Effects of different stunning methods on carcass characteristics and the conversion of muscle to meat in commercially farmed Nile crocodiles (*Crocodylus niloticus*)

By

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Declaration

I, Natasha van den Bergh, declare that the dissertation, which I hereby submit for the degree MScAgric (Animal Science) Production Physiology and Product Quality at the University of Pretoria, is my own work and has not previously been submitted by me for a degree at this or any other tertiary institution.

SIGNATURE:

DATE: 15 October 2023

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Abstract

From the 1950s to the 1960s crocodilians were hunted for their skins, which led to many wild populations being killed. Subsequently, all crocodilians were added to Appendix I or II of the Convention on International Trade of Endangered Species of Wild Fauna and Flora. Restocking wild populations by crocodilian breeding started in 1974, and African countries began showing an interest in commercial crocodilian production in 1984. In South Africa, Nile crocodiles (*Crocodylus niloticus*) are the preferred species farmed. The crocodilian farming industry mainly produces skins, while meat is the main by-product. Alternative income methods, like meat production, should be considered to increase the feasibility of the industry.

There is little knowledge about the conversion of muscle to meat and meat quality in Nile crocodile carcasses. Furthermore, various stunning methods are accepted and used in the commercial crocodilian industry. However, the effect of these methods on crocodilian welfare and meat quality are unknown. This study was conducted to determine baseline values for the conversion of muscle to meat and some physicochemical parameters (i.e., thaw loss, cooking loss, shear force, and fatty acid composition) of farmed Nile crocodiles. This study also assessed the effects of preferred stunning methods on commercially farmed Nile crocodiles' meat quality. Lastly, this study investigated the effects of different anatomical locations on the conversion of muscle to meat.

This study was conducted in May 2023 on a commercial crocodile farm in the Limpopo Province, South Africa. Twenty female Nile crocodiles were stunned and slaughtered from a single pen using two stunning methods (i.e. free bullets and electrical stunning). Carcass temperature and pH measurements and samples for muscle metabolomic analyses were taken from three anatomical locations (i.e. the *transversospinalis capitus*, *longissimus dorsi*, and *ilio-ischiocaudalis* muscles). Samples for the analysis of physicochemical characteristics were taken from the *ilio-ischiocaudalis* muscle within the tail. Lastly, live weight, blood loss during bleeding, cold carcass weight, and cut weights (i.e., the forequarter, rib casing, hindquarter, and tail tip) were measured.

This study showed significant differences in the carcass pH at each time interval between the stunning methods. Significant differences in carcass pH were seen at 6, 9, and 12 hours post mortem between the anatomical locations. Moreover, significant post mortem differences were seen in the glucose and glucose-6-phosphate concentrations between the stunning methods. The anatomical locations showed significant post mortem differences in the glycogen, glucose, and glucose-6-phosphate concentrations. Furthermore, the stunning methods showed no significant differences in the tail meat's thaw loss, cooking loss, or shear force. This study further showed that the primary fatty acids in the intermuscular and intramuscular fat of the tail are oleic, palmitic, and linoleic acids and that the content of individual fatty acids differ between these tissues. Lastly, a dressing percentage of 61.25% was found, and the tail cut was the highest yielding cut.

The results of this study showed that the free bullets stunning method caused less stress than the electrical stunning method. However, the stunning method did not significantly affect the meat quality parameters. Thus, both stunning methods produced similar and acceptable carcass and meat quality. This study further indicated that the tail had a slower glycolytic rate than the neck and body. Thus, the tail may have more slow-twitch muscle fibres than the neck and body.

Abbreviations, acronyms, and units

List of all abbreviations and acronyms

Adenosine diphosphate	ADP
Adenosine monophosphate	AMP
Adenosine triphosphate	ATP
Agricultural Research Council	ARC
Aluminium	Al
American Veterinary Medical Association	AVMA
Amyloglucosidase	AGS
Analysis of variance	ANOVA
Approximately	ca.
Barium	Ba
B-Nicotinamide dinucleotide monohydrate salt	NAD
Caesium	Cs
Calcium	Ca
Carbon dioxide	CO_2
Convention on International Trade in Endangered Species	CITES
Copper	Cu
Creatine phosphate	CP
Crocodile Specialist Group	CSG
Dark, firm, and dry	DFD
Department of Environment and Science	DES
Essential amino acid index	EAAI
Et cetera	etc.
Fat free dry matter	FFDM
Fatty acid methyl ester	FAME
Fatty acid	FA
Food and Agriculture Organization	FAO
Gallium	Ga
Glucose-6-phosphate	G6P
Glycolytic potential	GP
Hafnium	Hf
Hydrochloride	HCl
Intermuscular fat	INTMF
Intramuscular fat	IMF
International Business Machines Corporation, Statistical Package for the Social Sciences	IBM SPSS
International Crocodilian Farmers Association	ICFA
International Union for Conservation of Nature, Species Survival Commission	IUCN-SSC
Iron	Fe
Least significant difference	LSD
L-lactate dehydrogenase	LDH
Magnesium	Mg
Magnesium sulphate	$MgSO_4$
Monounsaturated fatty acid	MUFA
Natural Resource Management Ministerial Council	NRMMC
Nicotinamide adenine dinucleotide phosphate	NADP

Not-significant	NS
Omega-3	n-3
Omega-6	n-6
Oxygen	O_2
Pale, soft, and exudative	PSE
Perchloric acid	$HClO_4$
Phosphorous	P
Polyunsaturated fatty acid	PUFA
Potassium	K
Potassium hydroxide	KOH
Rubidium	Rb
Saturated fatty acid	SFA
Significance	Sign.
Sodium	Na
Sodium hydroxide	NaOH
South African Bureau of Standards	SABS
South African National Standards	SANS
Standard deviation	SD
Standard error	SE
Versus	vs
Water	H_2O
Zinc	Zn
Zirconium	Zr

<u>List of all units</u>

Amperes	A
Centimetres	cm
Degrees	^{o}
Degrees Celsius	^oC
Grams	g
Hours	h
Kilograms	kg
Kilogram-force	kgf
Metres	m
Metres squared	m^2
Microlitres	µL
Micrometres	µm
Micromoles	µmol
Milligrams	mg
Millilitres	mL
Millimetres	mm
Minutes	min
Nanometres	nm
Normality	N
Per cent	%
Pound-force per square inch	psi
Rands	R
Revolutions per minute	rpm
Seconds	s
Voltages	V

Definitions

Acute stress: This refers to a short-term stressor causing a stress response (Romero, 2004).

Anaerobic metabolism: This is a metabolic pathway occurring when there is a lack of O_2 or when the cellular demand for O_2 is greater than the amount supplied by the cardiovascular system. Energy is produced by the conversion of glycogen to pyruvate, which is subsequently converted into lactate instead of CO_2 and H_2O as with aerobic metabolism (NRMMC, 2009).

Captive breeding: Involves keeping adult crocodilians in a controlled environment, producing eggs, and rearing progeny in captivity (FAO, 1989; MacGregor, 2006; Brien *et al*., 2007; Beyeler, 2011; Manolis & Webb, 2016).

Chronic stress: This refers to a long-term stressor causing overstimulation of the stress response (Romero, 2004).

Crocodile: Refers to "true" crocodiles, i.e. members of the family Crocodylidae (IUCN-CSG, 2023).

Crocodilian: Refers to all members of the Order Crocodylia (NRMMC, 2009; IUCN-CSG, 2023).

Dressing: Refers to separating the carcass into edible and inedible parts (SABS, 2009).

Electrical stunning/immobilisation: This is a method utilised to immobilise animals by applying an electrical charge of high amperage to the animal (NRMMC, 2009).

Exsanguination: This refers to severing the major blood vessels (i.e. carotid arteries and jugular veins) (Riaz *et al*., 2021).

Hatchling: A crocodilian < one-year-old (SABS, 2009; Manolis & Webb, 2016).

Juvenile: A crocodilian > one-year-old and between 0.75 and 2 m long (SABS, 2009).

Manual capture/immobilisation: This entails capturing crocodilians by looping a rope on the end of a pole over the top jaw of the crocodilian (Franklin *et al*., 2003; Isberg, 2016).

Pithing: This pertains to inserting a sharp instrument into the brain (DES, 2008; NRMMC, 2009; SABS, 2009; Expert Panel, 2013), which ensures swift destruction of the brain. This method is only acceptable for unconscious crocodilians or directly after decapitation (Expert Panel, 2013).

Poikilothermic: This refers to an animal that does not regulate its body temperature (Furstenburg, 2008; Tosun, 2013) but maintains it by basking in the sun and lying in water (Furstenburg, 2008; Van der Westhuizen, 2019; IUCN-CSG, 2023).

Ranching: Entails collecting eggs, hatchlings, or juveniles from nature and rearing them in captivity (FAO, 1989; MacGregor, 2006; Brien *et al*., 2007; Furstenburg, 2008; Beyeler, 2011; Cawthorn & Hoffman, 2016; Manolis & Webb, 2016; Pooley, 2016; IUCN-CSG, 2023).

Steatotheca: This is a compact, white, visceral fat body located near the heart and is ca. the size of the heart, though its size might be correlated to the state of nutrition. This fat body is not equivalent to brown fat (only present in mammals) (Huchzermeyer, 2003; Osthoff *et al*., 2014).

Stocking density: Refers to the number of animals per unit area.

Stressor: Any unforeseeable stimulus within an animal that leads to a stress response (Romero, 2004; Mader, 2006).

Stress response: This refers to changes in physiology, hormones and behaviour that allow animals to overcome stressors (Romero, 2004).

Stunning: This is a practical method implemented during or at the start of slaughter, causing immobilisation or unconsciousness of animals. Stunning can cause the death of animals or not (Riaz *et al*., 2021).

Ultimate pH: The final pH attained by muscle tissue (measured at 24 h post mortem) (Hultin, 1984).

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Chapter 1

Introduction

The production cost of Nile crocodile farming for skins is increasing. Thus, an alternative income is required to increase the feasibility of the industry (Hoffman *et al*., 2000). Crocodilian meat is a delicacy in certain countries (Adan, 2000; Hoffman & Cawthorn, 2012). The tail meat is cut into fillets and marketed to high-class restaurants or sold abroad. The remaining portion of the carcass is deboned and sold abroad, marketed as goulash, or fed to crocodilians (Hoffman *et al*., 2000). Consumers knowledge about crocodilian meat is limited, and they consider eating it as an adventure (Hoffman *et al*., 2000; Huang *et al*., 2018).

Worldwide, 24 crocodilian species exist (IUCN-CSG, 2023). These crocodilians all form part of the Order Crocodylia, which comprises crocodiles, alligators, gharials, and caiman (MacGregor, 2006; NRMMC, 2009; Grigg *et al*., 2015; Cawthorn & Hoffman, 2016). This dissertation references species other than Nile crocodiles due to the limited research on their conversion of muscle to meat (carcass pH- and temperature-changes post mortem and post mortem muscle metabolomics) and meat quality. Furthermore, no previous research has been done on the effect of acute stress on the conversion of muscle to meat and meat quality in Nile crocodiles. Caimans are referred to since they are in the same Order (Crocodylia) as Nile crocodiles (IUCN-CSG, 2023), and broilers are referred to since they share some similarities with Nile crocodiles in the white musculature of their meat (Huchzermeyer, 2003; Jayasena, 2013). Lastly, reference is also made to fish as their meat is also classified as white meat (Zaukuu *et al*., 2020).

Various stunning methods are currently accepted in the crocodilian farming industry. This leads to farms utilising numerous methods to stun these animals at farms. These methods include penetrative and non-penetrative captive bolts, free bullets, a percussive blow to the head, electrical stunning and decapitation (Expert Panel, 2013; Manolis & Webb, 2016; AVMA, 2020; Martelli *et al*., 2020). However, it should be noted that very little is known about the effect these methods have on the meat quality of crocodilians. Thus, studying the effect of stunning methods on the conversion of muscle to meat and carcass characteristics will largely contribute to the crocodilian farming industry in terms of meat quality for the consumer and animal welfare.

Past studies regarding stress in crocodilians indicated that stress would lead to substandard growth and viability of animals, disease, inferior skin quality, and infertility (Brien *et al*., 2007; Isberg & Shilton, 2013). Huchzermeyer (2003) stated that acute stress causes the permeability of the intestinal blood capillaries to increase, ultimately resulting in septicaemia. In broilers, increased transportation time accelerates anaerobic metabolism of glycogen, especially in muscles containing more fast glycolytic fibres (type IIb), which results in lactate accumulation within the muscle. Finally, leading to a rapid decline in pH post mortem while the carcass temperature is still high (Wang *et al*., 2017).

Previous studies on caiman and Nile crocodiles indicated differences in meat quality parameters between various cuts. These include differences in the fatty acid (FA) composition between different cuts in Pantanal caiman (*Caiman crocodilus yacare*), with the back fillet and thigh having a higher omega-3 (n-3) FA content than the tail and sirloin (Fernandes *et al*., 2017). Huang *et al*. (2018) showed that the polyunsaturated fatty acid (PUFA) and saturated fatty acid (SFA) content is higher in the anterior ventral meat of Spectacled caiman (*Caiman crocodilus*) compared to other cuts. Vicente-Neto *et al*. (2010) showed that the tail of Pantanal caiman has the most SFAs, monounsaturated fatty acids (MUFAs), and the lowest n-6/n-3 ratio compared to other cuts. However, the FA composition in South African Nile crocodiles has not been studied extensively. In terms of chemical composition,

it is seen that the moisture content in the tail of Pantanal caiman is lower than in the other cuts, but its fat content is higher than in other cuts (Fernandes *et al*., 2017). Various studies have shown differences in the chemical composition of Nile crocodile meat in different cuts (Hoffman *et al*., 2000; Černíková *et al*., 2015). Fernandes *et al*. (2017) showed that the thigh and back fillet is redder (higher chroma a* value) compared to other cuts, and in Nile crocodiles, Černíková *et al*. (2015) have shown that the shoulder has the lowest L* (lightness) values and the highest a* values. Differences in pH between different portions of Spectacled caiman have been shown (Huang *et al*., 2018), while pH values and pH change post mortem in Nile crocodiles were similar in the tail and leg meat (Hoffman *et al*., 2000). A difference in amino acid composition between different portions of Spectacled caiman and Nile crocodiles has been shown (Černíková *et al*., 2015; Huang *et al*., 2018). In Nile crocodiles, the cheek is the least tender meat, and the neck is the most tender, while cohesiveness and adhesiveness are very similar among different cuts (Černíková *et al*., 2015).

This study aims to determine baseline values for the conversion of muscle to meat in Nile crocodile carcasses. Furthermore, this study aims to determine whether there is a difference in the conversion of muscle to meat between different anatomical locations. The study also aims to determine which stunning method is more appropriate in terms of animal welfare and meat quality. Lastly, this study aims to determine whether there is a difference in the FA composition between intramuscular fat (IMF) and intermuscular fat (INTMF) within the tail.

The hypotheses of this study were as follows:

$H1_0$ = There is no significant difference in the conversion of muscle to meat (carcass pH- and temperature-changes post mortem and post mortem muscle metabolomics) between different anatomical locations of the Nile crocodile carcass.

$H1_a$ = There is a significant difference in the conversion of muscle to meat (carcass pH- and temperature-changes post mortem and post mortem muscle metabolomics) between different anatomical locations.

$H2_0$ = There is no significant difference in the conversion of muscle to meat (carcass pH- and temperature-changes post mortem and post mortem muscle metabolomics) and physicochemical characteristics in Nile crocodiles stunned by different methods.

$H2_a$ = There is a significant difference in the conversion of muscle to meat (carcass pH- and temperature-changes post mortem and post mortem muscle metabolomics) and physicochemical characteristics in Nile crocodiles stunned by different methods.

$H3_0$ = There is no significant difference in the fatty acid composition between intramuscular fat and intermuscular fat within the tail of Nile crocodiles.

$H3_a$ = There is a significant difference in the fatty acid composition between intramuscular fat and intermuscular fat within the tail of Nile crocodiles.

Chapter 2
Literature Review

2.1 History of commercial Nile crocodile farming in South Africa

In the early- and mid-19th century, rampant hunting of Nile crocodiles for their skins took place across Africa (Furstenburg, 2008; Cawthorn & Hoffman, 2016; ICFA, 2022). Nile crocodiles were seen as vermin or pests because they preyed on human beings and livestock (Manolis & Webb, 2016; Pooley, 2016; ICFA, 2022). During a meeting held by the Crocodile Specialist Group (CSG) in 1986, crocodile ranching or farming was described as crucial in securing these animals' future in Africa since the conflict between humans and crocodiles were increasing rapidly (Pooley, 2016).

Farming of Nile crocodiles within South Africa began in the 1960s, mainly for the use of their skins (Hoffman *et al*., 2000; ICFA, 2022). Later, farms were used to restock and supplement wild populations (ICFA, 2022). Farmers were permitted to collect a specific number of eggs from wild populations and incubate these eggs for slaughter or marketing in 1963. In 1975, South Africa became a member state of the Convention on International Trade in Endangered Species (CITES), listing all crocodilians on Appendix I or II (Hoffman & Cawthorn, 2012; Manolis & Webb, 2016; ICFA, 2022). Species listed in Appendix I cannot be traded internationally except if the animal is bred in captivity (FAO, 1989; Isberg, 2016; Manolis & Webb, 2016; IUCN-CSG, 2023). In comparison, Appendix II comprises species which may be traded sustainably, though they can become endangered if the trade in these animals is not strictly regulated (MacGregor, 2006; Isberg, 2016; Manolis & Webb, 2016). In 1992, more than 40 crocodile farms were established in South Africa (Hoffman *et al*., 2000) and CITES allowed skin trading from South African crocodiles (ICFA, 2022).

Crocodilian farming can occur as ranching or captive breeding (MacGregor, 2006; Brien *et al*., 2007; Cawthorn & Hoffman, 2016; Manolis & Webb, 2016; ICFA, 2022; IUCN-CSG, 2023), though, in South Africa, captive breeding is the only form used (Louw, 2023). Tosun (2013) and Manolis & Webb (2016) stated that closed-cycle captive breeding operations are mainly used at crocodilian farms. Here, farms do not provide any incentive for the protection and preservation of native populations of crocodilians (Brien *et al*., 2007).

The main product of the crocodilian farming industry is skin (Hoffman *et al*., 2000; Mader, 2006; Isberg, 2016; IUCN-CSG, 2023). Alternative income methods are required for farmers to be profitable due to an increase in the cost of production (Hoffman *et al*., 2000). Meat production and

tourism were integrated as components of the industry (Adan, 2000; Hoffman *et al*., 2000). In South Africa, crocodilian meat is considered a delicacy (Adan, 2000; Huchzermeyer, 2003; Hoffman & Cawthorn, 2012). The tail meat is sold as fillets to restaurants or exported, while the remainder of the carcass is deboned and exported, sold as goulash, or fed to crocodilians (Hoffman *et al*., 2000). Crocodilian farming further contributes to job creation, increment of wild populations, and the provision of environmental education, and plays a vital role in tourist attraction (Luthada-Raswiswi *et al*., 2019).

The building, maintenance, and operation of crocodilian abattoirs are costly. If crocodilian meat is ultimately used for human consumption, abattoir management should be regulated strictly, and there are further responsibilities such as packaging, shipping, labelling, and record-keeping (Luthada-Raswiswi *et al*., 2019).

The crocodilian farming industry receives criticism because some crocodilians are raised in unitised pens. Some pens are compact and can even be shorter than the total length of the crocodilian. These pens do not allow movement, stretching, or supply cover nor provide appropriate water depth. Furthermore, these pens are covered by a wired arch roof. Unitised pens prevent the crocodilian from turning around and thermoregulating, which is inappropriate (NSPCA, 2018).

2.2 Crocodilian farming practices

2.2.1. Housing and stocking density

The species, national and climatic context (Manolis & Webb, 2016), and the size of the crocodilians determine the type of housing used at crocodilian farms (NRMMC, 2009; SABS, 2009). Space and budget are the most restrictive factors in deciding how to construct the pens. Crocodilian pens can be used for exhibition or not. All pens should be safe and secure and provide for the health and well-being of the animals. The pens not utilised to display crocodilians can be built with less costs. In addition to the minimal requirements, pens for exhibition must be pleasing to the eye and ensure the public's safety. The pens not used for public display can be designed to house more animals and have less construction and maintenance costs (Brien *et al*., 2007).

The pens must have walls or fences of a height, depth and design that do not allow crocodilians to escape (Brien *et al*., 2007; SABS, 2009). Recommendations for fence heights are given as 1.5 m (without an overhang) or 1.2 m (with a 300 mm overhang) for adults, 1.2 m (without an overhang) or

1 m (with a 300 mm overhang) for juveniles, and 1 m for hatchlings. Adult and juvenile pens should also be extended below the surface to ensure the crocodilians do not escape (SABS, 2009).

The houses of grower crocodilians are built with insulated concrete blocks, metal, or wood (FAO, 1989; Masser, 1993). The water within the pools of pens is kept warm, ideally 32 ºC, with pipes that run through the pool water from a central heating system. The pen floor is often made of concrete (Masser, 1993; Revol, 1995; Huchzermeyer, 2003), which is scrubbed daily (Revol, 1995), and the pool is cleaned and the water replaced at least once every year (SABS, 2009). Some houses have earthen floors, which lessen heat loss (Masser, 1993). However, earthen pools lead to increased difficulty in finding and monitoring the health of single animals due to varying depths, murky water and crocodilians digging tunnels in the banks (Brien *et al*., 2007). These pens are designed with ca. 1/3rd of the pen being above the average water level and is used for feeding and basking, and 2/3rd of the pen consists of a pool of ca. 0.3 m deep at the drain (Masser, 1993). The pool has a slope to ease cleaning (Masser, 1993; Brien *et al*., 2007; NRMMC, 2009; SABS, 2009).

NRMMC (2009) and SABS (2009) state that the pool should be of adequate length and width to allow animals to swim. The pool should further have an adequate depth for the complete submergence of the animal (Brien *et al*., 2007; NRMMC, 2009; Manolis & Webb, 2016). SABS (2009) states that the water area should be at least 25% of the total pen area. However, Brien *et al*. (2007) state that the pool should make up 50% of the enclosure while leaving sufficient dry land for all the animals to lay outstretched.

Crocodilian pens increase in size as the animals grow, and these pens can be nearly any size (Masser, 1993). The adult size of the species or the maximum size of the animal housed should determine the area of the enclosure. Some authors have mentioned minimum area requirements as follows. An area of twice the length of the adult for the species to be housed (width and depth) is required for one crocodilian. For each animal added to the pen, the pen size should increase, with the pool increasing by 20% and the land area increasing by 10%. Furthermore, the pen size should be adjusted according to the landscape features, which reduces the available land area (Brien *et al*., 2007). SABS (2009) states that adult crocodilians should have a minimum dry land area of 10 m^2/crocodilian. Revol (1995) noted that the least amount of space needed for hatchling crocodilians is 0.09 m^2/crocodilian, for yearlings 0.18 m^2/crocodilian, and for 2-to-3-year-old animals 0.3 m^2/crocodilian. More specific to Nile crocodiles, Webb *et al*. (2021) stated that an area of 1.24 m^2/grower Nile crocodile is more appropriate than 0.41 or 2.60 m^2/grower Nile crocodile.

Many factors determine the number of crocodilians that can be housed safely within a pen (Brien *et al*., 2007). Though the ideal stocking density for crocodilians has not been established (Mader, 2006), some recommendations exist. SABS (2009) states that crocodilian pens should provide enough space for the animals to have normal physiological functioning, and the stocking density should minimise death, injury, and disease. All crocodilians should have access to different pen components (i.e. land and water; cool and warm; and food). High stocking densities lead to stress, aggressive behaviour, and injuries in crocodilians (Huchzermeyer, 2003; Brien *et al*., 2007). Low stocking densities will also lead to crocodilians undergoing stress, fighting, and being injured. Furthermore, low stocking density increases costs because of the demand for costly space (Huchzermeyer, 2003). However, the FAO (1989) states that crowding is not harmful to juveniles if size segregation, proper feeding, and cleaning are maintained. Crocodilians should be placed in groups according to size (Revol, 1995; Brien *et al*., 2007; NRMMC, 2009) to reduce bullying and fighting (Brien *et al*., 2007).

Plasma corticosteroid levels in crocodilians are influenced by stocking density. Hatchlings should be stocked at a number that could still be accommodated once they reach the pre-slaughter juvenile stage. The formula, $p = (n\sqrt{m})/5$, can be used to determine the appropriate stocking density for crocodilians. Where p is the pen size (m^2), n is the number of crocodilians in the pen, and m is the average mass of a crocodilian within the pen. The divisor can be specific to species, where divisor 5 applies to the Nile crocodiles (Huchzermeyer, 2003).

2.2.2. Feeding and growth

The diet of crocodilians has been deduced by determining the contents of the stomach (Huchzermeyer, 2003; Wallace & Leslie, 2008; Grigg *et al*., 2015), as well as by observing the behaviour of these animals (Huchzermeyer, 2003). The diet of wild populations changes as the animals grow (Masser, 1993; Huchzermeyer, 2003; Furstenburg, 2008; Tosun, 2013). Furthermore, as the animal grows, the size of the prey increases (Huchzermeyer, 2003; Mader, 2006; IUCN-CSG, 2023).

