groups for any of the meat quality parameters evaluated (Table 4.4). The low R square value calculated also indicates that any possible differences would have been due to in-group variation rather than the groups' differences. The various quality parameters showed no differences in the Proc GLM analysis or for the Fisher least square means test. Due to the low R square values for all the related parameters, a PCA plot analysis was done to ensure data validity and to elucidate possible data associations. The associations between quality parameters were as expected, and are shown in Figure 4.1. Strong associations between colour readings and quality parameters were found. These quality parameters include pH and drip loss, cooking loss and shear force. However, centroid positioning of the main effects of loading density once again indicated no differences between treatments. The high and low density groups were not grouped specifically in the same location or distanced from one another as would be expected with association differences. This indicated no discernible association between groups. The change in

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shelf life was also plotted and the centroid positioning was still localised around the axes in a random manner with no identifiable association between experimental groups and quality parameters.

Table 4.4: Means of all physical quality parameters (± SE), grouped according to time post *mortem*

Time post mortem (days)	PH	Shear force	L*	a*	b*	N
4	6.3 ± 0.05	35.0 ± 1.18	29.7 ± 0.23	13.6 ± 0.21	8.1 ± 0.16	90
6	6.4 ± 0.05	32.1 ± 1.28	30.3 ± 0.26	14.6 ± 0.21	9.9 ± 0.18	90
8	6.4 ± 0.06	34.5 ± 1.36	30.0 ± 0.23	14.0 ± 0.25	9.9 ± 0.20	90

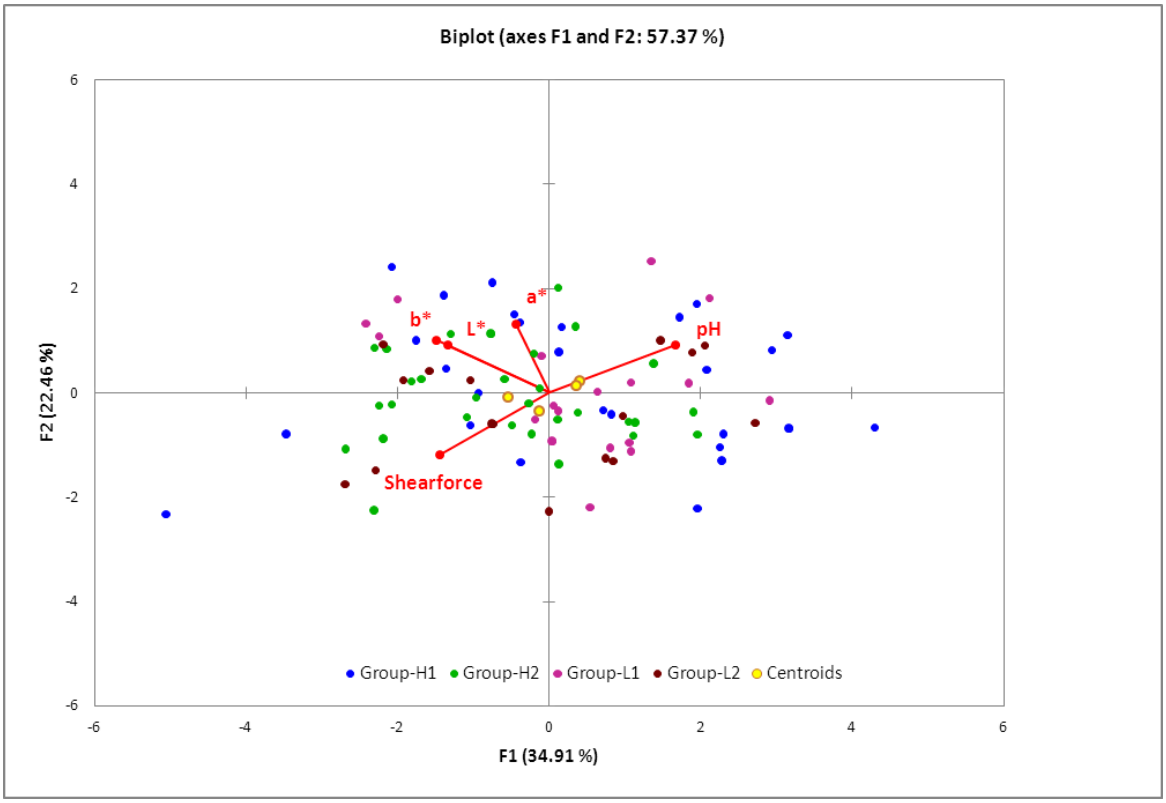

Figure 4.1: PCA plot depicting density centroid association with different meat quality parameters of ostriches transported at different densities (H1, H2, L1 and L2).

