Effects of production systems and canola meal supplementation on carcass and meat quality characteristics of spent laying hens

by

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Submitted in fulfillment of the requirements for the degree of

MASTER OF SCIENCE IN ANIMAL SCIENCE

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2018

DECLARATION

I hereby declare that this dissertation is the outcome of my own study. I have accordingly acknowledged in the text where the work of others has been used.

……

FAROUK SEMWOGERERE

I hereby certify that this statement is correct

……..

Prof V. MUCHENJE

ABSTRACT

This study evaluated the carcass and meat quality traits of spent laying hens as influenced by production systems (conventional cages and free range) and the inclusion/exclusion of canola meal in their diets. A total of 30 free range and 60 battery cage reared Lohmann Brown-Elite spent laying hens were obtained from a commercial egg producer. The 30 free range hens (53 weeks of age) and 30 of the caged hens (40 weeks of age) were fed a conventional diet, while the remaining 30 caged hens (48 weeks of age) were fed a diet supplemented with canola meal (20%). Carcass, portion and organ weights were determined. Physical attributes and proximate composition were analyzed for, with additional fatty acids and sensory profiles being determined for the effects of canola meal inclusion in the diet. Caged hens had heavier ($P \leq 0.05$) warm and cold carcasses, thigh, wing and feet compared to free range hens. The percentages of the breast (26.1 ± 0.51 vs. 28.3 ± 0.28), drum, breast bones, breast thaw and cooking losses and thigh cooking loss were lower ($P \leq 0.05$) for caged hens than for free range hens. Free range hens had heavier ($P \leq 0.05$) gizzards (33.9 ± 1.04 vs. 30.5 ± 0.73) and bones and a lower ($P \leq 0.05$) breast meat percentage (47.3 ± 0.94 vs. 51.7 ± 1.35). Meat redness (a*) (0.54 ± 0.222 vs. 1.40 ± 0.135) and hue angle value, skin redness (a*), breast and thigh, Warner-Bratzler shear force (WBSF) values (breast: 12.37 ± 0.411 vs. 17.10 ± 0.751, thigh: 29.68 ± 0.306 vs. 39.75 ± 0.826), breast moisture and thigh ash content were lower ($P \leq 0.05$) for caged hens than free range hens. Caged hens had higher ($P \leq 0.05$) thigh thaw loss and breast ash content than free range hens. Canola-fed hens had higher ($P \leq 0.05$) drum percentages, breast bone weights and percentages, with lower ($P \leq 0.05$) thigh and breast meat percentages. Canola-fed hens had lower ($P \leq 0.05$) thaw losses, skin yellowness (b*) and Chroma values, breast fat content with higher cooking losses, skin redness (a*) and hue angle value, as well as breast WBSF (12.37 ± 0.411 vs. 15.43 ± 0.600). Palmitic acid, stearic acid, heneicosanoic acid acid, palmitoleic acid, saturated fatty acids (SFA) (34.0 ± 0.56 vs.

38.7 ± 0.71), n-6:n-3 polyunsaturated fatty acids (PUFA) ratio (5.5 ± 0.13 vs. 7.2 ± 0.28), atherogenic index (IA), thrombogenic index (IT), delta-5 desaturase (D5D, elongase index and thiosterase index were lower ($P \leq 0.05$) for canola-fed hen breast meat. Breast meat from conventionally fed hens had lower ($P \leq 0.05$) myristic acid, lignoceric acid, nervonic acid, alpha-linolenic acid (ALA), docosahexaenoic acid (DHA), PUFA:SFA ratio (0.7 ± 0.05 vs. 0.9 ± 0.02), n-3 PUFA (3.4 ± 0.31 vs. 5.1 ± 0.17), hypocholesterolemic:Hypercholesterolaemic (h/H), stearoyl-CoA desaturase 16 (SCD16) and stearoyl-CoA desaturase 18 (SCD18). The breast meat from conventionally fed hens had lower ($P \leq 0.05$) metallic flavor than that from canola-fed hens. Strong positive correlations were observed for overall aroma with chicken ($r = 0.965$, $P < 0.001$) and brothy aroma ($r = 0.827$, $P < 0.001$); overall aroma with overall flavor ($r = 0.680$, $P < 0.001$), chicken flavor ($r = 0.668$, $P < 0.001$) and brothy flavor ($r = 0.548$, $P = 0.006$); initial juiciness with sustained juiciness ($r = 0.771$, $P < 0.001$) and tenderness ($r = 0.537$, $P = 0.007$); sustained juiciness with tenderness ($r = 0.790$, $P < 0.001$) and chewiness with residue ($r = 0.783$, $P < 0.001$). Whilst strong negative correlations were observed for: sustained juiciness with chewiness ($r = -0.655$, $P = 0.001$) and residue ($r = 0.783$, $P < 0.001$) and for tenderness with chewiness ($r = -0.845$, $P < 0.001$) and residue ($r = -0.855$, $P < 0.001$). Results of this study highlight that a free range production system when compared to a conventional cage system increased undesirable carcass and physical meat traits of spent laying hens. On the other hand, canola meal inclusion incorporates beneficial health aspects without affecting the sensory profile of meat derived from spent laying hens, both groups of hens being reared in battery cage system. Spent laying hen breasts can be consumed as a functional food (especially canola-fed) since the fat content and composition was observed to be close to that which is recommended for a healthy diet.