Crocodilians are carnivores and will almost exclusively feed on animal protein in nature (FAO, 1989; Huchzermeyer, 2003; Furstenburg, 2008; Beyeler, 2011; Grigg *et al*., 2015). Younger individuals essentially eat invertebrates (crayfish, insects, snails, and crustaceans) (FAO, 1989; Revol, 1995; Huchzermeyer, 2003; Mader, 2006; Furstenburg, 2008; Wallace & Leslie, 2008; Tosun, 2013; Van der Westhuizen, 2019). As the crocodilians grow, they start to eat vertebrates (fish, tadpoles, and tiny frogs), with the proportion of fish increasing with age (FAO, 1989; Revol, 1995;

Huchzermeyer, 2003; Mader, 2006; Wallace & Leslie, 2008). Adult crocodilians will eat mammals (muskrats and nutria), with large crocodilians also eating reptiles, small crocodilians, and birds (Huchzermeyer, 2003; Mader, 2006; Furstenburg, 2008; Tosun, 2013).

Captive crocodilians can be fed pellets from the hatchling phase to slaughter, where they are raised under controlled thermal conditions and protected from stressors. The feeding of pellets has the advantages of reducing feed wastage, being hygienic (Huchzermeyer, 2003), can be stored without refrigeration for more extended periods, having proper nutrient supplements, weighing ca. 20% of wet meat, saving on electricity for storage, fuel, and labour for handling and transportation (Manolis & Webb, 2016). Hatchlings are commonly fed ground, minced, or chopped feed (Revol, 1995; NRMMC, 2009). It has been noted that crocodilians prefer chunks, but by grinding or mincing feed, supplements can be mixed into the feed more effectively (DES, 2008; NRMMC, 2009). It is suggested that crocodilians are given bite-sized feed to reduce the mess made during feeding (Brien *et al*., 2007).

Crocodilians should be given a diet with suitable levels of protein (Brien *et al*., 2007; SABS, 2009). In captivity, crocodilians are given fish, butcher's meat, poultry, and carcasses (FAO, 1989; Revol, 1995; Brien *et al*., 2007), minced/finely diced red meat, or abattoir offal (Manolis & Webb, 2016); a standard vitamin supplement is also used widely (Revol, 1995; DES, 2008). However, a variety in the diet of crocodilians is beneficial for optimum results (FAO, 1989; Brien *et al*., 2007). A varying diet is easily attainable in hatchlings due to the small amount of feed needed. Crocodilians can also be given livers and hearts, with priority given to hatchlings (FAO, 1989). Though it should be noted that feeding captive crocodilians live prey is not allowed, this does not include live fish in the pool (SABS, 2009). Very fatty meat should be avoided, as it may be regurgitated after consumption. Furthermore, hatchlings should not be given bone unless crushed (FAO, 1989).

The size, age, diet, and environmental conditions determine the amount of vitamins and minerals required (NRMMC, 2009; SABS, 2009). A calcium-rich diet can eliminate abnormalities and bone disorders and improve skin quality. The results of a poor diet include reduced fertility, restricted growth, disorders of teeth and bones, and a reduction in disease immunity (Brien *et al*., 2007). Most dietary deficiencies in fish-fed crocodilians are associated with calcium, vitamin E/selenium, and vitamin A (DES, 2008). Thus, if calcium, vitamins, and minerals are not within the diet, it should be added (FAO, 1989; NRMMC, 2009; SABS, 2009; Manolis & Webb, 2016).

Red meat is high in phosphorous, and rickets will develop (due to a calcium deficiency) in crocodilians fed only red meat unless calcium is added. Furthermore, crocodilians not kept in direct sunlight should be given a supplement of vitamin D_3 to aid in the homeostasis of calcium (Manolis

& Webb, 2016). Usually, calcium is added in a palatable form (such as bone meal) at 1-2% of the crocodilian's weight. Moreover, vitamin supplements should be given fresh to crocodilians and stored within refrigerators since vitamin A oxidises and degrades easily (DES, 2008). Supplying additional macro-minerals is unnecessary if the crocodilians are fed meat containing bones (Huchzermeyer, 2003; Manolis & Webb, 2016).

Feeding should occur after cleaning the pen and water since crocodilians eat from the floor. The feed should be limited to how much crocodilians will eat within 30 minutes (Huchzermeyer, 2003). Feeding should occur outside the pools (Revol, 1995; Huchzermeyer, 2003). The feed should be distributed over a wide area in rows instead of piles to avoid fighting (FAO, 1989; Masser, 1993; NRMMC, 2009; SABS, 2009). The leftover feed should be removed within 12 hours after feeding (Huchzermeyer, 2003; SABS, 2009). Every hatchling should have access to feed and be taught how to feed (SABS, 2009).

Crocodilians should be given enough feed, and this should consider the season and size of the animals (SABS, 2009). It should be noted that overfeeding will waste money and can cause gout (Masser, 1993). Crocodilians raised outdoors will have a variable feed intake depending on the temperature and season (DES, 2008; NRMMC, 2009). Hatchling crocodilians of ca. 40-70 g consume 5-10% of their body weight per week, and as they grow, the intake will increase to 20-30% of their body weight per week (FAO, 1989). Juvenile crocodilians usually eat 15-20% of their body weight per week at temperatures of ca 32 ºC (Revol, 1995; Brien *et al*., 2007; DES, 2008; NRMMC, 2009). Sub-adult crocodilians consume ca. 8-10% of their body weight per week (Brien *et al*., 2007).

Certain farms start to feed hatchling crocodilians directly after hatch, while other farmers keep them in the incubator for up to a week before they begin feeding the animals. Some believe that leaving the crocodilians within the incubator for a few days stimulates the utilisation of the yolk, which can aid in detecting compromised hatchlings (Manolis & Webb, 2016). In practice, it is customary to feed hatchlings daily (Revol, 1995; Huchzermeyer, 2003; Brien *et al*., 2007; DES, 2008; NRMMC, 2009; Manolis & Webb, 2016); some species may be fed at this rate for the first year of life. Alternatively, feeding can be lessened to 4-5 days weekly, depending on the animal's size. Older animals are frequently fed less, e.g. 2-4 times per week (Brien *et al*., 2007; Manolis & Webb, 2016).

Feeding should not occur in the early morning after a cold night but rather when the crocodilian has attained its optimal core temperature (Huchzermeyer, 2003; Brien *et al*., 2007). The optimum temperature for feeding crocodilians is between 25 and 35 ºC (Mader, 2006). Crocodilians kept outdoors should not be provided with feed when they are unable to maintain a body temperature > 25

°C (Huchzermeyer, 2003; Mader, 2006; SABS, 2009). Crocodilians should be fed when the temperature is > 21 °C, and feeding should cease at < 21 °C (Tosun, 2013). Crocodilians stop eating and become inactive when they have a low body temperature. In captivity, Nile crocodiles do not show interest in food when the air or the water temperature is < 15.6 °C. At temperatures < 7.2 °C, crocodilians cannot move properly or keep their balance in water (FAO, 1989).

A farm's economic success depends mainly on the interaction between the survival rate, growth rate and time to slaughter. Should the animals be stress-free, well-fed, and reared under optimal temperatures in controlled farming conditions; the animals will attain higher growth rates than in the wild (Manolis & Webb, 2016).

It is noted that the average Nile crocodile will reach ca. 2 m in length and weigh ca. 37 kg within four years. During these four years, the Nile crocodile will have eaten ca. 260 kg of feed (FAO, 1989) and have a growth rate of ca. 300 mm/annum (Furstenburg, 2008). A crocodilian grows rapidly during the first months after hatching (Revol, 1995; Brien *et al*., 2007). Nile crocodiles typically have lengths of 55 cm at three months, 85 cm at six months, and 110 cm at one year (Revol, 1995). Crocodilians can convert high proportions of their feed into muscle (Mader, 2006; Manolis & Webb, 2016).

Incubation conditions affect the growth after hatching, survival and raising success. In some species, the initial size of hatchlings is a poor predictor of growth rates to one year of age since smaller hatchlings grow faster than larger ones. Furthermore, male crocodilians grow faster than female crocodilians. However, sex-specific growth rates are frequently not evident in optimum conditions until the crocodilians are older than one year (Manolis & Webb, 2016).

The rate of growth can differ depending on the diet given to the animals (Huchzermeyer, 2003; Alexander & Marais, 2013; Manolis & Webb, 2016). The growth rate further depends on temperatures, feed intake (FAO, 1989; Revol, 1995; Alexander & Marais, 2013; Tosun, 2013), and a management routine that minimises disease and stress (FAO, 1989). It is stated that the optimum temperature for crocodilian growth is ca. 30 to 34 °C (Brien *et al*., 2007; Beyeler, 2011; Tosun, 2013), with a relative humidity of 60-90% (Beyeler, 2011). Sub-optimal temperatures can lead to physiological problems, disease, reduced growth rates and increased mortality (Manolis & Webb, 2016). During lean times, growth may cease and even become negative. In many species, growth may continue indefinitely throughout life, although it does tend to slow with age (Alexander & Marais, 2013).

2.2.3. Methods of capture/immobilisation for wild and farmed crocodiles

There are two types of capture methods for crocodilians – direct and indirect (NRMMC, 2009). When deciding which method to use for capture the animal's size (Mader, 2006; NRMMC, 2009; SABS, 2009; Manolis & Webb, 2016), the context of the capture and the personnel's skill (Mader, 2006; Manolis & Webb, 2016), the enclosure type and the distance the crocodilian should be moved (SABS, 2009) should be considered. Different methods of capture based on the length of the crocodilian are indicated in **Table 2.1**. It is important to note that capturing and restraining crocodilians will trigger an acute physiological stress response, ultimately leading to the release of adrenaline and corticosterone and increasing glucose catabolism (Franklin *et al*., 2003).

Table 2.1 Appropriate capture method for crocodilians based on their length (adapted from Manolis & Webb, 2016)

Capture method	Crocodilian length (m)
Direct	
Nets	> 0.5
Tongs	< 0.9
Noose	1.0-1.5
Electrical stunning	1.0-2.4
Skin harpoon	1.0-5.0
Treble hook/noose	> 1.0
Hand capture	< 1.5
Rope	> 1.5
Indirect	
Cage trap	> 1.5
Baited digestible hook	> 1.5
Snare/baited noose	> 2.0

This literature review will discuss manual capture and electrical immobilisation in further detail.

Manual capture/immobilisation

Manually captured animals struggle while being handled until exhausted (Davis *et al*., 2000; Franklin *et al*., 2003; NRMMC, 2009; Manolis & Webb, 2016). By struggling, the crocodilian can injure itself, other crocodilians, or its handlers (NRMMC, 2009; Manolis & Webb, 2016). Furthermore, these animals will not eat for a few days after manual capture (Davis *et al*., 2000; Franklin *et al*., 2003), and their growth rate can also be affected (Franklin *et al*., 2003).

Due to struggling during capture, anaerobic metabolism takes place, and acidosis can occur (Franklin *et al*., 2003; NRMMC, 2009; IUCN-CSG, 2023). In large crocodilians, lactate build-up can lead to death unless assisted respiration is used to clear CO_2 from the lungs, and to provide O_2 (Manolis & Webb, 2016).

Electrical stunning/immobilisation

In South Africa, electrical stunners are frequently used on commercial crocodile farms to handle Nile crocodiles (Pfitzer *et al*., 2014). An electrical stunner consists of electrodes at the end of a rod (Davis *et al*., 2000; Franklin *et al*., 2003; Manolis & Webb, 2016). This stunner uses a combination of high amperage and low voltage, which decreases the risk of damaging the skin and electrical shock of the crocodile (Davis *et al*., 2000). During electrical stunning, a charge (ca. 80 to 160 V) is given to the crocodile for ca. three to 11 seconds at the back of the crocodile's neck (Davis *et al*., 2000; Franklin *et al*., 2003; NRMMC, 2009; Manolis & Webb, 2016). This causes the crocodile to be unconscious and immobilised for five to 10 minutes (Davis *et al*., 2000; Franklin *et al*., 2003; NRMMC, 2009; Pfitzer *et al*., 2014; Manolis & Webb, 2016). Davis *et al*. (2000) state that the electrical stunner is most effective when the crocodilian is in water. It is important to note that only two applications of electrical stunning are allowed the day prior to slaughter (Manolis & Webb, 2016).

Pfitzer *et al*. (2014) compared corticosterone, glucose, lactate, and other parameters between Nile crocodiles captured manually and electrically. They found that the concentration of lactate was higher in manually captured crocodiles than in electrically stunned crocodiles. Furthermore, the time taken to capture animals by the manual method is longer than that of the electrical stunning method (Pfitzer *et al*., 2014). A study by Franklin *et al*. (2003) on *Crocodylus porosus* showed that the concentration of haematocrit, haemoglobin, and lactate increases after manual capture and electrical stunning. However, the extent of the change is lower, and the recovery time is quicker in electrically stunned animals than in manually captured animals. Furthermore, glucose and corticosterone concentrations did not increase in electrically stunned animals (Franklin *et al*., 2003). Davis *et al*. (2000) stated that there are no noticeable adverse effects due to stunning. It is further said that animals captured by electrical stunning are less stressed than those captured manually (Franklin *et al*., 2003; Pfitzer *et al*., 2014; Manolis & Webb, 2016). Davis *et al*. (2000) also compared the pre-slaughter stress levels of stunned animals with the pre-slaughter stress levels of animals shot within the water and captured manually, though these results were inconclusive.

2.2.4. Acceptable stunning methods

There is a worldwide expectation that crocodilians should be slaughtered humanely. This means that the animal should be stunned or immobilised to swiftly render the animal unconscious, followed by slaughter, so that the process occurs with the least amount of pain (DES, 2008; Manolis & Webb, 2016). Two types of stunning methods are used, namely mechanical and chemical (Expert Panel, 2013). However, it should be noted that if the meat is used for human consumption, it is prohibited to use any immobilising agents or other drugs on the animal (DES, 2008; NRMMC, 2009; Manolis & Webb, 2016). Thus, this literature review will only discuss accepted mechanical stunning methods.

Electrical stunning causes the immobilisation and unconsciousness of crocodilians (Davis *et al*., 2000; Franklin *et al*., 2003; NRMMC, 2009; Pfitzer *et al*., 2014; Manolis & Webb, 2016) and is thus also a stunning method in addition to a capture method. However, it is followed by the use of penetrative- and non-penetrative captive bolt pistols, free bullets, a percussive blow to the head or decapitation when crocodilians are subsequently slaughtered.

Penetrative- and non-penetrative captive bolt pistol

Gunpowder and compressed air trigger captive bolt pistols (Expert Panel, 2013) and should supply adequate energy to puncture the skull when using a penetrative captive bolt or cause a lethal stun when using a non-penetrative captive bolt (Expert Panel, 2013; Manolis & Webb, 2016). When using the captive bolt gun, the animal should be immobilised manually or electrically (Expert Panel, 2013; Manolis & Webb, 2016; Martelli *et al*., 2020). During this procedure, the pistols are aimed at the brain cavity (Expert Panel, 2013; Manolis & Webb, 2016).

The captive bolt pistol can be used on any size of crocodilian. The size of the crocodilian will determine the proper gun charge that must be used to ensure that the skull is penetrated (Expert Panel, 2013; Manolis & Webb, 2016). After stunning with the captive bolt pistol, pithing can ensure death, although it is unnecessary (Expert Panel, 2013; Martelli *et al*., 2020).

Free bullets

This method involves shooting a crocodilian in the middle of the eyes or through the side or back of the cranium with a firearm (DES, 2008; NRMMC, 2009; SABS, 2009; Manolis & Webb, 2016). The size of the crocodilian will determine the type and size of the firearm. Some examples of which calibre firearm to use for a specific length of crocodilian are given in **Table 2.2**. According to Martelli

et al. (2020), pithing is not necessary after shooting due to the instant destruction of the brain, though it is frequently carried out. In contrast, the South African Bureau of Standards (SABS, 2009) and Manolis & Webb (2016) state that it is required to pith the brain after shooting.

Table 2.2 Calibre firearm to be used for a specific length of crocodilian (NRMMC, 2009)

Crocodilian length (m)	Firearm calibre
≤ 2	.22 short (low velocity)
2-3	.22 long/.22 Win Mag (medium velocity)
> 3	≥ .222 Centre-fire (high velocity)

It may be necessary to immobilise the animal before shooting (Martelli *et al*., 2020). The margin of error for missing the target is reduced by minimising the distance between the crocodilian and the shooter (Expert Panel, 2013). Farms that use firearms should comply with national and local firearms legislation (Manolis & Webb, 2016). Personnel trained in firearms should shoot the crocodilians (Expert Panel, 2013; Manolis & Webb, 2016; AVMA, 2020). One should ensure the bullet's trajectory is away from nearby personnel (NRMMC, 2009; Manolis & Webb, 2016). High-velocity calibres should not be used in confined spaces due to the risk of a bullet ricocheting (Manolis & Webb, 2016).

Percussive blow to the head

This method pertains to striking a crocodilian over the head with a solid instrument using adequate strength, which will cause a rapid loss of consciousness and/or death (Expert Panel, 2013; AVMA, 2020). This method requires the immobilisation of the animal. However, this method is unsuitable for crocodilians larger than 3 kg (Martelli *et al*., 2020) or in cases where many animals are stunned and slaughtered in a short period (Expert Panel, 2013; Martelli *et al*., 2020). Only experienced operators may perform this method. Directly after striking the crocodilian, a method to destroy the brain should be used (Expert Panel, 2013; AVMA, 2020).

Spinal cord severance or decapitation

Spinal cord severance is performed by separating the neck between the cranium and the first cervical vertebra with a sharp object (metal chisel/knife) (DES, 2008; NRMMC, 2009; Expert Panel, 2013). Following the severance of the spinal cord, the brain should be destroyed instantly by pithing (DES, 2008; NRMMC, 2009; Expert Panel, 2013; Manolis & Webb, 2016; AVMA, 2020; Martelli *et*

al., 2020). In Zimbabwe and Zambia, spinal pithing is also a requirement (Manolis & Webb, 2016). When using decapitation, the animal should be immobilised; electrical immobilisation is recommended (Martelli *et al*., 2020).

2.2.5. Skinning, dressing and meat processing

Crocodilians are slaughtered as soon as they reach a length of 1.5-2.0 m (Adan, 2000), between the ages of 2-3 years (Louw, 2023; Revol, 1995). On commercial crocodilian farms, the carcasses are separated into trunks, feet, and tails (Huang *et al*., 2018). Crocodilian tail meat and trunks were sold at R25-50/kg and R8-35/kg, respectively, in 2005 (Furstenburg, 2008).

After slaughter, skinning should occur as soon as possible (Manolis & Webb, 2016). Skinning should be done in a cool, shaded area (FAO, 1989; Manolis & Webb, 2016). The opening lines should be made in such a manner as to ensure that the final skin shape complies with market standards. If crocodilians are slaughtered for processing, the carcasses can be hung in the shade or a cool room, facilitating exsanguination and cessation of post mortem movements (Manolis & Webb, 2016).

Curing and preservation inhibit the microbial deterioration of raw skin until the skin is converted into leather. This is done by dehydrating the skin (saturating it with raw salt) or preserving it in a brine solution (Revol, 1995; Manolis & Webb, 2016). Usually, fine- to medium-grain salt is rubbed into the skin (Masser, 1993; Manolis & Webb, 2016) (half the weight of the skin or 1-2.5 cm deep, to absorb water from the skin) (FAO, 1989; Manolis & Webb, 2016). Within 24-48 h of initial salting, the salt is removed and discarded, and the skin is resalted with a fresh layer of salt. However, care should be taken not to dry out the skin completely, as this can lead to brittle skin or cracking, thus downgrading the skin quality. Some farms use a brine solution to initially preserve skins (Manolis & Webb, 2016).

For storage, skins can be rolled from the head skin to the tail tip to prevent folding. Furthermore, the sides can be folded inward and the skin rolled (FAO, 1989; Manolis & Webb, 2016) to ensure that the keratin is on the outside and the underside is not exposed (Manolis & Webb, 2016). After rolling, the skin should be stored in a cool, climate-controlled facility (Masser, 1993; Manolis & Webb, 2016), which will avoid fold marks that reduce the skin's value. Farms usually store skins in a refrigerated room at 5-10 ^{o}C (Manolis & Webb, 2016).

The number and quality of skins will determine the viability of a crocodilian farm. Skins are graded at the salted skin stage (Manolis & Webb, 2016) on a 5-point scale, with grade 1 having no marks,

scratches, or defects and grade 5 includes rejects (Beyeler, 2011; Manolis & Webb, 2016). For each point lower grade, the skin has 25% less value (Revol, 1995; Furstenburg, 2008; Beyeler, 2011).

Grading criteria include imperfections and whether the imperfections are in the area of primary importance (known as the 'pattern') (FAO, 1989; Revol, 1995; Manolis & Webb, 2016). Imperfections include scratches, stretched skin (Masser, 1993; Manolis & Webb, 2016), erosion of scale edges, double-scaling, cuts, parasite trails, localised pitting, infections in/between scales, and severe pattern abnormalities (Manolis & Webb, 2016).

Crocodilian skin prices are based on the skin's grade (Furstenburg, 2008; Beyeler, 2011) and width (cm) (FAO, 1989; Furstenburg, 2008; Beyeler, 2011). Species with small scutes have the most significant value (FAO, 1989; Adan, 2000). The width of the skin is measured from the third inside osteoderm across to the other side. The horn-back, back-strap, and belly are significant cuts of crocodilian skin (Beyeler, 2011). Crocodilian leather is divided into 'classic' and 'non-classic' categories based on leather quality (MacGregor, 2006). The skins that are cut classically (belly) have the most value, having high dorsal cut lines (Revol, 1995). Classic skins have high value and low volume, and non-classic skins, usually caiman skins, have low value and high volume (MacGregor, 2006).

2.3 Conversion of muscle to meat

Directly following slaughter, blood stops circulating, thus ceasing the transportation of O_2 to muscles. This leads to anaerobic metabolism (Hultin, 1984; Stajkovic *et al.*, 2019). Exsanguination is followed by a delay period. Few rigor bonds are formed during this period, and the muscle is extensible. The adenosine triphosphate (ATP) levels remain constant throughout this period (Lonergan *et al.*, 2010).

Once O_2 is unavailable, the muscle cells can no longer produce ATP through aerobic respiration. During the delay period, creatine phosphate (CP) is still available. Thus, the muscle cells can produce ATP from the CP reserves (Hultin, 1984; Lonergan *et al.*, 2010). Muscles can also produce ATP through a reaction catalysed by adenylate kinase, whereby two molecules of adenosine diphosphate (ADP) are converted to one molecule of adenosine monophosphate (AMP) and one molecule of ATP (Hultin, 1984). However, during anaerobic conditions, the primary source of ATP is glycolysis, whereby glycogen or glucose is catabolised to glucose-6-phosphate (G6P), which is ultimately converted to lactate and ATP. The lactate produced corresponds roughly to the amount of ATP

synthesised and hydrolysed. The pH in muscle tissue decreases as ATP is hydrolysed due to the production of a hydrogen ion (Hultin, 1984; Lonergan *et al*., 2010; Stajkovic *et al*., 2019). Thus, the amount of lactate that has built up is a useful measure of the amount of acid produced by the hydrolysis of ATP. As ATP is broken down, more ATP is synthesised by the glycolytic enzymes, which are under cellular control to maintain a high level of ATP (Hultin, 1984). Rigor develops with the decrease in pH (Lonergan *et al*., 2010).

Two factors lead to the cessation of glycolysis. This includes a decrease in pH to such a degree that the glycolytic enzymes are inhibited. In certain species, glycolysis may cease due to the depletion of substrates for glycolysis (i.e. glycogen). Once the substrate for glycolysis is depleted or the muscle's pH becomes unfavourable, ATP production stops. Though the enzymes breaking down ATP will continue to function. Thus, ATP is gradually decreased within the muscle (Hultin, 1984).

With the declination of ATP levels, actomyosin cross-bridges cannot disassociate, ultimately decreasing muscle extensibility (Lonergan *et al*., 2010; Stajkovic *et al*., 2019). ATP degrades to AMP, ADP, inosine, inosine monophosphate and hypoxanthine. ADP and ATP plasticise thin and thick filaments of muscles. In the presence of ADP and ATP, thin and thick filaments are prevented from interacting. However, once ADP and ATP are depleted, the thin and thick filaments will interact (Hultin, 1984). This leads to muscles losing their extensibility. This state is referred to as rigor mortis (Hultin, 1984; Stajkovic *et al*., 2019). Rigor mortis is generally accompanied by contractions. The time taken to attain rigor mortis depends on multiple factors, such as the species, the muscle under consideration, handling of the muscle following slaughter, the temperature the muscle is held at, etc. Prior to rigor mortis, muscle is generally tender when cooked. After rigor mortis, the muscle becomes tough (Hultin, 1984). Rigor bonds are formed at an increasing rate, potentially leading to myofibrils shortening. This phase is called the rapid onset phase (Lonergan *et al*., 2010).

