Egyptian goose (*Alopochen aegyptiacus*): Sensory, biochemical and physical meat quality as affected by gender, diet and ageing

Greta Geldenhuys

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Promoter: Prof Louwrens C. Hoffman

Co-Promoter: M. Muller

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DECLARATION

By submitting this dissertation electronically, I declare that the entirety of the work contained therein is my own, original work, that I am the sole author thereof (save to the extent explicitly otherwise stated), that reproduction and publication thereof by Stellenbosch University will not infringe any third party rights and that I have not previously in its entirety or in part submitted it for obtaining any qualification.

This dissertation includes four original papers published in peer-reviewed journals and books and five unpublished publications. The development and writing of the papers were the principle responsibility of myself and for each of the cases where this is not the case, a declaration is included in the dissertation indicating the nature and extent of the contribution of co-authors.

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NOTES

This thesis is presented in the format prescribed by the Department of Food Science, Stellenbosch University. The structure is in the form of one or more research chapters (papers prepared for publication) and is prefaced by an introduction chapter with the study objectives, followed by a literature review chapter and culminating with a chapter for elaborating a general discussion and conclusions. Language, style and referencing format used are in accordance with the requirements of the *International Journal of Food Science and Technology*. This thesis represents a compilation of manuscripts where each chapter is an individual entity and some repetition between chapters has, therefore, been unavoidable.

Results from this dissertation that have been published in the following journals:

- Geldenhuys, G., Hoffman, L.C. & Muller, M. (2013a). Gamebirds: A sustainable food source in Southern Africa? *Food Security*, **5**, 235-249.
- Geldenhuys, G., Hoffman, L.C. & Muller, M. (2013b). Aspects of the nutritional value of cooked Egyptian goose (*Alopochen aegyptiacus*) meat, compared to other well-known fowl species. *Poultry Science*, **92**, 3050-3059.
- Geldenhuys, G., Hoffman, L.C. & Muller, M. (2013c). The effect of season, sex and portion on the carcass characteristics, pH, colour and proximate composition of Egyptian Goose (*Alopochen aegyptiacus*) meat. *Poultry Science*, **92**, 3283-3291.
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- Geldenhuys, G., Hoffman, L.C. & Muller, M. (2012b). Sensory profile of Egyptian goose (*Alopochen aegyptiacus*) meat and the influence of season. International Union of Food

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- Geldenhuys, G., Hoffman, L.C. & Muller, M. (2012c). The feeding activities of Egyptian geese - The influence on the sensory quality and utilisation of the meat. South African Wildlife Management Association (SAWMA) Symposium, Bela-Bela, South Africa; 16-19 September 2012. Oral presentation.
- Geldenhuys, G., Hoffman, L.C. & Muller, M. (2013). The effect of season, gender and portion on the carcass characteristics, pH, colour and proximate composition of Egyptian Goose (*Alopochen aegyptiacus*) meat. International Congress of Meat Science and Technology (ICoMST), Izmir, Turkey; 18-23 August 2013. Oral presentation.
- Geldenhuys, G., Hoffman, L.C. & Muller, M. (2014). Histological characterisation of the fibre types in the *M. Pectoralis* of Egyptian geese: A Southern African wildfowl species. International Congress of Meat Science and Technology (ICoMST), Punta Del Este, Uruguay; 17-22 August 2014. Poster presentation.

SUMMARY

In Southern Africa, the hunting of wildfowl species has increased considerably in the past few years. Crop farmers incur major financial losses due to the feeding activities of Egyptian geese (*Alopochen aegyptiacus*); consequently a large number of geese are hunted in an attempt to reduce the damage caused. With the current absence of scientific information baseline research investigating the meat quality of this species is essential.

The sensory profile of Egyptian goose meat was found to be very distinct in relation to the characteristics of other well-known fowl species. It has very strong game aroma and flavour attributes with a prominent metallic aftertaste. The intense aroma and flavour notes were linked to the substantially higher iron content, as well as the high overall polyunsaturated fatty acid content as revealed by chemical profiling. The trained sensory panel also found the meat to be very tough (high shear force) compared to the other species.

To identify the factors which may affect the overall consistency of the meat quality, the influence of three main effects namely; season (grain vs. non-grain diet), gender and portion was investigated. This revealed that season had the largest effect and harvesting periods should therefore be considered. The main issue is the higher intramuscular fat (IMF) content in winter (July), as well as the substantial difference in the fatty acid profiles of the two seasons. The forage vs. grain based diets during certain periods of the year leads to variation in the content of key fatty acids in the meat i.e. oleic acid, linoleic acid and α-linolenic acid. In winter, the meat had a characteristic, prominent game and metallic aroma while the summer (November) profile was governed by “sweet-oily-duck” and beef-like sensory notes. The fatty acid differences also result in variation between the omega 6 to omega 3 ratios of the seasons; the portions from winter are within the recommendations (ratio<5) and those from summer not. Regarding gender, the females had a lower carcass yield but higher IMF content. The female breast portion was also more tender (lower shear force).

In attempting to elucidate the toughness of the meat, possible causes have been proposed. The pH decline in the *pectoralis* muscle occurs quite rapidly and it is possible that the high rigor temperature (>20 °C) may contribute to the increased toughness. Regardless of the proteolytic enzyme activity during the rigor period, the meat is still tough at 36 h post mortem and the proteolytic contribution may be overshadowed by the background toughness, i.e. the connective tissue content and fibre structure. The latter was confirmed when the breast portions were aged for 14 days and no change (decline) in the shear force values was observed even though myofibrillar degradation did occur (during ageing). Given the lack of a decline in the shear force, the aging of Egyptian goose meat as a means of improving the overall toughness cannot be proposed without further research.

The study in its entirety provides substantial proof that the commercial utilisation of Egyptian goose meat is feasible.

OPSOMMING

Die jag van wildsvoël spesies in Suider-Afrika het aansienlik toegeneem in die laaste paar jaar. Graanboere lei jaarliks groot finansiële verliese as gevolg van die voedingsgedrag van kolganse (*Alopochen aegyptiacus*). Gevolglik word 'n groot hoeveelheid ganse elke jaar geoes om sodoende die skade te verminder. Met die huidige tekort aan wetenskaplike inligting is grondslag navorsing rakende die vleiskwaliteit van hierdie wildsvoël spesie noodsaaklik.

Die sensoriese profiel van kolgansvleis is baie uniek in vergelyking met die vleis van ander bekende voël spesies. Dit word gekenmerk deur die sterk wildagtige aroma en geur tesame met 'n baie prominente metaal nasmaak. Hierdie intense aroma en geur hou verband met 'n baie hoër yster-inhoud, asook 'n hoë poli-onversadigde vetsuur profiel soos uitgewys deur die chemiese karakteriserings studie. Die opgeleide sensoriese proepaneel het ook die vleis van hierdie voël spesie beskou as baie taai in vergelyking met die vleis van ander spesies.

Ten einde te bepaal watter faktore die algehele variasie in vleiskwaliteit sal beïnvloed, is drie hoofeffekte naamlik, seisoen (graan teenoor nie-graan dieet), geslag en porsie ondersoek. Die verskeie studies het uitgewys dat seisoen die grootste invloed het en daarom sal die periodes waarin ganse geoes word in ag geneem moet word. Die hoër intramuskulêre vetinhoud in winter (Julie), asook die aansienlike verskil in die vetsuur profiele van die twee seisoene is die vernaamste verskille. Die weiding (hoofsaaklik gras) teenoor graan diëte wat gevolg word in sekere dele van die jaar lei tot variasie in die inhoud van belangrike vetsure (oliensuur, linoleïensuur en α-linoleensuur) in die vleis. In die winter het die vleis die kenmerkende en prominente wild geur en metaalagtige nasmaak getoon terwyl die profiel in die somer (November) hoofsaaklik bestaan het uit "soet-olierige-eend" en beesvleis geure. Die vetsuur verskille lei ook tot 'n verskil in die omega-6 tot omega-3 verhouding van die seisoene; in die winter is die porsies binne die aanbevole voedingsvereistes (<5) terwyl die somer porsies nie aan hierdie vereistes voldoen het nie. Rakende die invloed van geslag, het die vroulike voëls 'n laer karkas massa getoon tesame met 'n laer intramuskulêre vetinhoud. Die borsie van die vroulike kolganse was ook sagter.

In 'n poging om die taaiheid van kolgansvleis te verklaar, is 'n paar moontlike oorsake voorgestel. Die na-doodse pH daling in die borsspier vind redelik snel plaas, daarbenewens is dit ook moontlik dat die hoë temperature (>20 °C) waartydens rigor mortis plaasvind, kan bydrae tot die taaiheid. Ongeag die werking van die proteolitiese ensieme tydens die rigor periode was die vleis steeds taai 36 uur na dood, daarom was die proteolitiese bydrae moontlik oorskadu deur die agtergrond-taaiheid, dit wil sê die bindweefsel inhoud en vesel struktuur. Laasgenoemde is bevestig toe die borsspiere verouder is vir 'n tydperk van 14 dae. Geen verandering (afname) in die instrumentele taaiheid is waargeneem nie, selfs al het miofibrillêre afbraak plaasgevind. Aangesien

daar geen afname in instrumentele taaiheid opgemerk is nie kan veroudering van kolgansvleis, met die doel om die taaiheid te verbeter, nie aanbeveel word voor verdere navorsing nie.

In geheel voorsien hierdie studie beduidende motivering rakende die moontlike kommersiële aanwending van kolgansvleis.

TABLE OF CONTENTS

CHAPTER 1

Introduction

Gamebirds are a group of wild fowl species that are hunted mainly for recreational purposes but have also been utilised as a valuable food source (Little & Crowe, 2011). The hunting (wingshooting) of these species is a popular but also very traditional activity practiced in numerous countries around the world, particularly those in Europe. Although the hunting of wildfowl species has increased considerably over the past few years, the South African industry is not as established since wingshooting is mainly regarded as a sporting or leisure activity. Another drawback is the fact that the potential for the commercial utilisation of the meat has not been recognised. The meat is mainly consumed by the hunters themselves or donated to the local communities (Geldenhuys *et al*., 2013). That being said, a rough estimation based on the number of shotgun shells sold indicates that around 2 million gamebirds are shot annually in South Africa. Berutti (2005) is of the opinion that the gamebird industry in total, based on a study into the birding ecotourism of South Africa, is worth a value of approximately R300-R800 million per annum (1 US$ = R10.69).

The hunting of wildfowl has not only increased substantially in recent years but a large number of birds are hunted annually as they are considered to be agricultural pests (Geldenhuys *et al*., 2013). Crop farmers incur major financial losses due to the feeding activities of Egyptian geese (*Alopochen aegyptiacus*). In the late 1980's Mangnall and Crowe (2001; 2002) investigated the population dynamics and the physical and financial impact of Egyptian geese on cereal crops in the Agulhas Plain region of the Western Cape, South Africa. They found that Egyptian geese are mainly a problem between the periods of April to July (germination of the wheat) and October to November (harvesting of the wheat). Based on the quantification by Mangnall and Crowe (2002) in 1997 the geese were responsible for a total of R385 000 of damage to surface seeds, growing crops and windrows on the farmlands in this district. During 1998, damage to the growing plants in this area was estimated to be worth a total value of R420 000. It should be noted that this value is dated as there has been a dramatic increase in the value of cereals since this loss was calculated 17 years ago e.g. producer prices in 1997 for wheat was R817/ton vs. R2878/ton in 2012 (DAFF, 2013). The increase in the population numbers of this species and the consequent financial implication for crop farmers therefore generates a potential for utilisation of the meat and possible recovery of some of the financial losses due to the damage (Berruti, 2005).

Limited research is available regarding the meat quality of Southern African gamebird species (Geldenhuys *et al*., 2013). The consumer acceptability of meat is largely determined by the tenderness, juiciness and flavour (Risvik, 1994; Warriss, 2000; Wiklund *et al*., 2003); attributes which

are determined by several intrinsic and extrinsic factors. Although the views and opinions of the general public with regards to the eating quality of meat vary, it is essential to ensure the overall uniformity in terms of meat quality (Wiklund *et al*., 2003). This is where the challenge lies concerning Egyptian goose meat.

So as to provide a product with a consistent eating quality it is essential to scientifically evaluate the biochemical factors involved in meat quality. As no scientific knowledge is available regarding the various extrinsic (season/diet, gender, ageing, etc.) and intrinsic (portion/muscle, fibre type, rigor mortis, etc.) factors that influence the meat quality characteristics such as yield, sensory attributes and the chemical composition, these need to be quantified.

An initial descriptive phase is required where Egyptian goose meat is compared to other well-known fowl species in order to profile the sensory attributes and chemical composition thereof. The use of reference species will provide the opportunity to identify the quality of Egyptian goose meat in relation to the meat from other fowl (game and domestic) consumed on a regular basis in South Africa. When an initial profile is in place, further research investigating the effect of season (grain vs. non-grain diet), gender and portion on the carcass yield, physical characteristics, chemical composition and sensory profile will become more feasible. Since gamebird meat is generally perceived as being tough compared to the meat from domestic animals, the biochemical factors which determine the toughness of meat should also be explored. This research will not only provide new insight into the nutritional characteristics of Egyptian goose meat which is, to date, not available in literature but may also reveal certain aspects of the quality which may require improvement.

Ultimately, this may disclose the commercial viability of Egyptian goose meat and indirectly promote the commercial utilisation of the meat. The latter will not only benefit the development of the South African gamebird industry, but may also assist in improving the situation regarding Egyptian geese, the damage they cause to croplands and the consequent financial implication for farmers. From a more comprehensive perspective, the world is also faced with the universal issue of having to drastically improve food security (FAO, 2009). In order to overcome this problem effective action is required. Therefore, the meat from this unconventional and underutilised species can be a valuable food source for many people, especially in the rural areas of South Africa (Geldenhuys *et al*., 2013). For this reason, scientific knowledge of the overall meat quality, particularly the nutritional value, of Egyptian geese will be beneficial.

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CHAPTER 2

Literature review:

Gamebirds: A sustainable food source in Southern Africa?*

ABSTRACT

In order to alleviate the current food security situation the world is faced with, it is essential to investigate meat sources, which have the potential to be utilised in a sustainable manner. In Africa, malnutrition is a very common occurrence especially with regard to protein intake, therefore increasing protein consumption in developing countries, such as those in Africa, will be beneficial. In Southern Africa, there is an opportunity to utilise the meat of sport hunted wildfowl, with the aim of incorporating it into the commercial market. By expanding the range of meat products available, the Southern African population will be provided with valuable, additional protein sources. However, before the utilisation of wildfowl meat can be realised, there are certain challenges to overcome in order to ensure meat with the best possible quality reaches the consumer. The areas that warrants scientific research ranges from; investigating the intrinsic and extrinsic factors that may have an influence on the ultimate meat quality to, exploring possible techniques of improving the eating quality of wildfowl meat. The insight this may provide surrounding the quality of African wildfowl meat will indirectly increase the commercial viability thereof and thus contribute to food security.

Keywords: Wildfowl, Utilisation of gamebird meat, Egyptian geese, Guineafowl, Meat quality, Food security

* Geldenhuys, G., Hoffman, L.C. & Muller, M. (2013). Gamebirds: A sustainable food source in Southern Africa? *Food Security*, **5**, 235-249.

1 INTRODUCTION

The current situation that the world is faced with, regarding food security, not only calls for critical evaluation of ways to approach this problem, but effective action in order to improve food security. In 2009, it was estimated that 1.02 billion people in the world did not have a diet which provides the minimum nutritional requirements (FAO, 2009). As emphasized by Von Braun (2007), in Sub-Saharan Africa specifically, there has been a 26% increase in the amount of food-insecure individuals since 1992. In emerging countries such as those in Africa, meat can be utilised to decrease undernourishment and improve the food security situation (McNeill & Van Elswyk, 2012). Therefore, as the consumption of animal-protein in Africa between 1995 and 2005 was only 17% of what the recommended protein intake should be (FAO, 2011), increasing meat as a food source would be beneficial. One of the strategies suggested by Godfray *et al*. (2010), with the aim of overcoming this food security challenge, is to reduce the wastage of food. This, however, is mainly aimed at the loss of food products due to a lack of infrastructure and appropriate food related knowledge within developing countries. Nonetheless, reducing food wastage is not only related to products that are already part of the production chain but also to food (meat) that is not utilised as a food source yet. For this reason, it is vital to explore meat sources with the potential to be utilised in a sustainable manner. In Southern Africa, specifically South Africa, there is such an opportunity to utilise the meat of sport hunted wildfowl, especially with regards to Egyptian geese (*Alopochen aegyptiacus*) and guineafowl (*Numida maleagris*) as well as various wild pigeon breeds.

Gamebird hunting originated with the ancient Egyptians who used falcons, dogs and other weapons and techniques in order to outsmart these birds (Viljoen, 2005). In Europe, with the invention of the shotgun, gamebird hunting developed into a major sport with a vast amount of traditions, prestige and ethics involved (Viljoen, 2005). It is an activity that became popular, as well as a tradition, in numerous countries around the world and gamebird meat certainly forms part of the culinary customs of these countries. Gamebird dishes seem to be especially popular in countries such as Britain and France. However, in Southern Africa the gamebird industry and gamebird hunting or wingshooting have not yet developed into its full potential. More specifically, the sustainable utilisation of gamebirds is an aspect which has not been accomplished and this can be beneficial for South Africa if successfully implemented (Berruti, 2005).

Gamebirds are currently responsible for creating some new challenges as certain species such as the Egyptian goose is regarded as being agricultural pests due to the damage caused by these species and the subsequent financial losses for crop farmers (Mangnall & Crowe, 2001; 2002).
Another challenge is the fact that there is such limited information regarding the quality (physical and nutritional) of gamebird meat. In addition, ensuring the safety of the meat is an area of concern that

needs to be addressed as the contamination of the meat due to the shooting activities or improper slaughtering and cooling procedures can be hazardous. These aspects are essential to investigate in order for the utilisation of gamebird meat to be a success.

2 GAMEBIRDS AND THEIR CLASSIFICATION

Gamebirds are a group of birds which are hunted mainly for recreational reasons (Viljoen, 2005; Little & Crowe, 2011) but have also been utilised as a food source. This is mainly due to their ability to endure continuous harvesting (Little & Crowe, 2011). The expression "gamebird" describes a very broad collection of birds and Little and Crowe (2011) grouped these bird species into land fowl and waterfowl. Species of the following orders (Table 1) are regarded as gamebirds: Galliformes, Anseriformes, Columbiformes, Pterocliformes, and Charadriformes (snipes) (Viljoen, 2005). Viljoen (2005) noted that all birds currently classified as gamebirds have three things in common, namely; they all have a very high rate of reproduction, they exhibit a sporting behaviour which demands the necessity of a certain skill for the successful hunting of these birds and all are edible.

Table 1 The orders classified as gamebirds and the common names of the species belonging to each order (Viljoen, 2005)

Order	Common names
Galliformes	Partridge
	Francolin
	Quail
	Guineafowl
Anseriformes	Duck
	Geese
Columbiformes	Pigeons
	Doves
Pterocliformes	Sandgrouse
Charadriiformes	Ethiopian snipe (*Gallinago nigripennis*)

3 THE GAMEBIRD INDUSTRY: INTERNATIONAL AND SOUTHERN AFRICAN

Initially Europe dominated the industry; however, according to Viljoen (2005) the hunting of gamebirds not only thrives in Europe but also in the USA where it has developed into an industry responsible for generating millions of dollars worldwide. The author states that in the USA more than 38 million gamebirds are shot each year and that the gamebird hunters outnumber the common hunter by far.

Gamebird hunting is the major contributor to the hunting industry in the REGHAB (Reconciling Gamebird Hunting and Biodiversity) countries in Europe as gamebirds are the species predominantly being hunted (Martinez *et al*., 2002). In the United Kingdom (UK) this industry contributes to the economy of the country by creating 26 000 employment opportunities which is responsible for a financial revenue of R 5 billion (8.2 R = 1 U$) annually (SA Wingshooters Association, 2005). According to the independent report by the Public and Corporate Economic Consultants (PACEC) (2006), approximately 19 million gamebirds were shot in the UK in 2004 alone of which the majority is incorporated into the country's food chain.

It appears that the international gamebird industry is thriving and according to Viljoen (2005) the success is accompanied by the on-going management and conservation of the bird populations. The gamebird industry in Europe is centred on family traditions, especially in countries such as France and Spain (Martinez *et al*., 2002).

Internationally the circumstances are very different from that experienced in Southern Africa. Captive-bred birds is a key part of the international industry, especially in Europe where large quantities of gamebirds are bred on an annual basis in order to be released into the wild (put and take) (Berruti, 2005; Viljoen, 2005). In the UK these captive bred birds are predominantly pheasants (80%), but Red-legged partridge (16-17%), Grey partridge and ducks are also reared for this purpose (ADAS, 2011). According to the Agricultural Development Advisory Service (ADAS, 2011), the UK Game Farmers Association estimates that a quantity of 20-30 million birds is reared for release into the wild each year. Apart from the financial advantage this holds for the landowners the motivation behind the use of this method is the fact that it is believed to reduce the stress on the wild gamebird populations (Viljoen, 2005). This method also offers ethical hunting and inter-breeding occurs between the released birds that survive the hunting season and the wild birds. Subsequently, the gamebird population numbers are enhanced (Viljoen, 2005). Viljoen (2005), however, states that in view of the current information with regard to the captive breeding of native South African Gamebirds the use of the technique in this country seems to be unfeasible. This is primarily due to the low survival rate of captive-bred species in the wild and the fact that these bred birds are not fit for wingshooting as they do not have adequate sporting behaviour (Viljoen, 2005). Berruti (2005), on the other hand, is of the opinion that the use of captive-bred gamebirds in South Africa can be favourable for the gamebird habitats. However, caution needs to be taken and the North American and European methods should only be used as guidelines.

Also, in Southern Africa and in South Africa in particular, gamebird hunting has not been that popular even though hunting, in general, is considered to be a large part of the South African culture (van Rooyen, 2010). Although game bird hunting or wingshooting, nationally, is becoming more popular it is still mainly regarded as a sporting or leisure activity (Viljoen, 2005; SA Wingshooters

Association, 2005). Furthermore, one of the main drawbacks for the South African industry is the fact that the potential to utilise the meat has not yet been recognised. The meat is mostly used for consumption by the hunters themselves while the landowner also generally receives a portion of the shootings and donations are also made to the local communities.

There is very limited information available with regard to the South African gamebird industry. Berruti (2005) in a research document regarding the recreational and professional hunting in South Africa emphasizes this fact when it is stated that there is no specific estimation of the amount the industry is valued at. It was determined that a quantity of 500 000 gamebirds were shot in the year of 1997 (Berruti, 2005). Furthermore, according to the SA Wingshooters Association the number of gamebird hunters in South Africa were believed to be 20 000 individuals in 2005. Berutti (2005), however, did indicate a rough estimation of the industry's total worth in 2005, based on a study into the birding ecotourism of South Africa. This study estimates that the gamebird industry in total is worth a value of approximately R300-R800 million per annum in addition to the gundog industry which is also estimated at R200 million per year. Presently, it is estimated that around 2 million gamebirds are shot annually.

Another interesting fact recognised by Berruti (2005), which also proves the value of gamebirds is the illegal poisonings occurring on farms. It is predicted that between 174 000 and 428 000 gamebirds are poisoned in South Africa every year. The harvesting of gamebirds for their meat is believed to be the reason behind these poisonings. This, however, is not a suitable method for utilisation because of the health concerns, illegality of these activities and some other concerns in terms of other wildlife (Berruti, 2005).

4 POPULAR GAMEBIRDS HUNTED IN SOUTHERN AFRICA

Southern Africa has a large variety of native gamebirds. This includes; two guineafowl, six partridge, two quail, five francolin, four sandgrouse, eight pigeon and dove, one snipe, as well as 16 species of waterfowl (Viljoen, 2005).

According to Berruti (2005), the Rock pigeon, Namaqua sandgrouse, Helmeted guineafowl, Swainson's francolin, Grey-wing partridge and some of the duck species depending on the area and season, are the main gamebird species hunted in terms of the value of the birds and the population numbers in South Africa. Three out of a total of 44 gamebird species in South Africa i.e. the Yellow-throated sandgrouse (*Pterocles gutturalis*), Delegorgue's Pigeon (*Columba delegorguei*) and the African Pygmy goose (*Nettapus auritus*) cannot be utilised because of their distribution which is limited and their habitats that are threatened (Viljoen, 2005). Table 2 indicates the gamebird species found in South Africa along with the status of the population numbers within the country.

Table 2 Traditional gamebirds of Southern Africa and their population status (adapted from Viljoen 2005; Berruti 2005)

Common name	Species name	Status
Partridges		
Coqui Partridge	*Francolinus coqui*	Stable
Crested Partridge	*Francolinus sephaena*	Improving
Grey-wing Partridge	*Francolinus africanus*	Stable
Shelley's Partridge	*Francolinus shelleyi*	Improving
Red-wing Partridge	*Francolinus levaillantii*	Improving
Orange River Partridge	*Francolinus levaillantoides*	Improving
Francolins		
Red-billed Francolin	*Pternistis adspersus*	Stable
Cape Francolin	*Pternistis capensis*	Stable
Natal Francolin	*Pternistis natalensis*	Improving
Swainson's Francolin	*Pternistis swainsonii*	Improving
Red-necked Francolin	*Pternistis afer*	Unknown
Quails		
Common Quail	*Coturnix coturnix*	Stable
Harlequin Quail	*Coturnix delegorguei*	Stable
Guineafowls		
Crested Guineafowl	*Guttera pucherani*	Improving
Helmeted Guineafowl	*Numida meleagris*	Improving
Sandgrouse		
Namaqua Sandgrouse	*Pterocles namaqua*	Stable
Burchell's Sandgrouse	*Pterocles burchelli*	Improving
Yellow throated Sandgrouse	*Pterocles gutturalis*	Unknown
Double-banded Sandgrouse	*Pterocles bicinctus*	Stable
Pigeons and Doves		
Rock Pigeon	*Columba guinea*	Improving
Rameron Pigeon	*Columba arquatrix*	Improving
Red-eyed Dove	*Streptopelia semitorquata*	Improving
Delegorgue's Pigeon	*Columba delegorguei*	Declining
Laughing Dove	*Streptopelia senegalensis*	Improving
Cape Turtle Dove	*Streptopelia capicola*	Improving
Mourning Dove	*Streptopelia decipiens*	Stable
Green Pigeon	*Treron calva*	Improving
Ducks and Geese		
White-faced Duck	*Dendrocygna viduata*	Improving
Egyptian Goose	*Alopochen aegyptiacus*	Improving
South African Shelduck	*Tadorna cana*	Improving
Yellow-billed Duck	*Anas undulata*	Improving
African Black Duck	*Anas sparsa*	Stable
Cape Teal	*Anas capensis*	Stable
Hottentot Teal	*Anas hottentota*	Stable
Red-billed Teal	*Anas erythrorhyncha*	Improving
Cape Shoveller	*Anas smithii*	Improving
Southern Pochard	*Netta erythrophthalma*	Stable
Spurwing Goose	*Plectropterus gambensis*	Improving
Fulvous Duck	*Dendrocygna viduata*	Improving
White-backed Duck	*Thalassornis leuconotus*	Stable
Knob-billed Duck	*Sarkidiornis melanotos*	Improving
African Pygmy Goose	*Nettapus auritus*	Declining
Moccoa Duck	*Oxyura maccoa*	Unknown
Snipes		
Ethiopian Snipe	*Gallinago nigripennis*	Unknown

Southern Africa's two most popular and prevalent gamebird species is the Egyptian goose as well as the Helmeted Guineafowl. These species are not only very familiar under the local African people but several authors have emphasized their extremely widespread distribution and high population numbers in Southern Africa. In Zimbabwe for example, the guineafowl is farmed on an extensive scale by local villagers (Saina *et al*., 2005) and there is market potential for this species in developing countries (Madzimure *et al*., 2011).

5 WINGSHOOTING

Wingshooting is the term that is generally used for gamebird hunting and is profoundly different from the hunting of mammals or furred game animals (Berruti, 2005). This is because of the difference in the hunting techniques, habitats of the species, as well as the biological characteristics and the economical (industry) and social aspects of the two different types of hunting (Berruti, 2005).

The main motivation behind the hunting and harvesting of gamebirds are the financial and recreational aspects involved. However, it can also be beneficial in the management of gamebird population numbers (Viljoen, 2005). The harvesting of excess gamebirds for management purposes should occur late autumn to early winter, at the beginning of the hunting season when the breeding activities are at its lowest. This ensures lower population numbers before winter arrives and consequently more food sources are available. This technique allows for a larger survival rate throughout the winter months which results in the population entering the breading season in a good condition thus having an improved capability of production (Viljoen, 2005).

The period (season) each gamebird species may be hunted is dependent on the breeding season and the population numbers in each region. The amount of gamebirds that may be shot is also regulated by daily bag limits. In the Western Cape province, species such as Egyptian geese, with an improving population status, can be hunted throughout the year and has a bag limit of 10 geese per day (Western Cape Nature Conservation Board, 2010).

The techniques involved in gamebird hunting vary according to the specific type of gamebirds hunted for instance; upland gamebirds (terrestrial) vs. waterfowl. Waterfowl are generally shot on the flight routes onto or from the feeding areas such as croplands where they feed on the grains (Mangnall & Crowe, 2001). A double-barrelled shotgun (12-gauge) is mainly used as most gamebirds have to be shot in-flight.

6 FACTORS THAT INFLUENCE THE EATING QUALITY OF GAMEBIRD MEAT

The term meat quality is based on the consumer's preference and the characteristics they require a meat product to have. It is widely recognized that meat quality is influenced by several factors such as animal genetics, pre and post slaughter conditions, nutritional characteristics, environmental

conditions (Lefaucheur, 2010) as well as the measurements taken to ensure meat safety. More specifically, the eating quality of meat involves three main attributes namely; tenderness, juiciness and flavour which are the major contributors to the consumers' acceptability of meat (Risvik, 1994; Warriss, 2000). However, nowadays consumers also demand that meat is lean and that it provides adequate nutritional requirements (Hoffman & Wiklund, 2006).

6.1 Physical activity, carcass portions and fibre types

Similar to all game species; gamebirds are very active. The levels of physical activity do, however, vary from species to species. For example, Egyptian geese are waterfowl which are accustomed to various types of exercise such as terrestrial (walking/running), aquatic (swimming and diving) and aerial (flying long distances to forage). As Shewell (1959) noted, they will rather move into the water when threatened and will only fly away when they are caught by surprise. This combination of the different types of activities compared to that of terrestrial birds such as the Guineafowl and the Pheasants ensure that the muscles of the breast and leg of Egyptian geese are different in terms of the muscle fibre composition. Muscles responsible for long-term, systematic activity (such as retaining posture) is comprised of red, type I, slow oxidative (SO) fibres that contracts slowly and continuously. The muscles responsible for locomotive behaviour consist of fast twitch fibres. The muscles which are used during rapid movements/locomotive behaviour and bursts of activity consists of white, type IIb fast glycolytic (FG) fibres, but this muscle is very easily fatigued (George & Berger, 1966; Warriss, 2000). The type IIa, fast oxidative glycolytic (FOG) muscle fibres are resilient to fatigue and used during locomotion because of their fast contracting ability – these would typically be dominant in birds that fly long distances such as pigeons. George and Berger (1966) states that intermediate fibres become more prevalent when there is an increase in the sustainability of the rapid movements.

6.1.1 Breast

The breast portion (*M. pectoralis* and *M. supracoracoideus*) is situated in the front breast area of the carcass where the sternum provides a surface for this muscle. The breast portion of poultry is sold with the sternum intact (whole breast) or the muscle can be removed and sold as a deboned portion. The large breast muscle (*pectoralis*) is mainly used during flying when it is responsible for the downward movement of the wings and the *M. supracoracoideus* is the muscle which raises the wing during the upward movement (Dial, 1992; Poore *et al*., 1997; Swatland, 2000; Biewener, 2011). The breast muscle of gamebirds such as Egyptian geese which fly long distances will therefore endure a higher level of activity compared to that of terrestrial gamebird species. This *M. pectoralis* in volant species mainly consists of red, FOG fibres and a small amount of white, FG fibres (Butler, 1991).

Although George and Berger (1966) claim that ducks have a combination of FG, FOG and SO in the *M. pectoralis*, the study by Rosser and George (1986) found that the *M. pectoralis* in Anseriformes (ducks and geese) consists of red, FOG fibres and white, FG fibres. This is believed to be a result of the fast muscle contracting requirements for movement of the wings (Rosser & George, 1986). Baeza *et al*. (2000) also reported that the breast muscle of Mule ducks consisted of type IIa (88%) and type IIb (12%) muscle fibre types. The breast muscle's fibre composition of terrestrial gamebirds (Galliformes) such as Guineafowl and Pheasant will be very different from that of volant bird species. Terrestrial gamebirds will run rather than fly but if required they will rapidly take off but only fly for a very short distance (Kaiser & George, 1973). Kiessling (1977) reported that the breast muscle of Guineafowl hens only consisted of 10% red fibres. Similarly for pheasants, Kiessling (1977) also noted that their breast muscle consists of 17% red fibres and Hofbauer *et al*. (2010) emphasizes the fact that this muscle is primarily comprised of white, type II, glycolytic fibres. This high amount of white fibres are responsible for the rapid take off and flight acceleration while the low red fibre count suggests that the movement/flapping of the wings can only be sustained for a short period (Kaiser & George, 1973).

6.1.2 Leg

The leg consists of two portions; the thigh is the proximal part (closer towards the body) situated at the top of the leg and the drumstick which is the bottom or distal part (away from the body) of the leg (Swatland, 2000). The leg muscles are locomotive muscles which are used during activities such as walking by terrestrial gamebirds and waterfowl species also use these muscles when swimming and diving (Butler, 1991; Turner & Butler, 1988). The leg muscles therefore consist of a combination of SO, FOG and FG fibre types depending on the muscle and its activity.

The thigh which is the proximal part of the leg is removed at the hip joint of the carcass and separated from the drumstick at the knee joint. The femur bone forms part of this portion. The main muscles of the thigh portion consist of amongst others the *sartorius*, *iliotibialis*, *semitendinosus*, *biceps femoris* and *semimembranosus* which are located in the lateral position of the leg (Swatland, 2000). The main medial muscles in the thigh are the *ambiens*, *adductor longus*, *piriformis* and the *obturator internus* (Swatland, 2000).

The drumstick is the distal part of the leg and is separated from the thigh at the knee joint between the femur and the tibia (Swatland, 2000); the latter is the bone which forms part of the drumstick portion. The *gastrocnemius* and *peroneus longus* are the two main muscles of the drumstick (Swatland, 2000).

6.1.3 Fibre types and meat quality

The composition of the muscles/portions regarding the types of fibres they are composed of is a vital determinant of meat quality (Lefaucheur, 2010). Muscle fibre type can affect the colour, tenderness, juiciness and even the flavour of meat and as such, may have a detrimental effect on the consumer acceptability of meat.

Tenderness is probably the attribute which is most affected by muscle fibres. The total number of fibres, the cross sectional area and diameter of the fibres (size) as well as the composition of the muscle fibre types play a significant role in the tenderness of meat (Lefaucheur, 2010). Several studies have found correlations between fibre size and shear force/tenderness of meat (Hiner *et al*., 1953; Tuma *et al*., 1962; Crouse *et al*., 1991). It is suggested that muscle comprised of larger fibres produce meat (pre-maturation) that is less tender while more tender meat generally contain smaller fibres (Crouse *et al*., 1991). Red, Type I, oxidative muscle fibres have the smallest size whereas Type IIa, oxidative-glycolytic muscle fibres have an intermediate size and white, Type IIb, oxidative muscles have the largest fibre size (Oshima *et al*., 2009; as cited by Lefaucheur, 2010). Muscle fibre diameter is also related to sarcomere length. Herring *et al*. (1965) reported that a decrease in sarcomere length, due to the shortening of the muscle, results in a larger muscle fibre diameter and therefore less tender meat. Several other studies are in agreement with Herring *et al*. (1965) regarding the influence of sarcomere length on the tenderness of meat (Locker & Hagyard, 1963; Purchas, 1990). Furthermore, literature also indicates a negative relationship between the amount of fibres present in muscle and the tenderness of meat. Ryu and Kim (2005) found the number of muscle fibres to be positively correlated with shear force (low tenderness). Carpenter *et al*. (1963) explained that a decrease in the muscle fibre size results in a possible increase in the number of muscle fibres present therefore more connective tissue and ultimately less tender meat. This also clarifies the inverse relationship between total fibre number and cross-sectional area (size) of fibres as reported by several studies (Larzul *et al*., 1997; Ryu & Kim, 2005).

The tenderization (ageing) of meat is another of the aspects where muscle fibres are important to consider (Huff-Lonergan *et al*., 2010). The muscle fibre composition is involved in this process as the variation in the calpain/calpastatin and cathepsin activity (due to the amount of these proteotylic enzymes present) within different fibres affects the toughness of meat. It is believed that type IIb muscle fibres (fast twitch) have higher amounts of calpains compared to the inhibitor for this proteolytic enzyme; calpastatin (Ouali & Talmant, 1990). Therefore a higher rate of tenderization exists in muscles containing higher amounts of type IIb fibres (Ouali & Talmant, 1990; Lawrie & Ledward, 2006). Alternatively, type I, red muscle fibres contain higher concentrations of calpain II and calpastatin but they are less susceptible to proteolysis because of the lower post mortem calcium

concentrations in the red muscles which favour the activity of the proteolytic enzymes. However, both Ouali and Talmant (1990) and Sazili *et al*. (2005) noted that type I muscle fibres contain higher concentrations of calpastatin promoting inhibition of the proteolytic activity of calpain leading to less tenderization taking place in muscle with high levels of type I fibres. An additional aspect where fibre composition is involved is the occurrence of cold shortening in muscles. Oxidative muscle is prone to cold shortening as there is a more rapid loss in the ability of the sarcoplasmic reticulum to sequester calcium (Lawrie & Ledward, 2006; Huff-Lonergan *et al*., 2010). There is also variation in the collagen content of different muscle fibre types. It has been found that type I, oxidative fibres have a higher collagen content compared to type IIb glycolytic fibres (Kovanen *et al*., 1984; Rodrigues *et al*., 1996; Nakamura *et al*., 2003). This is also linked to the fact that there is an increase in the concentrations of intramuscular collagen in the muscles of animals that are physically more active pre-slaughter (Lewis *et al*., 1989).

The fibre composition is not only involved in the tenderness of meat but can be linked to the flavour of meat as well. Type I, oxidative fibres have a high proportion of phospholipids (Lefaucheur, 2010) and it is widely accepted that these predominantly long chain polyunsaturated fatty acids play a significant role in the flavour formation process (Wood *et al*., 2003). Due to their susceptibility to oxidation, these fatty acids may also cause negative flavour characteristics. However, muscles comprised of type I fibres also have a higher intramuscular fat content as oxidative fibres use fat as an energy source during metabolism (Wood *et al*., 2003; Lawrie & Ledward, 2006). Therefore it is postulated that these muscles may produce meat with a more prominent flavour as fat is responsible for conveying flavour during mastication (Melton, 1990).

Intramuscular fat being higher in red muscles may also have a possible effect on juiciness. This is attributable to the stimulation of saliva secretion by intramuscular fat during mastication of the meat (Lawrie & Ledward, 2006). Meat composed of high levels of type I fibres are also susceptible to producing DFD (dry, firm, dark) meat if the animals are subjected to stress pre-slaughter (Lawrie & Ledward, 2006). The low concentration of stored glycogen in the red muscles results in the rapid depletion of glycogen and therefore meat with a high ultimate pH. The high ultimate pH causes the meat to have a very high water holding capacity which reduces the juiciness of the meat upon cooking; a negative aspect for consumer acceptability.

6.2 Season

The seasonal changes that generally have an influence on meat quality are linked to the variation in the diet as well as the breeding periods and winter months. Concerning gamebirds, these are elements that cannot be controlled as they are not raised within a domestic environment. It is therefore essential to consider season in terms of the possible utilisation of wildfowl meat.

6.2.1 Variation in the diet

In regions where croplands are abundant, the diets of certain gamebirds vary on account of the grain season. Guineafowl is one of the gamebird species which depend on harvested grains and maize as part of their dietary intake (Little & Crowe, 2011). In South Africa it has also been reported that pigeons cause huge economic losses to sunflower crops, however this period is also when large numbers are hunted for sport. The meat derived normally goes to the local population for consumption. Another species relying heavily on the grain season is Egyptian geese which mainly feed on aquatic plants, aquatic invertebrates and green terrestrial plant materials but seedlings and growing crops also form part of their diet (Maclean, 1988; Viljoen, 2005). Halse (1984) reported that during certain periods they also rely on Bermuda grass, alga and pondweeds as food sources. This diet, however, is very different during the grain harvesting season. The geese travel long distances in order to forage on grain seeds found on croplands in the harvesting period, especially in the Western Cape where a vast amount of crop farms are present (Mangnall & Crowe, 2001; 2002). The difference in the diet of Egyptian geese during these two periods; July (forage based diet) compared to the grain based diet of November, may result in a difference in the fatty acid profile of the meat from the respective periods.

In monogastric birds, the dietary constituents (lipids) are directly incorporated into the tissue lipids with minimum modification, therefore, it is postulated that the difference in the diet can have an effect on the fatty acid profile and the latter will be a direct reflection of the diet (Wood & Enser, 1997; MacRae *et al*., 2005). The fatty acid profile is considered to be one of the major determinant factors in the characteristic aroma and flavour profile of meat as the thermal degradation of the lipids is one of the key processes in producing the aroma volatiles (Mottram, 1998). Though, not all of the fatty acids influence the flavour of meat to the same extent and a wider range of lipid derived aroma or flavour volatiles are produced from unsaturated fatty acids because of their higher susceptibility towards oxidation (Mottram & Edwards, 1983). Unsaturated fatty acids have the ability to rapidly oxidize, particularly PUFA with their increased number of double bonds. This is important in the flavour development process during cooking (Wood *et al*., 2003). The process of flavour formation; covering the involvement of the fatty acids present in raw meat, the flavour compounds produced upon cooking and the consequent aroma and flavour volatiles released, have not to date been investigated on gamebird meat. This is an area of research that is vital to quantify before the link between the differences in season (diet), fatty acid profiles and the sensory characteristics can be made within gamebird meat.

6.2.2 Breeding season

The annual breeding periods of wild birds have a large impact on their overall body condition, especially with regard to the females (Raveling, 1979; Reinecke *et al*., 1982). For example; the peak breeding season of Egyptian geese in the Western Cape are between late winter and early summer during the months of August and October (Viljoen, 2005). It is therefore postulated that regardless of the difference in the diet, the geese will have the best possible body condition, with high energy reserves, by the end of July as they have prepared for the breeding season. During the breeding period the energy reserves will be depleted but by the end of October/early November Egyptian geese start to forage on grain seeds (high energy source) during the harvesting season and the energy reserves are gradually restored. Consequently, the breeding season may not only have a significant influence on the body size and mass but also on the intramuscular fat content of Egyptian goose meat. Similar theories have been studied on the fluctuations in the body condition due to the annual cycle of Black ducks (Reinecke *et al*., 1982) and Canada geese (Raveling, 1979). It is therefore essential that breeding periods of gamebirds are considered in terms of meat quality.

6.3 Age and sex

The influence of animal age on the eating quality of meat is mainly restricted to two factors; collagen content and intramuscular fat which may have an effect on the tenderness, juiciness and flavour attributes. There is a tendency for meat originating from older animals to be juicier, to have more flavour but to be less tender. According to Lawrie and Ledward (2006), with an increase in the age of the animal a decrease in tenderness is observed. This phenomenon occurs because of an increase in the polypeptide cross links of the collagen as well as a decrease in its solubility upon heat treatment or cooking. This theory has been verified by numerous studies which correlated age with the tenderness of meat (Xiong *et al*., 2007; Schönfeldt & Strydom, 2011). Along with the changes in the connective tissue with age there also seem to be an increase in the flavour intensity of the meat (Lawrie & Ledward, 2006). This is associated with an increase in the intramuscular fat deposition as the animal matures. As fat is involved in conveying the flavour during mastication, a higher intramuscular fat content ensures better transmission of flavour and therefore increases the flavour intensity (Melton, 1990). Chartrin *et al*. (2006) noted a more prominent flavour in the breast portion of Mule ducks with higher intramuscular lipid levels. However, Lawrie and Ledward (2006) states that there is not only an increase in the intramuscular fat content of the meat but an alteration in the composition of the fatty acid profile also occurs. The fatty acid profile becomes more saturated which is also associated with an improved flavour. Though intramuscular fat is an important aspect of flavour formation, it is also involved in the sustained juiciness of meat (Lawrie & Ledward, 2006). The

higher intramuscular fat levels in the meat from older animals may therefore also increase the sustained juiciness.

The most significant difference regarding meat quality and gender is the variation in fat deposition between male and female animals. It is generally accepted that female animals tend to store fat more readily (Lawrie & Ledward, 2006). This results in the meat having a higher intramuscular fat content and ultimately, due to the diluting ability, the higher fat may have an influence on the tenderness as well. Baeza *et al*. (2001) reported that Guineafowl meat from female fowl was found to be more tender and less stringy by a sensory panel.

During the slaughter process, the sex of gamebirds can be determined without any difficulty by means of the reproductive organs. However, limited literature is available regarding the age determination of wild birds. This makes the incorporation of age, as a factor of influence, in research investigating meat quality difficult if not impossible.

6.4 Stress and meat quality

The quality of meat is determined by several factors of which the post-mortem metabolism is one of the key aspects. The rate and extent of the pH decline in post-mortem muscle is fundamental in the transformation of muscle into meat. This post mortem acidification process is directly linked to the amount of glycogen available in the muscle which can be influenced by several intrinsic (muscle type, species, variability between individuals) and extrinsic factors (environmental temperature and stress) (Lawrie & Ledward, 2006). Ante-mortem stress is one of the extrinsic elements that has a major effect on the glycogen metabolism and ultimate pH of the muscle (Remignon *et al*., 1998; Lawrie & Ledward, 2006); consequently defining the meat quality attributes such as tenderness, juiciness and colour. The two conditions responsible for this negative impact on the meat quality is known as dry, firm and dark (DFD) meat and pale, soft and exudative (PSE) meat. Both conditions occur as a result of stress i.e. chronic and acute.

DFD meat is characterised by a high ultimate pH (pH>6), a result of very low levels of available muscle glycogen post mortem which is a consequence of chronic stress. DFD meat has an increased water binding capacity and therefore the absorption of light is high resulting in very dark, firm and dry meat (Hoffman, 2000). This condition is also known to have a detrimental effect on the tenderness of meat as the high pH creates a very rigid structure. The research by Purchas (1990) also concluded that the tenderness of meat decreases as the ultimate pH increases from 5.5 to 6.2 while Yu and Lee (1986) found that at an ultimate pH between 5.8 and 6.3, tenderness is at its lowest. This is mainly related to the influence of the ultimate pH on the activity of the proteolytic enzymes and this pH range is not optimum for proteolytic activity. According to Remingnon *et al*. (1998), the ultimate muscle pH of Japanese quail is highly correlated with stress pre-slaughter and the glycogen levels are depleted

ante-mortem, subsequently resulting in low lactic acid production. It is thus expected that the meat from gamebird species will also have a tendency towards the DFD condition; especially since most birds are shot when in flight, to and from the roosting sites which causes a certain amount of exercise in the breast muscles.

PSE meat occurs because of a rapid post mortem decline in the pH of the muscle which is induced by acute, ante-mortem stress (Warriss, 2000). The rapid decrease and low ultimate pH of the muscle together with temperatures that are still fairly high produces the following characteristics of PSE meat: denaturation of the proteins, low water holding capacity and a pale colour due to an increased scattering of light. In general, the PSE condition is well-known in meat from genotypes of pigs which are very susceptible to stress. However, characteristics that suggest the occurrence of PSE have also been reported in poultry (Barbut, 1997; Woelfel *et al*., 2002; Zhu *et al*., 2011) and turkey meat (Barbut, 1996; Molette *et al*., 2003). The high content of white, glycolytic fibres present in the muscles of poultry, turkey and other Galliformes birds, with high amounts of available glycogen, have the tendency to rapidly acidify post mortem (Warriss, 2000; Taylor, 2004). It is thus postulated that if these gamebirds are wounded during the hunt there is a possibility that the PSE condition may be found in the meat.

Research quantifying the impact of stress on the meat quality of Southern African wildfowl is restricted and is therefore an area requiring extensive investigation. An understanding of the pre and post mortem biochemical activities, relating to stress, will be beneficial in terms of improving or adapting the shooting procedures to ensure the best possible meat quality.

6.5 Safety

The production of game meat for the commercial market varies a great deal from the use of domestic livestock for meat production. The manner in which the animals are killed, the evisceration process as well as the cooling procedures are elements of concern with regard to the safety of the meat (Gill, 2007). During game meat production these elements are more difficult to control as the environment in which the meat is sourced provides for certain challenges. This particularly applies to gamebirds where the method of killing is the factor causing the greatest concern regarding safety. Shotgun shells may damage the intestinal cavity resulting in microbial contamination of the meat; Paulsen *et al*. (2008) investigated the effect of shot shell wounding on hunted, uneviscerated Pheasant and only found a loss of hygienic quality at day seven (stored at 0-4 °C) by the presence of *Escherichia coli* (>1 log10 cfu/g). Alternatively, emphasis can also be placed on disposing gamebird carcasses where gunshot damage to the intestinal cavity has occurred so as to ensure that contaminated meat are not consumed. Immediate cooling of the shot birds is also an area of concern, especially in Southern Africa with high ambient temperatures. To ensure effective cooling of the birds in the field is a

challenge and it is thus essential for a practical approach in order to overcome this challenge (refer to section 8.2 “Post mortem handling”).

It is, however, not only safety in terms of microbial contamination that needs to be considered but lead shot contamination is another controversial topic. Lead is toxic and affects several systems within the human body (WHO, 2010). The major source of lead intake by humans is the ingestion of lead pellets or fragments of pellets that is embedded in the tissue of hunted gamenirds (Scheuhammer *et al*., 1998; Johansen *et al.*, 2004; Mateo *et al*., 2007). Furthermore, it is also believed that residual lead, originating from hunting activities, is ingested by gamebirds as they confuse the pellets for seeds or other food (Mateo, 2009; Thomas, 2010). This results in secondary lead intake by humans when the tissue is consumed. For this reason certain countries such as Denmark, Norway and The Netherlands have banned the use of lead shots for hunting purposes. In other countries, the use of lead-free shots is compulsory for all waterfowl hunting as extensively discussed by Mateo (2009) and Thomas and Guitart (2010). However, the process of implementing the use of non-toxic, lead-free shots as alternatives is progressing slowly.

The legislation regarding meat safety in South Africa (Meat Safety Act, no. 40 of 2000), does not provide for hunted, wild gamebirds but only for those that have been domesticated such as Guineafowl, Pheasant and Partridge. This Act also currently prohibits the acceptance and slaughter of dead animals in an abattoir. It is therefore vital that the legislation is altered so as to provide for the commercial utilisation of gamebird meat and the areas of concern involved.

6.6 Traditional ageing of wildfowl

The traditions and ethics involved in gamebird hunting also include the preparation and handling of the birds before consumption. By tradition, gamebirds are usually hanged; uneviscerated without being plucked, in a cool environment for some time before further preparation and consumption (Barnes, 1976). This technique is customary, particularly in Britain and several other countries. It is believed that this traditional ageing or hanging of the birds not only increases the tenderness of the meat but is involved in the flavour formation process as well (Barnes, 1980). It is interesting to note that different gamebird species are handled differently with regard to the time it is hanged for. Table 3 describes the recommendations for the hanging of some gamebirds shot in Britain. According to Barnes (1980), the ambient temperatures, as well as the age of the gamebird are factors of influence. This is important to consider as the season in which certain gamebirds are hunted will determine the ambient temperature and therefore affect the hanging process. Barnes (1980) noted that the development of the game characteristics is related to the autolytic changes which occur in the muscles when hanged.

Table 3 Methods of handling and hanging of some British gamebirds (adapted from Barnes 1980)

Gamebird	Hanging technique
Partridge	Young birds, 3-5 days; older birds, 10 days or more
Pheasant	13-14 days according to age and weather conditions
Wild duck	Eaten fresh or hanged for up to 2 days
Grouse	3 – 10 days according to age and weather conditions

Theoretically, the term ageing or conditioning is defined as the post rigor storage of unprocessed meat, without any microbial deterioration, above the point of freezing and is therefore the natural process of tenderisation (Warriss, 2000; Lawrie & Ledward, 2006). The biochemical changes, involved in tenderisation during the ageing of gamebird meat, are very complex and interrelated. The two main tenderisation processes occurring in post rigor muscle are denaturation and proteolysis (Lawrie & Ledward, 2006). As the oxygen supply to the muscle is terminated, after death, there is no energy available to preserve the muscle structure and denaturation of the proteins follow. However, tenderisation mainly results from proteolysis by the protease systems present in the muscles which are responsible for the breakdown of the protein structure. There are four systems identified thus far which include calpains, cathepsins, proteasomes and caspases (Kemp & Parr, 2012). Of these systems, the calpains and cathepsins are the two which have been studied extensively and their involvement in tenderisation have widely been recognised (Ouali, 1992). Koohmaraie (1996) reported that μ-calpain is the most important factor in the post mortem proteolytic tenderisation of meat. This biochemical process of tenderisation can significantly be influenced by factors such as pH, temperature, muscle fibre composition, ionic strength and protein oxidation (Huff-Lonergan *et al*., 2010).

The temperature of pre and post rigor muscle determines the rate of the pH decline and the degree of muscle shortening (Huff-Lonergan *et al*., 2010) while it is suggested that the pH regulates both the activation and inactivation of the proteolytic enzymes (Melody *et al*., 2004; Maddock Carlin *et al*., 2006). However, it is not only temperature and pH that may affect the ageing process but, as mentioned, fibre type can also be linked to tenderisation as there is variation in the content of proteolytic enzymes within oxidative and glycolytic muscle fibres (Ouali & Talmant, 1990; Sazili *et al*., 2005). It is also believed that protein oxidation may interfere with the tenderisation process and as such should also be considered. Protein oxidation leads to the denaturation and aggregation of the myofibrillar proteins together with the inactivation of the proteolytic enzymes and consequently may influence the ability of meat to become more tender over time (Rowe *et al*., 2004; Huff-Lonergan *et al*., 2010).

As Barnes (1980) noted, an alteration in the flavour during hanging/ageing of gamebirds may also arise. It is suggested that ageing enhances the desirable gamey flavour of the meat. In 1972, Wasserman gave emphasis to the influence of ageing on the precursors of flavour in beef and stated that both microbial and enzymatic changes are responsible for this phenomenon. The proteins become more susceptible to enzymatic protease, due to denaturation post mortem, and leads to the development of peptides as well as free amino acids. The formation of other substances such as ammonia, H_2S, carbonyls and ribose also contribute to flavour alteration. Several authors have found that ageing of beef has a positive effect on flavour; increasing the intensity (Miller *et al*., 1997; Daszkiewicz *et al*., 2003) and Campo *et al*. (1999) also reported that specifically livery flavour was enhanced. Although it appears as if ageing, in general, increases the desirable sensory characteristics of meat, negative consequences can also occur. Lipid oxidation has a major influence on meat quality and may ultimately cause a rancid flavour (Elmore & Mottram, 2009).