Effect of lairage time on weight losses and meat quality

Weight loss results

In Trial 2, there were no significant differences (P>0.05) between live weights prior to loading, or intact carcass weight at slaughter (Table 4.4). Dressing percentage as a function of the end weight did not

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differ significantly (P>0.05) between groups. Null hypothesis was accepted for non-cumulative values and rejected for cumulative values. Cumulative loss as a percentage was calculated for the different weighing time points (Table 4.5) and showed significant differences for percentage loss at time point one between L0hr and the rest of the groups (Table 4.5). Significant differences were also noted between L48hr and L48hr *ad lib.* for cumulative percentage loss at time point 4 (48hr). All other time points showed no differences between groups. Carcass percentage was also found to be significantly different between L0hr and L48hr as a function of the initial weight. However group L48hr and L48hr *ad lib.* did not differ significantly for carcass percentage as function of dead weight but did for initial loading weight. This showed that an increased time period in lairage does affect live weights (at point of slaughter) and cause live weight losses. This supports the postulation by Wolmarans (2011) that lairage causes weight losses and feed availability influences live weight.

Table 4.5: Mean weights (± se) for ostriches standing for increasing lairage times at different time points and key process periods.

Group	L0hr	L24hr	L48hr	L48hr *ad lib.*
Loading weight (kg)	104.9 ± 2.59	103.7 ± 2.39	102.9 ± 3.65	106.8 ± 1.79
Dead carcass weight (kg)	100.5^{a} ± 3.72	97.9^{a} ± 3.68	95.3^{a} ± 4.21	101.0^{a} ± 3.02
Carcass weight (kg)	49.2^{a} ± 1.67	47.5^{a} ± 1.65	45.9^{a} ± 1.89	48.5^{a} ± 1.36
Dressing percentage as function of loading weight	49.0 ^{a} ± 0.48	47.7 ^{a} ± 0.47	46.61 ^{b} ± 0.67	47.22 ^{a} ± 0.65
Dressing percentage as function of dead weight	49.1^{a} ± 0. 90	48. 6^{a} ± 0.01	48.4^{a} ± 0.01	48.1^{a} ± 0.01
Differences in weight (kg) : Time point 1	4.0 ^{a} ± 0.30	0.58 ^{b} ± 0.29	0.74 ^{b} ± 0.42	1.63 ^{b} ± 0.42
Differences in weight (kg): Time point 4			1.56 ^{a} ± 1.56	-1.15 ^{b} ± 2.45

^{a,b} LSMeans within rows with different letters differ at P < *0.05*

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Muscle pH

All birds from this investigation, irrespective of their lairage time, had pH_u levels higher than those of ostriches not transported, as Wolmarans (2011) recorded an average mean pH of 5.77 ± 0.053 for these birds. Only 2 out of the 91 ostriches had pH_u levels lower than 5.8 for all lairage times (Table 4.6). Group L0hr, L48hr and L48hr *ad lib.* had lower pH_u range percentages with ~80% between 5.8 and 6.2. Group L24hr had the highest range with ~73% being higher than 6.2 which can be classified as DFD. Groups L48hr and L48hr *ad lib.* had 60% and 75% birds, respectively in the 5.8~5.99 pH range which can be classified as the standard pH_u range for transported ostriches.

