EVALUATION OF REJECTED WET CARCASS SYNDROME LAMB MEAT FOR HUMAN OR ANIMAL CONSUMPTION

By

MELISSA HATTING

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Department of Sustainable Food Systems and Development

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Bloemfontein

Supervisor: Prof. A. Hugo

Co-Supervisor: Prof. C.J. Hugo

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DECLARATION

I, Melissa Hatting, declare that this thesis for the Master of Science in Agriculture majoring in Food Science at the University of the Free State titled “Evaluation of rejected Wet Carcass Syndrome lamb meat for human or animal consumption” is my own work, has not been submitted before for any degree or examination at any other university, and that all sources I have used or quoted have been indicated and acknowledged by complete references. The language, formatting and reference style of this thesis are in accordance with the requirements for the Meat Science journal.

Signed 

Melissa Hatting

Student number: 2016002772

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GLOSSARY OF ABBREVIATIONS

a^*	Redness
ABP	Animal By-Product
A:G	Albumin:Globulin
AI	Atherogenicity Index
AOAC	Association of Official Analytical Chemists
b^*	Yellowness
CIE	Commission International on Illumination
CO_2	Carbon dioxide
CP	Crude protein
DM	Dry matter
DNA	Deoxyribonucleic acid
EPA	Eicosapentaenoic acid
FAME	Fatty acid-methyl esters
g	Gram
kg	Kilogram
KOH	Potassium hydroxide
L^*	Lightness
MAP	Modified atmosphere packaging
MUFA	Monounsaturated fatty acids
N	Newton
N_2	Nitrogen
n-3	Total omega-3 fatty acids
n-6	Total omega-6 fatty acids
n-6/n-3	Total omega-6 fatty acids (n-6)/Total omega-3 fatty acids
OM	Organic matter
PA	Pyrrolizidine alkaloids
PSE	Pale, soft, exudative
PUFA	Polyunsaturated fatty acids
PUFA:SFA	Polyunsaturated fatty acids: Saturated fatty acids
PUFA:MUFA	Polyunsaturated fatty acids: Monounsaturated fatty acids
SA DAFF	South African Department of Agriculture, Forestry and Fisheries
SF	Shear force
SFA	Saturated fatty acid
REA	Ribeye area

WCS	Wet Carcass Syndrome

ABSTRACT

Wet carcass syndrome (WCS) is a condition found among sheep and is characterised by a 'wet' appearance of the subsurface meat at slaughtering. These carcasses are considered unfit for further use, resulting in financial loss to farmers and the industry as a whole. No preventions or cause have been determined for this syndrome, although WCS seems to be associated with winter/drought conditions. The current study compared twenty WCS carcasses to twenty unaffected normal carcasses to determine the quality and safety of the affected meat. Parameters tested included fat thickness, water holding capacity, colour, proximate analysis, fatty acid composition, water activity, pH and microbial load. A shelf-life study was performed on two products, *i.e*., lamb chops for human consumption and pet mince for the pet food industry.

From morpho-physical examinations, WCS carcasses had measurements with significantly higher cold mass, external length, shoulder and buttock circumference than the normal carcasses. No significant differences were, however, found in the fatness and conformation code, showing that WCS-affected carcasses were physically larger, but retained the same level of fat around the outside of the carcass. Measurements taken between the 12^{th} and 13^{th} rib, showed normal carcasses had significantly higher fat thickness (45 mm and 110 mm), while WCS carcasses had significantly higher eye muscle width, depth, area and perimeter. The pH, temperature, water activity and water holding capacity had no significant differences. Proximate analysis included dry matter, moisture, protein, fat, organic matter and ash. All of these parameters showed higher levels in the normal vs WCS carcasses, except for moisture content which was higher in WCS vs normal carcasses. Nine of the fatty acids showed significant differences, where all but one, arachidic acid, had higher levels in the WCS, than in normal carcasses. From surface swabs, there were no differences in the number of microbes recovered from the surface of the WCS carcasses versus normal carcasses.

Two products were formulated, lamb chops for human consumption and pet mince for the pet food industry. A shelf-life study (microbial load, colour and pH) determined the quality over a period of six days for lamb chops and 10 days for pet mince. The microbial load and pH on both products showed no significant differences between the WCS and normal carcasses, suggesting that WCS meat had the same microbial quality and was just as safe as that from a normal carcass. The colour analysis of the lamb chops showed no differences in the lightness nor the redness, although there was a difference in the blue/yellow colour on day 0, implying a greenish tint involved with WCS. This phenomenon, however, was only seen on day 0 and not throughout the 6-day experimental period.

The study confirmed the safety of WCS meat for both human and animal consumption, although the wet surface appearance, soft texture and loose subcutaneous fat, is expected to still deter consumers from

purchasing such meat. Processing of WCS meat into products like pet mince, will support new commercial ventures and lessen the financial impact of the condition on the livestock industry.

Keywords: Wet Carcass Syndrome, sheep, quality, human consumption, pet consumption

Chapter 1 Introduction

Wet carcass syndrome (WCS) is a condition found mostly among sheep, *Ovis aries* (Order: *Artiodactyla*, Family: *Bovidae*), and can be described as a build-up of fluid under the skin, which leads to an unaesthetic appearance of the carcass meat. During the pre-slaughter process, the animal seems to be clinically stable and shows no signs of a defect. The carcass, however, appears to be "wet" after the skin is removed during the slaughter process (Van der Westhuizen et al., 2019).

The condition is mainly observed in South Africa's Northern Cape Province and Namibia's southern region. The cases also seem to increase during the autumn and winter seasons (Van der Westhuizen et al., 2019). Although many hypotheses have been tabled to explain the cause of WCS, there has been no definite cause confirmed. Possible causative factors associated with WCS, include overhydration (Joubert et al., 1985), stress during transport (Brock et al., 1983), allergies (Hattingh et al., 1983), washing of carcasses under high pressure (Brock et al., 1983), provision of salt licks (Joubert et al., 2012), weather conditions (low rainfall) linked to geographical location (Brock et al., 1983; Jansen & Pretorius, 1986) and diet-related factors (Joubert et al., 1985). The most recent study involved a genetic approach, where two deoxyribonucleic acid (DNA) markers positioned on the X chromosome of affected male animals, were most likely associated with WCS and warrants further investigation (Van der Westhuizen et al., 2019).

According to red meat safety regulations, governed by the South African Department of Agriculture, Forestry and Fisheries (SA DAFF) under the Meat Safety Act No. 40 of 2000 (SA DAFF, 2000), meat with an abnormal condition is ‘unsafe for human and animal consumption’. This results in the burning of the affected carcass without any experimental tests conducted. Given the non-processing of WCS carcasses, farmers and the meat industry as a whole, suffer significant financial losses. During the period 21 - 27 November 2022, the average selling price of lamb meat (Class A2) was R96.63/kg and the average carcass mass was 31.05 kg (Red Meat Industry Forum, 2022). These figures relate to a loss of more than R2 000 per affected sheep carcass.

A similar condition to WCS can be found in pork, referred to as pale, soft, and exudative (PSE) meat. PSE meat, however, may be sold and is accepted by the consumer, unlike WCS meat (MacLennan & Phillips, 1992). This infers a possibility that WCS meat can be utilized for human or animal consumption, leading to less financial losses to farmers and the meat industry. It may also lead to potentially new industries like pet food, fertilizers, etc.

Due to the abnormal physical appearance and possible shortened shelf-life, carcasses affected by this condition are generally not accepted for further processing. The most plausible explanation for the alleged shortened shelf-life is that the surface of moistened meat is an ideal environment for microorganisms to grow, thus compromising the quality and safety thereof (Van der Westhuizen et al., 2019). However, no microbial studies and limited physio-chemical (pH, water activity, lipid stability, colour) studies have been performed on WCS carcass cuts or WCS products to determine the quality and safety of the meat.

The aim of this study was to determine whether the meat from WCS carcasses may be used for human and/or animal consumption.

The objectives of the study were to:

- Compare the carcass characteristics, functional properties and microflora from normal versus WCS-affected lamb meat carcasses.

- Compare the microbiological shelf life, colour shelf life and lipid stability of lamb loin chops from normal versus WCS-affected carcasses, during refrigerated display.

- Compare the microbiological shelf life of minced meat as a pet food, manufactured from normal versus WCS-affected meat, during refrigerated storage.

Null hypothesis

- It is to be expected that a large difference in the microbial, functional and chemical properties of normal vs wet carcass syndrome meat be observed. With the expectation that WCS meat quality should be inferior to normal meat quality.

Chapter 2 Literature Review

2.1. Introduction

Similar to other livestock, sheep are prone to various conditions and diseases, which have an effect on the quality of the meat. Wet carcass syndrome (WCS) is a condition found primarily in sheep, which affects the appearance of their carcasses. It can be identified by the accumulation of a watery fluid under the skin, which is only visible after removal thereof. The meat is rendered inedible and destroyed, which imply financial losses to the producer (Van der Westhuizen et al., 2019). A similar condition to WCS can be found in pork, referred to as pale, soft, and exudative (PSE) meat. PSE is thought to be a stress related condition (MacLennan & Phillips 1992), where the meat has a light grey colour, soft texture and low water-holding capacity. Thus, moisture loss from between the muscles can be observed; this is similar to WCS, but PSE is accepted by consumers.

The aims of this literature review are to provide some history and background to what WCS is, the factors that may cause WCS, the influence of WCS on meat quality (especially on the microbial and colour characteristics), the prevention of WCS, safety and legislative aspects and possible uses of WCS meat.

2.2. History and Background

Wet carcass syndrome is a condition found primarily in sheep, which has a detrimental effect on the appearance of their carcasses. The animal seems to be clinically healthy during the pre-slaughter phase and displays no signs of a defect. However, the carcass tends to be “wet” following the removal of the skin during the slaughter process (Fig. 2.1; Van der Westhuizen et al., 2019), with subcutaneous accumulation of watery fluid (Brock et al., 1983). Areas on the carcass that are most affected, are the brisket, flanks, hindquarters, sides and back (Brock et al., 1983; Hattingh et al., 1983). Watery fluid is also found in the intramuscular connective tissue layers of both the flank and subscapular area. Affected carcasses do not dry off with overnight cooling (Joubert et al., 1985).

According to Voster (2019), WCS may not show up directly after slaughter but can manifest over time; thus, the carcass can be loaded, which is normal immediately after slaughter, but can be wet at the point of arrival. With good ventilation during the drying off stage, the carcasses can be dried off, but the carcasses still have a dull appearance. The groin has a green shine and a glassy appearance on the back of the shoulder, which makes it less aesthetic. The fluid appears to come from the connective tissue, especially the fatty tissue (Voster, 2019).

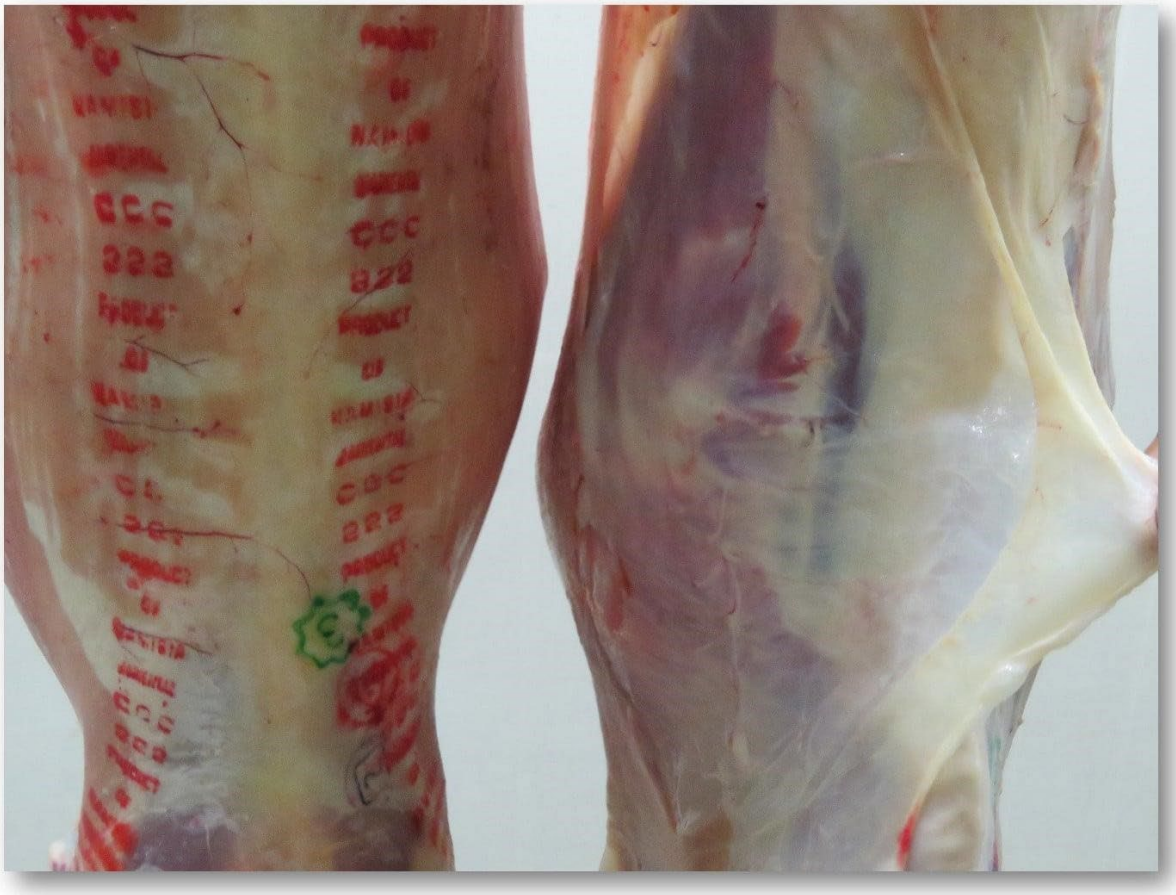


Fig. 2.1. Difference between a normal (left) and a wet carcass syndrome carcass (right) where the adipose tissue can be pulled away from the carcass (Van der Westhuizen et al., 2019).

The wet carcass condition occurs in all breeds of sheep, although the condition was first observed in the Dorper breed and Dorper crosses; possibly because this breed is more frequently slaughtered at a younger age (Van der Westhuizen et al., 2019). Unofficial WCS slaughter figures show that some abattoirs have higher numbers of WCS carcasses, whereas other abattoirs in the same area have no reported incidences. The condition is observed to be prevalent in areas where the level of grazing is low, despite a high quantity available to animals. Wet carcass syndrome is typically observed in the autumn and winter, particularly after droughts or after cycles of rainfall in the spring, followed by reduced rainfall throughout the rest of the summer (Van der Westhuizen et al., 2019). A similar 'winter trend' is reported for WCS among cattle, although less common (Webb et al., 2020).

Wet carcass syndrome carcasses are generally not admitted for further processing, because of the abnormal physical appearance and possible shortened meat shelf-life. The most logical reason for a suspected shortened shelf-life is that the moistened meat surface is a suitable habitat for the growth of microorganisms. Furthermore, there is an occupational hazard involved with the cutting of moist carcasses, as a band saw draws further into the meat and may result in operator injuries (Van der Westhuizen et al., 2019).

The first incidence of WCS was recorded in 1981 at an abattoir in Krugersdorp, Gauteng (Jansen, 1991). The number of WCS carcasses has been increasing at abattoirs since 1981 when it was first reported in the Annual Report of the South African Department of Agriculture. It was estimated that from 1981 to 1984, R1.5 million was lost due to WCS (Joubert et al., 1985). Recently, the financial loss was estimated at *ca*. R1 500 per affected carcass (Du Pisani, 2019). During 2010, it was estimated that the meat industry sustained financial losses of around R27 million as a result of the rejection of undesirable, slimy carcasses and the downgrading of trimmed carcasses (Van der Westhuizen et al., 2019). South Africa's Northern Cape Province and Namibia's southern region have been listed as geographic regions with a high WCS incidence.

Initial investigations did not explain the cause of the syndrome. Other early cases were identified at an abattoir in Port Elizabeth (Eastern Cape) as well as in Upington (Northern Cape) during a slaughter trial, where it was discovered that the syndrome prevalence increased when lick blocks were provided. The thought then emerged that the increased consumption of salt caused an excessive consumption of water and that this over-hydration could be responsible for WCS (Joubert et al., 2012).

2.3. Factors causing WCS

Various factors have been mentioned as possible causes, including over-hydration, stress during transport, allergies, compulsory dip, washing of carcasses under high pressure, condensation in cooling facilities and provision of feed blocks, which will be elaborated on in the next paragraphs.

Hattingh et al. (1983) conducted a study on WCS, where they investigated the composition of the plasma and interstitial fluid (fluid surrounding body cells) of the sheep. Their findings showed that the potassium levels of the plasma of unaffected live, unaffected slaughtered and affected slaughtered sheep, differed. Compared to living animals, potassium levels were substantially higher in slaughtered sheep, with or without WCS. The concentration of potassium in ovine wet carcass and regular slaughtered sheep was not significantly different. However, the interstitial fluid differed between the normal sheep and wet carcass sheep. The wet carcass fluid had a significantly higher potassium content and albumin-globulin (A:G) ratio, but had a lower protein content and colloid osmotic pressure than the unaffected sheep. These authors concluded that wet carcass syndrome is the result of an increase in capillary blood vessel permeability, combined with an increased mean capillary hydrostatic pressure. Histamine (nitrogenous compound involved in local immune response), or substances similar to histamine, can induce any of those changes. Therefore, it would seem that there is a moderate allergic mechanism involved and that anti-histamine treatment may be helpful.

Since the syndrome cannot be seen in the live animal, the analyses of pre-slaughter blood specimens were correlated with results from post-slaughter data. The total protein serum, A:G ratio and electrolyte levels of WCS meat were normal. Liver specimens showed normal levels of vitamin A but significantly higher levels of vitamin E (Brock et al., 1983), an essential vitamin in maintaining the integrity of cell membranes (Bowen, 2020). Moreover, the interconnected tissue fluid showed less protein and more albumin than that of unaffected sheep. These results supported the findings of Hattingh et al. (1983). Although no data were presented, Brock et al. (1983) mentioned the following as improbable causes: washing the carcasses with too high water pressure, incorrect cooling, feed available at the abattoir and the possibility of stress during transport.

Shortly after the publications by Hattingh et al. (1983) and Brock et al. (1983), a study was published by Joubert et al. (1985), in which the results showed that WCS was linked to dehydration followed by over-hydration (Table 2.1). The condition was exacerbated (+28%) when animals had access to a feeding lick before slaughter. The study stated that the wet and glassy appearance was due to macroscopic changes in the connective tissue of subcutaneous-, subscapular- and intermuscular areas because these areas expand easily and therefore, may become ‘overfilled’ (Fig. 2.2a and b). The authors concluded that the cause of WCS is over-hydration, a notion supported in the Annual Report of the Directorate Veterinary Services (SA DAFF, 1984). Hyper-hydration, over-hydration or water intoxication, affects brain function when excessive water consumption forces the normal electrolyte balance in the body outside safe limits (https://en.wikipedia.org/wiki/Water_intoxication, 2023). In contrast, the study by Brock et al. (1983) found the electrolyte levels of affected carcasses to be normal.

Joubert et al. (1985) conducted a similar experiment on a farm near Askham, Northern Cape (over a period of six weeks), where 300 sheep were divided into two flocks. These two flocks, each containing 150 sheep, were placed in adjoining camps; the one flock was provided feed and pasture for feeding, while the other only had pasture. At the end of the six-week period, half of each group (75) were taken to an abattoir where salt licks were made available. The other group was taken to an abattoir where only hay was available. Table 2.2 shows the results from that experiment, which clearly indicates that the salt licks were the cause of WCS.

Table 2.1

Effects of water deprivation followed by over hydration and salt supplementation on ovine carcasses (adapted from Joubert et al., 1985).