It is important to note that these aspects, specifically concerning gamebird meat, have not yet been investigated. Therefore, research measuring the post mortem pH profile and enzyme activity of the muscles as well as the fibre typing of the main portions will provide information which could clarify certain textural aspects of gamebird meat such as tenderness. By investigating the ageing process, especially the role of the enzymes involved and the effect on flavour, a method to produce gamebird meat with optimum quality can be developed. This will indirectly increase the commercial acceptability of the meat and the commercial utilisation of these bird species. The latter will not only benefit the development of the South African gamebird industry, but may also assist in improving the situation regarding gamebirds, the damage they cause to croplands and the consequent financial implication for farmers. Ultimately this is also an opportunity to utilise new sources of meat in attempting to alleviate the problems regarding food security in Southern Africa.

7 GAMEBIRDS: A SUSTAINABLE FOOD SOURCE?

7.1 Utilisation in Southern Africa

According to Berruti (2005) the African Gamebird Research, Education and Development Trust (AGRED) believes that South African Gamebirds are definitely a natural underutilised resource and this organisation is of the opinion that they should be utilised in an ethical and sustainable manner as this will be beneficial for South Africa. Berruti (2005) defines sustainable utilisation as being able to make use of gamebirds by means of the continuous, successful harvesting/hunting of this group of birds. The author also answers the question of the ability of gamebirds to be a sustainable resource by stating that, with adequate management of the population numbers the utilisation of these species is viable. This is especially because of the high reproduction rate of gamebirds and the fact that the

utilisation should be based upon removal (harvesting) of the surplus gamebirds from the populations (Berruti, 2005). Furthermore, gamebird hunting is legal in South Africa with regard to species that are extensively found throughout the country (Berruti, 2005). Berruti (2005) does however state that for sustainable utilisation and the development of the gamebird hunting industry, it is vital to have the appropriate policies and legislations for this type of hunting.

Viljoen (2005) emphasizes the fact that, in general, the feasibility of gamebirds as a resource in South Africa has not been noticed. Most farmers do not realise that gamebird hunting can provide an additional income. This is because it is either considered to be mediocre compared to big game hunting or the farmers are oblivious of the fact that there are gamebird species on their land. Furthermore, in the past, large numbers of gamebirds have been poisoned in order to eliminate these species as they are generally regarded as pests (Viljoen, 2005). According to Berruti *et al*. (2004), an estimated 176 000 to 470 000 gamebirds in South Africa are illegally poisoned on an annual basis. However, gamebirds are not only killed because they are pests but also for consumption by the farm workers (Maphasa, 1966; Berruti, 2005) and if gamebirds can be harvested in a sustainable manner it will lead to a radical decline in the poisonings (Berruti, 2005). Currently gamebird utilisation in South Africa is on the rise as the hunting of other game species is becoming more expensive (Viljoen, 2005). The fact that there is an upsurge in the popularity of gamebird hunting can be very positive as the utilisation of gamebirds, the hunting activities on the farms and the availability of the meat can definitely lead to some major benefits for the South African economy and the meat industry.

7.2 Gamebirds regarded as agricultural pests

In general, farmers in South Africa seem to have a negative connotation towards certain gamebird species because of the perceived damage that they do to crops. However, in South Africa there have been limited studies on the quantification of the damage caused by gamebirds and the financial implication thereof for farmers (Mangnall & Crowe, 2002). Various gamebird species are considered to be agricultural pests of which the Rock pigeon (*Columba guinea*) and the Egyptian goose (*Alopochen aegyptiacus*) seem to be the two species most often referred to as being a problem in terms of agriculture (Berruti, 2005).

Van Niekerk and Ginkel (2004) reported that rock pigeons and red-eyed doves feed on ripening sunflower seeds and were responsible for 20-30% sunflower seed losses in the year 2000 in the Highveld region of South Africa. In another similar study, the national damage caused by these two species is believed to be approximately 8.4% of the potential crop income (R197 million) annually (van Niekerk, 2009).

Similarly, crop farmers in the Western Cape consider Egyptian geese to be a very serious agricultural pest, subsequently having a negative impact on the agricultural economy of this region.

This problem, however, creates an opportunity to improve food security which has been overlooked by farmers (Viljoen, 2005). The Egyptian goose is one of the leading gamebirds hunted in Southern Africa (Viljoen, 2005). As a result there is enormous potential as the wingshooting of this gamebird could provide farmers with an additional income if the meat is sufficiently utilised (Mangnall & Crowe, 2001).

The studies by Mangnall and Crowe (2001; 2002) quantified the damage caused by geese to grain fields in the Agulhas plain region of the Western Cape of South Africa. The population dynamics and the physical and financial impact of Egyptian geese on cereal crops over the period of 1997 and 1998 were investigated. They cause damage during three stages of crop production: shortly after sowing when the surface seeds are still in the ground, early stages of growing - April to July (germination); and when the crops have been cut - October to November; and are ready to be harvested (windrows) (Mangnall & Crowe, 2002). The largest number of geese was found during June and July when the Geese foraged on the growing plants (<25 cm) in the croplands. During October and November the geese return to the croplands and feed on barley and wheat seeds in the windrows. The geese are also responsible for damage due to the loss of grains as a result of stepping on the windrows (Mangnall & Crowe, 2002). Mangnall and Crowe (2001; 2002) reported that there was a substantial increase in the numbers of Egyptian geese in the late 1980's and early 1990's. This is believed to be a result of the Caledon Southern Association Malters factory which was established in the 1970's and is responsible for a substantial increase in crop production in this region (Mangnall & Crowe, 2002). The surveys completed by local farmers prove that since the early nineties the Egyptian goose numbers have increased. Mangnall and Crowe (2002) emphasize that with the increase in numbers farmers will continue to suffer damage and financial losses until measures are taken to reduce the damage. Therefore, it is suggested that when flocks of geese larger than 150 birds remain on viable crops for more than a week, farmers should resort to wingshooting activities in order to decrease the numbers and therefore reduce the damage (Mangnall & Crowe, 2001). The increase in the population numbers of this species and the consequent financial implication for crop farmers thus generates a potential for utilisation of the meat and possible recovery of the financial losses due to the damage (Berruti, 2005).

8 CHALLENGES AND THE WAY AHEAD

It is evident that there is an enormous potential to utilise certain Southern African wildfowl species, as a food source, to assist the current state of food security, especially in Africa. Sustainable utilisation will not only benefit the development of the South African gamebird industry, but may also assist in improving the situation regarding the damage specific gamebird species cause to croplands and the consequent financial implication for farmers. However, before the sustainable utilisation of gamebird

meat can be a success, there are several challenges, which are essential to overcome in order to ensure the availability of gamebird meat with the best possible quality.

8.1 Limited information on the meat quality of Southern African wildfowl

Limited research is available regarding the meat quality of Southern African gamebird species. The information available is mainly centred on culinary customs rather than scientific investigations (Hofbauer & Smulders, 2011). The gamebird industry in South Africa is becoming more popular and information on the overall meat quality of the birds hunted is vital.

It is generally accepted that consumers prefer meat to be tender, juicy and flavourful (Risvik, 1994; Warriss, 2000; Wiklund *et al*., 2003); attributes which are determined by several intrinsic and extrinsic factors. Although these characteristics are those that the general public look for when purchasing meat, it is always a challenge for the meat industry to provide the desired product. As stated by Wiklund *et al*. (2003) the views or opinions of consumers in terms of the acceptability and eating quality of meat vary from population to population. However, an essential aspect of consumer acceptability is the overall uniformity in terms of meat quality; i.e. the product should have a consistent eating quality at all times (Wiklund *et al*., 2003). This is where the challenge lies with meat originating from gamebirds.

Therefore, an aspect that warrants research is quantifying the perception or attitude of consumers towards gamebirds as a meat source. At the same time, there is a need to scientifically evaluate those biochemical activities as well as the effect of the interventions performed on domesticated fowl to ensure an acceptable quality of gamebird meat. As very little scientific knowledge exists about the extrinsic (e.g. season/diet, gender) and intrinsic (muscle portion, fibre types, development of rigor mortis, activation of enzymes) factors that influence the meat quality (e.g. yield, organoleptic attributes, and chemical composition) of gamebirds, these also need to be quantified. This will allow insight into certain aspects of the meat quality which may require improvement. Consequently, it will be possible to provide consumers with a product having the best possible quality.

8.2 Post mortem handling

Another ambiguous area with regard to possible wildfowl utilisation is the handling of the shot gamebirds during/after the wingshooting activities. In South Africa, the handling of gamebirds is somewhat different compared to the traditional European customs. Generally the birds are not handled correctly in order to ensure meat with the best possible quality, for example there are delays in the cold storage of the shot gamebirds where they are placed on the back of the transporting vehicle, at ambient temperatures, for 3-6 h until the hunt is finished.

Concerning the slaughtering and storage of gamebirds, it is believed that several different methods are currently used during organised shoots. The birds are normally eviscerated by the staff and given to the hunters at the end of the day. The carcasses are then either placed into a freezer, usually with the feathers intact or kept fresh in the refrigerator for immediate use. Less often the gamebirds are kept uneviscerated and refrigerated. If proper utilisation of the meat for incorporation into the commercial market is a possibility, serious measurements will have to be taken in order to establish the appropriate post mortem handling and slaughtering methods for these hunted fowl. Research on the general handling practices during the hunt and thereafter as well as the microbiological quality of the hunted birds in the Southern African context is an area that warrants more research.

8.3 Potential farming with wildfowl species for meat production

The raising of wildfowl within a farming environment is not a modern activity and dates back to ancient times when settlers and explorers transported wild birds back to their home countries to be used for meat and egg production (Ratcliffe & Crowe, 1991; Cooper, 1995). Today, the domestic production of gamebird species is applied globally and countries such as those in Europe, Asia as well as the USA are leaders in this industry (Leech *et al*., 2003). Domestic production of gamebirds in Southern Africa is less successful. Nonetheless, there has been some domestication of Guineafowl originating in West Africa (Ratcliffe & Crowe, 1991; Little, 1997). Guineafowl farming has also been taking off in certain other developing countries such as Botswana (Moreki & Seabo, 2012), Zimbabwe (Saina *et al*., 2005) and Nigeria (Obike *et al*., 2011). This is in an attempt to increase meat production and therefore human protein consumption by means of farming with this, more resilient species compared to poultry (Agwunobi & Ekpenyong, 1990).

Introducing new wildfowl species into domestication may not only contribute to the food security situation in Africa but may also be beneficial to the economy. According to Moreki and Seabo (2012), the domestication of Guineafowl in Botswana will potentially create new employment opportunities and therefore assist in reducing the situation regarding poverty in this country. It is therefore postulated that increasing the manifestation of gamebird farming in Southern Africa, by applying the appropriate scientific knowledge, will lead to the successful incorporation of meat from new species into the commercial market.

Gamebird species which show potential for domestication are mainly of the orders Galliformes and Anseriformes (Cooper, 1995). Amongst these identified species is the Hartlaub's duck (*Cairina hartlaubi*) and the Egytpian goose (*Alopochen aegyptiacus*) both found in Africa. Regarding Egyptian geese, Mongin (1991) reports that this fowl has a semi-domesticated status as it has been partially domesticated in some parts of Africa but intensive breeding with this species has not yet occurred.

9 CONCLUSIONS

In attempting to emphasise the vast potential of Southern African wildfowl as a food source, this review has provided information detailing both the international and South African gamebird industries. It revealed the major factors of influence in terms of the eating quality of gamebird meat, which involve the physical activity of the different portions and muscle fibre types, diet, breeding, age, sex as well as the post mortem handling/ageing of the meat. The safety issues i.e. shot contamination, involved in producing gamebird meat are also discussed. The question of gamebirds and their potential to be utilised in a sustainable manner is answered by providing substantial arguments to prove the viability of this resource. Certain gamebirds, i.e. Egyptian geese and Guineafowl, are identified as having particular potential to be utilised as a food source in an attempt to alleviate the current food security issue in Africa. The fact that there is such limited information on the meat quality of gamebirds, the handling practices during and after the shooting activities as well as potential farming possibilities are recognised as the challenges that are essential to overcome before wildfowl can successfully contribute to food security.

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CHAPTER 3

Aspects of the nutritional value of cooked Egyptian goose (*Alopochen aegyptiacus*) meat, compared to other well-known fowl species*

ABSTRACT

There is no scientific research regarding Egyptian goose (*Alopochen aegyptiacus*) meat, therefore a descriptive chemical analysis to establish the nutritional characteristics of the breast portion is described. Meat from guineafowl, pekin duck, ostrich and broiler chicken were used as reference. The high intramuscular fat content of Egyptian goose meat (5.6 g/100 g) may be linked to the fact that this species relies on fat for heat insulation and buoyancy. Egyptian goose meat is very high in PUFA (39.5%). The P/S ratio is within the recommendations (>0.4) and the *n*-6/*n*-3 ratio is also below the suggested value of 5. The high iron content of 7.5 mg/100 g is the differentiating factor within the mineral compositions and is related to the physical activity endured by the breast muscle of Egyptian geese. This study provides new insight into the nutritional characteristics of a meat species providing crucial information which is, as of yet, not available in literature.

Keywords: Gamebirds, Egyptian geese, Chemical composition, Fatty acids, Minerals

* Geldenhuys, G., Hoffman, L.C. & Muller, M. (2013). Aspects of the nutritional value of cooked Egyptian goose (*Alopochen aegyptiacus*) meat, compared to other well-known fowl species. *Poultry Science*, **92**, 3050-3059.

1 INTRODUCTION

Internationally, gamebird hunting has developed into a multimillion dollar industry which is particularly popular in countries such as the USA as well as Europe (Viljoen, 2005). In South Africa, this is still an emerging industry and in 2010, it was estimated that a quantity of 2 million gamebirds were shot annually (J. Van Giessen, South African Wingshooting Association, South Africa, personal communication). The gamebird hunting industry in South Africa is becoming more popular and information on the nutritional composition of the hunted birds is vital for this industry (Geldenhuys *et al*., 2013). The Egyptian goose (*Alopochen aegyptiacus*) is one of the leading gamebirds hunted in Southern Africa (Viljoen, 2005). According to Mangnall and Crowe (2001; 2002) and Viljoen (2005), Egyptian geese population numbers have increased considerably during the past two decades and are still rising in the Western Cape, South Africa. However, farmers are suffering from financial losses due to their extensive foraging on croplands and this gamebird is thus responsible for a negative impact on the agricultural economy of this region. For this reason, wingshooting of the geese is recommended but the commercial utilisation of the meat has not yet been achieved.

Detailed scientific data concerning the chemical characteristics of Southern African gamebird species is limited and before Egyptian goose meat can be introduced as a marketable product, information regarding the nutritional value is essential. This is particularly important since consumers are becoming more aware of the health aspects involved in food consumption and are demanding that red meat products have the required nutritional characteristics (Ngapo & Dransfield, 2006). Troy and Kerry (2010) also emphasizes the fact that the perception of food quality is shifting towards nutrition, well-being and health. As red meat products, such as Egyptian goose meat are frequently associated with having a presumed, high fat content and the consumption thereof connected to certain human health issues (Demeyer *et al*., 2008) the positive contribution of meat towards human nutrition is often overlooked (Troy & Kerry, 2010; McNeill & Van Elswyk, 2012). Red meat is not only a major source of animal protein but provides some of the key dietary micronutrients i.e. iron, zinc, selenium, vitamin A, vitamin B12 and vitamin B9 (folic acid) (Biesalski & Nohr, 2009; McNeill & Van Elswyk, 2012). It is therefore imperative to obtain insight into the nutritional aspects of the meat from this wildfowl species.

In order to have a clear understanding of the benefits and shortcomings, as pertaining to the nutritional value, it is necessary to compare these characteristics with those of other familiar/popular fowl consumed in South Africa. Amongst the gamebirds hunted on a regular basis in South Africa, guineafowl (*Numida meleagris*) is a very popular, terrestrial gamebird species of which there is some available scientific literature regarding the meat quality (Tlhong, 2008; Hoffman & Tlhong, 2012). The meat of other domestic fowl species such as pekin duck (*Anas platyrhynchos*, *domestica*), ostrich

(*Struthio camelus*) and broiler chicken are also consumed on a regular basis in South Africa. By means of this comparison, the positive and negative nutritional aspects of Egyptian goose meat will be evident.

There are several factors that determine the chemical profile of meat and these factors are also responsible for the unique chemical characteristics that exist between meat from different species. Diet is one of the major influential factors and variation in the dietary constituents between game and domestic species may result in a difference in the chemical composition, especially the fatty acid profile of the meat. The diet of Egyptian geese consists mainly of green plant material, small mammals, aquatic invertebrates, aquatic vegetation and insects (Viljoen, 2005). Guineafowl forage on bulbs and stems of plants, grass seeds, harvested grains, maize, as well as insects (Little & Crowe, 2011). Domestic species such as pekin duck, ostrich and broiler chicken are raised within a farming environment and a standard commercial feed is often used for these species. Furthermore, when comparing game and domestic birds, the extent of physical exercise the different birds/species are subjected to will have a direct influence on the meat quality i.e. the intermuscular fat (IMF) and iron (Fe) content which is due to the difference in the constituents of muscles between active and inactive animals (Lawrie & Ledward, 2006).

Given that no scientific data is available on the meat quality of Egyptian geese, this study was conducted to quantify the nutritional value of the meat from this underutilised gamebird species. It also allows for critical evaluation of the nutritional composition as a comparison is made between Egyptian goose meat and that of other familiar game and domestic fowl. Furthermore, this research will provide valuable nutritional data for the food composition databases.

2 MATERIALS AND METHODS

2.1 Sampling and slaughtering

The gamebirds Egyptian geese (*Alopochen aegyptiacus*) and guineafowl (*Numida meleagris*) were shot during August 2010 on Mariendahl Agricultural Experimental Farm, Western Cape, South Africa. A double barrelled shotgun was used during the wingshooting activities (ethical clearance number: 10NP_HOF01). The geese and guineafowl were collected in the field and placed in a refrigerator (4 °C) over-night (± 12 h); where after the slaughtering procedures were carried out manually. Firstly, the head was removed at the base position, between the C1 and C2 vertebrae. Then both of the feet were removed at the ankle joint (intertarsal joint) together with the removal of the tip of each wing from the wrist region (carpal joint). Skinning involved the cutting from the neck to the tail region on the ventral side of the body, followed by the removal of the skin containing the feathers from the body. The fowl were then eviscerated by means of an incision in the abdominal muscles. The broiler

chickens were slaughtered according to the commercial, standard procedures which include; immobilisation by electrical stunning (50-70 volts; 3-5 s), followed by exsanguination, de-feathering and evisceration (DAFF, 2006). The breasts (*M. pectoralis*) were removed from the respective bird carcasses and the meat was vacuum-packed in a polystyrene bag and frozen at -18 °C for approximately 6 weeks. The pekin duck breasts, ostrich fan fillets (*M. iliofibularis*) and moon steaks (*M. femorotibialis*) were derived from different birds, sourced from commercial producers, was frozen immediately after deboning at -18 °C for 6 weeks.

2.2 Experimental units

The experimental layout is indicated in Table 1. The experimental units were the following; the breast portion of Egyptian geese, guineafowl, pekin duck and broiler chicken together with ostrich fan fillet (*M. iliofibularis*) and ostrich moon steak (*M. femorotibialis*). There were thus six meat treatments (five species with the ostrich having two different muscles sampled) with six samples per treatment. The meat samples used were cooked in pre-heated (160 °C) conventional ovens (Defy, Durban, South Africa, Model 835) connected to a computerised monitoring system for temperature regulation (Viljoen *et al*., 2001). The samples were removed when a core temperature of 75 °C were reached and left to acclimatise to room temperature (21 °C). The chemical analyses were performed on the cooked left breasts (*M. pectoralis*) of the different bird carcasses. A strip was removed down the centre of the cooked ostrich fan fillet (*M. iliofibularis*) and moon steak (*M. femorotibialis*) samples which were used for the chemical analyses.

Table 1 Sample set and experimental units

Meat treatments	Cuts used	Number of birds analysed
Egyptian goose	Breast	6
Guinaefowl	Breast	6
Pekin duck	Breast	6
Ostrich	Fan fillet	6
Ostrich	Moon steak	6
Broiler chicken	Breast	6

2.3 Chemical analysis

2.3.1 Sample preparation.

After the six meat treatments (6 replications/birds per treatment) were cooked, each sample was homogenised, vacuum sealed and placed in a -18 °C freezer for 4 weeks until the chemical analyses

were performed. The samples were thawed at 4 °C for 12 h before each analysis. All of the analyses were performed in duplicate.

2.3.2 Proximate analyses.

The moisture content (%) was determined by using a 2.5 g homogenised cooked meat sample according to the Association of Official Analytical Chemist's Standard Techniques (AOAC, 2002a) method 934.01. The ash content (%) of the moisture free sample was determined by the official AOAC (2002b) method 942.05. The chloroform/methanol (1:2 v/v) extraction method stipulated by Lee *et al*. (1996) was used to determine the total lipid (%) (IMF) of a 5 g homogenised cooked meat sample. To establish the total crude protein content (%) the Dumas combustion method 992.15 (AOAC, 2002c) was applied. A 0.15 g defatted, dried and finely grounded meat sample was analysed using a Leco Nitrogen/Protein Analyser (FP – 528, Leco Corporation). The Leco was calibrated with EDTA samples (Leco corporation, 3000 Lakeview Avenue, St. Joseph, MI 49085-2396, USA, Part no. 502-092, Lot no. 1055) before each of the analysis sessions. The results were expressed in % Nitrogen (N). The N was multiplied with a conversion factor (6.25) in order to determine the crude protein (%) present in the meat sample. All samples were analysed in duplicate and the mean value was used for the statistical analyses. The accuracy of all the proximate analyses in the laboratory were verified by a National inter-laboratory scheme (AgriLASA: Agricultural Laboratory Association of South Africa) where blind samples are analysed once every three months to control and ensure the accuracy and repeatability of the procedures used.

2.3.3 Fatty acid analysis.

The fatty acid profile was determined after the homogenised cooked meat samples were defrosted. A 2 g sample was extracted by the use of a chloroform:methanol (2:1; v/v) solution according to the method described by Folch *et al*. (1957). The solvents used for extraction contained 0.01% butylated hydroxytoluene (BHT) which functioned as an antioxidant. The meat sample together with the extraction solvent was homogenised by means of a polytron mixer (WiggenHauser Homogenizer, D-500 fitted with a standard shaft 1; speed setting D). In order to quantify the individual fatty acids present within the meat sample Heptadecanoic acid (C17:0) was used as an internal standard (catalogue number H3500, Sigma–Aldrich Inc., 3050 Spruce Street, St. Louis, MO 63103, USA). A 250 µL sub-sample of the extracted lipids was transmethylated for 2 h at 70 °C and a methanol/sulphuric acid (19:1; v/v) solution (2 mL) was used as the transmethylating agent. The mixture was cooled to room temperature followed by the extraction of the fatty acid methyl esters (FAME) with water and hexane by transferal of the top hexane phase to a spotting tube and then drying it under nitrogen. Hexane (50 µL) was then added to the dried FAME sample after which 1 µL

was injected into the gas-chromatograph. The FAME was determined with a Thermo Finnigan Focus gas-chromatograph (Thermo-Electron Corporation, Rodano, Milan, Italy) equipped with a flame ionized detector and a 60 m BPX70 capillary column (internal diameter of 0.25 mm, 0.25 µm film, SGE International, Ringwood, Victoria, Australia). The gas flow rate of the carrier, hydrogen, was 30 mL/min. The following temperature settings where applied: initial temperature of 60 °C, injector and detector 220 °C and 260 °C respectively and a final temperature of 160 °C. The GC injection volume was 1 µL with a run time of approximately 45 min. By comparing the FAME of the meat samples with a standard FAME mixture (Supelco, 37 Component FAME mix C4-C24, Cat, no. 47885-U. Supelco, North Harrison Rd, Bellefonte, PA 16823-0048, USA) the FAME levels were identified. The results were recorded as percentage (%) of the total fatty acids.

2.3.4 Mineral analysis

The minerals analysed were calcium, potassium, magnesium, sodium, iron, copper, zinc, manganese, phosphorus, boron and aluminium. The mineral content of a 0.5 g defatted, dried and finely ground meat sample was determined. Ashing of the sample occurred at 460-480 °C for 6 h, followed by the cooling of the sample and addition of 5 mL of 6 M HCl. The sample was placed in an oven (50 °C) for 30 min where after 35 mL of distilled water was added, the solution was filtered and distilled water was added to obtain a final volume of 50 mL (AGRILASA, 2007). An iCAP 6000 Series Inductive Coupled Plasma (ICP) spectrophotometer (Thermo-Electron Corporation, Rodano, Milan, Italy) fitted with a vertical quartz torch and Cetac ASX-520 auto sampler was used to measure the elements. The concentrations of the elements were calculated by means of iTEVA Analyst software (Thermo-Electron Corporation, Rodano, Milan, Italy). The Argon gas flow rate was 2-5 mL/min and the settings for the instrument included the following: camera temperature -27 °C, generator temperature 24 °C, optics temperature 38 °C, radio frequency power 1150 W, pump rate 50 rpm, aux gas flow 0.5 L/min, nebulizer 0.7 L/min, coolant gas 12 L/min and normal purge gas flow. The wavelengths for the elements were the following: Al 167.079 nm, B 249.773 nm, Ca 317.933 nm, Cu 324.754 nm, Fe 259.940 nm, K 766.490 nm, Mg 285.213 nm, Mn 257.610 nm, Na 589.592 nm, P 177.495 nm and Zn 213.856 nm. The minerals were recorded as mg/100 g dry meat sample. After the analysis of 11 samples, standards of high, medium and low range were analysed for quality control.

2.4 Statistical analysis

Experimentally the study consisted of a randomised block design with six meat treatments and six replications per treatment. The chemical data was subjected to an analysis of variance (ANOVA). The Shapiro-Wilk test was performed to test for normality (Shapiro & Wilk, 1965). All of the outliers were identified and removed before final analysis of the ANOVA's. The t-Least Significant Differences

(LSD) were calculated at a 5% significance level to compare the treatment means. Results were defined as being not significant at $P>0.05$ and significant at $P\leq0.05$. SAS statistical software (Statistical Analysis System 2006, Version 9.2, SAS Institute Inc., Cary, NC, USA) was used for the analyses of variance (ANOVA).

3 RESULTS

3.1 Proximate composition

The results of the proximate analysis (Table 2) on the cooked meat samples indicate that broiler chicken and guineafowl were the species with the highest moisture content (65.7 and 64.5 g/100 g, respectively). The moisture content of ostrich fan fillet and moon steak did not differ ($P>0.05$) from guineafowl or Egyptian goose. Pekin duck and Egyptian goose with mean values of 61.8 and 62.2 g/100 g for moisture respectively, were the treatments with the lowest moisture contents.

In terms of protein content, ostrich fan fillet had the highest value (32.7 g/100 g). It was higher ($P\leq0.05$) than that of Egyptian goose and broiler chicken with the latter having the lowest protein content. Guineafowl, ostrich moon steak and pekin duck both had average protein contents which did not differ ($P>0.05$) from ostrich fan fillet or Egyptian goose.

Egyptian goose (5.9 g/100 g) and pekin duck (5.8 g/100 g) were higher ($P\leq0.05$) in intramuscular fat content compared to the other treatments. Ostrich fan fillet and moon steak, broiler chicken and guineafowl had IMF contents which were more than 2 g/100 g lower ($P\leq0.05$) than that of Egyptian goose and pekin duck. These four treatments did not differ ($P>0.05$) from each other.

The different species did not differ ($P>0. 05$) in the amount of ash (g/100 g) present in their cooked muscle.

Table 2 The means values (±SD[1]) for the proximate analyses (g/100 g) of the meat derived from six meat treatments

	Species						LSD[2] P=0.05
	Egyptian goose n=6	**Guineafowl n=6**	**Ostrich fan fillet n=6**	**Ostrich moon steak n=6**	**Pekin duck n=6**	**Broiler chicken n=6**	
Moisture	62.2^{c} ± 2.3	64.5^{ab} ± 1.5	63.0^{cb} ± 2.6	63.2^{cb} ± 2.3	61.8^{c} ± 0.7	65.7^{a} ± 0.9	2.0
Protein	30.9^{cb} ± 2.6	31.9^{ab} ± 1.9	32.7^{a} ± 2.2	32.5^{ab} ± 1.6	31.4^{ab} ± 0.6	29.8^{c} ± 1.4	1.6
Fat	5.9^{a} ± 1.9	3.2^{b} ± 0.9	3.8^{b} ± 0.7	3.8^{b} ± 0.8	5.8^{a} ± 0.6	3.7^{b} ± 0.8	1.2
Ash	1.7^{a} ± 0.3	1.6^{a} ± 0.4	1.6^{a} ± 0.5	2.0^{a} ± 0.5	1.7^{a} ± 0.3	1.6^{a} ± 0.4	0.5

$^{a-c}$Means in rows with different superscripts differ at P≤0.05.
[1]SD (standard deviation); [2]LSD (least significant difference).

3.2 Fatty acid composition

The fatty acid composition of different species differs. The mean scores and standard deviations (±SD) for the fatty acid composition of the six cooked meat treatments are presented in Table 3. The fatty acid composition is expressed as a percentage of the total identified fatty acids present. Although all of the fatty acids are presented in the tables, only specific fatty acids will be discussed. Considering the overall saturated fatty acid (SFA) content of the species; ostrich moon steak (42.6%), ostrich fan fillet (42.5%), guineafowl (43.1%) and Pekin duck (41.0%) were significantly higher (P≤0.05) in SFA compared to the other species. Egyptian goose and broiler chicken differed (P≤0.05) from each other in terms of SFA with broiler chicken having the lowest content (31.9%). It is noticeable that individual SFA differed between the species (Table 3). Palmitic acid (C16:0) was the major SFA in all the species with the Pekin duck, ostrich fan fillet, ostrich moon steak and guineafowl having the highest concentration, although they did not differ (P>0.05) from each other. This fatty acid was the lowest (P≤0.05) in Egyptian goose and broiler chicken treatments. There were no differences (P>0.05) between ostrich moon steak, ostrich fan fillet, guineafowl and Egyptian goose in terms of the concentration of stearic acid (C18:0). Pekin duck and broiler chicken did not differ (P>0.05) from each other but the latter did have a lower (P≤0.05) percentage compared to the other four treatments.

Regarding the total monounsaturated fatty acids (MUFA), Pekin duck (34.2%) has the highest (P≤0.05) concentration. Broiler chicken (22.7%) and Egyptian goose (23.7%) had the lowest concentrations of MUFA and did not differ (P≤0.05) from each other. Pekin duck dominated with regard to the content of oleic acid (C18:1cis-9) having a higher (P≤0.05) concentration (30.7%) of this fatty acid compared to the other treatments.

Broiler chicken (43.8%) and Egyptian goose (39.5%) has a higher (P≤0.05) total polyunsaturated fatty acid (PUFA) content than the other species. Pekin duck had the lowest (P≤0.05) percentage of PUFA (24.8%).

Although broiler chicken had the highest total PUFA content, this was only due to the very high concentrations of two of the individual fatty acids; linoleic acid (C18:2 *n*-6*c*) and γ-linolenic acid (C18:3*n*-6). The amount of these fatty acids within broiler chicken was much higher and thus increased the total PUFA content. Egyptian goose, guineafowl, ostrich moon steak and ostrich fan fillet had higher concentrations in more of the individual PUFA than broiler chicken. Egyptian goose had a substantially higher (P≤0.05) α-linolenic acid (C18:3 *n*-3) (8.57%) and docosadienoic acid (C22:2) (0.16%) content. Homo-γ-linolenic acid (C20:3 *n*-6) was the highest (P≤0.05) in the ostrich fan fillet and moon steak whilst arachidonic acid (C20:4 *n*-6) was higher (P≤0.05) in the gamebird species; Egyptian goose (10.5%) and guineafowl (8.80%). Egyptian goose together with the ostrich

fan fillet and moon steak were also found to be higher in eicosapentaenoic acid (C20:5 *n*-3; EPA). The percentages of docosapentaenoic acid (DPA; C22:5 *n*-3) were significantly higher (P≤0.05) in ostrich moon steak but did not differ (P>0.05) from guineafowl.

The polyunsaturated to saturated fatty acid ratio (PUFA/SFA) was higher (P≤0.05) in broiler chicken (1.40). Egyptian goose had a ratio of 1.03. This ratio did not differ (P>0.05) between the other species and ranged between 0.61-0.71.

Considering the omega 6 to omega 3 ratio (*n*-6/*n*-3), broiler chicken proved to have the highest (P≤0.05) ratio of 18.4 compared to the other species. Egyptian goose had the lowest ratio (2.20) of *n*-6 to *n*-3 fatty acids.

Table 3 The mean scores (±SD[1]) for the fatty acid composition (% of total fatty acids) of the meat derived from the six meat treatments

Fatty acid	Species						LSD[2] P=0.05
	Egyptian goose n=6	Guineafowl n=6	Ostrich fan fillet n=6	Ostrich moon steak n=6	Pekin duck n=6	Broiler chicken n=6	
SFA							
C14:0	0.262^{c} ± 0.076	0.420^{a} ± 0.079	0.374^{ab} ± 0.116	0.358^{ab} ± 0.076	0.318^{bc} ± 0.047	0.309^{bc} ± 0.098	0.09
C15:0	0.102^{c} ± 0.005	0.118^{bc} ± 0.014	0.149^{a} ± 0.018	0.133^{ab} ± 0.013	0.069^{d} ± 0.013	0.111^{c} ± 0.019	0.02
C16:0	19.839^{b} ± 1.597	25.597^{a} ± 1.716	25.938^{a} ± 2.435	25.256^{a} ± 3.119	26.760^{a} ± 1.807	20.223^{b} ± 3.613	2.95
C18:0	16.920^{a} ± 3.639	16.046^{a} ± 2.578	15.078^{ab} ± 1.655	17.145^{a} ± 1.966	12.533^{bc} ± 1.026	10.965^{c} ± 1.051	2.68
C20:0	0.109^{b} ± 0.041	0.132^{ab} ± 0.036	0.064^{c} ± 0.005	0.106^{b} ± 0.022	0.166^{a} ± 0.035	0.100^{bc} ± 0.021	0.04
C21:0	0.170^{c} ± 0.027	0.327^{b} ± 0.067	0.300^{b} ± 0.044	0.338^{b} ± 0.064	0.284^{b} ± 0.035	0.600^{a} ± 0.107	0.07
C22:0	0.139^{a} ± 0.024	0.134^{ab} ± 0.026	0.108^{cd} ± 0.014	0.132^{abc} ± 0.023	0.112^{bc} ± 0.027	0.087^{d} ± 0.016	0.03
C24:0	0.925^{a} ± 0.299	0.338^{c} ± 0.077	0.441^{bc} ± 0.207	0.549^{bc} ± 0.087	0.663^{b} ± 0.308	0.435^{bc} ± 0.138	0.25
MUFA							
C14:1	0.016^{a} ± 0.018	0.022^{a} ± 0.030	0.033^{a} ± 0.026	0.020^{a} ± 0.022	0.041^{a} ± 0.009	0.015^{a} ± 0.026	0.03
C16:1 *n*-7	1.347^{d} ± 0.646	2.003^{cd} ± 1.053	4.082^{a} ± 0.680	3.451^{ab} ± 0.638	2.802^{bc} ± 0.624	1.465^{d} ± 1.018	0.81
C18:1 *n*-9*t*	0.103^{b} ± 0.017	0.117^{b} ± 0.032	0.204^{a} ± 0.022	0.209^{a} ± 0.031	0.191^{a} ± 0.027	0.091^{b} ± 0.029	0.03
C18:1 *n*-9*c*	21.785^{bc} ± 4.441	23.801^{b} ± 2.740	22.644^{bc} ± 1.870	21.050^{bc} ± 1.869	30.703^{a} ± 1.810	20.687^{c} ± 2.103	2.93
C20:1 *n*-9	0.201^{c} ± 0.056	0.305^{a} ± 0.067	0.264^{ab} ± 0.043	0.294^{a} ± 0.042	0.280^{ab} ± 0.059	0.223^{bc} ± 0.025	0.06
C22:1 *n*-9	0.072^{bc} ± 0.044	0.090^{abc} ± 0.016	0.093^{ab} ± 0.029	0.119^{a} ± 0.033	0.057^{c} ± 0.021	0.059^{c} ± 0.034	0.03
C24:1 *n*-9	0.110^{c} ± 0.040	0.181^{b} ± 0.047	0.315^{a} ± 0.067	0.358^{a} ± 0.065	0.149^{bc} ± 0.049	0.116^{bc} ± 0.047	0.07
PUFA							
C18:2 *n*-6*t*	0.046^{b} ± 0.003	0.048^{b} ± 0.014	0.069^{a} ± 0.017	0.066^{a} ± 0.009	0.042^{b} ± 0.012	0.063^{a} ± 0.013	0.01
C18:2 *n*-6*c*	14.627^{b} ± 1.427	14.888^{b} ± 2.300	15.926^{b} ± 0.850	14.502^{b} ± 2.249	15.711^{b} ± 1.119	35.412^{a} ± 2.509	2.31
C18:3 *n*-6	0.106^{c} ± 0.046	0.197^{b} ± 0.070	0.180^{b} ± 0.041	0.178^{b} ± 0.016	0.114^{c} ± 0.024	0.348^{a} ± 0.076	0.06
C18:3 *n*-3	8.571^{a} ± 2.319	1.727^{c} ± 0.666	2.168^{c} ± 0.556	1.957^{c} ± 0.582	0.518^{d} ± 0.046	3.885^{b} ± 0.963	1.17
C20:2	0.040^{a} ± 0.001	0.050^{a} ± 0.016	0.051^{a} ± 0.013	0.058^{a} ± 0.017	0.055^{a} ± 0.023	0.039^{a} ± 0.028	0.02
C20:3 *n*-6	0.269^{d} ± 0.135	0.502^{c} ± 0.107	1.360^{a} ± 0.248	1.518^{a} ± 0.16	0.794^{b} ± 0.091	1.005^{b} ± 0.284	0.23
C20:3 *n*-3	0.172^{a} ± 0.070	0.119^{bc} ± 0.039	0.093^{c} ± 0.021	0.108^{bc} ± 0.038	0.023^{d} ± 0.012	0.147^{ab} ± 0.044	0.05
C20:4 *n*-6	10.455^{a} ± 1.625	8.792^{a} ± 2.384	6.633^{b} ± 0.957	6.693^{b} ± 0.801	6.740^{b} ± 1.172	3.374^{c} ± 0.806	1.72
C20:5 *n*-3	1.637^{a} ± 0.709	0.454^{b} ± 0.104	1.409^{a} ± 0.710	1.655^{a} ± 0.604	0.112^{b} ± 0.018	0.162^{b} ± 0.036	0.56
C22:2	0.157^{a} ± 0.046	0.065^{cd} ± 0.039	0.088^{bc} ± 0.021	0.114^{b} ± 0.030	0.053^{cd} ± 0.027	0.040^{d} ± 0.036	0.04
C22:5 *n*-3	0.973^{bc} ± 0.392	1.490^{ab} ± 0.631	1.152^{bc} ± 0.539	1.992^{a} ± 0.310	0.356^{d} ± 0.100	0.795^{dc} ± 0.329	0.53
C22:6 *n*-3	0.761^{b} ± 0.278	1.901^{a} ± 0.646	0.575^{bc} ± 0.354	0.590^{bc} ± 0.231	0.275^{c} ± 0.162	0.894^{b} ± 0.324	0.42
SFA[3]	38.489^{b} ± 2.537	43.113^{a} ± 2.71	42.451^{a} ± 1.293	42.573^{a} ± 1.317	40.985^{a} ± 1.629	31.920^{c} ± 2.340	2.31
MUFA[4]	23.728^{cd} ± 5.083	26.613^{bc} ± 3.736	27.636^{b} ± 2.456	25.769^{bcd} ± 1.920	34.223^{a} ± 2.376	22.657^{d} ± 3.040	3.51
PUFA[5]	39.467^{a} ± 3.901	30.274^{b} ± 3.370	29.913^{b} ± 2.964	30.046^{b} ± 4.417	24.792^{c} ± 2.370	43.758^{a} ± 6.414	4.76
PUFA/SFA[6]	1.025^{b} ± 0.062	0.705^{c} ± 0.095	0.706^{c} ± 0.085	0.691^{c} ± 0.149	0.606^{c} ± 0.071	1.406^{a} ± 0.131	0.12
***n*-6/*n*-3[7]**	2.200^{e} ± 0.770	4.369^{cd} ± 0.423	4.848^{c} ± 1.405	3.621^{d} ± 0.677	18.356^{a} ± 1.348	6.416^{b} ± 0.551	1.20

[a-e]Means in rows with different superscripts differ significantly at P≤0.05.
[1]SD (standard deviation); [2]LSD (least significant difference); [3]SFA (saturated fatty acids); [4]MUFA (mono-unsaturated fatty acids); [5]PUFA (polyunsaturated fatty acids); [6]PUFA/SFA (polyunsaturated fatty acid/saturated fatty acid ratio); [7]*n*-6/*n*-3 (omega 6/omega 3 ratio).

3.3 Mineral composition

The mean concentrations and standard deviations (±SD) for the mineral composition of the six meat treatments are presented in Table 4. The most significant result in terms of the mineral composition is the iron (Fe) content. It is evident that Egyptian goose meat had an elevated iron content, higher ($P \leq 0.05$) than the other species. The Fe content is approximately 3.3, 3.9 and 2.9 mg/100 g higher than that of ostrich fan fillet, ostrich moon steak and pekin duck respectively and more than 5 mg/100 g higher than what was found in guineafowl and broiler chicken. Broiler chicken and guineafowl proved to have the lowest iron content with the former being lower ($P \leq 0.05$) than the latter. Regarding the phosphorus and potassium content of the meat; the highest contents were found in broiler chicken and the lowest in ostrich fan fillet and guineafowl. The sodium content of pekin duck was higher ($P \leq 0.05$) than the rest with guineafowl having the lowest content. The manganese content of Egyptian goose meat was higher ($P \leq 0.05$) compared to the other treatments and the copper content of Egyptian goose and pekin duck did not differ ($P > 0.05$) from each other but was also higher than the other treatments. The lowest manganese content was found in the ostrich moon steak. Broiler chicken and guineafowl had the lowest copper contents, broiler chicken being slightly lower although there was no difference ($P > 0.05$). The zinc and boron contents were the highest in the ostrich treatments. Guineafowl and broiler chicken had the lowest zinc content ($P \leq 0.05$) while Egyptian goose, guineafowl and pekin duck had the lowest boron content. The effect of species was not significant ($P > 0.05$) in relation to the aluminium content. The results for magnesium and calcium are similar to that of aluminium except for broiler chicken and pekin duck which was higher ($P \leq 0.05$) in magnesium and calcium respectively, compared to the other species.

Table 4 The mean scores (±SD[1]) of the mineral composition (mg/100 g dry basis) for the six different meat treatments

Mineral	Species						LSD[2] P=0.05
	Egyptian goose n=6	Guineafowl n=6	Ostrich fan fillet n=6	Ostrich moon steak n=6	Pekin duck n=6	Broiler chicken n=6	
Phosphorus	192.5^{b} ± 15.6	182.4^{bc} ± 18.4	179.3^{c} ± 9.80	181.7^{c} ± 6.5	186.5^{bc} ± 6.4	208.7^{a} ± 16.0	11.0
Potassium	180.1^{ab} ± 19.1	162.5^{c} ± 15.0	171.5^{bc} ± 9.6	180.1^{ab} ± 8.3	169.3^{bc} ± 13.8	189.5^{a} ± 20.8	11.0
Calcium	12.3^{b} ± 1.74	11.9^{b} ± 1.8	11.6^{b} ± 1.80	11.6^{b} ± 2.0	17.3^{a} ± 1.4	10.7^{b} ± 1.5	2.0
Magnesium	32.5^{b} ± 2.3	30.2^{b} ± 5.0	32.6^{b} ± 1.3	30.7^{b} ± 1.0	31.4^{b} ± 2.0	36.7^{a} ± 2.7	3.0
Sodium	22.0^{bc} ± 6.0	15.8^{d} ± 2.2	20.6^{c} ± 0.6	24.5^{b} ± 1.9	29.0^{a} ± 1.9	18.9^{cd} ± 2.2	3.2
Iron	7.5^{a} ± 0.59	1.8^{d} ± 0.6	4.2^{b} ± 0.40	3.6^{c} ± 0.4	4.6^{b} ± 0.8	1.4^{e} ± 0.2	0.5
Copper	0.5^{a} ± 0.14	0.2^{cd} ± 0.1	0.3^{bc} ± 0.03	0.3^{bc} ± 0.03	0.4^{ab} ± 0.2	0.1^{d} ± 0.02	0.1
Zinc	2.1^{bc} ± 0.40	1.2^{d} ± 0.3	2.3^{b} ± 0.2	5.5^{a} ± 0.4	1.9^{c} ± 0.2	1.2^{d} ± 0.2	0.2
Manganese	0.1^{a} ± 0.01	0.04^{bcd} ± 0.01	0.04^{bc} ± 0.01	0.03^{d} ± 0.002	0.04^{b} ± 0.01	0.03^{cd} ± 0.004	0.01
Boron	0.03^{b} ± 0.004	0.03^{b} ± 0.01	0.03^{a} ± 0.01	0.03^{a} ± 0.003	0.03^{b} ± 0.004	0.03^{ab} ± 0.003	0.003
Aluminium	2.8^{a} ± 2.2	3.1^{a} ± 1.9	4.3^{a} ± 0.9	4.4^{a} ± 1.0	2.7^{a} ± 1.8	3.2^{a} ± 1.6	1.9

$^{a-d}$Means in rows with different superscripts differ significantly at P≤0.05.
[1]SD (standard deviation); [2]LSD (least significant difference).

4 DISCUSSION

The results of the various chemical analyses which were performed within this study indicate that the meat from Egyptian geese is somewhat unique compared to the other more well-known fowl. This is due to the major difference in some of the key chemical factors such as the IMF content, fatty acid profile and Fe content of Egyptian goose meat.

4.1 Fat and moisture – Why is the waterfowl species so different?

The average fat content of Egyptian goose meat of 5.9 g/100 g (Table 2) was not only higher than the other bird species in this study, but is also high when compared to that of ungulate game species which generally have an IMF of less than 3% as indicated by von la Chevallerie (1972) and Hoffman and Wiklund (2006). This significantly higher IMF content of the meat from Egyptian geese, as well as pekin duck, could be associated with the fact that both are aquatic birds. The increased fat deposition on an intramuscular level may be involved in body insulation for these waterfowl species of which water forms an essential part of their being. According to De Vries and van Heerden (1995), water has a 25 times greater heat capacity than air. Therefore, when animals are in contact with water (waterfowl) the thermal conductivity between the animal and the water is increased (De Vries & van Heerden, 1995). For this reason, aquatic birds generally have a thick subcutaneous fat layer for heat insulation as the ability of fat to conduct heat is poor (Evans, 1972; O'Malley, 2005). Smith (1962) states that fat is essential in insulation since its heat insulation ability is three times that of water. However, no research specifically indicating that the IMF content of waterfowl species is related to or involved in heat insulation could be found. In a study investigating the effect of wind protection and the patterns of airflow on cattle in an outside feedlot, Mader *et al*. (1997) found that the carcasses of cattle which was raised in an area exposed to these environmental conditions, not only had a larger ($P \leq 0.05$) fat thickness but also increased ($P \leq 0.05$) IMF (marbling fat) values. It is therefore suggested that the increased IMF content of Egyptian goose meat may be related to heat insulation. Another contributing factor to the higher fat content of these species could be the matter of buoyancy as this mechanism is an additional advantage of fat deposition (Pond, 1978). Waterfowl species such as Egyptian geese spend a lot of time floating on the water and therefore buoyancy is required. Buoyancy is the propensity of a fluid (water) to lift a body which is submerged in this fluid upwards (Cullerne, 2009) and is related to the density characteristics where the body floats in the fluid when the density of the body is less than that of the fluid (Giancoli, 1998). For this reason certain constituents of the body with a lower density than water, such as fat, will have a positive effect on the floating ability of the waterfowl. This theory proposes that the higher IMF of the waterfowl species may be linked to the involvement of fat in the heat insulation and buoyancy mechanisms of the

animal. It is based on the fact that, although fat deposition initially occurs subcutaneously, the body will not resort to using these reserves until it is absolutely necessary. This means that when the animal is in a cold environment (water) other lipid sources will be used for energy metabolism instead (Evans, 1972) i.e. IMF. It is therefore hypothesised that fat depots, such as IMF, may have a possible higher content to assure availability of reserves for energy metabolism.

Egyptian goose meat also had a higher IMF content compared to the 3.39 g/100 g present in the breast muscle of wild mallard ducks (*Anas platyrhynchos*) (Cobos *et al*., 2000). The proximate results indicated that there was a negative relationship between the moisture content and IMF (g/100 g) which is in agreement with the findings of Alfaia *et al*. (2010). The cooking process causes moisture loss which results in a significant increase in the IMF content of the cooked compared to the raw meat. As the proximate analysis was performed after completion of the cooking process, meat with a higher cooking loss will have a higher intramuscular fat content (Alfaia *et al*., 2010). This is another possible explanation for the high fat (g/100 g) of Egyptian goose meat and this theory could also be similar with regards to the pekin duck and both of the ostrich treatments. Muscle that mainly consists of red fibres, such as the breast muscle of Egyptian geese, are generally higher in total lipid content and more specifically PUFA content (Lawrie & Ledward, 2006); a phenomenon that may also contribute to the higher IMF values.

The important question, however, is; what is the impact of the high fat content? The IMF content of Egyptian goose meat may have an influence on the sensory properties. Fat is involved in the secretion of saliva in the mouth during mastication (Lawrie & Ledward, 2006) therefore; it is possible that a correlation may exist between high IMF and sustained juiciness of the meat. Even though the IMF content of Egyptian goose meat is fairly high, MacRae *et al*. (2005) and MacAfee *et al*. (2010) emphasises the fact that, in terms of human health, the fatty acid profile of the meat is more important especially the ratio of the polyunsaturated (PUFA) to saturated fatty acids (SFA) present.

4.2 Fatty acid profile – Game (Egyptian goose) vs domestic

It is evident that all of the muscles/species had a significantly different fatty acid profile (Table 3). The main factors influencing the fatty acid composition of meat are species differences and the variation in their diets (Wood & Enser, 1997). In monogastric animals, such as gamebirds and poultry, diet is a key factor, since the fatty acid profile of the intramuscular lipids is a reflection of the dietary constituents (Wood & Enser, 1997; Coetzee & Hoffman, 2002; MacRae *et al*., 2005). The mean fatty acid percentages (Table 3) indicate that the profile of Egyptian goose meat was dominated by the presence of PUFA contributing 39.5% to the total fatty acids. The diet of Egyptian geese is mainly composed of green plant material, growing crops, aquatic vegetation and aquatic invertebrates (Viljoen, 2005). This forage based diet is of a more unsaturated nature, due to the higher linolenic

acid content (Marmer *et al*., 1984; Enser *et al*., 1998; Ward *et al*., 2003) compared to that of the other domestic species which received a standardised commercial feed. The major individual PUFA within the profile seems to be α-linolenic acid (C18:3 *n*-3) and arachidonic acid (C20:4 *n*-6).

The high PUFA and lower SFA content results in Egyptian goose meat having a high PUFA to SFA ratio (P/S) (1.03). This value is in agreement with the findings of Cobos *et al*. (2000) in a study on the breast muscle of wild ducks indicating a P/S of 1.17. The only other species with a higher P/S than Egyptian goose meat is broiler chicken (1.41), however, the range of PUFA within broiler chicken differs according to the diet fed to these birds. Linoleic acid (C18:2 *n*-6) constituted 35.4% of the total PUFA (43.8%) present in broiler chicken and the percentage values for some of the other PUFA were smaller when compared to that of Egyptian goose meat. The P/S is considered to be important in terms of human health as SFA are the fats generally associated with having negative effects on human health, while monounsaturated fatty acids (MUFA) and PUFA are more favourable (Luciano, 2009). It is thus believed that a reduction in the intake of SFA together with an increase in the P/S may decrease the occurrence of cardiovascular disease in humans (Gidding *et al*., 2006). According to Raes *et al*. (2004) the P/S should not be below 0.7 but Scollan *et al*. (2006), Durand *et al*. (2005) and Wood *et al*. (2008) indicates that the recommended dietary intake of P/S must be >0.4. Considering these recommendations it is evident that Egyptian goose meat has an acceptable P/S ratio of 1.03. The omega 6 to omega 3 (*n*-6/*n*-3) fatty acids is another vital aspect with regards to human health. Smolin *et al*. (2003) explains that the *n*-6 and *n*-3 fatty acids, linoleic and α-linolenic acid are essential as they cannot be synthesized by the human body and are used for the production of other important omega fatty acids. Therefore, both *n*-6 and *n*-3 fatty acids are beneficial, however, the ratio in which these fatty acids are consumed must be considered, since an increased intake of *n*-6 may decrease the levels of HDL cholesterol consequently leading to health risks (Smolin *et al*., 2003). It is suggested that the *n*-6/*n*-3 ratio of meat should be below 5 (Raes *et al*., 2004; Durand *et al*., 2005; Scollan *et al*., 2006). This value for Egyptian goose meat is below the recommendation which can be ascribed to a very high content of C18:3 *n*-3.

The question also arises of why there is such variation in the fatty acid profiles of the two gamebird species; Egyptian goose and guineafowl. This can be ascribed to the fact that these two types of gamebirds have different diets. The diet of guineafowl is much more “domestic”, in a sense, with seeds and grains as the main food source (Little & Crowe, 2011). Guineafowl meat had a higher SFA content which can mainly be ascribed to the higher palmitic acid (C16:0) levels present. It can also be assumed that the main dietary PUFA, in this case, is linoleic acid (C18:2 *n*-6); this assumption is supported by the higher content of this fatty acid (Table 3) in the guineafowl meat compared to that of Egyptian geese. Guineafowl also had much lower levels of α-linolenic acid (C18:3 *n*-3) compared to Egyptian goose. Likewise, this also applies to the difference in the profile of Egyptian goose meat,

compared to the other domestic (farmed) birds used in this study which receives a standardised commercial feed primarily composed of grains and seeds.

It can be postulated that the overall fatty acid composition will be an influential factor with regard to the flavour characteristics of Egyptian goose meat (Hornstein & Crowe, 1960; 1963), particularly since PUFA is associated with the game attributes found in meat. These aspects warrant further research.