Table 4.6: End pH tabulated and grouped according to levels and trial groups described as percentage of N.

pH category	Time Group								n=91	Total percentage
	L0hr		L24hr		L48hr		L48hr *ad lib*			
	n=29	%	n=31	%	n=15	%	n=16	%		
pH > 6.2	4	13.4	13.	41.9	3	20.0	2	12.5	22	24.2
pH 6 - 6.2	13	44.8	10	32.3	3	20.0	2	12.5	28	30.8
pH 5.8 - 5.99	12	41.4	7	22.3	9	60.0	12	75.0	40	43.9
pH < 5.8	0	0.0	1	3.2	0	0.0	0	0.0	1	1.1

Transport distance and length has been shown to affect live weight losses and meat quality (Wolmarans, 2011). These losses were caused by transport but without a rest period after transport which could repair or limit the losses incurred. Taking this into account, the lairage trial all birds transported on the same route, and by the same transport company, day and density in attempt to limited these factors.

Quality parameters

No differences were found between groups for any specific day's shelf life using Fishers least square means test. Table 4.7 depicts the quality parameters for any specified day's shelf life as all lairage groups showed no differences. Changes in fresh meat parameters such as pH, drip loss and colour

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measurements were noted and as expected to change with deterioration of meat quality due to the aging process (Lawrie 1998). A postulation could be made that birds either don't experience stress to an extent that it would affect meat quality or were all exposed to the same level of stress. pHu means were also noted to be non-significant between the lairage groups. This, combined with the low previously mentioned R square values across all parameters, indicates that further investigation needs to be conducted into the associations between parameters. A PCA plot was then drawn to identify possible associations between the various parameters and main effects tested and measured.

Table 4.7: Means (± se) of quality parameters groups according to time after slaughter with accompanying standard error.

Time post mortem (days)	pH	Drip loss (%)	Cooking loss (%)	Shear force (N)	L*	a*	b*	N
4	6.02±0.02	0.55±0.04	35.92±0.24	45.69±0.88	33.2±0.16	20.91±0.15	12.22±0.10	1362
6	6.09±0.02	1.35±0.05	37.61±0.31	43.87±0.82	33.01±0.18	18.22±0.12	11.77±0.09	1365
8	6.07±0.02	2.36±0.07	36.49±0.37	40.24±0.80	32.47±0.17	18.22±0.20	10.87±0.09	1365
10	6.1±0.01	4.26±0.28	34.34±0.47	37.91±0.65	31.98±0.20	18.22±0.19	10.75±0.10	1365

Both the PCA and DA results of the different lairage groups, ignoring the time after slaughter, indicated no significant differences between lairage groups (Figures 4.2 and 4.3). The DA plot (Figure 4.2) was less explanatory and showed less information than the PCA plot (Figure 4.3).

The PCA plot was therefore used for statistical inference; 66.2% of the variation was explained by the first two Principle Components, F1 and F2, which would to a certain degree accurately indicate any association between the groups. Group L24hr was noted to be different in relation to grouping and association but not significantly ($P>0.05$) so, and also became less associated with the factors that are connected with fresh meat with increase in time as seen in Figure 4.2. This was also noted for all lairage groups. The relative position of group L24hr's centroids in the PCA could then be attributed to the relative grouping of its pH_u in comparison to the other groups.

pH_u was noted to decrease in association with group centroids over time and had a strong negative association with both shear force, drip loss and the L* values. This corresponds with literature (Lawrie, 1998), where increased pH levels corresponds with lower oxymyoglobin levels. pH changes are associated with the texture change that decreases the intra-fibre space, decreasing the amount of light reflected. The increase of myoglobin concentration experienced in wild animals and

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ostriches also influence the levels of light absorbed by the meat, thereby influencing the colour values. This explains the association between the centroids for these subsequent factors. The negative association between shear force and pH could be attributed to the inhibition of calpastatin at a high pH (Lawrie, 1998). This can also explain the association of pH with cooking loss percentage (Figure 4.3) which is subsequently affected by the loss of texture caused by the proteolysis of the calpain system, lowering the water holding capacity of the meat during cooking.