Following death, muscles tend to contract. This is due to a reduction in the ability of muscle cells to regulate the Ca^{2+} levels within the sarcoplasm. During anaerobic conditions, mitochondria cannot retain Ca^{2+}, thus leading to a calcium build-up within the sarcoplasm. Furthermore, the sarcoplasmic reticulum cannot function efficiently enough to maintain a low Ca^{2+} concentration to prevent contractions (Hultin, 1984).

The toughness of meat decreases with storage time. The decrease in toughness is referred to as the resolution of rigor. The breakdown of Z-discs is essential in the post rigor tenderisation of meat. There is evidence that the action of proteolytic enzymes (i.e. calpains, cathepsins, caspases, proteosomes) destroys Z-discs. Cathepsins are a group of proteases associated with lysosomes, and they function

optimally at an acidic pH (Hultin, 1984). An illustration of the conversion of muscle to meat is given in **Figure 2.1**.

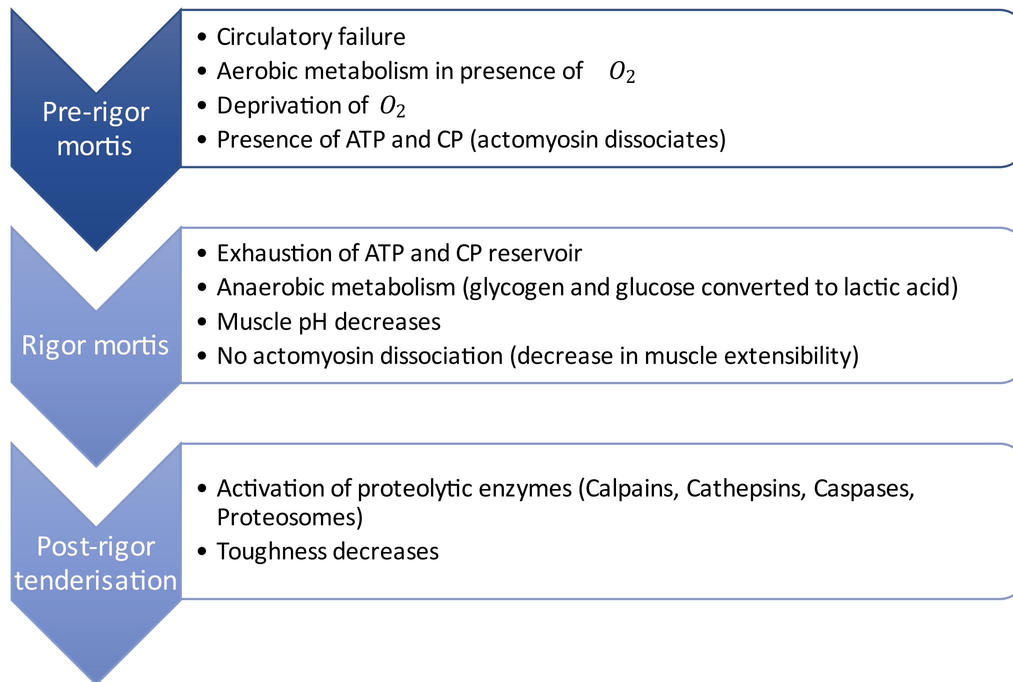

Figure 2.1 Illustration of the conversion of muscle to meat (adapted from Madhusankha & Thilakarathna, 2021)

2.4 Meat quality parameters

Meat quality is determined by multiple facets (Bekhit *et al.*, 2014). When assessing meat quality, consumers consider meat tenderness (Bailey, 1972; Lonergan *et al.*, 2010; Bekhit *et al.*, 2014). Meat texture can be measured by the Warner Bratzler or Volodkovitch technique (Bailey, 1972). It is important to note that muscle is mainly comprised of fibrous proteins, which provide structure to the muscle (Bailey, 1972; Lawrie & Ledward, 2006). The nature of proteins and the arrangement and length of sarcomeres determine the tenderness of meat (Bailey, 1972; Lawrie & Ledward, 2006; Mandal & Pal, 2014). Furthermore, the structure and/or number of collagens and elastin in meat influences meat tenderness (Bekhit *et al.*, 2014). Bailey (1972) further states that the number of collagen crosslinks determines the tenderness of the meat. Additionally, the age of the animal affects meat toughness, where older animals give tougher meat than younger animals, and vice versa (Bailey, 1972; Mandal & Pal, 2014). According to Bekhit *et al.* (2014), the tenderness of meat is primarily determined by the maturity and amount of connective tissue, muscle contraction, and the degree of

tenderisation that takes place during aging. The relative contribution of these facets to meat tenderness varies based on the species, age, muscle type, processing conditions, and cooking method (Bekhit *et al*., 2014).

The temperature after slaughter will also affect the tenderness of the meat. At a temperature of ca. 15 ºC, minimum contraction will occur. Extensive contraction will occur at temperature < 10 ºC (Bailey, 1972; Hultin, 1984; Lawrie & Ledward 2006; Lonergan *et al*., 2010). This is referred to as cold-shortening and is most apparent within red muscles. Cold-shortening leads to the increase of meat toughness to ca. 60% of the rest length. Thin and thick filaments overlap completely at ca. this degree of shortening (Hultin, 1984). Lastly, at temperatures > 25 ºC, contractions due to rigor are less rapid (Bailey, 1972; Lawrie & Ledward, 2006). Of further importance is the ultimate pH reached by meat. In chicken the ultimate pH is ca. 6.2 and in fish the ultimate pH ranges between 6.6 and 6.7. However, a rapid drop in pH below these levels while the carcass is still warm leads to conditions such as pale, soft and exudative meat (Hultin, 1984).

The muscle fibre type (Klont *et al*., 1998; Lee *et al*., 2010; Bekhit *et al*., 2014), size and number can influence the meat quality, where larger muscle fibres lead to less tender meat and vice versa (Lee *et al*., 2010). Furthermore, the fibre type composition varies between muscles (Klont *et al*., 1998). The fibre type composition is determined by the physiological function of muscles in the live animal (Bekhit *et al*., 2014), the location of the muscle within the body, animal age, weight, and breed of animal. There are three muscle fibre types, namely red muscle fibres (slow-twitch fibres), intermediate muscle fibres, and white muscle fibres (fast-twitch fibres) (Klont *et al*., 1998). Muscle fibre types can further be divided into type I, type IIA and type IIB as determined by histochemical methods (Klont *et al*., 1998; Lee *et al*., 2010).

Various physical and chemical factors affect the pigment myoglobin's chemistry (Winstanley, 1979). Furthermore, myoglobin's chemistry ultimately affects the colour of meat (Winstanley, 1979; Lawrie & Ledward, 2006; Mandal & Pal, 2014). Myoglobin, an oxygen carrier within muscles, varies in colour from purple to red to brown. The amount of myoglobin in a muscle is based on the physiological need of the muscle (Winstanley, 1979), with the amount of myoglobin causing different colour intensities in meat (Winstanley, 1979; Lawrie & Ledward, 2006; Mandal & Pal, 2014). Furthermore, the activity of the muscle affects the amount of myoglobin, with higher activity levels leading to more myoglobin and vice versa (Winstanley, 1979). The animal's age also determines the colour of the meat since the concentration of myoglobin increases as age increases (Winstanley, 1979; Mandal & Pal, 2014). Mandal & Pal (2014) further states that the colour of meat can be influenced

by stress in the animal. Parameters of colour include L* (lightness or brightness), a* (red-green), b* (yellow-blue), chroma (intensity of colour) and h_{ab} (hue angle) (Černíková *et al*., 2015).

The proximate composition of meat includes moisture, crude protein, ether extract, ash, and crude fibre content. Moisture is determined by drying a known sample weight at 100 ºC. The crude protein content is calculated utilising the Kjeldahl method, where nitrogen content is multiplied by 6.25. The ether extract content is determined by extracting the sample with petroleum ether for an exact period; hereafter, the solvent is evaporated, and the remaining residue is the ether extract. Ash is determined by igniting a known sample weight at 550 ºC; the remaining residue is known as ash. Lastly, the crude fibre content is determined by treating the residue after ether extraction with boiling acid and alkali (Van Tonder, 2019).

2.5 Crocodilian meat quality and carcass characteristics

2.5.1. Carcass characteristics

A carcass yield of 65% and meat yield of 30-40% can be expected from crocodilians. However, there can be differences between species, sexes, sizes of crocodilians (Huchzermeyer, 2003), availability of food, handling techniques while cutting, and hormonal changes (Hoffman *et al*., 2000; Kluczkovski Junior *et al*., 2015). Hoffman *et al*. (2000) found the dressing percentage of Nile crocodiles to be 56.5%, Kluczkovski Junior *et al*. (2015) found a dressing percentage of 57.02% for Black caiman (*Melanosuchus niger*), and Medeiros *et al*. (2021) found a dressing percentage of 59.7% for Pantanal caiman.

In Nile crocodiles, the tail, legs, torso, and neck make up 32.6 ± 0.82, 16.8 ± 0.82, 39.0 ± 0.67, and 11.9 ± 0.97% of the carcass weight, respectively (Hoffman *et al*., 2000). In Pantanal caiman of intermediate weight (5.9-6.9 kg), the tail, sirloin fillet, back fillet, and thigh have yields of 14.2%, 7.3%, 5.2%, and 5.0%, respectively (Medeiros *et al*., 2021). The tail has the highest yield compared to other cuts in Pantanal caiman (Fernandes *et al*., 2017; Medeiros *et al*., 2021) and Black caiman (Kluczkovski Junior *et al*., 2015). Pantanal caiman showed no differences in carcass yields between male and female animals (Medeiros *et al*., 2021). In contrast, Fernandes *et al*. (2017) found that the female Pantanal caiman yields more than the male Pantanal caiman.

2.5.2. Meat pH, colour, and texture

The initial post mortem pH value may differ due to species, sex, diet, season, activity level, muscle type, and stress caused by capture or stunning and slaughter (Huang *et al*., 2018). Černíková *et al*. (2015) determined the pH of Nile crocodile meat within the dorsal and ventral tail, neck, shoulder, leg, and cheek after 24 h of storage at 4 ºC, as 5.75 to 7.11. Hoffman *et al*. (2000) determined that the pH of Nile crocodile tail and leg meat is 6.67 ± 0.258 and 6.53 ± 0.186 at 24 h post mortem. The pH at 24 h post mortem within various anatomical locations of the Nile crocodile carcass is indicated in **Table 2.3**.

Table 2.3 pH in various anatomical locations of Nile crocodile carcasses after 24 h of storage at 4 ± 1 ºC (adapted from Černíková *et al*., 2015)

Anatomical location	pH
Dorsal tail	5.75 to 6.25
Ventral tail	6.18 to 6.82
Neck	6.17 to 6.66
Shoulder	6.71 to 7.11
Leg	6.59 to 6.91
Cheek	6.24 to 6.77

In the Nile crocodile carcass, the highest pH measurement after 24 h of storage is in the leg and shoulder, and the lowest is in the dorsal part of the tail (Černíková *et al*., 2015). Hoffman *et al*. (2000) found no difference in pH between the leg and tail meat at any time post mortem. In a study by Huang *et al*. (2018), the highest post mortem pH value was seen in the anterior ventral meat of Spectacled caiman.

The temperature of the environment greatly affects the rate of pH decline post mortem since crocodilians are poikilothermic. The rate of pH decline pattern is similar within the tail and leg at each time interval post mortem. However, there is a decrease in the pH of leg meat at 12 h post mortem, which is not seen in the tail. The pH of leg and tail meat decreases towards 48 h post mortem. This decreasing pH indicates that rigor mortis is incomplete when carcasses are processed (Hoffman *et al*., 2000).

Crocodile meat is white and firm and has a flavour ranging between chicken, fish, and veal (Huchzermeyer, 2003) and a texture like pork or veal (FAO, 1989). Hoffman *et al*. (2000) state that the tail and neck of crocodiles are lighter than the legs, with the tail being white to pink and the legs

being darker. Colour parameters were measured in different anatomical locations of the Nile crocodile carcass by Černíková *et al*. (2015), and are indicated in **Table 2.4**.

Table 2.4 Colour measurements in different anatomical locations of Nile crocodile carcasses after 24 h of storage at 4 ± 1 ºC (adapted from Černíková *et al*., 2015)

	L*	a*	b*	h_{ab}
Dorsal tail	58.6 to 62.2	-1.63 to -0.22	6.49 to 8.32	272 to 282
Ventral tail	45.1 to 56.7	0.21 to 3.14	7.18 to 9.48	66.4 to 88.4
Neck	53.9 to 59.1	0.11 to 4.05	6.70 to 11.4	68.9 to 89.1
Shoulder	33.4 to 40.0	10.3 to 15.0	5.97 to 9.89	28.0 to 39.8
Leg	43.0 to 45.3	6.31 to 12.6	6.16 to 9.63	27.1 to 54.6
Cheek	55.8 to 62.2	-1.71 to 1.08	2.28 to 6.39	28.7 to 87.3

A study by Černíková *et al*. (2015) found the lowest lightness (L*) values and highest redness (a*) values in the shoulder of Nile crocodiles. In contrast, the dorsal tail and cheek had the highest lightness and lowest redness values. The study further found that the yellowness (b* values) was similar in various carcass locations. Lastly, the shoulder and legs have similar hue angle (h_{ab}) values, which are lower than those in the ventral tail and neck (Černíková *et al*., 2015). In Pantanal caiman, lightness is similar among the cuts and sexes. The male animals have more yellow meat than the female animals. In both sexes, the thigh and back fillet is more reddish than the sirloin and tail (Fernandes *et al*., 2017).

In a study by Balowski *et al*. (2015), sensory analysis (tenderness, springiness, cohesiveness, chewiness, juiciness, stringiness, the perceptibility of connective tissue, flavour intensity, and odour intensity) was done on crocodile tail meat (*m. longissimus*) from Zimbabwe and compared to kangaroo meat. These parameters were given a result based on a seven-point scale (1-7). The outcomes of the study were scores of 7.00 ± 0.25 for tenderness (1 being tough and 7 being tender), 6.00 ± 0.01 for springiness, 2.75 ± 0.25 for cohesiveness, 6.00 ± 0.25 for chewiness, 5.00 ± 0.25 for juiciness, 7.00 ± 0.25 for stringiness, 1.00 ± 0.25 for perceptibility of connective tissue, 6.25 ± 0.01 for flavour intensity, and 1.50 ± 0.25 for odour intensity of crocodile meat.

Hoffman *et al*. (2000) determined the cooking loss of the tail, legs, torso, and neck of Nile crocodiles as 31.45 ± 1.61%, 29.64 ± 2.48%, 23.19 ± 5.35%, and 32.07 ± 2.33%, respectively. In comparison, Balowski *et al*. (2015) determined a cooking loss of 18.94 ± 0.35% for crocodile tail meat from Zimbabwe. **Table 2.5** shows the texture parameter values for different anatomical locations of Nile crocodile carcasses.

Table 2.5 Texture parameters for different anatomical locations of Nile crocodile carcasses

	Tail	Dorsal tail	Ventral tail	Neck	Shoulder	Leg	Cheek
Hardness (Newtons)	55.05 ± 10.08	183 to 223	196 to 238	140 to 169	172 to 208	191 to 255	259 to 276
Cohesiveness (-)	0.323 ± 0.051	0.37 to 0.45	0.38 to 0.49	0.34 to 0.47	0.49 to 0.61	0.46 to 0.53	0.56 to 0.62
Adhesiveness (Newtons/s)	-	0.29 to 0.34	0.21 to 0.29	0.32 to 0.38	0.21 to 0.30	0.24 to 0.31	0.22 to 0.30
Reference	Balowski *et al.* (2015)	Černíková *et al.* (2015)	Černíková *et al.* (2015)	Černíková *et al.* (2015)	Černíková *et al.* (2015)	Černíková *et al.* (2015)	Černíková *et al.* (2015)

Cooking loss is the lowest in the torso compared to other anatomical locations in Nile crocodile carcasses (Hoffman *et al*., 2000). Černíková *et al*. (2015) indicated that the hardness is similar in the dorsal tail, ventral tail, shoulder, and legs. Furthermore, the neck is less hard than the parts mentioned above, and the cheek has the highest hardness value. Moreover, cohesiveness and adhesiveness are similar in all anatomical locations of the Nile crocodile carcass (Černíková *et al*., 2015).

2.5.3. Proximate composition

Various studies have determined Nile crocodile meat's moisture, protein, fat, ash, and fibre content in different anatomical locations. Luthada-Raswiswi *et al*. (2019) also determined the proximate composition of meal from various anatomical locations in Nile crocodile carcasses; these parameters' values are indicated in **Table 2.6**.

Table 2.6 Proximate composition (g/kg) of different anatomical locations in Nile crocodile carcasses

Description	Moisture	Protein	Fat	Ash	Fibre	Reference
Tail						
Raw	70.17 ± 1.896	21.09 ± 0.809	8.85 ± 2.715	0.59 ± 0.155	-	Hoffman *et al.* (2000)
Cooked	65.01 ± 2.189	28.07 ± 1.254	5.44 ± 2.599	0.66 ± 0.340	-	Hoffman *et al.* (2000)
Dorsal	66.1 to 68.8	15.9 to 17.1	13.3 to 15.5	-	-	Černíková *et al.* (2015)
Ventral	74.3 to 76.6	14.3 to 16.2	4.41 to 5.9	-	-	Černíková *et al.* (2015)
Legs						
Raw	73.39 ± 0.757	22.40 ± 0.959	4.04 ± 1.163	0.36 ± 0.079	-	Hoffman *et al.* (2000)
Raw meal	12.40 ± 0.88	85.06 ± 0.25	3.63 ± 0.26	3.24 ± 0.41	0.01 ± 0.07	Luthada-Raswiswi *et al.* (2019)
Cooked	68.29 ± 0.563	26.90 ± 0.963	3.76 ± 1.268	0.97 ± 0.615	-	Hoffman *et al.* (2000)
Cooked meal	12.39 ± 0.81	84.55 ± 1.69	6.22 ± 0.12	3.23 ± 0.16	0.02 ± 0.07	Luthada-Raswiswi *et al.* (2019)
	71.4 to 73.9	16.6 to 18.7	4.97 to 7.02	-	-	Černíková *et al.* (2015)
Torso						
Raw	67.07 ± 2.238	21.88 ± 0.702	9.11 ± 1.696	0.65 ± 0.200	-	Hoffman *et al.* (2000)
Raw meal	8.73 ± 2.72	81.05 ± 1.30	4.12 ± 0.07	2.74 ± 0.41	0.03 ± 0.06	Luthada-Raswiswi *et al.* (2019)
Cooked	64.71 ± 4.010	25.12 ± 1.570	8.19 ± 5.710	0.42 ± 0.079	-	Hoffman *et al.* (2000)
Cooked meal	11.04 ± 1.17	82.03 ± 0.23	8.13 ± 0.42	2.83 ± 0.58	0.11 ± 0.02	Luthada-Raswiswi *et al.* (2019)
Neck						
	71.8 to 75.8	14.0 to 16.1	0.96 to 1.50	-	-	Černíková *et al.* (2015)
Raw meal	9.75 ± 1.18	82.11 ± 0.17	8.45 ± 0.35	3.32 ± 0.46	0.04 ± 0.06	Luthada-Raswiswi *et al.* (2019)
Cooked meal	12.03 ± 2.18	80.02 ± 0.39	8.22 ± 0.10	3.08 ± 0.32	0.26 ± 0.02	Luthada-Raswiswi *et al.* (2019)
Shoulder						
	74.8 to 78.0	15.8 to 17.4	0.47 to 1.01	-	-	Černíková *et al.* (2015)
Cheek						
	74.1 to 76.5	17.2 to 19.6	0.48 to 1.53	-	-	Černíková *et al.* (2015)

The proximate composition of meat can vary based on the age of the animal, the sex, the weight, and the cut of meat (Huang *et al*., 2018). It is noted that there are differences in the proximate composition between the muscles of animals due to the muscle fibre type and the type of exercise the muscle does (Vicente-Neto *et al*., 2010). A study by Hoffman *et al*. (2000) showed that the protein and fat content differ in various anatomical locations of the Nile crocodile carcass. It is shown that the neck of Nile crocodiles has high protein content, whereas the tail and legs have a high fat content (Hoffman *et al*., 2000). Černíková *et al*. (2015) showed that the least amount of water and the highest amount of fat is found in the dorsal tail. The highest amount of crude protein is found in the cheek. Furthermore, the neck, shoulder, and cheek have the least fat (Černíková *et al*., 2015). Vicente-Neto *et al*. (2010) also showed that the tail of *Caiman crocodilus yacare* has lower moisture, protein and ash content and a higher fat content than the neck. Luthada-Raswiswi *et al*. (2019) also found differences in the crude protein content between anatomical locations.

In various species, it has been seen that proximate composition is different among the sexes, e.g. at similar slaughter weights, males have a lower amount of fat and a higher amount of lean meat compared to females. Furthermore, the age of animals affects the proximate composition, e.g. meat from older animals tend to be more tough compared to meat from younger animals (Mandal & Pal, 2014). Within Pantanal caiman, the least amount of moisture and the highest amount of lipid is found in the tail compared to other cuts in both males and females at approximately two years of age. The thigh of male Pantanal caiman has lower protein content than other cuts of males. However, crude protein content is similar between the sexes. Furthermore, females contain more fat within the tail and thigh than males. Lastly, the thigh of males has lower ash content than the tail of males and the sirloin of females (Fernandes *et al*., 2017).

2.5.4. Mineral content

Hoffman *et al*. (2000), Swanepoel *et al*. (2000), Du Preez *et al*. (2016), Luthada-Raswiswi *et al*. (2019) and Van der Westhuizen (2019) ground various parts of the Nile crocodile carcass's meat to form a meal for the analysis of mineral composition. **Table 2.7** indicates the mineral content of various anatomical locations of the Nile crocodile carcass, as seen by various studies.

Table 2.7 Mineral content (mg/kg dry mass) of various anatomical locations in Nile crocodile carcasses

	Neck	Leg	Torso	Tail	Tail	Tail	Muscle
K	38.20 ± 1.77	39.67 ± 0.30	37.23 ± 1.69	2 423 ± 205.5	7 600	-	-
Na	8.72 ± 0.61	11.24 ± 0.16	10.98 ± 0.42	282 ± 27.1	11 000	-	-
Ca	2.30 ± 0.02	1.42 ± 0.06	2.11 ± 0.03	68 ± 9.0	2 800	-	-
Mg	1.69 ± 0.12	1.54 ± 0.08	1.62 ± 0.08	185 ± 15.3	1 000	-	-
Zn	0.15 ± 0.09	0.35 ± 0.05	0.20 ± 0.02	11 ± 2.0	68	0.27 to 5.8	39.4 to 109.7
Fe	0.12 ± 0.02	0.22 ± 0.02	0.21 ± 0.05	3 ± 0.4	210	18 to 523	156.0 to 615.0
Al	0.10 ± 0.02	0.13 ± 0.03	0.15 ± 0.02	7 ± 2.1	13	1.7 to 33	73.5 to 367.8
Cu	0.07 ± 0.02	0.09 ± 0.03	0.06 ± 0.04	-	6.9	0.014 to 2.8	7.9 to 12.6
P	-	-	-	1 939 ± 129.2	-	-	-
Reference	Luthada-Raswiswi *et al.* (2019)	Luthada-Raswiswi *et al.* (2019)	Luthada-Raswiswi *et al.* (2019)	Hoffman *et al.* (2000)	Van der Westhuizen (2019)	Du Preez *et al.* (2016)	Swanepoel *et al.* (2000)

Van der Westhuizen (2019) found no difference in the mineral content of the tail muscle between wild male and female Nile crocodiles sampled from the Kruger National Park. Furthermore, it is shown that Rb and Cs increase with increasing crocodile mass. Moreover, Zn, Ga, Ba, Zr and Hf decrease with increasing mass of crocodile (Van der Westhuizen, 2019). Swanepoel *et al.* (2000) found that the amount of Fe is correlated to the size of the crocodile, with larger crocodiles having higher amounts of Fe and vice versa in the muscle tissue. In contrast, Fe is lower in the fat tissue of larger crocodiles and vice versa. Furthermore, the Fe content differs between the locations sampled (Swanepoel *et al.*, 2000). Du Preez *et al.* (2016) found that larger animals have lower metal concentrations than smaller animals within muscle tissue. Luthada-Raswiswi *et al.* (2019) found differences in the concentrations of K, Na, Ca, Mg, Zn, and Al between the various anatomical locations of meal from Nile crocodile carcasses.

2.5.5. Fatty acid composition

Hoffman *et al*. (2000) reported a mean SFA content of 37.72%, a total mean MUFA content of 51.09%, a total PUFA content of 10.74%, an omega-3 FA value of 1.69%, an omega-6 FA value of 9.05%, and a PUFA/SFA ratio of 0.28 in the tail meat of Nile crocodile carcasses. The primary FAs in the tail of Nile crocodiles are oleic and palmitic acid, with stearic, linoleic and palmitoleic acids present in significant concentrations (Hoffman *et al*., 2000). **Table 2.8** indicates the FA composition of various anatomical locations in Nile crocodile carcasses.