4.3 Fe is the differentiating factor within the mineral profile

Several factors could be responsible for the variation within the overall mineral content of the species. In this case the main influence is the fact that this study consisted of different species. Considering the mineral contents of the six different meat treatments (Table 4), it is clear that the Fe content was significantly higher in the red meat types compared to guineafowl and broiler chicken known for having a whiter coloured meat. The most apparent result, however, was the elevated Fe level (7.46 mg/100 g) of Egyptian goose meat (Table 4). This result is consistent with the study by Khalifa and Nassar (2001) where higher levels of Fe were present in game duck species than domestic ducks. The Fe values of the game ducks ranged between 4.22-6.19 mg/100 g meat and are more or less twice the amount present in domestic ducks. The high Fe levels of Egyptian goose meat is attributed to an elevated myoglobin content as the breast muscle of this gamebird endures a high level of physical activity on a regular basis. The *pectoralis* muscle in volant birds mainly consists of red type IIa, fast oxidative glycolytic (FOG) fibres together with a small percentage of type IIb, fast glycolytic (FG) fibres (Butler, 1991; Baeza *et al*., 2000). Type IIa fibres are aerobic thus having a high myoglobin content for oxygen supply. This also explains the elevated Fe contents of the meat with an overall higher concentration of red fibres. Meat is considered to be a very good source of Fe since 50-60% is in the heme form and is therefore more readily absorbed (Luciano, 2009). It is also speculated that the high Fe levels of the meat from this species may have an effect on the palatability as studies regarding the sensory properties of meat have found correlations between Fe content and metallic/liver flavour (Yancey *et al*., 2006). The Fe content could also have a detrimental effect on the flavour of the meat as it is considered to be a pro-oxidant in the flavour formation process. Excluding the results pertaining to Fe, the overall mineral compositions of the meat from the different species did not vary considerably. The data, however, did reveal that, besides the high Fe content, Egyptian goose meat also contains high levels of zinc (Zn) and copper (Cu) (Table 4). The concentrations of both Fe and Cu is higher than what was found in beef, lamb, ostrich, pork, chicken and turkey meat in the study by Lombardi-Boccia *et al*. (2005). Phosphorus was found to be the most abundant mineral present in Egyptian goose meat, followed by potassium and magnesium.

5 CONCLUSIONS

This study quantified the chemical profile of Egyptian goose meat in relation to that of other well-known species. A high IMF content may be linked to the fact that this species is a waterfowl. However, the fatty acid profile rather than the total fat content should be considered in terms of human health. In general, Egyptian goose meat is very high in PUFA. The P/S and *n*-6/*n*-3 ratio of Egyptian goose meat is within the health recommendations. With regard to the mineral composition of Egyptian goose meat, the high Fe content is the major differentiating factor and is mainly related to a higher level of physical activity. This research provides new insight into the nutritional characteristics of Egyptian goose meat which is, to date, not available in literature. It is suggested that further research evaluates the effects of diet, gender and age on the chemical composition of this gamebird species. However, cognisance should also be taken of the fact that it would be very difficult to quantify these effects, especially as pertaining to age and gender, as it is extremely challenging to classify wild birds according to the categories whilst they are in flight.

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CHAPTER 4

Sensory profiling of Egyptian goose (*Alopochen aegyptiacus*) meat*

ABSTRACT

No sensory profile information is available for Egyptian goose (*Alopochen aegyptiacus*) meat. The aim of this study was to conduct descriptive analysis in order to establish the sensory attributes of the breast portion of this species. Meat from guineafowl, Pekin duck, ostrich and broiler chicken were used as reference species. Egyptian goose meat had a very intense game aroma, game flavour and metallic aftertaste, mainly attributable to the muscle's high percentage of polyunsaturated fatty acids and Fe. Egyptian goose meat was also low in tenderness and high residue; this may be due to the high level of physical exercise endured by the breast muscle. Egyptian goose meat proved to be similar to ostrich meat regarding appearance (dark, red colour) and low tenderness, but differed from guineafowl and broiler chicken, the latter two meat types illustrated a higher degree of juiciness and tenderness. These results of Egyptian goose meat can now be used for further sensory studies as it is important to also establish the influence of extrinsic factors such as season and gender on the meat quality of this waterfowl species.

Keywords: Gamebird, Meat quality, Descriptive sensory analysis, Egyptian goose, *Alopochen aegyptiacus*, Fatty acids

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1 INTRODUCTION

Globally, unusual animal species have been increasingly utilised as valuable sources of meat (Hoffman & Wiklund, 2006; Hoffman & Cawthorn, 2012). Irrespective of its contribution to human nutrition, the consumption thereof is becoming popular amongst modern day consumers. These unconventional meat sources include a wide variety of wild bird species, especially those that are either widespread or considered to be agricultural pests. However, the significance of these meat sources has been overlooked particularly in rural Southern Africa.

The Egyptian goose, a waterfowl species, is native to Africa south of the Sahara and the Nile Valley. In South Africa, Egyptian geese are found in regions with inland water, along the coastline and in close proximity to croplands that they utilise for foraging (Viljoen, 2005). Egyptian geese are renowned for flying great distances. This species is also one of the leading gamebirds hunted in South Africa (Viljoen, 2005). The research by Mangnall and Crowe (2001, 2002) and Viljoen (2005) stresses the fact that population numbers have increased considerably and are still rising, especially in the Western Cape, South Africa. Consequently, farmers suffer financial losses due to damage on croplands. This situation could, however, be beneficial to farmers as wingshooting of this gamebird could provide farmers with an additional income if the meat is sufficiently utilized (Mangnall & Crowe, 2001).

Another common gamebird is the guineafowl (*Numida meleagris*). Guineafowl is considered to be the most abundant gamebirds in South Africa (Viljoen, 2005; Little & Crowe, 2011) and is also well known for being used in traditional cooking. Contrary to gamebirds, domestic birds such as ostrich, Pekin duck and broiler chicken are mainly farmed with for meat production. Scientific-based knowledge regarding the quality of gamebird meat is limited and it is therefore important to gain insight into the full sensory profile thereof. Especially since the gamebird industry in South Africa is becoming more viable (Geldenhuys *et al*., 2013a) [Chapter2]. There are a limited number of studies in which the sensory characteristics of meat from different species are compared (Rodbotten *et al*., 2004). Shahidi (1998) describes that several of the flavour volatiles, which occur in meat from different species, are in fact similar; however, the quantity thereof varies from species to species. Sensory reference standards could therefore be valuable tools when characterizing the sensory profile of a product such as Egyptian goose meat. Reference standards may be food products, chemicals or other substances and are used to communicate the concept of product attributes, thus ensuring that sensory panellists have the same understanding of the nature of a sensory attribute (Drake & Civille, 2002). Ostrich and Egyptian geese are similar with regard to the appearance of the meat; both having dark, red meat. Pekin ducks and Egyptian geese are both waterfowl species; however, the former is a domestic bird while the latter is a gamebird. Broiler chicken meat is

regarded as having the least variation in terms of quality and is therefore considered to be a good reference standard when conducting sensory analysis of meat. This is due to the genetic selection and controlled environment under which domesticated animals such as broiler chickens are reared, resulting in a decrease in the intrinsic variation of the sensory attributes.

The diet of Egyptian geese is mainly forage-based, consisting of growing crops, green plants, aquatic vegetation, invertebrates and insects (Viljoen, 2005). Guineafowl forage on bulbs and stems of plants, grass seeds, harvested grains, maize, as well as insects (Little & Crowe, 2011). Domestic species such as Pekin duck, ostrich and broiler chicken usually receive a standard commercial feed. The physical and chemical characteristics of meat influence the sensory profile thereof, and it is widely regarded that the fatty acid composition of the diet can have a major influence on the flavour of meat (Hornstein & Crowe, 1960, 1963; Mottram, 1998; Wood *et al*., 2003; Calkins & Hodgen, 2007). In addition, the presence of certain minerals such as iron could also have an effect on the flavour of meat (Yancey *et al*., 2006). For instance, high iron content in meat has been linked to a metallic/livery flavour. Furthermore, when comparing game and domestic birds, the extent of physical exercise the different species are subjected to will have a direct influence on the sensory quality of the meat, mainly due to the difference in muscle constituents of active and inactive animals (Lawrie & Ledward, 2006). By investigating all the influential factors, i.e. chemical and physical, it is possible to conduct regression analysis to determine which of the latter intrinsic chemical and physical attributes predict specific sensory attributes of Egyptian goose meat. This will provide the necessary insight to understand the factors driving the sensory quality of meat.

In view of this, the objective of this study was to fingerprint and describe the sensory profile of Egyptian goose meat in comparison to other well-established species which are consumed on a regular basis in South Africa. The sensory, physical and proximate characteristics, together with the fatty acids and minerals, were determined, where after multivariate analyses were conducted to determine the drivers of sensory meat quality, as well as to quantify the potential of Egyptian goose meat for the meat industry.

2 MATERIALS AND METHODS

2.1 Experimental layout, sampling and slaughtering

The experimental layout is indicated in Table 1. The design consisted of six meat treatments which included the breast portion of Egyptian geese, guineafowl, Pekin duck and broiler chicken together with ostrich fan fillet (*M. iliofibularis*) and ostrich moon steak (*M. femorotibialis*). There were six samples per treatment. The different species and muscles were selected based on the fact that this is a descriptive study and that the samples should be representative of each species. As such, the extrinsic (diet etc.) and intrinsic (muscle differences) factors that may be influential are recognized and accepted as being characteristic of each sample.

Table 1 Sample set and experimental units

Meat treatments	Cuts used	Number of birds analyzed
Egyptian goose	Breast	6
Guinaefowl	Breast	6
Pekin duck	Breast	6
Ostrich	Fan fillet	6
Ostrich	Moon steak	6
Broiler chicken	Breast	6

The gamebirds Egyptian geese (*Alopochen aegyptiacus*) and guineafowl (*Numida meleagris*) were harvested during August 2010 on Mariendahl Agricultural Experimental Farm, Western Cape, South Africa (-33° 51' 1.9074"; 18° 49' 21.1476"). A double barrelled shotgun was used during the wingshooting activities (ethical clearance reference number: 10NP_HOF01). The geese and guineafowl were collected in the field and placed in a refrigerator (4 °C) over-night (± 12 h) where after the slaughtering procedures were carried out manually as described by Geldenhuys *et al*. (2013b) [Chapter 3]. The broiler chicken carcasses were slaughtered according to the acceptable standard slaughtering methods used for commercial chickens (Department of Agriculture, Forestry and Fisheries [DAFF], 2006). The breasts (*M. pectoralis*) were removed from the respective bird carcasses and each meat sample was individually vacuum-packed in a polystyrene bag and frozen at -18 °C for approximately 6 weeks. The Pekin duck breasts (*M. pectoralis*), ostrich fan fillets (*M. iliofibularis*) and moon steaks (*M. femorotibialis*) were sourced from commercial producers and also frozen at -18 °C for approximately 6 weeks. Sensory analysis was performed on the right breast (*M. pectoralis*) of the carcass, while the physical measurements were performed on the left breast. The

two portions used for the analyses were treated as an entity and cooked together. Two strips were removed down the centre of the cooked ostrich fan fillet (*M. iliofibularis*) and moon steak (*M. femorotibialis*) samples, one of which was used for the sensory analysis and the other for the instrumental measurements.

Four reference standards were also prepared and used during the training phase of descriptive sensory analysis (Corollaro *et al*., 2013). The reference standards included commercial free range chicken, beef sirloin, beef rump, as well as the *Longissimus thorasicus et lomborum* muscle of locally harvested blesbok (*Damaliscus pygargus phillipsi* - a free ranging wild ungulate). The reference samples enabled the panellists to calibrate their sensory perception during the training sessions, thereby allowing them to recognise and score all of the attributes tested in the respective meat samples.

2.2 Sample preparation

Sensory analysis was conducted on the six meat treatments (six different muscles/species) with six replications per treatment. The samples were randomly selected for each of the six replications. The vacuum-packed, frozen meat samples were thawed for 36 h in a refrigerator (4 °C) prior to each of the pre-determined sensory analysis sessions. The two breast meat samples of each bird were treated as one entity and placed together inside in an oven bag (Glad®), while one ostrich fan fillet and ostrich moon steak sample were placed in separate oven bags, respectively. No salt (NaCl) or any other seasoning was added to any of the meat treatments throughout the sensory analyses. The oven bags and meat samples were then placed on stainless steel grids which were fitted on an oven roasting pan. Thermocouple probes attached to a handheld digital temperature monitor (Hanna Instruments, South Africa) were placed in the centre of each of the meat samples (AMSA, 1995). The prepared samples were then placed in two conventional ovens (Defy, Model 835), pre-heated to 160 °C (AMSA, 1995). The ovens were connected to a computerized monitoring system responsible for regulation of the temperature (Viljoen *et al*., 2001). The meat samples were removed from the oven when a core temperature of 75 °C was reached (AMSA, 1995). The samples were cooled for 15 min where after they were cut into 1 cm x 1 cm cubes, individually wrapped in aluminium foil and placed into glass ramekins coded with randomized three-digit codes. The coded ramekins, each containing two wrapped meat cubes, were then placed in a preheated industrial oven (Hobart, France) at 100 °C for 10 min after which they were removed and immediately served to the sensory panel for analysis.

2.3 Descriptive sensory analysis

Descriptive sensory analysis (DSA) was performed on the six meat treatments (six different muscles/species). A panel of eight judges, based upon previous experience with sensory analysis of

meat, was selected. The panellists were trained according to the guidelines for sensory analysis of meat by the American Meat Science Association (AMSA, 1995) and the generic descriptive sensory analysis technique as described by Lawless and Heymann (2010).

The panel undertook six training sessions and during each of these training sessions the panellists received 1 cm x 1 cm cubes of meat from the four reference standards, as well as the six meat treatments. Reference standards were chosen to illustrate the respective aroma and flavour attributes associated with Egyptian geese, as well as the other five treatments. Finally, the panel decided on 13 sensory attributes: game, chicken, ostrich and beef aroma and flavour, as well as metallic flavour, initial and sustained juiciness, tenderness (evaluated on first bite) and residue. The definitions for each of the attributes are described in Table 2.

The test re-test method was used for DSA. The panellists received the six treatments in a complete randomized order, while seated in individual tasting booths fitted with the software programme Compusense® five (Compusense, Guelph, Canada). The samples were analysed for the respective sensory attributes using an unstructured line scale anchored to zero (indicating “low intensity”) and 100 (indicating “high intensity”) (AMSA, 1995). The sensory analysis sessions took place inside a temperature-controlled (21 °C) and light-controlled (artificial daylight) room (AMSA, 1995). In order to cleanse and refresh their palates between samples, the panellists received distilled water (21 °C), apple quarters and water biscuits (Carr, UK).

Table 2 Definition and scale of each attribute used for the descriptive sensory analysis

Sensory attribute	Description	Scale
Game aroma[1]	Aroma associated with game meat as soon as the aluminium foil is removed	0 = Extremely bland 100 = Extremely intense
Chicken aroma[1]	Aroma associated with chicken as soon as the aluminium foil is removed	0 = Extremely bland 100 = Extremely intense
Ostrich aroma[1]	Aroma associated with ostrich as soon as the aluminium foil is removed	0 = Extremely bland 100 = Extremely intense
Beef aroma[1]	Aroma associated with beef as soon as the aluminium foil is removed	0 = Extremely bland 100 = Extremely intense
Game flavour[1]	Flavour associated with game meat prior to swallowing	0 = Extremely bland 100 = Extremely intense
Chicken flavour[1]	Flavour associated with chicken prior to swallowing	0 = Extremely bland 100 = Extremely intense
Ostrich flavour[1]	Flavour associated with ostrich prior to swallowing	0 = Extremely bland 100 = Extremely intense
Beef flavour[1]	Flavour associated with beef prior to swallowing	0 = Extremely bland 100 = Extremely intense
Metallic flavour[1]	Flavour associated with metal/liver prior to swallowing	0 = Extremely bland 100 = Extremely intense
Initial juiciness	The amount of fluid exuded from the cut surface when pressed between the thumb and forefinger	0 = Extremely dry 100 = Extremely juicy
Sustained juiciness	The level of juiciness perceived after the first 5 chews using the molar teeth	0 = Extremely dry 100 = Extremely juicy
First bite	The impression of tenderness perceived after the first 5 chews using the molar teeth	0 = Extremely tough 100 = Extremely tender
Residue	The amount of residue left inside the mouth after the first 10 chews	0 = None 100 = Abundant

[1] Aroma and flavour were analysed orthonasally and retronasally, respectively.

2.4 Physical measurements

2.4.1 pH

The pH of the six meat samples for each of the six replications was measured after thawing the meat for 36 h, immediately after removal from the packaging and before the start of the cooking process of every DSA session. The pH was measured by means of a Crison pH 25 handheld portable pH meter (Lasec (Pty) Ltd, South Africa) with an automatic temperature adjuster calibrated before each session with the standard buffers (pH 4.0 and pH 7.0) provided by the manufacturer.

2.4.2 Drip and cooking loss

Following removal of the muscles from the carcasses, the mass was recorded before being vacuum-packed and frozen (-18 °C) for approximately 6 weeks. Before each of the DSA sessions, the meat was thawed in a freezer at 4 °C for 36 h where after the meat was removed from the packaging, blotted dry with blotting paper and weighed (Radwag PS 750/C/2, Lasec SA, Cape Town, South Africa). This procedure was followed for each of the six replications. The drip loss of each sample was calculated as a percentage of the original mass of the meat sample before it was frozen.

The cooking loss of the meat samples was determined according to the method described by AMSA (1995). The difference in the weight of each of the uncooked and cooked samples was calculated as the percentage of cooking loss.

2.4.3 Colour

Instrumental colour measurements were taken at three randomly selected positions on the inside of a strip of cooked meat removed from the centre of each sample. The colour was recorded using a Colour guide 45˚/0˚ colorimeter (Catealogue no: 6805; BYK-Gardner, USA) to establish the L*, a* and b* values with L* indicating lightness, a* the red-green range and b* the blue-yellow range. The hue angle (h_{ab}) (°) and chroma value (C*) were also calculated using the a* and b* values as indicated by Honikel (1998).

2.4.4 Water holding capacity

The water holding capacity (WHC) was determined according to the method described by Trout (1988). A 0.5 g cooked meat sample was used using Lasec filter paper (grade 292, 90 mm diameter, part no. FLAS3205090) and a standard pressure of 588 N for 60 s. Using Image J Software (Version 1.41, 2009, http://rsbweb.nih.gov/ij/) the ratio between the outer (liquid) and inner (meat) purge areas was calculated to indicate the water holding capacity of each meat sample.

2.4.5 Shear force

The Warner Bratzler shear force test (WBSF), as described by Honikel (1998), was used to measure the instrumental shear force of the cooked meat samples. Each of the six treatments (six replications per treatment) was analysed for instrumental tenderness. Two adjacent 1 x 1 cm meat strips were cut parallel to the muscle fibre direction from the centre of the cooked meat samples, wrapped in aluminium foil and placed in the refrigerator (4 °C) for 24 h. The respective meat strips were then cut to obtain a total of six rectangular cubes with a length of 2 cm per cube. An Instron Universal Testing Machine (Instron UTM, Model 2519-107), attached to a Warner-Bratzler fitting, was used to determine the force necessary to shear the cooked rectangular meat cubes perpendicular to the muscle fibre direction. The WB fitting was a 1 mm thick triangular (V-notch) blade with a semi-circular cutting edge (radius of 0.508 mm). The UTM was operated with a 2 kN compression load cell. The shear test was performed at a speed of 200 mm/min. The shear force value of each of the samples was recorded in Newton (N). For statistical analyses, the mean of the six readings was used.

2.5 Chemical data

The chemical data (proximate, fatty acid and mineral composition) used in the multivariate analyses were obtained from Geldenhuys *et al*. (2013b) [Chapter 3]. This was possible as the data collected within this study and that of Geldenhuys *et al*. (2013b) [Chapter 3] were from the exact same samples.

2.6 Statistical analysis

The study consisted of a randomized block design with six meat treatments and six replications per treatment. The collected sensory data were pre-processed for further application in multivariate analyses using the following statistical techniques: PanelCheck Software (Version 1.3.2, http://www.panelcheck.com/) was used to monitor DSA panel performance. The sensory, physical and chemical data were also subjected to test-retest analysis of variance (ANOVA) using SAS® software (Statistical Analysis System 2006, Version 9.2, SAS Institute Inc., Cary, NC, USA) to test for the reliability of the panel. The Shapiro-Wilk test was performed to test for non-normality of residuals (Shapiro & Wilk, 1965). In the event of significant non-normality ($P \leq 0.05$), outliers were identified and residuals greater than 3 were removed. Correlation coefficients were calculated for the sensory, physical and chemical data by means of the Pearson's correlation coefficient (r) (Snedecor & Cochran, 1980). Principal component analysis (PCA), using the correlation matrix, was performed and used in conjunction with discriminant analysis (DA) in order to indicate and clarify the relationships between the sensory, physical and chemical data (Næs *et al*., 2010). The latter

multivariate analyses were conducted using XLStat software (Version 2012, Addinsoft, New York, USA).

3 RESULTS AND DISCUSSION

3.1 Aroma and flavour

The mean scores for the respective aroma and flavour attributes are illustrated in Fig. 1. It is clear that the aroma and flavour profile of Egyptian goose meat was very distinct compared to the other treatments. The trained panel found Egyptian goose meat to have a more ($P \leq 0.05$) intense game aroma (41.9), game flavour (48.4) and metallic flavour (28.2) compared to the other treatments. PCA plots are used in sensory analysis to demonstrate the relationships between different sensory attributes, as well as their association with other chemical or physical characteristics. The PCA bi-plot (Fig. 2) provides insight into the sensory attribute associations when comparing the different species. Although the PCA bi-plot only describes 51% of the variation, this is still high when it is taken into account that there are numerous extrinsic (and intrinsic) factors that could influence the sensory profile such as diet and age. The sensory attributes illustrated in the top left quadrant of the 1st principal component (PC1/F1) associate with Egyptian goose meat. This offers further evidence that the sensory profile of Egyptian goose meat was predominantly governed by game-like attributes, and the metallic flavour. According to Fig. 2 there is a reasonably strong correlation between game flavour and intramuscular fat (IMF%) ($r = 0.601$; $P = 0.0001$). Generally IMF is regarded as an essential driver of meat flavour (Melton, 1990). Studies by Hoffman *et al*. (2009) and Tshabalala *et al*. (2003) showed significant correlations between the amount of IMF present and meat flavour intensity. These findings are in agreement with our study as the high IMF content of Egyptian goose meat (Geldenhuys *et al*., 2013b) seemed to contribute to the reasonably intense game flavour.

According to Swanson and Penfield (1991) increased levels of PUFA in meat from game animals are also responsible for the distinct game characteristics. According to Geldenhuys *et al*. (2013b) the Egyptian goose meat samples used in this study illustrated high levels of PUFA. As indicated in Fig. 2, both game aroma and game flavour were highly correlated with omega 3 fatty acids (*n*-3) with values of 0.800 ($P < 0.0001$) and 0.701 ($P < 0.0001$). However, the correlation between the game characteristics and total polyunsaturated fatty acid (PUFA) content was low, and not significant.

In a study comparing the sensory attributes of 15 different species, Rodbotten *et al*. (2004), states that the flavour of game animals can be influenced by diet. In monogastric animals, such as gamebirds and poultry, diet is a key factor. The dietary lipids in the feed are directly linked to the fatty acid composition of the intramuscular lipids (Wood & Enser, 1997; MacRae *et al*., 2005). This applies

particularly to essential fatty acids which cannot be synthesized (Wood & Enser, 1997). The food supply of Egyptian geese is variable, but mainly forage-based (Viljoen, 2005). During the grain harvesting season they forage on crops such as wheat and barley (Maclean, 1988; Viljoen, 2005). The geese used in this study were, however, not harvested during the grain season and thus predominately consumed grasses, young green crops and aquatic vegetation. According to the DA plot (Fig. 3a), indicating the classification of the different meat samples based on the individual fatty acids (Fig. 3b), it is evident that the fatty acid composition of the respective species is quite diverse. The diet of Egyptian geese is of a more unsaturated nature, since grass- or forage-based diets are regarded as high in linolenic acid (18:3) (Manner *et al*., 1984; Enser *et al*., 1998; Ward *et al*., 2003). Another important aspect to consider is the abundance of specific individual fatty acids. In Fig. 3b moderate to strong correlations are illustrated between the game aroma and flavour attributes and some of the individual fatty acids. See Table 3 for significant correlations between specific PUFA's and game aroma and flavour. α-Linolenic acid (C18:3*n*-3) had the strongest correlation with game aroma and flavour, respectively. Although Yancey *et al*. (2006) suggest that specific fatty acids seem to result in game-like attributes, limited information could be found to substantiate the link between individual fatty acids and specific sensory notes. This is an area of research that requires further investigation. Free iron (Fe) in meat acts as an oxidative catalyst and during cooking the concentration thereof increases as the Fe containing proteins (myoglobin and haemoglobin) denature (Campo *et al*., 2003). High levels of polyunsaturated fatty acids are particularly susceptible to oxidation (Campo *et al*., 2003). With the increased Fe levels of Egyptian goose meat (Geldenhuys *et al*., 2013b) [Chapter 3] having a possible pro-oxidant effect, lipid oxidation might contribute to the intense game-like aroma and flavour. However, Yancey *et al*. (2006) suggest that game-like or livery flavours do not seem to be related to lipid oxidation.

It is interesting to note that there is such a major difference between the sensory profiles of the two gamebird species, i.e. Egyptian goose and guineafowl. The mean sensory scores (Fig. 1) for game aroma (0.62) and game flavour (1.01) of guineafowl meat were extremely low and barely detectable ($P \leq 0.05$) compared to that of Egyptian goose meat. The guineafowl meat compared well with broiler chicken in that it was high in chicken aroma (64.1) and chicken flavour (56.6). This phenomenon is also clearly visible on the PCA plot (Fig. 2) where there were moderate, negative correlations ($P \leq 0.05$) between the game and chicken sensory attributes. The very low game aroma or game flavour scores for guineafowl may be explained by the fact that the range of fatty acids found in guineafowl meat is different to that of Egyptian goose meat. This could be related to the difference in the diet in terms of linoleic and linolenic acid contents, where grain-based diets are high in linoleic acid and grass or forage-based diets are high in linolenic acid (Manner *et al*., 1984; Enser *et al*., 1998; Ward *et al*., 2003). Guineafowl feed on bulbs and stems of plants, grass seeds, harvested grains and

maize which may clarify the similarity between the sensory profiles of guineafowl and broiler chicken. Diet is thus also the key factor when comparing the sensory profile of Egyptian goose meat with that of the other domestic fowl species.

It is evident from the PCA plot (Fig. 2) that Egyptian goose meat is also associated with a strong metallic flavour. This sensory attribute definitely contributes to the unique sensory profile of this species. Very strong correlations were noted for metallic flavour, game aroma ($r = 0.928$; $P = <0.0001$) and game flavour ($r = 0.943$; $P < 0.0001$), respectively. The metallic flavour could be explained by the reasonably strong correlation ($r = 0.793$; $P <0.0001$) with iron (Fe) content. Geldenhuys *et al*. (2013b) [Chapter 3] reported a very high level of Fe (7.46 mg/100 g) in Egyptian goose meat and the metallic flavour may thus be ascribed to the presence of Fe. In literature, there seems to be an association between metallic flavour and a liver-like flavour in meat. Miller (2001) found that a metallic flavour is often connected to a liver flavour in beef and Mendell *et al*. (1998) reported a correlation between liver flavour and a metallic aftertaste. Rodbotten *et al*. (2004) defines metallic flavour as the flavour of ferrosulphate, whereas a liver flavour is associated with animal liver. It is possible that, in this study, metallic flavour could have been perceived as a combination of the two. Wild animals tend to have a more intense liver flavour than farmed animals (Rodbotten *et al*., 2004). Yancey *et al*. (2006) established that an increase in the total Fe content (myoglobin) resulted in a more intense liver flavour in beef. The fact that the geese were not bled after being shot might also have caused an increase in the intensity of the metallic flavour due to the presence of haemoglobin (blood) in the muscle. Yancey *et al*. (2006), however, found no significant correlation in terms of haemoglobin content and a liver-like flavour. Furthermore; the studies of Calkins and Hodgen (2007) and Yancey *et al*. (2006) revealed that the presence of long chain unsaturated fatty acids in the meat can also be responsible for the development of a liver-like flavour. Mendell *et al*. (1998) reports a significant correlation between metallic aroma and C18:3 fatty acids in forage fed beef and speculated that dietary fatty acids (C18:1 and C18:3) are responsible for the higher metallic aroma found. In our study this relates back to the fact that relatively strong correlations were found between metallic flavour and the long chain PUFA listed in Table 3 and indicated in Fig. 3b. Of these PUFA, α-linolenic acid (C18:3*n*-3) ($r = 0.788$, $P < 0.0001$) showed the highest correlation with metallic flavour.

An aspect that warrants further investigation is the effect of ageing on the flavour and aroma of Egyptian goose meat. This is particularly pertinent as there is a strong probability that most consumers would age the meat because of the low tenderness of the breast muscle (Fig. 1, Fig. 2 and Table 4). Strong associations with any of the other aroma and flavour attributes were absent, proving the dominance of the game-like and metallic sensory attributes in Egyptian goose meat. The aroma and flavour of Egyptian goose meat are thus quite unique when compared to the other fowl species.

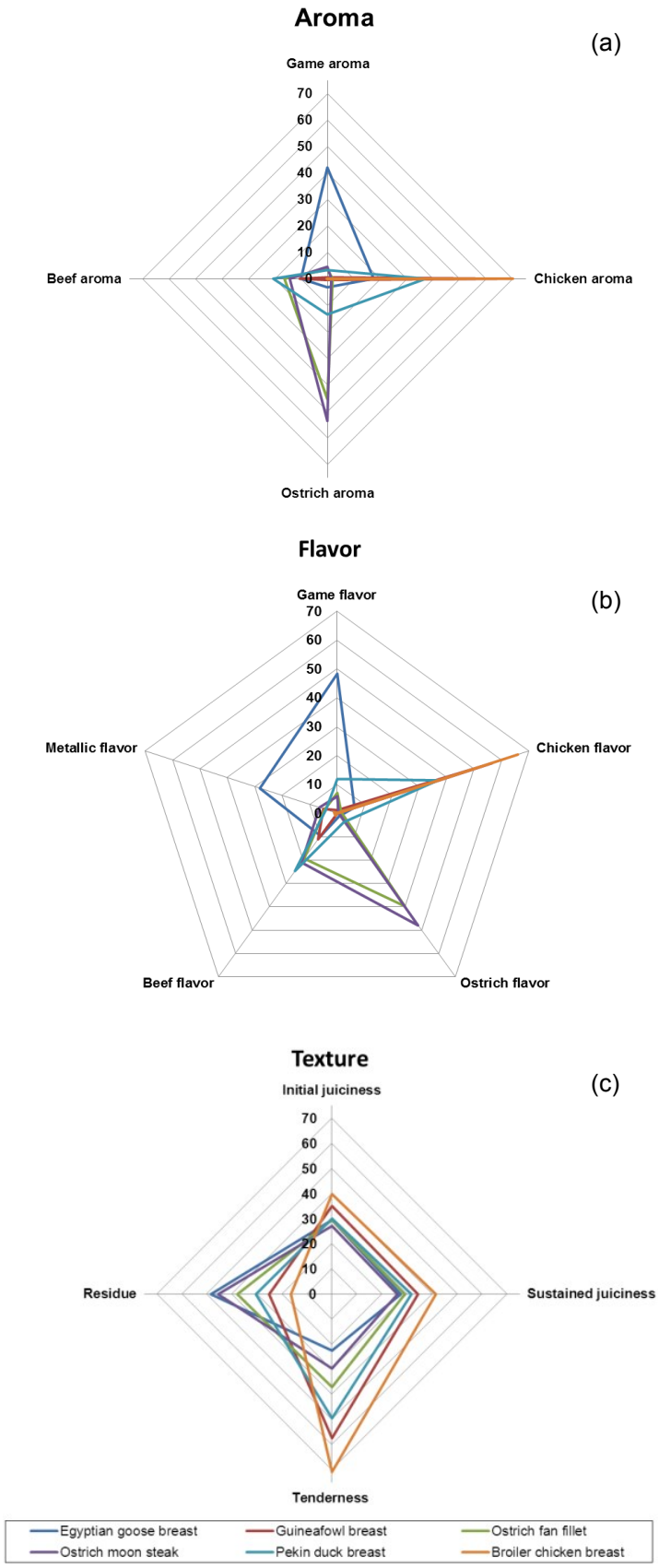

Figure 1 Spider plots illustrating the aroma (a), flavour (b) and texture (c) attributes derived from the six meat treatments. Unstructured line scale ranged from 0 = low intensity to 100 = high intensity.

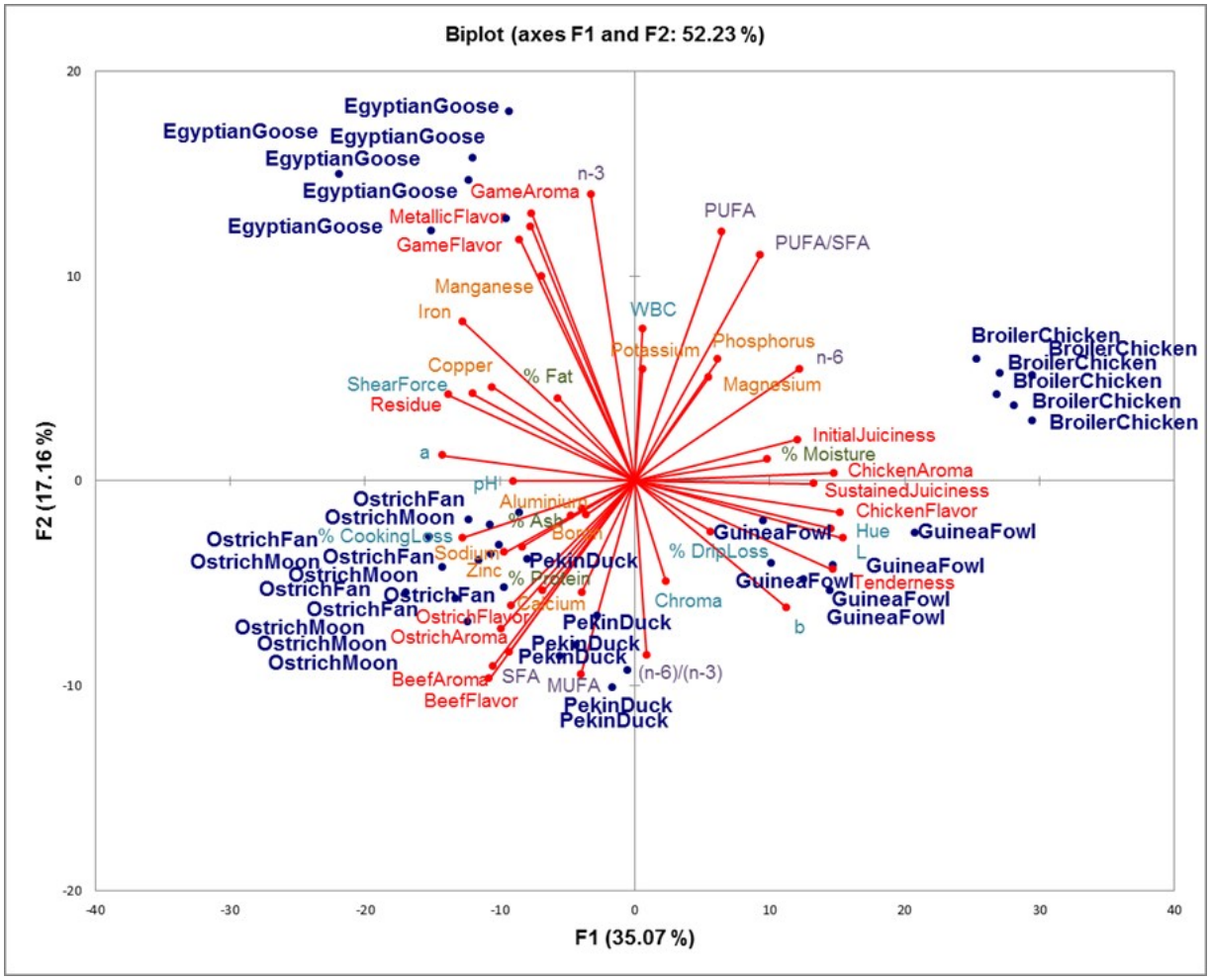

Figure 2 PCA bi-plot of sensory attributes, physical attributes, proximate composition, mineral composition and total fatty acids of the six meat treatments.

Table 3 Significant correlations between the game attributes and individual fatty acids

Fatty acids		Game aroma		Game flavour		Metallic flavour	
		r[1]	P-value	r[1]	P-value	r[1]	P-value
α-Linolenic acid	18:3 n-3	0.838	< 0.0001	0.766	< 0.0001	0.788	< 0.0001
Arachidonic acid	20:4 n-6	0.600	0.0001	0.560	0.000	0.590	0.000
Eicosatrienoic acid	20:3 n-3	0.445	0.007	-	-	0.388	0.019
Eicosapentaenoic acid (EPA)	20:5 n-3	0.434	0.008	0.377	0.024	0.431	0.009
Docosadienoic acid	22:2 n-6	0.660	< 0.0001	0.575	0.000	0.606	< 0.0001

[1] r (Pearson correlation coefficient).

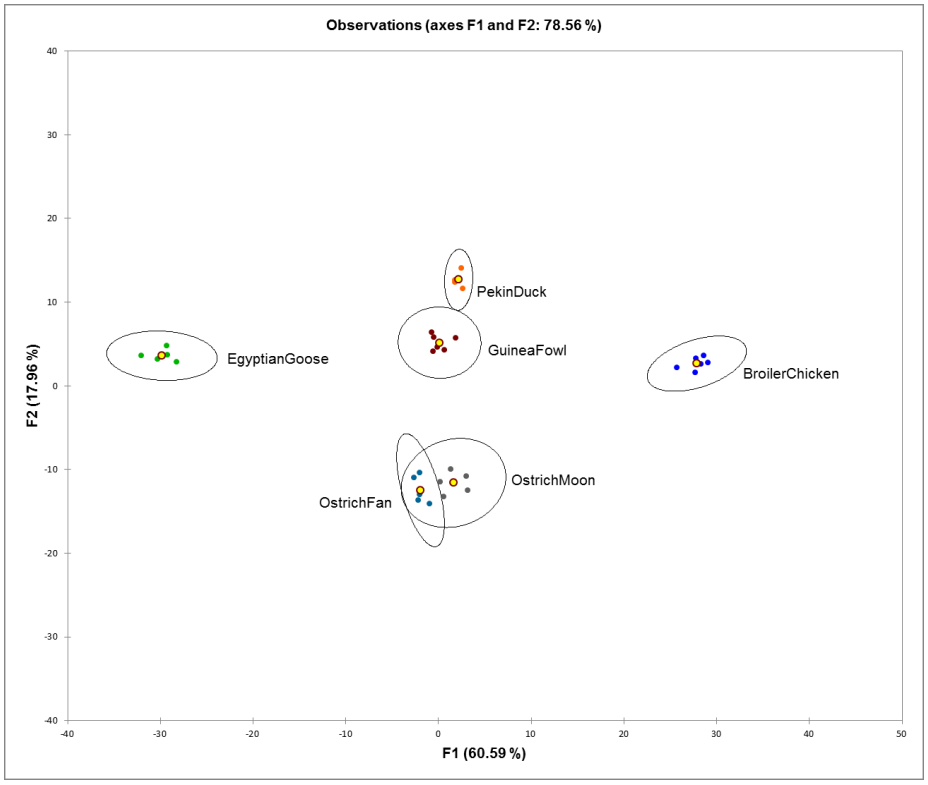

Figure 3a DA plot illustrating the classification of treatments based on the specific fatty acids.

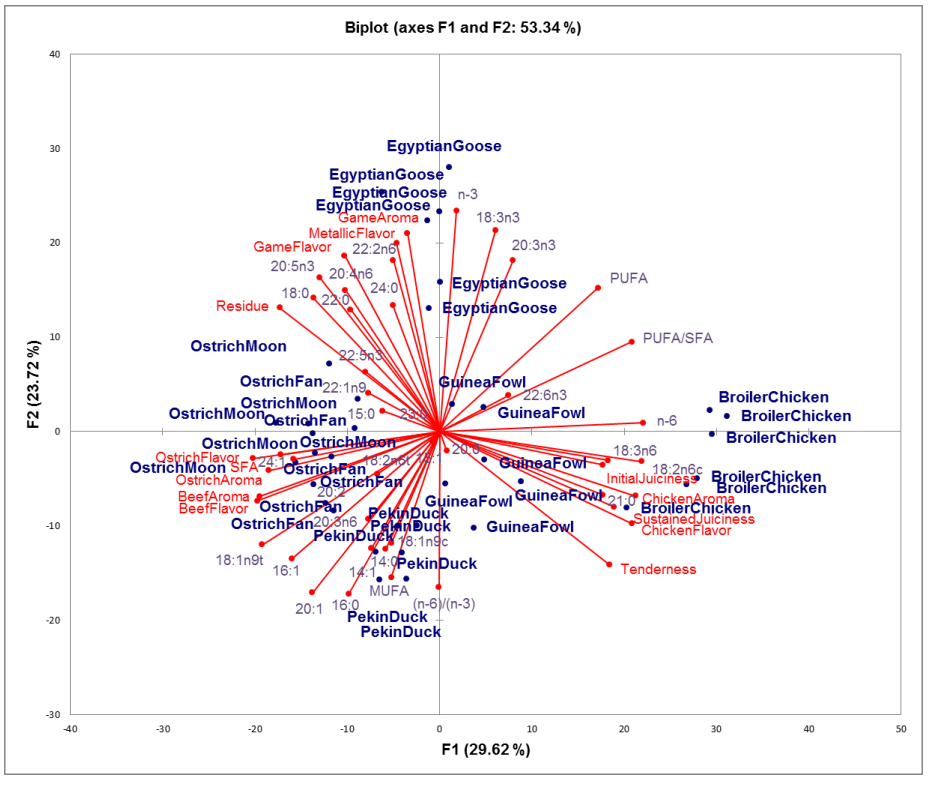

Figure 3b PCA bi-plot indicating the means for each individual fatty acid, as well as the sensory attributes of the six respective meat treatments.

3.2 Juiciness

Initial juiciness in meat is defined as the moisture released during mastication, whereas the stimulation of saliva secretion due to the presence of intramuscular fat is defined as sustained juiciness (Dryden & Maechello, 1970; Lawrie & Ledward, 2006). Egyptian goose meat received significantly lower sensory mean scores for initial and sustained juiciness (Fig. 1) compared to the broiler chicken and guineafowl samples. This is also illustrated in the PCA plot (Fig. 2) where broiler chicken and guineafowl associate with both forms of juiciness. It is also evident from Fig. 2 that there is a moderate correlation between percentage moisture and initial ($r = 0.618$; $P < 0.0001$) and sustained juiciness ($r = 0.583$; $P = 0.0002$), respectively. The juiciness is therefore a reflection of the moisture content (%) of the meat. As a result of the cooking process, Egyptian goose meat had a greater loss of moisture than guineafowl and broiler chicken (Table 4), this may explain the lower juiciness. This higher cooking loss is inconsistent with literature as muscle with a high ultimate pH (pH_u) generally produces meat with a relatively low cooking loss (Lawrie & Ledward, 2006). However, it could be argued that the lower drip loss due to the high pH_u ($r = -0.377$; $P = 0.024$) may have resulted in an increased amount of moisture available for release during cooking. This theory may explain the moderate correlation (Fig. 2) of a high pH with cooking loss ($r = 0.450$; $P = 0.006$). Thomas *et al*. (2004) also reported that the percentage cooking loss and drip loss showed an inverse trend and a high muscle pH usually results in less drip loss. Another indication of the juiciness of the meat is the WHC as this reflects the amount of fluid present in the meat after the cooking process. Fig. 2 illustrates a low, but significant correlation ($r = 0.370$; $P = 0.027$) between the percentage moisture and the WHC of the meat. The initial juiciness of meat is thus positively correlated to the water holding capacity which in turn is determined by the pHu of the muscle (Offer & Trinick, 1983).

Generally IMF contributes to sustained juiciness, but in this study, no significant correlation was observed between these two attributes (Fig. 2). The absence of a significant correlation between IMF and sustained juiciness may be related to the effect of cooking on the IMF (%) determination. The cooking process causes moisture loss which results in a significant increase in the IMF (%) in meat from the raw to the cooked state. In our study the proximate analysis was performed after completion of the cooking process, and in this instance meat with a higher cooking loss will naturally have a higher intramuscular fat content (Alfaia *et al*., 2010). The low tenderness of Egyptian goose meat (Fig. 1) could also have had a concealing effect on the perception of sustained juiciness due to the elevated fat content. Hoffman *et al*. (2007), in a study on Springbok (*Antidorcas marsupialis*) meat quality, found that with an increase in the shear force values (decrease in tenderness) the sustained juiciness decreased. It could thus be expected that with a decrease in the tenderness, the meat will become lower in juiciness due to impeded water release from the meat (Tshabalala *et al*., 2003).

3.3 Tenderness

Egyptian goose meat proved to have the lowest tenderness compared to the other treatments as indicated by the very low mean sensory tenderness (22.7) and residue (48.2) values (Fig. 1), as well as the shear force mean value of 48.7 N (Table 4). Fig. 2 also illustrates that Egyptian goose meat associated with a high shear force and high residue, respectively. It is perhaps of note that although Egyptian goose meat had the highest mean shear force value, it did not differ ($P>0.05$) from the ostrich moon steak and fan fillet.

According to Lewis *et al*. (1989) muscles with a high level of physical activity pre-slaughter will result in less tender meat because of an increased intramuscular collagen content. Egyptian geese are gamebirds and are known for flying long distances compared to the other domestic fowl in this study. Therefore the breast muscle (*pectoralis*), primarily used for flying (Biewener, 2011), has a higher level of activity resulting in less tender meat.

An important correlation illustrated by the PCA plot (Fig. 2) is that of shear force and pH ($r = 0.427$; $P = 0.009$). The low tenderness could also be related to the higher pH (5.95) of Egyptian goose meat (Table 4). Egyptian geese endure continuous physical exercise and were shot as they were flying back to the roosting sites from the areas where they were foraging. They may thus have covered a long distance. This exercise ante mortem causes the pH_u of the breast muscle to be relatively high as there is very few energy reserves left for lactic acid production (Lawrie & Ledward, 2006). The rate of pH fall and pH_u has a considerable effect on shear force and sensory tenderness of meat (Sales & Mellett, 1996). According to Purchas (1990) there is a decrease in tenderness as the pH_u increases from 5.5 to 6.2. Yu and Lee (1986) also concluded that between pH 5.8 and 6.3 the tenderness is at its lowest as this is not the optimal functioning pH for the proteolytic enzyme system, i.e. the calpains and cathepsins.

There also seems to be a trend in terms of cooking loss (%) and sensory tenderness, with a moderate to strong negative correlation ($r = -0.695$; $P < 0.0001$) existing between these two attributes (Fig. 2). Also, an increase in shear force is positively correlated to a high percentage cooking loss ($r = 0.648$; $P < 0.0001$). Egyptian goose meat and the ostrich treatments had significantly higher cooking losses (%) compared to the other treatments (Table 4) and were considered to be the least tender treatments by the sensory panel. In several studies, similar results were found (Silva *et al*., 1999; Hoffman *et al*., 2007, Hoffman *et al*., 2008) and a possible reason for this decreased tenderness could be the diluting effect of the bound moisture (Thomas *et al*., 2004).

3.4 Instrumental colour

In general game meat is considered to have a darker red colour when compared to domestic animals (Hoffman, 2000). The colour of Egyptian goose meat resembles that of ostrich and other ruminant

game species which is evident from the strong association of these species and the a* value (red colour) in the PCA plot (Fig. 2). The mean a* value (Table 4) is low for game species in general, but this value reflects that of cooked and not raw meat where the a* value is usually substantially higher. The dark colour of game meat is most likely the result of the higher level of physical activity in game species (Hoffman, 2000). The level and type of activity that a muscle is subjected to directly determines the fibre composition. Kiessling (1977) reported that the breast muscle of geese consists of approximately 80% red fibres and Baeza *et al*. (2000) found that the *M. pectoralis* of mule ducks consist of 88% type IIa fibres and 12% type IIb fibres. Lawrie and Ledward (2006) state that there is an amplification of the myoglobin content in the muscle during regular exercise, mainly to enhance its oxygen carrying capacity, therefore the dark red colour. In our study, the gamebird species were more active than the domestic birds. In Fig. 2 the high myoglobin content relates to the higher a* value (red colour) of Egyptian goose meat and there are negative correlations with the hue angle (less red colour) and the L* value (lightness). The association of Egyptian goose meat with a dark red colour is verified by the mean scores in Table 4. This species indicated a mean score of 9.82 for a*, which was not significantly different from that of ostrich proving the similarity in terms of colour between the two. Furthermore, the L* value of Egyptian goose meat (40.92) was significantly lower than the other meat treatments, especially broiler chicken (78.02), indicating a much darker meat colour.

In Fig. 2 there is a moderate correlation ($r = 0.434$; $P = 0.008$) between high pH and a high a* value (red colour). The PCA also illustrates a moderate negative correlation ($r = -0.534$; $P = 0.001$) between pH and L* value (lightness). These correlations verify the fact that pH may partly be responsible for the dark, red colour of Egyptian goose meat. Red (type I and IIa) muscles with a high level of exercise have a higher ultimate pH. The Egyptian geese used for this study were all shot while in flight, the breast muscles thus also experienced a certain amount of ante mortem stress. Although the meat is not classified as DFD ($pH<6.0$), the tendency towards this condition could be a contributing factor to the darker colour. There is a greater depletion of muscle glycogen, ante mortem, which is associated with stress and causes a higher pH_u (Lawrie & Ledward, 2006). Consequently, water is bound tightly, the structure of the muscle is firmer, scattering of light is low and the muscle surface appears to be darker (Warris, 2000). Therefore an inverse correlation between pH and lightness exists which relates to a darker coloured meat.

Where ostrich meat was very similar in appearance to Egyptian goose meat, it was entirely the opposite with guineafowl and broiler chicken. The association of guineafowl and broiler chicken with L*, b* and hue values were expected (Fig. 2). These associations are confirmed by the significantly different mean values (Fig. 1) for the colour variables. Compared to the other treatments, broiler chicken and guineafowl both had significantly higher L* and b* mean values and significantly lower a*

values. Guineafowl was more yellow (higher b*) and broiler chicken lighter (higher L*) in appearance. The difference in the colour of these two birds compared to the other species, can be attributed to the type of fibres present in the breast muscle. Both guineafowl and broiler chicken are birds that do not use their wing muscles very often. Broiler chickens never fly, whereas guineafowl will, on occasion fly short distances. Kiessling (1977) established that the guineafowl breast muscle consists mainly of fast twitch, type IIb, glycolytic, white fibres with only 17% red fibres present. This is typical for gallinaceous birds to rapidly take off and fly short distances.

Table 4 The average values (±SD[1]) of the physical measurements from the six meat treatments

	Species						LSD[2]
	Egyptian Goose	**Guineafowl**	**Ostrich Fan**	**Ostrich Moon**	**Pekin Duck**	**Broiler Chicken**	**P=0.05**
% Drip loss	$7.93^{b} \pm 3.66$	$10.59^{a} \pm 2.29$	$5.14^{c} \pm 0.67$	$8.41^{ab} \pm 1.42$	$10.51^{a} \pm 0.57$	$8.98^{ab} \pm 2.06$	2.38
% Cooking loss	$29.99^{b} \pm 4.91$	$22.89^{c} \pm 2.15$	$35.21^{a} \pm 2.90$	$35.78^{a} \pm 3.81$	$28.65^{b} \pm 2.54$	$22.94^{c} \pm 3.12$	3.55
pH	$5.95^{ab} \pm 0.11$	$5.70^{c} \pm 0.09$	$6.06^{a} \pm 0.12$	$5.94^{ab} \pm 0.08$	$6.02^{a} \pm 0.06$	$5.85^{b} \pm 0.12$	0.12
L*	$40.92^{d} \pm 3.34$	$68.17^{b} \pm 1.61$	$49.47^{c} \pm 0.97$	$51.76^{c} \pm 1.43$	$51.86^{c} \pm 2.72$	$78.02^{a} \pm 2.23$	2.50
a*	$9.82^{a} \pm 2.08$	$4.57^{c} \pm 0.65$	$9.13^{a} \pm 2.09$	$9.04^{a} \pm 1.59$	$7.30^{b} \pm 1.51$	$1.93^{d} \pm 0.64$	1.71
b*	$14.11^{e} \pm 0.61$	$18.45^{a} \pm 0.83$	$14.86^{d} \pm 0.68$	$15.89^{c} \pm 0.22$	$15.91^{c} \pm 0.46$	$17.36^{b} \pm 0.96$	0.56
Hue	$55.41^{d} \pm 6.53$	$76.38^{b} \pm 2.54$	$59.16^{d} \pm 6.22$	$60.49^{cd} \pm 4.71$	$65.51^{c} \pm 4.35$	$83.68^{a} \pm 1.96$	5.11
Chroma	$17.28^{b} \pm 0.92$	$19.56^{a} \pm 1.42$	$17.79^{b} \pm 1.28$	$18.33^{ab} \pm 0.65$	$17.54^{b} \pm 0.87$	$17.48^{b} \pm 0.99$	1.24
WBC[3]	$3.83^{a} \pm 0.66$	$3.77^{ab} \pm 0.38$	$3.37^{ab} \pm 0.27$	$3.34^{b} \pm 0.47$	$2.68^{c} \pm 0.25$	$3.39^{ab} \pm 0.33$	0.47
Shear Force (N)	$48.76^{a} \pm 13.65$	$25.72^{b} \pm 5.34$	$42.38^{a} \pm 12.23$	$41.71^{a} \pm 11.94$	$26.20^{b} \pm 4.62$	$18.10^{b} \pm 3.98$	8.83

[a-e] Means in rows with different superscripts are significantly different at P≤0.05. [1]SD (standard deviation); [2]LSD (least significant difference); [3]WBC (water binding capacity).

4 CONCLUSIONS

This study illustrated that the aroma and flavour attributes of Egyptian goose meat are very distinct when compared to the other species used in this study. The sensory profile of Egyptian goose meat has a very strong game aroma and game flavour, but also a distinctive metallic aftertaste. The presence of a substantial amount of Fe in the meat was responsible for the intense metallic flavour, while the high PUFA content could have been involved in producing intense game aroma and flavour notes. Egyptian goose meat is low in tenderness (high shear force), which is a result of the high level of physical activity endured by the breast muscle during flying. The low moisture content and high cooking loss explains the low initial juiciness thereof and regardless of the high fat content, Egyptian goose meat tends to be low in sustained juiciness.

This research essentially categorises the sensory profile of Egyptian goose meat in relation to that of other well-known fowl species consumed in South Africa. This allows for the potential incorporation of the meat as a product on the South African meat market. With an initial sensory profile in place, it is now possible to do further sensory research in order to determine the effect of factors such as gender and grain season (diet) on the meat quality of Egyptian geese.