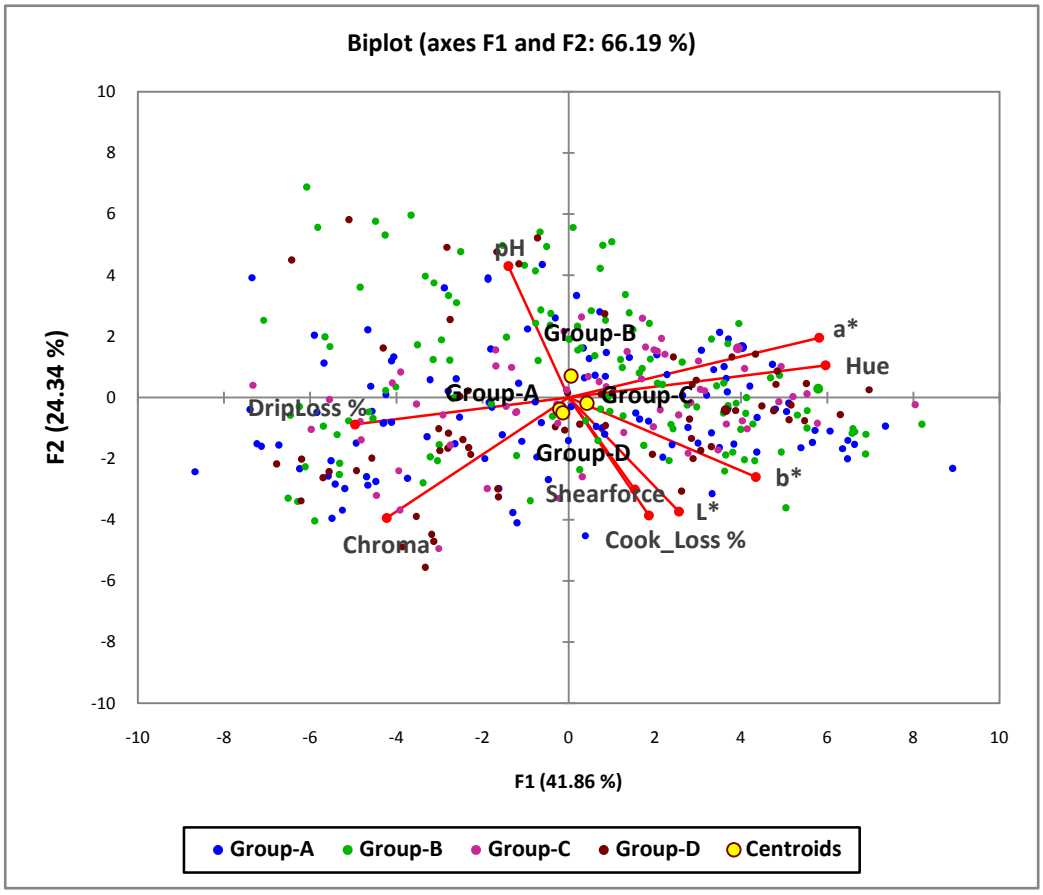

Figure 4.2: Bi-plot depicting different transport densities and meat quality parameters' associations – Group A - L0Hr ; Group B -L24hr; Group C -L48hr and Group D -L48hr *ad libitum*

The meat quality parameter relations changed with the decline in meat freshness (time *post mortem*) and not between lairage groups. Fresh meat is more closely associated with Chroma, a* values, and lower drip loss levels as seen in the comparison of the centroids on the F1 axes, when considering the time (shelf life) period. The in-group differences in centroid association with the meat quality parameters, when evaluating the shelf life, are shown on the F1 axes, This indicates a noted

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change in meat parameters with time progression, specifically between day 6 and 8 (Figure 4.3). This difference in association between in-group centroids for these time points could indicate that the shelf life changed the meat quality to levels that could be considered unacceptable, as seen for example with the higher drip loss on day 10 (Table 4.7).