Groups	Feed	Water	Licks	No. of sheep per group	Appearance of carcass			
					Immediately after slaughter		After 18 h in cool room	
					%Dry	%Wet	%Dry	%Wet
Control	Milled Lucerne hay	Free access	None	20	100	0	100	0
A	Milled Lucerne hay	Deprived for 52 h, then *ad lib* for last 18 h before slaughter	*Ad lib* for last 18 h before slaughter	25	0	100	20	80
B	Milled Lucerne hay	Deprived for 25 h, then *ad lib* for last 18 h before slaughter	None	25	28	72	52	48

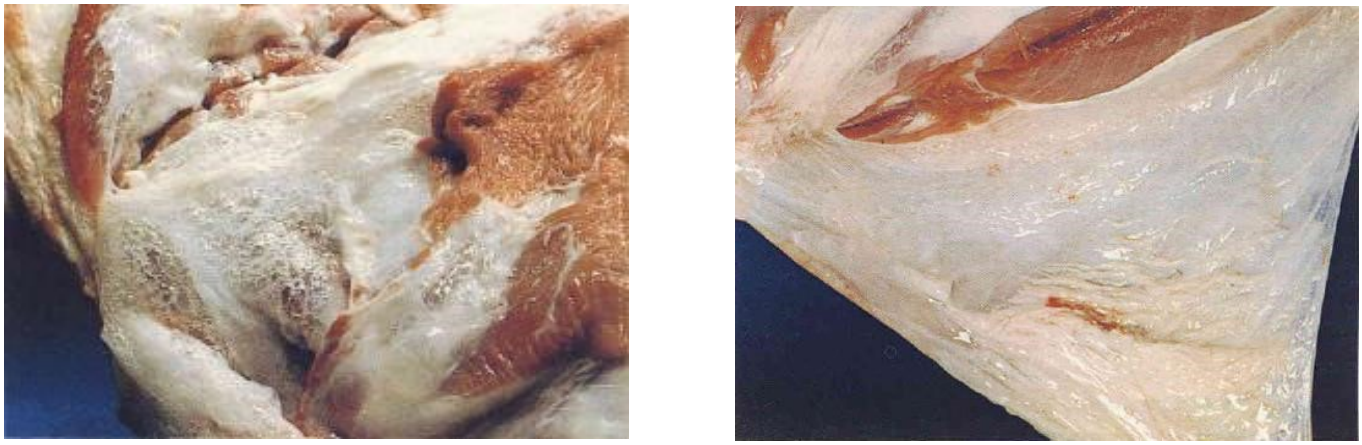


Fig. 2.2. (a) Wet carcass with intermuscular connective tissue layers displayed. (b) Hind leg with accumulation of watery fluid in subcutaneous tissues (Joubert et al., 1985).

Table 2.2

Relationship between salt licks and hay on WCS incidences (Joubert et al, 1985).

Results	**Salt licks- abattoir**		**Hay- abattoir**	
	Feed	Pasture	Feed	Pasture
Total sheep	75	75	75	75
% WCS	1.30%	12%	0%	0%

After the above-mentioned biochemical investigations, Jansen and Pretorius (1986) investigated a possible correlation between WCS and geographic distribution. The authors concluded that certain districts in the Northern Cape (Gordonia, Kuruman, Postmasburg, Hay and Prieska) were problem areas, and constitute mainly the grazing areas associated with the Orange-, Kuruman- and Molopo-rivers. According to their results, it seems as if there was a relationship between WCS and pasture composition and grazing condition. According to Brock et al. (1983), Gordonia and surrounding areas had the highest prevalence, while the South-Western area of the Free State suffers only sporadic incidences.

The condition persists in the Northern Cape, as a recent article by Du Pisani (2019) reported a spike in WCS cases in the Kalahari, northwest of Upington and southern Namibia during 2018. This correlates to the areas with the highest number of cases in the early to mid-1980s (Brock et al., 1983; Jansen & Pretorius, 1986), as depicted in Fig. 2.3.

Fig. 2.3. Locations where WCS are most prevalent (Van der Westhuizen et al., 2019).

Notably, this region is characterised by low annual rainfall and hot summers. Seasons with below average rainfall occur naturally, and during such years, grazing quality may be compromised. According to Voster (2019), WCS is more prevalent during drought, which may explain the spike in cases during 2018 in the Kalahari, northwest of Upington and southern Namibia. During that season, annual rainfall in the Northern Cape was <75% of the normal (Fig. 2.4; South African Weather Service, 2023). Under low rainfall conditions, borehole water becomes more concentrated and therefore saltier. As soon as the animal moves from a high-salt water source (brackish water) to a low-salt water source, the animal drinks more than what is needed (Voster, 2019). This is underscored by the study of Joubert et al. (1985), where the authors concluded that WCS is caused by over-hydration after a dehydration period. Moreover, the ingestion of feeds with a high salt content during rehydration, may worsen the condition.

According to Table 2.3, the handling and method of farming have an impact on the percentage of wet carcass incidences. Farmers who farmed ‘extensively’ or ‘relatively extensively’ reported 100% WCS, while those in the ‘average’ category reported 57.1% WCS. Farmers who farmed ‘relatively intensive’, only reported 26.7% WCS and those who famed ‘intensively’, reported no WCS. This showed that farmers who were committed to their farms and managed an intensive agri-business, had fewer incidences of WCS (Jansen & Pretorius, 1988).

Table 2.3

The relationship between farming intensity and WCS incidence (Jansen & Pretorius, 1988).

Input level	Wet carcass cases (%)	No wet carcass cases (%)
Extensive	100	0
Relatively extensive	100	0
Average	57.1	42.9
Relatively intensive	26.7	73.3
Intensive	0	100

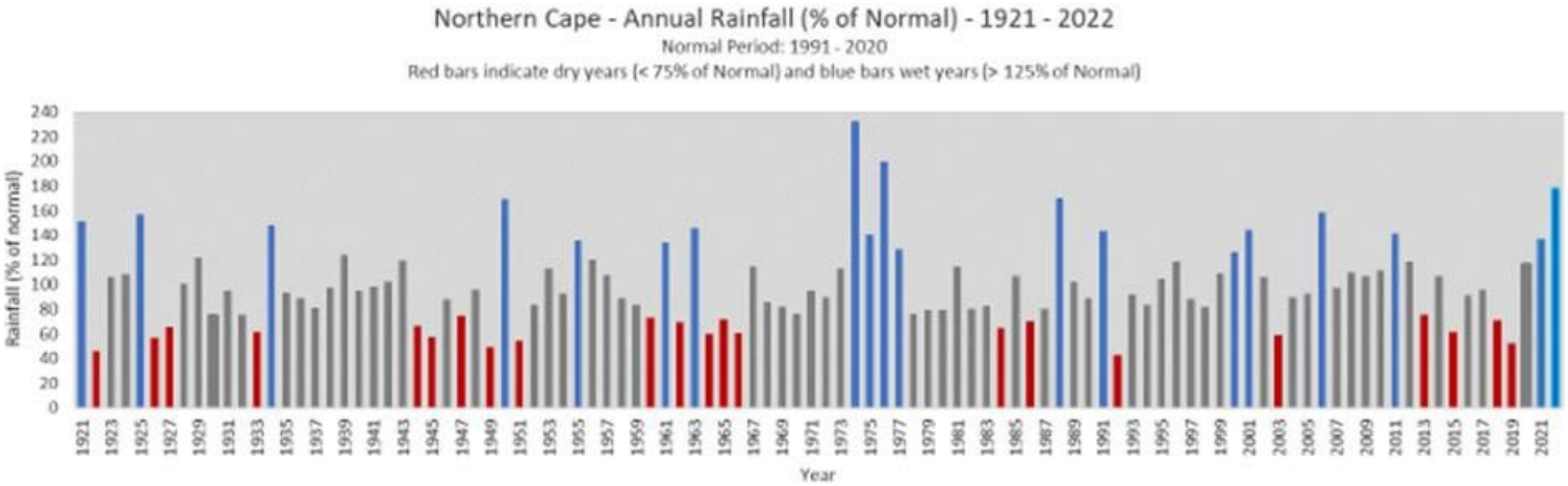


Fig. 2.4. Annual rainfall for the Northern Cape from 1921 to 2022 (South African Weather Service, 2023).

The geographical link to WCS, noted by Jansen and Pretorius (1986), may also involve a phytochemical component, *i.e*., within a dietary context. A study by Visser (2017) found notable differences in the nutritive value of two major veld types used for sheep-grazing in the Northern Cape (Grassveld versus Ranteveld). One of the thirteen parameters measured, was ingestion of crude protein; found to be low during winter, especially on Grassveld. Brock et al. (1983) reported *ca*. 80% lower total protein in the interconnective tissue fluid of WCS sheep compared to normal carcasses.

Another example of a chemical component in plants, consumed by sheep during natural grazing, is pyrrolizidine alkaloids (PAs). This is a group of naturally produced alkaloids based on a pyrrolizidine structure, with more than 6 000 plants identified to contain these PAs. The plant families *Apiaceae, Apocynaceae, Asteraceae, Boraginaceae, Fabaceae, Lamiaceae, Orchidaceae* and *Urticaceae* are commonly associated with PAs (Moreira et al., 2018; Wiedenfeld, 2014). Sheep are allowed to graze freely on the veld, which implies the consumption of a variety of plants and flowers, including those in the *Plantaginaceae, Malvaceae, Asteraceae* and different deciduous trees and shrubs. Unsaturated PAs, are hepatotoxic and can cause damage to the liver of humans and animals when consumed (Wiedenfeld & Edgar, 2011). Interestingly, liver samples of WCS-sheep showed significantly higher than normal levels of vitamin E (Brock et al., 1983), a deficiency of which, on the other hand, may lead to White Muscle Disease (Tustin, 1959). As stated by Bowen (2020): “Once in the circulation, vitamin E is liberated from chylomicrons, and much is taken up by the liver, where it is repackaged into very low-density lipoproteins and secreted again into blood. Ultimately, vitamin E is transported in the blood bound to a variety of lipoproteins, from which tissues throughout the body take it up”.

Another food/plant-related aspect, is the inadvertent consumption of microorganisms and/or their toxins during feeding. Two well-known conditions are ergotism, caused by the fungus *Claviceps purpurea* (*Clavicipitaceae*), especially on rye and other grasses (Wegulo & Carlson, 2011); and lupinosis, caused by the fungus *Diaporthe toxica* (*Diaporthaceae*) (previously *Phomopsis leptostromiformis*) on lupins (Van Warmelo et al., 1971). Ergot causes vasoconstriction by direct action on the muscles of the arterioles, which first reduce blood flow and later, lead to complete immobility with terminal necrosis of extremities (Osweiler, 2014). In some cases, the organism shows no external signs or symptoms on the host plant, (asymptomatic), then termed endophytic. These microorganisms do not cause any harm to the host (Clay & Schardl, 2002), but live in symbioses with the host plant, acquiring nutrition and shelter (Thrower & Lewis, 1973). In exchange, some endophytes may increase host resistance by producing bioactive alkaloids (Breen, 1994; Clay, 1996; Clay & Schardl, 2002; Latch, 1993; Wilkinson et al., 2000), enhance tolerance to drought stress (Kane, 2011; West, 1994), and/or improve host competitive ability (Clay et al., 1993).

However, consumption of some endophytes by animals may cause disease (Cheeke, 1995). For example, gousiekte (‘quick disease’), is a fatal cardiotoxicosis of ruminants caused by acute heart failure within eight weeks of ingestion. The etiological agent is endophytic *Burkholderia* bacteria, carried in the leaves of (South African) plant species of the genera *Fadogia, Pavetta* and *Vangueria* (all belonging to *Rubiaceae*) (Verstraete et al., 2011).

Likewise, fungal endophytes of the genus *Epichloë*, especially in grasses, produce toxic alkaloids associated with diseases like “fescue toxicosis”, “ryegrass staggers”, “sleepy grass” and “drunken horse grass” in livestock (Guerre, 2015). Consumption of plants harbouring endophyte-produced toxins could have a similar hepatotoxic effect to that of the above-mentioned PAs and may warrant further investigation as factors causing WCS.

The most recent study regarding WCS entailed a genetic approach, using a high-density single nucleotide polymorphism (SNP) assay to search genetic loci, which may predispose the animal to this condition (Van der Westhuizen et al., 2019). Although it was possible to map the genetic loci linked to PSE and red, soft and exudative meat (RSE) using candidate genes, there was no consistency between the position of these loci and that of WCS in the ovine genome. There was also no relationship between SNP located within these genes and WCS. In addition, there was no link of the phenotype (visible WCS) with autosomal genetic markers. The study, however, showed clear positive results, that WCS is most likely associated with at least two DNA markers found on the Xchromosomes of affected male animals. Subsequently, these two DNA markers were further connected to two potential genes, *i.e.*, *DMD* (linked to cell membrane permeability) and *HTR2C* (known to regulate the calcium ion pump). It was hypothesized that under stress (release of the stress hormone cortisol), the calcium ion pump opens, allowing liquid to flow through the cell wall. At/after slaughter, the pump does not shut down, facilitating more liquid loss and the manifestation of subsequent WCS. Notably, rams have one X-chromosome while ewes have two, possibly explaining this disparity between male and female animals. Random X-inactivation (Ahn & Lee, 2008) in the somatic cell of the female animal, may contribute to the probable co-expression of heterozygous genotypes. The possibility that variation in pre-slaughter stress (associated with the release of cortisol) may have an effect on the occurrence of WCS, complicates the issue because the genotypes of certain unaffected or normal carcasses also had those markers (Van der Westhuizen et al., 2019). These two complicating issues necessitate further testing to establish a genetic basis for WCS, conclusively. The relevant research group has proposed a follow-up study with a larger animal sample size.

2.4. Prevention strategies for WCS

Various experiments will have to be performed to determine what can be done at abattoir kraals regarding feed supply. The supply of hay is troublesome, given that it creates sewer system blockages. At the moment, however, as shown in Table 2.4, the provision of dry hay instead of lick blocks at the abattoir, seems to be the only feasible solution to the problem, as water cannot be removed. The State Veterinarian at Upington suggested making salt licks available *ad libitum* for stock in the veld. A few farmers verified that his advice has effectively minimized the occurrence of WCS (Joubert et al., 2012). Nonetheless, those results will need to be scientifically confirmed.

It is thought that if animals are accustomed to salt licks, they do not consume large quantities of lick at the abattoir. Animals that consume large quantities of lick in a short time period, tend to drink more water. Rapidly hydrating animals may contribute to WCS (Joubert et al., 2012). These statements are supported by the results in Table 2.4. The number of wet carcass cases decreased as the sheep became more accustomed to licking blocks. After three days without water, group 2 had access to licks for only two days, with a WCS incidence of 80%, versus a mere 20% in group 4, which had access to licks for four days.

Table 2.4 suggests a partial remedy to the WCS problem in that the carcasses show some recovery when cooled. Irrespective of feeding history, cooling of wet carcasses for 18 h resulted in improvements ranging from 20 – 52%. However, the sheep that received licks, and were confirmed to be wet, were at the lower end (20% reduction), while non-lick carcasses were at 52% (Joubert et al., 1985). As stated by Voster (2019), it is possible to limit excess watery fluid with good ventilation during the drying-off stage, but the carcasses will still have a dull appearance.

2.5 Safety and legislative aspects of WCS

Food hygiene legislation implies that every food manufacturer has a duty to ensure food safety by implementing and recording good hygienic practices and food safety management procedures. In South Africa, red meat safety is regulated by the Department of Agriculture, Forestry and Fisheries (DAFF) under the Meat Safety Act No. 40 of 2000 (SA DAFF, 2000). This act states ‘to provide for measures to promote meat safety and the safety of animal products; to establish and maintain essential national standards in respect of abattoirs; to regulate the importation and exportation of meat; to establish meat safety schemes, and to provide for matters connected therewith’.

Wet carcass syndrome is most probably considered under *abnormal condition* and is, therefore, unsafe for human and animal consumption. By definition, ‘unsafe for human and animal

consumption' means unsafe for human and animal consumption by reason of a disease, an *abnormal condition*, putrefaction, decomposition, contamination or residues, or by reason of exposure to, or contact with, a disease or putrefied, decomposed or contaminated material. It should be noted that the above-mentioned Act serves to regulate the formal sector, but has little impact on the informal sector.

Since this study also wants to explore the possibility of using the WCS meat to produce new products, it should be kept in mind that all new food products that contain meat, are subject to the following Acts and Regulations (SA DAFF, 2012):

- Agricultural Product Standards Act, 1990 (Act No. 119 of 1990) ○ Government Notice No. R. 342 – Regulations regarding the classification and marking of meat
- Foodstuffs, Cosmetics and Disinfectants Act, 1972 (Act No. 54 of 1972)
- Meat Safety Act, 2000 (Act No. 40 of 2000)
- Health Act, 1977 (Act No. 63 of 1977) ○ Government Notice No. R198 of 30 July 1999 – Regulations governing general hygiene requirements for food premises and the transport of food

Table 2.4

Wet carcass incidence as affected by prolonged versus limited access to lick blocks (adapted from Joubert et al., 2012).

Dorpers	**First 6 days**			**Rehydration day (Day 1)**		**Thereafter**						**Wet carcasses (%)**
						Day 2		Day 3		Day 4		
Group	Water (l)	Lick (g)	3 days without water, feed or lick	Water (l)	Lick (g)	Water (l)	Lick (g)	Water (l)	Lick (g)	Water (l)	Lick (g)	
1	2.4	-		7.7	9.7							60
2	2.5	-		6.5	522	2.7	615					80
3	2.4	-		8.3	645	2.2	642	3.2	940			60
4	2.4	-		7.7	490	1.8	530	2.2	972	2.4	853	20

2.6. Influence of WCS on meat quality

From a commercial point of view, quality is characterised and determined by factors relating to consumer demand, rather than that required by the industry. Presence of spoilage microorganisms and pathogens, colour stability, pH, cooking losses, tenderness, water holding capacity, chemical composition and fatty acid composition, are quality checks that are important in order to meet scientific and supply chain requirements. A high-end quality product is also affected by intrinsic and extrinsic factors, such as the age of the animal, gender, physiological state, post-mortem biochemistry of muscle and fat, feed contribution to flavour, carcass composition, protein and fat levels, the effect of genetics on tissues and metabolism, pre- and post-slaughter handling and storage (De Lima Jùnior et al., 2016).

2.6.1. Carcass characteristics

The classification of lamb carcasses is done according to the age and fatness of the carcass. This allows consumers and industry to pick the product best suited to their need. According to the Agricultural Product Standards Act No. 119 of 1990, the number of incisors is used to describe the age class, *i.e.*, A = 0 teeth, AB = 1–2 teeth, B = 3–6 teeth and C = more than 6 teeth (Fig. 2.5). A fatness code (Fig. 2.6), ranging from 0 (= no fat) to 6 (= extremely fatty), is determined by visual appeal. These classifications are combined, e.g., A2 indicates subcutaneous fat of 5.6% to 8.5% or 1 mm to 4 mm fat cover over the loin (SA DAFF, 1990).

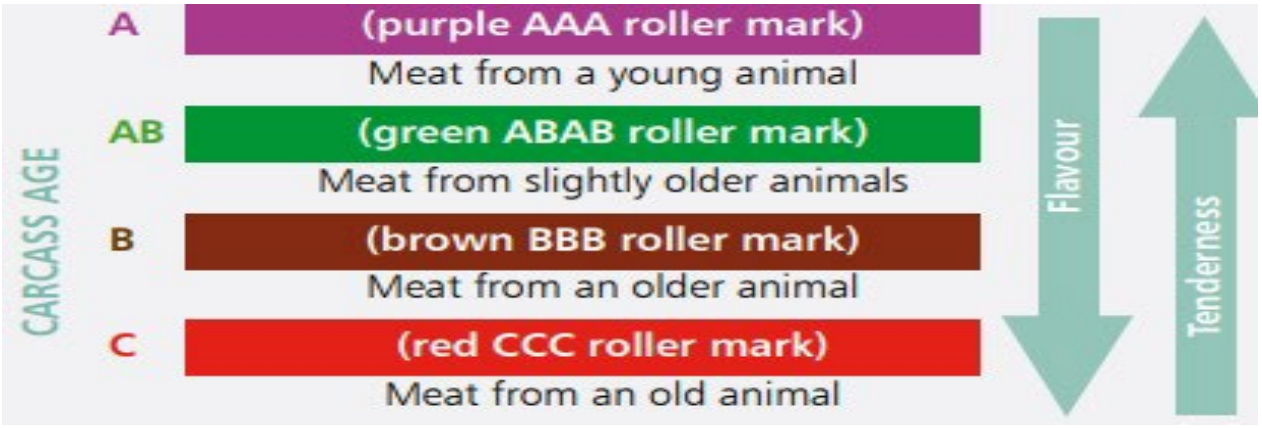


Fig. 2.5. Carcass age classification according to the Agricultural Product Standards Act, 1990 (SA DAFF, 1990).