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CHAPTER 5

The effect of season, gender and portion on the carcass characteristics, pH, colour and proximate composition of Egyptian goose (*Alopochen aegyptiacus*) meat*

ABSTRACT

The carcass yield, physical characteristics and proximate composition of Egyptian geese (*Alopochen aegyptiacus*), a Southern African gamebird species, have been studied. A total of 69 geese were harvested during two seasons; summer (n=36) and winter (n=33). This total group of geese consisted of 27 female birds and 42 male birds. Gender alone affected (P≤0.05) the live and carcass weights and the average muscle weights (g) of each portion was higher for the male fowl. The data does not indicate differences between the meat's physical characteristics on account of gender; however, the meat from the female birds did have a higher IMF (intramuscular fat content). Season (winter vs. summer) did not influence the average muscle weights (g) of the breast, thigh and drumstick portions but the IMF content of the birds hunted in winter was higher. Muscle colour and pH differed as a result of season with the summer meat having a higher pH and more vivid red colour compared to winter. The physical characteristics and the proximate composition of the breast, thigh and drumstick portions varied considerably. This is essentially connected to a difference in physical activity of the muscles in the portions. Overall, this study revealed that in order to ensure a consistent eating quality the harvesting periods of Egyptian geese should be considered.

Keywords: Egyptian goose, Yield, Chemical composition, Colour, pH

* Geldenhuys, G., Hoffman, L.C. & Muller, M. (2013). The effect of season, sex and portion on the carcass characteristics, pH, colour and proximate composition of Egyptian Goose (*Alopochen aegyptiacus*) meat. *Poultry Science*, **92**, 3283-3291.

1 INTRODUCTION

The gamebird industry and wingshooting in South Africa is becoming increasingly popular and the Egyptian goose (*Alopochen aegyptiacus*) is considered to be one of the leading gamebird species hunted (Viljoen, 2005; Geldenhuys *et al*., 2013). Egyptian goose meat is consumed regularly in South Africa by farmers and people in rural/farming communities that are familiar with this species. Crop farmers in the Western Cape, South Africa also consider Egyptian geese to be very serious agricultural pests, causing a negative impact on the agricultural economy of this region (Mangnall & Crowe, 2001; 2002). In 1997, Mangnall and Crowe (2002) estimated the total financial losses in the Agulhas Plain region of the Western Cape, South Africa to be R385 000 (9.86 R = 1 US $). In attempting to reduce the damage they cause, wingshooting of the geese are recommended. For this reason, utilisation of the meat is vital; however, with the current absence of available scientific literature, it is essential to investigate the meat quality of this waterfowl species (Geldenhuys *et al*., 2013). Information regarding the carcass yield, physical characteristics and proximate composition of Egyptian geese will give insight into the chemical composition and nutritional value, as well as the economic potential of the meat from this species.

The Egyptian goose is the second largest waterfowl species found in South Africa (Viljoen, 2005). The average live weight of adult Egyptian geese is estimated to be 2.35 kg for male fowl while the females are slightly smaller at 1.87 kg (Viljoen, 2005). Kamar (1962) reported that when Egyptian geese are raised within a domesticated environment they have an average weight of approximately 2.6 kg and 3.2 kg at six and 12 months of age, respectively. This waterfowl species is also large compared to other gamebirds such as the Guineafowl (1.1 - 1.2 kg) (Mareko *et al*., 2006; Hoffman & Thlong, 2012) and wild pheasants (0.9 - 1.3 kg) (Hofbauer & Smulders, 2011). The typical weight of domestic waterfowl species is somewhat different than that of wildfowl. For instance, Mule ducks already have an average live weight of 4.5 kg (males) and 4.3 kg (females) at an age of 13 weeks (Baeza *et al*., 2000). This is similar for Muscovy ducks with the males having an average weight of 4.6 kg and the females 2.9 kg (Baeza *et al*., 1998).

Because Egyptian geese are monogastric fowl, their diet is expected to be a major influential factor in terms of the meat quality. The diet of Egyptian geese mainly consists of grass or forage based material of which seedlings and growing crops form a large part of their intake (Halse, 1984; Viljoen, 2005). However, in early summer, during the South African grain harvesting period (November), Egyptian geese travel inland in order to forage on grain seeds found on croplands. Kokoszynski *et al*. (2008) found that supplementing the diet of farmed game pheasants with whole grains resulted in increased body, carcass and portion weights. This alteration in the diet may therefore have an effect on the overall meat quality i.e. the carcass yield, as well as the proximate

composition of the meat. The grain-based diet is high in energy compared to the more forage-based diet and it can be assumed that this change in diet may result in an increased body and muscle weight, as well as a difference in the chemical composition, especially the intramuscular fat content (IMF) of the meat.

It is also essential to consider the effect of the breeding season on the carcass yield and compositional characteristics of the meat. Breeding of Egyptian geese occurs throughout the year but peaks during the months of August to November (late winter to early summer) (Viljoen, 2005; Maclean, 1997), however, in the South-Western Cape Province breeding usually occurs mainly during August to October (Maclean, 1997). Hofbauer *et al*. (2010) emphasize the fact that female wild pheasants have a 15-40% reduced live weight compared to that of their male counterparts. It is also well known that the meat from females tend to have a higher IMF content (Lawrie & Ledward, 2006). This indicates that gender may be an influential factor regarding the production and meat yield of gamebirds; this aspect therefore requires investigation.

Age is known to affect the meat quality of animals (Lawrie & Ledward, 2006). Kokoszynski *et al*. (2011) and Baeza (1998) found that the body weights of domestically raised fowl are significantly influenced by age. Egyptian geese are gamebirds; therefore determination of the specific age is difficult due to the limited literature available on this aspect. Egyptian geese have the appearance of an adult after the first basic plumage (Clancey, 1967); as a result it is only possible to distinguish between adults, juveniles and ducklings. This makes the determination of the specific age of Egyptian geese almost impossible; consequently the effect of age on the meat quality cannot be quantified.

Variation in the meat quality is not only found as a result of diet, gender and age but also within the commercial portions (breast, thigh and drumstick) of a carcass. This tendency is closely related to the type of physical activity of the respective muscle groups in the live bird. Since Egyptian geese are very active waterfowls their movements are not only restricted to walking. Running, swimming, diving and flying also form part of their daily exercise routine. This is very different from the activities of terrestrial gamebirds (Guineafowl) and domestic fowls. It can thus be expected that the different portions will have different physical and chemical characteristics.

This study was therefore aimed at determining the effect of season (diet) and gender on the carcass yield, physical characteristics (pH and colour) and proximate composition of Egyptian goose meat. The variation of these attributes within the three commercial portions will also be investigated. This will not only give insight into the optimum harvesting period but will indicate whether season, gender and portion have a significant effect on these aspects of meat quality.

2 MATERIALS AND METHODS

2.1 Harvesting

Egyptian geese were harvested on the University of Stellenbosch's agricultural experimental farm, Mariendahl (ethical clearance reference number: 10NP_HOF01). The method of wingshooting was applied in order to successfully harvest these waterfowl by the use of double barrelled shotguns. A total of 36 mature geese were harvested during the month of July (winter) 2010. This group consisted of 14 females and 22 males. During November (summer) 2010 a further 33 mature geese, which included 13 female and 20 male geese, were harvested. The geese were collected in the field and held in a refrigerator (4 °C) for approximately 12 h before further processing. As it is impossible to determine gender in these birds from their external appearance, especially when flying, the numbers per gender were unbalanced as the birds were shot randomly.

2.2 Slaughtering and deboning

Slaughtering of the geese (winter and summer) took place in the meat science laboratory of Stellenbosch University. The slaughtering procedures were carried out manually. Firstly, the head was removed at the base position, between the C1 and C2 vertebrae. Then both of the feet were removed at the ankle joint (intertarsal joint) together with the removal of the tip of each wing from the wrist region (carpal joint). Skinning of the geese involved making an incision from the neck to the tail region on the ventral side of the body, followed by the removal of the skin containing the feathers from the body. The geese were then eviscerated by means of an incision in the abdominal muscles. After the slaughtering process, the dressed carcasses were hung over-night (±24 h) in a refrigerated area (4 °C), where after the neck was removed at the base of the junction between the neck and body (shoulder region) and the carcasses were halved by means of a portioning machine. The right side of each carcass was vacuum-packed separately, while the left sides of the carcasses were used within this study. Portioning of the halved left sides of the carcasses into the breast, thigh and drumstick followed. A cut was made at the knee joint to remove the drumstick (containing the *M. gastrocnemius*, *M. peronaeus longus*, *M. flexor perforans*) from the thigh; another cut was made at the junction between the thigh and the post-dorsal region of the carcass in order to remove the thigh (containing the *M. iliotibialis*, *M. semitendinosus*, *M. semimembranosus*, *M. biceps femoris*, *M. sartorius*) at the hip joint from the backbone. The *M. pectoralis* and *M. supracoracoideus* attached to the sternum and the clavicle was removed from the breast region of the carcass by cutting through the shoulder joint and around the *M. pectoralis* on the lateral side to detach the breast from the carcass. The three portions were weighed and then deboned by removing the fibula and tibiotarsus from the drumstick, femur from the thigh, as well as the sternum and clavicle from the breast.

Subsequently, each portion was weighed where after the physical measurements (pH and CIELab colour) were completed and each portion was packaged separately for the proximate analyses of this study. The meat samples were all frozen and stored at -18 °C until the chemical analyses were performed (approximately six months storage time for the samples harvested in winter and two months for the samples harvested in summer).

Carcass yield

Live weight, carcass weight and dressing percentage. The "live" weights of each of the geese (winter and summer) were recorded before the slaughtering process commenced. After slaughtering, the dressed carcasses were hung for approximately 24 h where after the weights of the individual carcasses (after the neck was removed) were recorded. The dressing percentage of each individual goose was determined by calculating the dressed carcass weight as a percentage of the live weight.

Portion yield. Following the portioning process into the breast, thigh and drumstick, the weights of the three portions (bone intact) of each individual goose (winter and summer) were recorded. The portions were then deboned, where after, the muscle and the bone of each portion was weighed individually. The muscle of each portion was calculated as a percentage of the carcass, as well as a percentage of the intact portion. The average total muscle weight of the three respective portions was also determined.

2.3 Physical measurements

The ultimate pH (pH_u) (48 h post mortem) of the breast, thigh and drumstick of each of the individual geese (July and November) were recorded 48 h post mortem. The pH of the thigh and drumstick portions was measured in the *M. iliotibialis* and the *M. gastrocnemius* respectively whilst the pH of the breast (*M. pectoralis*) was measured in the centre of the muscle. The pH was measured by means of a Crison pH25 handheld portable pH meter (Lasec (Pty) Ltd, Cape Town, South Africa) calibrated with the standard buffers (pH 4.0 and pH 7.0) provided by the manufacturer.

2.3.1 Colour

The instrumental colour measurements were taken 48 h post mortem, at three randomly selected positions on each of the three respective portions from winter and summer, according to the method described by Honikel (1998). The colour measurements of the breast muscle were performed on a cut of meat (2 cm), removed from the middle of the portion, across the fibre direction. The colour of the thigh and drumstick was measured on the inside of the deboned meat portion. The colour was recorded using a Colour guide 45˚/0˚ colorimeter (catalogue no: 6805; BYK-Gardner, USA) to establish the L*, a* and b* values with L* indicating the lightness, a* the red-green range and b* the

blue-yellow range. The hue angle (h_{ab}) (°) and chroma value (C^*) were calculated using the a* and b* values and the following equations:

$$h_{ab}=\tan^{-1}\left\{\frac{b^*}{a^*}\right\} \quad C^*=\sqrt{(a^*)^2+(b^*)^2}$$

2.4 Proximate analysis

Proximate analysis was performed on a homogenised meat sample (skin and subcutaneous fat removed) from the breast, thigh and drumstick portions, respectively. This was done for each of the geese (winter and summer). The moisture content (%) was determined according to the Association of Official Analytical Chemist's Standard Techniques (AOAC) method 934.01 (AOAC, 2002a) and the ash content (%) of the moisture free sample was determined by the AOAC method 942.05 (AOAC, 2002b). The chloroform/methanol (1:2 v/v) extraction method stipulated by Lee *et al*. (1996) was used to determine the total lipid (%) (IMF) of the raw meat sample. To establish the total crude protein content (%) the Dumas combustion method 992.15 (AOAC, 2002c) was applied. All proximate analyses in our analytical laboratory are controlled by a National Inter-laboratory Scheme (AgriLASA: Agricultural Laboratory Association of South Africa) where blind samples are analysed once every three months to control and ensure the accuracy and repeatability of the procedures used.

2.5 Statistical analysis

The carcass yield determination involved the main effects; season and gender, as well as the interaction between the two. The experiment thus consisted of 4 treatments (2 seasons X 2 genders) with approximately 36 replicates for each season and gender. The model for the carcass yield design is indicated by:

$$y_{ij} = \mu + s_i + s_j + (ss)_{ij} + \varepsilon_{ij}$$

The terms within the model are defined as; the overall mean (μ), the effect of season (s_i), the effect of gender (s_j), the effect of the interaction $(ss)_{ij}$ and ε_{ij}, the error associated with the effect of season and gender as well as, the interaction between the former and latter.

The analysis of the portion (muscle) as a percentage of the intact portion, physical (pH and colour) and proximate data required the use of a split-plot design. The carcasses were randomly selected for season and gender. Each of the carcasses was considered as one experimental unit and every carcass was further divided into the three different portions (breast, thigh and drumstick). Portion was therefore a sub-plot factor. The model for the split-plot design is indicated by:

$$y_{ij} = \mu + s_i + s_j + (ss)_{ij} + \eta_{ij} + p_k + sp_{ik} + sp_{jk} + ssp_{ijk} + \varepsilon_{ijk}$$

The terms within the model are defined as; the overall mean (μ), the effect of season (s_i), the effect of gender (s_j), the effect of the interaction $(ss)_{ij}$ and η_{ij} is the error associated with the effect of the whole-

plot. The effect of portion forms the sub-plot (p_k), followed by the interactions between; season and portion (sp_{ik}), gender and portion (sp_{jk}) and season, gender and portion (ssp_{ijk}) while ε_{ijk} indicates the error of the sub-plot.

All of the data were subjected to an analysis of variance (ANOVA). The Shapiro-Wilk test was performed to test for normality (Shapiro & Wilk, 1965). All of the outliers were identified and removed before the final analysis. The t-Least Significant Differences (LSD) were calculated at a 5% significance level. Results were defined as being not significant at $P>0.05$ and significant at $P\leq0.05$. SAS™ statistical software (Statistical Analysis System, Version 9.2, SAS Institute Inc., Cary, NC, USA) was used for the analyses of variance (ANOVA).

3 RESULTS

3.1 Carcass yield

As there were no significant interactions, the main effects (gender and season) will be discussed in detail. The carcass yield results are represented in Table 1. The live and carcass weights of the geese were significantly different between the two genders; both of these weights being higher ($P\leq0.05$) in the male fowl. The dressing percentage, however, did not differ ($P>0.05$) between genders. The average weight (g) of the breast, thigh and drumstick was higher ($P\leq0.05$) for the male fowl. The portion (muscle) as a percentage of the carcass and portion (muscle) as a percentage of the intact portion were not affected ($P>0.05$) by gender.

The live and dressed carcass weight, as well as the dressing percentage were not affected ($P>0.05$) by season. The average portion muscle weight were also similar ($P>0.05$) in terms of season. With regard to the muscle as a percentage of the intact portion, the breast and drumstick consisted of more ($P\leq0.05$) muscle in winter. The thigh did not differ ($P>0.05$) as a result of season for the latter.

Table 1 The mean values (±SD[1]) for the carcass yield of Egyptian geese as affected by season and gender

	Season		LSD[2] P=0.05	Gender		LSD[2] P=0.05
	Winter (July) n=36	Summer (November) n=33		Female n=27	Male n=42	
Live weight (g)	2413.4[a] ± 359.4	2470.9[a] ± 430.6	161.5	2165.7[b] ± 226.8	2614.0[a] ± 376.1	165.2
Dressed carcass weight (g)	1312.4[a] ± 221.4	1305.4[a] ± 241.1	93.7	1145.0[b] ± 102.2	1413.3[a] ± 226.6	95.9
Dressing (%)	54.4[a] ± 4.1	52.9[a] ± 3.7	1.9	53.1[a] ± 3.4	54.1[a] ± 4.3	2.0
Portion (muscle) (%) of dressed carcass						
Breast	35.5[a] ± 1.8	33.9[b] ± 2.1	1.0	34.7[a] ± 2.1	34.8[a] ± 2.1	1.0
Thigh	10.2[a] ± 1.2	9.5[b] ± 1.2	0.6	10.0[a] ± 1.3	9.8[a] ± 1.2	0.6
Drumstick	9.3[a] ± 0.7	8.8[b] ± 0.9	0.4	9.1[a] ± 1.0	9.0[a] ± 0.7	0.4
Muscle (%) of intact portion						
Breast	88.3[a] ± 2.8	76.8[b] ± 4.5	1.8	83.5[a] ± 7.7	82.6[a] ± 6.3	1.8
Thigh	85.0[a] ± 2.2	85.6[a] ± 3.0	1.3	85.7[a] ± 2.4	85.0[a] ± 2.8	1.3
Drumstick	75.6[a] ± 2.7	69.2[b] ± 3.0	1.4	72.2[a] ± 4.9	72.9[a] ± 3.8	1.4
Average portion muscle weight (g)						
Breast	233.2[a] ± 41.7	221.5[a] ± 44.9	18.0	198.8[b] ± 22.8	246.1[a] ± 43.3	18.4
Thigh	67.5[a] ± 15.4	61.8[a] ± 15.8	7.3	57.4[b] ± 9.5	69.9[a] ± 17.0	7.4
Drumstick	60.7[a] ± 11.3	57.1[a] ± 11.3	4.9	52.3[b] ± 7.4	63.3[a] ± 11.4	5.0

[a-b] Means in rows within main effect, with different superscripts are significantly different at P≤0.05. [1]SD (standard deviation); [2]LSD (least significant difference).

3.2 Physical characteristics

The results of the physical characteristics (colour and pH) as indicated in Table 2 were significantly affected by season. The L*, a*, b* and chroma values of the meat from the summer geese were higher (P≤0.05) compared to that of winter which indicated a higher (P≤0.05) hue value. The pH was also higher (P≤0.05) in the summer meat.

The colour and pH values within the different portions also varied. The results display that the thigh was the portion with the lightest colour, due to the higher (P≤0.05) L* value, in contrast to the much darker breast portion indicated by the lowest (P≤0.05) L* value. The thigh also had a higher (P≤0.05) a* value (red colour) and b* value (yellow colour) than the breast and the drumstick, the latter two potions did not differ (P>0.05) from each other. Similarly the hue and chroma values were higher (P≤0.05) in the thigh compared to the other portions, whilst the breast and the thigh did not (P>0.05) differ from each other. The pH of the thigh and drumstick portions was higher (P≤0.05) than that of the breast. Gender was not a significant factor (P>0.05) in terms of the colour or pH of the meat.

3.3 Proximate composition

The proximate composition as affected by season, gender and portion is presented in Table 3. Only season had an effect (P≤0.05) on the protein and intramuscular fat content. The meat from summer had a higher (P≤0.05) protein content compared to that from winter; the latter having a higher (P≤0.05) intramuscular fat content. No differences (P>0.05) in terms of season, were found within the moisture and ash contents.

The moisture and intramuscular fat contents of the meat were significantly influenced by gender. The meat from the male fowl had a higher (P≤0.05) moisture content while that of the females had a higher (P≤0.05) fat content.

It is evident that the proximate composition of the different portions varied. The moisture content within each of the respective portions differed (P≤0.05). The drumstick had the highest moisture percentage, followed by the breast and then the thigh with the lowest percentage. The protein content of the breast and drumstick was higher (P≤0.05) than that of the thigh. The breast and the thigh portions indicated the highest (P≤0.05) percentages of intramuscular fat. With regard to the ash content, the mean percentages of each portion were significantly different. The breast was the portion with the highest (P≤0.05) ash content and the thigh had the lowest (P≤0.05) percentage of ash.

Table 2 The mean values (±SD[1]) for the physical characteristics (CIELab colour and pH_u) of Egyptian geese as affected by season, gender and portion

		Number of birds (n)	L*	a*	b*	Hue	Chroma	pH
Season	*Winter*[3]	36	30.06[b] ± 2.93	14.84[b] ± 1.79	9.38[b] ± 1.60	32.06[a] ± 3.06	17.62[b] ± 2.22	5.84[b] ± 0.17
	Summer[4]	33	31.79[a] ± 3.00	18.54[a] ± 2.60	10.19[a] ± 1.66	28.65[b] ± 3.93	21.23[a] ± 2.71	5.99[a] ± 0.11
	LSD[2]		0.90	0.77	0.49	1.33	0.80	0.06
Gender	*Female*	27	31.01[a] ± 3.15	16.94[a] ± 2.87	10.04[a] ± 1.62	30.51[a] ± 3.84	19.77[a] ± 2.97	5.90[a] ± 0.17
	Male	42	30.80[a] ± 3.04	16.40[a] ± 2.89	9.59[a] ± 1.69	30.38[a] ± 3.94	19.07[a] ± 3.09	5.92[a] ± 0.15
	LSD[2]		0.92	0.79	0.50	1.36	0.82	0.06
Portion	*Breast*	69	28.61[c] ± 2.37	16.57[b] ± 2.86	9.58[b] ± 1.89	29.87[b] ± 3.66	19.18[b] ± 3.2	5.85[b] ± 0.17
	Thigh		32.57[a] ± 2.85	17.22[a] ± 2.82	10.43[a] ± 1.37	31.32[a] ± 3.75	20.24[a] ± 2.78	5.95[a] ± 0.13
	Drumstick		31.48[b] ± 2.55	16.04[b] ± 2.90	9.30[b] ± 1.53	30.11[b] ± 4.14	18.61[b] ± 2.99	5.93[a] ± 0.16
	LSD[2]		0.67	0.61	0.48	0.85	0.71	0.03

[a-c] Means in columns, within main effect, with different superscripts are significantly different at P≤0.05. [1]SD (standard deviation); [2]LSD (least significant difference); [3]Winter (July); [4]Summer (November).

Table 3 The mean percentages (±SD[1]) for the proximate composition of Egyptian geese as affected by season, gender and portion

		Number of birds (n)	Moisture	Protein	Fat	Ash
Season	*Winter*[3]	36	72.86[a] ± 2.02	20.15[b] ± 1.49	4.57[a] ± 1.13	1.14[a] ± 0.12
	Summer[4]	33	72.30[a] ± 1.98	20.75[a] ± 1.74	4.10[b] ± 1.11	1.17[a] ± 0.13
	LSD[2]		0.72	0.56	0.44	0.03
Gender	*Female*	27	71.98[b] ± 2.06	20.34[a] ± 1.73	4.65[a] ± 1.11	1.16[a] ± 0.13
	Male	42	72.99[a] ± 1.89	20.50[a] ± 1.58	4.15[b] ± 1.13	1.15[a] ± 0.13
	LSD[2]		0.74	0.57	0.45	0.03
Portion	*Breast*	69	72.56[b] ± 1.31	20.81[a] ± 1.39	4.43[a] ± 1.27	1.23[a] ± 0.13
	Thigh		72.08[c] ± 2.77	19.44[b] ± 1.39	4.63[a] ± 0.96	1.07[c] ± 0.10
	Drumstick		73.15[a] ± 1.51	21.07[a] ± 1.65	3.97[b] ± 1.09	1.17[b] ± 0.10
	LSD[2]		0.43	0.33	0.24	0.03

[a-c] Means in columns, within main effect, with different superscripts are significantly different at P≤0.05. [1]SD (standard deviation); [2]LSD (least significant difference); [3]Winter (July); [4]Summer (November).

4 DISCUSSION

4.1 Carcass yield

Even though it was expected that the carcass yield of Egyptian geese would be influenced by both season and gender; only gender significantly affected the live and carcass weight of Egyptian geese. Similar results have been found in ungulate game species (Hoffman, 2000) but also in wild gamebirds such as pheasants (Hofbauer *et al*., 2010) and bobwhite quails (Ballard *et al*., 1994). This trend is not only evident in the live and carcass weight of the geese but can also be seen in the average muscle weight (g) of the various portions (Table 1). The somewhat higher standard deviation (SD) values for the live weights may have been confounded by a possible variation in the age of the birds. However, it is more likely that this SD is typical of species that has had no artificial selection pressures applied as similar results have been reported for SD values regarding the weight characteristics of wild ungulates (Hoffman *et al*., 2009).

If the carcass characteristics are considered in a more detailed manner (Table 1), season does in fact have an influence. This is apparent in Table 1 which illustrates that the muscle of each portion as a percentage of the dressed carcass was significantly higher in July (winter). July is the month prior to the peak breeding period. The body condition of wild birds, especially females, is known to be influenced by the different stages of breeding (Raveling, 1979; Reinecke *et al*., 1982). This suggests that by the end of July the females were in an excellent condition, with high amounts of stored energy, as provision for the breeding period has thus been made. Throughout this breeding period the energy reserves of the female geese will be depleted. It is important to note that, due to courtship and attending to their mates during egg-laying and incubation, male birds also experience substantial losses in body reserves during the breeding season (Hohman, 1986). However, by the end of October or early November (summer) the geese start to feed on grain seeds during the harvesting season – the harvesting season of wheat usually peaks around November in South Africa. These grains are very high in energy and the body reserves of the geese will therefore be gradually restored. In our study, the summer harvesting of the geese occurred in early November and at that stage the reserves had not yet been restored on an intramuscular level, resulting in a lower muscle yield. This theory should also apply to the muscle as a percentage of the intact portion (Table 1). These results in terms of the breast and drumstick portions do correspond as the percentage muscle were higher ($P \leq 0.05$) in winter, though, the thigh was not affected ($P > 0.05$). Although this is a valid theory, the fact that there were no differences between the average muscle weights of winter and summer (Table 1) create uncertainty regarding this concept. It was therefore necessary to investigate the bone weights as this seemed to be the only other factor that could have been involved in the lower portion

muscle yield in summer (Table 1). In fact, the weights (g) of the breast (65.9 vs. 29.9) and drumstick (24.8 vs. 18.7) bones were higher (P≤0.05) in summer but the weight of the thigh (10.8 vs. 9.6) bone was higher (P≤0.05) in winter. These findings may be linked to the changes that occur in the bone biology of female fowl, before/during egg-laying, where medullary bone is produced rather than structural bone (Whitehead, 2004). This change in the bone structure is due to the reproductive requirements of calcium for eggshell production as the medullary bone, particularly those present in the leg bones, provides the extra calcium necessary (Whitehead, 2004). Larison *et al*. (2001) investigated the mineral sequestration in the leg bones of female White-Tailed Ptarmigan and found that the bone mineral density (BMD), as well as the calcium content of the leg bones rapidly increased several weeks before egg-laying, resulting in a 70% higher BMD in pre-laying birds. It can thus be assumed that the thigh bones (Femur) of the female Egyptian geese in this study underwent the same process and this could possibly clarify the higher mass of the thigh bone (Femur) in July. This postulation together with the results of the bone weights correspond with the results regarding the percentage muscle of the intact portion (Table 1) and explains the fact that no difference was found between the average muscle weights of the July and November portions (Table 1).

Our findings have shown that seasonality may only have a significant, economic impact if the carcasses are sold as an entity. Perhaps a more viable option will be to only provide deboned portions of Egyptian geese. The data does, however, also show that gender may be of greater influence and will create more variation within the carcass characteristics. It is virtually impossible to determine sex while shooting; therefore gender will be a difficult aspect to control.

4.2 Physical characteristics

Gender was not a significant factor regarding the physical characteristics, i.e. colour and pH of the muscles, which is usually more influenced by seasonality, different portions as well as stress factors. The results (Table 2) indicate that, in terms of the seasonal effect, the meat from the geese hunted in summer was lighter (P≤0.05) and more yellow (P≤0.05) compared to that of winter. It is possible that the high L* value of the meat can be connected to the grain based diet of November. It is known that fat particles, with a high melting point (more saturated) clustered together appear to be whiter than fat with a lower melting point (more unsaturated) (Wood *et al*., 2003). Studies have also shown that the colour of the subcutaneous and intramuscular fat of grass-fed beef become whiter when the feed intake becomes grain-based (Forrest, 1981; Strachan *et al*., 1993; French *et al*., 2000). Fat colour is also correlated to the carotenoid content of the forage and animals that feed on grass-based diets generally have higher levels of this compound present in their fat, which results in a more yellow and less white colour. However, the a* and b* values of the meat from November did not correlate well with this theory but when the hue angle and chroma value, which encompass both these values, are

considered the November meat had a much more vivid, red colour as opposed to the July meat situated more towards the yellow area on the colour wheel. The colour profile is also related to the pH_u which was significantly higher in November. This suggests that the geese may have been more active during the summer period as they forage to rebuild body condition after the breeding season/winter, resulting in an increased myoglobin content and red coloured meat (Lawrie & Ledward, 2006).

The instrumental colour results also revealed that the breast and drumstick portions differed from the thigh which was lighter (higher L* value) and less red in colour (higher hue angle), however, the red colour was more vivid (higher chroma value). The lighter colour may be related to the higher ($P \leq 0.05$) intramuscular fat content (Table 3). Priolo *et al*. (2001) explains that fat is lighter in colour compared to muscle and therefore contributes to a higher L* value. The lighter colour can also be correlated to the composition of the muscle fibre types; waterfowl species use the leg muscles for walking, swimming and diving and it therefore consists of a combination of type I (red, slow, oxidative), type IIb (red, fast, oxidative-glycolytic) and type IIa (white, fast, glycolytic) fibres (Turner & Butler, 1988; Butler, 1991). The higher intramuscular fat content together with the high a* value suggests that the thigh consisted of a greater proportion of type I, red, oxidative fibres as muscles predominantly containing these fibres store fat rather than glycogen for energy metabolism (Wood *et al*., 2003; Lawrie & Ledward, 2006). Although the thigh portion had a higher hue angle (less red), the a* value was significantly higher than that of both the breast and the drumstick. This may be clarified by the correspondingly high b* value (yellow colour) due to the increased fat content, the latter being responsible for the higher hue angle. The high a* value together with the higher pH of the thigh portion confirms that Egyptian geese do walk more than fly. Especially since the pH of the drumstick is also higher ($P \leq 0.05$) than the breast.

The overall colour of meat is a very important aspect of consumer acceptability and is used as an indication of the quality (Troy & Kerry, 2010). It is one of the first attributes recognised by the customer and is therefore critical in the decision making process. An aspect that is noteworthy is the overall dark colour of Egyptian goose meat as a dark coloured meat is often unfavourable in terms of consumer preference (Von la Chevallerie, 1972). Another essential aspect is the uniformity of the meat; it should always have a consistent quality (Wiklund *et al*., 2003). Although significant differences were found as a result of the season and portion effects, it is questionable whether these differences are substantial enough to be recognised by the average consumer.

4.3 Proximate composition

The proximate composition (Table 3) was significantly influenced by season, gender and portion. It appears as though the breeding period was responsible for the increased ($P \leq 0.05$) IMF content of the

meat harvested in July. The peak breeding period of Egyptian geese is between August and October in the Western Cape of South Africa. The body fat or energy levels of female wildfowl is highly influenced by the breeding period and in the days preceding egg-laying these levels are very high as the geese have been preparing their body for the strain it will endure during this period (Raveling, 1979; Reinecke *et al*., 1982). This is verified by the fact that female geese had an overall higher ($P \leq 0.05$) IMF of 4.6% when compared to that of the males. However, it is also generally accepted that the muscle of female animals do contain a higher IMF content (Lawrie & Ledward, 2006). The negative relationship that exists between the fat and moisture content of muscle is also found within the gender effect of this study where the moisture content of the male muscles was higher ($P \leq 0.05$) due to the lower ($P \leq 0.05$) IMF present. This trend between IMF and moisture can also be seen in the proximate data of the various portions.

There is a higher incidence of IMF in the thigh and breast portions of Egyptian geese. The breast muscle (*pectoralis*) of Anseriformes is used during flying, which requires fast, sustained muscle contraction, and therefore consists of red, FOG (fast, oxidative-glycolytic) together with a small amount of FG (fast, glycolytic) fibres (George & Berger, 1966). Muscle fibres with an oxidative metabolism have a higher intramuscular fat content as red, oxidative fibres use fat as an energy source during metabolism (Wood *et al*., 2003; Lawrie & Ledward, 2006). A similar explanation applies to the thigh which is comprised of a combination of the three main fibre types but these muscles are also used for postural activity which indicates that slow, type I, oxidative fibres are present. However, anatomically the thigh portion may have more physical capacity in order to accommodate intramuscular fat deposition.

Even though the ash content was not affected by season and gender it did in fact differ ($P \leq 0.05$) between portions with the breast muscle having the highest amount of ash. The ash content is an indication of the total amount of minerals present in muscle and the quantity thereof can vary as a result of species differences, hormones, age, gender, region and diet (Keeton & Eddy, 2004). Additionally, Doornenbal and Murray (1981) found that the mineral concentration of beef varies considerably between different muscles. The variable ash contents of the breast, thigh and drumstick portions of Egyptian geese may therefore be linked to the inherent muscle effect. Although, studies investigating terrestrial avian muscles have found contradictory results in terms of the ash content of the leg and breast muscles (Zarkadas *et al*., 1987; Hofbauer *et al*., 2010). These studies reported that the leg muscles of pheasants and chickens were higher in ash compared to the breast muscles. It can therefore be postulated that the ability of Egyptian geese to fly long distances may somehow be involved in the higher ash content of the breast muscle. There is limited literature regarding this matter and further research is necessary to confirm this theory.

The main concern in terms of the proximate composition is the fact that both season and gender have a significant influence on the IMF content of the meat. Variation in the IMF content will not only influence the sensory attributes such as flavour and tenderness, but the nutritional value as well. It is therefore essential that these factors are kept constant in order to ensure a consistent eating quality.

5 CONCLUSIONS

This study revealed several factors which are important to consider regarding the potential, commercial utilisation of Egyptian goose meat. Gender affects the carcass characteristics and IMF content of the meat which should be taken into account for economical and nutritional reasons, respectively. The main issue is seasonality as there are some differences in the quality of the meat from geese hunted during the summer and winter periods in South Africa. This particularly concerns the IMF content of the meat and selected physical parameters but it is questionable whether the physical differences will be recognised by the average consumer. Even so, the results indicate that in order to ensure a consistent eating quality the harvesting periods should be considered and kept constant.

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CHAPTER 6

The fatty acid, amino acid and mineral composition of Egyptian goose meat as affected by season, gender and portion

ABSTRACT

With the current absence of scientific information on the nutritive aspects, it is essential to investigate the fatty acid, mineral and amino acid composition of Egyptian geese, as well as the factors of influence. The forage vs. grain based diets of Egyptian geese during certain periods of the year lead to variation in the content of the key fatty acids such as oleic acid, linoleic acid and α-linolenic acid. The differences in these fatty acids results in variation between the *n*-6/*n*-3 ratios of the seasons; the portions from July (winter) are within the health recommendations (ratio <5) and those from November (summer) not. This study indicates that Egyptian goose meat does not only vary in nutritional composition but season may also have a substantial effect on the flavour profile and ultimate uniformity of the meat. The season and portion effects were, however, interlinked but the general tendency shows that the portions, especially the breast and thigh do differ concerning the major fatty acids. No substantial differences were found in the mineral composition of the breast portion on account of season and gender, however, there were some variation in certain amino acids such as lysine and arginine due to season/diet. This research provides essential information that should be considered not only regarding the everyday consumption of Egyptian goose meat, but also the potential utilisation and ultimate consistency of this meat product.

***Keywords*:** Gamebirds, Egyptian geese, Chemical composition, Fatty acids, Amino acids, Minerals

1 INTRODUCTION

A large variety of unconventional species are valuable food sources for many individuals worldwide (Hoffman & Wiklund, 2006; Hoffman, 2008; Hoffman & Cawthorn, 2012). The consumption of meat from these species is an important source of essential nutrients, especially within the rural areas of developing countries. Regardless of the invaluable contribution of non-traditional meat sources towards food security, the meat from these species is also becoming more popular amongst modern consumers and tourists (Hoffman *et al*., 2003). Wild species which are either extensively found or that are often referred to as being agricultural pests have the potential to be sustainably harvested for food production (Cooper, 1995; Geldenhuys *et al*., 2013a).

In recent years, the hunting of wildfowl species in South Africa has increased considerably (Geldenhuys *et al*., 2013a) [Chapter 2]. This is mainly due to the growing popularity of this sport. However, some wildfowl species are also hunted to reduce the population numbers as they are considered to be agricultural pests. Crop farmers in South Arica incur major financial losses due to the feeding activities of Egyptian geese (*Alopochen aegyptiacus*) (Mangnall & Crowe, 2001; 2002). Consequently, a large number of geese are hunted annually in order to reduce the damage caused. The meat generated is mainly consumed by the hunters or donated to the local, rural communities because, as of yet, it has not been introduced into the commercial market. In order to market Egyptian goose meat; accurate, scientific information regarding the nutritional characteristics of this species and the factors of influence, i.e. season and gender, is vital. This is particularly important since consumers nowadays demand lean meat with adequate nutritional requirements (Hoffman & Wiklund, 2006).

The fatty acid composition of red meat has certainly been a controversial topic in terms of human health (Orellana *et al*., 2009; MacAfee *et al*., 2010). Research has shown that the ratios of polyunsaturated fatty acids to saturated fatty acids (PUFA/SFA) and omega 6 to omega 3 (*n*-6/*n*-3) polyunsaturated fatty acids (PUFA) are the major risk factors associated with cardiovascular disease. It is recommended that the intake of saturated fatty acids (SFA), trans fatty acids (TFA) and cholesterol are lowered while an increase in the consumption of omega 3 fatty acids, specifically eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) is beneficial. Factors such as the diet of gamebirds related to seasonal variation as well as gender may have quite an impact on the nutritional characteristics of meat.

Contrary to its fatty acid composition, the role of meat as a protein source is very unambiguous. Meat provides all of the amino acids which are essential for human nutrition (Pereira & Vicente, 2013). Additionally, meat is also an excellent source of minerals especially iron, zinc, selenium and phosphorus (Biesalski & Nohr, 2009; McNeill & Van Elswyk, 2012; Pereira & Vicente, 2013).

These nutritional constituents not only contribute in a dietetic capacity but they can also significantly influence the sensory characteristics and palatability of meat. The fatty acid composition plays a key role in the aroma and flavour profile (Wood *et al*., 2003; Calkins & Hodgen, 2007). It is also evident that certain minerals, specifically iron is associated with an increased metallic or livery flavour in meat (Yancey *et al*., 2006).

At present there is an absence of accurate nutritional information on the meat of wildfowl species particularly Egyptian geese. The research by Geldenhuys *et al*. (2013c) shows that the chemical composition of Egyptian goose meat is very distinct compared to other well-known fowl species and it is speculated that the dietary differences are responsible. However, further investigation is required as factors such as season, gender and portion should be considered. This study therefore investigates the nutritional aspects of Egyptian goose meat which may be influential in terms of human health. It also considers the effect of factors such as season, gender and portion on the fatty acid, amino acid and mineral composition of Egyptian goose meat. This will not only provide insight into the nutritional value of a species of which little information is available but will also allow for a better understanding of the factors which may influence the nutritional value as well as the sensory profile of the meat.

2 MATERIALS AND METHODS

2.1 Harvesting and processing

Egyptian geese were harvested on the University of Stellenbosch's agricultural experimental farm, Mariendahl (ethical clearance reference number: 10NP_HOF01). The method of wingshooting was applied in order to successfully harvest these waterfowl by the use of double barrelled shotguns. The geese were collected in the field and held in a refrigerator (4 °C) for approximately 12 h before further processing.

Slaughtering of the geese (winter and summer) took place in the meat science laboratory of Stellenbosch University. The geese were manually slaughtered and deboned according to the technique described by Geldenhuys *et al*. (2013b) [Chapter 5].

2.2 Sampling

The sample set is illustrated in Table 1. Meat samples for the various chemical analyses were collected from geese that were harvested during winter (July) and summer (November) of 2010. Nine male and nine female birds from each of the respective seasons were selected based on the method described in section 2.6 (Statistical analysis). The fatty acid analysis was conducted on each of the three main portions (breast, thigh and drumstick) whilst the amino acid and mineral analyses were only conducted on the breast portion.

To quantify the effect of diet/season on the muscle chemical (especially fatty acid) composition, the crop contents from 6 birds per season were collected, pooled and analysed for fatty acids.

Table 1 Sample set for analyses on Egyptian goose meat samples

	Winter (July)		**Summer (November)**	
	Male	**Female**	**Male**	**Female**
Number of birds analysed (n=36)	9	9	9	9
Portions analysed for:				
Fatty acid composition	Breast, Thigh and drumstick			
Amino acid composition	Breast			
Mineral composition	Breast			

2.3 Fatty acid analysis

The fatty acid profile was determined after the homogenised meat and crop content samples were defrosted. A 2 g sample was extracted by the use of a chloroform:methanol (2:1; v/v) solution according to the method described by Folch *et al*. (1957). The extraction solvents contained 0.01% butylated hydroxytoluene (BHT) which operated as an antioxidant. The meat sample with the extraction solvent was homogenised by means of a polytron mixer (WiggenHauser Homogenizer, D-500 fitted with a standard shaft 1; speed setting D). Heptadecanoic acid (C17:0) (catalogue number H3500, Sigma–Aldrich Inc., 3050 Spruce Street, St. Louis, MO 63103, USA) was used as an internal standard to quantify the individual fatty acids present within the meat sample. Transmethylation of a 250 µL sub-sample of the extracted lipids occurred at 70 °C for 2 h and a methanol/sulphuric acid (19:1; v/v) solution (2 mL) was used as the transmethylating agent. The mixture was then cooled to room temperature followed by the extraction of the fatty acid methyl esters (FAME) with water and hexane by transferal of the hexane phase to a spotting tube, after which it was dried under nitrogen. Hexane (50 µL) was then added to the dried FAME sample and 1 µL was injected into the gas-chromatograph. The FAME was determined with a Thermo Finnigan Focus gas-chromatograph (Thermo-Electron S.p.A, Rodana, Milan, Italy) equipped with a flame ionized detector and a 60 m BPX70 capillary column (internal diameter of 0.25 mm, 0.25 µm film, SGE International, Ringwood, Victoria, Australia). The gas flow rate of the carrier, hydrogen, was 30 mL/min. The following temperature settings where applied: initial temperature of 60 °C, injector and detector 220 °C and 260˚C respectively and a final temperature of 160 °C. The GC injection volume was 1 µL with a run time of approximately 45 min. By comparing the FAME of the meat samples with a standard FAME mixture (Supelco, 37 Component FAME mix C4-C24, Cat, no. 47885-U. Supelco, North Harrison Rd, Bellefonte, PA 16823-0048, USA) the FAME levels were identified. The results were recorded as percentage (%) of the total fatty acids.

2.3.1 Fatty acid composition of crop contents

The fatty acid composition of the dietary constituents obtained from the crop contents of the geese was also analysed according to the method described in section 2.3 and is presented in Table 2. The results reflect the composition of six pooled samples (n=6) from each respective season therefore statistical differences cannot be shown.

Table 2 Fatty acid composition (%) of the crop contents of Egyptian geese harvested in winter and summer

Fatty acid	Winter (July)	Summer (November)
SFA		
14:0	0.404	0.073
15:0	0.250	0.011
16:0	16.679	6.813
18:0	3.186	1.017
20:0	0.363	0.066
21:0	0.000	0.013
22:0	1.118	0.073
24:0	0.443	0.038
23:0	0.000	0.000
MUFA		
14:1	0.241	0.000
15:1	0.000	0.000
16:1	2.464	0.053
18:1 *n*-9t	0.170	0.009
18:1 *n*-9c	8.432	75.005
20:1	0.470	0.437
22:1 *n*-9	0.193	0.040
24:1	0.000	0.031
PUFA		
18:2 *n*-6*t*	0.142	0.008
18:2 *n*-6*c*	16.779	15.587
18:3 *n*-3	46.453	0.548
18:3 *n*-6	0.637	0.023
20:2	0.000	0.039
20:4 *n*-6	0.281	0.024
20:3 *n*-6	0.000	0.000
20:3 *n*-3	0.085	0.004
20:5 *n*-3	0.165	0.017
22:2 *n*-6	0.313	0.028
22:5 *n*-3	0.413	0.024
22:6 *n*-3	0.319	0.020
SFA[1]	22.443	8.102
MUFA[2]	11.970	75.575
PUFA[3]	65.587	16.322
PUFA/SFA[4]	2.922	2.015
(*n*-6)/(*n*-3)[5]	0.383	25.547

[1]SFA (saturated fatty acids); [2]MUFA (mono-unsaturated fatty acids); [3]PUFA (polyunsaturated fatty acids); [4]PUFA/SFA (polyunsaturated fatty acid to saturated fatty acid ratio); [5]*n*-6/*n*-3 (omega 6 to omega 3 ratio).

2.4 Amino acid analysis

Dried and defatted muscle samples (0.1 g) were hydrolysed in a glass hydrolysis tube with 6 mL (6N) hydrochloric acid (HCl) and 15% phenol sealed in a vacuum after flushing with nitrogen gas. These samples were placed in an oven for 24 hours at 110°C. Following the hydrolysis, samples were stored at -20 °C in Eppendorf tubes until further analysis commenced.

The amino acid profile of each sample was determined by the use of a Dionex high performance liquid chromatography (HPLC) unit. The amino acids were prepared for injection into the HPLC by the filtration of 1 mL of hydrolysed protein sample through a 33 mm Millex-HV 0.45 µm filter 55 into a second Eppendorf tube. The filtered sample (10 µL) was pippeted into an Erlenmeyer flask and the following was added: 4 mL distilled water, 800 µL Borate buffer, 10 µL NorValine. These sample solutions (1 mL) were then injected in the HPLC with RF2000 Fluorescence detector and a Nova-Pak C18 4 µm, 3.9 x 150 mm column using Chromeleon 6.80 software. The results were read as amount of moles per mL sample and converted to g/100 g meat sample.

2.5 Mineral analysis

The minerals analysed were calcium, potassium, magnesium, sodium, iron, copper, zinc, manganese, phosphorus, boron and aluminium. The mineral content of a 0.5 g defatted, dried and finely ground meat sample was determined. Ashing of the sample occurred at 460-480 °C for 6 h, followed by the cooling of the sample and addition of 5 mL of 6 M HCl. The sample was placed in an oven (50 °C) for 30 min where after 35 mL of distilled water was added, the solution was filtered and distilled water was added to obtain a final volume of 50 mL (Method 6.1.1 AGRILASA, 2007). An iCAP 6000 Series Inductive Coupled Plasma (ICP) spectrophotometer (Thermo Electron Corporation, Rodana, Milan, Italy) fitted with a vertical quartz torch and Cetac ASX-520 auto sampler was used to measure the elements. The concentrations of the elements were calculated by means of iTEVA Analyst software. The Argon gas flow rate was 2-5 mL/min and the settings for the instrument included the following: camera temperature -27 °C, generator temperature 24 °C, optics temperature 38 °C, RF power 1150 W, pump rate 50 rpm, aux gas flow 0.5 L/min, nebulizer 0.7 L/min, coolant gas 12 L/min and normal purge gas flow. The wavelengths for the elements were the following: Al 167.079 nm, B 249.773 nm, Ca 317.933 nm, Cu 324.754 nm, Fe 259.940 nm, K 766.490 nm, Mg 285.213 nm, Mn 257.610 nm, Na 589.592 nm, P 177.495 nm and Zn 213.856 nm. The minerals were recorded as mg/100 g dry meat sample. After the analysis of 11 samples, standards of high, medium and low range were analysed for quality control.

2.6 Statistical analysis

In order to obtain a representative sample set, the samples (nine birds for season and gender respectively) were selected based on the proximate composition of an initial group of birds (n=69). Ward's clustering (XL Stat software, Version 2011, Addinsoft, New York, USA) was used to group the birds into 9 clusters, within each respective gender and season, based on the proximate data. From each of the clusters one bird/carcass was selected to form the sample set.

The amino acid and mineral compositions of the breast portion involved the main effects; season and gender, as well as the interaction between the two. These analyses were performed on nine birds/breast portions (n=9) from the two respective seasons (July and November) and genders. The model for the amino acid and mineral composition design is indicated by:

$$y_{ij} = \mu + s_i + g_j + (sg)_{ij} + \varepsilon_{ij}$$

The terms within the model are defined as; the overall mean (μ), the effect of season (s_i), the effect of gender (g_j), the effect of the interaction $(sg)_{ij}$ and ε_{ij}, the error associated with the effect of season and gender as well as, the interaction between the former and latter.

The analysis of the fatty acid data required the use of a split-plot design. Each of the carcasses was considered as one experimental unit and every carcass was further divided into the three different portions (breast, thigh and drumstick). Portion was therefore a sub-plot factor. The model for the split-plot design is indicated by:

$$y_{ij} = \mu + s_i + g_j + (sg)_{ij} + \eta_{ij} + p_k + sp_{ik} + gp_{jk} + sgp_{ijk} + \varepsilon_{ijk}$$

The terms within the model are defined as; the overall mean (μ), the effect of season (s_i), the effect of gender (g_j), the effect of the interaction $(sg)_{ij}$ and η_{ij} is the error associated with the effect of the whole-plot. The effect of portion forms the sub-plot (p_k), followed by the interactions between; season and portion (sp_{ik}), gender and portion (gp_{jk}) and season, gender and portion (sgp_{ijk}) while ε_{ijk} indicates the error of the sub-plot.

All of the data were subjected to an analysis of variance (ANOVA). All of the outliers were identified and removed before final analysis of the ANOVA's. The t-Least Significant Differences (LSD) were calculated at a 5% significance level to compare the treatment means. Results were defined as being not significant at $P>0.05$ and significant at $P\leq0.05$. SAS™ statistical software (Statistical Analysis System, Version 9.2, SAS Institute Inc., Cary, NC, USA) was used for the analyses of variance (ANOVA). A Principle Component Analysis (PCA) and Discriminant Analysis (DA) were performed and used in order to indicate and clarify the relationship between the portion effect and the individual fatty acids present (Næs *et al*., 2010). The multivariate analysis was conducted using XLStat software (Version 2012, Addinsoft, New York, USA).

3 RESULTS AND DISCUSSION

3.1 Fatty acid composition

The significant interactions between the main effects, regarding the fatty acid composition (%) of Egyptian goose meat, are presented in Table 3. The variation in season as well as portion had the largest effect on the fatty acid profile. There were no significant interactions between season, gender and portion (SxGxP) and season and gender (SxG). The main effect of gender also did not have a significant influence on the fatty acid profile of Egyptian goose meat. Although all of the affected fatty acids are listed in the tables only the main fatty acids will be discussed. The fatty acid composition of the breast, thigh and drumstick portions, expressed as mg/g meat will not be discussed but is added as Addendum A to this chapter for use in compiling human nutritional data bases.

The individual fatty acids which were significantly affected by the interaction of gender and portion (GxP) are listed in Table 4. The percentage of palmitic acid (C16:1 *n*-7) was found to be higher ($P \leq 0.05$) in the female drumstick compared to the other portions. The concentrations did, however, not differ ($P > 0.05$) from the male drumstick. The female drumstick and male thigh portions had higher concentrations of eicosenoic acid (C20:1 *n*-9) but not significantly higher than the male drumstick. The γ-linolenic acid (C18:3 *n*-6) content was also higher ($P \leq 0.05$) in the female drumstick portion.

The significant interactions between season and portion (SxP) are presented in Table 5. The percentage of oleic acid (C18:1 *n*-9) (49%) was highest ($P \leq 0.05$) in the thigh portion from the geese harvested in summer. The summer thigh portion had equally high levels (17%) of linoleic acid (C18:2 n-6) which did not differ significantly from the breast (16%) and drumstick (15%) portions. Overall the winter meat were higher in α-linolenic acid (C18:3 *n*-3) compared to those from summer and the thigh portion contained the highest ($P \leq 0.05$) percentage (19%) of this fatty acid. The total saturated fatty acid (SFA) concentration was highest in the winter breast portion (36%), however, it did not differ ($P > 0.05$) from the winter (32%) and summer (34%) drumsticks. The summer thigh portions also proved to have the highest ($P \leq 0.05$) total concentration (51%) of monounsaturated fatty acids (MUFA) as well as the highest ratio (15.7) of omega 6 to omega 3 (*n*-6/*n*-3) fatty acids. The latter ratio is high compared to that of the winter breast portion with a ratio of 1.9. This is because of the increased linoleic acid (C18:2 *n*-6) and decreased α-linolenic acid (C18:3 *n*-3) content of the summer portions.

The main effect of season on the fatty acid profile of Egyptian goose meat is presented in Table 6. All of the fatty acids affected by season were present in higher ($P \leq 0.05$) levels in winter. These include all of the long chain polyunsaturated fatty acids, specifically arachidonic acid (C20:4 *n*-6) as well as the total polyunsaturated fatty acids (PUFA).

The fatty acids which were affected by the variation in portion are listed in Table 7 and this variation is visualised by the Discriminant Analysis (DA) plot in Fig. 2. The main fatty acids affected was arachidonic acid (C20:4 *n*-6) as well as the total PUFA; both were present in higher ($P \leq 0.05$) percentages in the breast portion of Egyptian geese. The total PUFA content did, however, not differ ($P > 0.05$) between the breast and drumstick portions.

So as to have a better understanding of the results, the PCA bi-plot (Fig. 1) containing the fatty acid data of the breast portion provides for a more visual representation but also allows for a broader perspective. PCA plots are used to demonstrate the relationships between different attributes, in this case the fatty acid profile, as well as their association with the treatments/samples. The PCA bi-plot (Fig. 1) illustrates the clear discrepancy between the fatty acid profiles of the meat from the two harvesting periods. The fatty acids illustrated in the left half of the 1st principal component (PC1/F1) associate with the breast meat harvested in winter. The driving forces behind this strong association are mainly the PUFA and SFA contents. The breast portions from the geese harvested in summer are mostly found in the right half and they are closely associated with MUFA and oleic acid (C18:1 *n*-9*c*).