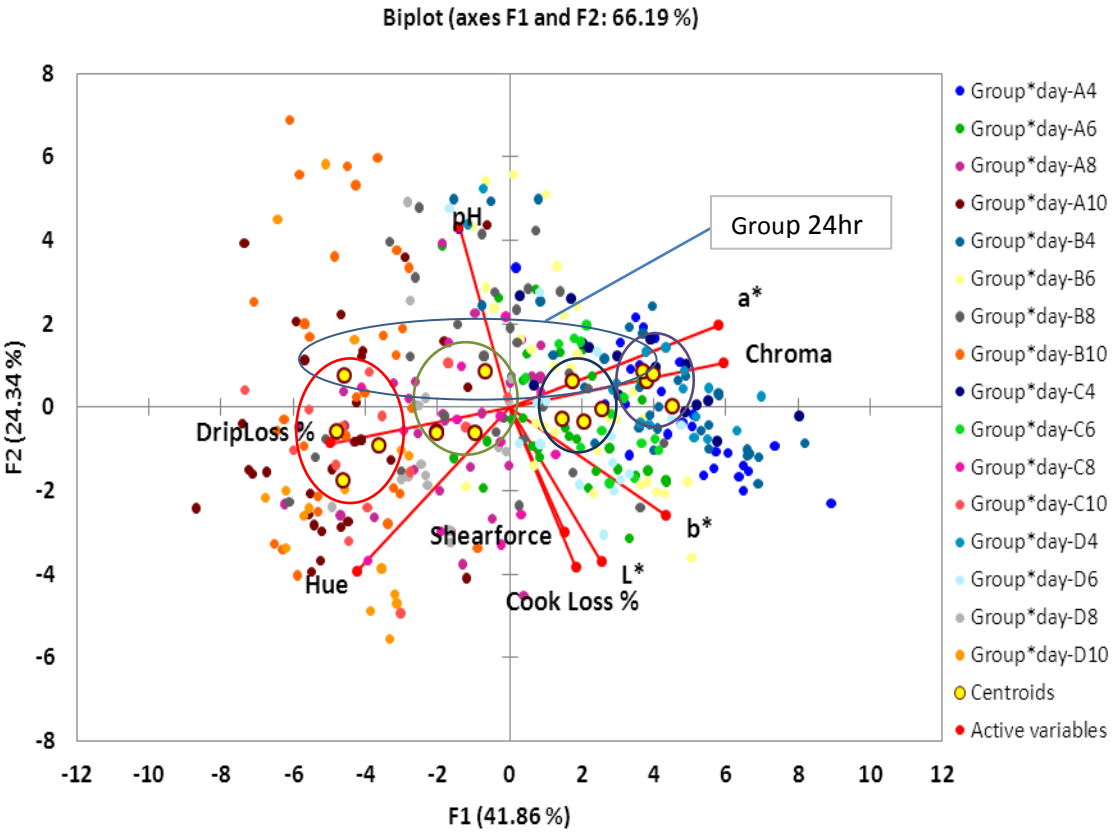

Figure 4.3: Biplot depicting Group*Day centroids for quality parameters shelf life test Group A -L0Hr ; Group B -L24hr; Group C -48hr and Group D -L48hr *ad libitum*

Group centroid location over time also indicated a possible inflection point between days 6 and 8 for fresh meat, as previously stated. The values associated with a decrease in shelf life supports this postulation (Figure 4.3). Hue increases and drip loss percentage and pH association decreased respectively, shown on the F1 and F2 axis for the time progression. This is indicative of changes expected in meat with the progression of time as pertaining to microbial and texture changes (Borch, 1996).

The decrease in colour acceptability also increased with time as indicated by the association of hue increasing with a comparable decrease in Chroma. This is supported by the strength of

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association between hue and Chroma parameters on the F1 axis shown in the PCA plot. The increased b* and L* association could be indicative of the systematic change in colour values. The association between shear force, b* and L*, are strongly localised in the same quadrant. This association could be because factors such as pH, myoglobin content and WBC are all factors influencing these colour factors and are also known to affect shear force (Lawrie 1998).

The Group*day centroids are displayed on the PCA plot (Figure 4.2). The Group centroids for each day were relatively weak in their association with one another. This increased over time. This change was noted for all attributes. Of the different lairage groups, group L48hr exhibited the least amount of association shift on both axes, but no significant differences when analysed existed between the groups.

Conclusion

The results indicate that no significant differences in ostrich meat quality were observed between different transport densities for muscle quality parameters with the PCA plot indicating no associations. This would indicate that meat quality parameters are relativity unaffected by the transport densities tested in this investigation.