Table 2.8 Fatty acid composition of various anatomical locations in Nile crocodile carcasses

	Tail	Tail	Tail	Fat	Abdominal	Steatothecum
% Fat	-	77.4 ± 10.7	1.8 ± 0.3	78.9 ± 8.7	73.3 ± 7.5	70.8 ± 10.9
% FFDM	-	8.6 ± 2.1	24.1 ± 0.3	9.3 ± 2.2	8.5 ± 1.9	9.0 ± 1.8
% Moisture	-	14.0 ± 9.7	74.1 ± 0.4	11.8 ± 6.7	18.6 ± 6.8	20.2 ± 10.4
FAME (% of total FAs)						
SFAs						
Lauric	0.04 ± 0.019	0.2 ± 0.3	-	-	0.2 ± 0.3	0.1 ± 0.2
Myristic	0.75 ± 0.066	3.8 ± 0.8	0.3 ± 0.1	0.4 ± 0.0	3.9 ± 0.9	3.8 ± 0.9
Pentadecyclic	0.27 ± 0.029	1.0 ± 0.3	0.1 ± 0.0	0.1 ± 0.0	1.0 ± 0.2	1.0 ± 0.2
Palmitic	25.38 ± 3.008	27.9 ± 1.9	20.2 ± 0.1	20.6 ± 0.2	28.2 ± 1.7	28.7 ± 1.6
Margaric	-	0.9 ± 0.2	0.1 ± 0.0	0.1 ± 0.1	1.0 ± 0.2	1.0 ± 0.2
Stearic	9.89 ± 1.700	8.1 ± 2.5	7.9 ± 0.4	5.7 ± 0.1	8.5 ± 2.7	9.3 ± 2.6
Arachidic	1.38 ± 0.530	0.2 ± 0.1	0.1 ± 0.1	0.1 ± 0.0	0.2 ± 0.1	0.2 ±0.1
Behenic	-	0.1 ± 0.1	-	-	0.1 ± 0.1	0.1 ± 0.1
Total SFA	-	42.3 ± 3.3	28.7 ± 0.4	26.9 ± 0.2	43.2 ± 3.7	44.3 ± 3.0
MUFAs						
Myristoleic	-	0.1 ± 0.1	-	0.1 ± 0.0	0.1 ± 0.1	0.1 ± 0.1
Palmitoleic	5.85 ± 0.184	7.5 ± 1.6	3.1 ± 0.3	4.1 ± 0.1	7.4 ± 1.8	6.3 ± 1.7
Heptadecenoic	-	0.3 ± 0.3	-	0.1 ± 0.0	0.2 ± 0.2	0.1 ± 0.2
Elaidic	-	0.3 ± 0.2	0.1 ± 0.0	0.1 ± 0.1	0.2 ± 0.1	0.2 ± 0.1
Oleic	43.05 ± 2.056	28.6 ± 6.4	27.3 ± 2.1	34.2 ± 0.3	29.4 ± 5.7	30.8 ± 8.1
Vaccenic	-	4.1 ± 1.5	2.6 ± 0.2	2.0 ± 0.1	4.1 ± 1.6	3.8 ± 1.6
Eicosenoic	2.19 ± 0.811	0.2 ± 0.2	0.2 ± 0.1	0.3 ± 0.0	0.2 ± 0.2	0.3 ± 0.2
Total MUFAs	-	41.2 ± 5.2	33.2 ± 2.2	40.7 ± 0.3	41.7 ± 3.7	41.6 ± 6.5
PUFAs						
Linoleic	9.05 ± 6.898	5.5 ± 2.0	29.6 ± 0.3	28.7 ± 0.3	5.4 ± 2.2	5.6 ± 2.9
γ-linolenic	-	0.3 ± 0.2	0.2 ± 0.1	0.3 ± 0.0	0.2 ± 0.2	0.2 ± 0.2
α-linolenic	-	3.0 ± 1.2	1.6 ± 0.1	1.9 ± 0.1	3.0 ± 1.2	2.3 ± 1.1
Eicosadienoic	-	0.3 ± 0.2	0.3 ± 0.1	0.3 ± 0.0	0.3 ± 0.2	0.2 ± 0.2
Eicosatrienoic	-	0.4 ± 0.2	0.4 ± 0.1	0.3 ± 0.0	0.4 ± 0.2	0.4 ± 0.3
Arachidonic	-	1.3 ± 0.4	4.2 ± 1.1	0.8 ± 0.1	1.3 ± 0.5	1.0 ± 0.5
Eicosaptaenoic	0.48 ± 0.007	0.8 ± 0.6	0.2 ± 0.1	0.1 ± 0.0	0.6 ± 0.3	0.4 ± 0.3
Docosapentaenoic	0.31 ± 0.035	1.2 ± 0.6	0.5 ± 0.4	0.1 ± 0.1	1.0 ± 0.6	0.7 ±0.6
Docosahexaenoic	0.90 ± 0.021	2.6 ± 1.8	1.1 ± 0.3	0.1 ± 0.0	2.2 ± 1.5	1.5 ± 1.8
Total PUFAs	-	15.6 ± 5.6	38.1 ± 1.8	32.4 ± 0.4	14.7 ± 5.6	12.4 ± 6.6
Total omega-3 FA		8.0 ± 3.8	3.8 ± 0.6	2.4 ± 0.1	7.3 ± 3.4	5.4 ± 3.6
Total omega-6 FA	-	7.6 ± 2.5	34.3 ± 1.4	30.0 ± 0.3	7.4 ± 2.8	7.1 ± 3.4
Reference	Hoffman *et al.* (2000)	Osthoff *et al.* (2014)	Osthoff *et al.* (2010)	Osthoff *et al.* (2010)	Osthoff *et al.* (2014)	Osthoff *et al.* (2014)

The diet of crocodilians influences the FA composition of the meat (Hoffman *et al.*, 2000; Huchzermeyer, 2003; Osthoff *et al.*, 2010; Vicente-Neto *et al.*, 2010). Furthermore, the FA composition determines the firmness of fat (Osthoff *et al.*, 2010), specifically the length of the FAs

and the presence or absence of double bonds in the FAs (Webb & O'Neill, 2008). Meat from crocodiles fed a fish-based diet contains more FAs with 20 carbons or longer chain FAs than that of crocodiles fed chicken or beef (Osthoff *et al*., 2010). Osthoff *et al*. (2014) further stated that the species, sex, nutrition, and environment affect the FA composition. The fat content differs according to the season, the diet, and the exercise intensity of the animal (Kluczkovski Junior *et al*., 2015).

Hoffman *et al*. (2000) and Osthoff *et al*. (2014) indicated that the FA composition does not differ between various types of adipose tissues. However, eicosapentaenoic acid is higher in the steatotheca (Osthoff *et al*., 2014). Osthoff *et al*. (2010) noted differences in the FA composition between the intramuscular and adipose fat in captive Nile crocodiles, with intramuscular fat having less oleic and more arachidonic acid. In Pantanal caiman, the tail has the highest SFAs and MUFAs content and a higher omega-6/omega-3 ratio than the other cuts. The back fillet contains more PUFAs, followed by the sirloin, thigh, and tail. The back fillet and thigh have more omega-3 FAs than the tail and sirloin (Fernandes *et al*., 2017). Huang *et al*. (2018) found greater amounts of SFAs and PUFAs in the anterior ventral meat than in other parts of the carcass. Due to the higher amount of oleic acid in the tail of Spectacled caiman, a higher amount of MUFAs is seen in this cut (Huang *et al*., 2018). Vicente-Neto *et al*. (2010) showed that the SFA content is similar between the tail and neck of Pantanal caiman and that the tail has more MUFAs than the neck. Furthermore, the neck had a higher PUFA content than the tail (Vicente-Neto *et al*., 2010). In terms of FA composition, the abdomen and tail fat are more similar to each other than either is to the steatotheca. The amount of tail fat in crocodiles is related to their nutritional state (Osthoff *et al*., 2014).

Osthoff *et al*. (2014) showed differences in the FA composition between the sexes in Nile crocodiles; this difference is due to the higher PUFA content in female animals than male animals. It is further shown that the fat of male animals is more saturated than the fat of female animals. The most differences between the sexes are in pentadecyclic, palmitoleic, vaccenic, stearic, oleic, and margaric acids. The females have more palmitoleic and vaccenic acids but less oleic acid than the males. Lastly, females have higher omega-3 and omega-6 FAs and more uneven FAs than males (Osthoff *et al*., 2014).

2.5.6. Amino acid composition

Regarding amino acids, Nile crocodile meat has the greatest amount of asparagic and glutamic acids, followed by lysine; leucine is also present in great amounts (Černíková *et al*., 2015). **Table 2.9** shows the amino acid composition of different anatomical locations in Nile crocodile carcasses.

Table 2.9 Amino acid composition (g/kg) of different anatomical locations in Nile crocodile carcasses

	Tail	Dorsal tail	Ventral tail	Neck	Shoulder	Leg	Cheek
Asparagic acid	-	14.5 to 18.5	12.4 to 14.9	12.0 to 14.8	12.9 to 14.1	13.0 to 14.8	15.9 to 17.4
Threonine	3.291 ± 0.057	5.29 to 7.95	4.39 to 6.06	4.32 to 6.53	4.76 to 6.26	5.48 to 6.10	5.89 to 6.74
Serine	2.817 ± 0.066	5.56 to 6.33	3.13 to 5.51	3.78 to 5.63	4.20 to 5.45	4.39 to 5.38	4.35 to 5.84
Glutamic acid	-	24.4 to 28.9	19.4 to 22.9	22.8 to 24.6	23.2 to 25.3	24.6 to 27.6	26.2 to 28.2
Glycine	4.056 ± 0.239	8.69 to 11.9	5.45 to 6.84	6.49 to 7.88	6.78 to 8.55	7.79 to 8.75	7.83 to 9.44
Proline	-	4.85 to 6.90	4.24 to 5.97	5.29 to 6.09	5.80 to 6.78	5.91 to 6.84	5.72 to 6.93
Alanine	4.533 ± 0.086	5.46 to 7.90	5.76 to 7.62	6.98 to 8.59	6.34 to 8.33	7.71 to 9.88	7.41 to 9.30
Valine	3.471 ± 0.087	6.83 to 8.88	4.89 to 6.95	5.88 to 7.44	5.78 to 7.14	5.35 to 6.96	6.12 to 8.11
Methionine	2.060 ± 0.045	4.23 to 5.94	4.80 to 6.58	4.25 to 6.57	4.10 to 5.65	4.84 to 5.86	5.61 to 7.12
Isoleucine	3.557 ± 0.113	6.76 to 9.46	5.15 to 6.65	5.72 to 6.61	5.38 to 6.89	6.36 to 7.18	5.92 to 7.47
Leucine	6.430 ± 0.133	7.23 to 10.7	8.40 to 10.3	9.07 to 10.4	9.08 to 11.3	9.74 to 11.9	11.4 to 13.8
Tyrosine	2.597 ± 0.076	5.16 to 6.41	3.39 to 4.72	3.81 to 5.25	3.76 to 5.12	4.37 to 5.58	4.04 to 5.52
Phenylalanine	2.913 ± 0.081	5.11 to 6.63	3.67 to 5.24	4.45 to 6.09	4.38 to 5.65	4.86 to 6.27	5.45 to 6.57
Histidine	2.147 ± 0.073	4.77 to 6.10	3.44 to 4.92	3.51 to 4.95	3.42 to 5.32	3.92 to 4.94	3.27 to 5.23
Lysine	6.971 ± 0.152	10.1 to 12.3	9.56 to 11.8	9.20 to 11.4	10.4 to 12.4	12.4 to 14.5	12.3 to 14.5
Arginine	6.346 ± 1.469	7.69 to 8.91	6.30 to 8.39	6.64 to 8.97	7.26 to 9.74	8.25 to 10.2	8.45 to 10.0
Cysteine	-	1.92 to 2.56	1.88 to 2.57	2.27 to 2.83	2.04 to 2.86	2.34 to 2.77	1.86 to 2.79
EAAI	-	104 to 114	93.3 to 103	84.1 to 93.0	82.8 to 91.5	96.1 to 106	114 to 126
Reference	Hoffman *et al.* (2000)	Černíková *et al.* (2015)	Černíková *et al.* (2015)	Černíková *et al.* (2015)	Černíková *et al.* (2015)	Černíková *et al.* (2015)	Černíková *et al.* (2015)

The highest essential amino acid index (EAAI) values are found in the cheek and dorsal tail and the lowest in the neck and shoulder of Nile crocodiles. Valine, leucine, and threonine are limiting amino acids in the crocodile carcass. Similar amino acid concentrations are seen in the ventral tail,

neck, shoulder, and leg, which is marginally lower than the amino acid concentration in the dorsal tail and cheek. The limiting essential amino acid within the ventral tail, shoulder, leg, and cheek is valine. While threonine is the limiting essential amino acid in the neck, and leucine is the most limiting in the dorsal tail (Černíková *et al*., 2015).

2.6 Stress and its effect on meat quality

When an animal is exposed to a stressor, it will respond through the fight or flight response (Davis *et al*., 2000; Mader, 2006). This includes adrenaline and glucocorticoid secretion by the hypothalamic-pituitary-adrenal axis (Romero, 2004; Mader, 2006; Isberg & Shilton, 2013; Ganswindt *et al*., 2014). From being exposed to a stressor to the increase in circulating glucocorticoids takes ca. 3-5 minutes in birds and mammals and can be even longer in other vertebrates (Romero, 2004). In reptiles, the glucocorticoid corticosterone is typically secreted (Huchzermeyer, 2003; Romero 2004; Mader, 2006; Isberg & Shilton, 2013). Thus, corticosterone is commonly used as an indicator of stress (Romero, 2004; Manolis & Webb, 2016). This reaction is like that which occurs in other reptiles, avian and mammalian species (Huchzermeyer, 2003; Isberg & Shilton, 2013). It should be noted that the ambient temperature can cause quantitatively and qualitatively different stress responses, e.g. at 30 ºC, crocodilians might have an elevated stress response compared to at 20 ºC (Lance *et al*., 2000). Furthermore, female crocodilians commonly have lower levels of stress steroids than male animals (Elsey *et al*., 1990).

Common stressors at crocodilian farms include the inability of hatchlings or juveniles to find cover, transport, fear (Huchzermeyer, 2003), cold and changing temperatures, overheating, inadequate stocking densities (Revol, 1995; Huchzermeyer, 2003), overcrowding, disruption (Masser, 1993; Huchzermeyer, 2003), and inappropriate feeding practices (Masser, 1993), capture, restraint (Huchzermeyer, 2003; NRMMC, 2009), and handling (Revol, 1995; Huchzermeyer, 2003; NRMMC, 2009). When numerous stressors co-occur, they can have an additive effect. This depends on the severity and duration of the stress (Huchzermeyer, 2003).

Stress can lead to disturbed behaviour, which includes anorexia, hydrophobia and excessive lithophagy (Huchzermeyer, 2003; Manolis & Webb, 2016). Piling, decreased food consumption (Masser, 1993; Revol, 1995), stargazing, and aggressive behaviour are indicators of stress (Masser, 1993). Furthermore, crocodilians exposed to stress can have poor growth and survival, disease, reproductive failure (Brien *et al*., 2007), inferior skin quality (Brien *et al*., 2007; Isberg & Shilton, 2013) and poor immune function (Revol, 1995; Isberg & Shilton, 2013).

Chronic stress can occur in crocodiles housed at inappropriate stocking densities, where fleeing from one another is complicated by a lack of space. Continual stress can negatively affect a crocodilian's production, reproduction, and immune system (Huchzermeyer, 2003; Isberg & Shilton, 2013; Ganswindt *et al*., 2014) and can alter wound healing (Isberg & Shilton, 2013).

Chronic stress causes very little acidification of meat post mortem (Winstanley, 1979; Mandal & Pal, 2014; Stajkovic *et al*., 2019). This leads to enzymic activity using up oxygen, consequently forming a small layer of oxymyoglobin, with purple myoglobin predominating. The less acidic pH causes meat to look darker than usual (dark, bluish-red) due to an alteration of the light-scattering properties (Winstanley, 1979). Furthermore, a less acidic pH leads to less tender meat with a higher water-holding capacity. This condition is referred to as dark, firm, and dry (DFD) meat (Mandal & Pal, 2014; Stajkovic *et al*., 2019).

Due to acute stress, the permeability of blood capillaries within the intestine of crocodilians increases. This permits the entry of intestinal bacteria into the bloodstream, leading to septicaemia. Generally, the immune system will manage this once blood corticosteroid levels return to normal. However, in cases where the crocodilian is slaughtered while still in the acute septicaemic stage, bacteria distributed throughout the body remain in the meat. Thus, handling before slaughter should be kept to a minimum if necessary. This is part of the reasoning behind recommending the shooting of crocodilians within their pens without prior handling. However, it should be noted that the corticosteroid response to an acute stressor is delayed, which allows for a swift handling procedure without restraint or live transport (Huchzermeyer, 2003).

Acute stress results in rapid meat acidification post mortem (Winstanley, 1979; Mandal & Pal, 2014), which entails pH falling below 5.9 while the carcass is warm. This leads to protein denaturation, giving the meat a looser texture than usual and allowing more light to reflect, resulting in paler meat than normal (Winstanley, 1979; Stajkovic *et al*., 2019). This is termed pale, soft, and exudative (PSE) meat, which has a lower pH value than average and a paler colour with poor water-holding capacity (Mandal & Pal, 2014; Stajkovic *et al*., 2019). In other species, it is noted that acute stress prior to slaughter, due to the stunning method used, may affect the bleeding/exsanguination process or meat quality (Mandal & Pal, 2014).

An example of short-term stress's effect on meat quality in chickens will be explained since no previous study on the effect of short-term stress on meat quality in the order Crocodylia has been done before. Increased transportation time of broilers accelerates the post mortem anaerobic glycolysis of muscle glycogen, especially in muscle containing more fast glycolytic fibres (type IIb),

which results in lactate accumulation and a rapid post mortem pH decline while the carcass temperatures are still high. Breast muscle from broilers transported for 3 h has a lower pH, higher cooking loss, and higher shear force values at 24 h post mortem than broilers transported for 0.5 h. Increased transportation time also leads to decreased pH values at 45 min post mortem, increased L* value, and increased drip loss of the breast muscle at 24 h post mortem. However, the increased transportation time does not affect the overall quality traits of thigh meat (Wang *et al*., 2017).

Chapter 3
Experimental Design and Statistical Analysis

Ethical approval for this study was given by the University of Pretoria Animal Ethics Committee, with ethics reference number NAS273/2022. Section 20 approval was obtained on the 7th October 2022 from the Department of Agriculture, Land Reform & Rural Development.

3.1 General management of crocodiles

This project was conducted during the last month of Autumn (8 May to 10 May) 2023 at a commercial crocodile farm in South Africa, in the Province of Limpopo. The coordinates of the farm are 24º53'29.3"S, 28º17'10.4"E.

The project used Nile crocodiles hatched out on the farm at a temperature of ca. 30 ºC. This resulted in all crocodiles for the project being female since crocodiles do not have sex chromosomes, and the sex is determined by the temperature at which the egg is incubated (Huchzermeyer, 2003). Female animals are produced when eggs are incubated at ≤ 30 ºC, and eggs incubated at ≥ 34 ºC produce male animals (FAO, 1989; Revol, 1995).

Crocodiles were randomly selected for this project from a single pen housing 521 crocodiles. The pen had a perimeter of 90.53 m and an area of 489.96 m^2, which brought the stocking density to 0.94 m^2/crocodile or 1.063 crocodiles/m^2. The pen had two pools with 162 870 litres of water in total, covering a total area of 162.87 m^2.

The crocodiles were fed a diet of finely minced, fat-free chicken from 3 weeks post-hatching to 12 months of age. After 12 months, the crocodiles were switched to a diet of minced chicken with a supplement (Salmonella inhibitor). The feed was withdrawn 24 h before slaughter. At slaughter, the crocodiles were ca. 36 to 40 months old.

3.2 Stunning and slaughter of crocodiles

The crocodiles were stunned and slaughtered within the pen according to standard operating procedures. At the time of the stunning, the temperature was 16.8 ºC, and the humidity was 99% and it was raining. Prior to stunning, one pool from the pen in which the crocodiles were stunned was

emptied to ca. ¼ of its volume. All crocodiles for the project were stunned within the single emptied pool.

Before stunning, ten buckets were weighed, and the weight was noted as 0.65 kg for each bucket. These buckets were subsequently used to place the crocodile carcasses in for weight measurements. (Note that all weight measurements occurred in a closed cooler truck to prevent rain from entering the buckets).

Trained personnel performed two approved stunning methods (i.e. free bullets and electrical stunning) followed by slaughter. The free bullets method entailed shooting the crocodile through the brain with a .22 rifle, with a silencer attached. CCI sub-sonic .22 long rifle, lead hollow points bullets were used, with a grain of 40 (Annexure A, plate 2). During the electrical stunning method, an electrical stunner was used to stun a crocodile at the back of the head. The stunner was powered by a generator placed in the pen's corner. The stunner consisted of two electrodes at the end of a pole (Annexure A, plate 3). The stunner was set to a voltage of 110 V, and the electrical current was calculated as 3.52 A using the formula in **Figure 3.1**. Directly after stunning for approximately 5-7 seconds, the neck was severed between the cranium and the first cervical vertebra, and the brain was destroyed by pithing.

$$\frac{A_{max}}{V_{max}} = \frac{A_{input}}{V_{input}}$$

$$\frac{8}{250} = \frac{A_{input}}{110}$$

$$A_{input} = 3.52\ A$$

Figure 3.1 Formula for calculating the electrical current (A) of the electrical stunner

Ten crocodiles were stunned at first within the pool by the free bullets method. Each crocodile was sexed before bleeding, and all animals were determined to be female. Directly, after sexing, the crocodiles were slaughtered by making a cut of ca. 3 cm on top of the head (caudal to the cranium) with a sterilised knife, and the crocodiles were placed into separate buckets for bleeding. The crocodile with the bucket and blood flowing into the bucket was weighed directly after placement into the bucket to determine the animal's live weight (calculated using the formula in **Figure 3.2**). The carcasses were left at an angle of ca. 45° in the bucket to bleed out for 20 min, and each carcasses' tail tip was tagged with a skin tag (Annexure A, plate 9) for subsequent identification. After 20 min

of bleeding, the bucket with blood was weighed to determine the weight of blood loss (calculated using the formula in **Figure 3.3**). After bleeding, these carcasses were hung on a rail in a cooler truck by inserting a sterilised hook into a cut made through the tail for transportation to the abattoir on the farm.

Live weight (kg) = whole carcass before skinning and dressing – bucket weight * *(whole carcass includes the head, body, tail, skin, feet, and intestines)*

Figure 3.2 Formula for calculating the live weight (kg) of crocodiles

Blood loss (kg) = weight of the bucket and blood – weight of the bucket

Figure 3.3 Formula for calculating blood loss (kg)

After the blood loss of the first ten carcasses was weighed, the buckets were washed with water and dried out, and the generator for the electrical stunning method was started. The electrical stunning method was performed to stun another ten crocodiles within the same pool as those stunned by the free bullets method. After stunning, slaughter, and pithing, the crocodiles were sexed, and all animals were determined to be female. After sexing, the carcasses were placed into a bucket for bleeding. Directly after placement into the bucket, the carcass, with the bucket and blood flowing into the bucket, was weighed to determine the animal's live weight (according to the formula in **Figure 3.2**). The carcasses were left at an angle of ca. 45º in the bucket to bleed out for 20 min, and each carcasses' tail tip was tagged with a skin tag for subsequent identification. After 20 min of bleeding, the bucket with blood was weighed to determine the weight of blood loss (calculated according to the formula in **Figure 3.3**).

After bleeding the last ten carcasses, these carcasses were also hung on a rail in the truck and taken directly to the abattoir on the farm, where the carcasses were skinned and dressed.

3.3 Skinning and dressing

On arrival at the abattoir, the carcasses were examined within the truck for signs of contamination or abnormalities that may affect the meat's soundness. After inspection, the carcasses were taken into the carcass wash area. In the wash area, the carcasses (still having the skin attached) were scrubbed

on a stainless-steel table with premixed food-grade approved detergent using soft nylon bristle brushes. The mouths were also cleaned and washed out.

Afterwards, the carcasses were dipped in two basins with food-grade approved chlorine solution to disinfect the skin. Each carcass was submerged in each of the basins for ± 30 seconds. The chlorine solution comprised 35 g chlorine Pharma-Link tablets dissolved in 25 litres of water. After washing the carcasses, each carcass was hung by a hook through the tail on a rail within the abattoir to allow for air drying. Once hung, a disposable paper towel was inserted into the cloaca to prevent intestinal leakage.