DEDICATION

To my son, Fadil Semwogerere for the positive energy he always brings to me; to my siblings, Hakim Kinene and Shabirah Nakitende for the emotional support; and Josephine Nanfuma, for bringing a new life into my life.

ACKNOWLEDGEMENTS

I would like to extend my special appreciation and acknowledgement to the Almighty Allah for life, opportunities and guidance this life. He is the supreme mentor and controller of my life and has made everything go well through this time of research and report writing.

Special thanks to Prof Louw Hoffman and Prof Voster Muchenje for financial opportunities extended to me through amongst others, the DST/NRF SARChI Chair in Meat Science bursary. Without this bursary, it would have been impossible for me to complete my studies. My deepest gratitude goes to my supervisors, Prof Voster Muchenje, Prof Louw Hoffman and Dr. Jeannine Marais, for the positive critiques, invaluable support and enthusiastic encouragement during my studies. The timely response and late-night WhatsApp conversations with Dr. Jeannine Marais cannot go unacknowledged; thank you.

Thanks also go to my colleagues from the Food Security and Meat Science group, Department of Livestock and Pasture Science, University of Fort Hare; Sharon Lungu, Wongalethu Tyeni, Dr. Trymore Moyo, Thuthuzelwa Stempa, Zimkitha Soji and Dr. Lizwell Mapfumo, with special thanks to the 'UFH maties' (Leo Mahachi and Chido Chakanya) for their technical, moral and emotional support during my studies.

My gratitude also goes to the Department of Animal Sciences, University of Stellenbosch support staff and laboratory assistants, Mrs. Adele Carstens, Mrs. Lisa Uys, Mrs. Beverly Ellis, Mr. Michael Mlambo and Ms. Janine Booyse for their tireless willingness to help and provide guidance in all laboratory and logistics solutions. Lastly, to Stellenbosch students, Daniel van der Merwe, Pumi Shange, Fundeka Ndyoki, Caitlin Firth, Mark Kirstein, Retha Engels, Karla Pretorius, Mari and Alretha van Heerden; your support at the abattoir and meat science laboratory was invaluable; Pumi and Caitlin sensory, I thank you indeed. Special thanks to Nulaid Company who donated the birds.

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ACRONYMS

ADF	Acid Detergent Fiber
AI	Atherogenic Index
ALA	Alpha-Linolenic Acid
BHT	Butylated Hydroxytoluene
CM	Canola Meal
D5D	Delta-5 Desaturase
D6D	Delta-6 Desaturase
DHA	Docosahexaenoic Acid
DM	Dry matter
DSA	Descriptive Sensory Analysis
FA	Fatty Acid(s)
FAME	Fatty Acid Methyl Esters
IMF	Intramuscular Fat
IT	Thrombogenic Index
LSD	Least Significant Difference
ME	Metabolisable Energy
MUFA	Monounsaturated Fatty Acids