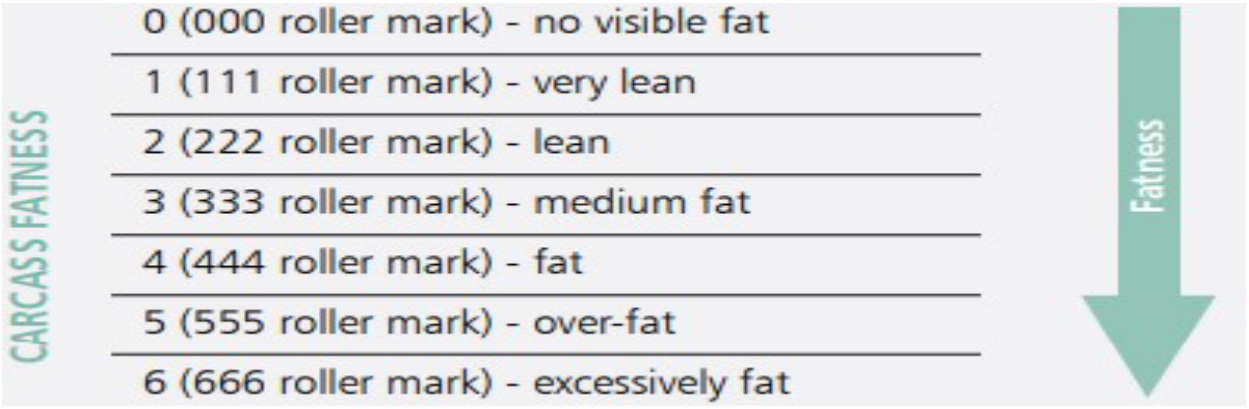


Fig. 2.6. Fatness code classification according to the Agricultural Product Standards Act, 1990 (SA DAFF, 1990).

A conformation code is used to indicated the quality of the carcasses in terms of the size of high-priced cuts available. Carcasses with shorter legs and a bulkier body, are believed to be higher in quality than those with longer legs (Kirton & Pickering, 1967).

Fat thickness, measured between the 12^{th} and 13^{th} rib, is used to calculate the yield grade of lamb carcasses. Carcasses with a fat thickness less than 2.54 mm are ideal, carcasses with more than 7.62 mm of fat, are unwanted. The more fat a carcass has, the more excess fat has to be trimmed away, thus, a more labour-intensive process has to be followed and the cutability of the carcass decreases due to the high waste percentage. A 50% cutability means that 50% of carcass weight will become retail cuts (Mouch, 2010). However, too little fat can lead to dehydration and shrinkage during transportation and storage of meat (Van der Westhuizen et al., 2019).

The ribeye area indicates the total muscle mass of a carcass, the area size is directly related to the carcass weight (Greiner, 2008). Measurements taken between the 12^{th} and 13^{th} rib include: fat thickness (45 mm), fat thickness (110 mm), eye muscle width (mm), eye muscle depth (mm), eye muscle area (mm^2), eye muscle perimeter (mm), $pH_{24\ hours}$, $Temp_{24\ hours}$ (˚C), water activity (a_w), and water holding capacity (WHC).

In a Canadian study (Juárez et al., 2018), the carcass characteristics by average measurements of 155 lambs were determined and are indicated in Table 2.5.

Table 2.5.

Average measurements of 155 Canadian lambs (Juárez et al., 2018).

Measurements	Mean
Cold Mass (kg)	24.4
External length (cm)	64.4
Shoulder circumference (cm)	-
Buttock circumference (cm)	68.9
Ribeye area (cm^2)	21.9
Ribeye length (mm)	58.7
Ribeye width (mm)	30.1
Fat depth (mm)	6.5

Sheep carcasses can be divided into three major parts: muscle (meat, referred to as lamb in young animals and mutton in older animals), bone and fat. The ratio of these three parts, determines the quality of the carcass. The optimal carcass should have minimum bone, a maximum level of muscle and average levels of fat. Muscle weight comprises around 74% water; the other 26% is protein, lipids, minerals and vitamins. The bone mass is the support structure and consists of hard and calcified cells. The fat is the energy source and found inside muscles and under the skin (Sebsibe, 2008).

When the animal dies, the glycogen is converted into lactic acid, which causes the pH to decrease. If there is insufficient glycogen present in the animal, the animal will lack sufficient levels of lactic acid, and the pH will stay elevated, resulting in dark meat with a lower quality. The meat is described as having a dark colour, rough texture, reduced tenderness and unpalatable flavour. Bacterial growth may increase at high pH levels and therefore, the shelf-life will be reduced (https://www.mla.com.au /research-and-development/animal-health-welfare-andbiosecurity/ parasites).

2.6.2. Proximate analysis

The proximate analysis includes % dry matter (DM), % moisture, % protein, % fat, % organic matter (OM) and % ash. These tests are performed to determine the macromolecules in the meat, which can then be used to compare the nutritional value between different cuts, feed and animals (Karakök et al., 2010). The average proximate analysis values of Dorper sheep found by Villatoro et al. (2021), are indicated in Table 2.6.

Table 2.6.

Average proximate analysis values of untrimmed loins of Dorper sheep (Villatoro et al., 2021).

Proximate analysis tests	Value (%)
Dry Matter	23.34
Moisture	70.94
Protein	21.35
Fat	5.83
Ash	0.91
Carbohydrates	1.09

2.6.3. Fatty acid composition

Fatty acids are related to nutritional value and sensory attributes. During the cooking process, the thermal oxidation of these fatty acids contributes to the meat aromas (Khan et al., 2015). Fatty acids in meat products can be classified as either saturated or unsaturated fatty acids and are mostly triglycerides and phospholipids; they are described by their carbon chain length and number of double bonds (Fig. 2.7). Fatty acids which have no double bonds are saturated fatty acids (SFA), while monounsaturated fatty acids (MUFA) have one double bond and polyunsaturated fatty acids (PUFA) have two or more double bonds. Red meat consists mostly of myristic (C14:0), palmitic (C16:0) and stearic (C18:0) acids, which are all SFA. Palmitoleic (C16:1) and oleic (C18:1) acids are MUFA, while linoleic (C18:2), linolenic (C18:3), and arachidonic (C20:4) acids are PUFA (Dinh et al., 2021).

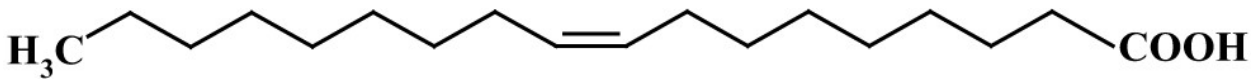


Fig. 2.7. Typical features of fatty acids (ACD/ChemSketch, 2020).

The fatty acid profile is important to consumers because the fat in meat can have a negative effect on consumer health, as heart disease can be linked to meat with a high SFA content (Wood et al., 1999). Thus, consumers who follow a healthy diet, prefer meat with less SFA and more omega-3 fatty acids. The biohydrogenation by microorganisms of unsaturated fatty acids results in lamb meat having low levels of PUFA and an excess in SFA (Beriain et al., 2000). The fatty acid composition of meat is affected by genetic factors and dietary nutrition (Skele, 2022). The fatty acid composition of 80 *Musculus (M.) longissimus dorsi* loin chops from conventional production

systems in South Africa, is presented in Table 2.7, while the molar % (w/w) and gravimetric (mg/g) content of cis-and transfatty acids in these lamb chops are shown in Table 2.8 (Webb, 2021).

Table 2.7

Fatty acid composition of *M. longissimus dorsi* loin chop from conventional production systems in South Africa (n = 80) (Webb, 2021).

Lipid fraction	Content (% ± SD)
Intramuscular fat	13.95 ± 4.18
SFA	52.13 ± 1.22
MUFA	38.98 ± 2.65
PUFA	3.24 ± 0.857
CLA	0.70 ± 0.148
TFAs	4.65 ± 1.284
TFA's/100 g lamb	0.22 g

Table 2.8

The molar % (w/w) and gravimetric (mg/g) content of cis-and trans-fatty acids in lamb chops *(M. longissimus dorsi* samples) from conventional production systems in South Africa (n = 80) (Webb, 2021).

Fatty acid composition	Molar % ± SD (w/w)	Gravimetric content ± SD (mg/g meat)
$C_{12:0}$	0.146 ± 0.057	0.081 ± 0.040
$C_{14:0}$	3.368 ± 0.673	1.742 ± 0.710
$C_{14:1}$	0.084 ± 0.024	0.050 ± 0.024
$C_{16:0}$	24.23 ± 1.788	16.612 ± 4.562
$C_{16:1}$	1.023 ± 0.192	0.908 ± 0.279
$C_{17:0}$	1.774 ± 0.525	0.545 ± 0.152
$C_{18:0}$	22.30 ± 3.321	10.740 ± 2.373
$C_{18:1}$ (n-11*t*)	3.910 ± 1.256	1.735 ± 0.584
$C_{18:1}$ (n-9*c*)	33.27 ± 2.809	24.348 ± 5.668

$C_{18:1}$ (n-11*c*)	0.631 ± 0.114	0.608 ± 0.124
$C_{18:2}$ (n-6*t*)	0.046 ± 0.014	0.019 ± 0.007
$C_{18:2}$ (n-6*c*)	2.029 ± 0.772	1.918 ± 0.385
$C_{18:2}$ (n-10*t*, n-12*c*)	0.592 ± 0.133	0.314 ± 0.147
$C_{18:2}$ (n-10*c*, n-12*c*)	0.006 ± 0.001	0.003 ± 0.001
$C_{18:2}$ (n-9*c*, n-11*t*)	0.013 ± 0.008	0.008 ± 0.006
$C_{18:3}$ (n-6)	0.026 ± 0.005	0.022 ± 0.004
$C_{18:3}$ (n-3)	0.331 ± 0.069	0.176 ± 0.056
$C_{20:0}$	0.131 ± 0.026	0.052 ± 0.011
$C_{20:1}$ (n-9)	0.060 ± 0.016	0.034 ± 0.006
$C_{20:2}$	0.029 ± 0.007	0.030 ± 0.006
$C_{22:0}$	0.011 ± 0.004	0.018 ± 0.004
$C_{20:4}$ (n-6)	0.081 ± 0.020	0.520 ± 0.147

2.6.4. Colour characteristics

Colour is one of the most important factors in the meat industry as consumers ‘shop with their eyes’, and if the colour of meat is slightly off to what they are used to, they will regard it as spoiled and/or not fresh. Colour only, does not prove that meat has spoiled, but rather a combination of foul odour, sticky or tacky feeling, sliminess and colour. Consumers expect fresh and high-quality lamb/mutton to be a light red to brick red colour, if there is any colour change, the consumer will relate it to bacterial growth. In a retail scenario, discolouration of packaged fresh meat is known as “loss of bloom” and can be caused by various factors and not only bacterial growth (Seideman et al., 1983).

Meat can change colour during display, when the myoglobin pigments on the meat surface are exposed to oxygen, transitioning from predominantly purple deoxymyoglobin, to red oxymyoglobin and lastly brown metmyoglobin (Calnan et al., 2014). According to Voster (2019), the WCS carcasses have a dull appearance and the groin has a green shine and/or glassy colour. This already classifies the meat as spoiled and the carcasses are discarded before any tests are performed, or proof is provided.

The colour of meat is expressed as *L**, *a** and *b**, which is defined by the International Commission on Illumination (CIE). Where *L** represents the lightness, *a** is the redness/greenness and the *b** is the yellowness/blueness. The *a** and *b** values are used to determine the chroma and hue of the meat. The *L** value is linked to the pH of the meat, where a decrease in the pH results in an increase in the lightness (greater luminosity). The *a** value is related to the level of myoglobin present in the meat. The redness of the meat decreases over time as the myoglobin is oxidized to metmyoglobin (red to brown). The *b** value of meat tends to increase as the meat matures, because the meat becomes darker in colour (Tarsitano et al., 2013).

2.6.5. Microbial characteristics

The nutritional composition of meat plays a significant role in the microbial quality of the meat. Microorganisms require important nutrients for growth and metabolic functions; these nutrients include water, an energy source, nitrogen, vitamins and minerals. The quantity and type of nutrient differ according to the species of microorganism. Meat has a high lipid, protein, vitamin and mineral content, with low carbohydrate levels. Minerals are required in low quantities and include manganese, calcium, phosphorus, iron, magnesium, sulphur and potassium. Foodborne microorganisms can derive their energy from the glycogen that is present in the muscle, while some microbes are able to use fat as a source of energy (Batt & Robinson, 2014).

Generally, meat is subject to contamination by *Salmonella, Clostridium botulinum* (*C. botulinum*)*, Staphylococcus aureus* (*S. aureus*)*, Clostridium perfringens* (*C. perfringens*)*, Bacillus cereus* (*B. cereus*)*, Escherichia coli* (*E. coli*)*, Campylobacter jejuni* (*C. jejuni)* and/or *Listeria monocytogenes* (*L. monocytogenes*) (Hobbs & Roberts, 1993; Rani et al., 2017). Murugkar et al. (1993) inspected mutton samples sourced in Bombay, India, and reported *E. coli, S. aureus, B. cereus, C. perfringens* as well as coliforms. A study in Morocco found *S. aureus, E. coli* and *C. perfringens* on samples of lamb collected during summer and winter seasons (Cohen et al., 2006). Another study by Abd (2009), reported that staphylococci, *Corynebacterium*, streptococci, *Micrococcus, Salmonella, E. coli* and yeast were the most prevalent in lamb carcasses. Likewise, animal by-products (ABPs) can also be contaminated by microorganisms, as in the case with goat meat ‘sarapatel’ (Brasil et al., 2014) and beef/pig offal (Lee & Lee, 2016).

The water activity (a_w) of food can be described as the amount of available water ranging from 0 to 1, where 0 is absolute zero water available, and 1 is pure water (https://foodcrumbles.com/wateractivity-in-food-the-theory/ Retrieved on 27 June 2023). This is the water that is available for microorganisms to grow, not to be confused with moisture content or water-holding capacity. The moisture content of food is expressed as a percentage that’s related to the

total weight of the product (https://blog.kett.com/bid/362219/moisture-content-vs-water-activity-use-both-to-optimize-foodsafety-and-quality, retrieved on 27 June 2023), whereas the water-holding capacity is the ability of the meat muscle to hold water (Huff-Lonergan, 2015).

Spoilage and pathogenic bacteria grow at an a_w higher than 0.85. Meat products usually have an a_w of 0.95-0.99, thus making it ideal for bacterial growth (Gall, 2017). The optimum, minimum and maximum water activity levels needed by the most common pathogens are presented in Table 2.9.

Table 2.9

Minimum, optimal and maximum growth water activity ranges of selected bacterial pathogens (Gall, 2017).

Pathogen	Minimum a_w	Optimal a_w	Maximum a_w
Salmonella spp.	0.94	0.99	> 0.99
Staphylococcus aureus	0.83	0.98	> 0.99
Escherichia coli	0.95	0.96	-
Listeria monocytogenes	0.92	-	-
Clostridium perfringens	0.97	0.95 to 0.96	0.93

- No data provided by authors

In contrast to PSE-related studies (Caldara et al., 2014), no investigation has been performed on the microbial composition of WCS meat *per se*. From previous literature, it is known that WCS meat shows an excess of watery fluid in the dorsal (upper) areas of the carcass, as well as in the hind legs and flanks. This excess of water can act as a breeding ground for certain microorganisms since it does not dry off overnight. The handling of meat at different stages of the distribution chain, may also affect meat quality and subsequent consumer health (Rani et al., 2017). As the high water activity of WCS meat may predispose such meat to microbial contamination post-slaughter, proper handling becomes crucial.

The prevention of contamination of food products during handling and processing is controlled by the Hazard Analysis Critical Control Point (HACCP). In South Africa, the Hygiene Management System (HMS) is regulated under the Meat Safety Act 40 of 2000, which is based on HACCP principles (Katsande & Govender, 2014). The HMS is implicated by the SA DAFF to control the risk of contamination or cross contamination during the processing of meat at abattoirs (Govender & Genis, 2010).

A qualified meat inspector has to approve carcasses after the slaughtering process, and stamp each carcass as approved or condemned. Carcasses suspected to have WCS, are removed from the line and left to dry overnight. A veterinarian then inspects the carcasses the next day and finally condemns them as WCS carcasses. In South Africa, these inspections are usually performed on a

visual bases, since frequent microbial testing is not compulsory. The Department of Veterinary Public Health (DVPH) performs monthly tests during audits, using standards (Table 2.10) set by the European Union (EU). Bigger retail markets, however, are known to perform their own microbial tests using their own standards (Katsande & Govender, 2014).

Standards set by the SA DAFF, state that the total bacteria count on meat carcasses should be in a limit of 3.5 to 5 log cfu/cm^2. The coliform and *Enterobacteriaceae* counts should be less than 2.5 log cfu/cm^2. Both *E. coli* and *S. aureus* should fall within the limits of 0 to 2 log cfu/cm^2 (SA DAFF, 2018).

2.7. Possible uses of WCS meat

According to Walsh (2014), data in 2012 showed that an estimated extra 10% of the live weight of bovine animals is being consumed when compared with 2008. This trend also reduced the carbon footprint of the meat industry by about 26%. Markets have also changed significantly due to new Animal By-Product (ABP) regulations and improved export opportunities. Generally, an increase in consumption of so-called 5th quarter products (red offals and ABP), soft bones and tendons as well as the uptake of ABP for pet-food, fertilizer and energy generation, is evident (Alao et al., 2017). Optimal utilisation of WCS carcasses will feed into these (positive) statistics and generate some level of income for affected farmers.

Table 2.10

Microbial limits of meat and meat products set by the European Union (Kyprianou, 2007).

Food category	Micro-organisms	Limits
Carcasses of cattle, sheep, goats and horses	Aerobic colony count	3.5 to 5.0 log cfu/cm^2 daily mean
	Enterobacteriaceae	1.5 to 2.5 log cfu/cm^2 daily mean
	Salmonella	Absence in the area tested per carcass
Minced meat	Aerobic colony count	5 x 10^5 to 5 x 10^6 cfu/g
	E. coli	50 to 500 cfu/g

Since no microbial quality and safety evaluations have been performed to date, the safety of WCS meat is unknown. However, even if found to harbour a higher and/or more 'toxic' microbial load compared to normal meat, strategies like hurdle technology (Leistner, 2000), may be deployed for meat preservation. This approach is an intelligent combination of impediments or hurdles, which ensures microbial safety and stability, as well as the nutritional quality of food products. Interventions include high temperature during processing, low temperature during storage, lowering the pH, lowering the a_w, or redox potential, or adding preservatives (Leistner, 2000). On the other hand, if various tests are conducted, and the results prove that there are no microbial and/or chemical toxins in the meat, with an acceptable shelf-life, then the solid meat and bone can be separated from the liquid fat. The solid meat and bone can be pressed to form a meal, which can be utilised in products such as stock feed for livestock production, pet food and/or as a fertiliser (from blood and bone). The liquid fat can be used in the food industry for dripping, industrial margarine, frying fats, stock-feed and pet food. Other uses for the fat include soap manufacturing or processed products such as glycerine (https://businessrecycling.com.au/recycle/meat, retrieved 2021).