Table 3 The P-values[1] indicating the impact of season, gender and portion on the fatty acid composition (% total fatty acids) of Egyptian goose meat

Fatty acid	SxGxP[2]	GxP[3]	SxP[4]	SxG[5]	Season	Gender	Portion
SFA							
14:0	0.8113	0.2315	**0.0208**	0.7214	**0.0147**	0.2140	**0.0024**
15:0	0.8250	0.1354	**0.0223**	0.5905	**0.0013**	0.5763	**0.0001**
16:0	0.9335	0.9827	0.2321	0.5249	0.7460	0.2167	0.1007
18:0	0.4226	0.5808	**0.0068**	0.6391	**0.0534**	0.1737	**<.0001**
20:0	0.4378	0.3515	**0.0199**	0.5420	**0.0751**	0.3038	**<.0001**
21:0	0.1806	0.0828	**0.0151**	0.2367	0.1912	0.5209	**<.0001**
22:0	0.9507	0.5499	**0.0288**	0.6238	**0.0466**	0.2013	**<.0001**
23:0	0.1717	0.8118	0.9281	0.5470	0.8796	0.4053	0.4166
MUFA							
14:1	0.8864	0.1328	0.3173	0.9317	**0.0030**	0.9602	**0.0001**
16:1 *n*-7	0.7681	**0.0393**	0.1957	0.9100	**0.0017**	0.5798	**<.0001**
18:1 *n*-9*c*	0.7398	0.5065	**0.0405**	0.0831	**0.0013**	0.9645	**0.0004**
20:1 *n*-9	0.3295	**0.0009**	**0.0029**	0.2269	0.8161	0.6532	**0.0004**
22:1 *n*-9	0.2362	0.1036	0.2471	0.2894	**0.0473**	0.9455	**<.0001**
24:1 *n*-9	0.4408	0.9545	0.7478	0.5880	0.1281	0.3299	**<.0001**
PUFA							
18:2 *n*-6*c*	0.5033	0.3636	**0.0333**	0.0953	**0.0068**	0.6279	0.1315
18:3 *n*-3	0.3053	0.8566	**<.0001**	0.5739	**<.0001**	0.5002	**0.0004**
18:3 *n*-6	0.1084	**0.0081**	**0.0148**	0.1185	0.2865	0.3242	**0.0002**
20:2	0.2480	0.6371	**0.0140**	0.5228	0.3477	0.7086	**<.0001**
20:3 *n*-6	0.5391	0.4087	0.1040	0.2745	**0.0273**	0.5843	**<.0001**
20:3 *n*-3	0.5138	0.2039	0.0699	0.3520	**<.0001**	0.7117	**<.0001**
20:4 *n*-6	0.1946	0.1353	0.3042	0.4924	**0.0449**	0.1738	**<.0001**
20:5 *n*-3	0.8723	0.1470	**<.0001**	0.5884	**<.0001**	0.7301	**<.0001**
22:2	0.5467	0.6112	**0.0376**	0.8758	0.1158	0.4132	**<.0001**
22:5 *n*-3	0.9951	**0.0245**	0.8253	0.7606	**0.0122**	0.8979	**<.0001**
22:6 *n*-3	0.1397	0.0807	0.6244	0.3486	**0.0090**	0.4924	**<.0001**
SFA[6]	0.8256	0.9479	**0.0466**	0.4584	0.1706	0.7017	**0.0231**
MUFA[7]	0.7416	0.5455	**0.0543**	0.0826	**0.0019**	0.9549	**0.0006**
PUFA[8]	0.2512	0.2621	0.5012	0.0600	**0.0006**	0.8024	**0.0046**
PUFA/SFA[9]	0.5388	0.3067	0.6820	0.2409	0.4001	0.4461	0.5432
n-6/n-3[10]	0.7743	0.7437	**0.0069**	0.8027	**0.0089**	0.6900	**0.0184**

[1]P-values in bold indicate significance at P≤0.05. [2]Interaction between harvesting season, gender and portion type (SxGxP); [3]interaction between gender and portion type (GxP); [4]interaction between harvesting season and portion type (SxP);[5]interaction between harvesting season and gender (SXG); [6]SFA (saturated fatty acids); [7]MUFA (mono-unsaturated fatty acids); [8]PUFA (polyunsaturated fatty acids); [9]PUFA/SFA (polyunsaturated fatty acid/saturated fatty acid ratio); [10]*n*-6/*n*-3 (omega 6/omega 3 ratio).

Table 4 The means (±SD[1]) of the fatty acids (% total fatty acids) of Egyptian goose meat significantly affected by the interaction between gender and portion

Fatty acid	Female			Male			LSD[2] P=0.05
	Breast	Drumstick	Thigh	Breast	Drumstick	Thigh	
16:1 *n*-7	1.584^{bc} ± 0.846	2.308^{a} ± 0.916	1.075^{d} ± 0.683	1.306^{cd} ± 0.761	1.917^{ab} ± 0.844	1.464^{bcd} ± 0.704	0.457
20:1 *n*-9	0.251^{bc} ± 0.112	0.355^{a} ± 0.109	0.219^{c} ± 0.144	0.226^{c} ± 0.093	0.310^{ab} ± 0.124	0.336^{a} ± 0.202	0.063
18:3 *n*-6	0.082^{b} ± 0.026	0.106^{a} ± 0.046	0.060^{c} ± 0.045	0.069^{cb} ± 0.022	0.081^{b} ± 0.038	0.073^{cb} ± 0.031	0.018
22:5 *n*-3	0.868^{b} ± 0.473	1.264^{a} ± 0.708	0.405^{c} ± 0.559	0.878^{b} ± 0.552	1.003^{b} ± 0.628	0.596^{c} ± 0.505	0.229

$^{a-d}$Means in rows with different superscripts differ significantly at P≤0.05.
[1]SD (standard deviation); [2]LSD (least significant difference).

Table 5 The mean scores (±SD[1]) of the fatty acids (% total fatty acids) of Egyptian goose meat significantly affected by the interaction between season and portion

Fatty acid	Winter (July)			Summer (November)			LSD[2]
	Breast	Drumstick	Thigh	Breast	Drumstick	Thigh	P=0.05
14:0	0.271^{ab} ± 0.107	0.314^{a} ± 0.103	0.301^{a} ± 0.136	0.214^{bc} ± 0.089	0.331^{a} ± 0.159	0.162^{c} ± 0.09	0.080
15:0	0.085^{a} ± 0.02	0.099^{a} ± 0.026	0.089^{a} ± 0.035	0.061^{b} ± 0.024	0.092^{a} ± 0.044	0.043^{b} ± 0.036	0.019
18:0	14.293^{a} ± 3.92	11.229^{b} ± 4.651	7.320^{c} ± 4.433	10.075^{b} ± 4.429	10.579^{b} ± 6.333	3.736^{d} ± 3.795	1.636
20:0	0.092^{bc} ± 0.029	0.113^{a} ± 0.052	0.084^{cd} ± 0.051	0.066^{d} ± 0.032	0.106^{ab} ± 0.065	0.040^{e} ± 0.04	0.018
21:0	0.174^{a} ± 0.06	0.158^{a} ± 0.062	0.117^{c} ± 0.067	0.158^{a} ± 0.081	0.153^{a} ± 0.061	0.06^{b} ± 0.072	0.026
22:0	0.107^{a} ± 0.041	0.096^{ab} ± 0.046	0.066^{d} ± 0.047	0.072^{cd} ± 0.043	0.085^{bc} ± 0.053	0.030^{e} ± 0.031	0.015
18:1 *n*-9*c*	24.375^{d} ± 9.769	30.764^{cd} ± 11.811	33.619^{bc} ± 10.567	40.382^{b} ± 18.984	35.910^{bc} ± 14.079	49.719^{a} ± 13.988	6.850
20:1 *n*-9	0.220^{c} ± 0.075	0.314^{ab} ± 0.106	0.328^{a} ± 0.177	0.258^{bc} ± 0.123	0.351^{a} ± 0.128	0.227^{c} ± 0.18	0.063
18:2 *n*-6*c*	13.563^{bc} ± 2.496	11.483^{cd} ± 3.182	9.151^{d} ± 4.62	16.430^{ab} ± 7.098	14.949^{ab} ± 5.307	17.084^{a} ± 0.537	2.919
18:3 *n*-3	10.635^{c} ± 4.306	13.154^{b} ± 8.269	18.942^{a} ± 9.388	3.551^{d} ± 2.23	4.992^{d} ± 3.405	3.103^{d} ± 4.442	2.221
18:3 *n*-6	0.076^{b} ± 0.016	0.091^{ab} ± 0.034	0.082^{ab} ± 0.04	0.074^{b} ± 0.031	0.096^{a} ± 0.052	0.051^{c} ± 0.031	0.018
20:2	0.055^{bc} ± 0.015	0.064^{ab} ± 0.025	0.053^{bc} ± 0.031	0.045^{cd} ± 0.017	0.075^{a} ± 0.039	0.032^{d} ± 0.024	0.015
20:5 *n*-3	1.488^{a} ± 0.699	0.782^{b} ± 0.405	0.518^{c} ± 0.374	0.397^{c} ± 0.295	0.217^{d} ± 0.165	0.075^{d} ± 0.077	0.167
22:2	0.111^{a} ± 0.058	0.104^{a} ± 0.056	0.072^{b} ± 0.054	0.074^{b} ± 0.05	0.096^{a} ± 0.071	0.034^{c} ± 0.036	0.019
SFA[3]	35.840^{a} ± 4.74	31.663^{ab} ± 7.216	30.589^{bc} ± 5.278	29.611^{bc} ± 8.797	33.854^{ab} ± 11.981	26.420^{c} ± 8.879	4.887
MUFA[4]	26.561^{d} ± 10.162	33.806^{c} ± 11.903	35.947^{bc} ± 10.29	42.114^{b} ± 18.66	39.056^{bc} ± 13.609	50.952^{a} ± 13.893	6.630
***n*-6/*n*-3[5]**	1.856^{b} ± 1.53	1.857^{b} ± 2.284	1.367^{b} ± 2.35	5.429^{b} ± 3.181	4.642^{b} ± 3.654	15.709^{a} ± 22.224	5.260

[a-d]Means in rows with different superscripts differ significantly at P≤0.05.
[1]SD (standard deviation); [2]LSD (least significant difference); [3]SFA (saturated fatty acids); [4]MUFA (mono-unsaturated fatty acids); [5]*n*-6/*n*-3 (omega 6 to omega 3 ratio).

Table 6 The mean scores (±SD[1]) of the fatty acids (% total fatty acids) of Egyptian goose meat affected by season

Fatty acid	Winter (July)	Summer (November)	LSD[2] P=0.05
14:1	0.027[a] ± 0.020	0.014[b] ± 0.016	0.008
16:1 *n*-7	1.895[a] ± 0.822	1.323[b] ± 0.845	0.340
22:1 *n*-9	0.081[a] ± 0.034	0.060[b] ± 0.049	0.021
20:3 *n*-6	0.230[a] ± 0.107	0.169[b] ± 0.119	0.054
20:3 *n*-3	0.190[a] ± 0.094	0.072[b] ± 0.05	0.044
20:4 *n*-6	6.253[a] ± 3.955	4.316[b] ± 3.537	1.900
22:5 *n*-3	1.045[a] ± 0.631	0.626[b] ± 0.554	0.321
22:6 *n*-3	0.503[a] ± 0.303	0.297[b] ± 0.267	0.150
PUFA[3]	35.263[a] ± 7.525	25.998[b] ± 10.543	4.994

[a-b]Means in rows with different superscripts differ significantly at P≤0.05.
[1]SD (standard deviation); [2]LSD (least significant difference); [3]PUFA (polyunsaturated fatty acids).

Table 7 The mean scores (±SD[1]) of the fatty acids (% total fatty acids) of Egyptian goose meat significantly affected by portion

Fatty acids	Breast	Drumstick	Thigh	LSD[2] P=0.05
14:1	0.015[b] ± 0.019	0.030[a] ± 0.02	0.017[b] ± 0.015	0.007
22:1 *n*-9	0.080[a] ± 0.033	0.082[a] ± 0.051	0.049[b] ± 0.037	0.014
24:1 *n*-9	0.084[a] ± 0.046	0.087[a] ± 0.051	0.052[b] ± 0.048	0.013
20:3 *n*-6	0.270[a] ± 0.11	0.215[b] ± 0.082	0.114[c] ± 0.1	0.029
20:3 *n*-3	0.155[a] ± 0.101	0.140[a] ± 0.08	0.098[b] ± 0.096	0.019
20:4 *n*-6	7.693[a] ± 3.886	5.672[b] ± 3.032	2.488[c] ± 2.66	0.768
22:6 *n*-3	0.413[b] ± 0.233	0.562[a] ± 0.334	0.225[c] ± 0.235	0.071
PUFA[3]	32.976[a] ± 9.722	30.842[ab] ± 8.931	28.073[b] ± 11.557	2.870

[a-c]Means in rows with different superscripts differ significantly at P≤0.05.
[1]SD (standard deviation); [2]LSD (least significant difference); [3]PUFA (polyunsaturated fatty acids).

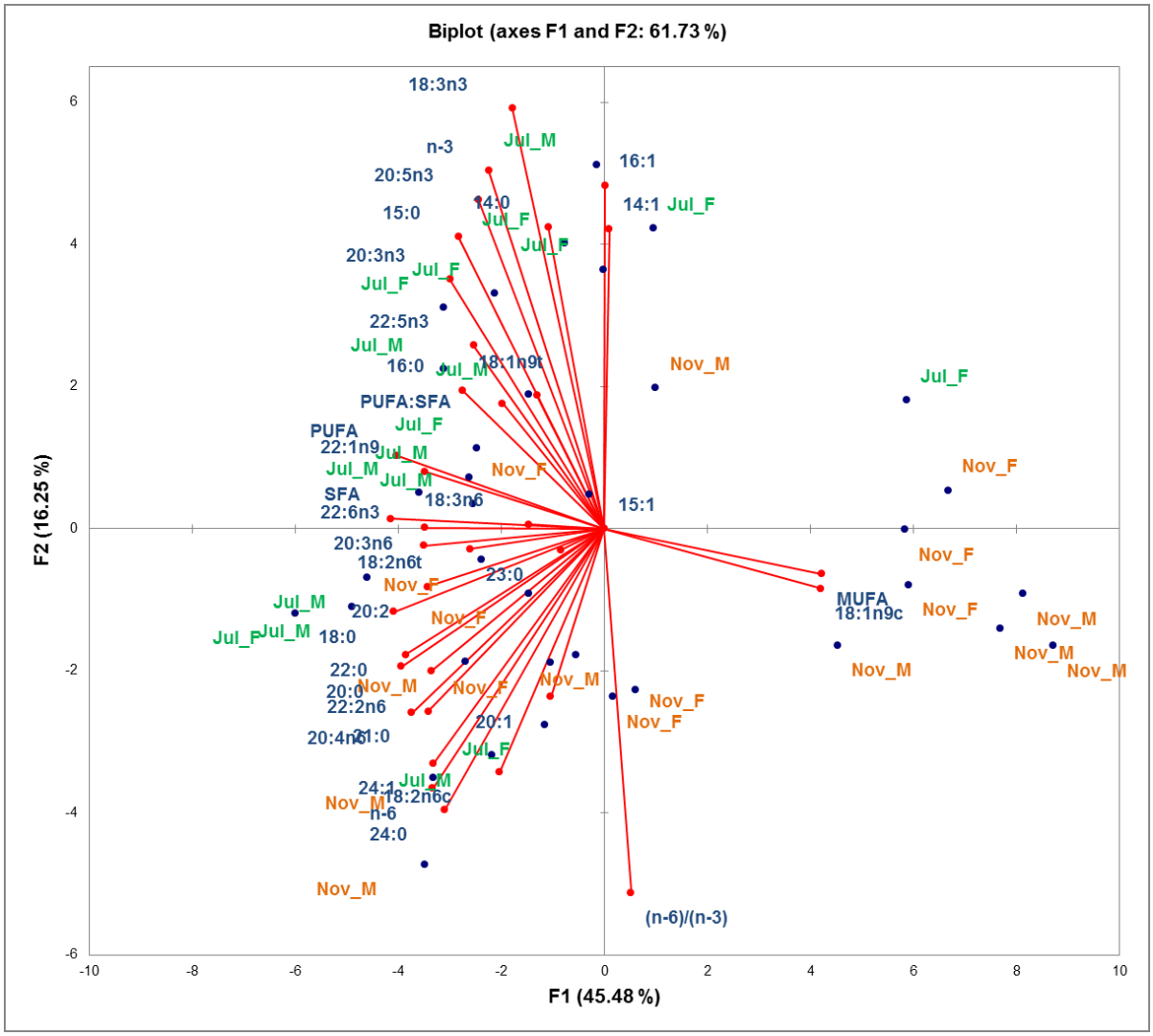

Figure 1 PCA bi-plot of the individual fatty acids in the breast portion as affected by season and gender. Jul_M (July male); Jul_F (July female); Nov_M (November male); Nov_F (November female).

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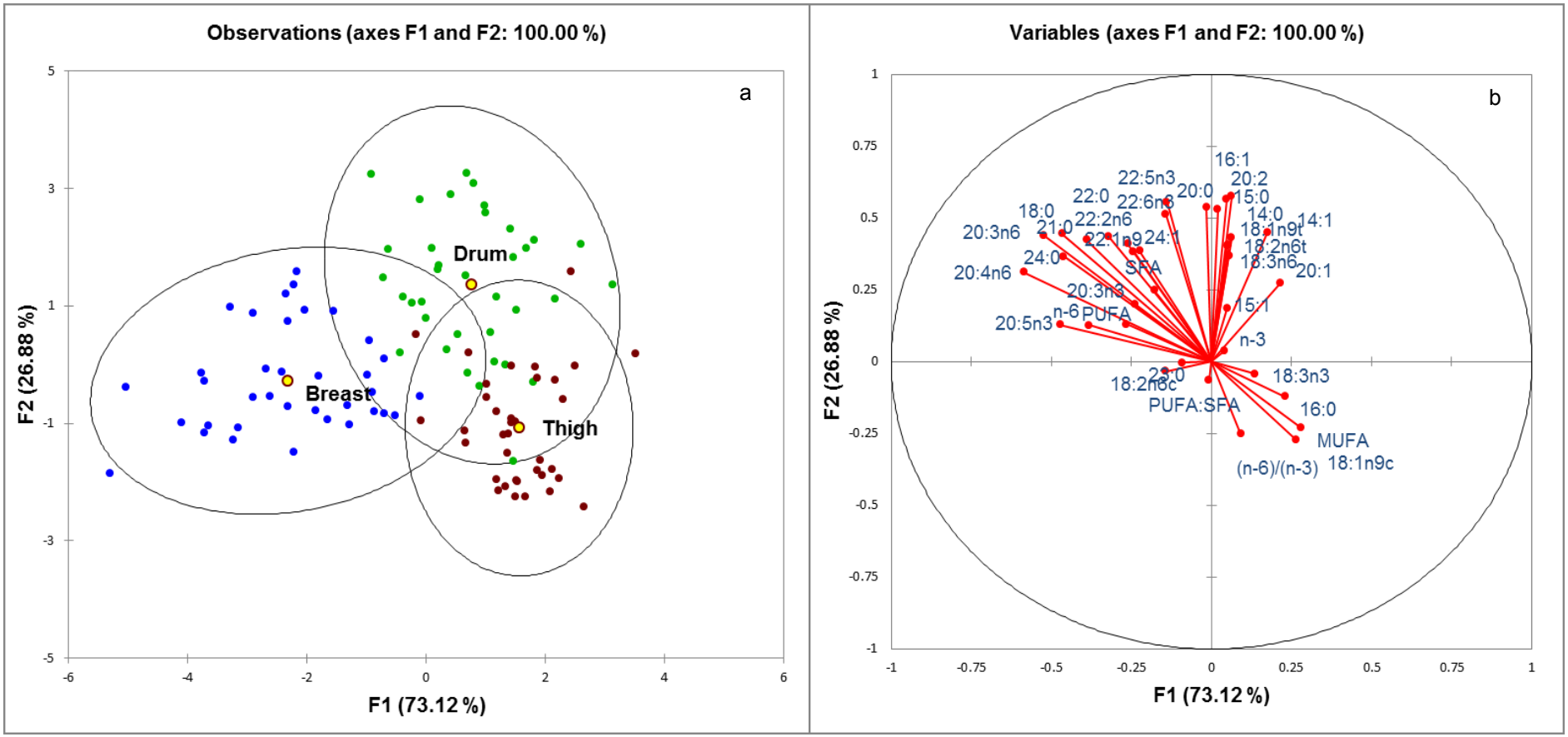

Figure 2 DA plot (a) and the discriminant loadings for the variables (b) illustrating the effect different portions have on the fatty acid composition of Egyptian goose meat.

3.1.1 Season/diet

The fatty acid composition of meat is dependent on several factors of which diet is one of the major elements involved. The diet of Egyptian geese varies considerably between the winter and summer months in South Africa. Their feeding activities are closely related to the grain (wheat and barley) season and during early summer (November) their diet mainly consists of grain seeds during the harvesting period. Throughout the rest of the year the diet is mainly forage based. They feed on aquatic plants, aquatic invertebrates and other aquatic vegetation; green terrestrial plant materials, seedlings as well as growing crops also form part of the dietary intake of these waterfowl (Maclean, 1988; Viljoen 2005). Halse (1984) reported that during certain periods (seasons) they also rely on Bermuda grass, alga and pondweeds as food sources.

The major contributors towards the fatty acid profile of Egyptian goose meat are palmitic acid (C16:0), stearic acid (C18:0), oleic acid (C18:1), linoleic acid (C18:2 *n*-6) and α-linolenic acid (C18:3 *n*-3). Of these, oleic acid (C18:1), linoleic acid (C18:2 *n*-6) and α-linolenic acid (C18:3 *n*-3) are the fatty acids expected to show the greatest variation in terms of season/diet differences. The meat from animals that feed on grain based diets generally have a very high oleic acid (C18:1) and linoleic acid (C18:2 *n*-6) content (Enser *et al*., 1998; McDonald *et al*., 2002). In contrast, animals with mainly a green forage or grass based diet is expected to produce meat with a very high α-linolenic acid (C18:3 *n*-3) content due to the high presence of this fatty acid in the diet (Marmer *et al*., 1984; Enser *et al*., 1998; McDonald *et al*., 2002; Ward *et al*., 2003; Poulson *et al*., 2004). The discrepancy within the fatty acid composition, due to the dietary differences, is particularly evident in monogastric animals where the dietary constituents are incorporated into the tissue lipids in a more direct manner (Wood & Enser, 1997; MacRae *et al*., 2005). This theory coincides with the findings of our study and the clear trend is depicted by the PCA-biplot (Fig. 1) containing the fatty acid data of the breast portion. All three portions from Egyptian geese hunted in winter had a substantially higher percentage ($P \leq 0.05$) of α-linolenic acid (C18:3 *n*-3) (Table 5). The high percentage of this fatty acid in the winter portions, together with the fact that five of the individual long chain PUFA were also substantially higher in winter (Table 6) results in the total PUFA being 9% higher ($P \leq 0.05$). The crop content's fatty acid composition (Table 2) of the geese harvested in winter confirms that the diet was approximately 49% higher in total PUFA compared to that of summer. The percentage of α-linolenic acid (C18:3 *n*-3) present was particularly high (46% in winter compared to 0.5% in summer). α-Linolenic acid (C18:3 *n*-3) is classified as an essential fatty acid because of its inability to be synthesised by the body (Bezard *et al*., 1994). It is also a precursor for the formation of most of the longer chain PUFA by alternate, desaturation-elongation reactions (Bezard *et al*., 1994). The incidence of certain fatty acids in the diet is likely to influence the synthesis of others by means of competitive inhibition (Bezard *et*

al., 1994). For instance, an increased intake of α-linolenic acid will, in theory, limit the desaturation of linoleic acid into the *n*-6 desaturation products. The higher incidence of the longer chain *n*-3 PUFA (eicosapentaenoic acid, docosapentaenoic acid and docosahexaenoic acid) in winter (Table 6) is therefore characteristic of this grass based diet.

In contrast, the summer portions showed and overall higher percentage of oleic acid (C18:1), specifically the thigh portion which contained much higher (P≤0.05) levels that ultimately contributes to the higher total MUFA (Table 5). This difference was less evident regarding linoleic acid (C18:2 *n*-6), however the thigh portion from the geese hunted in summer did have a significantly higher content of this fatty acid. According to Chung and Ohm (2000), the main fatty acid in cereal grain lipids is oleic acid (C18:1) and this is clearly reflected in the profile of the crop content (Table 2). The geese harvested in summer contained 75% of this fatty acid compared to 8.4% in winter.

The variation within the content of the key fatty acids may influence the n-6/n-3 ratio of the meat. This is reflected in the summer thigh portion which had a very high (P≤0.05) ratio (15.7) compared to its counterpart in winter (1.4). Similarly, the breast portion from summer had a ratio of 5.4 compared to 1.6 in winter. The *n*-6/*n*-3 fatty acid intake of humans should be balanced and provided in adequate amounts so as to optimise the biological function of these nutrients. The n-6/n-3 ratios of all three portions from the geese harvested in winter are within the recommended requirements (<5) with regards to human health (Bezard *et al*., 1994; Raes *et al*., 2004; Durand *et al*., 2005; Scollan *et al*., 2006). However, the breast and drumstick portions from summer had *n*-6/*n*-3 ratios (5.4; 15.7) that do not comply with the recommendations. Although not significantly affected by the factors investigated, the polyunsaturated to saturated fatty acid ratio (PUFA/SFA) of the meat from both harvesting seasons were above the required limit of 0.4-0.7 (Raes *et al*., 2004; Scollan *et al*., 2006; Durand *et al*., 2005; & Wood *et al*., 2008).

Altogether, the results show that there is a clear link between bird diet and the fatty acid composition of the meat. The forage vs. grain based diets of Egyptian geese during certain periods of the year leads to variation in the content of the key fatty acids. This not only results in variation within the nutritional composition but may also have a substantial effect on the flavour profile and ultimate uniformity of the meat. The PCA-biplot (Fig. 1) of the breast portion indicates exactly what was expected in terms of the fatty acid profiles of winter vs. summer. The breast portion of Egyptian geese constitutes approximately 35% of the intact, dressed carcass (Geldenhuys *et al*., 2013b) [Chapter 5] and will therefore largely contribute to the nutrient intake when an entire carcass is cooked and consumed. It is thus important to consider the composition of the breast portion as a contributing factor.

3.1.2 Portion

Another determining factor of the fatty acid profile is the inherent muscle composition and therefore the variation between different portions/muscles. The composition of the fibres, which is linked to the physical capacity/activity and metabolic requirements of the muscle, may have an influence on the type of fatty acids present. More specifically, the phospholipids contained in the cell membranes (Alasnier *et al*., 1996; De Smet *et al*., 2004; Costa *et al*., 2008) and neutral lipids (triacylglycerols) in the cytosolic droplets in the muscle fibres (Costa *et al*., 2008) may vary on account of muscle fibre type. Fibre type is not the only inherent muscle characteristic presumed to have an influence on the fatty acid composition. There are lipids present in muscle which are not specifically related to the metabolic capacity thereof. These lipids include the neutral lipids (triacylglycerols) confined in the adipocytes which are positioned between muscle fibres or in the interfascicular region (Costa *et al*., 2008). Therefore, the fluctuations in the neutral and phospholipid content and ratios thereof largely determine the fatty acid profile of the meat.

It is evident from the DA plot (Fig. 2a) that overall the three portions did not differ significantly. Although the centroids overlapped there was still a clear grouping of the respective portions. This grouping is clarified by the Discriminant Loadings plot (Fig. 2b) of the variables were the main fatty acids responsible for the specific grouping of the portions are illustrated. The thigh portion is situated towards the total MUFA and oleic acid (C18:1 *n*-9) while the breast portion is positioned more in the direction of the total PUFA and the long chain PUFA such as arachidonic acid (C20:4 *n*-6) and eicosapentaenoic acid (20:5 *n*-3). Considering the portion effect on the fatty acids the results (Table 7) indicate that the breast portion indeed contained a higher ($P \leq 0.05$) percentage of total PUFA, three of the long chain PUFA and two of the long chain MUFA. The thigh portion had the lowest percentages of these fatty acids. Similar results have been reported by Baeza *et al*. (2010) on the higher MUFA content of chicken thighs when compared to the breast muscle with higher PUFA and SFA levels. Alternatively, the Egyptian goose drumstick was high in the short chain SFA such as myristic acid (C14:0). This variation in the composition of the portions may be explained in two ways; the metabolic muscle fibre composition which mainly affects the membrane lipids (phospholipids).and the composition of the main lipid fractions of the portion, specifically the neutral lipids (triacylglycerols) which is not related to fibre type. It is postulated that the muscles in the thigh portion consists of a combination of oxidative and glycolytic fibres for postural and locomotive activity compared to the breast which is mainly comprised of fast, oxidative-glycolytic fibres for sustained, fast activity during flight (Geldenhuys *et al*., 2013a; Geldenhuys *et al*., 2013b, Chapter 8). The general consensus seems to be that fibres with a smaller diameter (oxidative and oxidative-glycolytic) which require the muscle to have more of a membrane structure as well as fibres which also contain more mitochondria

(oxidative) are subsequently rich in phospholipids (Alasnier *et al*., 1996; Costa *et al*., 2008; Lefaucheur, 2010). The phospholipid fractions are usually high in long chain PUFA (Alasnier *et al*., 1996; De Smet *et al*., 2004; Lawrie & Ledward, 2006) which may be the reason for the increased overall PUFA content of the breast compared to the thigh. Even though the metabolic capacity of the muscle may be a determining factor, its influence on the fatty acid composition may be minor and not have a biological effect. It is more likely that the amount and composition of the neutral lipids (triacylglycerol) are responsible for the differences. Baeza *et al*. (2010) describes that birds predominantly synthesise MUFA such as oleic acid and that these fatty acids are stored as triacylglycerides in the adipose tissue. Muscles/portions, such as the thigh, may anatomically have more physical capacity to allow for increased fat deposition within the adipocytes (Geldenhuys *et al*., 2013b) [Chapter 5]. Unlike the breast portion, the thigh is also composed of several small muscles and the physical structure may allow for the presence of more triacylglycerol adipocytes. De Smet *et al*. (2004) describes that triacylglycerols contain much lower amounts of PUFA compared to phospholipids and that the composition can also be influenced by dietary factors particularly in monogastric animals. An increase in fat deposition within the adipocytes leads to dilution of the PUFA content by the other fatty acids. This theory may link to the fact that in summer, with a grain based diet, the thigh portions had the highest levels of C18:1 *n*-9 and C18:2 *n*-6 (Table 5).

Although the main effect of gender was not significant there were interactions ($P \leq 0.05$) between gender and portion for four of the fatty acids (Table 4). The female drumstick portion had the highest content of palmitic acid (C16:1 *n*-7), eicosenoic acid (C20:1 *n*-9), γ-linolenic acid (C18:3 *n*-6) and docosapentaenoic acid (C22:5 *n*-3). Female birds tend to have a more saturated fatty acid profile (MUFA) compared to male birds because of increased fat deposition in peripheral tissues (Baeza *et al*., 2010). There was, however, no fixed trend in terms of our results and fatty acid type.

The influence of fibre type on the fatty acid composition of meat is a controversial topic. In our study variation within the three portions were found especially regarding the major fatty acids. The difference between the thigh and breast portions is particularly interesting; however, it is difficult to clarify the exact causes. It is postulated that the difference in the physical structure of the muscle/portions have the greatest influence.

3.2 Amino acid composition

The amino acid composition (g/100 g meat) of the breast portion is presented in Table 8. There were no significant interactions between the main effects. The table therefore depicts the results pertaining to season and gender separately. The amino acid composition of the male and female breast portions did not differ significantly (P>0.05).

Season significantly affected (P≤0.05) all but the following four amino acids; methionine, threonine, aspartic acid, tyrosine. Those that were affected all had higher (P≤0.05) values in winter. The seasonal variation found within the amino acid composition may be diet related, especially the following amino acids which were significantly higher in winter: arginine, histidine, leucine, lysine, alanine, glycine and serine. The content of all of these amino acids also differed (P≤0.05) on a g/100 g protein basis. The fact that the geese mainly feed on cereal grains during summer (November) may have resulted in the lower incidence of these amino acids in the meat. This is substantiated by McDonald *et al*. (2002) and D'Mello (1993) which state that grain proteins can be limiting in the amount of certain amino acids such as lysine and arginine. The differences regarding the amino acids and season/diet coincides with the findings of Hoffman *et al*. (2007) where variation was found regarding the content of certain amino acids in springbok (*Antidorcas marsupialis*) meat related to region/diet. The other amino acids that differed significantly between seasons (g/100 g meat) did not vary on a g/100 protein basis and the proximate composition was therefore of influence here.

In a 2007 report by the World Health Organisation (WHO, 2007) regarding the protein and amino acid requirements in human nutrition it is stated that there are nine amino acids which are essential for humans. These include; histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, valine and tryptophan. The two main essential amino acids found in the Egyptian goose breast portion were leucine and lysine (Table 8) while the two main, non-essential amino acids were glutamine and aspartic acid. In comparison to domestic species i.e. ostrich, beef and chicken, as specified by Sales and Hayes (1996), the amino acid composition of the Egyptian goose breast portion is somewhat lower. Specifically compared to ostrich the average values of the two major essential amino acids present in the Egyptian goose breast portion; lysine (1.3 g/100 g meat) and leucine (1.4 g/100 g meat) are lower than ostrich with values of 1.8 and 1.6 g/100 g respectively. The amino acid values reported by Hoffman *et al*. (2005) for Impala (*Aepyceros melampus*) as well as by Hoffman and Ferreira (2004) for the common Duiker (*Sylvicapra grimmia*) are also much higher compared to the values found in this study.

Considering the human requirements (mg/kg per day) of amino acids listed in the World Health Organisation's report (WHO, 2007), the breast portion of Egyptian geese would still be a valuable source of these essential amino acids.

Table 8 Amino acid composition[1] (g/100 g meat) (±SD[1]) of breast meat in female and male Egyptian geese from different seasons

Amino acid	Season		LSD[2] P=0.05	Gender		LSD[2] P=0.05
	July (winter)	November (summer)		Female	Male	
Essential						
Histidine	0.234[a] ± 0.069	0.176[b] ± 0.078	0.053	0.201 ± 0.057	0.207 ± 0.096	0.053
Isoleucine	0.761[a] ± 0.085	0.667[b] ± 0.163	0.092	0.725 ± 0.134	0.702 ± 0.144	0.092
Leucine	1.441[a] ± 0.141	1.271[b] ± 0.218	0.128	1.376 ± 0.198	1.332 ± 0.207	0.128
Lysine	1.373[a] ± 0.153	1.162[b] ± 0.338	0.187	1.278 ± 0.274	1.252 ± 0.297	0.187
Methionine	0.388[a] ± 0.051	0.355[a] ± 0.075	0.045	0.383 ± 0.066	0.360 ± 0.065	0.045
Phenylalanine	0.748[a] ± 0.083	0.643[b] ± 0.148	0.085	0.704 ± 0.123	0.685 ± 0.139	0.085
Threonine	0.579[a] ± 0.146	0.522[a] ± 0.268	0.155	0.550 ± 0.226	0.550 ± 0.213	0.155
Valine	0.778[a] ± 0.092	0.673[b] ± 0.178	0.100	0.738 ± 0.145	0.711 ± 0.159	0.100
Non-essential						
Arginine	0.936[a] ± 0.123	0.836[b] ± 0.136	0.083	0.890 ± 0.129	0.882 ± 0.126	0.083
Alanine	0.831[a] ± 0.091	0.720[b] ± 0.137	0.083	0.776 ± 0.123	0.775 ± 0.136	0.083
Aspartic acid	1.464[a] ± 0.172	1.384[a] ± 0.216	0.137	1.439 ± 0.181	1.408 ± 0.215	0.137
Glutamine	2.443[a] ± 0.241	2.167[b] ± 0.495	0.275	2.330 ± 0.414	2.274 ± 0.420	0.275
Glycine	0.765[a] ± 0.098	0.658[b] ± 0.096	0.067	0.693 ± 0.097	0.726 ± 0.122	0.067
Serine	0.660[a] ± 0.066	0.570[b] ± 0.102	0.060	0.620 ± 0.092	0.608 ± 0.103	0.060
Tyrosine	0.625[a] ± 0.077	0.551[a] ± 0.135	0.078	0.597 ± 0.116	0.577 ± 0.117	0.078

[a-b]Means in rows, within main effect, with different superscripts are significantly different at P≤0.05.
[1]SD (standard deviation); [2]LSD (least significant difference).

3.3 Mineral composition

The mineral composition of the breast portion is indicated in Table 9. No significant interactions were found between the main effects. The table therefore contains the results as affected by season and gender respectively.

The mineral composition of meat can vary on account of genetic, physiological (gender) and environmental factors (diet) (Doyle, 1980). However, season did not have a significant influence on the mineral composition of the Egyptian goose breast portion. Also, the only mineral which was affected ($P \leq 0.05$) by gender was boron. The breast portion of the female geese had a slight, but significantly higher, amount of boron present. Although significant, this difference between female and male was very small (0.030 vs. 0.027) and the biological relevance thereof is therefore questionable.

Similar to the results found in Geldenhuys *et al*. (2013c) [Chapter 3] phosphorus was found to be the most abundant mineral present in Egyptian goose meat, followed by potassium and magnesium. The elevated iron (Fe) content (5.3 mg/100 g) of Egyptian goose meat is also evident. This high level of Fe is related to the metabolic capacity and fibre composition of the breast muscle as clarified in Geldenhuys *et al*. (2013c) [Chapter 3]. This muscle in volant birds mainly consists of red type IIa, fast oxidative glycolytic (FOG) fibres together with a small percentage of type IIb, fast glycolytic (FG) fibres (Butler, 1991; Baeza *et al*., 2000). Type IIa fibres are aerobic, thus having a high myoglobin content for oxygen supply. High Fe levels have previously been linked to the meat of game ducks when compared to domestic ducks (Khalifa & Nassar, 2001). When the Fe content in the Egyptian goose breast portion is compared to that of the ostrich *M. iliofibularis* the levels are also much higher than what was reported by both Brand (2006) and Sales and Hayes (1996). Another aspect of note is the fact that the breast portion of this wildfowl species also have a much higher Fe content in relation to the meat from wild ungulates such as springbok (Hoffman *et al*., 2007) and blesbok (Neethling, 2012). The average concentrations of both Fe and copper (Cu) (0.4 mg/100 g) is also higher than that found in beef, lamb, ostrich, pork, chicken and turkey meat (Lombardi-Boccia *et al*., 2005). Geldenhuys *et al*. (2014) [Chapter 4] debated the involvement and contribution of Fe in the overwhelming metallic flavour of Egyptian goose meat. It is interesting that no significant differences were found in terms of season and Fe levels and it would therefore be interesting to investigate the incidence of the metallic flavour due to seasonal variation.

Red meat is an excellent source of minerals especially iron, zinc, selenium and phosphorus (Biesalski & Nohr, 2009; McNeill & Van Elswyk, 2012; Pereira & Vicente, 2013). The increased bioavailability of certain minerals (iron and zinc) in meat is also beneficial for human health. Egyptian goose meat contains all of the minerals essential for human consumption (FAO, 2001).

Table 9 Mineral composition (mg/100 g dry basis) (±SD[1]) of the breast meat in female and male Egyptian geese from different seasons

Mineral	Season		LSD[2] P=0.05	Gender		LSD[2] P=0.05
	July (winter)	November (summer)		Female	Male	
Phosphorus	173.0 ± 16.7	167.7 ± 17.9	12.0	173.7 ± 17.1	166.9 ± 17.3	12.0
Potassium	165.7 ± 19.8	156.4 ± 18.4	13.0	164.7 ± 20.6	157.4 ± 18.0	13.0
Calcium	7.0 ± 1.1	7.4 ± 1.0	1.0	7.5 ± 1.2	6.9 ± 0.9	1.0
Magnesium	31.3 ± 2.8	31.0 ± 3.1	2.0	31.9 ± 3.0	30.3 ± 2.7	2.0
Sodium	21.1 ± 4.2	23.3 ± 3.9	2.7	20.9 ± 3.1	23.5 ± 4.7	2.7
Iron	5.3 ± 0.8	5.4 ± 1.2	0.7	5.3 ± 1.0	5.4 ± 1.0	0.7
Copper	0.4 ± 0.2	0.4 ± 0.1	0.10	0.40 ± 0.2	0.4 ± 0.1	0.1
Zinc	1.5 ± 0.2	1.5 ± 0.5	0.3	1.5 ± 0.2	1.6 ± 0.5	0.3
Manganese	0.06 ± 0.008	0.06 ± 0.011	0.006	0.06 ± 0.008	0.06 ± 0.011	0.006
Boron	0.03 ± 0.004	0.03 ± 0.005	0.003	0.03[a] ± 0.004	0.027[b] ± 0.004	0.003
Aluminium	2.6 ± 1.7	3.3 ± 1.6	1.1	3.2 ± 1.8	2.7 ± 1.5	1.1

[a-b]Means in rows, within main effect, with different superscripts are significantly different at P≤0.05.
[1]SD (standard deviation); [2]LSD (least significant difference).

4 CONCLUSIONS

Ultimately, it is clear that our study provides essential insight and information into the seasonal variation within the fatty acid profile of Egyptian goose meat. The composition of the fatty acids consumed is one of the key aspects of human health. The forage vs. grain based diets of Egyptian geese during certain periods of the year leads to variation in the content of the key fatty acids such as oleic acid, linoleic acid and α-linolenic acid. This difference in the key fatty acids results in variation in the n-6/n-3 ratios within season. This study indicates that Egyptian goose meat does not only vary in nutritional composition but season may also have a substantial effect on the flavour profile and ultimate uniformity of the meat. The season and portion effects were, however, interlinked but the general tendency shows that the portions, especially the breast and thigh do differ concerning the major fatty acids. No substantial differences were found in the mineral composition of the breast portion on account of season and gender, however there were some variation in certain amino acids such as lysine and arginine due to season/diet. This research provides essential information that should be considered not only regarding the everyday consumption of Egyptian goose meat but the potential utilisation and ultimate consistency of this meat product.

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6 ADDENDUM A

Table 10 The fatty acid composition of the breast, thigh and drumstick portions, expressed as mg/g meat

Fatty acid	Portion		
	Breast	**Drumstick**	**Thigh**
SFA			
14:00	0.115 ± 0.07	0.132 ± 0.064	0.11 ± 0.08
15:00	0.034 ± 0.019	0.038 ± 0.016	0.03 ± 0.022
16:00	8.73 ± 3.341	8.288 ± 2.955	10.597 ± 4.545
18:00	5.389 ± 2.263	4.183 ± 1.797	2.368 ± 1.778
20:00	0.035 ± 0.015	0.042 ± 0.018	0.027 ± 0.02
21:00	0.072 ± 0.031	0.06 ± 0.023	0.038 ± 0.03
22:00	0.039 ± 0.02	0.035 ± 0.018	0.02 ± 0.017
23:00	0.003 ± 0.009	0.004 ± 0.012	0.003 ± 0.008
MUFA			
14:01	0.008 ± 0.01	0.015 ± 0.017	0.008 ± 0.007
16:1 *n*-7	0.709 ± 0.532	0.897 ± 0.466	0.602 ± 0.398
18:1 *n*-9*c*	14.988 ± 9.59	13.691 ± 6.722	19.776 ± 8.174
20:1 *n*-9	0.108 ± 0.047	0.134 ± 0.057	0.127 ± 0.09
22:1 *n*-9	0.035 ± 0.017	0.033 ± 0.022	0.022 ± 0.018
24:1 *n*-9	0.036 ± 0.019	0.034 ± 0.02	0.023 ± 0.021
PUFA			
18:2 *n*-6*c*	6.509 ± 2.392	5.254 ± 1.995	5.885 ± 4.13
18:3 *n*-3	3.647 ± 3.408	3.927 ± 3.898	5.582 ± 6.158
18:3 *n*-6	0.035 ± 0.015	0.037 ± 0.019	0.028 ± 0.015
20:02	0.022 ± 0.008	0.027 ± 0.013	0.018 ± 0.012
20:3 *n*-6	0.12 ± 0.056	0.085 ± 0.036	0.049 ± 0.041
20:3 *n*-3	0.072 ± 0.052	0.056 ± 0.034	0.045 ± 0.045
20:4 *n*-6	3.344 ± 1.695	2.178 ± 1.072	1.015 ± 0.96
20:5 *n*-3	0.449 ± 0.411	0.209 ± 0.197	0.135 ± 0.168
22:02	0.038 ± 0.02	0.038 ± 0.021	0.022 ± 0.019
22:5 *n*-3	0.375 ± 0.234	0.426 ± 0.268	0.22 ± 0.242
22:6 *n*-3	0.183 ± 0.105	0.218 ± 0.126	0.093 ± 0.094
SFA[1]	14.695 ± 5.336	13.005 ± 4.138	13.309 ± 4.677
MUFA[2]	15.929 ± 9.718	14.961 ± 6.863	20.611 ± 8.103
PUFA[3]	14.848 ± 6.193	12.515 ± 4.967	13.117 ± 6.538
PUFA/SFA[4]	0.996 ± 0.168	1.019 ± 0.477	0.981 ± 0.485
***n*-6/*n*-3**[5]	4.354 ± 4.278	3.249 ± 3.319	8.538 ± 17.19

[1]SFA (saturated fatty acids); [2]MUFA (mono-unsaturated fatty acids); [3]PUFA (polyunsaturated fatty acids); [4]PUFA/SFA (polyunsaturated fatty acid/saturated fatty acid ratio); [5]*n*-6/*n*-3 (omega 6/omega 3 ratio).

CHAPTER 7

The influence of season on the sensory profile of Egyptian goose (*Alopochen aegyptiacus*) meat

ABSTRACT

The feeding activities of Egyptian geese especially in the Western Cape region of South Africa are becoming a problem for crop farmers. Wing shooting activities are recommended in order to reduce the damage caused. Therefore utilisation of the meat is vital. The opposing diet of the geese during the grain harvesting season compared to the diet during the rest of the year, as well as the gender will have an influence on the meat quality, especially the sensory profile. This is a follow up study of the initial sensory profiling of Egyptian goose meat [Chapter 4] in order to establish the effect of season and gender on the sensory profile. Descriptive sensory analysis together with physical measurements (pH, cooking loss, water holding capacity and shear force) and the proximate composition were used to investigate these effects. Season (diet) was the major influential factor with the meat from summer (November) associating with sweet-oily-duck and beef attributes in contrast to the strong association towards the game, metallic and fish attributes of the winter (July) meat. This is a result of the difference in the main fatty acids of the meat from the respective seasons; winter higher ($P \leq 0.05$) in PUFA (especially C18:3 *n*-3) and summer higher ($P \leq 0.05$) in MUFA (especially C18:1 *n*-9). Regarding gender, meat from female geese associated more with the sweet-oily-duck attributes whereas the males had more intense game and metallic attributes as well as being lower ($P \leq 0.05$) in tenderness (shear force). The proximate results did not indicate any other significant differences. This study established that season (diet) has a significant effect on the sensory profile of Egyptian goose meat and should be considered in terms of the utilisation and consumption of the meat.

Keywords: Egyptian goose meat, Game birds; Sensory analysis, Meat quality, Diet, Season, Gender

1 INTRODUCTION

The gamebird industry, as well as the sport of wingshooting is becoming increasingly popular, not only in South Africa but internationally as well (Little & Crowe, 1993; Viljoen, 2005; Geldenhuys *et al*., 2013a). Additionally, the population numbers of species such as Egyptian geese are rising and in some cases this gamebird is becoming a problem for crop farmers (Mangnall & Crowe, 2001; 2002). This is a result of the feeding activities of the geese where extensive foraging and damaging of crops occur at different stages of the grain season. Mangnall and Crowe (2002) emphasises this problem and quantified the damage caused by geese to the crop farms in the Agulhas plain region of the Western Cape, South Africa. The subsequent financial damage suffered by farmers is considered to be major. For this reason, gamebird wingshooting was recommended in order to manage the population numbers and, therefore, reducing the damage caused by the geese (Mangnall & Crowe, 2001). This situation, however, raises some unanswered questions as to what happens with the meat after the birds are shot, the quality thereof and its potential for utilisation.

The meat generated from the shooting activities are generally donated to the local communities or consumed by the hunters themselves. Therefore, proper utilisation of the meat is essential. There is very limited information on the quality of gamebird meat (Hofbauer & Smulders, 2011); and needless to say, there is no literature specifically on the quality of Egyptian goose meat. To address this problem, descriptive sensory profiling (Geldenhuys *et al*., 2014) [Chapter 4] was essential as the first step in the process of determining the overall meat quality. It is also vital to investigate the aspects such as season (diet) and gender, which may have an influence on the sensory profile in order to understand the potential of Egyptian goose meat as another meat option for consumption.

Egyptian geese are waterfowl abundant in the Western Cape region, as well as the rest of South Africa (Maclean, 1997; Viljoen, 2005). Areas with available inland water such as dams or wetlands are ideal environments for this species (Maclean, 1997; Viljoen, 2005). Their diet mainly consists of aquatic plants, aquatic invertebrates and other aquatic vegetation; green terrestrial plant materials, seedlings and growing crops also form part of the dietary intake of these waterfowl (Maclean, 1997; Viljoen, 2005). Halse (1984) reported that during certain periods (seasons) they rely on Bermuda grass, alga and pondweeds as food sources. This diet, however, is very different during the grain harvesting season. The geese travel long distances in order to forage on grain seeds found on croplands in the harvesting period, especially in the Western Cape where a vast amount of crop farms are present.

The difference in the diet during July (winter) (forage based diet) compared to the grain based diet of November (summer), resulted in a difference in the fatty acid profile of the meat from the respective periods [Chapter 6]. The fatty acid profile is considered to be one of the major determinant

factors in the characteristic aroma and flavour profile of meat as the thermal degradation of the lipids is one of the key processes in producing the aroma volatiles of meat (Mottram, 1998). Although not all of the fatty acids influence the flavour of meat to the same extent (Mottram & Edwards, 1983), a wider range of lipid derived aroma or flavour volatiles are produced from unsaturated fatty acids because of their higher susceptibility towards oxidation (Mottram & Edwards, 1983). Unsaturated fatty acids have the ability to rapidly oxidize, particularly polyunsaturated fatty acids (PUFA) with an increased number of double bonds. This is important in the flavour development process during cooking (Wood *et al*., 2004). For this reason, it is expected that the sensory profile of Egyptian goose meat, from birds harvested in the Western Cape region during July (winter) and November (early summer), will be very different.

The gender of the Egyptian geese can also be an influential factor in terms of the sensory attributes. The main difference as a result of gender is the fact that female animals generally tend to have an increased intramuscular fat content (Lawrie & Ledward, 2006), which may have an effect on the sensory attributes such as sustained juiciness, as well as the tenderness of the meat as perceived by a trained sensory panel. There is a correlation between intramuscular fat content and sustained juiciness of meat as the fat is responsible for the secretion of saliva during mastication which increases the sustained juiciness thereof (Weir, 1960; Lawrie & Ledward, 2006). The intramuscular fat content can also be correlated to an increased tenderness in meat (Sami *et al*., 2004; Chartrin *et al*., 2006). This is due to the diluting effect of the fat on the muscle fibres present in a specific area of the meat (Wood *et al*., 1999).

Although age is another important factor which influences the sensory quality of meat, especially with regard to tenderness, it is virtually impossible to determine the specific age of gamebirds, especially when in flight. Therefore, age will not be considered in this study.

Ultimately, season (diet) and gender must be considered when researching the eating quality of Egyptian goose meat. The eating quality of meat involves three main attributes namely; tenderness, juiciness and flavour which are the major contributors to the consumer acceptability of meat (Warris, 2000; Lawless & Heymann, 2010). Some consumers appreciate game meat for the wild/gamey flavour while this attribute can also be considered as negative (Wiklund *et al*., 2003). In South Africa, game meat is regularly consumed by different demographic groups (Hoffman *et al*., 2005). However, according to Wiklund *et al*. (2003), an essential aspect of consumer acceptability is the overall uniformity in terms of meat quality; i.e. the product should have a consistent eating quality at all times.

With the growing gamebird industry of South Africa and the problem situation in terms of financial losses and population numbers, it is important to utilise the meat. The aim of this study is therefore to investigate the effect of season and the difference in the composition of the diet, as well as gender on the sensory profile of Egyptian goose meat. If the extent of the variation in the meat

quality, due to these two factors is known, the true potential of this species as a meat product can be identified. The prospective production of Egyptian goose meat with the best quality will then be a possibility.

2 MATERIALS AND METHODS

2.1 Harvesting, slaughtering of birds and experimental units

The Egyptian geese were harvested on the University of Stellenbosch's agricultural experimental farm, Mariendahl. The method of wing shooting was applied in order to successfully harvest these waterfowl by the use of a double barrelled shotgun. A total of 36 birds were harvested during the month of July 2010 (winter). This group consisted of 14 females and 22 males followed by a total of 33 birds harvested during November 2010 (summer), which included 13 female and 20 male birds (ethical clearance reference number: 10NP_HOF01). The birds were collected after shooting and held in a refrigerator (4 °C) for approximately 12 h. Subsequently, the slaughtering process (Geldenhuys *et al*., 2013b) [Chapter 5] followed where the head, feet, wings and skin containing the feathers were removed. After the slaughtering process the carcasses were hanged over-night (approximately 24 h) in a refrigerated area (4 °C), where after, the carcasses were halved, the right sides of each carcass were vacuum-packed separately, while the left side of the carcasses were deboned into portions and packaged separately (Geldenhuys *et al*., 2013b) [Chapter 5]. The meat samples were all frozen and stored at -18 °C until the sensory analysis commenced and the chemical analysis were performed (approximately 6 months storage for winter samples and 2 months for summer samples).

The carcasses were randomly selected for each of the six replications (n=6), six male and female from winter and summer, respectively (24 birds in total). The vacuum-packed and frozen right carcasses were thawed in a refrigerator at a temperature of 4 °C, 36 h prior to each of the pre-determined sensory analysis sessions, followed by the removal of the breast muscle (*pectoralis*) from the carcasses. The left breast muscle of the carcasses were thawed at 4 °C (12 h) and used for the proximate analysis.

The experimental units (Table 1) included four meat treatments which consisted of a male and female sample from both of the seasons in 2010 (2 genders and 2 periods). An experimental unit consisted of six replications (n=6). The sensory analysis and physical measurements were performed on the right breast (*M. pectoralis*) of the carcass while the proximate analyses were performed on the raw left breast muscle of the carcass. The analyses were thus performed on 24 birds.

Table 1 Sample set and experimental units of birds used for the sensory comparisons

	Number of birds (n)	
	Winter (July)	**Summer (November)**
Female	6	6
Male	6	6

2.2 Sample preparation for descriptive sensory analysis

The breast portion of each bird was placed inside in an oven bag (Glad®). No salt (NaCl) or any other seasoning was added to any of the meat treatments throughout the sensory analyses. The oven bags and meat samples were then placed on stainless steel grids which were fitted on an oven roasting pan. Thermocouple probes attached to a handheld digital temperature monitor (Hanna Instruments, South Africa) were placed in the centre of each of the meat samples (AMSA, 1995). The prepared samples were then placed in two conventional ovens (Defy, Model 835), pre-heated to 160 °C (AMSA, 1995). The ovens were connected to a computerized monitoring system responsible for regulation of the temperature (Viljoen *et al*., 2001). The meat samples were removed from the oven when a core temperature of 75 °C was reached (AMSA, 1995). The samples were cooled for 15 min where after they were cut into 1 cm x 1 cm cubes, individually wrapped in aluminium foil and placed into glass ramekins coded with randomized three-digit codes. The coded ramekins, each containing two wrapped meat cubes, were then placed in a preheated industrial oven (Hobart, France) at 100 °C for 10 min after which they were removed and immediately served to the sensory panel for analysis.