ND	Not Detectable
NDF	Neutral Detergent Fiber
PCA	Principal Component Analysis
PUFA	Polyunsaturated Fatty Acids
SBM	Soybean Meal
SCD	Stearoyl-CoA
SE	Standard Error
SFA	Saturated Fatty Acids

Chapter 1

General Introduction

1.1 Background

The South African poultry industry is one of the prime subsectors of the agricultural production sector, yielding an approximately 24% of annual agricultural production (SAPA, 2014; 2016a). The poultry industry remains the most important source of animal protein, accounting for 42.8% (SAPA, 2016a) and 65% (excluding cow milk) (SAPA, 2012; 2016a) of all locally produced animal protein. The egg industry alone makes up 18% of the poultry industry (UEDE, 2013). This has made the egg industry the fourth largest animal product subsector in the agricultural sector after poultry meat, beef and cow milk, although eggs are still the cheapest animal protein source per unit weight compared to beef, chicken and pork (SAPA, 2016b). The lower prices have made eggs an important source of animal protein for low-income earners in the rural areas of South Africa (Tarwireyi and Fanadzo, 2013). In 2013, the production period of laying hens was 69 weeks. However, the production period was increased to 74 weeks in 2015 due to improvements in the genetics and management of laying hens (SAPA, 2016b). At the end of their laying cycle, laying hens now referred to as spent laying hens or off-layers are disposed of. Hens culled during the production period are also referred to as spent laying hens. The fate of spent laying hens is still largely unaccounted for in South Africa; as egg producers tend to sell these hens live to local traders at farm gates, at extremely low prices.

In developed countries, spent laying hen meat is processed into chicken products such as sausages, as their meat is considered to be of low quality (Souza *et al.*, 2011). In some cases spent laying hens are processed into animal feeds, chicken soup and traditional delicacy

recipes (Chuaynukool *et al.*, 2007; Hill, 2009). This is due to the fact that spent laying hens have less muscle and fat compared to the meat derived from broiler chickens. The South African layer flock consisted of 25.05 million hens in 2015 (SAPA, 2016b). This implies that on average the same number of spent laying hens must be culled and/or disposed of annually. This number of spent laying hens only accounts for 72.2% of the total spent hen population of the poultry industry with the rest being spent broiler breeder laying hens (ARC, 2016). The disposal of spent laying hens is a major economic problem for egg producers, second only to feed costs (Souza *et al.*, 2011). This is because, whether using point-of-lay pullets or raising pullets, the initial cost of buying or raising pullets is drastically reduced when spent laying hens can fetch a reasonable market price. The fixed cost of slaughtering a spent laying hen versus a broiler chicken is the same, but the value of the latter is higher than that of the spent laying hen, thus making the commercial slaughter of spent laying hens economically unviable; unless the value of a spent laying hen can be increased to higher than that of a broiler chicken per unit weight. The need to increase the economic value of spent laying hens has triggered scientific research into the feasible utilization thereof.

The production system of layers determines the method of feeding as well as the quality of the eggs and meat produced. In South Africa, laying hens are generally reared in the battery cage or free range systems (SAPA, 2016b). Over the past decade, the South African egg industry has undergone tremendous changes which include vertical integration. This has enabled large-scale production, making eggs a low-cost alternative to other protein sources. However, vertical integration pushes small-scale egg producers out of the main market channel (Sams, 2001). Small-scale producers in South Africa are now occupying the high-value niche of organic eggs under free range systems. The recent worldwide campaign against the conventional battery cage system, which is considered an inhumane way of rearing chickens, has given free range products a high value advantage (Neufeld, 2002; Miao

et al., 2005). Consumers also have a perception of free range chicken eggs and meat as being tastier, healthier, higher in protein and vitamins and lower in calories and polyunsaturated fats; hence they are willing to pay more for free range meat and eggs than battery cage produced eggs and meat (Miao *et. al.*, 2005; Rodić *et al.*, 2010; Napolitano *et al.*, 2010). Although literature exists on the high nutritive value of free range eggs, little is known on the meat quality of spent laying hens raised in free range systems.