2.8. Conclusions

Although several studies have been conducted in an attempt to find the cause of WCS, no physiological, genetic, environmental, or management system-effect could be conclusively identified as the causative agent of WCS. Notably, the condition is more prevalent in the Northern Cape and southern parts of Namibia; an arid region where cases are often noted in the autumn and winter, particularly after droughts or after cycles of rainfall in spring, followed by reduced rainfall throughout the rest of the summer. How these harsh conditions affect stress levels and/or food-plant diversity (harbouring possible hepatotoxins), and their potential link to WCS, may warrant further investigation.

Some studies have suggested a strong linkage to electrolyte imbalances. Recently, a molecular study identified two genes (*DMD* and *HTR2C*) on the X-chromosome, with *DMD* linked to cell membrane permeability and *HTR2C* known to regulate the calcium ion pump. It was hypothesized that under stress (release of the stress hormone cortisol), the calcium ion pump opens, allowing liquid to flow through the cell wall. At/after slaughter, the pump does not shut down, facilitating more fluid loss and subsequent WCS. The relevant research group has proposed a follow-up study with a larger animal sample size.

Since WCS meat does not dry off readily, the water activity will probably be high, and thus the meat will be more prone to bacterial growth, especially pathogenic bacteria, which grow in high a_w levels. Bacteria such as *E. coli, S. aureus, B. cereus, C. perfringens* as well as *Corynebacterium*,

streptococci, *Micrococcus*, *Salmonella* spp., *Campylobacter jejuni* and *Listeria monocytogenes,* are known contaminants of meat. The association of such and other contaminants with WCS meat, however, remains unexplored. If the microbial load and/or chemical composition of WCS meat is not significantly different from that of unaffected meat, new processing methods/markets may lead to optimised usage of such meat.

Chapter 3

Materials and Methods

3.1. Location

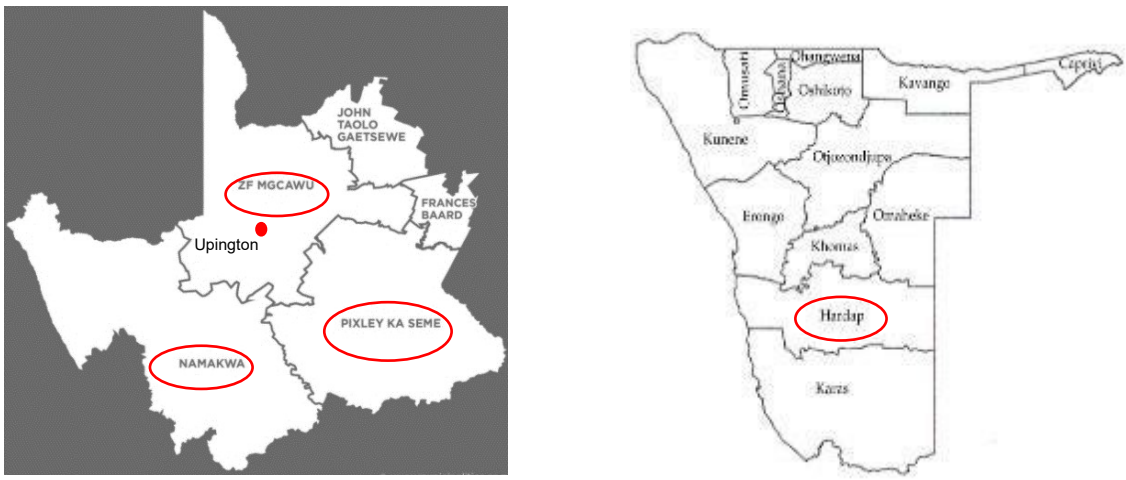
This study examined meat originating from the Northern Cape and southern part of Namibia, because WCS is more prevalent in these regions. All the carcasses were sourced from the KLK abattoir in Upington, Northern Cape (28.45°S/21.23°E). Table 3.1 shows the number and districts in the Northern Cape and Namibia where the 20 WCS and the 20 normal carcasses were obtained from. The first batch of carcasses was from the ZF Mgcawu district in the Northern Cape (Fig. 3.1a), and the next batch, from the Pixley Ka Seme district (Fig. 3.1a). The third batch was again from the ZF Mgcawu district. The fourth batch was from the Namakwa district in the Northern Cape (Fig. 3.1a), followed by the final batch from the district Hardap in Namibia (Fig. 3.1b). All samples from each sampling session were, as far as possible, from the same producer and slaughter batch. The left side rib (from rib 6) plus the loin cut, were removed from each carcass, refrigerated with ice bricks in cooler boxes and transported (564km) to the Meat Science laboratory of the University of the Free State on the same day. The following information was sourced from the abattoir:

- Farm and producer (origin of animal)
- Classification data
- Cold carcass mass
- $pH_{24\ hours}$
- carcass measurements, including carcass length, shoulder circumference and buttock circumference, were determined at the abattoir.

Table 3.1

Districts where the 40 sheep carcasses were obtained for this study.

Carcasses	Districts
1-8 (4 wet; 4 normal)	ZF Mgcawu (Northern Cape)
9-14 (3 wet; 3 normal)	Pixley Ka Seme (Northern Cape)
15-22 (4 wet; 4 normal)	ZF Mgcawu (Northern Cape)
23-32 (5 wet; 5 normal)	Namakwa (Northern Cape)
33-40 (4 wet; 4 normal)	Hardap (Namibia)



(a) (b)

Fig. 3.1. (a) Districts of the Northern Cape in South Africa, and (b) District of Namibia, where the carcasses were obtained for this study.

3.2. Experimental design

This study consisted of three major parts, which are depicted in Fig. 3.2:

- carcass measurements and surface swab sampling at the abattoir;
- removal of chops and microbial and physico-chemical analysis on the chops stored at 4 °C for six days to evaluate the possible use of WCS chops for human consumption;
- production of a fresh pet mince sausage stored at 4 °C for 10 days and evaluated in terms of physico-chemical and microbial quality, to evaluate the possible use of WCS meat as a pet food.

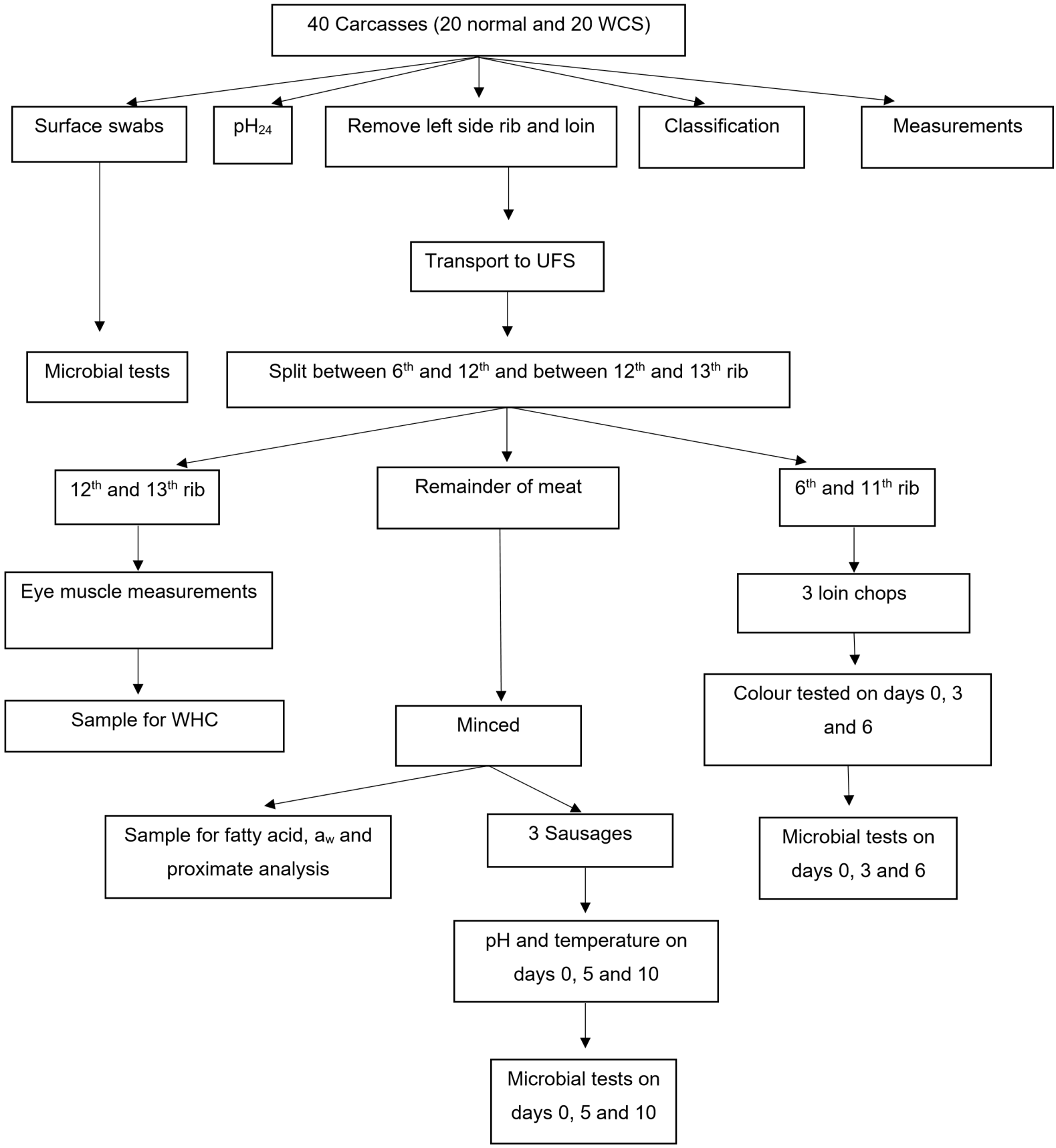


Fig. 3.2. Flow diagram depicting the experimental design.

3.3. Surface microbiological swabs

Before removing the left side rib and loin from each carcass, the carcass was swabbed using four sterile transport swabs (Lasec, Bloemfontein) for microbiological analyses (Fig. 3.3).

Fig. 3.3. Microbial swabs taken at KLK Upington Abattoir.

The swabs were taken from four locations on the carcass, namely the neck area, brisket area between the elbow and the midline cut, flank area and perineal area from the base of the tail to the hock, as suggested by VPN52 (Meat Safety Act, 2018; SA DAFF, 2018). A 2.5 X 2.5 cm square sterile template (Lasec HCPNT2905CS01; Bloemfontein) was used for obtaining the surface swab samples. All four swabs (per carcass), were placed in a 9 ml sterile buffered peptone (Oxoid CM0509) solution (10^{-1} dilution) in McCartney bottles. The samples were refrigerated with ice bricks in cooler boxes and transported to the Food Microbiology laboratory of the University of the Free State on the same day, to be evaluated within 24 h. A dilution series (10^{-2} to 10^{-5}) was prepared using McCartney bottles containing 9 ml of sterile 0.1 M phosphate buffer. One millilitre volumes of each dilution were pour-plated on different media, unless indicated otherwise. All Oxoid media were sourced from ThermoFisher (Pty) Ltd (Randburg, South Africa). The methods were performed according to standardized techniques (Harrigan, 1998). All evaluations were performed in duplicate on the various media.

Standard plate count agar (SPCA; Oxoid 0463) was used for the total bacterial count and plates were incubated at 32 °C for 48 h. After incubation, the colonies were enumerated by means of a colony counter.

Violet red bile lactose agar + 4-methylumbelliferyl-β-D-glucuronide (VRBA + MUG; Oxoid CM0978) was used for total coliform and *E. coli* counts and incubated at 37 °C for 24 h. The presence of *E.*

coli on VRBA plates was confirmed by fluorescence under ultraviolet light (366 nm; CAMAG Universal UV Lamp).

Enterobacteriaceae was enumerated on violet red bile glucose agar incubated at 37 °C for 24–48 h.

Spread-plating on Baird-Parker agar (BPA; Oxoid CM0275) with egg yolk tellurite supplement (Oxoid SR0054) was used for *S. aureus* enumeration and incubated at 37 °C for 24–48 h. *Staphylococcus aureus* typically forms colonies that are 1.0–1.5 mm in diameter, black, shiny, convex with a narrow white margin and surrounded by clear zones, extending 2–5 mm into the opaque medium.

Rose-bengal chloramphenicol agar (RBCA; Oxoid CM0549) with chloramphenicol supplement (Oxoid SR0078) was used for yeast and mould enumeration and incubated at 25 °C for 4 days.

MRS (Oxoid CM0361) agar was used for lactic acid bacteria counts and incubated at 32 °C for 24–48 h.

The *Pseudomonas* count was enumerated on *Pseudomonas* agar (Oxoid CM0559) with *Pseudomonas* C-F-C supplement (Oxoid SR103) and incubated at 25 °C for 24–48 h.

3.4. Meat quality measurements

Measurements taken include: Cold mass (kg), Fatness code, Conformation code, External length (cm), Shoulder circumference (cm) and Buttock circumference (cm). pH measurements were taken between the 12^{th} and 13^{th} rib, using a Hannah HI98163 pH meter reader (Labotec, Midrand, South Africa) to record pH measurements per carcass. Carcass evaluation was performed on the left side of each carcass. The rib and loin were split between the 12^{th} and 13^{th} rib. Fat depth was measured with a calliper, 45 and 110 mm from the mid-dorsal line and eye muscle area; between the 12^{th} and 13^{th} rib. Muscle depth and width were also measured between the 12^{th} and 13^{th} rib (Carson et al., 1999). The *Musculus longissimus* (*M. longissimus*) was traced onto transparent film in order to measure the surface area of the *M. longissimus* between the 12^{th} and 13^{th} rib (Edwards et al., 1989). The traced outline was scanned with a scale bar and the eye muscle area was measured using a video image analysis system (Kaiser Fototechnik GMBH and Co. KG, Germany). Images were captured using an EOS 80D camera (Canon, Japan), fitted with the EF 50 mm F1.4 USM lens, which was mounted on a Kaiser RS 1 copy stand (Kaiser Fototechnik GMBH & Co. KG, Germany). Image processing and measurement calculations were done by means of CellSens Entry v3.2 (20092021) imaging software (Olympus, Germany), described by Irie et al. (1996). Measurement calibrations were made using the Kaiser RS 1 measurement grid of the abovementioned copy stand.

Water-holding capacity (WHC) measures the amount of moisture (water) pressed out of an eye muscle sample (between 12th and 13th rib). A 400–600 mg fresh meat sample was placed on a filter paper (Whatman 4, Whatman International Limited, Maidstone, England), sandwiched between two perspex plates and pressed at a constant pressure for 5 min, according to the method described by Grau and Hamm (1953). Images were captured using an EOS 80D camera (Canon, Japan), fitted with the EF 50 mm F1.4 USM lens, which was mounted on a Kaiser RS 1 copy stand (Kaiser Fototechnik GMBH and Co. KG, Germany). Image processing and measurement calculations were performed by means of CellSens Entry v3.2 (2009-2021) imaging software (Olympus, Germany), described by Irie et al. (1996). Measurement calibrations were made using the Kaiser RS 1 measurement grid of the abovementioned copy stand. The WHC was expressed as the area of the meat, divided by the area of moisture (including the meat area).

3.5. Shelf-life study on loin chops

From all lamb carcasses the left 9th rib through the 12th rib was removed. Three loin chops from each lamb carcass were scraped clean and individually packed into a polystyrene tray, containing an absorbent pad and overwrapped with oxygen-permeable polyvinyl chloride (PVC) meat stretch wrap, and stored at 4 °C for 6 days, under fluorescent light. The following evaluations were performed on days 0, 3 and 6. The eye muscle area was swabbed for each chop (Fig. 3.4) and microbiological quality was monitored as explained above for surface microbiological swabs.

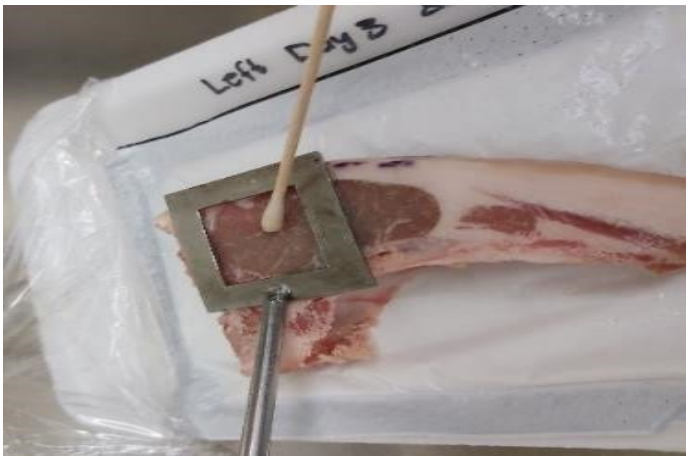


Fig. 3.4. Sterile template placed on chop, swab samples taken within the 2.5 X 2.5 cm^2 area.

The meat from each chop was left to bloom for 30 min and Commission International de l'Eclairage (CIE) colour coordinates (L^*, a^* and b^*) were measured in triplicate using a Minolta colorimeter (narich) (+60 red and -60 green) and yellow-blue b^* (+60 yellow and -60 blue) (Commission International de l'Eclairage, 1978) were recorded. Saturation index (SI) was determined according

to the formula: SI = $\sqrt{a^{*2} + b^{*2}}$ which is associated with meat colour intensity (Lanari et al., 1995). Hue angle was determined according to the formula $\tan^{-1}\left(\frac{b*}{a*}\right)$ (Ripoll et al., 2011).

3.6. Water activity

Homogenously mixed minced meat from the 6th to the 9th rib was filled into a water activity container (height of 5 mm and diameter of 39 mm) to the appropriate level and covered with a container lid. The water activity was determined using a Novasina Thermoconstanter TH 200 (Labotec, Midrand, South Africa) water activity meter. After equilibrium was reached with deionized distilled water, quadruplicate measurements per treatment group per replicate were recorded at a temperature of 25 °C. The results were reported as % relative humidity (% rH) and converted to a_w values by dividing each value by a factor of 100.

3.7. Proximate analysis

Proximate analysis (% protein, % fat, % moisture and % ash) was performed according to the Association of Official Analytical Chemists (AOAC, 2005) using deboned and minced meat from the 6th to the 9th rib (Fig. 3.1). A 100 g patty of each sample was formed, weighed and freeze-dried to a constant weight and a cryogenic tube was filled with mince to test for fatty acid composition.

Dry matter (DM) content of muscle tissue was analysed according to the AOAC method 934.01 for chemical procedures (AOAC, 1990). After drying, the samples were placed in desiccators.
Thereafter, the percentage moisture loss was calculated as follows:

% Moisture loss = [Weight loss after freeze drying (g) / Weight of test sample (g)] × 100

The crude protein (CP) content of freeze-dried muscle tissue was determined according to the AOAC method 990.03 for chemical procedures (AOAC, 1990) with a Leco® FP-528 instrument for nitrogen analysis. For each sample, 0.095 g of DM was accurately weighed and placed into aluminium foil cups that were sealed and placed on the carousel of the instrument, which performed sample analyses routinely. The principle of the Dumas method is that nitrogen (N_2) is freed by pyrolysis and subsequent combustion and is swept by carbon dioxide (CO_2) as carrier into the nitrogen analyser. The carbon dioxide is absorbed by potassium hydroxide (KOH) and the residual nitrogen volume measured. The nitrogen content is then converted to a protein equivalent by multiplying the percentage of nitrogen with a factor of 6.25 (McDonald et al., 2011). Protein values were recorded on a computer, which was connected to the scale, as well as the analysing instrument. The protein

equivalent was then calculated by the computer program from the numerical factor obtained, as described.