2.3 Descriptive sensory analysis

Descriptive sensory analysis (DSA) was performed on the four meat treatments (2 genders x 2 periods). A panel of eight judges, based upon previous experience with sensory analysis of meat, was selected. The panellists were trained according to the guidelines for sensory analysis of meat by the American Meat Science Association (AMSA, 1995) and the generic descriptive sensory analysis technique as described by Lawless and Heymann (2010).

The panel undertook four training sessions and during each of these training sessions the panellists received 1 cm x 1 cm cubes of meat from four reference standards, as well as the four meat treatments. Reference standards were chosen to illustrate the respective aroma and flavour attributes associated with Egyptian geese, as well as the other five treatments. The reference standards included commercial free range chicken, beef sirloin, beef rump, as well as the *Longissimus thorasicus et lomborum* muscle of locally harvested blesbok (*Damaliscus pygargus phillipsi* - a free ranging wild ungulate) (Geldenhuys *et al*., 2014) [Chapter 4]. The reference samples

enabled the panellists to calibrate their sensory perception during the training sessions, thereby allowing them to recognise and score all of the attributes tested in the respective meat samples.

During the training phase the panel used the descriptors obtained from Geldenhuys *et al*. (2014). These included the game, chicken, ostrich and beef aromas and flavours, as well as metallic flavour, initial and sustained juiciness, tenderness (first bite and residue). The panel also decided to add fish and sweet-oily-duck aromas and flavours to the final list of sensory descriptors. The definition for each of the attributes is described in Table 2. The test re-test method was used for DSA. The panellists received the four treatments in a complete randomized order, while seated in individual tasting booths fitted with the software programme Compusense® five (Compusense, Guelph, Canada). The samples were analysed for the respective sensory attributes using an unstructured line scale anchored to zero (indicating “low intensity”) and 100 (indicating “high intensity”) (AMSA, 1995). The sensory analysis sessions took place inside a temperature-controlled (21 °C) and light-controlled (artificial daylight) room (AMSA, 1995). In order to cleanse and refresh their palates between samples, the panellists received distilled water (21 °C), apple quarters and water biscuits (Carr, UK).

Table 2 Definition and scale of each attribute used for the descriptive sensory analysis

Sensory attribute	Description	Scale
Game aroma[1]	Aroma associated with game meat experienced as soon as the aluminium foil is removed	0 = Extremely bland 100 = Extremely intense
Chicken aroma[1]	Aroma associated with chicken experienced as soon as the aluminium foil is removed	0 = Extremely bland 100 = Extremely intense
Ostrich aroma[1]	Aroma associated with ostrich experienced as soon as the aluminium foil is removed	0 = Extremely bland 100 = Extremely intense
Beef aroma[1]	Aroma associated with beef experienced as soon as the aluminium foil is removed	0 = Extremely bland 100 = Extremely intense
Fish aroma[1]	Aroma associated with fish experienced as soon as the aluminium foil is removed	0 = Extremely bland 100 = Extremely intense
Sweet-oily-duck aroma[1]	A combined sweetish and oily aroma associated with duck meat, experienced as soon as the aluminium foil is removed	0 = Extremely bland 100 = Extremely intense
Game flavour[1]	Flavour associated with game meat prior to swallowing	0 = Extremely bland 100 = Extremely intense
Chicken flavour[1]	Flavour associated with chicken prior to swallowing	0 = Extremely bland 100 = Extremely intense
Ostrich flavour[1]	Flavour associated with ostrich prior to swallowing	0 = Extremely bland 100 = Extremely intense
Beef flavour[1]	Flavour associated with beef prior to swallowing	0 = Extremely bland 100 = Extremely intense
Fish flavour[1]	Flavour associated with fish prior to swallowing	0 = Extremely bland 100 = Extremely intense
Sweet-oily-duck flavour[1]	A combined sweetish and oily flavour associated with duck meat, experienced prior to swallowing	0 = Extremely bland 100 = Extremely intense
Metallic flavour[1]	Flavour associated with metal/liver prior to swallowing	0 = Extremely bland 100 = Extremely intense
Initial juiciness	The amount of fluid exuded from the cut surface when pressed between the thumb and forefinger	0 = Extremely dry 100 = Extremely juicy
Sustained juiciness	The level of juiciness perceived after the first 5 chews using the molar teeth	0 = Extremely dry 100 = Extremely juicy
First bite	The impression of tenderness perceived after the first 5 chews using the molar teeth	0 = Extremely tough 100 = Extremely tender
Residue	The amount of residue left inside the mouth after the first 10 chews	0 = None 100 = Abundant

[1] Aroma and flavour were analysed orthonasally and retronasally, respectively.

2.4 Physical measurements

2.4.1 pH

The pH of the four meat treatments, of each replication, was measured after thawing for 36 h, immediately after removal from the packaging and before the start of the cooking process of every sensory analysis session. The pH was measured by means of a Crison pH25 handheld portable pH meter (Lasec (Pty) Ltd, South Africa) calibrated before each set of readings with the standard buffers (pH 4.0 and pH 7.0) provided by the manufacturer.

2.4.2 Cooking loss

The cooking loss of the four meat treatments from each of the six replications were determined according to the method described by AMSA (1995). The weight (Radwag PS 750/C/2, Lasec SA, Cape Town, South Africa) of each treatment, as recorded before the cooking process, was used as the initial, raw weight of the samples. Following the cooking process the cooked meat samples were removed from the cooking bag and allowed to equilibrate to ambient temperature (±15 min) where after the samples were blotted dry with blotting paper and weighed. The difference in the weight of each of the samples was calculated as the percentage of cooking loss.

2.4.3 Water holding capacity

The water holding capacity was determined according to the method described by Trout (1988). A cooked meat sample from each of the four treatments and six replications were used. The sample was cut finely and 0.50 g weighed off and placed on top of a filter paper (Lasec, Paper Filter, grade 292, diameter 90 mm, part nr. FLAS3205090). The filter paper containing the meat sample was placed between two Perspex plates at a standard pressure of 588 N for 60 s. after which a photograph was taken of the filter paper showing the expelled liquid and meat areas. Using Image J Software (Version 1.41, 2009, http://rsbweb.nih.gov/ij/) the ratio between the outer (liquid) and inner (meat) purge area was calculated to indicate the water holding capacity of the 0.50 g meat sample.

2.4.4 Shear force

The shear force test (SF), as described by Honikel (1998), was used to analyse the instrumental shear force of the cooked meat samples. Each of the four treatments (six replications) was evaluated for instrumental tenderness. Two adjacent 1 x 1 cm meat strips were cut parallel to the muscle fibre direction from the centre of the cooked meat samples, wrapped in aluminium foil and placed in the refrigerator (4 °C) for 24 h. The meat strips were then cut to produce a total of five rectangular cubes each with a length of two centimetres and stored at 4 °C until the testing commenced. An Instron Universal Testing Machine (UTM) (Model 2519-107, Advanced Laboratory Solutions, United

Kingdom) attached with a Warner-Bratzler (WB) fitting was used in order to determine the force necessary to shear the cooked rectangular meat cube perpendicular to the muscle fibre direction. The WB fitting was a 1 mm thick triangular (V-notch) blade with a semi-circular cutting edge (radius of 0.508 mm). The UTM operated with a 2 KN compression load and compression extension of 20 mm. The shear test was performed at a compression speed of 200 mm/min. The shear force value of each of the samples was recorded in Newton (N).

2.5 Chemical analysis

2.5.1 Sample preparation

The proximate analysis was performed on the uncooked, left breast muscle of the geese used for the six replications of the descriptive sensory analysis. After the breast muscles were thawed (4 °C) homogenisation of the muscles followed. The samples were re-vacuum packed and frozen (-18 °C) until the proximate analysis commenced when the samples were thawed (4 °C) once more.

2.5.2 Proximate analysis

The proximate analyses were performed as described in Geldenhuys *et al*. (2013c) [Chapter 3]. The moisture content (%) was determined by the use of 2.5 g homogenised meat sample according to the Association of Official Analytical Chemist's Standard Techniques (AOAC) method 934.01 (AOAC, 2002a). The ash content (%) of the moisture free sample was determined by the official AOAC method 942.05 (AOAC, 2002b). The chloroform/methanol (1:2 v/v) extraction method stipulated by Lee *et al*. (1996) was used to determine the total lipid (%) (intramuscular fat) of a 5 g homogenised raw meat sample. To establish the total crude protein content (%) the Dumas combustion method 992.15 (AOAC, 2002c) was applied. A 0.15 g defatted, dried and finely grounded meat sample was analysed using a Leco Nitrogen/Protein Analyser (FP – 528, Leco Corporation). The Leco was calibrated with EDTA calibration samples (Leco corporation, 3000 Lakeview Avenue, St. Joseph, MI 49085-2396, USA, Part no. 502-092, Lot no. 1055) before each of the analysis sessions. The results were expressed in % Nitrogen (N). The nitrogen (%) was multiplied with a conversion factor (6.25) in order to determine the crude protein (%) present in the meat sample. All proximate analyses are controlled by a National inter-laboratory scheme (AgriLASA: Agricultural Laboratory Association of South Africa) where blind samples are analysed once every three months in order to control and ensure the accuracy and repeatability of the procedures used.

2.6 Statistical analysis

Experimentally, the study consisted of a randomised factorial block design with 4 treatments (2 seasons X 2 genders) and six replications. The study tested the effects of season and gender, as well as the interaction between the main effects. The trained panel consisted of eight judges and the six treatments were evaluated for the 17 sensory attributes established during the training sessions. The model for the experimental design is indicated by the following equation:

$$y_{ij} = \mu + \beta_j + s_i + g_k + (sg)_{ik} + \varepsilon_{ijk}$$

The terms within the model are defined as; the overall mean (μ), the effect of the block (β_j), the effect of season (s_i), the effect of gender (g_k), the effect of the interaction between season and gender $(sg)_{ik}$ and ε_{ijk} is the error associated with the effect of the block, season, gender and interaction of the former and latter.

The sensory, physical and proximate data were subjected to an analysis of variance (ANOVA). The Shapiro-Wilk test was performed to test for normality (Shapiro & Wilk, 1965). All of the outliers were identified and removed before final analysis of the ANOVA's. The t-Least Significant Differences (LSD) were calculated at a 5% significance level to compare the treatment means. Results were defined as being not significant at $P>0.05$ and significant at $P\leq0.05$. Correlations were made between the sensory attributes, physical characteristics and proximate composition by means of the Pearson's correlation coefficient. Principal Component Analysis (PCA) using a correlation matrix and Discriminant Analyses (DA) were performed to illustrate the relationships between the sensory, physical and proximate data (Naes *et al*., 2010). SAS™ statistical software (Statistical Analysis System, Version 9.2, 2006, SAS Institute Inc., Cary, NC, USA) was used for the analyses of variance (ANOVA) while the multivariate statistical analyses were performed using XL STAT™ statistical software (Version 2011, Addinsoft, New York, USA).

3 RESULTS AND DISCUSSION

3.1 Effect of season on the sensory profile

The major influential factor contributing to the difference in season was the variation in the diet. The effect of season/diet on meat quality is usually reflected in the chemical composition, especially the fatty acid profile, more so than the physical characteristics of the meat. This phenomenon is more so in monogastric than in ruminant animals. This is the reason why no differences ($P>0.05$) were found between the physical attributes, as well as the proximate composition, indicated by the Discriminant Analysis (DA) plots (Fig. 2b-c). In the respective DA plots, no trend was indicated between the four treatments, which was confirmed by the absence of any significant correlations ($P>0.05$) in Fig. 1a and within the mean results (Tables 3 and 4) which again showed no differences ($P>0.05$) between these attributes. Therefore, the physical and proximate chemical characteristics did not have a significant effect on the overall sensory profile and the significant difference in the treatments indicated by the DA plot in Fig. 1a, which is due to the effect of season, is mainly a result of the variation in the sensory attributes and those factors that influence it.

3.1.1 Aroma and flavour profile of July and November

The PCA bi-plot (Fig. 1a) and the DA plot (Fig. 1b) clearly indicate a difference in the treatments with regard to season in the 1st principal component (PC1/F1). Although the PCA bi-plot only describes 42% of the variation, this is still high when it is taken into account that there are numerous extrinsic (and intrinsic) factors that could influence the sensory profile. The meat from the winter and summer geese are in opposing positions; July associated with the attributes on the right side of the plot and November associated with the attributes on the left side of the plot. Strong negative correlations ($P\leq0.05$) (Table 5) between the attributes of the respective seasons, together with the sensory mean scores (Table 6), showing the significant differences between the main attributes of the two seasons, support this difference in the sensory profiles of the seasons. The geese harvested in winter mainly associated with the game, metallic and fish aroma and flavour attributes while the summer geese associated with the opposing sweet-oily-duck and beef aromas and flavours (Fig. 1a). The game, metallic and fish attributes also received higher ($P\leq0.05$) mean scores in July, while, the sweet-oily-duck attributes and beef flavour received higher ($P\leq0.05$) scores in November. Therefore, the major sensory driving forces behind the distinct variation in the sensory profiles of the geese were the differences in the aroma and flavour profiles between the treatments from winter and summer.

This difference in flavour is a direct result of the differentiation in the diet of the geese (Hornstein & Crowe, 1960; 1963) between these two time periods, which altered the fatty acid profile and consequently, the aroma and flavour profile of the meat were very different. Egyptian geese are

monogastric fowl and the fatty acid composition of the meat therefore reflects that of the dietary constituents (Wood & Enser, 1997; MacRae *et al*., 2005). Differences in the fatty acid profile in meat causes the formation of different flavours and aromas (Wood & Enser, 1997).

The association of the goose meat from winter with a very intense game aroma and flavour (Fig. 1a) was supported by the higher (P≤0.05) mean scores (Table 6) received for these two attributes and this is indicative of the diet. Swanson and Penfield (1991) found that increased levels of PUFA in meat from game animals are responsible for the distinct game characteristics. Geldenhuys *et al*. (2014) [Chapter 4] reported that the high PUFA content of Egyptian goose meat, compared to other well-known fowl species, may be the key contributing factor towards the dominating game characteristics found. Numerous studies have indicated that a predominantly green forage or grass-based diet is rich in α-linolenic acid (C18:3 *n*-3) (Marmer *et al*., 1984; Enser *et al*., 1998; Ward *et al*., 2003; Poulson *et al*., 2004). This is exactly what was reported in Chapter 6 of the diet of Egyptian geese during the winter months where the total PUFA content (%) was approximately 49% higher in total PUFA compared to that of November; the percentage of α-linolenic acid (C18:3 *n*-3) present was particularly high (46% compared to 0.5%). This results in high levels of this fatty acid being present in the muscle tissue of Egyptian geese during this period. Additionally the overall fatty acid composition (%) of the breast meat from July is listed in Chapter 6. In this study the results indicated a significantly higher percentage PUFA (35% compared to 26%) present in the meat harvested in July. Some of the individual fatty acids responsible for this higher (P≤0.05) PUFA content are homo-γ-Linolenic (C20:3 *n*-6), eicosatrienoic acid (C20:3 *n*-3), arachidonic acid (C20:4 *n*-6), docosapentaenoic acid (DPA) (C22:5 *n*-3) and docosahexaenoic acid (DHA) (C22:6 *n*-3). The breast portion specifically contained 10% α-linolenic acid (C18:3 *n*-3) in winter compared to the 3.6% in summer. The percentage of linoleic acid (C18:2 *n*-6) was also lower in July compared to November and the main PUFA's were linoleic acid (C18:2 *n*-6) and α-linolenic acid (C18:3 *n*-3) together comprising 64% of the total PUFA and 23% of the total fatty acids. There is, however, limited literature available on the role of individual fatty acids in the flavour formation of game characteristics and further research is required to establish the specific aroma volatiles involved in game flavour.

The respective fatty acid compositions emphasises the correlation between the fatty acid composition of the dietary constituents and the ultimate fatty acid profile of the meat. It can, therefore, be assumed that the higher overall PUFA content [Chapter 6] and the relationship between the specific polyunsaturated fatty acids are responsible for the distinct game sensory characteristics of winter (July).

Another factor contributing to the seasonal variation could be the relationship between the linoleic and α-linolenic acid content [Chapter 6]. In winter (July) there was a smaller difference in the percentages of these two fatty acids in the breast meat. Literature indicates that meat from cattle and

sheep, feeding on grass or forage based diets, has a stronger flavour compared to cereal-based diets which produce a milder flavoured meat (Enser, 1999). The variation is related to the main dietary fatty acid either being linolenic acid which creates the stronger flavour or linoleic acid which is responsible for the milder flavour of the meat (Melton, 1990; Sanudo *et al*., 1998; Enser, 1999). This is consistent with the results of this study; the "gamey" profile of the winter geese corresponds with the results found in the sensory profiling of Geldenhuys *et al*. (2014) [Chapter 4]. Although season was not investigated in Geldenhuys *et al*. (2014) [Chapter 4], the geese used in the sensory profiling research were harvested during this same period (winter) which explains their similarity towards the sensory profile of the geese from winter indicated in this chapter. In addition, the fatty acid analysis reported by Geldenhuys *et al*. (2013c) [Chapter 3] and by Geldenhuys *et al*. (2014) [Chapter 4] in the sensory profiling study also indicated that the Egyptian goose meat during this period was very high in PUFA content.

Another characteristic of the meat from winter is the perceptible metallic flavour, indicated by the higher (P≤0.05) mean score (Table 6). The strong association with metallic flavour found (Fig. 1a) can be correlated to the high iron (Fe) content present in Egyptian goose meat. In the previous sensory profile study, Geldenhuys *et al*. (2014) [Chapter 4] established that the high Fe content is responsible for the strong metallic flavour of Egyptian goose meat. The Fe content is related to the high level of physical exercise endured by the breast muscles of the geese. Lawrie and Ledward (2006) noted that there is an elaboration of the myoglobin content (Fe) in highly active muscles to ensure oxygen repletion. Furthermore, the studies of Yancey *et al.* (2006) and Calkins and Hodgen (2007) reveal that the presence of long chain unsaturated fatty acids in the meat can also be responsible for the development of a liver-like flavour. This is supported by the fact that Mendell *et al*. (1998) found a significant correlation between metallic aroma and C18:3 fatty acids in forage fed beef and speculated that a difference in the dietary fatty acids, C18:1 and C18:3, are responsible for the higher metallic attribute found. This relates back to the fact that the Egyptian goose breast meat from winter was significantly higher in the long chain PUFA C18:3 *n*-3 [Chapter 6].

The association of the winter meat with fish flavour (Fig. 1a), as well as the mean scores (Table 6) for this attribute, indicate that the panel found a significantly higher (P≤0.05) fishy taint in the goose meat from this season. This fishy taint can also be linked to the diet and the fatty acid profile. Enser (1999) noted that feeding high levels of eicosapentaenoic acid (EPA) (20:5 *n*-3) and docosahexaenoic acid (DHA) (22:6 *n*-3), produced a fishy flavour in meat. Campo *et al*. (2003) found that there is a significant correlation between fishy and linseed odours and the presence of C18:3. The fatty acid profile of the Egyptian goose breast meat [Chapter 6] not only indicates a higher percentage of α-linolenic acid (C18:3 *n*-3) but EPA as well. An overall higher level of DHA was also found in the meat from July. These fatty acids can therefore be linked to the fishy taint. The study by Campo *et al*.

(2003) also suggests that the high levels of Fe in Egyptian goose meat can also be a contributing factor to the ultimate presence of a fishy taint. By modelling the effect of fatty acids on odour development, Campo *et al*. (2003) found that the scores for fish, cod liver and linseed odours were significantly higher when Fe was added to a C18:3 fatty acid solution. This is related to the fact that Fe acts as a pro-oxidant in flavour formation reactions, especially with long chain unsaturated fatty acids due to their higher instability (Campo *et al*., 2003). However, the fishy aroma in this study seemed to disappear rapidly after cooking and resulted in a very low overall presence in the meat. The mean scores received (Table 6) was 0.44 (winter) and 0.09 (summer), and on a scale of 100 the presence of this attribute is negligible. This relates to the fact that statistically significant differences are not always relevant in terms of the ultimate influence on the sensory profile. There is a similar situation with the relevance of the chicken and ostrich attributes (Table 6) and the same conclusions can be made.

The goose meat harvested during summer was strongly associated with the sweet-oily-duck attributes and beef flavour. This association of the meat with these attributes resulted in the separate grouping on the left side of F1 on the PCA (Fig. 1a). This association is supported by the higher ($P \leq 0.05$) mean scores (Table 6) for sweet-oily-duck aroma and flavour, as well as, beef flavour during summer. This change in the typical aroma and flavour profile of the meat could be clarified by the fact that the main constituent of the diet during this period is grains, primarily wheat and barley. The research by Mangnall and Crowe (2001; 2002) emphasises the change in the feeding activities of the geese during the grain harvesting season. This grain or cereal based diet is considered to be of a more saturated nature, with a higher monounsaturated fatty acid (MUFA) content compared to the grass or forage based diet of winter. A diet such as this consequently resulted in meat with a higher MUFA and lower PUFA content [Chapter 6]. Grain diets generally consist of higher oleic acid (C18:1 *n*-6) and linoleic acid (C18:2 *n*-6c) content compared to grass based diets (Enser *et al*., 1998). This is not only reflected in the fatty acid profile of the crop contents but in the fatty acid composition of the breast meat from November as well [Chapter 6]. The profile of the crop content showed a high level of oleic acid (C18:1 *n*-9) in summer (75% compared to 8%), while the level of linoleic acid (C18:2 n-6c) was similar to that of winter. This is consistent with the profile of the breast meat which indicated a 16% higher content of oleic acid ($P \leq 0.05$) contributing to the increased MUFA ($P \leq 0.05$). Together oleic and linoleic acid comprised approximately 57% of the total fatty acids present in the breast meat from summer. In the research of Campo *et al*. (2003), meat with a high content of C18:1 and C18:2 fatty acids were found to be associated with an 'oily' characteristic. It is also believed that the 'sweet' sensory note found in meat could be associated with the development of lactones and has also been found in meat high in linoleic acid (C18:2 *n*-6*c*) due to an increase in the levels of 6-γ-dodecenolactone (Melton, 1990). Campo *et al*. (2003) also found a close association between the

sweet descriptor and mixtures of C18:1 and Fe, as well as C18:2 and Fe. They speculated that the pro-oxidant effect of the Fe assists in the ability of the flavour to be recognised. This 'sweet' characteristic of the summer geese was probably responsible for the relatively strong correlation (Table 5) of the sweet-oily-duck aroma ($r = 0.431$; $P = 0.035$) and flavour ($r = 0.405$; $P = 0.049$) with beef flavour. The panel associated beef with having a sweetish flavour and this may have been the reason for this association. To support this sweet association noted by the panel with beef flavour; Berry *et al*. (1980) described that a desirable flavour of beef was related to intense caramelised and sweet attributes. Overall the summer meat had a very mild flavour compared to the winter meat. The somewhat higher linoleic acid content in summer compared to winter may also have contributed to producing a milder flavoured meat (Enser, 1999). The results found during this study are in agreement with the findings of Campo *et al*. (2003) and Melton (1990), as the presence of the sweet and oily attributes were significant in the goose meat from summer. An increase in oleic and linoleic acid in the meat, due to a diet rich in grains, therefore explains the associated sweet-oily-duck attributes and beef flavour of this season.

The negative correlations between metallic flavour and the sweet-oily-duck attributes ($P \leq 0.05$) of summer (Fig. 1a and Table 5) indicate that the panel found the meat to have no association with metallic flavour whatsoever, compared to the winter geese. It is possible that the metallic flavour was concealed by the overwhelming sweet-oily-duck and beef attributes associated with the meat from this season (summer). Another possibility is that the metallic and game attributes are interlinked with regard to their ability to be recognised and without the game attributes, the metallic flavour is perceived as less intense by the panel. The mineral content results of Chapter 6 indicated that there was no significant difference ($P > 0.05$) in the Fe content of the breast muscle between the different seasons and, therefore, there may have been a concealing effect in terms of metallic flavour. The higher ($P \leq 0.05$) oleic acid and lower ($P \leq 0.05$) α-linolenic acid of the summer breast meat compared to that of winter could also have been responsible for the lower metallic flavour. Mendell *et al.* (1998) stated that a difference in the C18:1 and C18:3 dietary fatty acids are related to the metallic attribute where higher C18:3 levels correlate with this attribute.

In general, the breast meat from the geese harvested in summer tended to have a milder sensory profile with a sweet-like characteristic. These characteristics together with the fact that the gamey and metallic notes were less prevalent may ultimately mean that the sensory profile of the meat harvested in summer is more acceptable compared to that from winter. This is consistent with literature indicating that grain-based diets produce a more acceptable flavour compared to grass based diets in ruminant meats (Reagan *et al*., 1977; Melton, 1990). This is certainly an interesting finding as Chapter 6 concludes that the meat harvested in summer is the less healthy of the two

respective seasons. The difference in the aroma and flavour profile should be considered in terms of harvesting periods and producing meat with the best possible quality.

3.1.2 Texture attributes

As mentioned in the previous section, there were no significant differences (P>0.05) in the physical or proximate results as affected by season and no significant correlations were found between these and the sensory attributes in Fig. 1a and Table 6. Concerning the sensory attributes alone, the goose meat from winter associated with a high residue and high shear force on the PCA bi-plot (Fig. 1a); residue ($r = -0.943$; $P < 0.0001$) and shear force ($r = -0.688$; $P = 0.0001$) both negatively correlated (Table 5) to tenderness. In contrast, the goose meat from summer associated with tenderness. The mean scores (Table 6) confirm these correlations as summer received a slightly higher, although not significant (P>0.05), tenderness score while winter received a higher (P≤0.05) score for residue. This may be explained by the higher (P≤0.05) initial juiciness of summer (Table 6), as well as the association of this attribute with the summer meat on the PCA (Fig. 1a). There was also a very strong and significant correlation (Table 5) between sustained juiciness and tenderness ($r = 0.953$; $P < 0.0001$) confirming the perception that juicy meat is generally more tender. The amount of moisture in meat has a positive effect on the tenderness as the amount of water bound causes the dilution of the muscle fibres present in a certain area of meat (Thomas *et al*., 2004).

Table 3 The mean scores (±SD[1]) for the physical characteristics of Egyptian goose meat as affected by season and gender

	Season		Gender		LSD[2]
	July (Winter)	**November (Summer)**	**Female**	**Male**	**P=0.05**
pH	5.94[a] ± 0.25	5.93[a] ± 0.29	5.86[a] ± 0.17	6.02[a] ± 0.32	0.19
Cooking loss	30.21[a] ± 5.27	28.89[a] ± 4.34	28.80[a] ± 3.43	30.31[a] ± 5.87	3.60
WBC	4.06[a] ± 0.55	4.17[a] ± 0.51	4.00[a] ± 0.25	4.22[a] ± 0.69	0.39
Shear force	53.32[a] ± 16.77	54.05[a] ± 12.34	46.29[b] ± 7.63	61.08[a] ± 16.02	12.60

[a-b]Means in rows with different superscripts within season and gender respectively are significantly different at P≤0.05. [1]SD (standard deviation); [2]LSD (least significant difference).

Table 4 The means scores (±SD[1]) for the proximate analysis of Egyptian goose meat as affected by season and gender

	Season		Gender		LSD[2]
	July (Winter)	**November (Summer)**	**Female**	**Male**	**P=0.05**
Moisture	72.1[a] ± 1.11	72.8[a] ± 1.83	71.7[b] ± 1.02	73.2[a] ± 1.60	1.04
Protein	20.8[a] ± 1.77	20.5[a] ± 1.20	20.8[a] ± 1.61	20.4[a] ± 1.40	1.24
Fat	5.2[a] ± 1.51	4.3[a] ± 1.04	5.1[a] ± 1.19	4.4[a] ± 1.47	1.19
Ash	1.2[a] ± 0.19	1.2[a] ± 0.10	1.2[a] ± 0.08	1.3[a] ± 0.20	0.14

[a-b]Means in rows with different superscripts within season and gender respectively are significantly different at P≤0.05. [1]SD (standard deviation); [2]LSD (least significant difference).

Table 5 Correlation matrix showing Pearson correlation coefficients (r) and P-values[1] for the attributes discussed

Variables	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
1. Fish A	**1**	**0.433**	-0.032	-0.007	-0.237	-0.125	0.198	-0.378	-0.150	0.246	0.118	0.091	-0.051	0.069	0.012	-0.271	-0.158	-0.279	0.068	0.104	-0.238
2. Fish F	**0.035**	**1**	0.301	0.215	**-0.508**	**-0.605**	**0.647**	**-0.588**	**-0.519**	**0.703**	-0.165	-0.183	0.342	0.093	0.068	-0.339	0.184	0.002	-0.174	0.359	-0.005
3. Game A	0.880	0.153	**1**	-0.109	**-0.556**	**-0.416**	**0.529**	-0.344	**-0.664**	**0.537**	-0.264	-0.220	0.313	0.092	-0.039	0.195	0.098	-0.072	-0.020	0.191	-0.071
4. Beef A	0.975	0.313	0.612	**1**	**-0.436**	-0.035	0.207	-0.048	-0.385	0.286	-0.309	-0.323	0.382	0.224	0.045	-0.026	0.114	0.357	0.006	-0.133	0.205
5. Sweet Oily Duck A	0.265	**0.011**	**0.005**	**0.033**	**1**	0.377	**-0.639**	**0.431**	**0.869**	**-0.693**	0.348	0.348	**-0.468**	-0.032	-0.218	-0.169	-0.169	0.003	-0.080	-0.081	0.085
6. Initial J	0.559	**0.002**	**0.043**	0.871	0.069	**1**	**-0.496**	**0.561**	**0.515**	**-0.561**	0.157	0.101	-0.163	0.290	-0.341	0.120	0.242	0.321	0.025	**-0.563**	-0.078
7. Game F	0.354	**0.001**	**0.008**	0.332	**0.001**	**0.014**	**1**	**-0.655**	**-0.725**	**0.746**	-0.395	-0.324	**0.491**	0.210	-0.006	-0.220	0.139	0.128	-0.147	0.243	0.033
8. Beef F	0.069	**0.003**	0.100	0.823	**0.035**	**0.004**	**0.001**	**1**	**0.405**	**-0.737**	**0.454**	**0.428**	**-0.479**	0.019	0.071	0.180	-0.120	0.213	0.003	-0.343	0.144
9. Sweet Oily Duck F	0.485	**0.009**	**0.000**	0.063	**< 0.0001**	**0.010**	**< 0.0001**	**0.049**	**1**	**-0.705**	0.294	0.254	-0.400	-0.061	-0.167	-0.051	-0.071	0.023	0.052	-0.223	0.032
10. Metallic	0.247	**0.000**	**0.007**	0.175	**0.000**	**0.004**	**< 0.0001**	**< 0.0001**	**0.000**	**1**	**-0.554**	**-0.519**	**0.633**	0.074	0.129	-0.106	0.273	0.012	-0.194	0.371	-0.090
11. Sustained J	0.582	0.441	0.213	0.141	0.095	0.464	0.056	**0.026**	0.163	**0.005**	**1**	**0.953**	**-0.903**	0.172	-0.366	-0.304	**-0.646**	-0.279	0.024	0.023	-0.137
12. Tenderness	0.672	0.393	0.301	0.124	0.096	0.637	0.123	**0.037**	0.230	**0.009**	**< 0.0001**	**1**	**-0.943**	0.124	-0.336	-0.342	**-0.688**	-0.344	0.014	0.108	-0.155
13. Residue	0.812	0.102	0.137	0.066	**0.021**	0.448	**0.015**	**0.018**	0.053	**0.001**	**< 0.0001**	**< 0.0001**	**1**	0.066	0.256	0.137	**0.659**	0.399	0.010	-0.133	0.181
14. pH	0.749	0.665	0.668	0.292	0.881	0.169	0.325	0.931	0.775	0.733	0.421	0.562	0.761	**1**	**-0.665**	**-0.484**	0.007	**0.430**	-0.086	-0.282	0.232
15. % Cooking Loss	0.956	0.752	0.858	0.834	0.307	0.103	0.977	0.741	0.435	0.548	0.079	0.108	0.227	**0.000**	**1**	**0.427**	0.215	-0.008	0.040	0.120	-0.026
16. WBC	0.200	0.105	0.360	0.905	0.429	0.578	0.303	0.400	0.814	0.622	0.149	0.102	0.522	**0.017**	**0.037**	**1**	0.365	0.131	-0.105	0.033	-0.125
17. Shear Force	0.461	0.390	0.650	0.596	0.431	0.254	0.517	0.577	0.742	0.198	**0.001**	**0.000**	**0.000**	0.976	0.313	0.079	**1**	**0.581**	-0.258	-0.197	-0.086
18. % Moisture	0.187	0.994	0.739	0.087	0.988	0.126	0.550	0.317	0.917	0.955	0.187	0.100	0.054	**0.036**	0.969	0.542	**0.003**	**1**	-0.276	**-0.430**	0.057
19. % Protein	0.752	0.416	0.924	0.979	0.711	0.909	0.493	0.990	0.810	0.365	0.910	0.950	0.964	0.688	0.854	0.626	0.223	0.192	**1**	**-0.640**	-0.041
20. % Fat	0.629	0.085	0.372	0.537	0.705	**0.004**	0.252	0.101	0.295	0.074	0.915	0.615	0.534	0.182	0.575	0.880	0.355	**0.036**	**0.001**	**1**	0.054
21. % Ash	0.263	0.982	0.743	0.336	0.692	0.717	0.877	0.502	0.884	0.676	0.524	0.471	0.397	0.276	0.903	0.560	0.690	0.793	0.851	0.803	**1**

Numbers in the first row correlates with the numbers of the attributes in the first column. The letters following the attribute descriptors "A", "F" and "J" refers to aroma, flavour and juiciness respectively.
The upper right hand triangle of the table (illustrated in blue), separated by the diagonal divide indicates the correlation coefficients (r) while the bottom triangle indicates the corresponding P-values.
[1]All the values in bold are significant at a level of P≤0.05.

Table 6 The mean scores (±SD[1]) for the sensory attributes of Egyptian goose meat as affected by season and gender

Sensory attribute	Season		Gender		LSD[2]
	Winter (July)	Summer (November)	Female	Male	P=0.05
Game aroma	34.78[a] ± 8.72	29.73[b] ± 8.10	31.67[a] ± 9.14	32.87[a] ± 8.38	2.10
Chicken aroma	0.09[a] ± 0.23	0.08[a] ± 0.22	0.09[a] ± 0.22	0.08[a] ± 0.23	0.04
Ostrich aroma	0.09[a] ± 0.33	0.06[a] ± 0.22	0.08[a] ± 0.32	0.08[a] ± 0.25	0.07
Beef aroma	23.32[a] ± 10.99	22.73[a] ± 9.43	21.47[b] ± 9.43	24.57[a] ± 10.78	3.06
Sweet oily duck aroma	7.05[b] ± 11.57	17.22[a] ± 13.97	15.89[a] ± 14.08	8.52[b] ± 12.51	3.68
Fish aroma	0.52[a] ± 1.68	0.28[a] ± 1.10	0.39[a] ± 1.43	0.40[a] ± 1.41	0.48
Game flavour	41.97[a] ± 16.16	33.55[b] ± 14.89	36.07[b] ± 15.99	39.45[a] ± 16.04	3.23
Chicken flavour	0.10[a] ± 0.27	0.11[a] ± 0.42	0.15[a] ± 0.44	0.06[b] ± 0.21	0.09
Ostrich flavour	0.06[a] ± 0.21	0.07[a] ± 0.22	0.08[a] ± 0.25	0.05[a] ± 0.17	0.05
Beef flavour	14.03[b] ± 12.66	18.23[a] ± 10.06	16.01[a] ± 11.09	16.31[a] ± 12.09	2.30
Sweet oily duck flavour	3.73[b] ± 7.85	12.63[a] ± 11.85	10.39[a] ± 12.42	6.06[b] ± 8.91	2.84
Fish flavour	0.44[a] ± 1.02	0.09[b] ± 0.29	0.25[a] ± 0.67	0.28[a] ± 0.84	0.16
Metallic flavour	14.92[a] ± 9.03	8.39[b] ± 7.29	10.28[b] ± 8.07	12.96[a] ± 9.34	2.14
Initial juiciness	24.00[b] ± 8.16	30.43[a] ± 10.29	26.13[a] ± 9.14	28.32[a] ± 10.37	2.74
Sustained juiciness	25.51[a] ± 9.14	27.91[a] ± 9.75	29.00[a] ± 8.03	24.41[b] ± 10.31	3.63
Tenderness	41.06[a] ± 19.30	46.23[a] ± 19.39	49.34[a] ± 17.47	37.94[b] ± 19.78	8.66
Residue	37.98[a] ± 18.83	29.07[b] ± 19.84	26.05[b] ± 16.33	41.00[a] ± 20.21	7.93

[a-b] Means in rows with different superscripts are significantly different at P≤0.05. Means determined by an unstructured line scale (0 = low intensity, 100 = high intensity). [1]SD (standard deviation); [2]LSD (least significant difference).

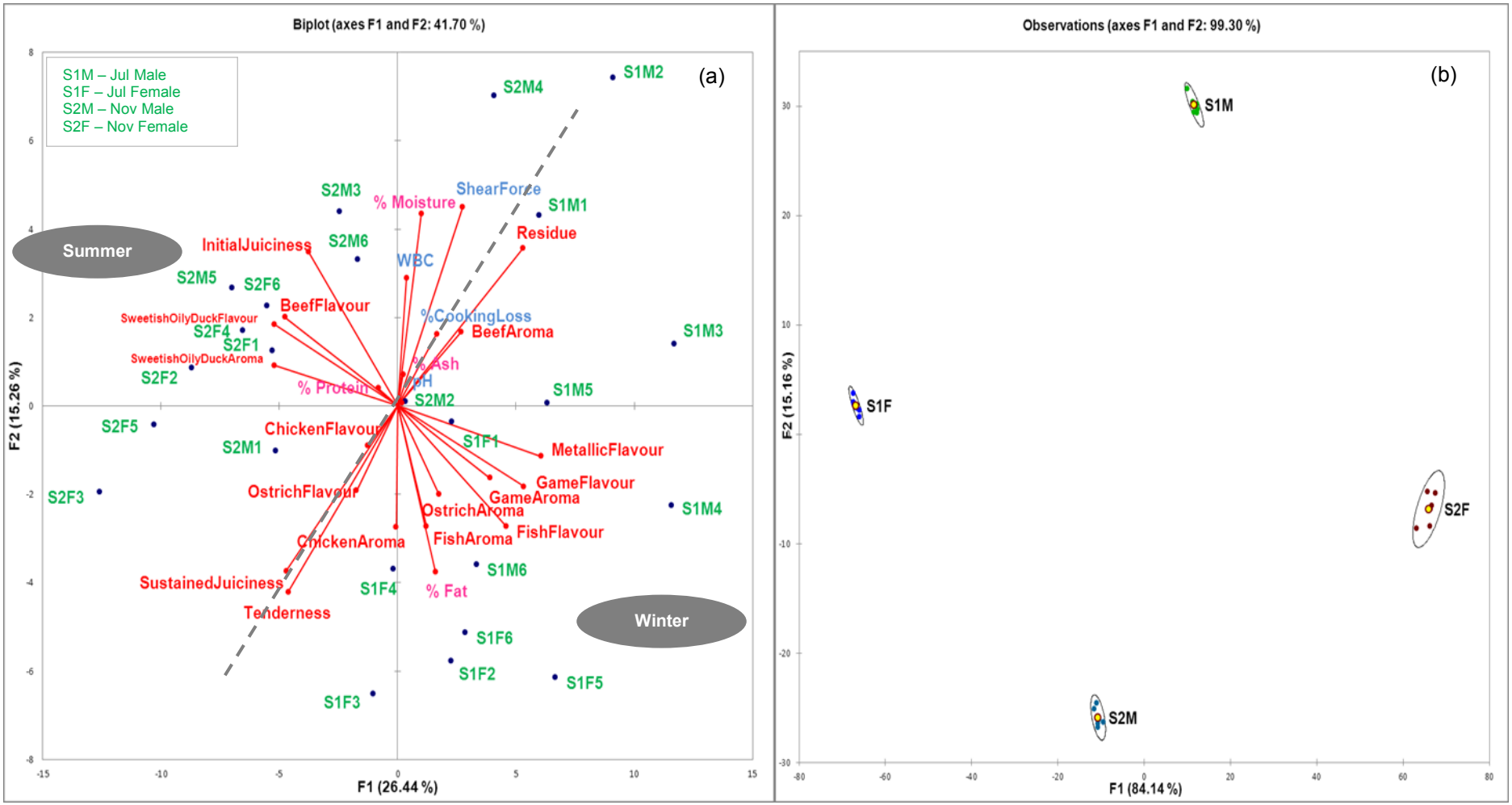

Figure 1 (a) PCA bi-plot and (b) DA plot of sensory attributes, physical characteristics and proximate analysis of Egyptian goose meat as affected by season and gender. Note the abbreviations used: S1 (Season 1 - July/winter); S2 (Season 2 – November/summer).

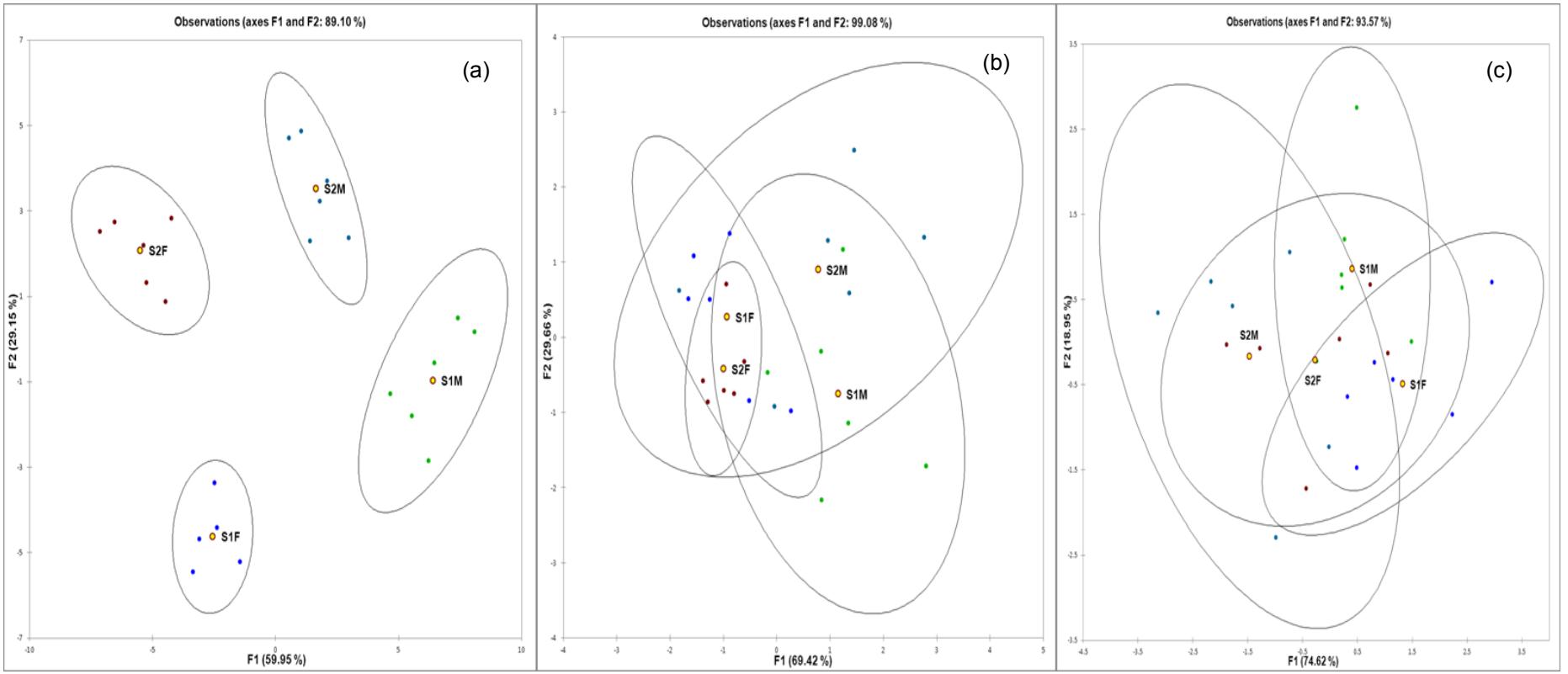

Figure 2 DA plots of the (a) sensory attributes, (b) physical attributes and (c) proximate composition of Egyptian goose meat as affected by season and gender. Note the abbreviations used: S1 (Season 1 - July/winter); S2 (Season 2 – November/summer).

3.2 Effect of gender on the sensory profile

The effect of gender on the sensory profile was less substantial in comparison to that of season. In Fig. 1a, there is somewhat of a grouping in terms of male and female within the two different seasons. This grouping within the PCA bi-plot was possibly due to the sensory differences, more so than due to the physical and chemical attributes. The females had a significantly higher (P≤0.05) mean score for both of the sweet-oily-duck attributes while the males were higher in beef flavour, as well as game and metallic flavour (Table 6). However, in the fatty acid composition of the breast meat [Chapter 6], no significant differences (P>0.05) were found for the related fatty acids (oleic, linoleic and linolenic acid) as a result of gender. The significantly higher metallic flavour of the male geese could be related to a higher level of physical activity resulting in an increase in myoglobin content (Fe) and therefore the increased metallic flavour. However, according to Chapter 6 the mineral composition of the breast did not indicate a significant difference between the Fe content of the different genders. The chicken flavour seems to be significantly higher in the females but the situation is similar to that of the fish flavour with the mean scores being 0.15 (female) and 0.06 (male) and on a scale of 100 the relevance of such a low presence in the meat is questionable.

The only significant difference (Table 3) in the mean score results of the physical data was the higher (P≤0.05) shear force of the male geese compared to that of the females which relates well with the significantly lower (P≤0.05) sensory tenderness (Table 6) and higher (P≤0.05) residue thereof. The lower tenderness is linked to a possible higher level of physical activity in breast muscle of male geese. Consequently there is an increase in the intramuscular collagen content which is responsible for the increase in the shear force of the meat (Smith & Carpenter, 1970; Lewis *et al*., 1989) from the male geese. Another contributing factor may be the higher fat content of the females, it was not significantly higher, but fat content affects the tenderness of meat in that it has a diluting effect on the muscle fibres and collagen present in a certain area of meat (Wood *et al*., 1999). Similarly moisture could also have been involved (Thomas *et al*., 2004), but the male geese had a significantly higher (P≤0.05) moisture (%) content (Table 4) which is a contradicting result in terms of high shear force and low tenderness. This high moisture is due to the lower intramuscular fat content of the males as there is an inverse relationship between moisture and fat content within muscle. This phenomenon corresponds well with this study as there was a negative, but low correlation (Fig. 1.a and Table 5) between the percentages of intramuscular fat and moisture ($r = -0.430$; $P = 0.036$). Furthermore, no association was found between the significantly higher moisture content of the males and the juiciness as perceived by the trained panel.

4 CONCLUSIONS

The research regarding the feeding activities of Egyptian geese and the quantification of the damage caused by this species to the crop lands in the Western Cape region generated the idea of exploring the potential of Egyptian goose meat as a product on the South African meat market. This study established that the grain harvesting season had a major effect on the overall sensory profile of the meat.

The geese harvested in winter (July) had the characteristic and overwhelming game and metallic attributes, similar to that found in Geldenhuys *et al*. (2014) [Chapter 4]. In contrast, the meat from the geese harvested in summer (November) had a very pleasant sweet-oily-duck and beef sensory profile with no overpowering game or metallic notes. This variation in the aroma and flavour profile is a result of the difference in the diet of the geese during these time periods; the grain based diet of summer responsible for higher levels of oleic and linoleic acid and lower PUFA content compared to winter.

With regard to gender, the females associated with the sweet-oily-duck attributes while the males were more closely related to the beef, game and metallic attributes. The texture attributes showed that there was variation in the tenderness and residue as affected by gender. The meat from male geese tended to be significantly less tender with a higher shear force.

It can thus be concluded that seasonality has the largest effect on the sensory profile and will most probably also influence the consumer acceptability of Egyptian goose meat. This aspect needs to be considered in the possible harvesting and utilisation of the meat from this species. It will, however, be challenging to find the best balance of healthy meat with the most acceptable sensory profile.

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CHAPTER 8

Histological characterisation of the fibre types in the *M. pectoralis* of Egyptian geese (*Alopochen aegyptiacus*)

ABSTRACT

The Egyptian goose is a Southern African wildfowl species which is hunted on a regular basis. The utilisation of the meat is imperative, but with the current absence of scientific literature it is vital to investigate the meat quality, as well as the factors of influence thereupon. One of the key aspects of meat quality is the composition of the muscle fibres. The *pectoralis* muscle of Egyptian geese is mainly comprised of fast, oxidative-glycolytic fibres (84%) with a cross-sectional area of 1283.9 μm^2. A small percentage of fast, glycolytic fibres (16%) are also present. This is attributable to the strenuous requirements of long distance flight. The large proportion of small, fast, oxidative-glycolytic fibres may be responsible for the perceived toughness of Egyptian goose breast meat.

Keywords: Fibre type, Egyptian geese, Avian *pectoralis*, Volant species, Meat quality

1 INTRODUCTION

In Southern Africa, the gamebird industry and the sport of wingshooting is becoming increasingly popular. The Egyptian goose (*Alopochen aegyptiacus*) is one of the leading gamebird species hunted (Viljoen, 2005). This wildfowl species is also considered to be a very serious agricultural pest by crop farmers in the Western Cape, South Africa (Mangnall & Crowe, 2002). In an effort to manage the population numbers and thereby reduce the damage they cause, wingshooting of the geese is recommended. The utilisation of the meat is therefore imperative, but with the current absence of scientific literature on the meat quality and composition of this species, it is vital to investigate the meat quality, as well as the factors of influence.

The composition of muscle fibres is a critical determinant of meat quality (Lefaucheur, 2010). Muscle fibre type can influence the colour, tenderness, juiciness and even the flavour of meat, which in turn may affect the consumer acceptability of the meat. The attribute which is most affected by muscle fibre type is the tenderness of meat. Factors such as the total number of fibres, the cross sectional area and diameter of the fibres, as well as the composition of the muscle fibre types are significant in terms of meat tenderness (Lefaucheur, 2010).

It is thus necessary to determine the muscle fibre composition of the breast portion (*M. pectoralis*) of Egyptian geese. This will provide valuable insight regarding the perceived toughness of the meat [Chapter 4 and Chapter 7]. Investigating the fibre types present in this muscle will also provide vital information in terms of the type of activity (locomotive/flight and postural) this muscle is used for. This will assist in explaining other characteristics such as the colour, pH and intramuscular fat content of the breast portion as reported by Geldenhuys *et al*. (2013a) [Chapter 5].

2 MATERIALS AND METHODS

2.1 Harvesting

Egyptian geese were harvested on the University of Stellenbosch's experimental farm, Mariendahl (ethical clearance: 10NP_HOF01), by the use of double barrelled shotguns. A total of 3 mature geese were harvested and collected in the field.

2.2 Sample preparation

Superficial muscle samples were removed (1 h post mortem) from the sternobrachialis region of the right *pectoralis* muscle within the cranial third of the muscle. Each block of muscle (0.7 x 0.5 x 0.5 cm) was mounted on a cardboard square with the muscle fibre orientation perpendicular to the cardboard surface. Muscle specimens were coated with Jung tissue freezing medium (Leica Biosystems, Midrand, South Africa) and frozen in iso-pentane cooled to -160 °C by liquid nitrogen

(Dubowitz, 1985) and stored at -80 °C for approximately one week. Serial cross-sections (12 µm) were cut from each muscle sample using a cryostat (Leica, Germany) maintained at -25 °C and mounted onto microscope slides (Lasec, SA).

2.3 Immunohistochemistry and NADH histochemistry

Three series sections from each sample were stained for immunofluorescence by a modified version of the method described by Rosser *et al*. (1996). Sections were first blocked in a solution of 5% natural goat serum (NGS), 1% bovine serum albumin (BSA) and phosphate buffered saline (PBS) for 20 min at ambient temperature (21 °C). The sections were then incubated with the primary antibodies; F59 and S46 (Developmental Hybridoma Studies Bank, University of Iowa, USA), respectively at 4 °C overnight. The primary antibodies were used at the dilution of 1:20 in blocking solution. The sections were then rinsed in PBS and incubated at room temperature for 1 h in a 1:200 dilution of secondary antibody (Alexa Fluor 488 goat anti-mouse IgG, Life Technologies, Carlsbad, USA) in PBS. The sections were then rinsed in PBS and fixed in 4% paraformaldehyde (PFA) for 3 min, after which a cover slip was mounted on the sections with fluorescent mounting media (DAKO, Denmark). So as to ensure that the immunoflorescent stain was effective and the antibodies specific, a serial section of the *sartorius*, a thigh muscle containing both slow and fast fibres, was stained as a control.

In order to distinguish between the two fast fibre isoforms (fast, oxidative-glycolytic and fast glycolytic) two sections from each series were stained for B-nicotinamide adenine dinucleotide (NADH) activity (Dubowitz, 1985). Slides were incubated in dinitroblue tetrazolium (NBT) (0.12 g in 60 mL, 0.05 M Tris buffer) and NADH (0.1g in 60 mL, 0.05 M Tris buffer) (Sigma-Aldrich, St. Louis, USA) for 30 min at 37 °C. Post incubation, the slides were rinsed with distilled water and washed in increasing concentrations of acetone (30%, 60% and 90%). This was followed by rinsing with distilled water and mounting of the cover slips with DPX mountant (Sigma-Aldrich, St. Louis, USA). All slides were kept at 4 °C until ready to view.

2.4 Fibre identification, counting and cross-sectional area

All slides were visualised and photographed at 4x magnification using an Olympus Cell^R Microscope system (Olympus Biosystems GMBH) with an F-view-II cooled CCD camera (Soft Imaging systems). A Xenon-Arc burner (Olympus Biosystems GMBH) was used as the light source. Photos were taken at three locations on each of the sections. The three locations coincided on each of the serial sections. The muscle fibres were identified (Rosser *et al*., 1996) and counted on each of the three locations. The cross-sectional area (CSA) was determined on 100 muscle fibres within a designated

rectangular area on each of the three locations by using Image J software (Version 1.41, 2009, http://rsbweb.nih.gov/ij/).

2.5 Statistical analysis

The data from each of the three areas obtained from the three *pectoralis* muscle samples (n=9) were subjected to an analysis of variance (ANOVA) and the t-Least Significant Differences (LSD) were calculated at a 5% significance level. Differences between the variables were accepted as being significant at P≤0.05. SAS™ statistical software (Version 9.2, USA) was used for the ANOVA.

3 RESULTS

The staining of the Egyptian goose pectoralis muscle for immunofluorescence revealed the presence of only fast twitch, oxidative-glycolytic (FOG/type IIa) and glycolytic (FG/type IIb) fibers. This is evident in Fig. 1 which indicates the positive labeling of the fast (F59) antibody (Fig. 1b) compared to the negative labeling of its slow (S46) counterpart (Fig. 1c) within the serial sections. For this reason, the staining of the sections for NADH activity (Fig. 1d) was necessary in order to differentiate between the two fast isoforms; FOG and FG present in the breast portion (pectoralis muscle).