The carcasses were then moved to the dressing area. Each carcass's head, feet, skin, and intestines were removed. The head was removed just behind the cranium before the first cervical vertebra, and the feet were removed where the carpus and ulna meet. The tail tip with the tag was left on each carcass to allow for identification. After removing the head, feet, skin, and intestines and intestinal fat, the carcass was moved to a cooler room, where the temperature was 8-12 ºC. In the cooler room, the carcasses were spaced to allow proper cooling (see Annexure A, plates 5 and 6).

3.4 Measurements and sampling

Carcass pH and temperature measurements were taken at 2, 4, 6, 9, 12, 25, 36, and 48 h post mortem. These measurements were done using Bluetooth-enabled Hanna Food pH probes connected to a smartphone with the Hanna Lab application. This application displays the pH and temperature measured by the pH probe connected to the smartphone (see Annexure A, plate 10). The probes were calibrated each morning before use, using standard buffers (pH 4.01 and 7.01) from Hanna Industries. The measurements were taken directly in the carcass from the neck, body, and tail. An attempt was made to take measurements, particularly from the *transversospinalis capitus muscle*, the *longissimus dorsi muscle*, and the *ilio-ischiocaudalis muscle*, as indicated in **Figure 3.4**. A slit was made in the carcass at each location, and the probe was inserted. The measurements were written in a notebook once the application indicated that the readings were stable for the measurements. Measurements were taken from both the left and right sides of each carcass.

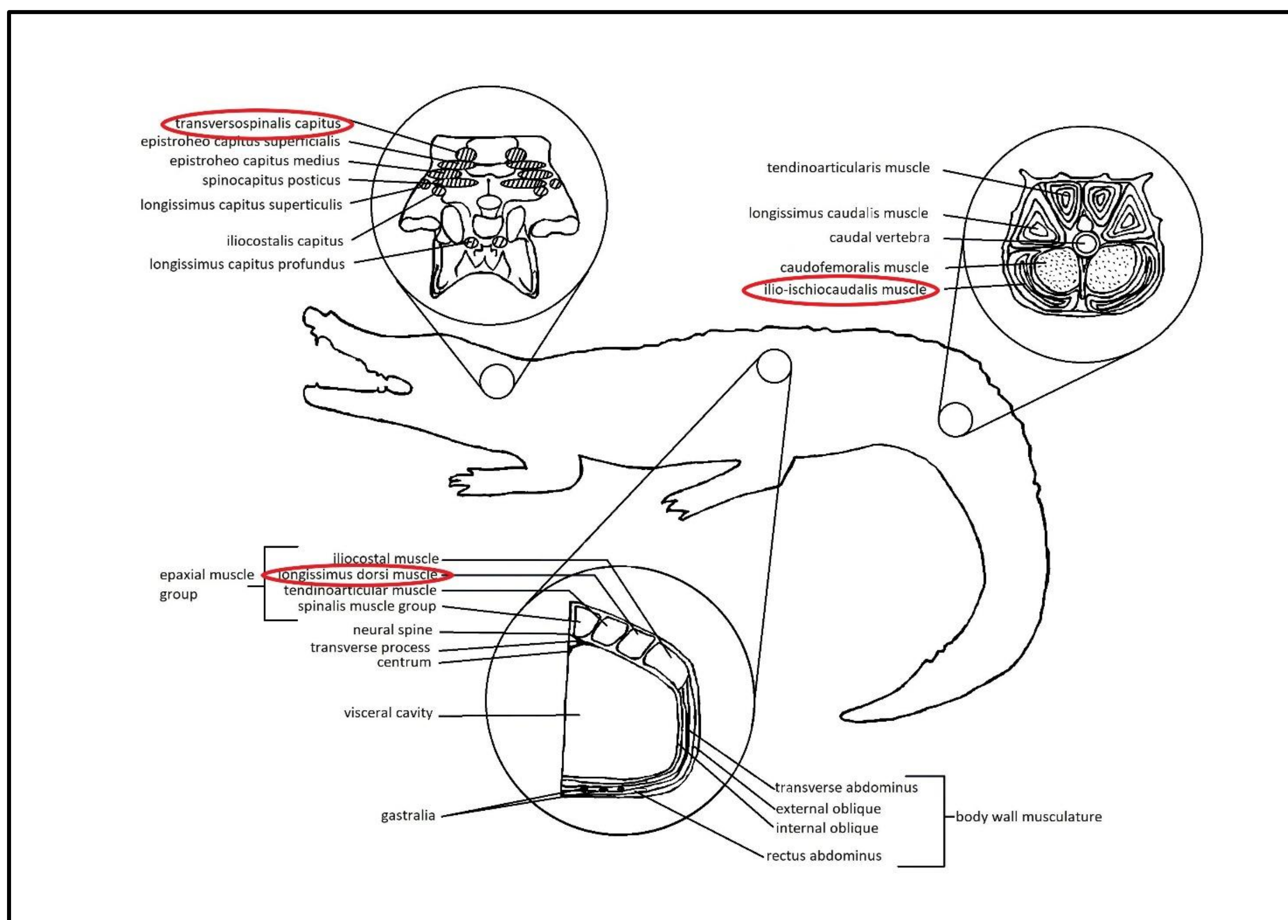

Figure 3.4 Illustration of muscles within the neck, body, and tail of crocodilians (adapted from Grigg *et al*., 2015)

At 25 h post mortem, the cold carcass (excluding head, skin, and intestines) was weighed to calculate the dressing percentage (calculated according to the formula in **Figure 3.5**). Furthermore, 25 h post mortem samples were taken for physicochemical (i.e. thaw loss, cooking loss, shear force, and fatty acid composition) and muscle metabolomics analyses.

Dressing percentage (%) = (cold carcass weight/live weight) x 100

Figure 3.5 Formula for calculating the dressing percentage (%)

Sixty samples were collected form the tail (*ilio-ischiocaudalis muscle*), the body (*iliocostal*, *longissimus dorsi*, and *tendinoarticular muscles*), and the neck (*transversospinalis capitus*, *epistroheo capitus superficialis*, and *epistroheo capitus medius muscles*). These samples were from one-half of each carcass, thus a total of three samples per carcass. These samples had dimensions of ca. 3 x 3 x 3 cm (thickness x width x length) and were used for the analysis of muscle metabolites.

After collection, the samples were placed into polyethene bags with an identification tag. The samples were then placed into a liquid nitrogen container for flash freezing.

Twenty samples (one from each carcass) were collected from the *ilio-ischiocaudalis muscle* at 25 h post mortem to analyse the thaw loss, cooking loss, and shear force. These samples were ca. 1 x 8 x 8 cm (thickness x width x length). After collection, the samples were placed into polyethene bags with an identification tag and then on ice in a polystyrene cooler box.

Forty samples were collected from the tail (20 from the *ilio-ischiocaudalis muscle* and 20 from the lipid surrounding the *caudofemoralis muscle*). These samples had dimensions of ca. 5 x 5 x 5 cm (thickness x width x length) and were used for fatty acid analysis. These samples were placed into polyethene bags with identification tags and then on ice in a polystyrene cooler box.

At 48 h post mortem, the carcasses were divided into cuts (i.e. forequarters, rib casings, hindquarters, tails, and tail tips). An illustration of the cuts is given in **Figure 3.6**. The tail tip consisted of the skin at the end of the tail. The tail was removed posterior to the last caudal vertebra, and the hindquarter consisted of the part between the first lumbar vertebra and the sacrum. The rib casing was cut from the last cervical to the last thoracic vertebra. Lastly, the forequarter was cut from the last cervical vertebra to where the head was removed at the first cervical vertebra. These cuts were weighed (kg) on a scale within the abattoir, and the percentage yield (%) of these cuts was calculated according to the formula in **Figure 3.7**.

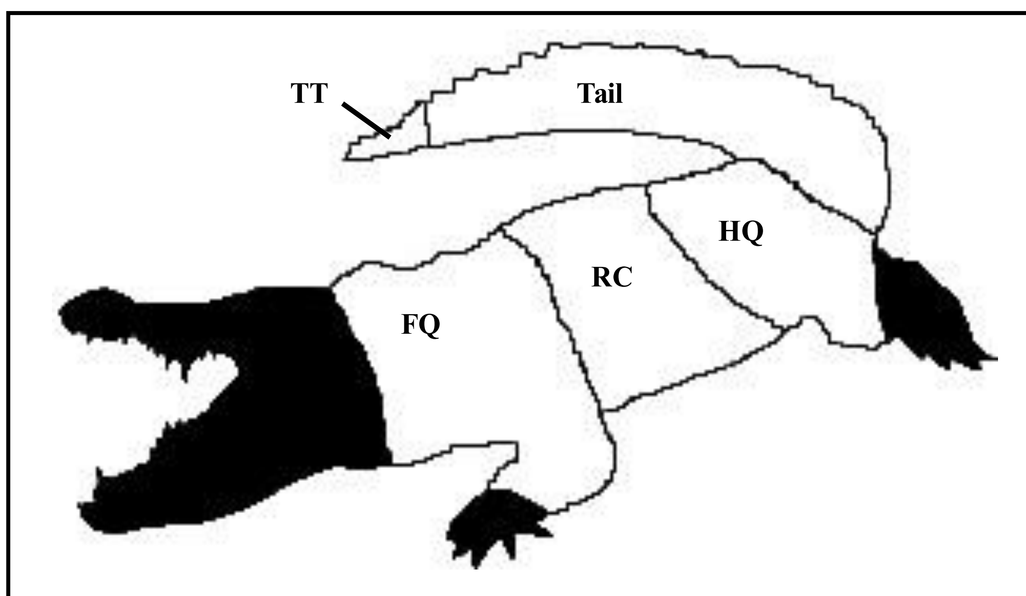

Figure 3.6 Illustration of cuts made
** FQ-Forequarter, RC- Rib casing, HQ-Hindquarter, TT-Tail tip*

Percentage yield (%) = (cut weight/live weight) x 100

Figure 3.7 Percentage yield (%) of selected cuts

3.5 Muscle metabolomics

Upon arrival of the 60 samples, 3 x 3 x 3 cm samples, at the Animal Production Institute of the ARC at Irene, the samples were placed into a freezer at -80 ºC for storage until analysis. It was noted that two samples defrosted before arrival at the ARC and were thus not analysed. Extraction was done according to the method used by Dalrymple & Hamm (1973). Subsequent lactate, glycogen, glucose, CP, ATP and G6P (Bergmeyer, 1974) analyses were performed. A brief explanation of these methods is given in the section below.

Below is a list of the enzymes used for sample extraction and metabolite analyses.

1. Amyloglucosidase (AGS) from *Aspergillus niger* (rabbit), 100 mg.
2. Adenosine-5-diphosphate, disodium salt dehydrate, 1 g.
3. Adenosine-5-triphosphate disodium salt trihydrate, 1 g.
4. Creatine kinase, 100 mg.
5. Glucose-6-phosphate dehydrogenase, 5 mg.
6. Hexokinase, 5 mg.
7. L-lactate dehydrogenase (LDH) from rabbit muscle, 10 mg.
8. B-nicotinamide adenine dinucleotide monohydrate salt (NAD), 1 g.
9. Nicotinamide adenine dinucleotide phosphate (NADP), 100 mg.

Sample extraction

For glycogen analysis, 0.1 mL 0.6 N perchloric acid ($HClO_4$), 50 µL 1 N potassium hydroxide (KOH), and 1 mL AGS was pipetted into a glass test tube (16 mm outer diameter) and stored in a fridge at ca. 4 ºC until glycogen analysis was performed (This preparation will later be referred to as the AGS solution).

To extract the sample, 10 mL of cold 0.6 N $HClO_4$ and 2 g of the sample (cut from the frozen sample) were placed into a plastic test tube (30 mm outer diameter). The exact sample weight was noted for use in calculations. After noting the sample weight (g), the sample was homogenised and centrifuged at 10 000 rpm for 15 min. The supernatant was decanted into a clean plastic test tube (30 mm outer diameter).

100 µL of the supernatant was pipetted into the AGS solution prepared above and placed into a water bath (Labotec, Laboratory circulator, model 103) at 40 ºC for 2 h. After 2 h of heating the reaction was stopped by pipetting 0.5 mL 0.6 N $HClO_4$ into the test tube.

A few drops of methyl orange indicator were added to the supernatant in the plastic test tube. The supernatant was neutralised by adding 5.4 N KOH (the solution turned yellow from pink once neutralised). After neutralisation, the solution was filtered using filter paper and a glass filter to remove the precipitate and stored at ca. 4 ºC until analysis. The volume (mL) of the filtered solution was noted for use in calculations.

L-lactate

For l-lactate analysis, a spectrophotometer (Agilent 8453 UV-Visible Spectroscopy system) was used, with Agilent chemstation software. The spectrophotometer was set to a wavelength of 340 nm and a temperature of 37 ºC, and both lamps were switched on. The lactate buffer (consisting of 83.33 mL hydrazine hydrate + 38 g glycine + 800 mL distilled H_2O) was placed into a heat bath (37 ºC) to heat up before the start of the analysis and the spectrophotometer was blanked against air.

After the setup of the spectrophotometer, a quartz cuvette of 1 cm was placed into the slot of the spectrophotometer. Firstly, 2.5 mL lactate buffer, 200 µL NAD solution, and 20 µL 0.6 N $HClO_4$ were pipetted into the cuvette, and the solution was mixed, and a reading was taken immediately. Thereafter, 20 µL of LDH solution was pipetted into the cuvette, and the solution was mixed and left to stand for 30 min before taking another reading.

Once the steps in the previous paragraph were completed, another quartz cuvette of 1 cm was placed into the slot of the spectrophotometer. 2.5 mL lactate buffer, 200 µL NAD solution, and 20 µL of the previous prepared, filtered sample (in the plastic test tube) were pipetted into the cuvette. The solution was mixed, and a reading was taken immediately. Thereafter, 20 µL LDH solution was pipetted into the cuvette, and the solution was mixed and left to stand for 30 min before taking another reading.

Glycogen and glucose

A spectrophotometer (Agilent 8453 UV-Visible Spectroscopy system) was used for glycogen analysis, with Agilent chemstation software. The spectrophotometer was set to a wavelength of 340 nm and a temperature of 37 ºC, and both lamps were switched on. The glycogen buffer (consisting of 56 g triethanolamine hydrochloride + 1 g $MgSO_4.7H_2O$ + 120 mL 1 N KOH) was placed into a heat

bath (37 ºC) to heat up before the start of the analysis and the spectrophotometer was blanked against air.

After the setup of the spectrophotometer, two microcuvettes were placed into two slots of the spectrophotometer. 1 mL of glycogen buffer and 50 µL of the previously prepared sample (in the glass test tube) were pipetted into the first microcuvette. 1 mL of glycogen buffer and 50 µL of the previously prepared, filtered sample (in the plastic test tube) were pipetted into the second microcuvette. Both these solutions were mixed and left to stand for 5 min before taking a reading. After taking the first reading, 5 µL of hexokinase was pipetted into each cuvette, and the solution was mixed and left to stand for 10 min before taking another reading.

Creatine phosphate, ATP, and Glucose-6-Phosphate

For the analysis of CP, ATP, and glucose-6-phosphate (G6P), a spectrophotometer (Agilent 8453 UV-Visible Spectroscopy system) was used, with Agilent chemstation software. The spectrophotometer was set to a wavelength of 340 nm and a temperature of 37 ºC, and both lamps were switched on. The triethanolamine hydrochloride (HCl) buffer (consisting of 9.3 g triethanolamine HCl + 22 mL 1 N NaOH + 800 mL distilled H_2O) was placed into a heat bath (37 ºC) to heat up before the start of analysis and the spectrophotometer was blanked against air.

After the setup of the spectrophotometer, a quartz cuvette of 1 cm was placed into the slot of the spectrophotometer. 2.5 mL triethanolamine HCl buffer, 100 µL NADP, 100 µL $MgCl_2$, 20 µL adenosine-5-diphosphate, disodium salt dehydrate and 50 µL of previously prepared, filtered sample (in the plastic test tube) was pipetted into the cuvette. The solution was mixed and left to stand for 5 min before taking a reading. After reading, 5 µL of G6P suspension was pipetted into the cuvette, and the solution was mixed and left to stand for 5 min before taking another reading. After reading, 100 µL glucose was pipetted into the cuvette, and the solution was mixed, and a reading was taken immediately. After reading, 5 µL of hexokinase was pipetted into the cuvette, and the solution was mixed and left to stand for 5 min before taking a reading. After reading, the solution was left to stand for another 20 min before reading again. After reading, 10 µL creatine kinase was pipetted into the cuvette, and the solution was left to stand for 10 min before reading.

Glycolytic potential

The lactate, glycogen, glucose and G6P concentrations determined above were used to calculate the glycolytic potential (GP) according to the formula in **Figure 3.8**.

Glycolytic potential (µmol/g) = 2 ([glycogen] + [glucose] + [G6P]) + [lactate]

Figure 3.8 Formula for calculating the glycolytic potential (µmol/g) (Monin & Sellier, 1985)

3.6 Physicochemical characteristics

Thaw loss, cooking loss, and shear force

After the 20, 1 x 8 x 8 cm samples arrived at the Animal Production Institute of the ARC at Irene, the samples were vacuum-packed and frozen in a chest freezer at -20 ºC for a minimum of 72 h. The samples were thawed after freezing for 24 h at 4 ± 1 ºC. After thawing, the bag with exudate and raw meat was weighed. Thereafter, the bag with exudate was weighed. Lastly, the bag itself was weighed. The weight of the raw meat was calculated according to the formula in **Figure 3.9**, and the thaw loss was calculated as the percentage leakage during thawing according to the formula given in **Figure 3.10**. After weighing the initial bag, a new bag was weighed, and the meat sample was vacuum-packed in the new bag with its identification tag for cooking.

Raw meat (g) = (bag weight + exudate + raw meat) – (bag weight + exudate)

Figure 3.9 Formula for calculating weight of raw meat (g)

Thaw loss (%) = (exudate/raw meat) x 100

Figure 3.10 Formula for calculating thaw loss (%)

After vacuum-packing, the samples were placed into a water bath (Labotec, Laboratory circulator, model 103) (see Annexure A, plates 11 and 12). The water bath was preheated to 75 ºC for 50 min (the same technique used by Hoffman *et al*., 2000). After removal from the water bath, the bag with the cooked meat and fluid was weighed. Afterwards, the bag with fluid was weighed, and the cooked meat weight was calculated according to the formula in **Figure 3.11**. The cooking loss was calculated according to the formula in **Figure 3.12**. Moreover, the cooking loss as a percentage of raw meat was calculated according to the formula in **Figure 3.13**. After removal from the bag, the cooked meat samples were weighed to verify the weight of the cooked meat sample and placed onto plates with their identification tag.

Cooked meat (g) = (bag weight + fluid + cooked meat) – (bag weight + fluid)

Figure 3.11 Formula for calculating cooked meat weight (g)

Cooking loss (g) = (bag weight + cooked meat + fluid) – (bag weight + cooked meat)

Figure 3.12 Formula for calculating cooking loss (g)

Cooking loss (%) = [(raw meat – cooked meat)/ raw meat] x 100

Figure 3.13 Formula for calculating cooking loss (%) as a percentage of raw meat

The samples were placed in an air-controlled room for cooling at 16 ºC for 1 h and turned to cool for another 30 min. After cooling, the samples were cut into six 10 mm wide strips, with the width being verified using a Mastercraft digital vernier calliper (Annexure A, plate 16). The Warner-Bratzler shear force method was performed to determine the meat tenderness. The shear force values were determined using a Warner-Bratzler shear device (Annexure A, plate 15) mounted on an Instron Universal Testing Machine (Model 4301, Instron Ltd, Buckinghamshire, England) (Annexure A, plate 17) with Bluehill 2 version 2.35 software. The crosshead speed of the Instron was set to 200 mm/min. The values were determined by shearing through the centre of each strip perpendicular to the muscle fibre direction. Only samples from the tail (*ilio-ischiocaudalis muscle*) were analysed for shear force. An illustration is given in **Figure 3.14** of the steps taken to determine the thaw loss, cooking loss and shear force of each sample.

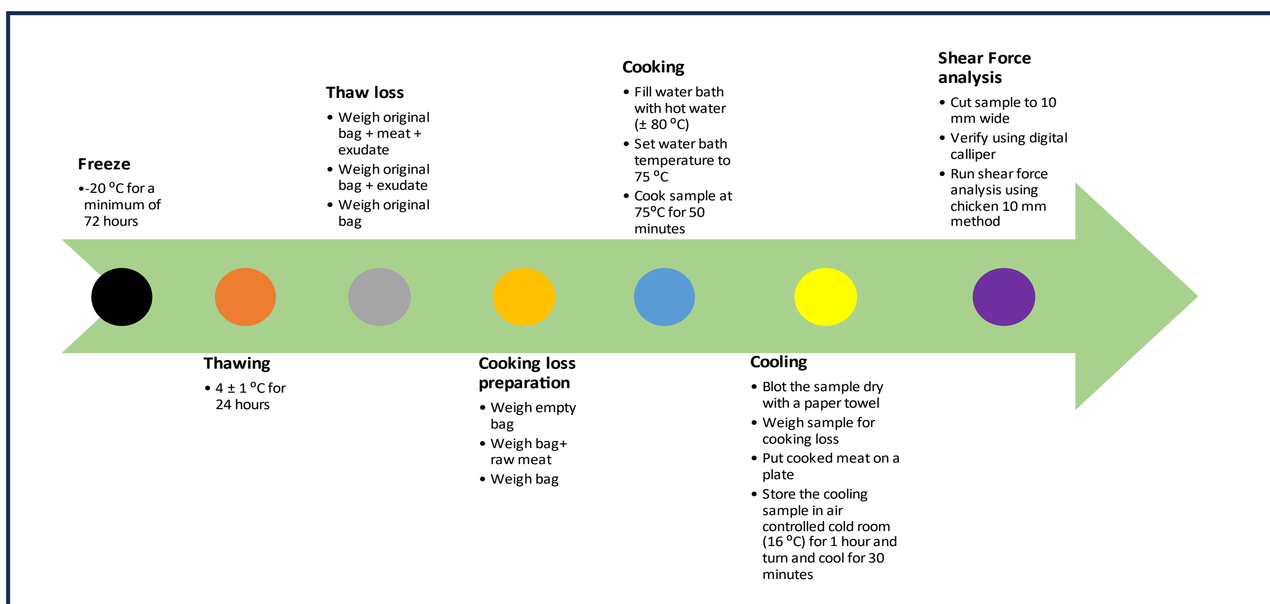

Figure 3.14 Steps for determining thaw loss, cooking loss and shear force (2023, P. Makatu, Pers. Comm, ARC, Irene)

Fatty acid composition

Dissected tail meat samples for FA composition analyses were stored (-20 ºC) and prepared for subsequent analyses at the University of Free State. Lipids from intramuscular fat (1 g) and intermuscular fat (5 g) within the tail 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 under vacuum, and the extracts were dried overnight in a vacuum oven at 50 ºC, using phosphorous pentoxide as a moisture absorbent.

Total extractable fat from the tissues was determined gravimetrically and expressed as per cent fat (w/w) per 100 g tissue. The fat free dry matter (FFDM) content (expressed as % FFDM (w/w) per 100 g tissue) was determined by weighing the residue on a pre-weighed filter paper used for Folch extraction after drying by determining the difference in weight. The moisture content of the tissue was determined by subtraction (100% - % lipid - % FFDM) and expressed as % moisture (w/w) per 100 g tissue. The extracted fat from the tissue was stored in a polytop (glass vial, with a push-in top) under a blanket of nitrogen and frozen at -20 ºC pending fatty acid analyses.

A lipid aliquot (20 mg) from the tissue was transferred into a Teflon-lined screw-top test tube using a disposable glass Pasteur pipette. Fatty acids were transesterified to form methyl esters using 0.5 N NaOH in methanol and 14% boron trifluoride in methanol (Park & Goins, 1994). Fatty acid methyl esters (FAMEs) from lipids were quantified using a Varian 430 flame ionisation gas chromatograph with a fused silica capillary column, Chrompack CPSIL 88 (100 mm length, 0.25 mm inner diameter, 0.2 µm film thickness). Analysis was performed using an initial isothermic period (40 ºC for 2 min). Thereafter, the temperature was increased at a rate of 4 ºC/min to 230 ºC. Finally, an isothermic period of 230 ºC for 10 min followed. FAMEs n-hexane (1 µL) were injected into the column using a Varian CP 8400 Autosampler. The injection port and detector were both maintained at 250 ºC. Hydrogen, at 45 psi, functioned as the carrier gas, while nitrogen was employed as the makeup gas. Galaxy Chromatography Software 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). All other reagents and solvents were of analytical grade and obtained from Merck Chemicals (Pty Ltd, Halfway House, Johannesburg, South Africa). Fatty acids were expressed as the proportion of each individual fatty acid to the total of all fatty acids in the sample. The following fatty acid combinations were calculated: omega-3 (n-

3) fatty acids, omega-6 (n-6) fatty acids, total saturated fatty acids (SFA), total monounsaturated fatty acids (MUFA), total polyunsaturated fatty acids (PUFA), PUFA/SFA ratio and n-6/n-3 ratio.