The ether extract (fat) content of the freeze-dried muscle tissue was determined by means of the Soxhlet extraction method (No 920.39), using petroleum ether (40–65 °C boiling point) as a solvent (AOAC, 1990). A 10 g sample (DM-basis) was closed inside a pre-weighed Schleicher and Schull No. 589/2 filter paper and carefully placed within the extraction thimble. The thimble was then closed with a clean cotton wool plug and placed inside the glass extractor that was connected with the preweighed glass flask before pouring the 150 ml solvent (petroleum ether) gently into the thimble. The extractor unit was placed into the individual heating units and connected with the water-cooling unit. The heating was set to ensure a droplet speed of approximately five to six drops of solvent per second and the samples were extracted for six hours. After the extraction process, glass flasks were removed from the heating units and all remaining solvent was damped off over a warm water bath before drying overnight in an oven at 60 °C. After drying, each glass flask containing the lipid fraction, was placed inside a desiccator until reaching room temperature and weighed to the 4th decimal. The fat fraction of muscle tissue was calculated as follows:

% Fat = [Weight of fat (g DM) / Weight of test sample (g DM)] × 100

Ash content of the freeze-dried muscle tissue was determined according to the AOAC method 942. 05 (AOAC, 1990). Ash content was determined by complete incineration of each sample using a muffle furnace. Approximately 2.5 g of the sample (DM basis) was weighed in porcelain crucibles. A porcelain lid was then placed on each crucible containing the sample and placed in a cold muffle furnace and heated to a constant temperature of 600 °C. Samples were kept at this temperature for three hours before switching the furnace off and allowing it to cool before the oven could be opened and samples handled. The samples were subsequently transferred to desiccators to cool and weighed immediately afterwards. The ash content was calculated as follows:

% Ash = [Weight of ash (g DM) / Weight of test sample (g DM)] × 100

The weight of the individual crucibles was deducted to determine the weight of the ash and the weight of the test sample, as all crucibles did not have the same weight. After chemical analysis of the freeze-dried material all results were recalculated to an “as is” basis.

3.8. Fatty acid profile determination

Total lipid from a minced loin sample (6th to the 9th rib) were quantitatively extracted, according to the method of Folch et al. (1957), using chloroform and methanol in a ratio of 2:1. An antioxidant, butylated hydroxytoluene was added at a concentration of 0.001% to the chloroform: methanol mixture. A rotary evaporator was used to dry the fat extracts overnight in a vacuum oven at 50 °C, using phosphorus pentoxide as a moisture adsorbent. Total extractable intramuscular fat was determined gravimetrically from the extracted fat and expressed as percent fat (w/w) per 100 g tissue. The fat free dry matter (FFDM) content was determined by weighing the residue on a preweighed filter paper, used for Folch extraction, after drying. By determining the difference in weight, the FFDM could be expressed as % FFDM (w/w) per 100 g tissue. The moisture content of the muscle and BF was determined by subtraction (100% - % lipid - % FFDM) and expressed as % moisture (w/w) per 100 g tissue. The extracted fat was stored in a polytop (glass vial, with push-in top) under a blanket of nitrogen and frozen at –20 °C pending fatty acid analyses.

A lipid aliquot (±30 mg) of muscle lipid were converted to methyl esters by base-catalysed transesterification, in order to avoid CLA isomerisation, with sodium methoxide (0.5 M solution in anhydrous methanol) during 2 h at 30 °C, as proposed by Park et al. (2001), Kramer et al. (2002) and Alfaia et al. (2007). Fatty acid methyl esters (FAMEs) from muscle were quantified using a Varian 430 flame ionization GC, with a fused silica capillary column, Chrompack CPSIL 88 (100 m length, 0.25 mm ID, 0.2 µm film thickness). Analysis was performed using an initial isothermic period (40 °C for 2 min). Thereafter, temperature was increased at a rate of 4 °C/min to 230 °C. Finally, an isothermic period of 230 °C for 10 min followed. FAMEs n-hexane (1 µl) was injected into the column using a Varian CP 8400 Autosampler. The injection port and detector were both maintained at 250 °C. Hydrogen, at 45 psi, functioned as the carrier gas, while nitrogen was employed as the makeup gas. Galaxy Chromatography Data System Software (Varian) recorded the chromatograms.

Fatty acid methyl ester samples were identified by comparing the retention times of FAME peaks from samples with those of standards obtained from Supelco (Supelco 37 Component Fame Mix 47885-U, Sigma-Aldrich Aston Manor, Pretoria, South Africa). Conjugated linoleic acid (CLA) standards were obtained from Matreya Inc. (Pleasant Gap, Unites States). These standards included: cis-9, trans-11 and trans-10, cis-12-18:2 isomers.

Fatty acids were expressed as the proportion of each individual fatty acid to the total of all fatty acids present in the sample. Fatty acid data were used to calculate the following ratios of FAs: total SFAs; total MUFAs; total PUFAs; PUFA/SFA; Δ^9 desaturase index (C18:1*c*9/C18:0); total omega-; total

omega-6; total omega-3; and the ratio of omega-6 to omega-3 (*n*-6)/(*n*-3) FAs. Atherogenicity index (AI) was calculated as: AI = (C12:0 + 4 x C14:0 + C16:0)/(MUFA + PUFA) (Chilliard et al., 2003).

3.9. Shelf-life study on pet food

Pet mince was separately manufactured from the frozen WCS and normal carcasses using the remainder of the loin cut (from rib 13 to the end of the loin) after the removal of the chop and the meat from ribs 6 to 12. Defrosted meat was comminuted through a 13 mm mincing plate, while the additives (Table 3.2) were mixed with the ice water and left to stand for 5 min, to allow for hydration and the water-soluble additives to dissolve properly. This mixture was thoroughly mixed, before being minced through a 4.5 mm mincing plate. Plastic casings were filled with the sausage mixture, using a manual sausage filler. This resulted in a single, continues roll of sausage that was cut into three individual sausages for each treatment (Fig. 3.5) and stored at 4 °C for 10 days under fluorescent light.

Table 3.2

Formulation used for the production of pet mince packaged as a sausage.

Ingredients	Inclusion %
Lamb Meat	93.50
Yeast extract	1.00
Salt	0.50
Water	5.00
Total	**100.00**

Fig. 3.5. Pet mince sausages made from normal (left) and WCS (right) meat.

On days 0, 5 and 10 the microbial quality of the minced sausage was evaluated, where 10 g of the meat was placed in a 532 ml WhirlPak stomacher bag (Lasec PLSCB01065WA), 90 ml of buffered peptone water was added and the sample was homogenized (AME Stomacher Lab-Blender 400, JHB) for 1 min. This was the 10^{-1} dilution. The 10^{-1} dilutions were further diluted to 10^{-5} using 9 ml of sterile 0.1 M phosphate buffer. The samples were then plated using the pour plate method on various media as described earlier.

The pH and temperature measurements were taken by inserting the probe into the meat and reading the value for all three sausages on days 0, 5 and 10. pH and temperature measurements were done directly by using a Hannah HI98163 pH meter reader (Labotec, Midrand, South Africa) to record triplicate pH and temperature measurements per sausage at room temperature. Each day before use, the pH meter was calibrated with standardized buffers (Merck, Johannesburg, South Africa) with pH values of 4.01 and 7.00, respectively.

3.10. Statistical analyses

All results were captured in spread sheets in Microsoft Excel 2018. Upon completion of all analyses where the attributes between the normal and wet carcass meat were compared, a two-sample t-test was used (NCSS Statistical Software package, version 11.0.20, 2018). Where the interaction between meat type and storage time was investigated, an analysis of variance (ANOVA) procedure was used (NCSS Statistical Software package, version 11.0.20, 2018). In this case the Tukey-Kramer multiple comparison test ($\alpha = 0.05$) was carried out, to identify significant differences between the treatment means (NCSS Statistical Software package, version 11.0.20, 2018). Differences were considered statistically significant at the $P < 0.05$ level.

Correlations were preformed to determine the linear relationship between the dependent and independent variables, by using Microsoft Excel XLSTAT 2016.

3.11. Ethical Clearance

Permission to perform research under section 20 of the animal diseases act, 1984 (act no 35 of 1984; SA DAFF, 1984), was obtained from the South African Department of Agriculture, Forestry and Fisheries. Ethical clearance was also obtained from the University of the Free State's Environmental and Biosafety Research Ethics Committee with clearance number UFS-ESD2020/0132/1111

Chapter 4 Results and Discussion

4.1. Occurrence

The occurrence of WCS per month, from 2019 until 2022 at KLK abattoir, is given in Table 4.1. The highest frequency of WCS was observed in the months of May, June, July and August of 2019, 2021 and 2022. This corresponded with the finding that WCS is more prevalent during autumn and winter seasons (Van der Westhuizen et al., 2019). In the year 2019, a total of 352 carcasses were condemned as WCS. The following year (2020) a drastic decrease in the number of WCS cases can be seen with only 43 carcasses condemned. However, in 2021, the number slowly increased again to 125 WCS cases, followed by a further increase in 2022, to reach 683 cases.

Table 4.1

Monthly occurrence of wet carcass syndrome sheep received by KLK Abattoir Upington from 2019 to 2022.

	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Total
2019	14	25	47	14	13	22	43	62	35	4	31	42	352
2020	1	11	0	6	1	6	1	4	9	2	1	1	43
2021	0	3	7	6	30	41	17	1	7	2	0	11	125
2022	1	6	18	23	34	116	172	52	42	42	113	64	683

Fig. 4.1 shows the trend of rainfall (in orange) in the Upington area from 2019 to 2022. The rainfall decreased during the winter months and slowly increased during the spring months. This is a common trend of yearly rainfall in the Northern Cape. The blue bars indicate the total number of WCS cases that the KLK abattoir in Upington received every month during the four years. As stated in the literature review, the borehole water consumed by the sheep in these areas, becomes saltier under low rainfall. In 2019, Voster concluded that animals drink more water when they move from a high-salt water source (brackish water) to a low-salt water source. This corresponded to the idea that WCS is caused by over-hydration after a dehydration period (Joubert et al., 1985).

During winter (low rainfall conditions) fewer plants and less diversity are available for feeding and animals may consume certain plant species possibly associated with WCS. The PAs can be hepatotoxic and may cause liver damage to animals when consumed (Wiedenfeld & Edgar, 2011).

The WCS-sheep showed significantly higher than normal levels of Vitamin E, this could be because of a compromised liver caused by PAs (Brock et al., 1983). A potential link between WCS and PA intake may lie in the inability of a compromised liver to regulate ("repackage and secrete") vitamin E (Traber, 2013); a hypothesis for possible future investigation.

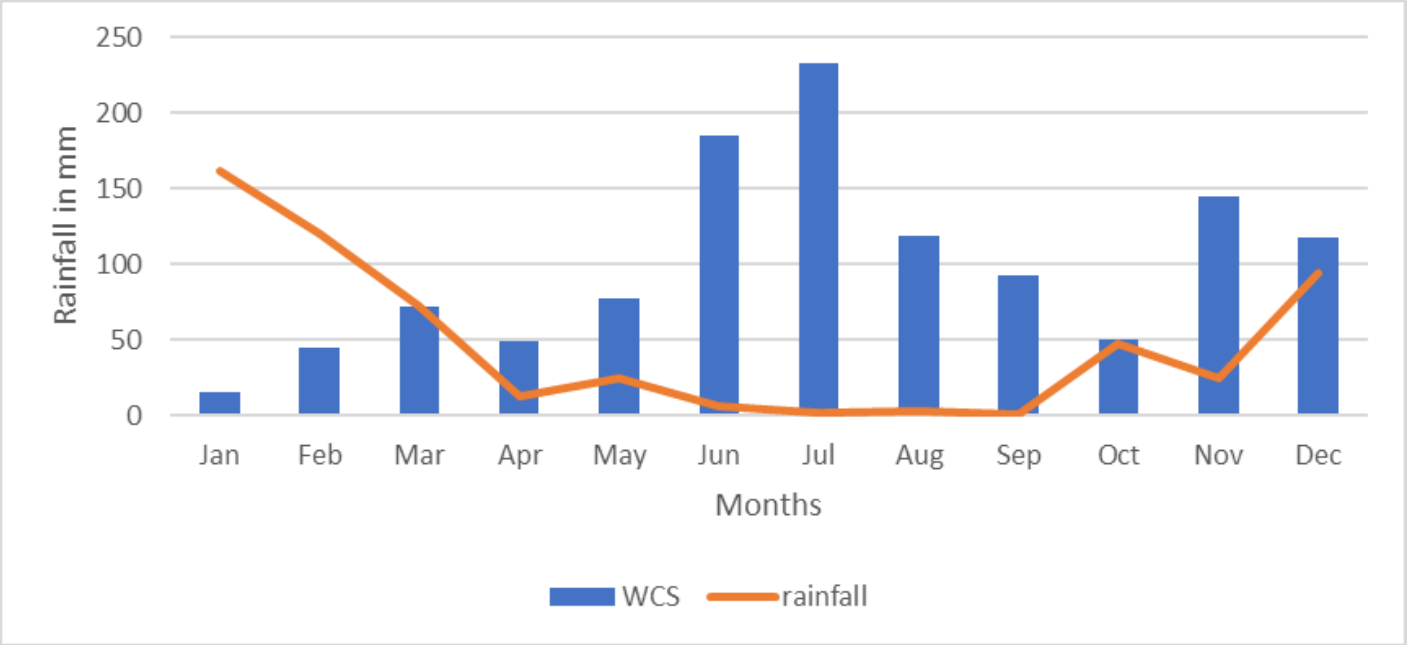


Fig. 4.1. Average trend of rainfall (mm) per month and Wet Carcass Syndrome cases over a 4-year period (2019 – 2022) at KLK abattoir in Upington.

The following graphs (Figs. 4.2 and 4.3), depict the relationship between the average occurrence of WCS cases and the rainfall and temperature during the years 2019, 2020, 2021 and 2022. The R^2 indicates the coefficient of correlation, also known as the 'goodness of fit', an R^2 of 1 would show a strong relationship between the variables, whereas $R^2 = 0$, would indicate a weak relationship (Hamilton et al., 2015). Figures 4.2 and 4.3 show a R^2-value of 0.5, indicating a 50% variability in the data. The findings indicated an indirect relationship between the occurrence and rainfall/temperatures throughout the four years, which corresponded with the findings by Voster (2019), who stated that WCS was more prevalent during dry seasons (low rainfall).

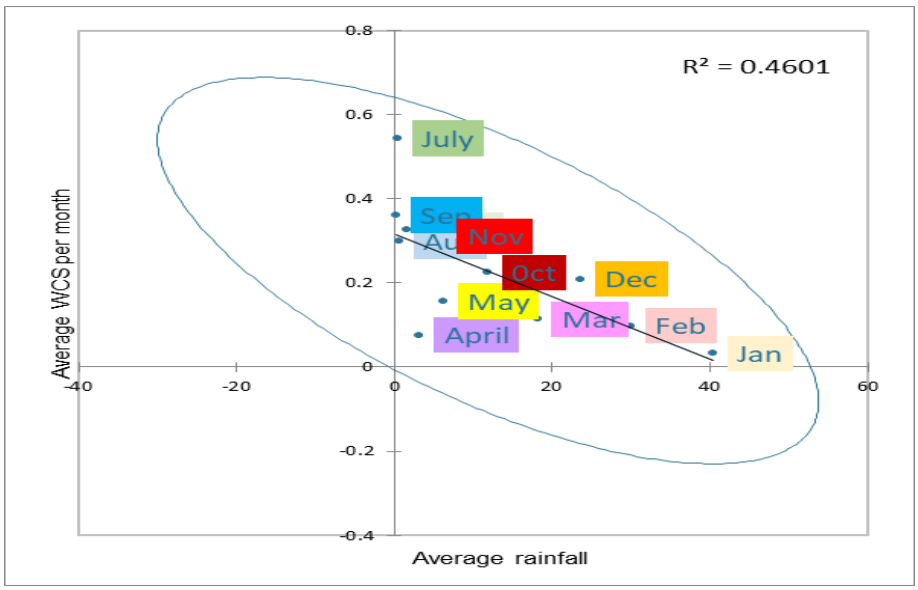


Fig. 4.2. Correlation between the average Wet Carcass Syndrome cases and average rainfall for 2019 to 2022 at KLK abattoir in Upington.

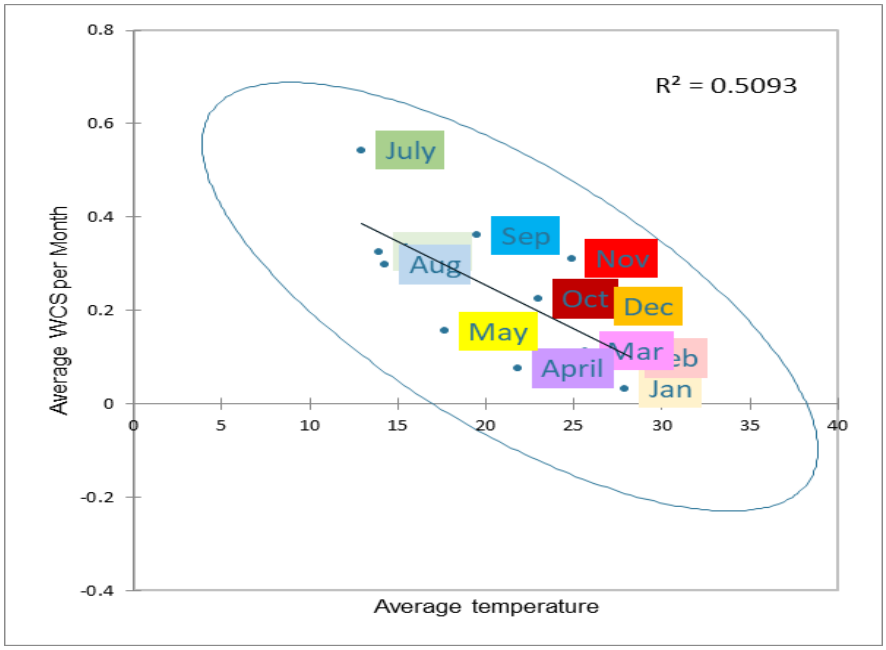


Fig. 4.3. Correlation between the average Wet Carcass Syndrome cases and average temperature for 2019 to 2022 at KLK abattoir in Upington.

4.2. Gender distribution

Table 4.2 shows the gender distribution of randomly selected normal carcasses and carcasses displaying WCS. The percentage of male and female for the normal, unaffected carcasses, were 30% and 70%, respectively. The WCS carcasses' gender distribution were 45% male and 55% female. This was an indication that there was a slightly higher occurrence of wet carcass syndrome among female, than male animals.

Table 4.2

Gender distribution of randomly selected normal carcasses and carcasses displaying Wet Carcass Syndrome at KLK abattoir in Upington (2019 to 2022).

Gender	Number (n)	Percentage (%)
Normal carcasses	20	
Female	14	70
Male	6	30
Wet carcasses	20	
Female	11	55
Male	9	45

4.3. Carcass measurements

Table 4.3 shows the carcass measurements of the normal and WCS carcasses taken after slaughtering. According to Table 4.4, the wet carcasses had a significantly ($p < 0.001$) higher cold mass than the normal carcasses. This is a very interesting observation since carcasses were selected randomly during each of the five sampling visits. It can be speculated that the higher cold carcass mass of wet carcasses could possibly be because of the excess water that the WCS carcasses hold.

The fatness code can be described as a minimum of 0 (no visible fat) and a maximum of 6 (excessively fat). There were no significant differences ($p = 0.168$) in the fatness code between the normal and WCS carcasses (Table 4.3). According to Table 4.3, there was no significant ($p = 1.00$) difference between the conformation codes of WCS and normal carcasses.

The external length, shoulder circumference, and buttock circumference demonstrated respective p-values of 0.014, 0.014 and 0.038, between the normal and the wet carcasses. That indicated significantly higher parameters for the WCS carcasses. The longer in length and larger

circumferences around the shoulders and buttocks of the WCS carcasses in this study, can be considered as positive carcass attributes for WCS carcasses (Kirton & Pickering, 1967).

Table 4.3

Characteristics and classification measurements (mean ± standard deviation) of randomly selected normal versus wet carcass syndrome-affected carcasses at KLK abattoir in Upington (2019 to 2022).