The quantification of the two fast isoforms revealed that the pectoralis muscle predominantly consists (P≤0.05) of FOG fibers (84.4%) (Table 1). These FOG fibers were much smaller than the FG fibers. This is indicated by the lower (P≤0.05) CSA and diameter (Table 1) of the FG compared to the FOG fibers.

Table 1 The mean (±SD[1]) fiber composition (%), fiber CSA and fiber diameter of the *pectoralis* muscle of Egyptian geese

	FOG[2]	FG[3]
Total (%)	84.4[a] ± 9.3	15.6[b] ± 9.3
CSA[4] (μm[2])	1283.9[b] ± 176.7	2793.3[a] ± 921.9
Diameter (μm)	40.3[b] ± 2.9	58.4[a] ± 12.9

[a-b]Means in rows with different superscripts differ significantly at P≤0.05. [1]SD (standard deviation); [2]FOG (fast, oxidative-glycolytic); [3]FG (fast-glycolytic), [4]CSA (cross-sectional area).

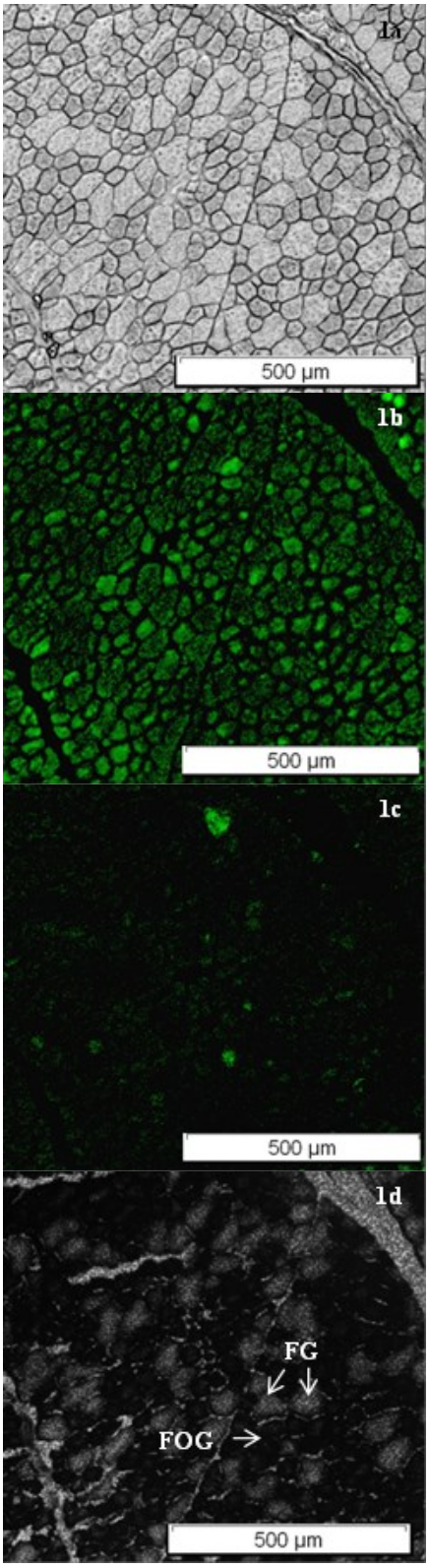

Figure 1 Serial sections of the Egyptian goose *pectoralis* muscle (a) Transverse section (b) immunocytochemical labelling by fast primary antibody (F59) (c) immunocytochemical labelling by slow primary antibody (S59) (d) histochemical demonstration of NADH activity and identification of FG vs. FOG fibers.

4 DISCUSSION

The *pectoralis* muscle of avian species, which rely on this muscle for long distance flight, is almost entirely composed of fast-twitch fibers (Rosser & George, 1986; Butler, 1991; Rosser *et al*., 1996). A rigorous requirement for rapid, sustained muscle contraction is placed on this muscle for long term flight. These requierments are responsible for the presence of mainly FOG fibers in the breast portion of volant species (Rosser & George, 1986). The small amount of FG fibers present is presumed to assist larger bodied birds when an increase in power is needed during take-off (Rosser & George, 1986). The results found in this study (Table 1) is in agreement with literature and shows that the pectoralis muscle of Egyptian geese is only comprised of fast-twitch fibers i.e. 84.4% FOG and 15.6% FG. Egyptian geese are volant gamebirds that fly long distances in order to forage on grain fields (Mangnall & Crowe, 2002). Their breast muscles therefore endure a much higher level of activity compared to terrestrial gamebird species such as guineafowl. Similar results have also been found in other Anseriforms (ducks and geese) such as mule ducks (Baeza *et al*., 2000), Canada geese (Rosser & George, 1987) and several other species (Rosser & George, 1986; Rosser *et al*., 1996).

Muscle fiber composition is recognised as one of the major determinants of meat quality. With that said, the definite relationship between fiber type and meat tenderness is still somewhat unclear (Maltin *et al*., 2003). The general consensus seems to be that muscles containing slow, type I fibers are more tender because of the smaller fiber diameter which results in it being less resistant to chewing or mechanical shearing (Purslow, 2005). However, some studies (Henckel *et al*., 1997; Maltin *et al*., 1997) have found that FOG (type IIa) fibers are smaller than the slow fibers present. This suggests that fiber size may vary on account of species, muscle and exercise and that the perception that slow fibers produce more tender meat may not be entirely correct. In light of all the controversy, Henckel *et al*. (1997) and Maltin *et al*. (1997) reported that the percentage of FOG fibers considerably affects tenderness. Another aspect to consider is the fact that with a decrease in fiber size there is a larger amount of fibers present, thus an increase in connective tissue (per unit area/volume) resulting in less tender meat (Carpenter *et al*., 1963). In the Egyptian goose pectoralis, the FOG fiber area of 1283.9 μm^2 (Table 1) is small compared to the CSA of fibers measured in studies on pork and beef (Henckel *et al*.,1997; Maltin *et al*.,1997; Vestergaard *et al*., 2000). This suggests that the small FOG fibers may be a contributing factor to the overall perceived toughness of Egyptian goose breast meat.

The high proportion of FOG fibers also clarifies the higher ($P \leq 0.05$) intramuscular fat percentage (Geldenhuys *et al*., 2013b) [Chapter 3] and dark, red colour (Geldenhuys *et al*., 2013a) [Chapter 5] of Egyptian goose breast meat compared to other species such as guineafowl and broiler chicken. Other important factors relating to fiber type and meat quality is the variation in the post

mortem muscle metabolism and the difference in the proteolytic enzyme/inhibitor concentrations. These factors may have an influence on the sensory tenderness of Egyptian goose meat and warrant further research.

5 CONCLUSIONS

The *pectoralis* muscle of Egyptian geese is mainly comprised of FOG fibres with a small percentage of FG fibers. This is attributable to the strenuous requirements of long distance flight. The large proportion of small, FOG fibers may be responsible for the perceived toughness of Egyptian goose breast meat.

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CHAPTER 9

Post mortem rigor development in the Egyptian goose (*Alopochen aegyptiacus*) breast muscle (pectoralis): Factors which may affect the tenderness

ABSTRACT

Baseline research investigating the toughness of Egyptian goose meat is required. To achieve this, the study therefore investigates the post mortem pH and temperature decline (15 min – 4 h 15 min post mortem) in the pectoralis muscle (breast portion) of this gamebird species. It also explores the enzyme activity of the Ca^{2+} dependant protease (calpain system) and the lysosomal cathepsins during the rigor mortis period. No differences were found for any of the variables between genders. The pH decline in the *pectoralis* muscle occurs quite rapidly (*c* = -0.806; ultimate pH = ~5.86) compared to other species and it is speculated that the high rigor temperature (>20 °C) may contribute to the increased toughness. No calpain I was found in Egyptian goose meat and the μ/m-calpain activity remains constant during the rigor period while a decrease in calpastatin activity was observed. The cathepspin B, B & L and H activity increased over the rigor period.

Keywords: Egyptian geese, Gamebirds, Rigor mortis, Post mortem muscle, pH, Calpains, Cathepsins

1 INTRODUCTION

The hunting of wildfowl species in Southern Africa has not only increased considerably in the past few years but a large number of birds are hunted annually as they are considered to be agricultural pests. Crop farmers incur major financial losses due to the feeding activities of Egyptian geese (*Alopochen aegyptiacus*), consequently the geese are hunted in order to reduce the damage caused (Mangnall & Crowe, 2001; 2002). To date, the meat generated is not utilised commercially but rather consumed by the hunters or donated to the local, rural communities. Therefore, potential exists for the meat from this game bird species to be utilised in a sustainable manner. However, there is an absence of accurate, scientific information regarding the meat quality of game birds (Geldenhuys *et al*., 2013a). Baseline research investigating the meat quality of this wild fowl species is therefore essential.

The sensory characteristics of Egyptian goose meat have been profiled (Geldenhuys *et al*., 2014) [Chapter 4] and when compared to other well-known fowl species such as ostrich, guineafowl and pekin duck, the meat was found to be very tough. In Chapter 7, significant differences were also found within the tenderness and shear force values of the breast portion of male vs. female geese. Meat tenderness is an important characteristic involved in the consumer acceptability of meat (Risvik, 1994; Warriss, 2000; Kemp & Parr, 2012). The low ultimate tenderness of Egyptian goose meat may have a negative impact on the acceptability and overall potential of this meat product. In order to clarify the low tenderness, valuable insight into the various biochemical processes occurring in post mortem muscle is therefore required.

The toughness of meat is determined by two aspects, the background toughness related to the characteristics of the connective tissue and the myofibrillar toughness (Hertzman *et al*., 1993). The latter is influenced by the development of rigor mortis and the natural tenderisation of the meat by means of proteolytic breakdown (Hertzman *et al*., 1993). During the rigor mortis period, when muscle is converted to meat there are several factors that determine the ultimate tenderness of the meat. Of these factors, the rate and extent of the post mortem pH decline (as a measurement of rigor mortis), as well as the activity of the proteolytic enzymes during this period, needs to be considered when exploring the possible reasons behind the toughness of the meat.

As of yet, there are four main protease systems associated with the post mortem proteolysis of muscle, i.e. calpains, cathepsins, proteasomes and caspases (Kemp & Parr, 2012). Of these, the involvement of the calpains has received the most attention by researchers. The calpains are Calcium (Ca^{2+}) dependant proteases that require Ca^{2+} for activation (Goll *et al*., 2013). The calpains are also inhibited by calpastatin the specific endogenous inhibitor (Goll *et al*., 2013). The contribution of this proteolytic system in meat tenderisation, especially calpain I, has been widely recognised (Sentandreu *et al*., 2002; Koohmaraie & Geesink, 2006). The lysosomal cathepsins is another

proteolytic system linked to meat tenderisation, especially during aging. The cathepsins (B, L and H) are cysteine proteases that are located in the lysosomes and have optimum activity at acidic pH levels (<6.0) (Sentandreu *et al*., 2002; Kemp *et al*., 2010). The cathepsins are released into the cytosol upon disruption of the lysosomal membranes which occur as a result of ion-exchange failure and depletion of ATP during the rigor period (Kemp *et al*., 2010). Because of the lower optimum pH, cathepsin enzymes are believed to contribute to tenderisation during the aging of meat.

In attempting to elucidate the low tenderness of Egyptian goose meat, this study investigates the post mortem pH and temperature decline in the *pectoralis* muscle (breast portion) of this gamebird species. It also explores the enzyme activity of the Ca^{2+} dependant protease (calpain system) and the lysosomal cathepsins (B, B & L and H) during the rigor mortis period.

2 MATERIALS AND METHODS

2.1 Harvesting

Egyptian geese (*Alopochen aegyptiacus*) were harvested on the University of Stellenbosch's experimental farm, Mariendahl (-33° 51' 1.9074"; 18° 49' 21.1476") (ethical clearance: 10NP_HOF01), by the use of double barrelled shotguns during November and December 2014. A total of 12 mature geese (6 female and 6 male) were harvested and collected in the field. The geese were harvested one at a time in order to simplify the sampling procedure.

2.2 Sampling

Immediately after death, an incision was made in the skin from the neck to the tail region on the ventral side of the body so as to expose the two breast muscles (*pectoralis*) of the geese. The carcasses were treated in a manner which is typical for the current handling practices of gamebirds during wingshooting expeditions in South Africa (Geldenhuys *et al*., 2013a) [Chapter 2]. The geese were therefore kept at ambient temperatures during the sampling period. Muscle samples were removed from the carcass (*pectoralis*) at the following nine post mortem (PM) categorical time periods; 15 min, 45 min, 1 h 15 min, 1 h 45 min; 2 h 15 min; 2 h 45 min, 3 h 15 min, 3 h 45 min and 4 h 15 min. The specific individual time points and length of the sampling time were selected based upon the observations made (regarding the pH decline and time at which the pH stabilises) during a pre-trial. Samples for the respective analyses, listed in Table 1, were removed at random locations within each of the breast portions. For the calpain and calpastatin determinations, two 5 g samples were removed from the left breast muscle per time period while two separate 2 g samples were removed for the pH and cathepsin determinations from the right breast muscle. Samples were packaged in plastic bags and snap frozen in liquid nitrogen immediately after they were taken and kept frozen at -80 °C until the biochemical analyses were performed. The μ/m-calpain and calpastatin

as well as the cathepsin B, B & L and H activity were only determined on the samples of five of the nine sampling periods while the pH was determined on all (Table 1).

Table 1 Sampling layout per bird for the analyses performed

Sample	Time period[1] (post mortem)	Left breast muscle (*pectoralis*)	Right breast muscle (*pectoralis*)
1	**15 min**	μ/m-Calpain and calpastatin	pH, Cathepsin B, B & L and H
2	45 min		
3	**1 h 15 min**		
4	1 h 45 min		
5	**2 h 15 min**		
6	2 h 45 min		
7	**3 h 15 min**		
8	3 h 45 min		
9	**4 h 15 min**		

[1]Time points illustrated in blue indicate the samples on which the μ/m calpain and calpastatin, as well as the cathepsin B, B & L and H analyses were performed.

2.3 Physical analyses

2.3.1 Temp

The internal, post mortem temperature of the right breast muscle was monitored by means of a LogTag Trex – 8 temperature recorder fitted with a ST100T-15 temperature probe (LogTag, Auckland, New Zealand). The temperature was recorded at each of the nine sampling periods (15 min – 4h 15 min post mortem).

2.3.2 pH

The post mortem muscle pH decline was determined by the iodoacetate method (Jeacocke, 1977). A frozen, 0.5 g meat sample was placed in a 5 mL solution of 5 mM Na-iodoacetate and 150 mM KCl (adjusted to pH 7 with KOH) and immediately homogenised. The pH of the homogenate was then measured with a calibrated Jenway 3510 bench top pH meter (IJEN351201, Lasec SA, Cape Town, South Africa). The pH determinations were performed in duplicate.

2.4 Calpain and calpastatin determination

The calpain system consisting of the calcium activated proteases; calpain-I, calpain-II and the inhibitor calpastatin, were extracted from the meat samples as described by Dransfield (1996) with minor adaptations. A 3 g sample of frozen meat was homogenized in 15 mL extraction buffer (75 mM Tris pH 7.8, 10 mM EDTA, 0.05% [v/v] 2-mercaptoethanol, 2 mM phenylmethylsulfonyl fluoride, 1 ul/L pepstatin A) at 4 °C. The homogenate was centrifuged at 10000 g and filtered. The volume of the filtrate was then made up to 20 mL with extraction buffer. The filtrate was adjusted to pH 7.5, with

care being taken to prevent it from dropping below pH 7 at any point. The protein concentration of the filtrate was determined by using the Biuret method (Gornall *et al*., 1949).

A Gilson apparatus (Fraction collector, FC204; peristaltic pump, Minipuls 3; detector, 112UV; valve actuator, Valvemate II; system interface, 506C) in conjunction with the automated Unipoint LC system software (version 4.0) was used to separate the calpastatin and calpain I from calpain II by means of the two-step gradient ion-exchange chromatography-method (Geesink & Koohmaraie, 1999). The sample was run through a 20 mL DEAE Sepharose (GE Healthcare Bio-sciences AB, C 10/10 column) packed column with 0.0 M, 0.175 M and 0.35 M NaCl-Tris buffers (pH 7.5) being run sequentially to separately elute the different fractions. Three protein peaks were obtained, one from each of the NaCl Tris buffers. Fractions containing these protein peaks were pooled. The fractions eluted with the 0.0 M buffer (fraction 1) contained calpastatin only while the 0.175 M (fraction 2) NaCl buffer fractions typically contain both calpastatin and calpain I. The latter fraction is used for the indirect determination of calpain I activity. The third fraction (0.35 M NaCl-Tris buffer) contained only calpain II.

Calpain activity was determined using an azo-casein assay (7.5 ug/mL azo-casien in 0.1 M tris buffer, 0.1 M calcium chloride and 0.05% [v/v] mercaptoethanol adjusted to pH 7.5 at 4 °C), with the reaction being stopped after 1 h using 10% trichloracetic acid. The sample was subsequently centrifuged at 4000 g and the absorbance measured at 366 nm. The calpain II activity was determined directly on aliquots from fraction 3 without any prior treatment. The inhibitory action of calpastatin was determined by assaying the proteolytic activity of the calpain II fraction (fraction 3) before and after the addition of heated aliquots of fraction 1 and 2. The heat process was used to inactivate the calpain I in the fractions. Calpain I activity was determined by assaying the total proteolytic activity of calpain I and II in combined aliquots of fraction 2 and 3 with the fraction 2 aliquot being either heated or unheated in order to inactivate calpain I (calpain I = [calpain I + calpain II – calpastatin]-[calpain II-calpastatin]). The heat treatment used in the determination of the calpastatin and calpain I activity entailed heating the aliquot to a temperature of 95 °C for 15 min and then cooling on ice prior to centrifuging at 4000 g for 15 min to precipitate the denatured proteins (Koohmaraie, 1990). The calpain and calpastatin determinations as described above are considered to be estimates and not exact determinations, as they are influenced by numerous factors such as protein extractability, the inseparability of calpastatin and calpain-I and stability of enzymes.

One unit of calpain activity is defined as an increase in absorbance at 366 nm of 1.0 per h at 25 °C. One unit of calpastatin activity was defined as the amount that inhibited one unit of calpain II activity. Data were expressed as units per milligram of extractable protein (specific activity).

No difference in the total proteolytic activity as assayed was found between the aliquots of fraction 2 that were heated and those that were unheated. This indicated that no calpain I activity was

present in the sample. This is in agreement with literature where it has been reported that calpain I as described for mammalian species is not present in avian muscle, with a different calcium dependant proteinase known as µ/m-calpain rather being found. The proteolytic activity detected in fraction 3 (0.35 M NaCl-Tris buffer, pH 7.5) with the azo-casein assay is therefore attributed to u/m-calpain.

2.5 Cathepsin B, B & L and H determination

One gram of frozen muscle sample was homogenized at 7500-8500 rpm for 30 s (Kinematica Polytron PT 2500 E – Lasec SA) in 2.5 ml of lysing buffer which consisted of 50 mM sodium acetate, 100 mM NaCl, 7.84 mM EDTA and 0.2% Triton X-100 (adjusted to pH 5 with acetic acid). The homogenate was stirred for one hour at 4 °C where after it was centrifuged (4 °C) for 40 min at 4024 g. The supernatant was subsequently filtered through Whatman number 1 filter paper. All samples were kept at either refrigerator temperatures (4 °C) or on ice throughout the extraction procedure.

The enzyme extract was assayed for cathepsin B, B and L, and H activity according to the procedures of Thomas *et al*. (2004) and van Jaarsveld *et al*. (1998); with minor alterations being made. The supernatant (25 µL), 50 µL of assay buffer consisting of 340 mM sodium acetate, 60 mM acetic acid, 3.14 mM EDTA and 8 mM DTT (adjusted to pH 5.5 using NaOH) and 10 µL 0.1% Brij were pipetted into wells in three black microplates, one for each of the enzymes (Greiner Cellstar 96 well black plates, Sigma Aldrich, St Louis, USA). The plates were then incubated for 2 min at room temperature. The enzyme specific substrates (B: Z-RR-AMC, 5 µM; B and L: Z-FR-AMC, 5µM; H: R-AMC, 10 µM, Bachem, Bubendorf Switzerland) were pipetted into an adjacent lane of wells on each plate. The plates were subsequently incubated inside the fluorescent plate reader (Varioskan version 3.01.15, Thermoscientific, Waltham, MA USA) for 5 min at 40 °C in order to preheat both the enzyme extract and the substrate to the correct temperature. The substrate was added to each well (25 µL), with the contents of the well being thoroughly mixed in the process. Fluorescence (excitation 360 nm, emission 460 nm) was measured every 30 s for a total of 5 min in order to obtain the initial slope of the progress curve (change in fluorescence vs. time (s) – see Fig. 1 for an example) (Varioskan version 3.01.15, Thermoscientific, Waltham, MA USA). The enzyme assays were performed in duplicate. The initial slope (linear portion of the progress curve; change in fluorescence per min) was used for calculating the activities of the enzymes. In order to calculate the specific enzyme activity (change in fluorescence per min per mg protein) a subsample of each enzyme extract was analysed for protein content using the DC protein assay kit II (Bio-Rad catalogue number 500-0112, California). Prior to enzyme determinations, a dilution series of the enzyme extracts was used in order to establish the concentration dependence of the three assays. The change in fluorescence per minute in the three assays was found to increase with an increase in the enzyme concentration.

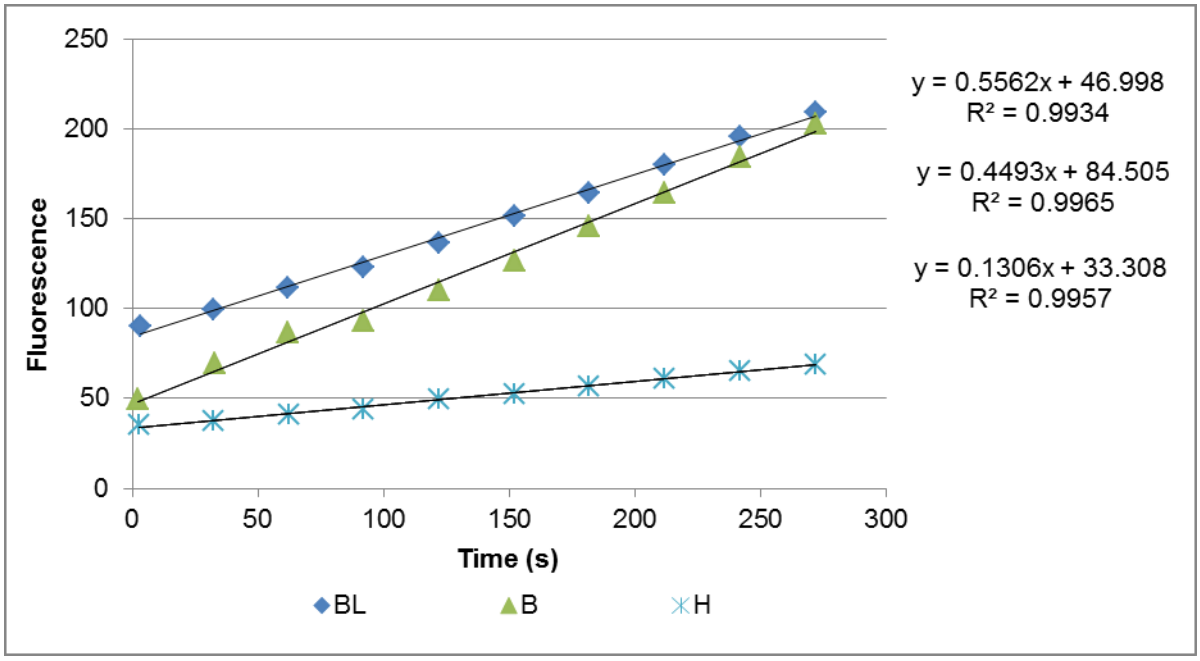

Figure 1 The initial slope of the progress curve (fluorescence vs. time) of the cathepsin B & L (BL), B and H activity with fitted linear trend lines and the R^2 values of the equation.

2.6 Statistical analysis

The data included the main effects; time post mortem and gender, as well as the interaction between the two. The experimental design was a completely random split plot with gender as main plot factor and post mortem time as subplot factor. The design included a total of 12 birds (n=12) consisting of six male and female birds, respectively which were selected at random. Regarding the pH and temperature data, the effect of time post mortem was analysed at the 9 post mortem time periods for each bird as listed in Table 1. The calpain, calpastatin, as well as the three cathepsin enzymes (B, B & L and H) were only analysed on five of the nine post mortem time periods (sections 2.4 and 2.5).

Univariate analysis of variance was performed on all of the variables accessed using GLM (General Linear Models) Procedure of SAS™ statistical software (Statistical Analysis System, Version 9.2, SAS Institute Inc., Cary, NC, USA). The model for the statistical design is indicated by the following equation:

$$y_{ijk} = \mu + g_i + (gr)_{ik} + t_j + (gt)_{ij} + \varepsilon_{ijk}$$

The terms within the model are defined as the overall mean (μ), the effect of gender (g_i), the correct error term for testing the main plot effect $(gr)_{ik}$, the effect of post mortem time (t_j), the effect of the interaction $(gt)_{ij}$ and the correct error term for testing the subplot effect, as well as the interaction between the former and latter (ε_{ijk}). The Shapiro-Wilk test was performed to test for normality (Shapiro & Wilk, 1965). All of the outliers were identified and removed before the final analysis. Fisher's Least Significant Differences (LSD) were calculated at a 5% significance level. A probability level of 5% was considered significant for all of the significance tests. Correlation coefficients were calculated between all of the variables by means of the Pearson's correlation coefficient (r).

Additionally non-linear regression analysis with post mortem time as independent variable was also performed for each individual bird to describe the rate of the pH and temperature decline over time. The regression function with the best fit (highest R^2 value) was selected as the appropriate regression function to describe trends. The model fitted for pH was the Mitscherlich function [$y = a\ (1-b\ e^{(ct)})$] where y is the dependant variable (pH), t the post mortem time hours, a is the asymptotic minimum pH, ($a + a*b$) is the pH at post mortem time 0 and c is the rate of pH change. The model fitted for temperature was an exponential function [$y = a\ e^{(bt)}$] where y is the dependant variable (temperature), t the post mortem time in hours, a is the temperature at post mortem time 0 and b is the rate of change for post mortem time (t) in hours.

Regression parameters obtained were used as input in an analysis of variance (ANOVA) comparing regression parameters for the genders. As no significant gender differences were observed, a single regression function was finally fitted to describe change over time for each variable.

3 RESULTS

There was no significant interaction between the main effects of post mortem time and gender for any of the variables and therefore the results of the two main effects are discussed separately.

Gender also did not have an influence (P>0.05) on any of the variables, i.e. pH (Table 2), temperature (Table 3) and calpain and cathepsin activity. The non-linear regression indicates that the overall rate of the post mortem pH decline (constant *c*) is -0.806 (Table 2; Fig. 2b). The average initial pH (15 min post mortem) was 6.71 where after it significantly decreased until a plateau was reached (Table 4). The temperature gradually declined at an overall rate (constant *b*) of -0.117 (°C/h) (Table 3 and Fig. 2a). The average internal temperature at 15 min post mortem was 38.9 °C where after the temperature gradually decreased to an average of 21.5 °C (Table 4). The temperature at each of the individual time points differed (P≤0.05).

The µ/m calpain, calpastatin and the calpastatin:µ/m calpain results are illustrated in Fig. 3. It is evident that while the µ/m calpain activity stayed constant (P>0.05) throughout the rigor period, the calpastatin activity together with the calpastatin:µ/m calpain did indeed show a gradual decrease. The 15 min post mortem activity was higher (P≤0.05) compared to the activity at 4 h 15 min post mortem.

The post mortem cathepsin B, B & L and H activity of the Egyptian goose *pectoralis* muscle is depicted in Fig. 4. It is evident from Fig. 4 that the trend indicates an overall increase in all cathepsin activities during the rigor period followed by a decrease occurring in cathepsin B & L activity; however this decrease was not significant. Activity values of all three enzymes are higher (P≤0.05) at 2 h 15 min, 3 h 15 min and 4 h 15 min post mortem compared to the first two time points. The activity of cathepsin B & L at 4 h 15 min post mortem did however not differ (P>0.05) from the activity at 15 min and 1 h 15 min post mortem.

Table 2 Mean values (±SE[2]) for the constants *a*, *b*, and *c* of the non-linear regression equation describing the change in the pH over time

	Constants for the non-linear regression equation $[y = a\,(1-b\,e^{(ct)})]^1$		
	a	**b**	**c**
pH overall average	5.825 ± 0.042	-0.194 ± 0.014	-0.806 ± 0.134
pH female	5.850[a] ± 0.018	-0.182[a] ± 0.02	-0.933[a] ± 0.066
pH male	5.757[a] ± 0.047	-0.228[a] ± 0.009	-0.783[a] ± 0.066

[1]*y* = pH at time *t*, *a* = asymptotic minimum pH; *e* = base of natural logarithm; (*a* + *a*b*) is the pH at post mortem time 0; *c* indicates the rate of change. R^2 (*a* = 0.149; *b* = 0.182; *c* = 0.115). [a]Means in columns with different superscripts differ at P≤0.05. [2]SE (standard error).

Table 3 Mean values (±SE[2]) for the constants *a* and *b* of the non-linear regression equation describing the change in the temperature over time

	Constants for the exponential equation $[y = a\,e^{(bt)}]^1$	
	a	**b**
Temperature overall average	38.652 ± 0.360	-0.117 ± 0.004
Temperature female	39.301[a] ± 0.314	-0.124[a] ± 0.005
Temperature male	38.051[a] ± 0.826	-0.111[a] ± 0.006

[1]*y* = temperature at time *t*, *a* = temperature at *t=0*, *b* = the rate of change. R^2 (*a* = 0.100; *b* = 0.141).
[a]Means in columns with different superscripts differ at P≤0.05. [2]SE (standard error).

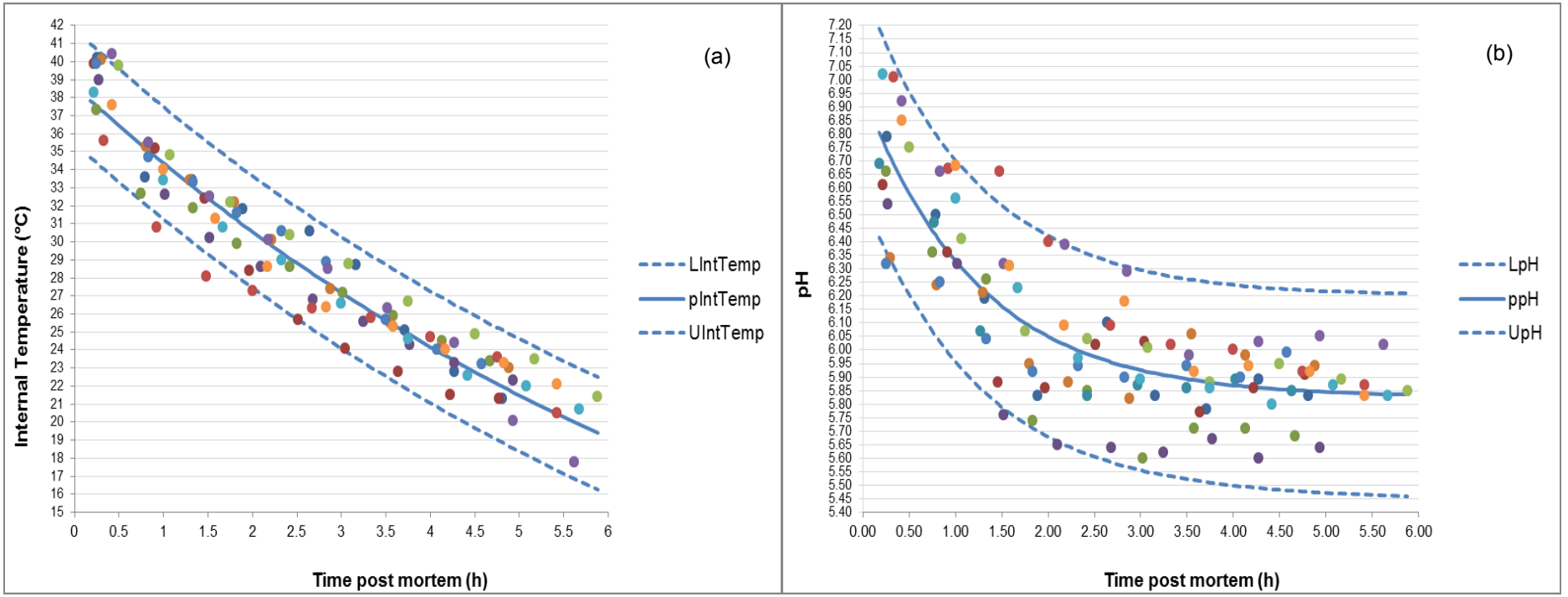

Figure 2 Predicted post mortem temperature (a) and pH (b) decline of the *M. pectoralis* from the 12 individual geese as well as the average.

Table 4 Mean values (± SE[2]) of the categorical change in pH and temperature over time

Sample	Post mortem time (h)	pH	Temperature (°C)[1]
1	0:15	6.71[a] ± 0.07	38.92[a] ± 0.44
2	0:45	6.46[b] ± 0.05	33.87[b] ± 0.42
3	1:15	6.17[c] ± 0.07	31.77[c] ± 0.46
4	1:45	5.99[d] ± 0.07	29.81[d] ± 0.46
5	2:15	5.98[d] ± 0.05	28.09[e] ± 0.53
6	2:45	5.86[e] ± 0.04	26.42[f] ± 0.45
7	3:15	5.88[e] ± 0.04	24.54[g] ± 0.31
8	3:45	5.87[e] ± 0.03	22.98[h] ± 0.38
9	4:15	5.85[e] ± 0.03	21.55[i] ± 0.46

[a-i]Means in columns with different superscripts differ at P≤0.05. [1]Note that the temperature decline was recorded on birds kept at ambient temperatures ($\bar{x}$ = 22.9°C; SE = 0.54). [2]SE (standard error).

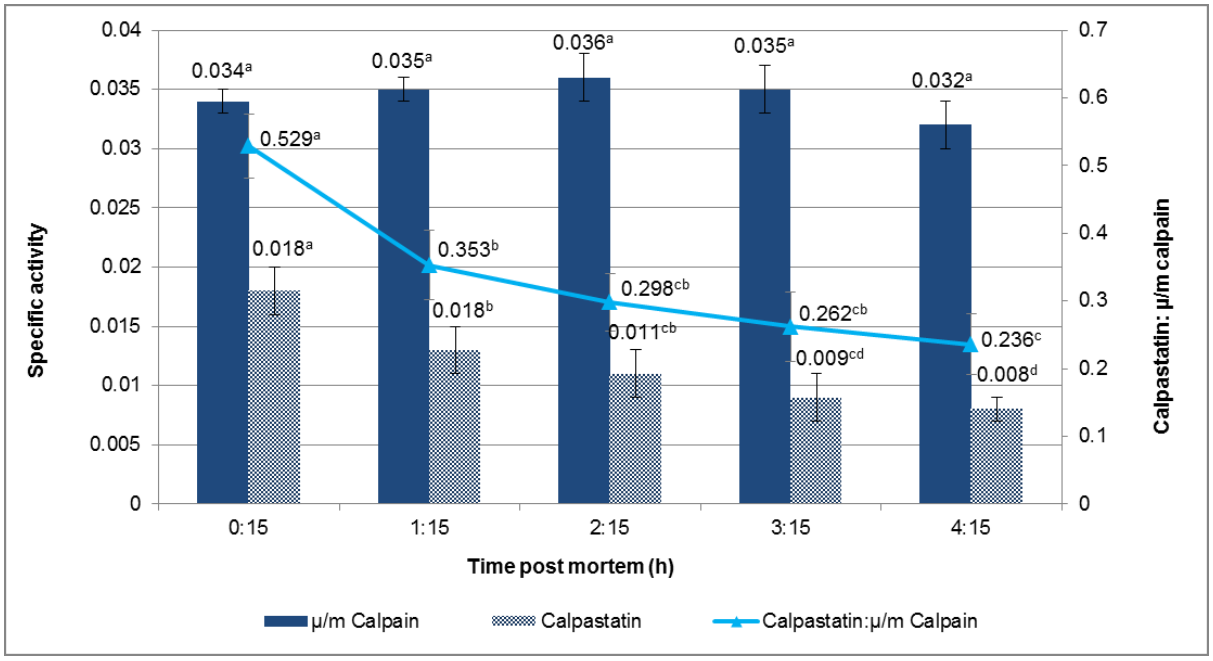

Figure 3 Bar chart illustrating the change in the average µ/m calpain and calpastatin specific activity as well as the µ/m calpain:calpastatin as affected by the time post mortem. [a-d]Different superscripts indicate significant differences between the mean values for each time point per calpain at P≤0.05.

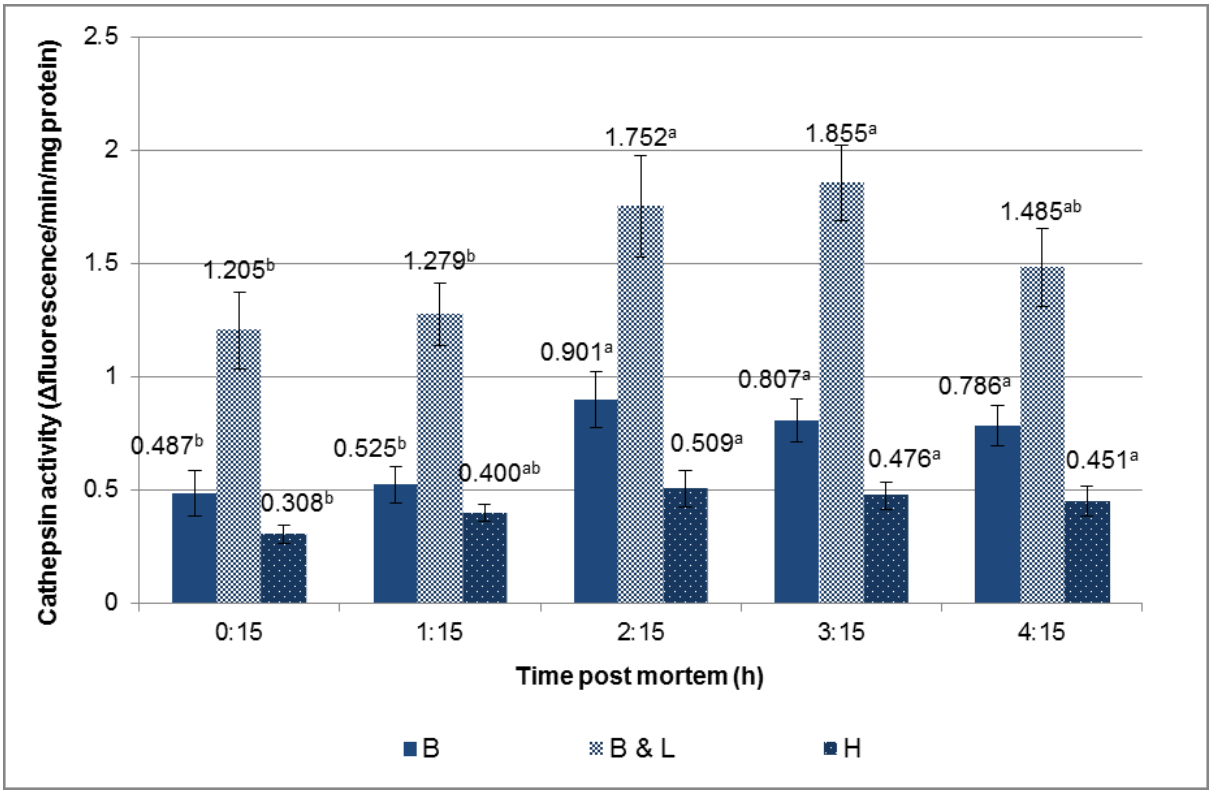

Figure 4 Bar chart illustrating the change in the average cathepsin B, B & L and H specific activity as affected by the time post mortem. [a-b]Different superscripts indicate significant differences between the mean values for each time point per cathepsin at P≤0.05.

4 DISCUSSION

The pH decline during rigor development in post mortem muscle is a complex and contradictory topic in literature. There are two aspects of pH which are believed to have an influence on the ultimate tenderness of meat, i.e. the rate at which the pH declines and the extent to which the pH declines (ultimate pH). The mean ultimate pH (~5.86) (Table 4) of the *pectoralis* muscle suggests that Egyptian goose meat should be categorized as having an intermediate ultimate pH between normal (pH <5.8) and extremely high (dark, firm and dry - DFD) (pH >6.2). The ultimate pH of the breast muscle found within this study coincides with the findings of Geldenhuys *et al*. (2013b) [Chapter 5]. The high ultimate pH was therefore expected, especially since most birds are shot when in flight, to and from their roosting sites. The long distance flight requires a certain amount of exercise by the breast muscles thereby increasing ante mortem glycogen depletion. This also relates to muscle fibre type. In Chapter 8, the fibre typing results showed that the *pectoralis* muscle mainly consists of type IIa, fast, oxidative-glycolytic fibres (84%) and type IIb, fast, glycolytic fibres (15.6%). Muscle which have a glycolytic metabolism are known to have higher amounts of stored glycogen (Lawrie & Ledward, 2006) so the fact that the muscle still had a fairly high ultimate pH (>5.8) confirms the ante mortem exercise and muscle glycogen depletion.

The rate of the post mortem pH decline is increased with an increase in muscle or ambient temperatures (Hertzman *et al*., 1993; Lawrie & Ledward, 2006; Huff Lonergan *et al*., 2010). This relationship is reflected by the strong, significant correlation ($r = 0.738$; $P < 0.0001$) between muscle pH and the internal muscle temperature in this study. The rate (Table 2 and Fig. 2b) of the average pH decline in the *pectoralis* muscle of Egyptian geese is fairly rapid (-0.806). The mean values as determined by the ANOVA performed on the categorical pH data (Table 4) indicates that the ultimate pH (~5.86) was reached after the fifth and sixth sampling period. This is apparent by the absence of significant differences between the mean values of the last four time points showing that a plateau was reached. The predicted pH decline at the specific/actual post mortem time points (Fig. 2b) indicates that a pH of ~5.86 was reached at approximately 3 h 45 min PM. The pH decline in post mortem muscle is frequently used as a measurement of rigor mortis. No literature could be found on the post mortem biochemical changes occurring in the meat from gamebirds. Also, it is difficult to directly compare this pH profile with that found in other studies on domestic fowl species because of the variation in the temperature decline; the geese being kept at ambient temperatures. The high temperature of the *pectoralis* muscles during rigor development, which is due to the common post mortem handling practices of gamebirds, is in actual fact the correct representation of the conditions occurring in the muscle. The results of our study therefore provide adequate insight into the post mortem biochemical changes in the muscle and the subsequent effect it may have on the tenderness.

So, irrespective of the temperature, the pH of the Egyptian goose *pectoralis* muscle showed a more rapid decline compared to that of ostrich muscles held at 0-4 °C, as reported by Sales and Mellet (1996). The ostrich muscles had an overall rate that ranged between -0.4 and -0.6 and reached an ultimate pH within 2-6 h post mortem depending on muscle type. Additionaly, Botha *et al*. (2008) found that ostrich muscle strips (kept at 7 °C) had a minimum pH (5.94) at 6.4 h post mortem. They did, however, also investigate the muscle strips held at 37 °C and found that a minimum pH (5.76) was reached within 4.8 h post mortem. This is still longer compared to the rigor mortis period of the Egyptian goose *pectoralis*. The rate of the pH decline in the *pectoralis* of Egyptian geese also occurs more rapidly in comparison to the *Longissumus thoracis et lumborum* muscle of wild ungulates such as the impala (*Aepyceros melampus*) (Hoffman, 2000), as well as the warthog (*Phacochoerus africanus*) as reported by Hoffman and Sales (2007). The post mortem pH profiling in these studies were, of course, also conducted at refrigeration temperatures (0-4 °C) as the carcasses were cooled immediately after slaughtering. The variation in the time taken to reach rigor mortis in the muscles of different species is related to a difference in the ATP levels below which rigor development occurs (Warriss, 2000).

The general consensus seems to be that an intermediate pH decline, in conditions which do not favour cold shortening, is beneficial in terms of tenderness (Marsh, 1987; Huff Lonergan *et al*., 2010). This is due to the early activation of the proteolytic enzymes. Lawrie and Ledward (2006) notes that a slower drop in pH is favourable as it allows for the muscle to be at an optimum pH together with near *in vivo* temperatures for a longer period and thereby increasing the activity and ultimate effect of the calpain system. Although, this depends on the overall efficiency, i.e. the amount and capacity of the calpain system present in the muscles of different species. It should also be noted that a rapid pH decline may lead to the denaturation of the sarcoplasmic and myofibrillar proteins which results in protein insolubility and increased toughness (Lawrie & Ledward, 2006).

4.1 Possible effect of the pH decline on muscle shortening and meat tenderness

Geldenhuys *et al*. (2014) [Chapter 4] reported that Egyptian goose breast meat (36 h PM) is very tough compared to the meat of other well-known fowl species such as the ostrich and guineafowl. This confirms that the pH decline in the *pectoralis* muscle may occur too rapidly which contributes to the ultimate toughness. However, the ultimate pH is still quite high and it is therefore questionable whether the rapid decline could have caused enough denaturation of the proteins for it to have had a substantial effect. Perhaps a more viable theory is the influence of the high muscle temperature during rigor development on the extent of muscle shortening, as well as the proteolytic enzyme activity. In meat the extent of rigor shortening of the myofibrils influences the tenderness thereof (Huff Lonergan *et al*., 2010). Short sarcomeres will result in tougher meat which will also age much less

effectively. Several studies have reported that a rapid decline in pH together with a slow drop in temperature (high rigor temperature) results in the meat being very tough (Locker & Hagyard, 1963; Hertzman *et al*., 1993; Tornberg, 1996). The lowest amount of rigor shortening (and thus more tender meat) occurs when muscle is allowed to go into rigor at temperatures in the range of 15-20 °C (Locker & Hagyard, 1963; Huff Lonergan *et al*., 2010). At temperatures below or above this range muscles may undergo cold and heat shortening, respectively, which will both result in meat with an increased toughness. Tornberg (1996) explained that for beef *Longissimus dorsi* the lowest amount of shortening was found within 10-15 °C, whilst Hertzman *et al*. (1993) also found that the amount and rate of muscle shortening was substantially higher at rigor temperatures in the range of 30-37 °C compared to 15 °C. Temperature has the same effect on rigor shortening in ostrich muscles where a significantly higher percentage shortening occurred when muscle was kept at 37 °C opposed to 7 °C (Botha *et al*., 2008). Even though pre-rigor excision of the Egyptian goose breast portions is not common practice and the muscles remained intact throughout the rigor period, this theory coincides with our findings and may clarify the increased ultimate toughness of the meat. The Egyptian geese were kept at ambient temperatures and the *pectoralis* muscles were therefore allowed to go into rigor within a temperature range of 20-39 °C. Considering the fact that the muscle reached an ultimate pH of ~5.86 after approximately 3 h 45 min PM (Table 4 and Fig 2b) the temperature decline (Fig. 2a) indicates that the *pectoralis* muscle was at ~25 °C when the pH stabilised. Furthermore, Thompson (2002) describes that for beef the pH/temperature window where heat shortening is likely to occur is when the muscle reaches a pH of <6.0 at a temperature of 35 °C or above. When this is related back to the Egyptian goose *pectoralis*, a pH of 6.0 was reached at approximately 2 h and 15 min post mortem at which stage the muscle temperature was at ~30 °C. Similar results have been found by Khan and Nakamura (1970), when investigating the effect of post mortem glycolysis on poultry tenderness. They reported that a rapid pH decline, where a pH of ~6.0 is attained while the muscle temperature is still very high, resulted in increased shear force values. The high rigor temperatures may therefore have a detrimental effect on the tenderness of Egyptian goose meat. However, further research investigating the influence of temperature on rigor shortening is required to verify this theory.

Regardless of the rate at which the pH drops, the extent to which it drops is also believed to have an effect on the tenderness. As there is an increase in the ultimate pH of the meat from 5.5 to 6.0, the tenderness decreases (Lawrie & Ledward, 2006; Jeremiah *et al*., 1991; Purchas, 1990). Devine *et al*. (1993) found lamb meat to be the least tender at an ultimate pH range of 5.8-6.2. There are two probable causes of this decreased tenderness. Firstly Purchas (1990) explains that there is a decrease in the sarcomere length as the ultimate pH increases to 6.3 which may result in the meat being tougher. Alternatively, Yu and Lee (1986) is of the opinion that an ultimate pH in this range is not optimum for either of the key proteolytic enzyme systems (calpains or cathepsins) and

tenderisation is therefore not favoured. It is evident that the ultimate pH of the Egyptian goose *pectoralis* muscle is ~5.86 which may therefore have contributed to the low tenderness. There are, however, other studies that have found increased sensory tenderness with an increase in ultimate pH (Silva *et al*., 1999) which makes it difficult to draw any concrete conclusions regarding the effect of the ultimate pH on the tenderness of Egyptian goose meat.

4.2 Possible effect of the proteolytic enzyme activity on tenderness

The efficacy of the post mortem proteolytic systems in muscle is linked to the ultimate tenderness of the meat. Recently, the contribution of the caspase and proteosome systems have been explored (Sentandreu *et al*., 2002; Ouali *et al*., 2006; Kemp *et al*., 2010) but the calpain and cathepsin systems are still considered to be involved in the weakening of the myofibrillar structure occurring in post mortem muscle. The amount of the enzymes and their inhibitors present in addition to the ratio of inhibitor/enzyme activity determine the ultimate efficacy of the enzyme system on protein breakdown.

4.2.1 The calpain system

In mammals the two main calpain isoforms present in muscle are calpain I (μ-calpain) and calpain II (m-calpain) (Goll *et al*., 2003). However, in bird species another calpain isoform, with a sequence intermediate to that of calpain I and calpain II (Sorimachi *et al*., 1995; Goll *et al*., 2003; Lee *et al*., 2007) exists. This has been described as μ/m-calpain. The Ca^{2+} concentration required (420 μM) for the activation of this calpain in chicken muscle is between those of calpain I and calpain II. Sorimachi *et al*. (1995) notes that the activity of μ/m-calpain is dominant in the muscle of avian species and the activity of other Ca^{2+} dependant proteinases is virtually undetectable. It has thus been suggested that μ/m-calpain performs the functions of both the calpain I (μ) and calpain II (m) types in the muscles of birds (Sorimachi *et al*., 1995). As a result of this difference in the calpain system, the extent to which the calpains are involved in the post mortem tenderisation process in bird species is still unclear (Wang *et al*., 2012). The absence of calpain I activity found in Egyptian goose meat during the extraction phase corresponds with the findings of Wang *et al*. (2013) and Li *et al*. (2012). This indicates that μ/m-calpain may be the main Ca^{2+} dependant proteinase in the post mortem muscle of Egyptian geese (Fig. 3).

This study indicated that there was no significant change in the μ/m-calpain activity over the rigor period. The calpastatin and calpastatin: μ/m-calpain ratio did, however, decrease ($P \leq 0.05$). When compared to the findings of Frylinck *et al*. (2009) the activity (U/g meat) of the Egyptian goose μ/m-calpain at 15 min post mortem compares well with their results for beef Calpain I at 1 h post mortem (2.06 U/g compared to 2.20 U/g for the Simmental crossbreed). In contrast, the calpastatin

activity in the Egyptian goose *pectoralis* muscle is somewhat different. In species such as beef and pork the calpastatin activity is usually found to be much higher than that of the calpains (Ouali & Talmant, 1990; Maddock Carlin *et al*., 2006; Frylink *et al*., 2009), which results in a calpastatin:calpain ratio greater than one. Goll *et al*. (2003) notes that muscle from domestic species normally contain calpastatin activity in surplus of the calpain activity.

The lack of change in μ/m-calpain activity, found in this study, may indicate that this enzyme is very stable during the rigor period and that the decrease in pH does not have a substantial effect on the activity within the 4 h 15 min post mortem window. However, in theory, once the calpains are activated in post mortem muscle they gradually lose their activity through autolysis. It is therefore accepted that a decrease in calpain activity indicates that the enzyme has been active and possibly contributed to protein degradation (Barnier *et al*., 1999; Huff Lonergan *et al*., 2010). This is supported by the fact that calpain activity is normally found to decline in port mortem muscle (Barnier *et al*., 1999; Wang *et al*., 2013; Li *et al*., 2012). The concept does not correspond to our findings as no significant decrease in the μ/m-calpain activity was found. Calpains are thought to be active in both the non-autolysed and semi-autolysed forms before they become completely inactivated by complete autolysis (Goll *et al*., 2003; Huff Lonergan *et al*., 2010). Brief autolysis reduces the amount of Ca^{2+} required for half-maximal activity of the semi-autolysed form. This could provide an alternative explanation for the fact that no decrease in activity was noticed over the rigor period. The slight ($P>0.05$) drop in activity at 4 h 15 min post mortem may indicate that more of the calpains had been inactivated by complete autolysis by this time point. It is therefore possible that a decrease in activity would have been noted if subsequent samples had been taken after the 4 h 15 min time frame.

Another important aspect of post mortem proteolysis that should be considered is the activity of the calpain inhibitor calpastatin. The rate of calpastatin degradation and inactivation is linked to the rate of proteolysis observed in meat (Geesink & Koohmaraie, 1999; Huff Lonergan *et al*., 2010). The decreasing ($P\leq0.05$) calpastatin activity observed during the rigor period may therefore be indirectly indicative of calpain activity as the calpains are known to degrade calpastatin. It is therefore possible the calpains may not only be active and involved in protein degradation but that the effectiveness of the calpain system may increase post mortem due to reduced inhibition by calpastatin.

However, regardless of the possible contribution of the μ/m-calpain to tenderisation, Egyptian goose meat at 36 h post mortem is still considered to be unacceptably tough [Chapter 4]. It is thus concluded that in the event that μ/m-calpain does in fact contribute to myofribrillar degradation post mortem, this effect is not substantial enough to be notable. The absence of calpain I in the muscle may also explain why Egyptian goose meat is so tough. Research have shown that calpain I is mainly responsible for early post mortem proteolysis in meat (Koohmariae & Geesink, 2006; Huff Lonergan *et al*., 2010).

4.2.2 The lysosomal cathepsins

The cathepsin activity (B, B & L and H) in the Egyptian goose *pectoralis* muscle increases as rigor develops (Fig. 4). From 2 h 15 min post mortem onwards, the activity is significantly ($P \leq 0.05$) higher and remains stable. This is in agreement with the results reported by Thomas *et al*. (2004) on ostrich muscle and Barnier *et al*. (1999) on reindeer muscle where the cathepsin activities were at a minimum during the initial stages of post mortem rigor development and then gradually increased during aging. This increase in cathepsin activity over time was associated with an increase in meat tenderness. Similar results have been found by Wang *et al*. (2013) on chicken muscle where low, unchanged activity during the first 3 h post mortem was attributed to a lack of lysosomal disruption up to that point. They postulated that after 3 h post mortem a sufficient decline in pH resulted in lysosomal release and the subsequent increase in free cathepsin activity. It can therefore be assumed that the cathepsin B, L and H may have some sort of contribution towards proteolytic breakdown, the value of this contribution will, however, only be known if the activity is correlated to tenderness over time.