3.7 Statistical analysis

This study was a completely randomised control study. A total of 10 replications (crocodiles) per treatment group (stunning method) were included. Genstat for Windows 22nd Edition (Genstat, 2022) was used to analyse the data and International Business Machines Corporation, Statistical Package for the Social Sciences (IBM SPSS) version 28 was used to verify the data. Means were compared at a significance level of 5%. A 2 x 3 factorial design (2 stunning methods and 3 anatomical locations) was used for the pH, temperature, and muscle metabolomic data. Furthermore, a 2 x 2 factorial design (2 stunning methods and 2 tissues) was used for the fatty acid data.

Carcass characteristics

The descriptive statistics were determined for live weight, blood loss, blood loss as % of the live weight, cut weights, cut weights as % of the live weight and cut weights as % of cold carcass weight data. For blood loss as % of the live weight, the "Student" t-test was used to test if there was a significant difference between the means of the stunning methods, and the live weight was included as a covariate.

Carcass pH and temperature

Live weight was included as a covariate for the carcass pH and temperature analysis. The pH and temperature data collected over time (repeated measurements) were analysed separately for each time period and combined, where time was included as a sub-plot factor in the analysis of variance (ANOVA). The residuals were examined for deviations from normality. Fisher's protected t-Least Significant Difference (LSD) was calculated to compare means of significant effects (Snedecor & Cochran, 1967). The R2 lines procedure (Hudson, 1966) was used to fit a two-straight-line (broken-stick) model for the relationship between the pH and -temperature data. Furthermore, the regression equation was calculated for the repeated measurements of pH data for each crocodile and the slopes were compared for significant differences.

Muscle metabolomics

The anatomical locations were added as a sub-plot factor to the ANOVA for the muscle metabolite data. Descriptive statistics were calculated for each parameter (i.e. glycogen, glucose, glucose-6-phosphate, ATP, CP, lactate, and glycolytic potential), and an ANOVA was conducted. The results were examined for deviations from normality, and Fisher's protected t-LSD was calculated to compare means of significant effects (Snedecor & Cochran, 1967). Live weight was not included as a covariate, as it did not show a significant difference.

Physicochemical characteristics

Live weight was included as a covariate for analysing thaw loss, cooking loss, and shear force. Descriptive statistics were calculated for each of these parameters, and the "Student" t-test was used to test if there was a significant difference in the means of thaw loss, cooking loss, and shear force between the 2 stunning methods.

For the FA data, live weight was included as a covariate and the tissue types were added as a sub-plot factor to the ANOVA. The residuals were examined for deviations from normality. Fisher's protected t-LSD was calculated to compare means of significant effects (Snedecor & Cochran, 1967).

Chapter 4
Results and Discussion

4.1 Carcass characteristics

Crocodilian carcasses are divided into various portions within the industry and between different studies. Some studies separate carcasses into tails, legs, torsos, and necks (Hoffman *et al*., 2000), while others separate carcasses into cheeks, shoulders, necks, legs, dorsal and ventral tails (Černíková *et al*., 2015). This makes it difficult to compare cut weights and yields between various studies. Furthermore, yields may differ depending on the species, the sex, the weight, the availability of food, the age of the crocodilian, the dressing technique and the skill of the operator separating the carcasses (Hoffman *et al*., 2000; Kluczkovski Junior *et al*., 2015; Fernandes *et al*., 2017).

In this study, carcasses were divided into forequarters, rib casings, hindquarters, tails, and tail tips at 48 h post mortem. Weights, weights as a percentage of the live weight, and weights of various portions as a percentage of the cold carcass weight of female Nile crocodiles of ca. 40 months old are indicated in **Table 4.1**.

Table 4.1 Weight and weight as a percentage of live weight and cold carcass weight of female Nile crocodiles (mean ± SD, n = 20)

Portion	Weight (kg)	% of Live Weight	% of Cold Carcass Weight
Live weight	8.895 ± 2.081	-	-
Blood loss	0.195 ± 0.100	2.332 ± 1.413	-
Cold carcass	5.418 ± 1.260	61.252 ± 6.815	-
Forequarter	1.308 ± 0.300	14.835 ± 1.901	24.207 ± 1.427
Rib casing	0.842 ± 0.258	9.448 ± 1.702	15.387 ± 2.016
Hindquarter	1.126 ± 0.266	12.743 ± 1.639	20.860 ± 2.103
Tail	1.730 ± 0.455	19.477 ± 2.694	31.757 ± 1.670
Tail tip	0.127 ± 0.028	1.455 ± 0.275	2.378 ± 0.386

The dressing percentage of female Nile crocodiles was calculated as 61.25%, which is higher than the dressing percentages of 56.5%, 57.02%, and 59.7% found by Hoffman *et al*. (2000), Kluczkovski Junior *et al*. (2015) and Medeiros *et al*. (2021) for Nile crocodiles, Black caiman, and Pantanal caiman, respectively. However, these differences may be due to differences in the skin removal and cooling regimes (Hoffman *et al*., 2000). Furthermore, this study only used female Nile crocodiles,

whereas Hoffman *et al*. (2000) did not note the sex of the animals. Moreover, Kluczkovski Junior *et al*. (2015) and Medeiros *et al*. (2021) examined Black caiman and Pantanal caiman, respectively. Thus, the differences between the current study and the studies by Kluczkovski Junior *et al*. (2015) and Medeiros *et al*. (2021) may be due to the different crocodilian species investigated.

This study showed a tail yield (31.8%) similar to the tail yield of 32.6% found by Hoffman *et al*. (2000) in Nile crocodiles. This study further showed that the tail had the highest yield compared to other cuts, followed by the forequarter, hindquarter, rib casing and tail tip in descending order. This was similar to the results of Kluczkovski Junior *et al*. (2015), Fernandes *et al*. (2017) and Medeiros *et al*. (2021), where it was also noted that caiman tails yielded the most in comparison to other cuts.

The blood loss weight during bleeding (195 g) was more than the (109 g) blood loss noted by Hoffman *et al*. (2000). Furthermore, the blood loss as % of live weight (2.3%) was higher than the blood loss as a percentage of live weight (1.3%) derived by Hoffman *et al*. (2000). These differences may be due to differences in bleeding time and method. Hoffman *et al*. (2000) bled crocodiles overnight in a cool room, while during this study, crocodiles were bled for 20 min directly after severing the jugular veins and carotid arteries in the truck before moving to a cool room within the abattoir. This study further compared the blood loss as a percentage of the live weight between the stunning methods used, i.e. free bullets and electrical stunning; these results are presented in **Table 4.2**.

Table 4.2 Means ± SE of blood loss as a percentage of the live weight between different stunning methods in female Nile crocodiles (n = 20)

	Stunning method		
	Free bullets	**Electrical stunning**	**Mean**
Blood Loss as % of Live Weight	2.267 ± 0.465	2.396 ± 0.465	2.332 ± 0.299
Significance	0.859		

** Live weight (8.895) as a covariate*

The results in **Table 4.2** show that the stunning method did not have a significant (P <0.05) effect on the blood loss as a percentage of live weight of female Nile crocodiles. Although no previous study in crocodilians compared the blood loss during bleeding between stunning methods, McNeal *et al*. (2003) indicated that the stunning method does not affect post mortem bleeding in broilers. Thus, this study and the study by McNeal *et al*. (2003) show that the stunning method does not affect post mortem bleeding.

4.2 Carcass pH and temperature

During this study, measurements of pH and temperature were taken post mortem of 20 female Nile crocodiles stunned by two methods. These measurements were taken directly in the carcass at three anatomical locations (i.e. neck, body, and tail), attempting to measure these parameters in the *ilio-ischiocaudalis muscle*, *transversospinalis capitus muscle*, and *longissimus dorsi muscle*. These measurements were taken at 2, 4, 6, 9, 12, 25, 36, and 48 h post mortem. During this study it was observed that crocodiles stunned by electrical stunning swiped their tails, followed by a full muscle contraction and combined with small muscle contractions, and lastly going into a relaxed state.

Previous studies measured post mortem carcass pH in various anatomical locations at 24 h post mortem (Černíková *et al*., 2015) or measured carcass pH over time in crocodiles stunned by one method (Hoffman *et al*., 2000). However, previous studies frequently did not use the same anatomical locations, as carcasses are divided into numerous cuts. Thus complicating comparisons between this study and previous studies for carcass pH measurements. Furthermore, no previous crocodilian study indicated the carcass's temperature at the time pH was measured post mortem, and comparison with other species is not possible as crocodilians are poikilothermic and other livestock species are poikilothermic.

No interaction was found between the stunning method and the anatomical location. In **Table 4.3**, carcass pH values are compared between the two stunning methods used. This data is further presented in **Figure 4.1** to demonstrate the rate of pH decline post mortem.

Table 4.3 Means ± SE of carcass pH measurements over time post mortem in female Nile crocodiles stunned by different methods (n = 60)

	Stunning method	
Time (h)	**Free bullets**	**Electrical stunning**
2	$6.99^{a} \pm 0.033$	$6.70^{b} \pm 0.033$
4	$6.93^{a} \pm 0.033$	$6.62^{b} \pm 0.033$
6	$6.92^{a} \pm 0.033$	$6.61^{b} \pm 0.033$
9	$6.80^{a} \pm 0.033$	$6.47^{b} \pm 0.033$
12	$6.74^{a} \pm 0.033$	$6.49^{b} \pm 0.033$
25	$6.44^{a} \pm 0.033$	$6.18^{b} \pm 0.033$
36	$6.23^{a} \pm 0.033$	$5.98^{b} \pm 0.033$
48	$6.04^{a} \pm 0.033$	$5.89^{b} \pm 0.033$

** Live weight (8.895) as a covariate*
**Means with different superscripts in a row differ significantly ($P < 0.05$)*

This study showed that the carcass pH differed significantly ($P < 0.05$) at each time interval post mortem between the two stunning methods, with the pH being lower in animals stunned by electrical stunning than in animals stunned by the free bullets method.

A lower initial and ultimate pH may be seen if an animal is exposed to acute stress, such as inappropriate stunning procedures (Støier *et al*., 2001; Daskalova & Pavlov, 2015). This lower initial pH may be due to increased muscle activity at the time of slaughter, which leads to an increase in anaerobic glycolysis, a rapid onset of rigor mortis and an acceleration of proteolytic activity. Furthermore, acute stress commonly leads to muscles reaching the ultimate pH faster than usual and muscles having a lower ultimate pH than average (Daskalova & Pavlov, 2015). Thus, from the results in **Table 4.3**, it can be concluded that the crocodiles stunned by electrical stunning experienced more stress than crocodiles stunned by the free bullets method. This can be confirmed due to the lower initial pH in crocodiles stunned by electrical stunning, thus indicating that they had accelerated anaerobic muscle metabolism directly after death.

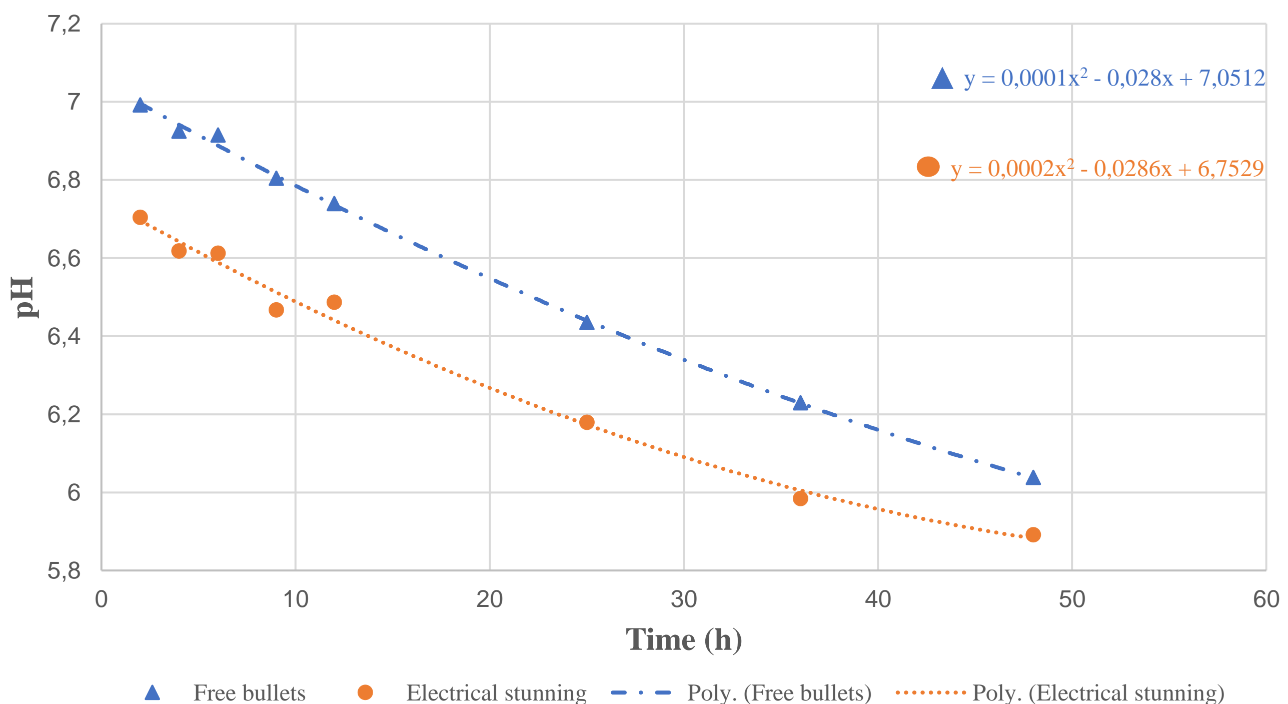

Figure 4.1 pH vs time (h) post mortem of female Nile crocodiles stunned by different methods
** Live weight (8.895) as a covariate*

Figure 4.1 shows that at 48 h post mortem, the crocodiles stunned by electrical stunning still had a significantly (P <0.05) lower pH than crocodiles stunned by free bullets. Furthermore, the slope of the pH decline within the graph were -0.137 and -0.121, respectively for the free bullets and the electrical stunning method. Thus, the rate of pH decline did not differ significantly (P <0.05) between the stunning methods. The ultimate pH could not be compared between the two stunning methods, as the time of rigor mortis is still unknown in Nile crocodiles (Hoffman *et al*., 2000). Based on the lower initial pH and lower pH at 48 h post mortem in crocodiles stunned by electrical stunning, it can be concluded that the free bullets method caused less stress than the electrical stunning method.

The post mortem carcass temperature in the muscle of female Nile crocodiles between two stunning method is shown in **Table 4.4**.

Table 4.4 Means ± SE of carcass temperature (ºC) measurements over time post mortem in female Nile crocodiles stunned by different methods (n = 60)

	Stunning method	
Time (h)	**Free bullets**	**Electrical stunning**
2	$15.8^{a} \pm 0.125$	$15.4^{b} \pm 0.125$
4	$12.0^{a} \pm 0.125$	$12.0^{a} \pm 0.125$
6	$7.5^{a} \pm 0.125$	$7.3^{a} \pm 0.125$
9	$7.0^{a} \pm 0.125$	$7.1^{a} \pm 0.125$
12	$4.0^{a} \pm 0.125$	$4.3^{a} \pm 0.125$
25	$4.1^{a} \pm 0.125$	$4.5^{b} \pm 0.125$
36	$2.3^{a} \pm 0.125$	$3.2^{b} \pm 0.125$
48	$0.1^{a} \pm 0.125$	$0.6^{b} \pm 0.125$

** Live weight (8.895) as a covariate*
** Means with different superscripts in a row differ significantly (P <0.05)*

The results in **Table 4.4** indicate that the initial temperature of the carcasses was 15.4 to 15.8 ºC. Although slightly lower than the environmental temperature of 16.8 ºC at the time of stunning and slaughter, this temperature was similar to the environmental temperature. This is expected as crocodiles are poikilothermic and regulate their body temperature by basking in the sun or lying in the water (Furstenburg, 2008; Van der Westhuizen, 2019; IUCN-CSG, 2023). The results further indicate that the temperature of the carcasses declined over time. Significant differences (P <0.05) in temperature were seen at 2 h, 25 h, 36 h, and 48 h post mortem. In **Figure 4.2**, the temperature decline post mortem is demonstrated as a graph to illustrate the rate of temperature decline post mortem.

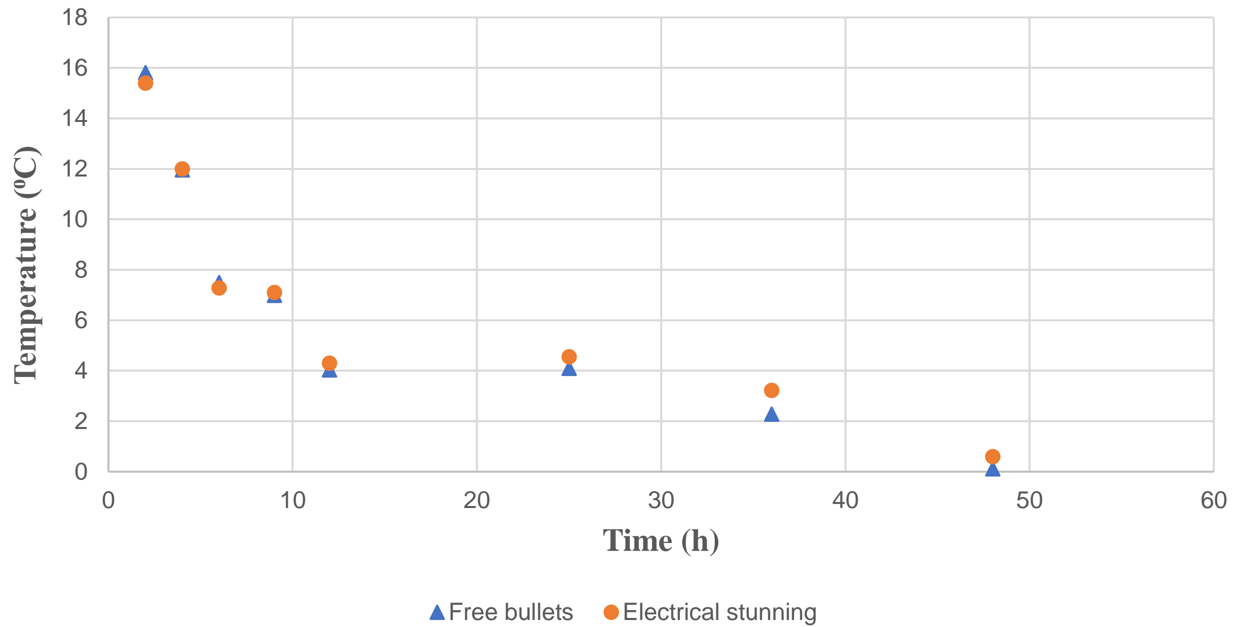

Figure 4.2 Temperature (ºC) vs time (h) post mortem of female Nile crocodiles stunned by different methods
** Live weight (8.895)*

In **Figure 4.2**, the temperature decline post mortem shows a non-linear pattern. Furthermore, the pattern of temperature decline was similar between the two stunning methods. However, this data cannot be compared to previous studies, as previous studies have not measured the temperature of crocodilian carcasses post mortem.

The muscle type is one factor that influences the rate and extent of pH decline post mortem (Huang *et al*., 2018). This study compared the carcass pH and temperature post mortem within three anatomical locations (i.e. neck, body, and tail). **Table 4.5** compares the carcass pH post mortem between the three anatomical locations, and **Figure 4.3** further demonstrates the rate of pH decline post mortem within the three anatomical locations.

Table 4.5 Means ± SE of carcass pH measurements over time post mortem in different anatomical locations of female Nile crocodiles (n = 60)

	Anatomical location		
Time (h)	**Neck**	**Body**	**Tail**
2	6.90^{b} ± 0.040	6.68^{a} ± 0.040	6.97^{b} ± 0.040
4	6.81^{b} ± 0.040	6.60^{a} ± 0.040	6.91^{b} ± 0.040
6	6.79^{b} ± 0.040	6.57^{a} ± 0.040	6.94^{c} ± 0.040
9	6.64^{b} ± 0.040	6.39^{a} ± 0.040	6.87^{c} ± 0.040
12	6.58^{b} ± 0.040	6.35^{a} ± 0.040	6.92^{c} ± 0.040
25	6.24^{a} ± 0.040	6.18^{a} ± 0.040	6.50^{b} ± 0.040
36	5.97^{a} ± 0.040	5.98^{a} ± 0.040	6.37^{b} ± 0.040
48	5.88^{a} ± 0.040	5.86^{a} ± 0.040	6.15^{a} ± 0.040

** Live weight (8.895) as a covariate*
** Means with different superscripts in a row differ significantly (P <0.05)*

The results in **Table 4.5** indicate that the tail had the highest initial pH, followed by the neck and the body. Significant differences (*P* <0.05) between all three anatomical locations are shown at 6 h, 9 h, and 12 h post mortem. The results further show that at 48 h post mortem, there was no longer a significant (*P* <0.05) difference in pH between the anatomical locations. However, the pH remained the highest in the tail, followed by the neck and body.

This study showed a similar pH value (6.24) in the neck at 25 h post mortem as Černíková *et al*. (2015), where a pH of 6.17 to 6.66 was seen at 24 h post mortem. Furthermore, a similar pH value was seen in the tail (6.50) at 25 h post mortem as by Hoffman *et al*. (2000) and Černíková *et al*. (2015), where pH at 24 h post mortem was 6.67 and 6.82, respectively.

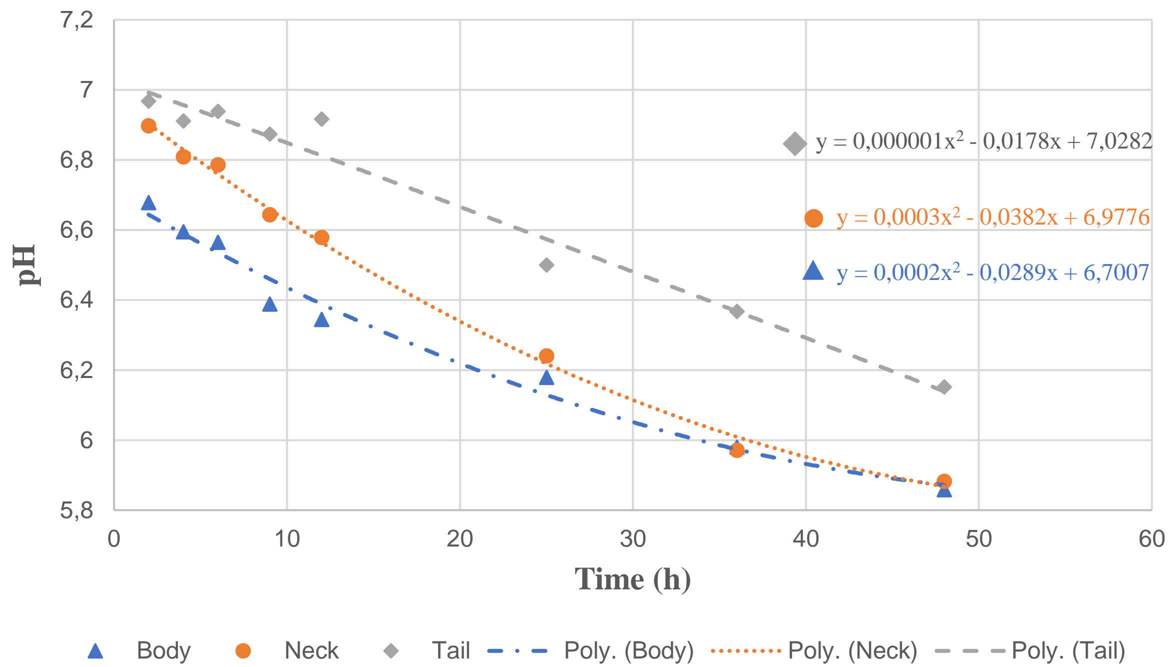

Figure 4.3 pH vs time (h) post mortem between various anatomical locations in female Nile crocodiles
** Live weight (8.895)*

The results in **Figure 4.3** show that the tail and body had a similar rate of pH decline. Furthermore, the tail had the highest pH values at each time interval post mortem. Meanwhile, the body had the lowest pH values at time intervals 2 h, 4 h, 6 h, 9 h, 12 h, and 25 h post mortem. Lastly, the neck had the fastest rate of pH decline, and its pH values were not significantly (P <0.05) different from the body at time intervals 25 h, 36 h, and 48 h post mortem.

This study compared the temperature decline post mortem between the three anatomical locations in **Table 4.6** and **Figure 4.4**.