Characteristic	Normal carcasses (n = 20)	Wet carcasses (n = 20)	Sign. level
Cold mass (kg)	14.45^{a} ± 1.47	17.63^{b} ± 2.67	p < 0.001
Fatness code	1.85^{a} ± 0.75	1.50^{a} ± 0.83	p = 0.168
Conformation code	2.85^{a} ± 0.37	2.85^{a} ± 0.49	p = 1.000
External length (cm)	53.58^{a} ± 3.30	56.28^{b} ± 3.32	p = 0.014
Shoulder circumference (cm)	66.33^{a} ± 5.78	70.85^{b} ± 5.29	p = 0.014
Buttock circumference (cm)	56.17^{a} ± 4.01	59.00^{b} ± 4.32	p = 0.038

Means with different superscripts in the same row differed significantly at the 5% test level.

4.4. Carcass measurements between the 12th and 13th rib

The carcass measurements between the 12th and 13th rib, were used to determine the yield, grade and quality of the meat, relating to the palatability of the lamb. In other words, the cutability, which refers to the percentage of closely trimmed and boneless cuts.

The measurements between the 12th and 13th rib are presented in Table 4.4. The fat thickness of the 1st position (45 mm) and 2nd position (110 mm) differed significantly (p < 0.011) and (p = 0.039), respectively. The wet carcasses had, in both cases, significantly thinner backfat compared to the normal carcasses. Thickness of fat affects the cutability of the meat, too little fat is also not desirable, because fat is needed to cover the carcass to avoid the meat drying out or shrink during cooling. The very thin fat layer on the wet carcasses was, therefore, a negative characteristic from a meat quality point of view.

The eye muscle width, depth, area and perimeter showed a significant difference between the normal and WCS carcasses (p < 0.05) (Table 4.4). The significantly higher values for these attributes from the wet carcasses were positive from a meat quality point of view. All of them implied a higher edible meat content in the WCS carcasses. The eye muscle area is also known as ribeye area (REA) and is used as a muscling indicator, describing the muscle to bone proportion, which usually range from

968 to 2581 mm^2 according to the United States Department of Agriculture (O'Rourke et al., 2010). The retail product yield increases as the area of the ribeye increases, showing a direct relationship. The REA is affected by the muscle ratio and weight of the live animal.

The pH, temperature, water activity (a_w) and water holding capacity (WHC) were all taken after 24 h and showed no significant differences between the normal and affected carcasses with p-values of 0.450, 0.251, 0.950 and 0.572, respectively (Table 4.4). One of the more interesting observations, was the lack of significant differences in water activity between normal and wet carcasses. The belief that WCS meat is more prone to bacterial growth due to its high water activity (Van der Westhuizen et al., 2019), therefore, does not hold. As mentioned in the literature review, pathogenic and spoilage bacteria grow at a a_w higher than 0.85 and meat has an average a_w of 0.95 – 0.99 (Gall, 2017). Table 4.5 showed that the a_w for normal and WCS-affected meat were exactly the same, dismissing the claim of a potential higher microbial load associated with WCS meat due to a higher a_w.

The water holding capacity (WHC), which was determined by the ratio of the liquid released by compressing the meat and the size of the meat particle used, also had no significant difference between the WCS and normal carcasses (p = 0.572) (Table 4.4). Water holding capacity influences the visual appearance of meat, which impacts the consumer who dislikes meat with a wet appearance (Warner, 2017).

Table 4.4

Carcass measurements (mean ± standard deviation) between the 12th and 13th rib of randomly selected normal versus wet carcass syndrome-affected carcasses at KLK abattoir in Upington (2019 to 2022).

Measurements	**Normal carcasses (n = 20)**	**Wet carcasses (n = 20)**	**Sign. level**
Fat Thickness (45 mm)	1.48^b ± 1.09	0.75^a ± 0.53	p = 0.011
Fat thickness (110 mm)	2.42^b ± 1.85	1.38^a ± 1.14	p = 0.039
Eye muscle width (mm)	53.67^a ± 4.46	57.07^b ± 4.58	p = 0.023
Eye muscle depth (mm)	24.75^a ± 3.30	28.96^b ± 4.03	p < 0.001
Eye muscle area (mm^2)	1047.65^a ± 212.43	1362.36^b ± 216.45	p < 0.001
Eye muscle perimeter (mm)	144.02^a ± 12.76	164.28^b ± 12.64	p < 0.001
$pH_{24\ h}$	5.65^a ± 0.10	5.68^a ± 0.12	p = 0.450
$Temp_{24\ h}$ (˚C)	7.85^a ± 2.85	8.99^a ± 3.34	p = 0.251
a_w	0.95^a ± 0.02	0.95^a ± 0.02	p = 0.950
Water Holding Capacity (Meat Area / Total Area) (mm)	0.35^a ± 0.04	0.35^a ± 0.05	p = 0.572

Means with different superscripts in the same row differed significantly at the 5% test level.

4.5. Proximate analysis

The proximate analysis is shown in Table 4.5, where the percentage dry matter (DM), moisture, protein, fat, organic matter and ash were calculated for carcasses unaffected and affected by WCS. The proximate analysis showed a significant difference for all the parameters tested ($p < 0.001$). The normal carcasses had a significantly higher percentage of DM, protein, fat, OM and ash compared to the wet carcasses. According to the findings of Villatoro et al. (2021), DM, moisture, protein, fat and ash were 23.34, 70.94, 21.35, 5.83, 0.91, respectively, in lamb carcasses.

However, the wet carcasses had a significantly ($p < 0.001$) higher moisture content than the normal carcasses (Table 4.5). According to Villatoro et al. (2021), the average proximate composition for raw lamb is 70.94% moisture. Both the normal and affected carcasses had a higher moisture content. Meat with high moisture and low DM content is expected to have less fat (Sawka, 1992).

The protein content for raw lamb is around 21.35% (Villatoro et al., 2021). Both the WCS-affected and normal carcasses had a lower quantity with normal carcasses having 20.71% and the affected carcasses 19.96%. South African Damara and Dorper lambs with lower fat percentage have higher moisture content (Tshabalala et al., 2003).

Table 4.5

Proximate composition (mean percentage ± standard deviation) of *M. longissimus thoracis* for randomly selected normal carcasses and carcasses affected with wet carcass syndrome.

Composition (%)	Normal carcasses (n = 20)	Wet carcasses (n = 20)	Sign. level
Dry matter (DM)	$24.72^{b} \pm 1.18$	$22.97^{a} \pm 0.75$	$p < 0.001$
Moisture	$75.28^{a} \pm 1.18$	$77.03^{b} \pm 0.75$	$p < 0.001$
Protein	$20.71^{b} \pm 0.62$	$19.96^{a} \pm 0.47$	$p < 0.001$
Fat	$2.56^{b} \pm 0.93$	$1.58^{a} \pm 0.82$	$p < 0.001$
Organic matter (OM)	$22.77^{b} \pm 1.23$	$21.35^{a} \pm 0.62$	$p < 0.001$
Ash	$1.95^{b} \pm 0.29$	$1.55^{a} \pm 0.31$	$p < 0.001$

Means with different superscripts in the same row differed significantly at the 5% test level.

4.6. Fatty acids composition

For a long time, red meat, such as lamb/mutton, was considered unhealthy because of the high fat content and saturated fatty acid content of the meat. Saturated fatty acids have been linked to various diseases such as obesity, cardiovascular diseases and hypertension (Webb & O'Neill, 2008). Professional health experts have advised consumers to increase their n-3 polyunsaturated fatty acids, while decreasing their intake of saturated fatty acids (Griel & Kris-Etherton, 2006).

The fatty acids are the main energy source of the body and give an indication of the animals' diet (Murariu et al., 2023). The fatty acid composition has an influence on the tissue firmness, affecting the hardness of the fat, which is due to the different melting points of the fatty acids. The shelf life of the meat is affected by fat oxidation, which increases the rancidity and deteriorate the colour of the meat. The fatty acid composition also affects the flavour, tenderness and juiciness of the meat (Wood, 2004).

Table 4.6 shows the different fatty acids composition in WCS-affected and WCS unaffected carcasses. The fatty acids which were significantly ($p < 0.05$) higher in WCS carcasses included: $C_{11:0}$, $C_{13:0}$, $C_{14:1}$ *c*9, $C_{18:2}$ *c*9,12 (n-6), $C_{18:3}$*c*9,12,15 (n-3), $C_{20:4}$*c*5,8,11,14 (n-6), $C_{20:5}$*c*5,8,11,14,17 (n3) $C_{22:5}$*c*7,10,13,16,19 (n-3). Fatty acid composition in WCS meat has never been evaluated before and the values, therefore, obtained in this study, could not be compared to any previous studies. One can only speculate the difference in fatty acids in phospholipids of the cell membranes may have an influence in the movement of the water between the cells of the normal vs WCS meat.

Hendecanoic acid ($C_{11:0}$) is an unsaturated fatty acid that can also be a food and plant toxin. It is primarily found in the black elderberry. These elderberries are known to be toxic to farm animals including sheep due to the cyanogenic glycosides and toxic alkaloids in the plant (Plant Addicts, 2023).

Tridecanoic acids ($C_{13:0}$) are known to be animal, food and plant toxins. They play a role in metabolizing plant products (National Center for Biotechnology Information, 2023a).

Myristoleic acid ($C_{14:1}$ *c*9) has been isolated from the plant *Serenoa repens* which has apoptosos-inducing and cytotoxic effects. This acid is also a plant metabolite and a carboxylesterase inhibitor (National Center for Biotechnology Information, 2023b).

Linoleic acid ($C_{18:2}$*c*9,12 (n-6)) is the largest n-6 fatty acid and occurs mostly in plant glycosides and acts as a plant metabolite. Grass fed lamb are high in linoleic acid which is essential for biological processes of sheep (Jauregui, 2023).

Alpha-linolenic acid ($C_{18:3}$ n-3) (ALA) is an essential fatty acid that is found in grasses, thus related to the animals' diet (Pattison, 1990). The ALA is essential for human growth and development, it is able to decrease the risk of heart disease by regulating the rhythm and pumping of the heart (https://www.webmd.com/vitamins/ai/ingredientmono-1035/alpha-linolenic-acid-ala, retrieved on 25 June, 2023).

Table 4.6

Composition of 23 (mean percentage fatty acid methyl esters of total fatty acids ± standard deviation) of *M. longissimus thoracis* for randomly selected normal carcasses and carcasses affected with wet carcass syndrome.

Fatty acid methyl esters (%)		Normal	Wet	Sign. level
Common name	**Abbreviation**	**carcasses (n = 20)**	**carcasses (n = 20)**	
Hendecanoic	$C_{11:0}$	0.39[a] ± 0.27	0.60[b] ± 0.24	p = 0.013
Lauric	$C_{12:0}$	0.11 ± 0.18	0.12 ± 0.18	p = 0.963
Tridecoic	$C_{13:0}$	0.31[a] ± 0.25	0.50[b] ± 0.21	p = 0.013
Myristic	$C_{14:0}$	3.06 ± 1.27	3.24 ± 1.67	p = 0.694
Myristoleic	$C_{14:1}$ *c*9	0.33[a] ± 0.15	0.50[b] ± 0.14	p = 0.001
Pentadecylic	$C_{15:0}$	0.62 ± 0.18	0.64 ± 0.22	p = 0.749
Palmitic	$C_{16:0}$	24.17 ± 2.30	23.58 ± 2.41	p = 0.436
Palmitoleic	$C_{16:1}$ *c*9	1.90 ± 0.42	1.76 ± 0.36	p = 0.242
Margaric	$C_{17:0}$	1.36 ± 0.28	1.30 ± 0.23	p = 0.433
Heptadecenoic	$C_{17:1}$ *c*10	0.69 ± 0.16	0.65 ± 0.18	p = 0.495
Stearic	$C_{18:0}$	20.16 ± 3.50	18.64 ± 2.05	p = 0.103
Vaccenic, trans	$C_{18:1}$ *t*11	2.16 ± 0.73	2.11 ± 0.75	p = 0.843
Oleic	$C_{18:1}$ *c*9	34.02 ± 2.93	32.62 ± 2.61	p = 0.118
Vaccenic, cis	$C_{18:1}$ *c*7	0.97 ± 0.17	1.07 ± 0.14	p = 0.059
Nonoadecanoic	$C_{19:0}$	0.09 ± 0.14	0.06 ± 0.14	p = 0.502
Linoleic	$C_{18:2}$ *c*9,12 (n-6)	3.71[a] ± 1.77	4.91[b] ± 1.54	p = 0.027
Arachidic	$C_{20:0}$	0.18[b] ± 0.20	0.06[a] ± 0.14	p = 0.036
α-Linolenic	$C_{18:3}$*c*9,12,15 (n-3)	1.11[a] ± 0.45	1.47[b] ± 0.59	p = 0.039
Conjugated linoleic acid	$C_{18:2}$*c*9*t*11 (n-6) (CLA)	0.52 ± 0.19	0.39 ± 0.26	p = 0.074
Behenic	$C_{22:0}$	0.50 ± 0.30	0.65 ± 0.47	p = 0.223
Arachidonic	$C_{20:4}$*c*5,8,11,14 (n-6)	1.39[a] ± 0.61	2.19[b] ± 0.93	p = 0.003
Eicosopentaenoic	$C_{20:5}$*c*5,8,11,14,17 (n-3)	0.62[a] ± 0.51	0.99[b] ± 0.56	p = 0.035
Docosapentaenoic	$C_{22:5}$*c*7,10,13,16,19 (n-3)	0.56[a] ± 0.37	0.97[b] ± 0.48	p = 0.004
Fatty acid ratios:				
Total saturated fatty acids (SFA)		51.66 ± 3.17	50.07 ± 3.50	p = 0.140
Total mono-unsaturated fatty acids (MUFA)		40.17 ± 2.90	38.74 ± 2.62	p = 0.109
Total poly-unsaturated fatty acids (PUFA)		8.17[a] ± 4.10	11.08[b] ± 3.76	p = 0.025
Total omega-6 fatty acids (n-6)		5.79[a] ± 2.67	7.57[b] ± 2.25	p = 0.028
Total omega-3 fatty acids (n-3)		2.38[a] ± 1.48	3.70[b] ± 1.83	p = 0.016
PUFA/SFA		0.16[a] ± 0.09	0.23[b] ± 0.09	p = 0.032
PUFA/MUFA		0.21[a] ± 0.12	0.29[b] ± 0.11	p = 0.036
n-6/n-3		2.60 ± 0.63	2.37 ± 0.68	p = 0.264

Atherogenicity Index	0.75 ± 0.15	0.72 ± 0.20	p = 0.540
Δ^9 Desaturase index	1.67 ± 0.32	1.77 ± 0.22	p = 0.253

Means with different superscripts in the same row differed significantly at the 5% test level.

Arachidic acid ($C_{20:0}$) was significantly (p = 0.036) lower in WCS carcasses than unaffected carcasses. Arachidic acid is a saturated fatty acid. Of all the fatty acids that showed a significant difference, this is the only one where the normal carcasses had a higher level than the wet carcasses.

Arachidonic acid ($C_{20:4}c$5,8,11,14 (n-6)) is a polyunsaturated fatty acid, that becomes an essential part of the animal diet if there is a deficiency in linoleic acid. This is because linoleic acid is converted to arachidonic acid.

Docosapentaenoic acid ($C_{22:5}c$7,10,13,16,19 (n-3)) is an essential fatty acid that supports the formation of healthy blood vessels and maintain platelet aggregation (XtendLife Natural Products, 2015).

Eicosapentaenoic acid ($C_{20:5}c$5,8,11,14,17 (n-3)) is an omega-3 polyunsaturated fatty acid that lowers the risk of heart disease (Brinton & Mason, 2017).

Of the 9 fatty acids that showed significant differences between WCS and normal carcass, eight of these had higher levels of fatty acids in the WCS, except for arachidic acid. The WCS carcasses had a significantly lower proximate fat content compared to normal carcass content. If meat contain less fat, the fatty acid profile becomes more unsaturated, which explained why the WCS had more unsaturated fatty acids that were higher than the normal carcass.

The ratio of PUFA/SFA is naturally 0.1 in ruminants. Research has been done to increase this to 0.4, which is recommend for healthy fat (Wood et al., 2004). In Table 4.7, the normal carcasses had a significantly (p = 0.032) lower value than WCS. This indicated that the WCS had more healthy fat compared to normal carcasses, however, both these values where above 0.12, which is classified as healthy meat (Hoffman et al., 2003).

4.7. Carcass microbial quality at abattoir

Swabs were taken from all WCS-affected and unaffected carcasses at the abattoir and evaluated for total bacteria count (TBC), *Enterobacteriaceae* count, lactic acid bacteria (LAB) count, *Pseudomonas* count, yeast and mould count, *Staphylococcus aureus* (*S. aureus*) count and *Escherichia coli* (*E. coli*) count. The microbial limits suggested by SA DAFF (2018) for carcasses and meat cuts of cattle, sheep, goats and horses for TBC, *Enterobacteriaceae* and *E. coli* are 3.5 – 5.0 log cfu/cm^2, 1.5 – 2.5 log cfu/cm^2 and 0 – 1 log cfu/cm^2, respectively.

The results are given in Table 4.7. There were no significant differences between the WCS-affected and WCS-unaffected carcasses in the counts of TBC, LAB, *S. aureus* and yeast and mould having p-values > 0.05. No *E. coli* or *Enterobacteriaceae* were detected in this study on any of the 40 carcasses (Table 4.7). *Enterobacteriaceae* and *E. coli* counts are indicators of fecal contamination of a food product (Health Protection Agency, 2009). The undetected numbers of these bacteria were indicative of very hygienic slaughtering processes at the KLK abattoir. The carcasses go through a washing process in the abattoir which could also explain a low microbial growth, however, this water could also lead to an increase in microbial growth if the carcasses were not dried quickly.

Since *Pseudomonas* spp. are regarded major spoilage bacteria of meat (Stellato et al., 2017) it was decided to include this bacterial parameter in this study. However, no *Pseudomonas* was detected in this study on any of the 40 carcasses evaluated at the abattoir (Table 4.7).

All the parameters were well below the legal limits of counts set by the South African Department of Agriculture, Forestry and Fisheries (SA DAFF, 2018). These findings contradicted the idea that the excess moisture of the WCS carcasses will result in high microbial counts (Van der Westhuizen et al., 2019).

Table 4.7

Microbial counts (mean log cfu/cm^2 ± standard deviation) of randomly selected normal carcasses versus Wet Carcass Syndrome-affected carcass surfaces.

Microbial count (log cfu/cm^2)	**Normal carcasses (n = 20)**	**Wet carcasses (n = 20)**	**Sign. level**
Total bacteria	2.01^a ± 0.88	2.00^a ± 0.85	p = 0.979
Lactic acid bacteria	0.77^a ± 0.44	0.85^a ± 0.65	p = 0.656
Staphylococcus aureus	0.29^a ± 0.36	0.32^a ± 0.54	p = 0.847
Yeast and mould	0.79^a ± 0.60	0.90^a ± 0.64	p = 0.958
Escherichia coli	ND	ND	NSA

Enterobacteriaceae	ND	ND	NSA
Pseudomonas	ND	ND	NSA

Means with different superscripts in the same row differed significantly at the 5% test level; ND = Not detected; NSA = Not statistically analyzed.

4.8. Shelf-life of lamb chops

The shelf-life of three loin chops, between the left 9th rib to the 12th rib, of each of the 40 lamb carcasses, stored at refrigeration temperature (4 ^{o}C) were evaluated by microbial quality parameters and colour analysis on days 0, 3 and 6. The aim was to determine whether WCS chops were comparable to normal carcass chops in terms of microbial and colour quality for human consumption.

4.8.1. Chops microbial parameters

There were no significant differences between the WCS-affected chops and normal carcass chops for the TBC, LAB, *S. aureus*, yeast and mould and *Pseudomonas* counts on days 0, 3 or 6 at 4 ^{o}C (Table 4.8). Coliforms and *Enterobacteriaceae* were not detected in any of the chops during the 6day storage period. This was an indication of good hygienic practices during the cutting of the chops (Health Protection Agency, 2009).