There is also a theory that a more rapid decrease in the post mortem pH at higher rigor temperatures may rupture the lysosomal membranes and therefore promote cathepsin activity early on (Moeller *et al*., 1976). The increase in the cathepsin activity in the Egyptian goose *pectoralis* may therefore be linked to the high rigor temperatures. A pH of 6.0 was reached at approximately 2 h and 15 min post mortem at which stage the muscle temperature was at ~30 °C. This is also the point where a significant increase ($P \leq 0.05$) in the cathepsin activity was observed (Fig. 4). Whipple *et al*. (1990), however, did not observe any significant differences in cathepsin activity due to increased rigor temperatures in beef. The effect of rigor temperature on the activity of both the lysosomal cathepsins and the Ca^{2+} dependant proteases warrant further research in order to make any substantial conclusions in this regard.

5 CONCLUSIONS

This study investigated the post mortem pH decline and proteolytic enzymes (calpain and cathepsin) activity during the rigor period. No differences were found for any of the variables between genders indicating that the higher tenderness of the breast portion of female geese [Chapter 7] may be due to other factors such as collagen content. The pH decline in the *pectoralis* muscle occurs quite rapidly compared to other species and it is speculated that the high rigor temperature may contribute to the increased toughness. Irrespective of the calpain and cathepsin activity during the rigor period, Egyptian goose breast meat is still tough at 36 h post mortem [Chapter 4] indicating that the contribution of the proteolytic enzymes during rigor, if any, is not substantial enough for it to be recognised. It is also important to consider the possibility that the contribution of the proteolytic enzymes may be overshadowed by the background toughness, i.e. the connective tissue content and fibre structure. Therefore, to make any concrete conclusions regarding the contribution of the calpain and cathepsin proteolytic systems in Egyptian goose meat it is essential to investigate the tenderness during ageing. The changes (if any) occurring in the physical myofibrillar structure will have to be explored in order to know if the proteolytic enzymes do in actual fact contribute.

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CHAPTER 10

The influence of post mortem ageing on the tenderness of Egyptian goose (*Alopochen aegyptiacus*) breast meat (*M. pectoralis*)

ABSTRACT

Egyptian goose breast meat has been found to be very tough compared to the meat of other well-known fowl species. In attempting to clarify the toughness of the meat, the physical and biochemical changes during post mortem ageing (14 days) was investigated. Although there was an increased cathepsin (B, B & L and H) activity together with a decrease ($P \leq 0.05$) in the myofibrillar fragmentation lengths (32 - 25 µm) with ageing, no change (decline) in the shear force values was observed. The higher ($P \leq 0.05$) shear force of the male breast portions may be linked to the higher ($P \leq 0.05$) concentrations of total and insoluble collagen. The ageing of Egyptian goose meat as a means of improving the overall toughness cannot be proposed without further research.

Keywords: Egyptian geese, Gamebirds, Meat quality, Ageing, Tenderness, Enzymes

1 INTRODUCTION

Gamebird hunting is not only a tradition but also an activity that became popular in numerous countries around the world, especially in Europe. In recent years, the hunting of gamebird species in South Africa has also increased considerably and many of these, specifically Egyptian geese (*Alopochen aegyptiacus*), are harvested in an attempt to reduce the damage they cause to crop lands (Geldenhuys *et al*., 2013a). Annually, crop farmers incur major financial losses due to their extensive feeding activities (Mangnall & Crowe, 2001; 2002). Egyptian goose meat is not utilised commercially but rather consumed by the hunters or donated to the local communities.

Based on the sensory profiling research by Geldenhuys *et al*. (2014) [Chapter 4], Egyptian goose meat is considered to be very tough compared to the meat of other fowl species (game and domestic) consumed on a regular basis in South Africa. In Chapter 7, variation was also found within the tenderness and shear force values of the breast portion of male compared to female geese. When exploring the development of rigor mortis in the *pectoralis* muscle the results of Chapter 9 suggests that heat shortening, due to a rapid decline in pH at high rigor temperatures, may contribute to the toughness. However, the toughness of meat is not only determined by the development of rigor mortis but the connective tissue also contributes to the background toughness (Hertzman *et al*., 1993; Takahashi, 1996; Sentandreu *et al*., 2002). The extent of the natural tenderisation of meat by means of proteolytic breakdown is an additional element that should also be considered (Hertzman *et al*., 1993). Therefore, in order to gain more of an understanding of all of the mechanisms involved in the toughness and to draw more concrete conclusions, it is essential to explore the natural ageing of Egyptian goose meat.

Hunted gamebirds are usually hanged, intact in a cool environment for some time before any further preparation or consumption thereof (Barnes, 1976). This is a traditional practice, particularly in Britain and several other European countries. In South Africa, the handling of gamebirds is rather different when compared to the traditional European customs (Geldenhuys *et al*., 2013a) [Chapter 2]. The carcasses are either frozen, usually with the feathers intact or kept fresh in the refrigerator for immediate use. In general, gamebirds are aged (non-eviscerated) much less frequently in Africa. The conditioning or ageing of meat is the storage (post rigor) of unprocessed meat, in conditions that do not favour microbial deterioration and which results in natural tenderisation (Warriss, 2000; Lawrie & Ledward, 2006). Complex and interrelated biochemical changes occur during the ageing of meat which ultimately increases the tenderness. Post mortem protein denaturation (Lawrie & Ledward, 2006), as well as other factors such as the non-enzymatic weakening of the myofibrillar structure (Takahashi, 1991) and programmed cell death or apoptosis (Herrera-Mendez *et al*., 2006; Ouali *et al*., 2006; Kemp & Parr, 2012) have been associated with meat tenderisation during ageing. However,

the main factor involved in meat tenderness and ageing of post rigor muscle is proteolysis and the subsequent weakening of the myofibrillar structure (Sentandreu *et al*., 2002; Lawrie & Ledward, 2006).

By investigating the ageing process, the extent of change in the instrumental tenderness and the myofibrillar fragmentation length (MFL) over time, as well as the role of the proteolytic enzymes (cathepsins) in this process can be monitored; this will allow for more accurate conclusions regarding the toughness of Egyptian goose meat. It is also important to identify the extent to which the collagen content and composition thereof contributes towards the background toughness of the meat. This study may thus provide valuable insight into a possible method to produce gamebird meat with an acceptable tenderness.

2 MATERIALS AND METHODS

2.1 Experimental outline

Two main factors were considered; gender and ageing period. The ageing periods included 1, 3, 7 and 14 days post mortem under refrigerated conditions. Six female and six male birds, respectively, were assigned to each ageing period (Table 1). The experimental outline therefore consisted of eight treatments and the experiment was replicated six times. Both of the breast portions (*M. pectoralis*) were used together and treated as one entity.

Table 1 Experimental outline

Ageing period (Day)	Number of birds (n=48)[1]	
	Female	Male
1	6	6
3	6	6
7	6	6
14	6	6

[1]The use of different birds for each ageing period within the six replicates was unavoidable due to the sample size required for the various analyses performed.

2.2 Harvesting, sampling and ageing

Egyptian geese (*Alopochen aegyptiacus*) were harvested on the University of Stellenbosch's experimental farm, Mariendahl (-33° 51' 1.9074"; 18° 49' 21.1476") (ethical clearance: 10NP_HOF01), by the use of double barrelled shotguns during March/April 2014. A total of 48 mature geese were harvested, collected in the field and placed within 6 h post mortem in the refrigerator (1-2 °C) over-night (± 12 h); by then the muscles had entered full rigor.

For the removal of the breast portions an incision was made in the skin from the neck to the tail region on the ventral side of the body so as to expose the two breast muscles (*pectoralis*) of the geese. The *pectoralis* muscles attached to the sternum and the clavicle were removed from the breast region of the carcass by cutting through the shoulder joint and around the *M. pectoralis* on the lateral side to detach the breast from the carcass. The two breast portions of each bird were packaged together in a polystyrene bag, vacuum-packed and aged at 1-2 °C for the allocated ageing period (Table 1). After the appropriate ageing time the samples were blast frozen (-40 °C) (Marcold Refrigeration co. (PTY) LTD, Cape Town, South Africa) and stored at -20 °C for approximately three weeks. Note that the meat was frozen following the respective ageing periods to facilitate the cooking and subsequent analyses of the samples together thereby reducing the variation in terms of the batch effect. The blast freezer was therefore used in order to minimise the effect of the freezing process on the meat (Leygonie, 2011).

2.3 Sample preparation

The vacuum-packed, frozen meat samples were thawed for 24 h in a refrigerator (1-2 °C) prior to each of the pre-determined cooking sessions. The meat samples were divided into six replicates, each consisting of the eight treatments (2 genders x 4 ageing periods). The samples of each replicate were cooked together. The left breast portion (*M. pectoralis*) of each of the treatments/birds was placed inside an oven bag (Glad®) while the other (right portion) was placed aside for the biochemical analyses (Section 2.5). The oven bags containing the meat samples were then placed on stainless steel grids which were put on an oven roasting pan. Thermocouple probes attached to a handheld digital temperature monitor (Hanna Instruments, South Africa) were placed in the centre of each of the meat samples (AMSA, 1995). The prepared samples were then placed in two conventional ovens (Defy, Model 835), pre-heated to 160 °C (AMSA, 1995). The ovens were connected to a computerised monitoring system responsible for regulation of the temperature (Viljoen *et al*., 2001). The meat samples were removed from the oven when a core temperature of 75 °C was reached (AMSA, 1995). The samples were cooled for 15 min where after the potions were individually wrapped in aluminium foil and placed in the refrigerator (1-2 °C) for approximately 24 h.

2.4 Physical analyses

2.4.1 pH

The pH of the meat samples for each of the six replications was measured after thawing the meat for 24 h, immediately after removal from the packageing and before the start of the cooking process. The pH was measured by means of a Crison pH 25 handheld portable pH meter (Lasec (Pty) Ltd, South

Africa) with an automatic temperature adjuster calibrated before each session with the standard buffers (pH 4.0 and pH 7.0) provided by the manufacturer.

2.4.2 Cumulative moisture and cooking loss

Following removal of the breast portions from the carcasses, the mass of both portions together was recorded before being vacuum-packed, aged (1-2 °C) for the allocated time periods and blast frozen. Before each of the cooking sessions, the meat was thawed in a refrigerator (1-2 °C) for 24 h where after the two portions were removed from the packaging, blotted dry with blotting paper and weighed (Radwag PS 750/C/2, Lasec SA, Cape Town, South Africa). This procedure was followed for each of the six replications. The moisture loss of each sample was calculated as a percentage of the original weight of the meat sample before it was aged and frozen.

The cooking loss was determined on the one breast portion according to the method described by AMSA (1995). The difference in the weight of each of the uncooked and cooked samples was calculated as the percentage of cooking loss.

2.4.3 Shear force

The Warner Bratzler shear force test (WBSF), as described by Honikel (1998), was used to measure the instrumental shear force of the cooked meat samples. Each of the eight treatments (six replications per treatment) was analysed for instrumental tenderness. Two adjacent 1 x 1 cm meat strips were cut parallel to the muscle fibre direction from the centre of the cooked meat portions. The respective meat strips were then cut to obtain a total of six rectangular cubes with a length of 2 cm per cube. An Instron Universal Testing Machine (Instron UTM, Model 2519-107), attached to a Warner-Bratzler fitting, was used to determine the force necessary to shear the cooked rectangular meat cubes perpendicular to the muscle fibre direction. The WB fitting was a 1 mm thick triangular (V-notch) blade with a semi-circular cutting edge (radius of 0.508 mm). The UTM was operated with a 2 kN compression load cell. The shear test was performed at a speed of 200 mm/min. The shear force value of each of the samples was recorded in Newton (N). For statistical analyses, the mean of the six readings was used.

2.5 Biochemical analyses

Following the thawing process (1-2 °C) of the aged and frozen meat samples, the right breast portion (*M. pectoralis*) of each treatment/bird per replicate was used for the biochemical analyses. Three 1 cm thick steaks (lateral cross sections) were cut from the raw breast portions. The samples were packaged separately in plastic bags and snap frozen in liquid nitrogen immediately after they were taken and kept frozen at -80 °C until the biochemical analyses were performed. The analyses were conducted on each of the treatments/birds per replicate. Note that the samples for biochemical

analyses were treated in the same manner as the samples that were cooked so as to reduce the variation.

2.5.1 Cathepsin B, B & L and H determination

One gram of frozen muscle sample was homogenized at an rpm of 7500-8500 for 30 s (Kinematica Polytron PT 2500 E – Lasec SA) in 2.5 mL of lysing buffer which consisted of 50 mM sodium acetate, 100 mM NaCl, 7.84 mM EDTA and 0.2% Triton X-100 (adjusted to pH 5 with acetic acid). The homogenate was stirred for one hour at 4 °C where after it was centrifuged (4 °C) for 40 min at 4024 g. The supernatant was subsequently filtered through Whatman number 1 filter paper. All samples were kept at either refrigerator temperatures (4 °C) or on ice throughout the extraction procedure.

The enzyme extract was assayed for cathepsin B, B and L, and H activity according to the procedures of Thomas *et al*. (2004) and van Jaarsveld *et al*. (1998); with minor alterations being made. The supernatant (25 µl), 50 µl of assay buffer consisting of 340 mM sodium acetate, 60 mM acetic acid, 3.14 mM EDTA and 8 mM DTT (adjusted to pH 5.5 using NaOH) and 10 µl 0.1% Brij were pipetted into wells in three black microplates, one for each of the enzymes (Greiner Cellstar 96 well black plates, Sigma Aldrich, St Louis, USA). The plates were then incubated for 2 min at room temperature. The enzyme specific substrates (B: Z-RR-AMC, 5 µM; B and L: Z-FR-AMC, 5µM; H: R-AMC, 10 µM, Bachem, Bubendorf Switzerland) were pipetted into an adjacent lane of wells on each plate. The plates were subsequently incubated inside the fluorescent plate reader (Varioskan version 3.01.15, Thermoscientific, Waltham, MA USA) for 5 min at 40 °C in order to preheat both the enzyme extract and the substrate to the correct temperature. The substrate was added to each well (25 µl), with the contents of the well being thoroughly mixed in the process. Fluorescence (excitation 360 nm, emission 460 nm) was measured every 30 s for a total of 5 min in order to obtain the initial slope of the progress curve (fluorescence vs. time – see Fig. 1 for an example) (Varioskan version 3.01.15, Thermoscientific, Waltham, MA USA). The enzyme assays were performed in duplicate. The initial slope (linear portion of the progress curve; change in fluorescence per min) was used for calculating the activities of the enzymes. In order to calculate the specific enzyme activity (change in fluorescence per min per mg protein) a subsample of each enzyme extract was analysed for protein content using the DC protein assay kit II (Bio-Rad catalogue number 500-0112, California). Prior to enzyme determinations, a dilution series of the enzyme extracts was used in order to establish the concentration dependence of the three assays. The change in fluorescence per minute in the three assays was found to increase with an increase in the enzyme concentration.

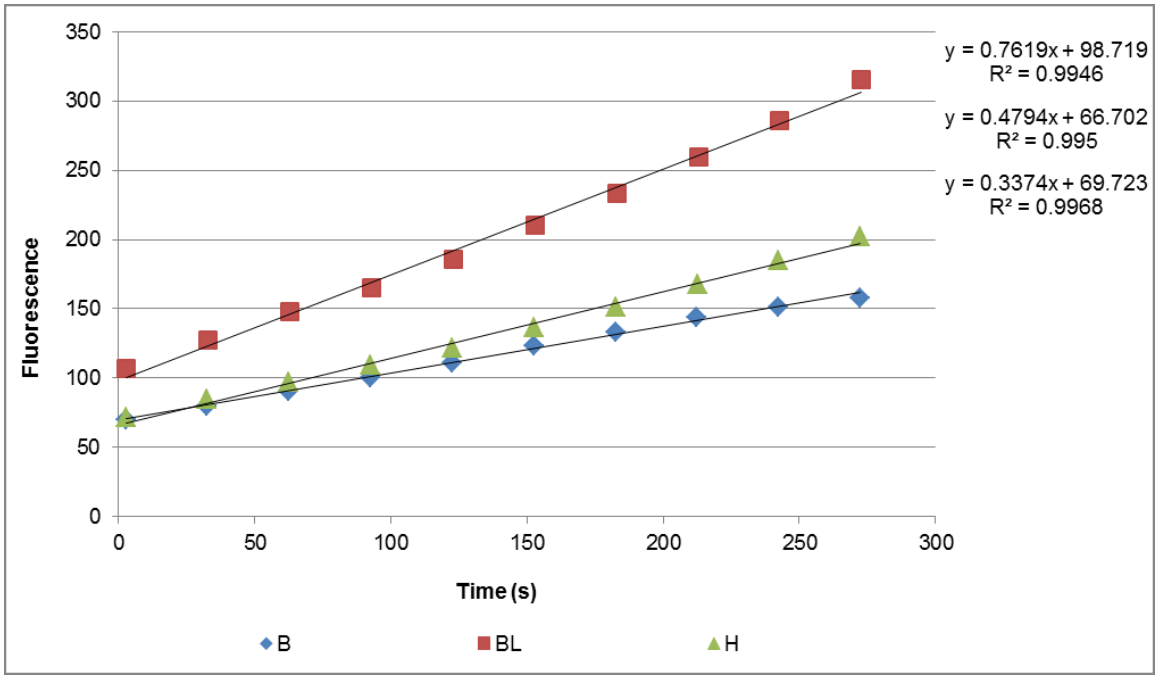

Figure 1 The initial slope of the progress curve (fluorescence vs. time) of the cathepsin B, B & L (BL) and H activity with fitted linear trend lines and the R^2 values of the equation.

2.5.2 Myofibril fragmentation length (MFL)

The MFL analysis was performed according to the method of Culler *et al*. (1978), with alterations being made according to Heinze and Bruggemann (1994). A portion of the frozen muscle sample was finely cut, without the inclusion of noticeable fat and connective tissue. Of this, 3 g was weighed off and added to 30 mL of 4 °C extraction buffer (0.02 M potassium phosphate buffer containing 100 mM KCl, 1 mM $MgCl_2$, 1 mM EDTA and 1 mM NaN_3). The sample was allowed to thaw inside the buffer for 60 s, where after it was homogenized at 20000 rpm for 30 s by the use of a Bühler HO4 homogeniser. The blade of the homogeniser was inverted so that the myofibrils were fragmented and not sliced.

The homogenised samples were centrifuged at 4 °C at 3000 rpm for 15 min where after the supernatant was discarded and the pellet re-suspended in another 30 mL of MFL extraction buffer and the centrifuge process was repeated. The supernatant was then again discarded and the pellet re-suspended in 10 mL of the extraction buffer. The suspension was filtered under vacuum through a 1000 µm polyethylene strainer, with 5 mL of extraction buffer used to wash the myofibrils through the strainer. The resulting filtrate was then filtered through a 250 µm polyethylene strainer.

The final filtrate was then mounted onto a microscope slide and examined using an Olympus BX40 microscope (400X magnifications). The fragments were measured using the analysis software package from Life Science, with 100 fragments measured per sample. The units of measurement were micrometer (µm).

2.5.3 Collagen content

For the determination of the total collagen content, 1 g samples taken from the centre of each sample were homogenised (Kinematica Polytron PT 2500 E – Lasec SA) in 10 mL of distilled water, where after 10 mL of 37% hydrochloric acid (HCl) was added and the homogenate was mixed thoroughly. This was then incubated at 120 °C for 3 h to allow for the complete hydrolysis of the proteins. After hydrolysis the sample was filtered to remove particles using filter paper (Whatman number 1). The filtrate (7.5 µL) from each sample was subsequently transferred to a clear 96 well microplate (Greiner Cellstar 96 well flatbottom plate, Sigma-Aldrich, St Louis USA). The liquid in the wells were then evaporated overnight (60 °C).

A sample (1 g) was taken from the same original sample as for the total collagen content and was placed in a 10 mL 0.3% sodium chloride solution and incubated at 90 °C for 2 h (Christensen *et al*, 2011). Subsequently, the sample was homogenised for approximately 30 s at 9-10000 rpm, after which it was centrifuged (Sigma 2-16 K, Wirsam scientific, Cape Town SA) for 12 min at 4500 g (at ambient temperature). A 500 µL sample of the supernatant was then withdrawn and placed in a

cryovial (Lasec SA) to which 500 µL of 37% HCl was added. After the sample was thoroughly mixed it was incubated at 120 °C for 3 h. A sub sample (70 µL) of each hydrolysed sample was then transferred to a clear 96 well microplate (Greiner Cellstar 96 well flatbottom plate, Sigma-Aldrich, St Louis USA). The wells were then evaporated overnight (60 °C).

Following the evaporation of the liquid, the hydroxyproline content of each well was determined by using a hydroxyproline assay kit (catalogue nr MAK008, Sigma-Aldrich, St Louis USA). This involved a reaction with a chloramine T/oxidation buffer mixture for 5 min at ambient temperature followed by a diluted DMAB reagent for 90 min (60 °C). The absorbance (560 nm) was then determined (Spectrostar Nano, BMG Labtech, Ortenberg, Germany). The absorbance value was then converted to a hydroxyproline content by the use of a standard curve and from there to collagen concentration was determined by multiplying by a factor of eight (based on the hydroxyproline content of collagen) (Kolar, 1990). The insoluble collagen was calculated by subtraction of the total and soluble collagen contents. Results are presented as mg/g meat.

2.6 Statistical analysis

The experiment consisted of a randomised factorial design with 8 treatments (4 ageing periods X 2 genders) and six replications. The study tested the effects of ageing period and gender, as well as the interaction between the main effects. The model for the experimental design is indicated by the following equation:

$$y_{ij} = \mu + \beta_j + a_i + g_k + (ag)_{ik} + \varepsilon_{ijk}$$

The terms within the model are defined as; the overall mean (μ), the effect of the block (β_j), the effect of ageing period (a_i), the effect of gender (g_k), the effect of the interaction between ageing period and gender $(ag)_{ik}$ whilst ε_{ijk} is the error associated with the effect of the block, ageing period, gender and interaction of the former and latter.

The data were subjected to an analysis of variance (ANOVA). The Shapiro-Wilk test was performed to test for normality (Shapiro & Wilk, 1965). All of the outliers were identified and removed before final analysis of the ANOVA's. The t-Least Significant Differences (LSD) were calculated at a 5% significance level to compare the treatment means. Results were defined as being not significant at $P>0.05$ and significant at $P\leq0.05$. Total and insoluble collagen was added to the model as a co-variant to cater for its effect on shear force. Correlations were made between the variables by means of the Pearson's correlation coefficient. SAS™ statistical software (Statistical Analysis System, Version 9.2, 2006, SAS Institute Inc., Cary, NC, USA) was used for the analyses of variance (ANOVA).

3 RESULTS

The significance of the main effects, as well as the interaction between the two is presented in Table 2. The pH, cumulative moisture loss (%), cooking loss (%) and collagen solubility (%) were not affected by either of the main factors or their interaction (Table 2). The individual variables which were affected ($P \leq 0.05$) by the interaction of ageing period and gender (ageing x gender) are listed in Table 3. None of the shear force values differed ($P > 0.05$) for the female geese throughout the ageing period. Nor did the meat from the male geese at day 1 and day 14 differ ($P > 0.05$) from that of the females, however, the breast meat from the males had higher ($P \leq 0.05$) shear force values at day 3 and day 7 of ageing compared to that of the females. Both the total and insoluble collagen showed similar trends to the values of the shear force. The meat from the male geese aged for 7 days had a higher ($P \leq 0.05$) total and insoluble collagen content while the other treatments did not differ ($P > 0.05$). The average collagen content was 3.1 ± 1.34 mg/g meat. The LSMeans (Table 3) of the shear force when the total and insoluble collagen were treated as co-variants also indicates the shear force was highest ($P \leq 0.05$) in the meat from the male geese aged for 3 and 7 days with no differences ($P > 0.05$) between the other treatments.

Table 4 indicates the physical and biochemical results for the two separate main effects. The pH, cumulative moisture (%) and cooking loss (%) did not differ ($P > 0.05$) within the main effects of gender and ageing period. The collagen solubility (%) did not differ ($P > 0.05$) for gender although the meat from day 7 showed a lower solubility.

The change in the cathepsin specific activity over the ageing period is illustrated in Fig. 2. The overall trend shows an increase in cathespin (B, B & L and H) activity with days of ageing. The activity of all three the proteolytic enzymes at day 7 was significantly higher followed by a decline at day 14.

The MFL (Fig. 3) of the meat becomes shorter during the ageing period. The MFL from day 7 and 14 is significantly shorter than the lengths of the day 1 and 3 samples.

Table 2 The P-values[1] indicating the impact of ageing period and gender on the physical and biochemical characteristics of Egyptian goose breast meat

	Ageing x gender[2]	Gender	Age
Physical analyses			
pH	0.5156	0.1345	0.3345
% Cumulative moisture loss	0.4578	0.7913	0.5162
% Cooking loss	0.9007	0.1308	0.4974
Shear force (N)	**0.0004**	**0.0055**	**0.0009**
Shear force (co-variant)			
Total collagen	**0.0030**	0.0877	**0.0134**
Insoluble collagen	**0.0035**	0.0844	**0.0181**
Biochemical analyses			
MFL	0.1573	0.5165	**0.0001**
Total collagen	**0.0548**	**0.0047**	0.2306
Insoluble collagen	**0.0405**	**0.0119**	0.1880
% Collagen solubility	0.1296	0.5508	0.1620
Cathepsin B	0.3084	0.9583	**0.0041**
Cathepsin B & L	0.5729	0.7351	**0.0098**
Cathepsin H	0.4267	0.5720	**0.0031**

[1]P-values in bold indicate significance at P≤0.05. [2]Interaction between ageing period and gender (Ageing x gender).

Table 3 The mean values (± SD[2]) of the shear force as affected by the interaction of ageing period[1] and gender

	Female				Male				LSD[3]
	1	**3**	**7**	**14**	**1**	**3**	**7**	**14**	**P=0.05**
Shear force (N)	50.03[c] ± 15.05	47.88[c] ± 16.34	51.43[c] ± 9.21	54.52[c] ± 19.73	41.59[c] ± 9.61	77.88[b] ± 26.63	98.92[a] ± 25.24	45.33[c] ± 11.81	20.53
Total collagen (mg/g meat)	2.352[b] ± 0.981	2.654[b] ± 0.689	2.188[b] ± 0.521	2.808[b] ± 1.593	2.921[b] ± 1.44	3.087[b] ± 1.298	5.283[a] ± 1.651	3.372[b] ± 1.533	1.565
Insoluble collagen (mg/g meat)	2.034[b] ± 0.881	2.288[b] ± 0.732	1.885[b] ± 0.546	2.436[b] ± 1.508	2.495[b] ± 1.147	2.701[b] ± 1.15	4.899[a] ± 1.652	2.607[b] ± 1.936	1.552
LSMeans (± SE[4]) of Shear force (N) with total and insoluble collagen as co-variants									
Total collagen	52.10[b] ± 7.42	49.12[b] ± 7.30	53.94[b] ± 7.51	55.34[b] ± 7.26	42.10[b] ± 7.24	77.93[a] ± 7.23	92.98[a] ± 8.71	47.25[b] ± 8.01	
Insoluble collagen	51.93[b] ± 7.42	49.08[b] ± 7.31	53.73[b] ± 7.51	55.31[b] ± 7.27	42.22[b] ± 7.26	77.94[a] ± 7.24	92.97[a] ± 8.87	47.64[b] ± 7.99	

[a-c]Means in rows with different superscripts are significantly different at P≤0.05. [1]Numbers (1, 3, 7, 14) refer to days of ageing. [2]SD (standard deviation); [3]LSD (least significant difference); [4]SE (standard error).

Table 4 The mean values (± SD[2]) of the physical and biochemical analyses as affected by gender and ageing period[1]

	Gender		LSD[3]	Ageing period[1]				LSD[3]
	Female	**Male**	**P=0.05**	**1**	**3**	**7**	**14**	**P=0.05**
Physical analyses								
pH	5.92[a] ± 0.15	5.99[a] ± 0.17	0.10	5.90[a] ± 0.15	5.95[a] ± 0.12	6.01[a] ± 0.19	5.97[a] ± 0.16	0.13
% Moisture loss	3.54[a] ± 1.30	3.77[a] ± 3.95	1.79	3.34[a] ± 2.49	2.87[a] ± 1.20	4.70[a] ± 5.01	3.70[a] ± 1.22	2.52
% Cooking loss	32.77[a] ± 4.85	35.02[a] ± 4.70	3.00	32.25[a] ± 4.28	34.01[a] ± 4.69	35.45[a] ± 6.04	33.86[a] ± 4.33	4.18
Biochemical analyses								
Total collagen (mg/g meat)	2.500[b] ± 0.992	3.666[a] ± 1.69	0.152	2.636[a] ± 1.211	2.871[a] ± 1.016	3.736[a] ± 1.994	3.090[a] ± 1.520	1.107
Insoluble collagen (mg/g meat)	2.160[b] ± 0.945	3.175[a] ± 1.738	0.776	2.264[ab] ± 1.004	2.494[ab] ± 0.944	3.392[b] ± 1.963	2.521[a] ± 1.657	1.097
% Collagen solubility	14.40[a] ± 5.10	16.65[a] ± 19.16	7.59	14.10[ab] ± 3.75	13.75[ab] ± 4.50	11.38[b] ± 5.36	22.87[a] ± 26.10	10.74

[a-c]Means in rows, within main effect, with different superscripts are significantly different at P≤0.05. [1]Numbers (1, 3, 7, 14) refer to days of ageing. [2]SD (standard deviation); [3]LSD (least significant difference).

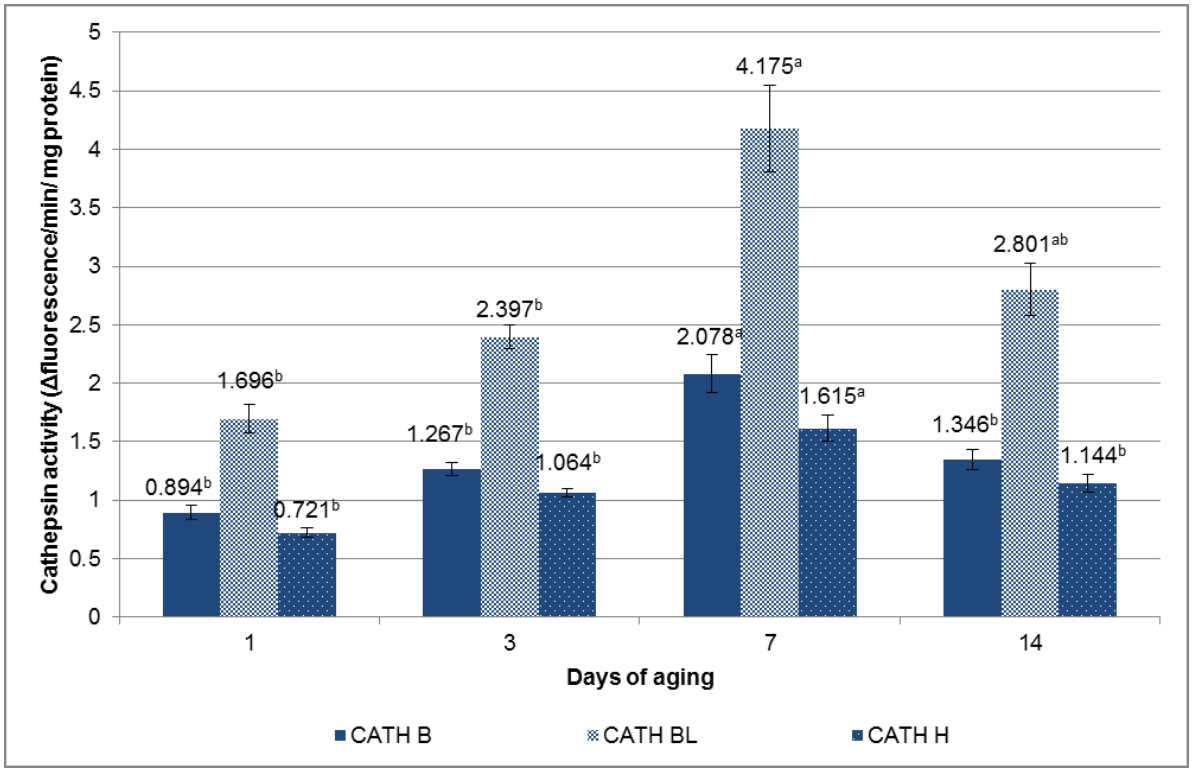

Figure 2 The change in the average cathepsin B, B & L and H specific activity as affected by the ageing period. [a-b]Different superscripts indicate significant differences between the mean values for each day of ageing per cathepsin at P≤0.05.

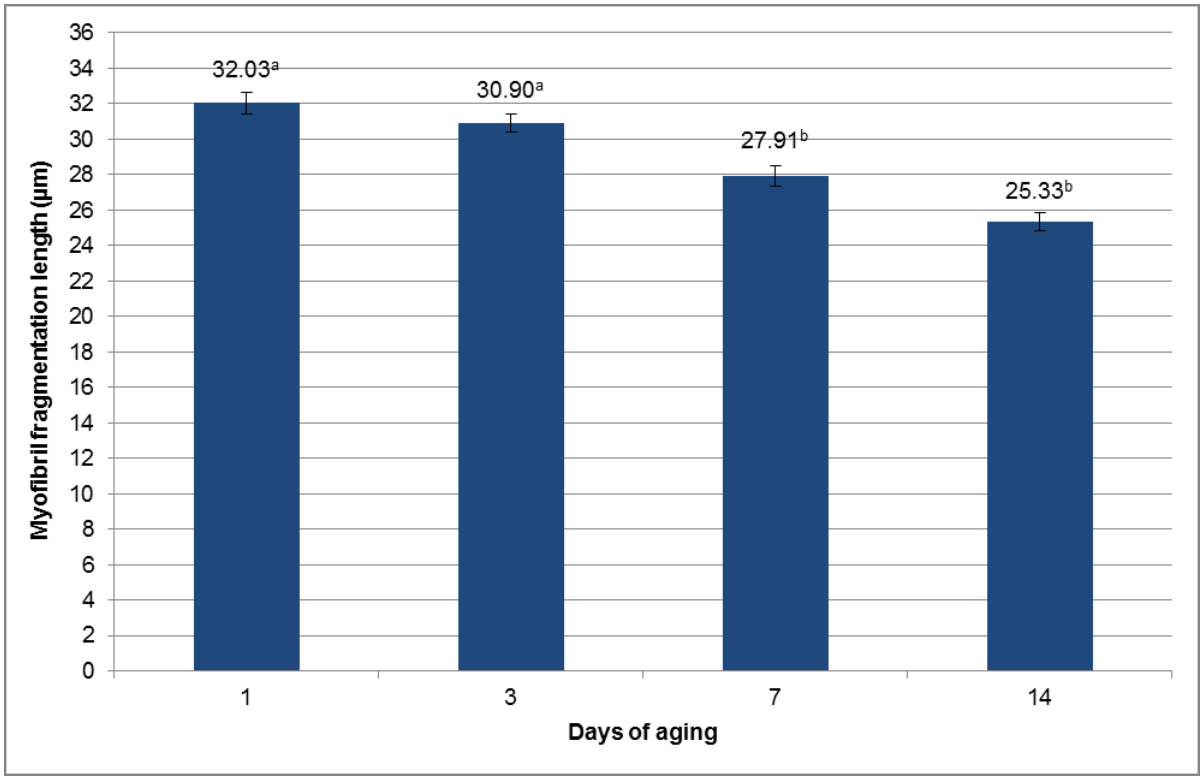

Figure 3 The myofibril fragmentation lengths as affected by days of ageing. [a-b]Different superscripts indicate significant differences between the mean values for each day of ageing per MFL at P≤0.05.

4 DISCUSSION

The factors contributing to the toughness of meat can be grouped into two; the connective tissue, of which mainly the total and insoluble collagen are responsible for the background toughness and secondly; those accountable for the myofibrillar toughness (Hertzman *et al*., 1993). The myofibrillar toughness is determined by the development of rigor mortis and the subsequent breakdown of the structural/myofibrillar proteins by the proteolytic enzymes.

The overall average shear force (58 N) compares well with that reported in Chapter 7, as well as by Geldenhuys *et al*. (2014) [Chapter 4]. The average total collagen content (3.1 mg/g meat) of the Egyptian goose breast portion (Table 4) relates well with that of the ostrich *M. illiofibularis* (2.99 mg/g) reported by Sales (1996). It is, however, much higher when compared to the 0.96-1.03 mg/g found by Brand (2006). The collagen solubility (15%) of the Egyptian goose breast meat is much lower compared to the meat of other domestic species such as ostrich (20%) (Brand, 2006) beef (21-27%) (Christensen *et al*., 2011) and pork (>35%) (Kristensen *et al*., 2002).

4.1 The effect of ageing

The weakening of the myofibrillar structure by endogenous peptidases results in an observed increase in the tenderness of meat during natural ageing (Ouali, 1992; Sentandreu *et al*., 2002). Therefore, as time progresses during the ageing of meat a gradual decrease and ultimate plateau in the instrumental shear force values are expected thereof (Calkins & Seideman, 1988; Takahashi, 1996; Barnier *et al*., 1999; Li *et al*., 2012; Lomiwes *et al*., 2014). For instance, with aged beef a maximum tenderness is observed after approximately eight to 14 days of ageing (Koohmaraie *et al*., 1991; Takahashi, 1996; Sentandreu *et al*., 2002; Koohmaraie & Geesink, 2006; Li *et al*., 2012).

Our study, however, shows contradictory results; with no decrease in the shear force values as the 14 day ageing period advances (Table 3). In fact, for the male geese, the meat aged for the intermediate periods (3 and 7 days) had higher ($P \leq 0.05$) values compared to the non-aged and 14 day aged meat. Nonetheless, considering the proteolytic enzyme activity there was indeed an increase in cathepsin activity (B, B & L and H) over the ageing period (Fig. 2) with activity peaking at 7 days. It is also evident that when the cathepsin activity is related back to the results obtained in the rigor mortis period [Chapter 9], there seems to be an increase in activity from the end of rigor (4 h 15 min post mortem) to 1 day of ageing. Thomas *et al*. (2004) reported a similar increase in the cathepsin (B, L and H) activity of aged ostrich (*M. illiofibularis*) with a substantial amount of activity left after 12 days. As time proceeds during ageing, progressive disruption of the lysosomal membranes occur, this may lead to an increase in cathepsin activity (Zeece *et al*., 1992). The drop in cathepsin activity at 14 days of ageing may therefore in some way be linked to the dissolution of lysosomal

disruption subsequent to 7 days of ageing. Although there is some uncertainty regarding the ultimate influence of the cathepsins in post mortem myofibrillar degradation (Koohmaraie, 1992; Sentandreu *et al*., 2002; Hopkins & Thompson, 2002), the observed increase in the cathepsin activity over the ageing period may suggest that it does in actual fact contribute.

The decrease in the MFL (32 - 25 µm) of the Egyptian goose *pectoralis* (Fig. 3) as ageing progresses coincides with the increase in the cathepsin activity observed, especially the much lower ($P \leq 0.05$) lengths of the myofibrils recorded on day 7 of ageing. This supports our theory that the increase in cathepsin activity over the ageing period may have been involved in myofibrillar degradation. Similar MFL results have been reported on beef by Frylinck *et al*. (2009) where the MFL decreased from 48 to 25 µm over a 14 day ageing period. This decrease in MFL was associated with a decrease in shear force observed over the ageing period. Although only investigated during the development of rigor mortis the contribution of the µ/m-calpain towards myofibrillar breakdown and the decrease in the MFL cannot be excluded. The results of Chapter 9 reported that µ/m-calpain activity was still reasonably high and had not yet started to decrease at the end of the rigor period. Therefore, the activity of µ/m-calpain over the ageing period and the possible influence thereof do warrant further research in order to draw any concrete conclusions in this regard. Even so, the decline in the lengths of the myofibrillar fragments confirms that proteolytic breakdown did indeed occur during the ageing of Egyptian goose meat. This, however, raises the question of why the decrease in the MFL of the aged muscles is not reflected in the shear force values. In attempting to clarify this, some theories can be proposed.

Firstly, it may be that the myofibrillar fragmentation was not substantial enough for the changes to be observed in the shear force measurements. It is also important to consider the fact that different birds were used for each of the ageing periods which may have increased the variation within this study and may have led to experimental bias. This could be a key factor especially since the age of the bird is another element that cannot be controlled when investigating the meat quality of Egyptian geese. All of the birds used were classified as being mature, but a more specific age determination was not viable as no work has yet been done on refining a method to determine the age of these gamebirds. It should also be noted that Egyptian geese are gamebirds and are generally shot while in flight which makes it even more difficult to control/select for age. Consequently, this variation in terms of different birds used, together with the possibility that there was a large discrepancy in age may have resulted in the weakening of the myofibril structure being overshadowed by the background toughness, i.e. the connective tissue content and composition. It is well recognised that the collagen composition of meat can be influenced by animal age with an increase in the polypeptide cross links resulting in a decrease in solubility upon heat treatment (Lawrie & Ledward, 2006). There can also be an increase in the concentration of total collagen in the muscles of animals that are physically more

active pre-slaughter (Smith & Carpenter, 1970; Lewis *et al*., 1989). Significant correlations were found between the shear force values and the total collagen (r=0.466; P=0.001), as well as the insoluble collagen (r=0.479; P=0.001) of the breast portion. In addition, when considering the specific total and insoluble collagen concentrations in the aged Egyptian goose breast portions (Table 3) a similar trend to the shear force results is observed. The male breast portions aged 7 days had higher levels (P≤0.05) of both total and insoluble collagen. No differences (P>0.05) were found in collagen between the other male portions as well as the female breast portions over the entire ageing period. The higher total and insoluble collagen concentration in the breast portions of this group of birds may indicate that they were physically more active and/or somewhat older. Upon the consideration of the total and insoluble collagen as co-variants to the shear force the only difference observed (for both factors) was the increase (P≤0.05) in the shear force of the male breast portions aged for 3 days (Table 3). Both the 3 and 7 day aged portions, therefore, had equally (P>0.05) high shear force values. This suggests that although the collagen content (especially insoluble collagen) may contribute to the high overall instrumental shear force (58 N) of Egyptian goose meat, other factors need to be considered in order to clarify the unaffected shear force values over time; a point further illustrated by the correlation values (although significant) being <0.5. The higher (P≤0.05) shear force values of the male breast portions aged for 3 and 7 days may be explained by the possible presence of large sinews (collagen) that were not necessarily included in the smaller muscle portion used in the chemical analyses for collagen determination. The size of the muscle fibres have also been implicated in meat tenderness, with an increase in fibre diameter observed with age resulting in higher shear force values (Tuma *et al*., 1962). It is therefore possible that the average age of the group of male birds used for the 3 and 7 day ageing periods was older compared to the rest. Another possibility is that increased sarcomere shortening due to increased temperatures occurred (as discussed in Chapter 9) during the rigor mortis period of the male breast samples aged for 3 and 7 days. Similar results have been reported by Devine *et al*. (1999) where the extent of tenderisation (enzymatic) was constant in all samples (at different rigor temperatures) but the tenderness varied on account of muscle shortening. They explain that MFL alone may therefore not be a comprehensive indicator of tenderisation during the ageing of meat as it does not report the tenderness in its entirety.

4.2 Effect of gender

The variation in the shear force values of the breast portions of female vs. male geese [Chapter 7] was also found within this ageing study. Gender (Table 2) was not only a significant factor concerning the instrumental shear force but also influenced (P≤0.05) the total and insoluble collagen content (mg/g meat). When the latter two variables were considered as co-variants to the shear force values, the gender effect was suppressed (Table 2). This suggests that the increased (P≤0.05) collagen

content (total and insoluble) in the male breast portions (Table 4) is responsible for the increased shear force values thereof. Gender was also not a significant factor regarding the post mortem pH decline and proteolytic enzyme activity during rigor mortis [Chapter 9] which indicates that the collagen content may be the only cause of the higher instrumental shear force and lower sensory tenderness observed by the trained panel in Chapter 7. It is quite possible that the male geese are more active and fly more compared to females and therefore have a higher amount of total collagen (Smith & Carpenter, 1970; Lewis *et al*., 1989).

5 CONCLUSIONS

Given the fact that there was no significant change (decline) in the shear force values, the ageing of Egyptian goose meat as a means of improving the overall toughness, cannot be proposed without further research. It can, however, be concluded that myofibrillar degradation does occur (over 14 days of ageing) but the relevance thereof needs to be clarified. In order to draw more concrete conclucions, the effect of ageing should be investigated on the same *pectoralis* muscle over time to minimise the variation. The higher shear force and lower sensory tenderness of the male breast portions as observed in Chapter 7 may be linked to higher concentrations of total and insoluble collagen.

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CHAPTER 11

General discussion and conclusions

With an emerging gamebird industry in South Africa, the hunting of wildfowl species has not only increased considerably in recent years but a large number of these birds are harvested annually as they are considered to be agricultural pests (Geldenhuys *et al*., 2013a) [Chapter 2]. The major financial losses that crop farmers suffer due to the extensive feeding activities of Egyptian geese (*Alopochen aegyptiacus*) substantiate the hunting of these birds in order to reduce the damage they cause. The increase in the population numbers of this species and the consequent financial implication for crop farmers therefore generates a potential for utilisation of the meat and possible recovery of some of the financial losses due to the damage. At present, the meat from wildfowl species, particularly Egyptian geese, are not commercially utilised or available for purchase. Consequently, an absence of available, scientific information that defines the meat quality characteristics of this wildfowl species provided the incentive for this baseline research.

A comparative study was the initial step and consisted of two aspects; chemical and descriptive sensory profiling. Well-known reference species (guineafowl, pekin duck, ostrich and broiler chicken) were used as reference standards in order to identify and categorise the sensory and chemical characteristics of Egyptian goose meat. The sensory profile was found to be very distinct in relation to the characteristics of the other wildfowl species (Geldenhuys *et al*., 2014) [Chapter 4]. Egyptian goose meat has very strong game aroma and flavour attributes with a prominent metallic aftertaste. The respective, intense aroma and flavour notes were linked to the substantially higher iron (Fe) content (7.5 mg/100 g meat), as well as the high overall polyunsaturated fatty acid (PUFA) content (39.5%) as revealed by the chemical profiling (Geldenhuys *et al*., 2013b) [Chapter 3]. The Fe content is attributed to the physical activity (flying) endured by the breast muscle of Egyptian geese while the variation in the fatty acid profiles of the species is diet related; the geese mainly having a grass/forage-based diet. The latter study also indicated that both the polyunsaturated to saturated fatty acid ratio (P/S) and the omega 6 to omega 3 ratio (*n*-6/*n*-3) of Egyptian goose meat is within the recommendations (P/S >0.4; *n*-6/*n*-3 <5) in terms of human health. The unique aroma and flavour profile, however, was not the only revelation regarding the sensory profile; because of the high level of physical activity endured by the breast muscle, the trained panel found the meat to be very tough (high shear force of 48N) compared to the other species. This initial sensory profile study created a platform for further research investigating the effect of the grain season (diet), as well as gender on the sensory profile and overall meat quality of Egyptian geese.

Although consumers prefer meat to be juicy, tender and flavoursome; these are attributes which are determined by several extrinsic and intrinsic factors. Consequently, an essential aspect of consumer acceptability is the overall uniformity of the meat quality. Ensuring a product with consistent eating quality is always a challenge, so identifying all of the factors which may affect the overall uniformity is very important in order to fully control the quality thereof. Therefore, following the initial sensory and chemical profiling, the influence of three main effects namely; season (grain vs. non-grain diet), gender and portion was investigated. Firstly, the impact of these factors on the carcass yield, physical characteristics and proximate composition was evaluated (Geldenhuys *et al*., 2013c) [Chapter 5]. The average dressed carcass weight of Egyptian geese is 1.31 kg with a dressing percentage of 53.6%. The average portion (muscle) weight of the breast, thigh and drumstick is 227.4 g, 64.7 g and 58.9 g respectively. This study was followed by the quantification of the chemical composition on a more in-depth level where the differences in the fatty acid, amino acid and mineral composition were determined. Finally, a descriptive sensory analysis was repeated to evaluate the effect of season (diet) and gender on the sensory profile.

Essentially, this research revealed that the factor which will have the largest effect on the consistency of Egyptian goose meat is seasonality. It is questionable whether the selected carcass parameters and physical characteristics, which differed on account of season, will be recognised by the average consumer. However, the main issue is the higher intramuscular fat (IMF) content in winter (July), as well as the substantial difference in the fatty acid profiles of the two seasons. The forage vs. grain based diets of Egyptian geese during certain periods of the year leads to variation in the content of the key fatty acids such as oleic acid, linoleic acid and α-linolenic acid. The differences in these fatty acids result in variation between the *n*-6/*n*-3 ratios of the seasons; the portions from winter (July) are within the health recommendations (ratio<5) and those from summer (November) are not. The composition of the fatty acids is not only one of the key aspects in terms of human health but it also has a considerable effect on the sensory profile of the meat, especially since Egyptian geese are monogastric birds. The geese harvested in winter (July) had the characteristic and overwhelming game and metallic attributes similar to that found in the comparative study (Geldenhuys *et al*., 2014) [Chapter 4]. In contrast, the meat harvested in summer (November) had a very pleasant profile with sweet-oily-duck and beef sensory attributes. The absence of intense game or metallic notes was surprising. The completely opposing sensory profiles of the respective seasons are directly linked to the fatty acids of the diet. The fatty acids are incorporated in the meat and are responsible for the distinct aroma volatiles produced upon cooking. The second main effect of gender was found to significantly affect the carcass characteristics and intramuscular fat content of Egyptian geese. The meat from the female geese associated more with the sweet-oily-duck attributes and was also more tender with a lower instrumental shear force. Irrespective of the tenderness differences,

gender is an aspect which will be very difficult, if not impossible to control. The variation between the different portions for the variables was expected as the muscle effect is one of the key causes of intrinsic variation. So these results indicate that in order to ensure a consistent meat product, the harvesting periods should be considered and kept constant. It will, however, be challenging to find the best balance of healthy meat with the most acceptable sensory profile; an area which warrants further research. It is also important to note that in terms of the ethics, the periods in which the geese are harvested should not fall within the peak breeding season (September). This would mean that the meat would only be available for 10/11 months of the year.

Following the comparative study (chemical and sensory), as well as the investigation into the influence of the three main effects (season/diet, gender and portion) on the meat quality, there was one more matter that needed to be addressed. Gamebird meat is generally perceived as being tough compared to the meat from domestic animals and this was certainly the case with Egyptian goose meat. In the initial, comparative study (Geldenhuys *et al*., 2014) [Chapter 4] the meat was found to be extremely tough in relation to the other well-known fowl species with a shear force value of 48N vs. the 18N of broiler chicken. The high shear force value was negatively correlated to sensory tenderness and the panel found Egyptian goose meat to be perceptibly tough. Significant differences were also found within the tenderness and shear force values of the breast portion of male vs. female geese. Meat tenderness is an important characteristic involved in the consumer acceptability of meat (Risvik, 1994; Warriss, 2000; Kemp & Parr, 2012). The low ultimate tenderness of Egyptian goose meat may have a negative impact on the acceptability and overall potential of this meat product. Therefore, those biochemical factors which determine the toughness of meat were also explored.

The toughness of meat is determined by two aspects; the background toughness related to the characteristics of the connective tissue and the myofibrillar toughness (Hertzman *et al*., 1993). The latter is influenced by the development of rigor mortis and the natural tenderisation of the meat by means of proteolytic breakdown (Hertzman *et al*., 1993). A fibre typing study of the *pectoralis* muscle (breast portion) verifies that this muscle is mainly comprised of fast, oxidative-glycolytic (FOG) fibres (84%) similar to that of other volant bird species (Rosser & George, 1986). It is speculated that the large proportion of small, FOG fibres may contribute to an increase in connective tissue and therefore the perceived toughness of Egyptian goose breast meat. Also, the pH decline in the *pectoralis* muscle occurs quite rapidly compared to other species and it is possible that the high rigor temperature (>20 °C) may contribute to the increased toughness. The carcasses were treated in a manner which is typical for the current handling practices of gamebirds during wingshooting expeditions in South Africa (Geldenhuys *et al*., 2013a) [Chapter 2]. They were therefore kept at ambient temperatures during the rigor period. Regardless of the calpain and cathepsin activity during the rigor period, Egyptian goose breast meat is still tough at 36 h post mortem (Geldenhuys *et al*.,

2014) [Chapter 4], indicating that the contribution of the proteolytic enzymes during rigor, if any, is not substantial enough for it to be recognised. It is also important to consider the possibility that the contribution of the proteolytic enzymes may be overshadowed by the background toughness i.e. the connective tissue content and fibre structure. The latter theory was confirmed when the breast portions were aged for a 14 day period and no change (decline) in the shear force values was observed over time. It can, however, be concluded that myofibrillar degradation does occur (over 14 days of aging) due to a significant decrease in the myofibrillar fragmentation lengths, but the relevance thereof needs to be clarified. Given the fact that there was no progressive decline in the shear force values over time, the aging of Egyptian goose meat as a means of improving the overall toughness, cannot be proposed without further research.

There are indeed aspects that require further research in order to strengthen the knowledge gained from this study. An aspect that should be investigated in more detail is the influence of temperature on rigor shortening, to verify the theory of its involvement in the toughness. It would also be recommended that the ageing process is studied in more detail, especially since one of the limitations of this study was the inability to use samples from the same bird for each of the ageing periods. This will allow for more concrete conclusions to be drawn regarding the ageing of Egyptian goose meat as a means of improving the toughness. Other means of tenderisation such as brine injection could also be explored which may provide valuable solutions towards the problem of Egyptian goose meat being so tough. Animal/bird age and its effect on the meat quality characteristics should also be considered seeing as it was not possible to determine age in this research. The latter recommendation may provide a platform to explore the potential farming with wildfowl species, specifically Egyptian geese, for meat production. Another equivocal area with regard to possible wildfowl utilisation is the handling of the shot gamebirds during/after the wingshooting activities. The post mortem handling practices will not only affect the meat quality but is also a safety concern. It will also be a viable option to investigate alternative harvesting procedures as it is not only very difficult to successfully hunt large numbers of birds but the use of lead shot pellets have also become a health concern. Also, the use of shotguns result in a percentage of the shot birds having perforated intestinal cavities which is a microbial risk. Finally quantifying the perception or attitude of consumers towards gamebirds as a meat source is also an aspect that would be worth investigating.

This study in its entirety provides essential baseline data regarding the basic sensory, physical and chemical characteristics of Egyptian goose meat whilst initial research into the extent to which the meat quality is influenced by season, gender and portion has also been explored. In attempting to elucidate the toughness of the meat, possible causes have been proposed. Ultimately, this research provides substantial proof that the commercial utilisation of Egyptian goose meat is feasible.

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