Over decades, soybean meal has been used in the poultry feed industry as a protein source. The ever increasing competition from humans and other livestock for soybeans has, in turn, made it scarce and more expensive (Messerschmidt *et al.*, 2014; Wickramasuriya *et al.*, 2015). This has triggered scientific research for alternative protein feedstuffs to be used in the formulation of poultry rations. Canola meal is an oilseed crop by-product that has been identified as suitable to partially replace soybean meal in poultry diets (Mikulski *et al.*, 2012). The inclusion of canola meal at a percentage greater than 20% in layer diets is known to cause physiological disorders due to high fiber and anti-nutritional factors: glucosinolate, eruca acid and tannins (Angelovicova and Angelovic, 2013; Messerschmidt *et al.*, 2014). Although canola meal has a low nutritional profile compared to soybean, it has a well-balanced amino acids profile and is high in essential oils. Canola meal is also high in essential vitamins and minerals (Chibowska *et al.*, 2000; Wickramasuriya *et al.*, 2015).

The use of canola meal in laying hen rations has shown to have no significant effect on egg production or hen mortality (Campbell *et al.*, 2007). Canola meal improves the nutritive value of egg yolk, including its fatty acid profile, by increasing the amount of n-3 polyunsaturated fatty acids (n-3 PUFA). Additionally, canola meal has been reported to lower the n-6 polyunsaturated fatty acids: n-3 polyunsaturated fatty acids ratio (Angelovicova and Angelovic, 2013), making eggs from canola meal-fed hens a functional food. The effects of

canola meal on the nutritional profile of eggs is well documented in the literature (Summers *et al.*, 1985; Ward *et al.*, 2009; Świątkiewicz *et al.*, 2010; Goldberg *et al.*, 2016). However, little is known about the effect of canola meal on the meat quality of laying hens. Therefore, this study seeks to investigate the effect of production systems and canola meal supplementation on the carcass and meat quality attributes of spent laying hens.

1.2 Problem statement

The utilization of spent laying hen meat is limited by the availability of information on the variation among carcass and meat quality attributes as influenced by intrinsic and extrinsic factors. South African egg producers are currently experiencing a difficulty with the disposing of spent laying hens at reasonable market prices. This is because spent laying hen sales are limited to local traders or middlemen who sell to consumers (DAFF, 2014). These entrepreneurs have established what are commonly known as 'spent hen' depots dealing in live spent birds and ungraded eggs. They buy live birds from the egg producers at low prices and sell at very high prices. This has made the sale of live birds a very lucrative venture (DAFF, 2014). However, it comes at the expense of the egg producers. Furthermore, commercial abattoirs do not slaughter spent laying hens, owing to the lack of a formal market for their meat.

Currently, many consumers are concerned about the quality of food they eat for their own health. Health-concerned consumers have driven the market into a more natural way of food (free range), triggering a reduction in the use of artificial chemicals in food production (Dyubele *et al.*, 2010) and an increase in the beneficial components (n-3 PUFA) of food to make it 'functional food' (Gül *et al.*, 2012). Additionally, consumers enjoy food with a unique taste and are willing to pay extra for so-called organically produced food (Okarini *et al.*, 2013). The aforementioned aspects have triggered production of table eggs and broiler

chicken meat from free range systems and the inclusion of canola meal supplementation in chicken diets in order to achieve organic and high n-3 PUFA content products, respectively.

Currently, there is a well-established market for free range and canola table eggs as well as broiler chicken meat, achieved through scientific research (González-Esquerra and Leeson, 2001; Hastings, 2003; Brouwer, 2015; Bertechini, 2017). Egg producers who are feeding canola meal as a supplement to layers or rearing hens in free range system are still struggling to tap into this high-value chicken meat market. This is mainly because of a lack of scientific knowledge on the carcass and meat quality of spent laying hens fed canola meal supplement or reared in free range production systems. More so, it is hypothesized that South African consumers are being exploited by the middlemen who market spent laying hen carcasses and portions as indigenous chicken, locally known as '*umleqwa*', on supermarket shelves. Consumers are willing to pay more for indigenous chicken meat as it is perceived to be tastier and produced in a more natural way (free range systems); there is thus deception to play here and exploitation of the system at the consumer's expense.