Table 4.6 Means ± SE of carcass temperature (°C) measurements over time post mortem in different anatomical locations of female Nile crocodiles (n = 60)

	Anatomical location		
Time (h)	**Neck**	**Body**	**Tail**
2	$15.6^{a} \pm 0.151$	$15.7^{a} \pm 0.151$	$15.5^{a} \pm 0.151$
4	$12.2^{b} \pm 0.151$	$11.4^{a} \pm 0.151$	$12.3^{b} \pm 0.151$
6	$7.6^{b} \pm 0.151$	$7.0^{a} \pm 0.151$	$7.6^{b} \pm 0.151$
9	$7.0^{a} \pm 0.151$	$7.1^{a} \pm 0.151$	$7.0^{a} \pm 0.151$
12	$4.2^{a} \pm 0.151$	$3.9^{a} \pm 0.151$	$4.3^{a} \pm 0.151$
25	$4.2^{a} \pm 0.151$	$4.4^{a} \pm 0.151$	$4.4^{a} \pm 0.151$
36	$2.8^{a} \pm 0.151$	$2.7^{a} \pm 0.151$	$2.7^{a} \pm 0.151$
48	$0.2^{a} \pm 0.151$	$0.0^{a} \pm 0.151$	$0.9^{b} \pm 0.151$

** Live weight (8.895) as a covariate*
** Means with different letters in a row differ significantly (P <0.05)*

The results in **Table 4.6** show that there were no significant (P <0.05) differences in the temperature post mortem between the different anatomical locations in Nile crocodiles, except at 4 h and 6 h post mortem where the neck and tail had a higher temperature than the body, and at 48 h post mortem the tail had a higher temperature than the back and neck.

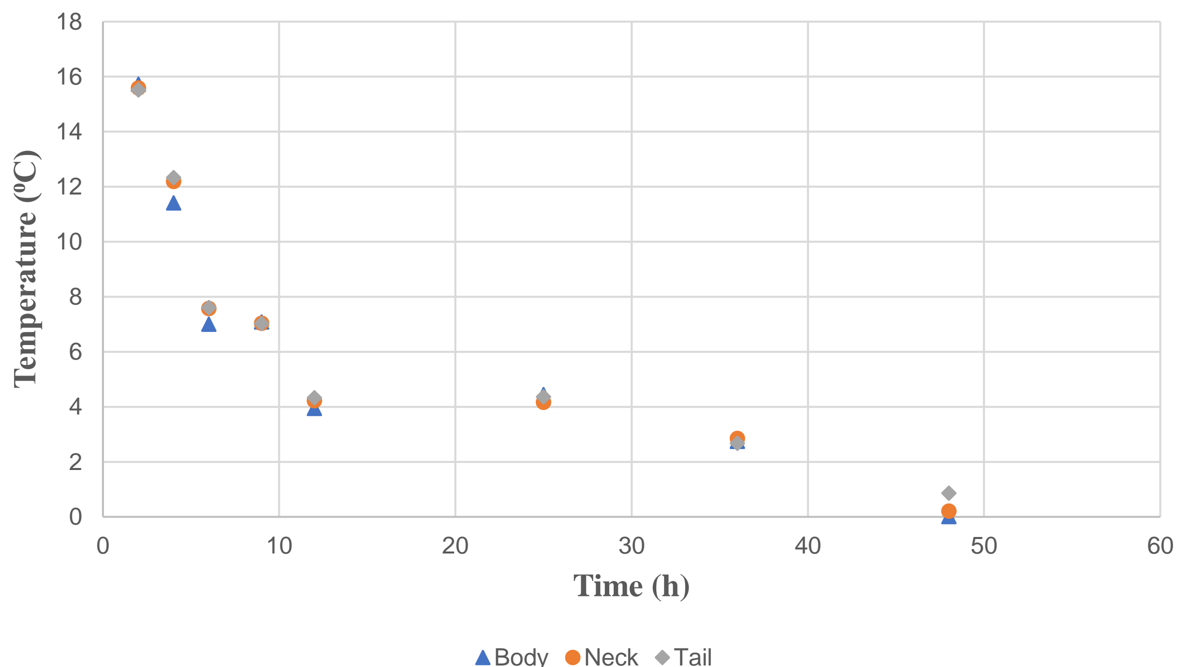

Figure 4.4 Temperature (°C) vs time (h) post mortem between various anatomical locations in female Nile crocodiles
** Live weight (8.895) as a covariate*

In **Figure 4.4**, a similar pattern and rate of temperature decline post mortem can be seen for the different anatomical locations.

The pH and temperature over time post mortem were also compared for each anatomical location between the two stunning methods, and these results are indicated in **Figure 4.5** and **Figure 4.6**, respectively.

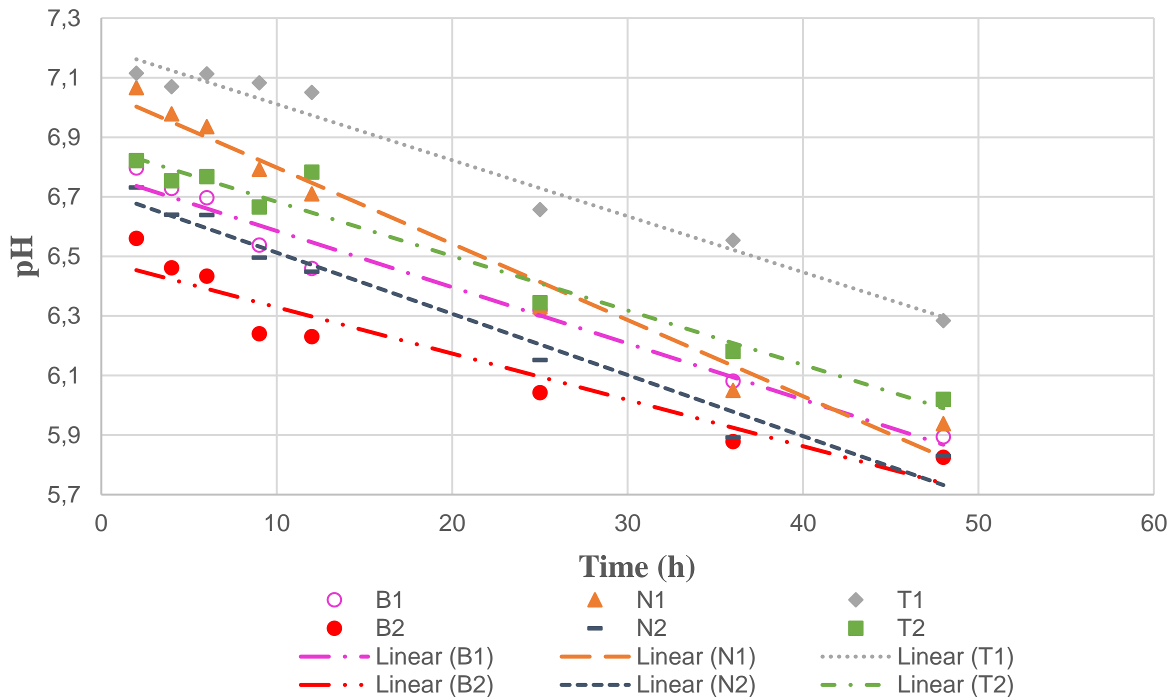

Figure 4.5 pH vs time (h) post mortem in three anatomical locations of female Nile crocodiles stunned by different methods
** 1-Free bullets, 2-Electrical stunning*
** B-Body, N-Neck, T-Tail*
** Live weight (8.895) as a covariate*

As seen in **Figure 4.5**, the pH was the highest in the tail and the lowest in the body at each time interval post mortem for both stunning methods. Furthermore, when comparing the stunning methods, each anatomical location demonstrated a lower pH at each time interval post mortem for the electrical stunning method than the free bullets method. Based on these results, it can be concluded that crocodiles stunned by electrical stunning had higher rates of anaerobic metabolism directly after

slaughter, resulting in a lower initial pH. Furthermore, a difference was seen in the pH at 48 h post mortem, with the tail having the highest pH and the body having the lowest pH.

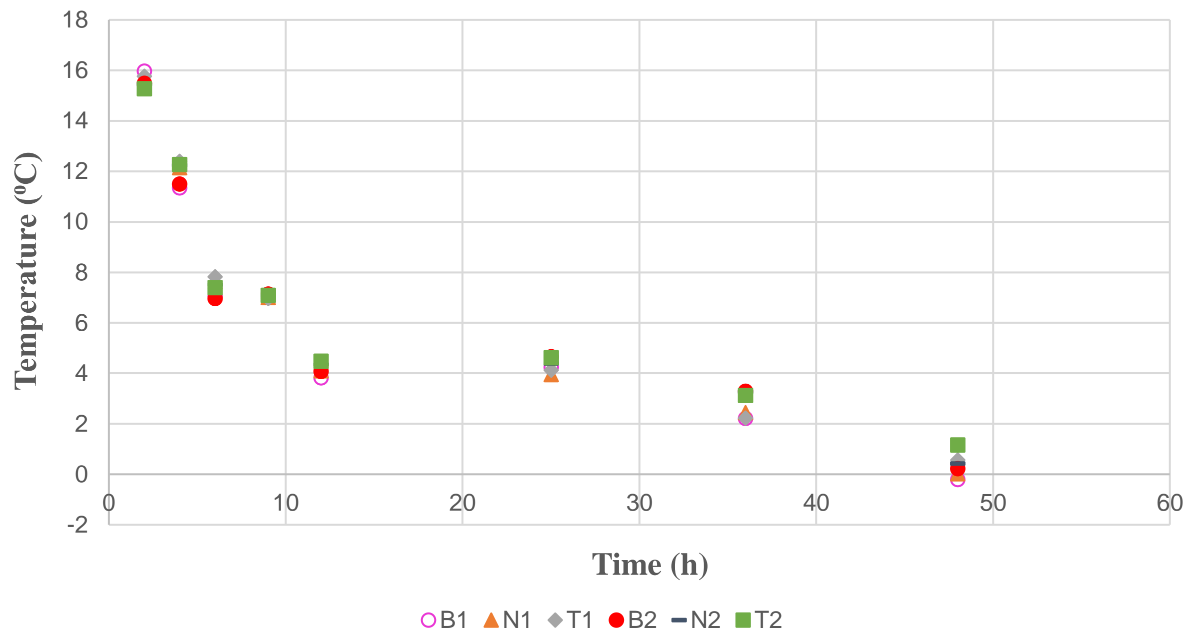

Figure 4.6 Temperature (ºC) vs time (h) post mortem in three anatomical locations of female Nile crocodiles stunned by different methods
** 1-Free bullets, 2-Electrical stunning*
**B-Body, N-Neck, T-Tail*
** Live weight (8.895) as a covariate*

It has been stated that red muscles have a higher ultimate pH than white muscles, Furthermore, red muscles are more sensitive towards stress than white muscles. Red muscles are expected to have a lower initial temperature and higher initial pH post mortem than white muscles (Henckel *et al*., 2000). Based on the results and the previous statements, it can be concluded that the tail of female Nile crocodiles consists of more red muscle fibres (slow-twitch fibres), while the neck and body consist of more white muscle fibres (fast-twitch fibres).

Furthermore, Choe *et al*. (2008) stated that muscles with a faster glycolytic rate (i.e. faster decrease in post mortem pH) have a lower glycogen content and higher lactate content at the early post mortem stage. The glycogen and lactate content of the various anatomical locations will be discussed in the next section.

Lastly, this section plots the post mortem pH against the -post mortem temperature for each stunning method in **Figure 4.7** and **Figure 4.8**.

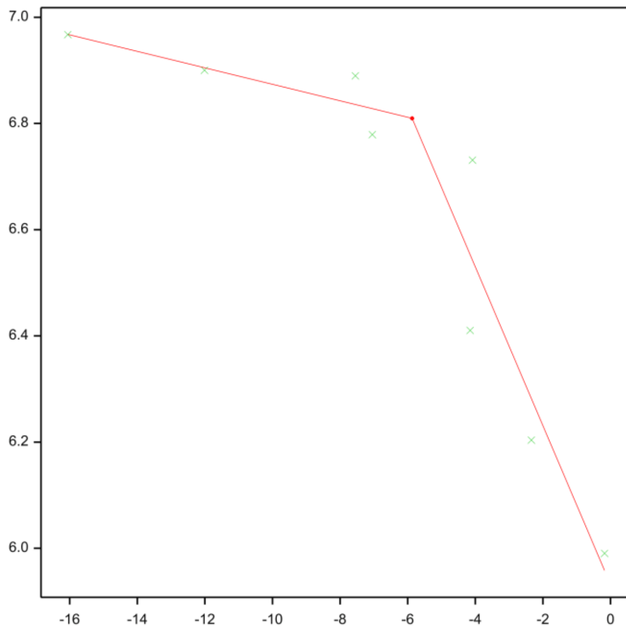

Figure 4.8 Broken-stick model: pH vs -temperature (ºC) post mortem in female Nile crocodiles stunned by free bullets

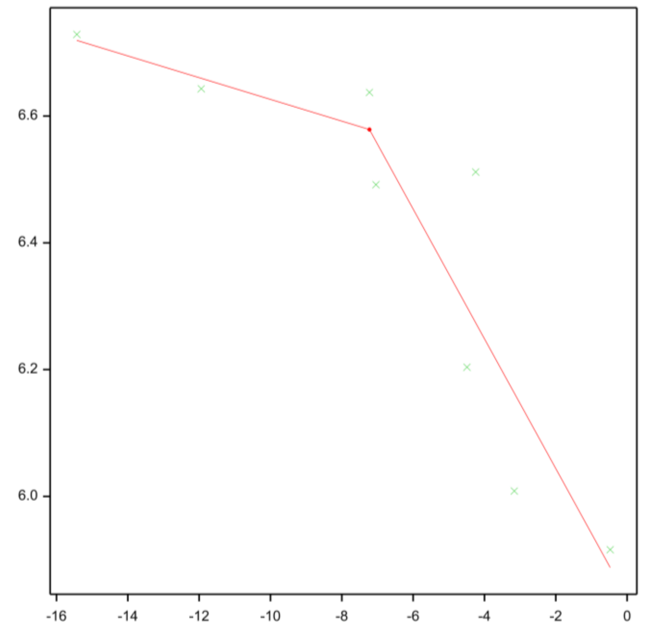

Figure 4.7 Broken-stick model: pH vs -temperature (ºC) post mortem in female Nile crocodiles stunned by electrical stunning

As seen in **Figure 4.7** and **Figure 4.8**, the pH fell as the temperature decreased. The slope of the first line in each figure was -0.0155 and -0.0171 for free bullets and electrical stunning, respectively. The breakpoint in each graph was at (-5.87;6.81) and (-7.225;6.578) for free bullets and electrical stunning, respectively. Lastly, the second line in each figure had a slope of -0.1495 and -0.1023 for free bullets and electrical stunning, respectively. These graphs indicate that animals stunned by electrical stunning had a lower temperature than animals stunned by free bullets. This is important, as Winstanley (1979) and Mandal & Pal (2014) stated that meat from animals undergoing acute stress has a lower pH while the carcass is still warm.

Thus, looking at the graphs above, one can conclude that stunning by electrical stunning caused a lower pH at the same temperature as stunning by free bullets. It should be noted however that this trial was done under cold conditions (16.8 ºC) and slaughtering in summer months where temperatures are higher can lead to an increased stress response which can cause a rapid pH drop, which can also lead to lower meat quality.

4.3 Muscle metabolomics

This study determined the concentrations (µmol/g) of various muscle metabolites (i.e. lactate, glucose, glycogen, G6P, ATP, CP, and glycolytic potential) at 25 h post mortem within the neck, body, and tail of female Nile crocodiles stunned by different methods. No previous study has been done on crocodilian meat's post mortem muscle metabolite concentrations. Thus, comparisons are made with other species, such as fish, since fish and crocodilian meat can both be classified as white meat (Huchzermeyer, 2003; Zaukuu *et al*., 2020).

This study compares the muscle metabolite concentrations (µmol/g) at 25 h post mortem of female Nile crocodiles stunned by different methods within **Table 4.7**. The muscle metabolite concentrations (µmol/g) at 25 h post mortem are further compared between the various anatomical locations of female Nile crocodile carcasses within **Table 4.8**.

Table 4.7 Means ± SD of muscle metabolite concentrations (µmol/g) at 25 h post mortem in female Nile crocodiles stunned by different methods (n = 60)

	Free bullets	Electrical stunning	Significance
Glycogen	13.10 ± 4.761	11.31 ± 4.817	0.266
Glucose	1.52 ± 0.474	2.11 ± 0.496	0.002
G6P	1.14 ± 0.705	2.04 ± 0.877	0.006
ATP	5.83 ± 0.924	5.85 ± 0.847	0.992
CP	3.81 ± 1.063	3.34 ± 1.096	0.188
Lactate	37.50 ± 9.719	42.56 ± 8.143	0.066
GP	69.01 ± 12.56	73.49 ± 11.37	0.161

Stress directly before stunning and slaughter increases the rate of glycolysis post mortem, ultimately leading to increased post mortem lactate concentrations (Støier *et al*., 2001; Qin *et al*., 2016). Thus, a decrease in the glycogen reserves, ATP and CP could also be expected in animals exposed to a stressor directly before slaughter (Qin *et al*., 2016).

Table 4.7 shows significant differences (P <0.05) in the glucose and G6P concentrations at 25 h post mortem between the two stunning methods. Specifically, the animals stunned by electrical stunning had higher glucose and G6P concentrations than those stunned by free bullets. This may be due to stress leading to increased glycogen turnover into glucose and G6P by glycogenolysis. This study did not show significant differences (P <0.05) in the glycogen, ATP, CP, lactate, and GP concentrations at 25 h post mortem between the two stunning methods. However, it is noteworthy that the glycogen and CP concentrations were slightly higher in animals stunned by the free bullets

method than in animals stunned by electrical stunning. Moreover, the lactate concentration was slightly lower in animals stunned by free bullets than those stunned by electrical stunning.

Table 4.8 Means ± SD of muscle metabolite concentrations (μmol/g) at 25 h post mortem in various anatomical locations of female Nile crocodile carcasses (n = 60)

	Neck	**Body**	**Tail**
Glycogen	$10.17^{a} \pm 4.449$	$11.83^{ab} \pm 4.937$	$14.33^{b} \pm 4.378$
Glucose	$1.63^{a} \pm 0.499$	$2.07^{b} \pm 0.548$	$1.75^{a} \pm 0.577$
G6P	$1.50^{a} \pm 0.861$	$1.94^{b} \pm 1.049$	$1.35^{a} \pm 0.735$
ATP	$5.81^{a} \pm 0.846$	$5.55^{a} \pm 0.892$	$6.16^{a} \pm 0.824$
CP	$3.34^{a} \pm 0.887$	$3.86^{a} \pm 0.767$	$3.48^{a} \pm 0.767$
Lactate	$40.76^{a} \pm 9.120$	$42.62^{a} \pm 8.569$	$37.03^{a} \pm 9.494$
GP	$67.36^{a} \pm 12.38$	$74.30^{a} \pm 12.76$	$71.90^{a} \pm 10.59$

** Means with different superscripts in a row differ significantly (P <0.05)*

The results in **Table 4.8** show significant differences (*P* <0.05) in the glucose, glycogen, and G6P concentrations at 25 hours post mortem between the various anatomical locations. More specifically, the body had the highest glucose concentration compared to the neck and tail, with the neck and tail having similar glucose concentrations. Glycogen had the highest concentration within the tail and the lowest within the neck, with the glycogen concentration in the body being similar to the neck and tail glycogen concentrations. Lastly, G6P concentrations were the highest in the body compared to the neck and tail, while the G6P concentrations were similar within the neck and tail.

Bendall (1978) stated that muscles with higher lactate concentrations early post mortem are more active during the process of death. Furthermore, Choe *et al*. (2008) stated that muscles with lower glycogen and higher lactate content have a faster glycolytic rate at the early post mortem period than muscles with higher glycogen and lower lactate content. Moreover, muscles having more type IIb fibres have a faster glycolytic rate in the early post mortem period (Choe *et al*., 2008). In sardines, it was seen that the CP concentration was lower in slow muscles than in fast muscles (Watabe *et al*., 1991). The slow-twitch muscle fibres mainly utilise aerobic metabolism, while fast-twitch muscles use anaerobic metabolism; this can further explain the higher glycogen and lower G6P and glucose concentrations in slow-twitch fibres (Wang *et al*., 2017).

The glycogen, glucose, and G6P concentrations (µmol/g) are further compared between the various anatomical locations of female Nile crocodiles stunned by different methods in **Figure 4.9**, **Figure 4.10**, and **Figure 4.11**.

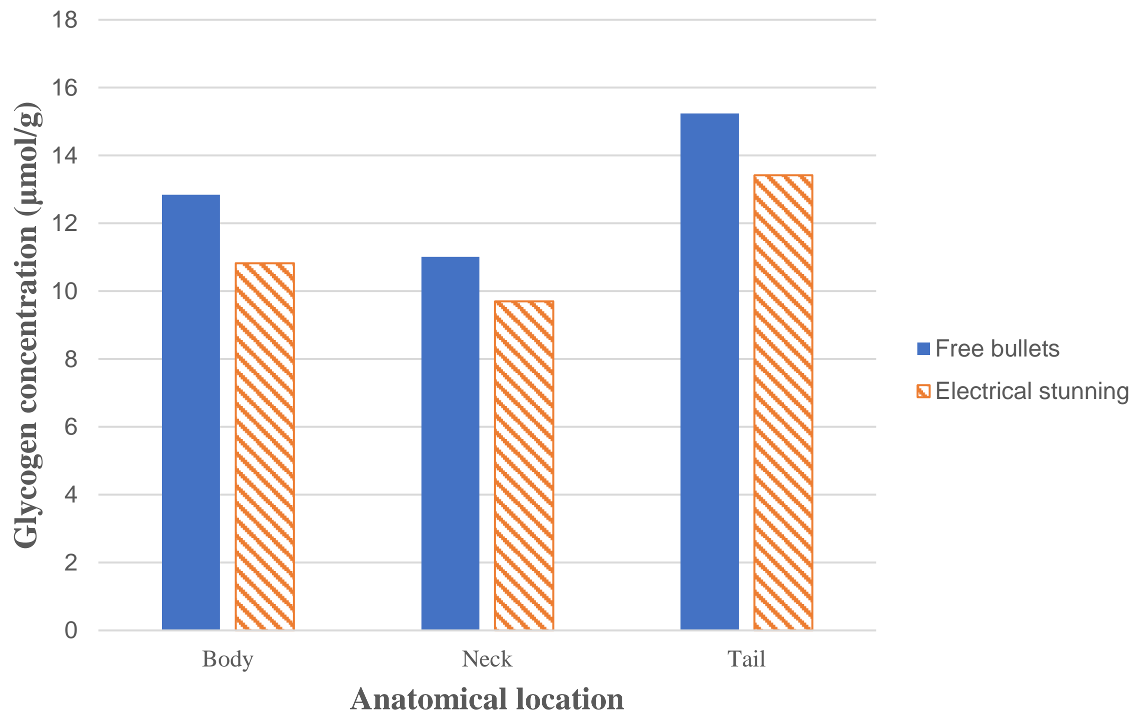

Figure 4.9 Glycogen concentration (µmol/g) at 25 h post mortem in various anatomical locations of female Nile crocodiles stunned by different methods

Figure 4.9 indicates that the glycogen concentration was similar between the different stunning methods within each anatomical location. It further indicates that the glycogen concentration was highest in the tail and the lowest in the neck of female Nile crocodiles stunned by each method.

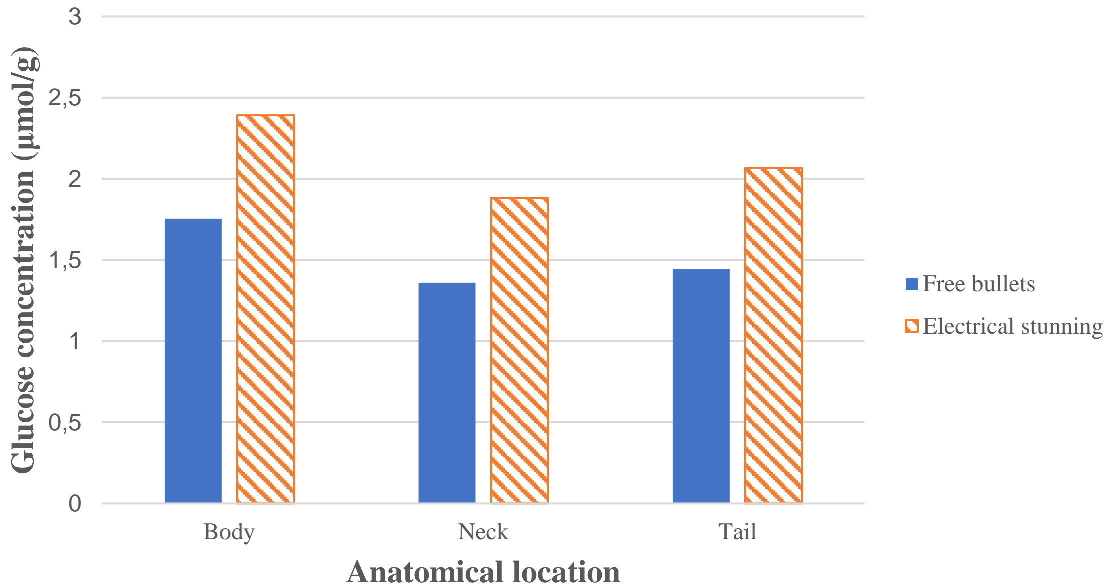

Figure 4.10 Glucose concentration (µmol/g) at 25 h post mortem in various anatomical locations of female Nile crocodiles stunned by different methods

In **Figure 4.10**, it is indicated that the glucose concentration (µmol/g) at 25 h post mortem was lower in the animals stunned by free bullets than in animals stunned by electrical stunning in each anatomical location. Furthermore, it is shown that the glucose concentration at 25 h post mortem was the lowest in the neck and highest in the body in animals stunned by each method.