The microbial limits suggested by SA DAFF (2018) for carcasses and meat cuts of cattle, sheep, goats and horses for TBC, *Enterobacteriaceae* and *E. coli* are 3.5 – 5.0 log cfu/cm^2, 1.5 – 2.5 log cfu/cm^2 and 0 – 1 log cfu/cm^2, respectively. The TBC for both normal and WCS-affected chops in this study ranged between 1.40 log cfu/cm^2 on day 0, to 1.08 log cfu/cm^2 on day 6. By fitting a regression line for TBC normal versus WCS at day 6 (Fig. 4.4), the TBC slope ($\frac{\Delta x}{\Delta y}$) = 1, implying that there were no differences between the wet and normal values at day 6. The correlation fit was 100% (R^2 = 1, p< 0.0001). *Enterobacteriaceae* and *E. coli* were not detected on days 0, 3 or 6. The microbial counts were much lower than the microbial limits set by SA DAFF (2018). The microbial quality of all the chops in this study were, therefore, very high and the microbial quality of the WCS affected chops were comparable to the normal chops during 6 days of refrigerated storage.

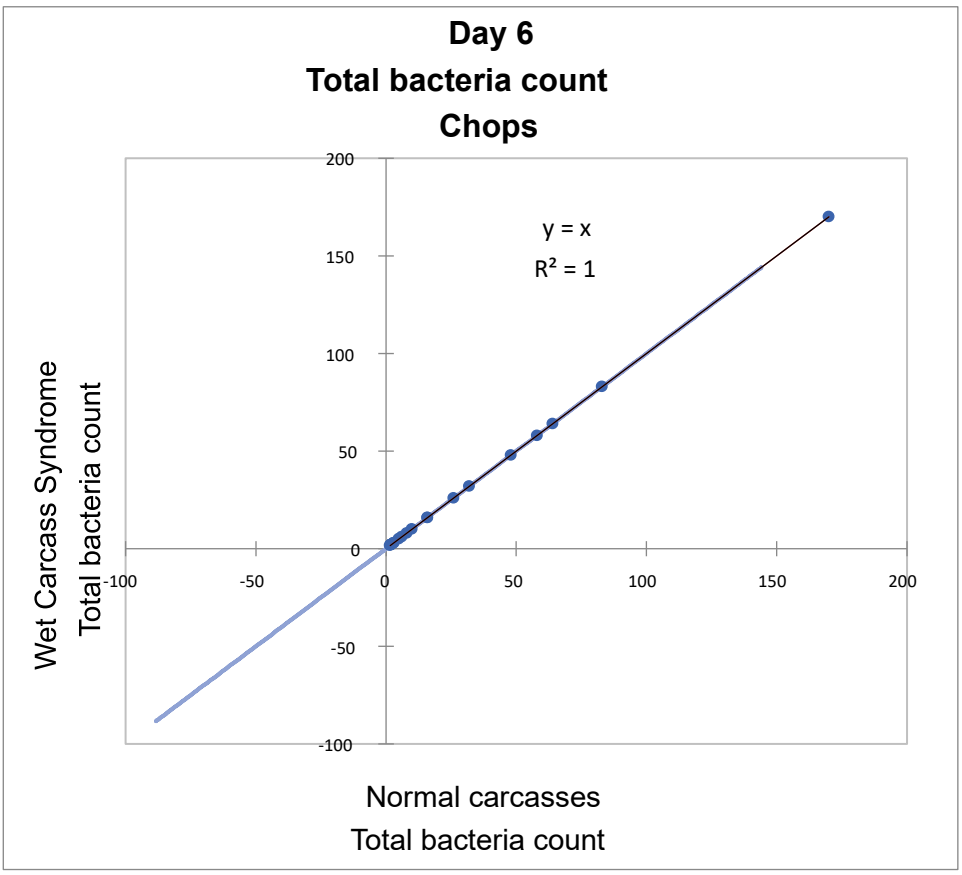


Fig. 4.4. Regression analysis of total bacterial count on day 6 for WCS vs normal carcasses.

Most lactic acid bacteria (LAB) grow in anaerobic conditions, but have a high tolerance to oxygen (Zotta et al., 2017). They are generally considered safe micro-organisms and not a major contributor to raw carcass spoilage. LAB can become dominant spoilage organisms when the growth of aerobic bacteria is inhibited under vacuum packed conditions (McSharry et al., 2021). The spoilage of meat by LAB can be seen by a change of colour, off-odour and slime on the surface of the meat (Karki, 2020). LAB are known to outcompete the growth of Gram-negative aerobic organisms, thus the growth of organisms such as pseudomonas are prevented (Egan, 1983; Li et al., 2023). The values for LAB in this study ranged between 0.38 log cfu/cm^2 on day 0, to 0.57 log cfu/cm^2 on day 6 for both normal and WCS-affected chops (Table 4.8). According the International Commission on Microbiology Specifications for Food (ICMSF) the limit of lactic acid bacteria count on fresh meat is 7 log cfu/cm^2 (Pothakos et al., 2015).

Pseudomonas counts do not normally form part of microbial quality parameters; however, it was decided to include it in this study. *Pseudomonas* are well-known spoilage bacteria of meat during refrigeration because of their psychrotolerant capabilities (Stellato et al., 2017). The counts of *Pseudomonas* in food should be < 2 log cfu/cm^2, this ensures acceptable sensory attributes and

increases the shelf life of the food (https://mb-labs.com/resources/pseudomonas-spoilage organism, 2023).

Staphylococcus aureus was present on both the normal and WCS-affected chops on days 0, 3 and 6 with low values, ranging between 0.13 log cfu/cm^2 on day 0, to 0.10 log cfu/cm^2 on day 6 (Table 4.8). Although no microbial limits are set for *S. aureus* by SA DAFF (2018), limits suggested by other studies range between < 1.0 – 3.0 log cfu/cm^2 (Shapton & Shapton, 1991). The presence of *S. aureus* would indicate contamination with skin or mucous membranes from either humans or animals (Ekici & Dümen, 2019; Wu et al., 2018).

Yeast and mould counts were also evaluated in this study since the presence of yeast and mould are associated with insufficient hygienic practices (Herrera, 2001). There are no microbial limits set for these organisms by SA DAFF (2018), however, according to Shapton and Shapton (1991) yeast and mould counts for fresh meat should be less than 3 log cfu/cm^2. While the contamination of meat carcasses by yeast and mould has been studied, the primary spoilage bacteria dominate most research (Dillon & Board, 1991). Spoilage by yeast and mould can be identified by bad taste, sliminess, off-odour, discolouration and rancidity (Karki, 2020). The yeast and mould counts in this study on both the normal and WCS-affected chops, ranged from 0.34 log cfu/cm^2 on day 0, to 0.20 log cu/cm^2 on day 6 (Table 4.8), which were much lower than what was proposed (Shapton & Shapton, 1991).

All the microbial analyses results indicated that both the normal and WCS-affected chops conformed to all suggested microbial limits and were of a very high microbial standard. No significant correlations have been detected for the other variables of the chops and could not conclude that WCS chops were significantly worse than normal chops. This study also indicated that the normal and WCS-affected chops microbial values were comparable to each other and may, therefore, be accepted for human consumption according to the limits set by SA DAFF (2018). However, from a visual point of view, the chops from the WCS-affected carcasses were not visually appealing (Fig. 4.5a) and will most likely be rejected by consumers. The fat can be seen as slimy and falling off the bone. The fat of normal carcasses is shown in Fig. 4.5b, which was firm and the shape of the chop was appealing. The meat of the WCS can, however, be processed and used in other forms, since the microbial quality will not be a limiting factor. It was, therefore, decided to produce a pet mince from the WCS-affected meat.

WCS lamb chops will immediately be rejected by consumers, thus processing the meat into products like pet mince is essential. Processed products can easily be manipulated to increase or decrease certain attributes like the pH using hurdle technology. Hurdle technology include chemical, physical

and biological factors that can be used on processed meat to limit microbial spoilages and enhance quality attributes (Mukhopadhyay & Gorris, 2014). These hurdles include temperature (low or high), acidity (pH), preservatives (i.e., nitrite), water activity (a_w), competitive microorganisms (i.e., lactic acid bacteria) and redox potential (Eh) (Gragg & Brashears, 2022).

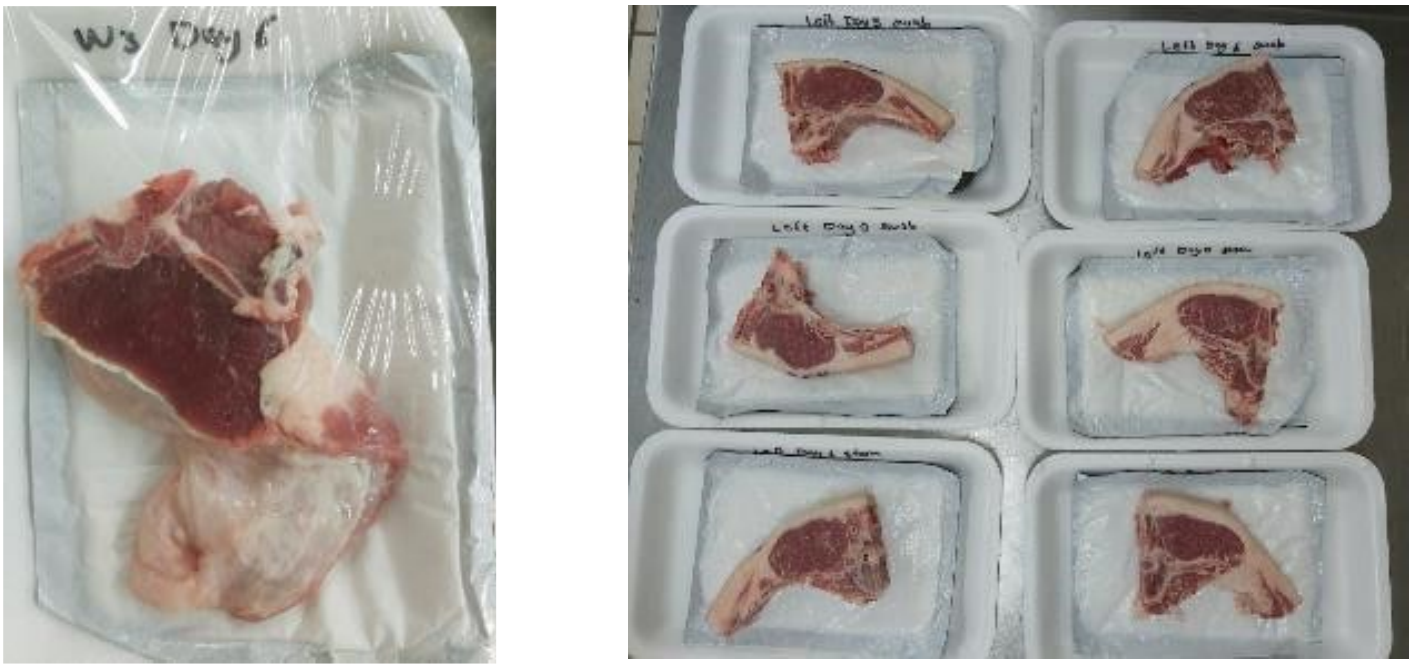


Fig. 4.5. (a) Lamb chop from a Wet Carcass Syndrome carcass, and (b) Lamb chop from normal carcasses.

Table 4.8

Microbial counts (mean log cfu/cm^2 ± standard deviation) of lamb chops from randomly selected normal carcasses versus Wet Carcass Syndrome affected carcasses, displayed for 6 days at 4 °C.

Days on display	**Treatment**	**TBC (log cfu/cm^2)**	**Lactic acid Bacteria (log cfu/cm^2)**	***S. aureus* (log cfu/cm^2)**	**Yeast and mould (log cfu/cm^2)**	***Coliforms* (log cfu/cm^2)**	Enterobacteriaceae **(log cfu/cm^2)**	Pseudomonas **(log cfu/cm^2)**
0	Normal carcass (n = 20)	1.38^a ± 0.62	0.38^a ± 0.22	0.13^a ± 0.17	0.33^a ± 0.31	ND	ND	0.24^a ± 0.19
	Wet carcass (n = 20)	1.40^a ± 0.56	0.44^a ± 0.36	0.17^a ± 0.18	0.34^a ± 0.40	ND	ND	0.23^a ± 0.18
3	Normal carcass (n = 20)	1.07^a ± 0.44	0.48^a ± 0.20	0.13^a ± 0.17	0.32^a ± 0.27	ND	ND	0.18^a ± 0.16
	Wet carcass (n = 20)	1.12^a ± 0.48	0.49^a ± 0.24	0.12^a ± 0.15	0.27^a ± 0.25	ND	ND	0.17^a ± 0.16
6	Normal carcass (n = 20)	1.02^a ± 0.64	0.55^a ± 0.30	0.14^a ± 0.18	0.20^a ± 0.23	ND	ND	0.13^a ± 0.17
	Wet carcass (n = 20)	1.08^a ± 0.67	0.57^a ± 0.29	0.10^a ± 0.17	0.28^a ± 0.29	ND	ND	0.14^a ± 0.17
Sign. Level		p = 0.152	p = 0.315	p = 0.899	p = 0.684	NSA	NSA	p = 0.235

Means with different superscripts in the same column differed significantly at the 5% test level; ND = Not detected; NSA = Not statistically analyzed.

4.8.2. Colour of Chops

Table 4.9 shows the colour analysis of the lamb chops from normal and WCS-affected carcasses. *L**, indicating lightness, on day 0, there were no significant differences in *L** values between the normal and the wet carcass syndrome meat. From day 3 onwards, the normal and wet carcasses had significantly ($p < 0.001$) higher *L** values compared to day 0, but there were no significant differences between the normal and WCS carcasses on days 3 and 6.

The *a**- value represents the redness of the meat and is also normally regarded the most important colour parameter in red meat. The normal and wet carcasses on day 0 had significantly ($p < 0.001$) higher *a** values (redder meat) at day 0 than the normal and wet carcasses on days 3 and 6. The heme protein, known as myoglobin, is responsible for the red colour of meat. The oxidation of this protein results in a colour change from red to brown, which explains the decrease in redness (a*) with time (Faustman et al., 2010). There were, however, no significant differences between the normal and WCS carcasses on days 0, 3 and 6.

On average, the *L** and *a** value in red meat, should be equal to or higher than 34 and 9.5, respectively, for consumers to approve of the colour. With values higher than 44 and 14.5, there is a 95% likelihood that consumers will approve of the product (Khliji et al., 2010). In Table 4.9, the *L**value was above 44 from days 0, 3 and 6, while the *a**-value was only above 14.5 on day 0 for the normal carcass chops. However, all the *a**-values were above 9.5, indicating that the colour was acceptable for both the normal and the WCS chops.

The *b** value represents the yellow/blue colour, where *b** positive is yellow and *b** negative is blue (King et al., 2023). On day 0 the normal carcasses chops had a significantly higher *b** value compared to WCS carcasses chops. No significant differences were observed on days 3 and 6 between the two treatments. The *b** value increased from day 0 to day 6.

The Chroma (saturation index) is known as the purity of colour, where high chroma level has no black, white or gray added to it. Chroma is calculated using the *a** and *b** values (King et al., 2023). The normal carcasses chops had a significantly ($p < 0.001$) higher chroma on day 0 than WCS carcasses chops. Days 3 and 6 had no significant differences between the normal and WCS carcasses chops, while the WCS showed no significant differences during the 6-day period. The hue angle represents the redness and yellowness on a range from 0 to 360° (Scalisi et al., 2022). The data showed no significant differences between the normal and WCS carcasses chops on days 0 and 6. On day 3, the WCS chops had a significantly higher value than the normal carcass chops.

As stated earlier, the moment meat is cut, it has a purple-red colour because of the presence of myoglobin. Meat is then exposed to oxygen for 30 min to ‘bloom’. This allows the myoglobin to react to the oxygen and form oxymyoglobin, resulting in the cherry-red colour that is seen in the store. To the naked eye, the colour seems to stabilize after the blooming process, but the reaction with oxygen is a continuous reaction (Seideman et al., 1983). Over time the oxymyoglobin is oxidized to metmyoglobin and form a brownish-green colour. Thus, the *a**-value will decrease and the *b**-value will increase as the red colour becomes brown-greenish (Moss, 2006). A study performed by Hood and Riordan (1973) showed that consumers rejected meat if 20% of the oxymyoglobin has been oxidized to metmyoglobin. These measurements can, however, not be used 100% accurately on consumer acceptance. A study on PSE pork chops, showed that consumers preferred the pork with PSE due to the pale colour. However, over time, the PSE pork started to show other signs of defects, such as drip loss, which resulted in consumers rejecting the meat (Moss et al., 2000).

Table 4.9

Physical colour properties (mean ± standards deviation) of lamb chops from randomly selected normal carcasses versus Wet Carcass Syndrome affected carcasses, displayed for 6 days at 4 °C.

Days on display	Treatment	Colour *L** - Value	Colour *a** - Value	Colour *b** - Value	Chroma (Saturation Index)	Hue Angle
0	Normal carcasses (n = 20)	$46.01^{a} \pm 3.06$	$15.16^{b} \pm 1.96$	$4.16^{b} \pm 1.52$	$15.72^{b} \pm 2.16$	$14.82^{a} \pm 3.91$
	Wet carcasses (n = 20)	$45.17^{a} \pm 2.52$	$14.44^{b} \pm 1.69$	$3.31^{a} \pm 1.52$	$14.82^{a} \pm 1.92$	$12.33^{a} \pm 4.04$
3	Normal carcasses (n=20)	$47.89^{b} \pm 2.90$	$12.62^{a} \pm 1.48$	$7.38^{c} \pm 1.19$	$14.66^{a} \pm 1.45$	$30.33^{b} \pm 4.69$
	Wet carcasses (n=20)	$48.50^{b} \pm 2.99$	$12.13^{a} \pm 1.07$	$8.00^{cd} \pm 1.19$	$14.56^{a} \pm 1.20$	$33.35^{c} \pm 4.11$
6	Normal carcasses (n=20)	$48.66^{b} \pm 3.32$	$11.99^{a} \pm 1.86$	$8.66^{d} \pm 1.36$	$15.13^{ab} \pm 1.28$	$36.00^{d} \pm 6.18$
	Wet carcasses (n=20)	$48.15^{b} \pm 3.09$	$11.75^{a} \pm 1.80$	$8.56^{d} \pm 1.52$	$14.61^{a} \pm 1.79$	$36.15^{d} \pm 5.98$
Sign. Level		$p < 0.001$	$p < 0.001$	$p < 0.001$	$p < 0.001$	$p < 0.001$

Means with different superscripts in the same column differed significantly at the 5% test level.

4.9. Pet mince

Since the meat from the WCS-affected carcasses is not regarded visually appealing for human consumption, it was decided to produce a pet mince from this meat, in the form of a fresh sausage, that can be consumed raw or can be cooked before consumption by pets. The microbial quality and pH of the fresh pet mince product were evaluated and compared to pet mince product from normal carcasses.

4.9.1. Microbial quality

Table 4.10 shows the microbial growth over a 10-day period for the pet mince. When considering the total bacterial count, there were no significant differences between the counts of the pet mince from the normal and the WCS carcasses over the 10-day period. The TBC on the pet mince of the normal carcasses were not significantly different over the 10-day storage period at 4 °C and ranged between 2.75 ± 0.39 log cfu/g on day 0, to 3.11 ± 0.91 log cfu/g on day 10.

However, the TBC on the WCS pet mince showed a significant increase ($p < 0.001$) from day 5 to day 10. According to EU Regulation No. 142/2011 for pet food, the total bacteria count should not exceed 6 log cfu/g (European Commission, 2011). Although none of the samples exceeded this limit from day 0 to day 10, the significant increase of TBC after day 5 of storage may be an indication that the pet mince product produced form WCS-affected meat will have a best shelf-life at 4 °C up to 5 days. At day 10 (Fig. 4.6) the regression for normal versus WCS TBC slope ($\frac{\Delta y}{\Delta x}$) = 0.97, an almost 100% ratio between the two variables, implying a similar bacterial load for the wet and normal pet mince products at day 10. The correlation fit was 88% ($R^2 = 0.8797$, $p < 0.0001$).