Lairage time was found to be a significant factor in the percentages losses calculated. Dressing percentages were also significant in relation to the weight taken at the first time point seen in Table 4.1. Results show that with a time period of 48hr lairage, weight losses and dressing out (yield based on initial live weight) differences of significance were found indicating that with increased time, the weight losses as percentages of the initial live weights, increases. Feed availability *ad libitum* was also found to minimise the dressing percentages loss for 48hr lairage: the group L48hr *ad lib.*, was not significantly different from L0hr or L24hr. Therefore, the data supports the postulation that feed availability does minimise weight losses and dressing percentages as calculated from live weight but no significant difference between lairage time and dead weight, and dressing percentage was found.

Quality parameter data, seen during the analysis of the PCA and DA plots, indicate the expected trend of shelf life change over time. This did not affect the relative close localisation of the centroids for either trial which would seem to indicate that there were no significant differences in association between groups. Group 24hr of Trial 1 was noted to stand slightly apart from the rest of the groups with the change in shelf life, although not statistically significant. This was attributed to the

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fact that its pH_u change was relatively stable over time. This does not indicate a specific trend in meat quality as the groups still had no significant differences between them. This could however indicate that group L24hr were chronically stressed but only further research into the subject can substantiate this postulation. Further substance is given to this postulation when considering percentage groups of groups L24hr and L48hr were found in the high pH range which could possibly be considered DFD. In comparison between L48hr and L48hr *ad lib*., the later was found have significantly fewer animals in the higher tier pH grouping than L48hr, indicating feed availability did help repair glycogen levels.

The availability of feed during the trial was found to be an effective method in combating the weight losses experienced for the period of this trial but no differences were noted in the expected stress induced factors concerning meat quality. Time in lairage also affects percentage losses in ostriches but only significantly after 48hr. Feed removal in lairage is not a contributing factor to weight losses for period <24hr. However, it did effect weight losses and dressing percentages for periods >24hr. No meat quality parameters were affected by transport density or lairage conditions or time. Current guidelines and responsibility for feed costs in lairage might need further investigation.

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Chapter 5

Conclusions

Standard ostrich behaviour descriptions are scarce due to the relative youth of commercial ostrich domestication. Even more so, the behaviour of birds during transport has not been described in the literature. Ostriches were noted to prefer leaning against objects to maintain their balance and found to favour the sides of the compartments in this regard. Orientation of ostriches during travel to counteract balancing issues was also noted. Further reactions to sound fluctuations were noted in both trials and some behavioural anomalies such as ducking their heads in an avoidance manner were recorded. This might be incorrectly classified as a reaction to light intensity changes when going under a bridge, rather than a reaction to sound changes. Analyses of bird reactions to sound stimuli indicate further research should be conducted with the focus on noise during transport and in lairage. Transport density was investigated and no differences between groups were found when considering weight losses or meat quality parameters. Carcass bruising was also found to be non-significant for the different loading density groups, with an exception in the low density group of 1 m^2/bird where the bruising was significantly higher than that of groups with a loading density of 0.56 m^2/bird. However, this bruising was limited to the neck region whilst the drum and chest bruising weights were similar and did not differ significant between the groups. Also, the total bruising weight removed did not differ significantly between the groups. The current guidelines for ostrich transport specifying density requirements of a minimum space of 0.5 m^2/bird and a decrease in density did not seem to affect any of the parameters tested.

In the lairage trial investigation the time spent in lairage and feed availability during this period were found to be an influencing factor on the various meat quality parameters tested. Differences were found in live weights between feeding and not feeding the birds during the lairage period. Live weight losses were also found to increase with lairage time which influenced the carcass weight percentages when calculated as a percentage of the bird weight upon arrival. Time in lairage had no effect on the meat quality. Although lairage had a stress effect on the birds, an evaluation of the pH groupings seemed to indicate that lairage time only had a minimal effect on the overall quality of the

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meat. The current guidelines are therefore effective in producing good meat quality in ostriches. However, live weight losses are affected by time in lairage which is of concern to both primary and secondary producers. The lack of consideration pertaining to sound in welfare regulations in both lairage and transport is also of concern and warrants further research.