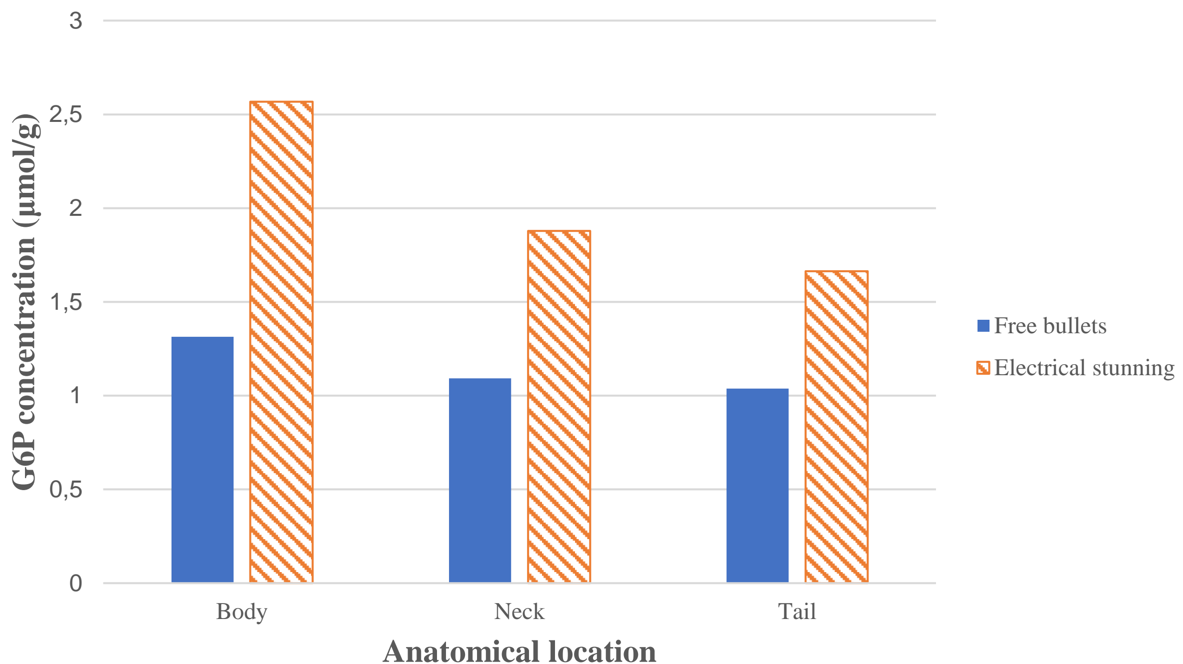

Figure 4.11 G6P concentration (µmol/g) at 25 h post mortem in various anatomical locations of female Nile crocodiles stunned by different methods

Figure 4.11 indicates that the G6P concentrations was highest in the body of female Nile crocodiles stunned by electrical stunning. In contrast, the G6P concentration was the lowest in the tail of female Nile crocodiles stunned by free bullets.

This section shows that glucose and G6P concentrations were affected by the stunning method. Animals stunned by electrical stunning had a higher glucose and G6P concentration than those stunned by free bullets. This may be due to an increase in muscle activity during the immediate post-slaughter period. This leads to an increase in the rate of glycogenolysis and glycolysis post mortem (Støier *et al*., 2001; Qin *et al*., 2016). Thus, based on the higher 25 h post mortem glucose and G6P concentrations it can be concluded that the electrical stunning method caused more stress to female Nile crocodiles than the free bullets method.

In terms of anatomical location, it was shown that the tail had the highest glycogen and lowest glucose and G6P concentrations. Moreover, the body had the lowest glycogen and highest glucose and G6P concentrations at 25 h post mortem. Thus, the tail was less active and had a slower glycolytic rate than the body early post mortem. Thus, based on these results, the *ilio-ischiocaudalis muscle* in

the tail contains more red muscle fibres (type I) than the neck and body, and the body contains more white muscle fibres (type II) than the neck and tail.

4.4 Physicochemical characteristics

Thaw loss, cooking loss and shear force

This study compared the thaw loss, cooking loss and shear force of the tail meat (*ilio-ischiocaudalis muscle*) from female Nile crocodiles stunned by free bullets and electrical stunning, shown in **Table 4.9**.

Table 4.9 Means ± SE of physicochemical characteristics of the tail meat from female Nile crocodiles stunned by different methods (n = 20)

	Stunning method		
	Free bullets	**Electrical stunning**	**Significance**
% Thaw loss	3.025± 0.306	2.302 ± 0.306	0.141
% Cooking loss	29.486 ± 0.715	30.820 ± 0.715	0.240
Shear force (kgf)	2.79 ± 0.118	2.769 ± 0.118	0.909

** Live weight (8.895) as a covariate*

This study showed no significant difference (*P* <0.05) in the thaw loss as a percentage of raw meat weight, cooking loss as a percentage of raw meat weight, and shear force values between the two stunning methods. Thus, the stunning method did not influence the meat quality as expected, and the use of a particular stunning method did not lead to more favourable meat quality. The cooking loss of 30.15% was slightly lower than the cooking loss of 31.45% found by Hoffman *et al*. (2000) for the tail of Nile crocodiles. Furthermore, the total mean shear force value of 2.78 kgf was lower than that of 4.35 kgf and 5.61 kgf found by Hoffman *et al*. (2000) and Balowski *et al*. (2015), respectively.

This trial was done under cold conditions (16.8 ºC) and slaughtering in summer months where temperatures are higher can lead to an increased stress response, which can exacerbate parameters such as purge and shear force.

Fatty acid composition

This study investigated the FA composition in the intermuscular fat (INTMF) and intramuscular fat (IMF) of female Nile crocodile tails. These crocodiles were ca. 40 months old and fed a chicken

diet. The fatty acid composition of INTMF and IMF in the tail of female Nile crocodiles stunned by different methods are shown in **Table 4.10**.

Previous studies have investigated the FA composition of Nile crocodile meat. Some of these studies investigated the FA composition of tail meat (Hoffman *et al*., 2000), while others investigated the FA composition of muscle and adipose tissue from Nile crocodile tail meat (Osthoff *et al*., 2010). Moreover, previous studies compared the FA composition between various anatomical locations, such as the abdomen and steatothecum (Osthoff *et al*., 2014). Previous studies also compared the FA composition between male and female Nile crocodiles (Osthoff *et al*., 2014), and others compared the FA composition between wild and captive Nile crocodiles (Osthoff *et al*., 2010).

It should be noted, however, that the diet of captive Nile crocodiles investigated by Osthoff *et al*. (2010) consisted of 80% chicken and 20% beef or horse meat. Furthermore, the diet of the captive Nile crocodiles studied by Hoffman *et al*. (2000) consisted mainly of carcass meal and raw chicken. Thus, these diets given in previous studies differ from the diet of chicken given in this study, which makes comparison between these studies difficult as the FA composition of crocodilians is affected by their diet (Hoffman *et al*., 2000; Huchzermeyer, 2003; Osthoff *et al*., 2010; Vicente-Neto *et al*., 2010).

Certain studies have also examined the FA composition in other crocodilian species, such as Spectacled caiman (Huang *et al*., 2018) and *Caiman crocodilus yacare* (Vicente-Neto *et al*., 2010). This also leads to difficulty comparing the results between these studies and the current study since FA composition is further affected by the species, sex, and environment (Osthoff *et al*., 2014).

Table 4.10 Fatty acid composition (mean ± SE, n = 40) of the INTMF and IMF tissues in the tail of female Nile crocodiles stunned by different methods

	Comparison between tissues			Comparison between stunning methods		
	INTMF	**IMF**	**Sign.**	**Free bullets**	**Electrical stunning**	**Sign.**
Chemical composition						
% Fat	82.0 ± 1.4	11.6 ± 1.4	***	46.1 ± 1.6	47.5 ± 1.6	NS
% FFDM	6.1 ± 0.3	18.7 ± 0.3	***	12.3 ± 0.3	12.4 ± 0.3	NS
% Moisture	11.9 ± 1.3	69.8 ± 1.3	***	41.7 ± 1.4	40.0 ± 1.4	NS
FAMEs (% of total FAs)						
SFAs						
Myristic	0.6 ± 0.0	0.5 ± 0.0	*	0.6 ± 0.0	0.5 ± 0.0	NS
Palmitic	22.8 ± 0.1	22.6 ± 0.1	NS	22.7 ± 0.1	22.7 ± 0.1	NS
Stearic acid	6.2 ± 0.0	6.3 ± 0.0	NS	6.3 ± 0.0	6.2 ± 0.0	NS
Total SFAs	29.6 ± 0.1	29.5 ± 0.1	NS	29.5 ± 0.1	29.5 ± 0.1	NS
MUFAs						
Palmitoleic	5.5 ± 0.0	5.6 ± 0.0	NS	5.6 ± 0.0	5.6 ± 0.0	NS
Oleic	38.2 ± 0.1	37.2 ± 0.1	***	37.7 ± 0.1	37.7 ± 0.1	NS
Vaccenic	2.2 ± 0.0	2.3 ± 0.0	**	2.2 ± 0.0	2.2 ± 0.0	NS
Eicosenoic	0.3 ± 0.0	0.2 ± 0.1	***	0.2 ± 0.0	0.2 ± 0.0	NS
Total MUFAs	46.2 ± 0.1	45.2 ± 0.1	***	45.8 ± 0.1	45.7 ± 0.1	NS
PUFAs						
Linoleic	21.6 ± 0.1	22.1 ± 0.1	***	21.8 ± 0.1	21.9 ± 0.1	NS
γ-Linolenic	0.1 ± 0.0	0.0 ± 0.0	**	0.0 ± 0.0	0.1 ± 0.0	*
α-Linolenic	1.5 ± 0.0	1.5 ± 0.0	NS	1.5 ± 0.0	1.5 ± 0.0	NS
Eicosatrienoic	0.2 ± 0.0	0.3 ± 0.0	***	0.2 ± 0.0	0.2 ± 0.0	NS
Arachidonic	0.8 ± 0.1	1.3 ± 0.1	***	1.1 ± 0.1	1.1 ± 0.1	NS
Total PUFAs	24.2 ± 0.1	25.3 ± 0.1	***	24.7 ± 0.1	24.8 ± 0.1	NS
Total omega-3 FAs	1.5 ± 0.0	1.5 ± 0.0	NS	1.5 ± 0.0	1.5 ± 0.0	NS
Total omega-6 FAs	22.7 ± 0.1	23.7 ± 0.1	***	23.1 ± 0.1	23.3 ± 0.1	NS
Fatty acid ratios:						
PUFA/SFA	0.8 ± 0.0	0.9 ± 0.0	***	0.8 ± 0.0	0.8 ± 0.0	NS
n-6/n-3	14.9 ± 0.1	15.4 ± 0.1	*	15.0 ± 0.2	15.3 ± 0.2	NS

** INTMF – intermuscular fat, IMF - intramuscular fat*
** Liveweight (8.895) as a covariate*
** P <0.05; ** P <0.01; *** P <0.001*

There was no interaction between the tissues and stunning methods. The results in **Table 4.10** show that the IMF had a significantly (*P* <0.05) higher moisture and FFDM content than the INTMF and a significantly (*P* <0.05) lower fat content than the INTMF. Furthermore, this study showed that the major FAs in both the INTMF and IMF were oleic, palmitic, and linoleic acids. This is similar to the results found by Hoffman *et al*. (2000), Osthoff *et al*. (2014) and Huang *et al*. (2018), where oleic, palmitic, and linoleic acids were also seen to be present in the greatest amount within crocodilian tail meat. This study further showed that the greatest difference between the FA of INTMF and IMF were in oleic, linoleic, and arachidonic acids. Furthermore, the IMF had a higher (*P* <0.05) PUFA and omega-6 FA content and a lower (*P* <0.05) MUFA content than the INTMF. This is similar to the results shown by Osthoff *et al*. (2010), which also showed that the IMF of Nile crocodile tails had a lower MUFA and higher PUFA content than the INTMF. This study did not show any differences in the FA composition between the stunning methods except for a difference in γ-linolenic acid. However, γ-linolenic acid was present in amounts less than 0.5% and the difference between stunning methods was only 0.1%, thus preventing any further comparisons.

Compared to the studies by Hoffman *et al*. (2000) and Osthoff *et al*. (2014), this study showed a lower SFA and MUFA content and a higher PUFA content. Furthermore, this study showed a lower MUFA content than the study by Hoffman *et al*. (2000). However, this study showed a higher SFA and MUFA content and a lower PUFA content than the study by Osthoff *et al*. (2010). Furthermore, Osthoff *et al*. (2014) showed a higher SFA (42.3%), similar MUFA (41.2%) and lower PUFA (15.6%) content in female Nile crocodiles than in this study. Lastly, this study showed a lower SFA and PUFA content and a higher MUFA content than caiman species (Vicente-Neto *et al*., 2010; Huang *et al*., 2018).

Moreover, this study found a n-3 FA and n-6 FA content of 1.5% and 22.7%, respectively, within the INTMF and a n-3 FA, n-6 FA content of 1.5% and 23.7%, respectively, within the IMF of the tail. This gives a n-6/n-3 ratio of 14.9 and 15.4 in the INTMF and IMF, respectively. This differs from the results found by Hoffman *et al*. (2000), where a n-3 FA content, n-6 FA content, and n-6/n-3 ratio of 1.7%, 9.1%, and 5.4, respectively, were found. Furthermore, Osthoff *et al*. (2010) found a n-6 FA content of 30.0% and 34.4% in the INTMF and IMF, respectively and an omega-3 FA content of 2.4% and 3.8% in the INTMF and IMF, respectively. Osthoff *et al*. (2014) found a higher n-3 FA content (8.0%) and a lower n-6 content (7.6%) compared to this study. Lastly, Huang *et al*. (2018) found a n-3 FA content (7.5%) higher than this study, a n-6 FA content (23.5%) similar to this study, and a n-6/n-3 ratio (3.1) lower than this study.

Lastly, this study showed a PUFA/SFA ratio of 0.8 and 0.9 in the INTMF and IMF, respectively. This differs from the results by Hoffman *et al*. (2000), Vicente-Neto *et al*. (2010) and Huang *et al*. (2018), where ratios of 0.3, 0.5, and 0.9, respectively, were found.

The results between the current study and the study by Hoffman *et al*. (2000), Osthoff *et al*. (2010) and Osthoff *et al*. (2014) may differ due to a difference in the diet given to the crocodiles, as the crocodiles in the study by Hoffman *et al*. (2000) where given a carcass meal in addition to chicken. The study by Osthoff *et al*. (2010) investigated the FA content of captive Nile crocodiles fed beef and horse meat in addition to chicken, and Osthoff *et al*. (2014) investigated the FA content of wild Nile crocodiles, which eat predominantly fish. Furthermore, the study by Hoffman *et al*. (2000) did not determine the sex of the crocodiles examined, while this study only examined the FA composition of female Nile crocodiles. Thus, differences between this study and the study by Hoffman *et al*. (2000) may further be due to a difference in the sex of the crocodiles. Lastly, differences between this study and the studies by Vicente-Neto *et al*. (2010) and Huang *et al*. (2018) may be due to the differences in species and sex since these studies investigated the FA composition in caiman species of different sexes.

Chapter 5
Conclusions

This study found a dressing percentage of 61.25% for female Nile crocodiles. It further showed that the forequarter, rib casing, hindquarter, tail, and tail tip yields 24.21%, 15.39%, 20.86%, 31.76%, and 2.3%, respectively. Thus, the tail has the highest yield in female Nile crocodiles, which is beneficial to the industry as the tail has the highest value. A blood loss as a percentage of live weight of 2.3% was found. However, the blood loss as a percentage of the live weight did not differ (P <0.05) between the stunning methods used in this project.

Carcass pH and temperature were measured over time in three anatomical locations between female Nile crocodiles stunned by free bullets and electrical stunning. These results indicated that crocodiles stunned by electrical stunning had a significantly (P <0.05) lower initial pH and lower pH at 48 h post mortem. The lower initial pH may be due to increased muscle activity during the stunning and slaughter procedure, leading to accelerated anaerobic muscle metabolism directly after slaughter. The results showed significant differences (P <0.05) in the carcass temperatures at 2 h, 25 h, 36 h, and 48 h post mortem between the stunning methods. Lastly, carcass pH was lower at a similar temperature in female Nile crocodiles stunned by free bullets than those stunned by electrical stunning. Thus, based on the pH and temperature results, it can be concluded that the free bullets method was more favourable compared to the electrical stunning method.

Regarding anatomical location, pH and temperature were measured in the neck, body, and tail. The pH was significantly (P <0.05) higher in the tail compared to the neck and body and significantly (P <0.05) lower in the body compared to the neck and tail. Thus, based on the pH results between the different anatomical locations, one can conclude that the tail consists of more slow-twitch fibres (red muscle fibres) than the neck and body. Moreover, the body has more fast-twitch fibres (white muscle fibres) than the neck and tail.

Muscle metabolomics were determined at 25 h post mortem in three anatomical locations between female Nile crocodiles stunned by free bullets and electrical stunning. These results indicated that animals stunned by electrical stunning had a significantly (P <0.05) higher glucose and glucose-6-phosphate concentration than those stunned by free bullets. The increased glucose and glucose-6-phosphate concentrations may be due to an increase in the rate of post mortem glycolysis, leading to a faster turnover of glycogen into glucose and glucose-6-phosphate post mortem. This increased rate

of glycolysis may be attributed to an increase in muscle activity during the stunning and slaughter procedure. Furthermore, this study showed that the tail had a lower (P <0.05) concentration of glycogen and a higher (P <0.05) concentration of glucose and glucose-6-phosphate. This is characteristic of muscles containing more slow-twitch fibres (red muscle fibres) than fast-twitch fibres (white muscle fibres).

Regarding physicochemical characteristics, Nile crocodiles in this study had a thaw loss of 2.66%, a cooking loss of 30.15%, and a shear force value of 2.78 kgf. Shear force values were similar between the two stunning methods used. Thus, the stunning method did not significantly (P <0.05) affect the meat quality of female Nile crocodiles. However, it should be noted that this study was done in cooler weather (16.8 ºC) and that one might expect slaughtering in summer months to lead to lower meat quality. Regarding FA composition, it can be concluded that the intermuscular and intramuscular fat differed in fatty acid composition. The greatest difference between these tissues were oleic, linoleic, and arachidonic acids. Moreover, the intramuscular fat contained fewer MUFAs and more PUFAs than the intermuscular fat. Lastly, the FA present in the greatest amount were oleic, palmitic, and linoleic acids.

Overall, this study showed that the free bullets method caused less stress than the electrical stunning method. However, in terms of meat quality, the stunning method did not have a significant effect and did not lead to more desirable meat quality for the consumer. Furthermore, this study showed that the anatomical locations differ in terms of the conversion of muscle to meat. This indicates that the tail may contain more slow-twitch muscle fibres than the neck and body. Lastly, this study indicated that fatty acid composition differs between the intermuscular and intramuscular fat of the tail in female Nile crocodiles.

Chapter 6
Critical evaluation and recommendations

Future studies may compare stunning methods/stress across sexes (if possible) as male and female animals respond differently to stress. However, this may be impractical if the farm only breeds one sex of crocodile, and proper planning should be done well in advance if such a study is done. Furthermore, this study can be repeated in the summer (crocodiles are more commonly slaughtered during this time) when temperatures are higher (25-35 ºC), as crocodiles might have an elevated stress response, and one may expect to see greater differences between stunning methods than observed in this study. It may also be useful to separate animals into size groups as smaller animals may react differently to stressors than larger animals.

A valuable contribution to the crocodilian farming industry would be to further assess the different stunning methods utilised in the industry, as this study only assessed the free bullets and electrical stunning methods, while there are still farms using percussive blow to the head and captive- and non-captive bolt pistols. Furthermore, it may be beneficial to study the effect of the free bullets method on crocodilians' mineral composition since it is unknown whether using lead bullets may lead to lead poisoning.

Future studies on Nile crocodile meat may benefit from using more valuable and used cuts than in this study, such as the tail, cheeks, and legs. Furthermore, the time that the ultimate pH is reached has yet to be determined, and extending the time period of pH and temperature measurement is recommended. Moreover, future studies can determine post mortem muscle metabolomics over time, which will further the knowledge of the post mortem changes in crocodilian muscle. Comparative studies between Nile crocodiles, Alligators, Caimans, and Saltwater crocodiles may be valuable to the industry as these animals remain different species, and their reaction to stress may differ (thus, cross referencing these species may not always be accurate). Future studies may also consider adding drip loss or purge to the parameters measured.

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Annexures

Annexure A: Photo plate

Plate 1: Scale

Plate 2: CCI bullets

Plate 3: Electrical stunner

Plate 4: .22 firearm with silencer

Plate 5: Crocodile carcasses in cool room (1)

Plate 6: Crocodile carcasses in cool room (2)

Plate 7: Example of tail

Plate 8: Example of body and neck

Plate 9: Example of skin tags

Plate 10: Hanna food pH probe and Hanna Lab application

Plate 11: Labotec water bath

Plate 12: Samples cooking in water bath

Plate 13: Weighing of cooked sample

Plate 16: Vernier calliper for checking strip size

Plate 14: Cut tail strips for shear force analysis

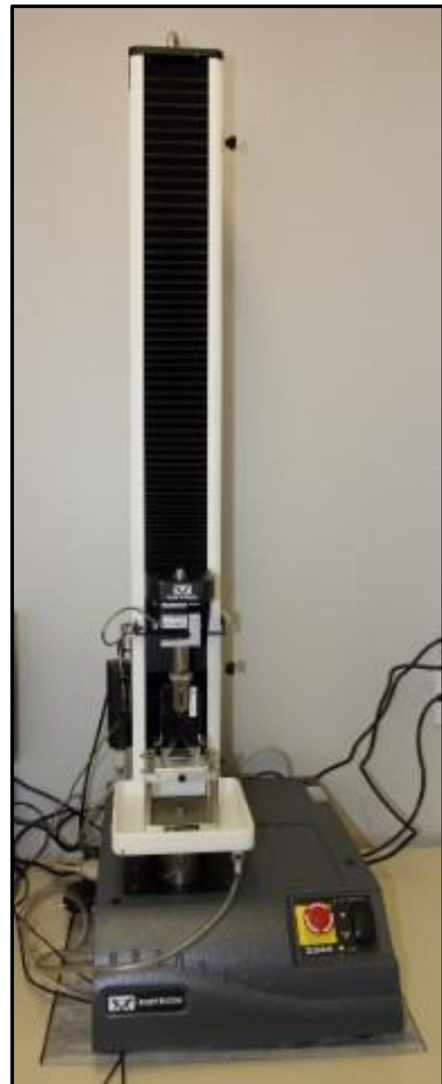

Plate 17: Instron machine

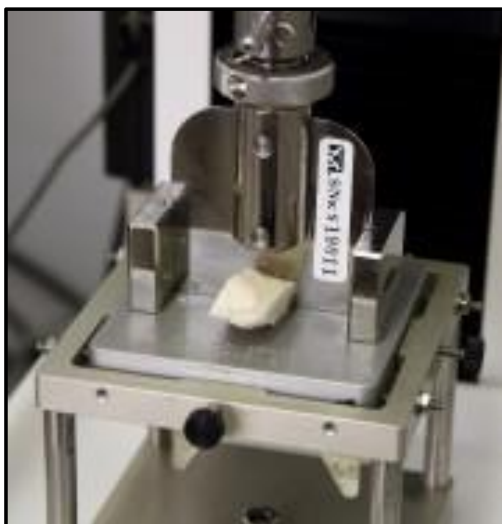

Plate 15: Warner Bratzler shear attachment

Annexure B: pH, temperature, and weight recording sheet

						ANIMAL NO.	**C**
SPECIES	Nile Crocodile						
DATE							
LIVE WEIGHT							
Cold carcass weight							
Dressing %							
Weight blood loss							
AGE							
SEX	M / F						
STUNNING METHOD	A / B						
Name of cut:	**Forequarter**	**Rib casing**	**Hindquarter**	**Tail**	**Tail Tip**		
Cut weights							
		Tail (Left)	**Tail (Right)**	**Body (Left)**	**Body (Right)**	**Neck (Left)**	**Neck (Right)**
	pH:						
	2 h						
	4 h						
	6 h						
	9 h						
	12 h						
	25 h						
	36 h						
	48 h						
	Temperature:						
	2 h						
	4 h						
	6 h						
	9 h						
	12 h						
	25 h						
	36 h						
	48 h						

Notes