1.3 Justification

Although there has been increased production of broiler chicken meat, production has not been able to meet the ever-increasing consumer demand which is triggered by the perceived benefits of chicken meat over other meat (Lyon *et al.*, 2010; Funaro *et al.*, 2014). The recent drought that hit South Africa (2015 – 2016) caused a 1.5% decline in broiler chicken production. This is currently having a devastating impact on food security and driving food prices higher (ARC, 2016). Spent laying hen meat is not regarded as a product of the egg industry. It is actually considered a by-product of laying flocks (Bell and Weaver, 2002; Kokoszynski *et. al.*, 2016).

In developed countries, spent laying hen meat quality attributes have been studied as a baseline for human consumption soups, canned meat, processed foods and traditional recipes (Lyon *et al.*, 2003; Chuaynukool *et al.*, 2007; Hill, 2009). The meat quality attributes of South African spent laying hens have not been intensively studied. This study intends to boost the marketing of spent laying hen meat by making the necessary information available; information that is required by consumers and the industry in order to make informed decisions on the utilization of spent laying hen carcasses and meat. For instance, commercial abattoirs usually offer higher prices (R54.52 per bird) than local traders (R30.01 per bird) for live broiler chickens (SAPA, 2016c). It is reasonable to assume that commercial abattoirs would be willing to offer better prices than local traders for live spent laying hens if a formal market is established.

1.4 Aim

The aim of this study was to investigate the effects of production systems and canola meal inclusion in layer diets on carcass and meat quality attributes of spent laying hens.

1.4.1 Objectives

Specific objectives were to:

1. Evaluate the effects of conventional battery cage and free range production systems on the carcass and meat quality attributes of spent laying hens.
2. Establish the effect of canola meal supplement on the carcass, meat and sensory quality characteristics of spent laying hens.

1.5 Hypothesis

1.5.1 Null hypothesis

H_0: There is no significant difference between the carcass and meat quality attributes of spent laying hens reared in conventional battery cages and free range production system.

H_0: There is no significant difference between in the carcass, meat and sensory quality attributes of spent laying hens fed on conventional and canola meal supplement diets.

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Chapter 2

Literature review

2.1 Introduction

In recent decades, there has been a significant increase in poultry meat production and consumption globally (Barbut, 2015; Food and Agriculture Organization (FAO), 2016). Global poultry meat production has registered a higher percentage of growth, at 625% (from 15.2 to 110.2 million tons), than red meat compared to red meat (pork 227% and beef 76%) from 1970 to 2014 (Kokoszynski *et al.*, 2016). This has made poultry meat the second highest produced and consumed meat after pork (Barbut, 2015; FAO, 2016). Poultry meat demand has superseded its production due to its consistently lower prices than red meat. The increased production of poultry meat is associated with a remarkable increase in the production of broiler chickens. Young broiler chicken meat accounts for an estimated 87% of total global poultry meat produced (Kokoszynski *et al.*, 2016). Rarely are adult, culled or spent chickens considered a source of poultry meat.

South African commercial abattoirs prefer to slaughter only broiler chickens. Spent laying hens are not considered a formal or commercial source of chicken meat due to a lack of market (SAPA, 2016). South Africa has recently been hit by a drought which has had a negative impact on the poultry industry, resulting in the broiler industry registering a negative growth of 1.5% in 2016 (ARC, 2016). The latter can explain the reduction in the percentage of production growth in South African poultry meat, from 3.3% (2015) to 0.9% in 2016. In addition, with the outbreak of Avian influence (H5N8) in June 2017 in South Africa (DAFF, 2017), it can be expected that the number of birds slaughtered will decrease further. The world's population is constantly increasing, posing a serious concern to the FAO, whose

mandate is food security. Food security entails obtaining and guaranteeing increased production of the best quality food for the population (FAO, 2016). Southern African countries are committed to increasing their animal protein sources by increasing poultry production (Tougan *et al.*, 2013). However, broiler chicken meat has not been able to meet the ever-increasing consumer demand for chicken meat. For instance, from 80,016 (2004) to 93,474 (2008) thousand tons (FAO, 2010).