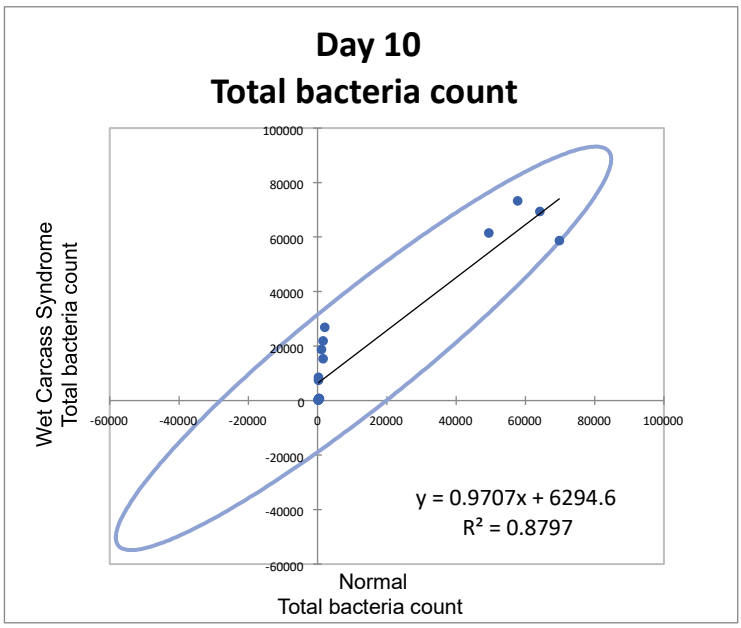


Fig. 4.6. Regression analysis of total bacterial count on day 10 for WCS vs normal carcasses.

The LAB counts in Table 4.10 showed no significant differences in LAB numbers for both normal carcass and WCF carcass pet mince, except for day 10 normal mince, which had a significantly ($p < 0.001$) lower LAB count compared to all other treatments. This could be because of a lab error. The regulations set by European Commission state the growth of LAB should not be higher than 6 log cfu/g. The growth during the 10-day period stayed well below this limit with numbers ranging between 1.61 ± 0.55 log cfu/g (normal pet mince on day 10) to 2.36 ± 0.64 log cfu/g (WCS pet mince on day 10) (European Commission, 2011).

Pseudomonas are proteolytic organisms, that break down protein, leading to off-flavours and odours. These organisms are known to cause slime and a green pigment on the surface of meat (https://mblabs.com/resources/pseudomonas-spoilage organism, 2023). *Pseudomonas* is considered a main spoilage organism in meat products, this is because they are psychrotolerant microorganisms which can grow at refrigerated temperatures (Wickramasinghe et al., 2019). There are no microbial limits available for *Pseudomonas* counts in meat products, but lower numbers will ensure a longer shelf life of the product (https://mb-labs.com/resources/pseudomonas-spoilage organism, 2023). There were no significant differences between the counts of *Pseudomonas* in WCS and normal carcass pet mince on any day of the 10-day storage period (Table 4.10). The only significant ($p = 0.002$) differences in *Pseudomonas* counts, were between the 5 days WCS pet mince and the Day 0 and Day 10 Normal and WCS pet mince.

Given the fact that LAB are known to outcompete and/or inhibit *Pseudomonas* (Egan, 1983; Li et al., 2023), it was interesting to note the association of these organisms in the current study. Through regression analysis, activity (counts) of the two organisms confirmed the negative relationship, although accounting for only 2% of the variation in the data for both normal and WCS scenarios (Fig. 4.7 and 4.8).

Staphylococcus aureus contamination is one of the leading causes of foodborne disease in the world. In the United States of America, *S. aureus* is on average involved in 241,000 food illnesses every year, while 12.5% of foodborne illnesses in China, is caused by *S. aureus* (Wu et al., 2018). The *S. aureus* count showed no significant differences between the normal and WCS pet mince on days 0, 5 or 10 (Table 4.11). Day 0 normal carcass pet mince had significantly ($p < 0.001$) lower *S. aureus* counts compared to day 10 of wet carcass samples. Day 0 WCS pet mince showed significantly higher ($p < 0.001$) *S. aureus* counts compared to day 5 of normal carcasses. Day 5 normal had significantly ($p < 0.001$) lower *S. aureus* counts compared to day 10 normal and wet carcass pet mince. Day 5 WCS had significantly ($p< 0.001$) lower *S. aureus* counts compared to day 10 WCS. The growth followed the same pattern as the TBC growth, with a decrease from day 0 to 5 and an increase from day 5 to 10. The growth of *S. aureus* was well under the limit of 2 log cfu/g (Kananub et al., 2020), showing no contamination risks.

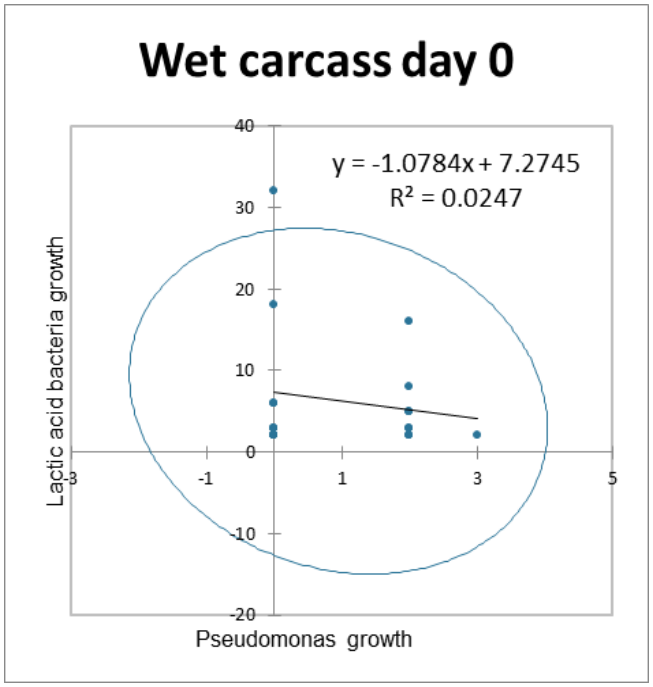


Fig. 4.7. Regressions analysis of counts of lactic acid bacteria vs *Pseudomonas* on wet carcass pet mince on day 0.

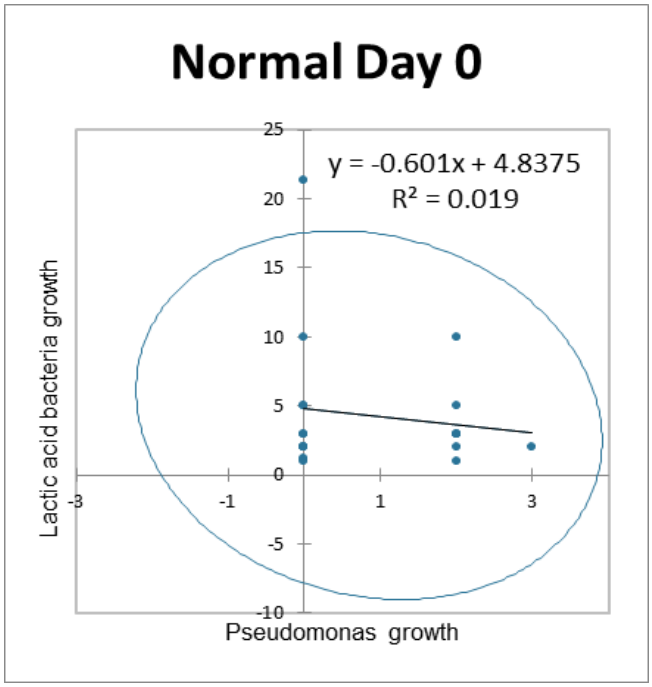


Fig.4.8. Regressions analysis of lactic acid bacteria vs *Pseudomonas* on normal carcass pet mince on day 0.

Coliforms are indicators of hygiene. Indicator organisms are used as markers. Their presence indicate possible contamination, occurrence of similar organisms (pathogens), poor processing and/or poor quality of raw materials. Coliforms are most widely used as measures of faecal contamination (Halkman & Halkman, 2014). The coliform count ranged from 0.39 ± 0.33 log cfu/g (WCS carcass pet mince on day 5) and 0.98 ± 0.69 log cfu/g (normal carcass pet mince on day 10) which conformed to microbial limits of < 2.7 log cfu/g (European Commission, 2005). The coliform count of both the normal and WCS carcass pet mince was significantly ($p = 0.014$) higher on day 10 than on day 5. This finding indicated that the shelf-life of both products is best up to 5 days of storage at 4 °C.

According to European Commission (EU) regulations, the numbers of *Enterobacteriaceae* should not exceed 3.7 log cfu/g (Serhan et al., 2022). The numbers of both the WCS and normal mince stayed well below these limits during the 10-day shelf-life test, with values ranging between 0.28 ± 0.35 log cfu/g (normal pet mince on day 5) to 0.78 ± 0.77 log cfu/ g (WCS pet mince on day 10).

There was also no significant difference between the WCS and normal carcasses *Enterobacteriaceae* counts over the 10-day storage period. There was, however, a significant ($p < 0.01$) increase in *Enterobacteriaceae* counts between day 5 of the normal carcass pet mince and day 10 of the normal carcass and wet carcass pet mince. This increase of numbers from day 5 to day 10, was another confirmation that the shelf-life of both the normal and the WCS carcass pet mince product was compromised after 5 days of storage at 4 °C.

According to the regulations set by the EU, yeast and mould counts exceeding 6 log cfu/g classifies a product as spoiled. The yeast and mould numbers in this study never exceeded 2 log cfu/g (European Commission, 2011). The only significant ($p < 0.05$) difference in yeast and mould counts for normal and WCS pet mince, was between Day 0 of the wet carcass mince (1.60 ± 0.65 log cfu/g) and day 5 (1.20 ± 0.30 log cfu/g) and day 10 (1.18 ± 0.26 log cfu/g) of the normal mince. This might be an indication that the normal carcass pet mince was less conducive for the growth of yeast and mould than WCS carcass pet mince over time with storage at 4 °C.

Table 4.10

Effect of randomly selected normal carcasses and carcasses affected with Wet Carcass Syndrome on the microbiological counts (mean log cfu/g ± standard deviation) of pet mince stored for 10 days at 4 °C.

Days on display	Treatment	Total bacteria (log cfu/g)	Lactic acid bacteria (log cfu/g)	*Staphylococcus aureus* (log cfu/g)	Coliforms (log cfu/g)	*Enterobacteriaceae* (log cfu/g)	*Pseudomonas* (log cfu/g)	Yeast and MOULD (log cfu/g)
0	Normal carcasses (n = 20)	$2.75^{ab} \pm 0.39$	$2.50^{b} \pm 0.73$	$0.94^{ab} \pm 0.45$	$0.53^{ab} \pm 0.53$	$0.44^{ab} \pm 0.38$	$0.20^{a} \pm 0.31$	$1.44^{ab} \pm 0.40$
	Wet carcasses (n = 20)	$2.97^{abc} \pm 0.49$	$2.54^{b} \pm 0.65$	$1.06^{bc} \pm 0.42$	$0.53^{ab} \pm 0.44$	$0.36^{ab} \pm 0.35$	$0.23^{a} \pm 0.30$	$1.60^{b} \pm 0.65$
5	Normal carcasses (n = 20)	$2.55^{ab} \pm 0.23$	$2.21^{b} \pm 0.21$	$0.62^{a} \pm 0.39$	$0.44^{a} \pm 0.40$	$0.28^{a} \pm 0.35$	$0.26^{ab} \pm 0.38$	$1.20^{a} \pm 0.30$
	Wet carcasses (n = 20)	$2.51^{a} \pm 0.27$	$2.11^{b} \pm 0.17$	$0.75^{ab} \pm 0.63$	$0.39^{a} \pm 0.33$	$0.33^{ab} \pm 0.41$	$0.57^{b} \pm 0.56$	$1.50^{ab} \pm 0.30$
10	Normal carcasses (n = 20)	$3.11^{bc} \pm 0.91$	$1.61^{a} \pm 0.55$	$1.11^{bc} \pm 0.43$	$0.98^{c} \pm 0.69$	$0.74^{b} \pm 0.61$	$0.15^{a} \pm 0.25$	$1.18^{a} \pm 0.26$
	Wet carcasses (n = 20)	$3.47^{c} \pm 1.05$	$2.36^{b} \pm 0.64$	$1.39^{c} \pm 0.44$	$0.91^{bc} \pm 1.14$	$0.78^{b} \pm 0.77$	$0.13^{a} \pm 0.23$	$1.45^{ab} \pm 0.19$
Sign. level		$p < 0.001$	$p < 0.001$	$p < 0.001$	$p = 0.014$	$p = 0.004$	$p = 0.002$	$p = 0.002$

Means with different superscripts in the same column differed significantly at the 5% test level.

4.9.2 Pet mince pH

The pH of the pet mince product from normal carcasses vs WCS-affected carcasses, stored at 4 ºC for 10 days, is indicated in Table 4.11. There were no significant differences between the normal and affected carcasses on any of the storage days. The pH slowly but significantly (p = 0.001), increased from day 0 to day 10. Meat spoils quicker at a higher pH (> 6.0) (Mathew & Jaganathan, 2017), which could be a reason for the higher total bacteria counts of both products on day 10 (Table 4.11).

Table 4.11 pH (mean ± standard deviation) of the pet mince sausages produced from randomly selected normal versus wet carcass syndrome-affected carcasses, stored for 10 days at 4 °C.

Days on display	Treatment	Product pH
0	Normal carcass (n = 20)	5.71^{a} ± 0.05
	Wet carcass (n = 20)	5.75^{ab} ± 0.10
5	Normal carcass (n = 20)	5.76^{ab} ± 0.06
	Wet carcass (n = 20)	5.79^{b} ± 0.08
10	Normal carcass (n = 20)	5.79^{b} ± 0.03
	Wet carcass (n = 20)	5.80^{b} ± 0.06
Sign. Level		p = 0.001

Means with different superscripts differed significantly at the 5% test level.

Chapter 5

General Discussion and Conclusions

The occurrence of WCS in sheep is considered a major food production constraint, as affected carcasses are discarded with no financial compensation for the farmer. Prior to this study, no in-depth investigation had been conducted to evaluate the quality of such carcasses and whether these carcasses may hold some value for alternative uses. One of the aims of this study was, therefore, to determine whether the meat from WCS carcasses may be used for human and/or animal consumption. A possible causative factor of WCS may be weather conditions. According to weather data of Upington, Northern Cape (during the test period), the occurrence of WCS carcasses showed an indirect relationship with rainfall and temperature. This confirmed the notion that WCS is more prevalent during winter and drought seasons (Van der Westhuizen et al., 2019). If this is a causing factor, no prevention can be taken against it. Therefore, confirming the safety and usefulness of such meat would be beneficial to farmers and reduce financial loss.

A total of 40 lamb carcasses, 20 normal and 20 WCS-affected carcasses, were sourced from farmers in the Northern cape and Namibia. Various parameters were tested throughout the study to determine the quality WCS-affected meat. These results were compared to normal unaffected meat, originating from the same farm and slaughtered and tested on the same days. Surface microbial swabs and carcass measurements were taken at the KLK Upington abattoir and the left rib and loin was transported to the University of the Free State. The following days, various tests were conducted, including: proximate analysis, water activity, water holding capacity, water activity, eye muscle measurements, fatty acid composition and microbial growth.

The first objective of the study was to compare the carcass characteristics, functional properties and microflora from normal versus WCS-affected lamb meat carcasses.

- Carcass characteristics

From the morpho-physical examinations, carcass measurements showed significant differences in cold mass, external length, shoulder and buttock circumference. No significant differences, however, were observed in the fatness and conformation code; thus, WCS-affected carcasses were physically larger, but still had the same amount of fat around the outside of the carcass. Measurements taken between the 12^{th} and 13^{th} rib showed significant differences in fat thickness (45 and 110 mm), eye muscle width, depth, area and perimeter. These measurements showed that the fat thickness of the

WCS carcasses were thinner, but the eye muscle measurements were significantly larger. No significant differences were found for pH, temperature, water activity and water holding capacity.

- Functional properties

All the parameters tested for proximate analysis (DM, protein, moisture, fat, OM and ash) showed significant differences. DM, protein, fat, OM and ash had higher levels in the normal vs WCS carcasses. The wet carcasses had a significantly higher moisture content than the normal carcasses, which could be explained by the excess moisture that the carcasses carried. These parameters are not associated with consumer preference, as most fresh meat packaging do not display these attributes. Fatty acid composition is important because of the linkage to sensory attributes and various diseases such as obesity, cardiovascular diseases and hypertension (Webb & O'Neill, 2008). Two important classes to look at are saturated and unsaturated fatty acids. Saturated fatty acids are deemed unhealthy fat, while unsaturated fatty acids are known as healthy fat. Most of these fatty acids are related to the sheep's diet and could possibly play a role in the syndrome, as 8 of the 23 fatty acids recorded were significantly higher in the wet carcasses.

- Microflora

The main reason for discarding WCS carcasses is the belief that the excess moisture will result in a high microbial load and thus be unsafe for consumption. Various microbial parameters were quantified: total bacteria count (TBC), *Enterobacteriaceae* count, lactic acid bacteria (LAB) count, *Pseudomonas* count, yeast and mould count, *S. aureus* count and *E. coli* count. Surface swabs were taken at the abattoir before further handling of the carcasses. These results showed no significant differences in microbial parameters between the normal and WCS carcasses.

The second objective of the study was to compare (1) the microbiological shelf life and colour shelf life of lamb loin chops from normal versus WCS-affected carcasses during refrigerated display and (2) the microbiological shelf life of minced meat as a pet food, manufactured from normal versus WCS-affected meat during refrigerated storage.

- Lamb loin chop

Two products were formulated, lamb chops for human consumption and pet mince for the pet food industry. A shelf-life study was conducted at 4 ^{o}C to determine the quality over a period of 6 days for lamb chops and 10 days for pet mince. The colour of the chops and pH of the pet mince was taken

throughout the shelf-life study. The microbial load (TBC, *Enterobacteriaceae*, LAB, *Pseudomonas*, yeast and mould, *S. aureus* and *E. coli* count) on the lamb chops showed no significant differences between the WCS and normal carcasses throughout the 6 days. The microbial load was well under the legal limit for all the micro-organism growth. This showed that the meat of the WCS had the same microbial quality and was just as safe as that from the normal carcasses.

The colour analysis on the chops showed no differences in the lightness (*L**) nor the redness (*a**). There was, however, a significant difference in the blue/yellow colour on day 0, implying a greenish tint involved with WCS. This phenomenon, however, was only seen on day 0 and not throughout the 6-day experimental period.

- Pet mince

As alternative, pet mince was explored as a possible use for the meat. Microbial and pH tests were conducted for 10 days. The microbial counts again showed no significant differences between the normal and WCS-affected sources. The pH showed a similar trend with no significant differences.

This concluded that, the meat from WCS-affected carcasses was safe for human and/or animal consumption up to 5 days of storage as none of the bacterial counts evaluated, were above microbial limits. However, given the visual appearance of WCS chops (and the danger that it holds during the cutting process), such chops will be rejected by consumers and not accepted as PSE meat is.

The null hypothesis is thus rejected, the quality of WCS meat is not inferior to normal meat quality.

Future studies:

- A follow up study to monitor pH/temperature fall and the glycolytic potential measurements.
- Visual and sensory analysis to determine if there is a taste difference in the meat and if there is a market for human consumption.
- Alternative uses like processing of WCS meat into products like pet mince or processed meat products for human/animal consumption will support new commercial ventures and lessen the impact of the condition on the livestock industry.
- Potential link between WCS and PA.
- Potential link between fatty acids found in plants (sheep's diet) and their potential link to wet carcass syndrome.

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