At the end of their production cycle, breeding broiler and layer chickens are referred to as spent hens. This definition also encompasses culled chickens from breeding or laying flocks. It is estimated that 25.5 million laying hens are culled from the egg industry of South Africa each year (ARC, 2016). In developed countries, spent laying hens are fully utilized for human consumption since scientific information on carcass and meat quality traits of spent laying hens is provided to consumers (Chuaynukool *et al.*, 2007). This is not the case in South Africa, where spent laying hens are marketed only as live birds on the informal market (SAPA, 2016). Although some literature exists on the carcass and meat quality traits of spent laying hens, this literature does not differentiate between carcass and meat quality attributes of spent laying hens reared outdoors (free range) or indoors (caged), nor does it specify the effects of diet on the nutritional composition of laying hens. Chicken meat is affected by a series of complex factors (Jaturasitha *et al.*, 2008). These factors can be divided into two; intrinsic (genetic, sex, muscle type and slaughter age) (Mourot, 2008) and extrinsic (feeds and feeding systems, breeding and slaughtering conditions, *post-mortem* biochemical changes and technological treatment) (Tougan *et al.*, 2013). Therefore, this review aims at highlighting the differences and similarities between carcass and meat quality traits of spent laying hens under different production systems and dietary composition (canola meal).

2.2 Production systems of laying hens

2.2.1 Battery cage system

The two distinct systems of rearing laying hens are the conventional cage and the free range systems (Dal Bosco *et al.*, 2012; Inci *et al.*, 2016). Although modern aviary designs for laying hens have been developed to reduce production costs while intensifying housing and easing management of commercial farms for laying hens, these confinements are not the best with respect to the ethological requirements of chickens (Mugnai *et al.*, 2011). Conventional cages are considered to have a negative effect on the welfare of laying hens and this system is under intense scrutiny (EC, 1999; Mugnai *et al.*, 2011). Cages have been shown to maximize egg production and minimize production costs (Inci *et al.*, 2016). This fulfils the first priority of food security, which is food availability (FAO, 2016); hence it is difficult to completely eradicate cages from poultry production systems. However, the continuous use of additives and/or animal by-products in cage production systems is posing health risks such as the presence of antimicrobial drug-resistant microbes in animals and consumers (Inci *et al.*, 2016).

Recently there has been an agitated demand by consumers for more animal-friendly farming systems (Funaro *et al.*, 2014). In some countries, the cage-based poultry production system has been banned and minimum welfare standards have been legislated for a few farmers wishing to retain this system (Keeling and Svedberg, 1999). The European Commission (EC) in the hen directive 1999/74/EC of 19 July 1999 (EC, 1999) also banned the use of cages for laying hens with effect from 1 January 2012. However, the same directive permitted the use of enriched cages with minimum standards, starting on 1 January 2002. Chapter 3, article 6 of the hen directive 99/74/EC provides the minimum space requirements for enriched cages: 750 cm^2 per hen; 15 cm per perches; 12 cm per feeding trough; 2 nipple drinkers or 2 drinking

cups per cage; 90 cm aisle width and 35 cm above the floor (EC, 1999). The cages should be fitted with suitable claw-shortening devices, a nest and litter. The spaces could also be increased to provide more comfort for the hens. Enriched cages have improved egg production although feed consumption is also increased (Englmaierová *et al.*, 2014) and conventionally caged hens still produce better quality eggs than hens in enriched cages, attributed to the more controlled farming conditions of caged hens (Tumova and Ebeid, 2003).

2.2.2 Free range system

The natural chicken diet entails grazing on pastures (Skřivan *et al.*, 2015). Pasture is an important component in the chicken diet as it provides trace nutrients such as carotenoids (Englmaierová *et al.*, 2013) and phytoestrogens. The latter may potentially offer health benefits to humans relating to breast and prostate cancer as well as cardiovascular diseases (Kalac, 2013; Skřivan *et al.*, 2015). Moreover, grass imparts a distinct fatty acid (FA) profile, carotenoids and vitamin E into chicken meat products (Holt *et al.*, 2011). The welfare of a chicken is drastically improved by foraging, feed selection and activity as a result of access to an outside area (Sales, 2014). Access to pasture also reduces supplementary feed intake by 5 to 15%, and therefore the total feeding costs (Skřivan and Englmaierová, 2014; Skřivan *et al.*, 2015). These factors have been the core reasons for advocating non-cage rearing of laying hens in the past few decades (Mugnai *et al.*, 2011).

The council directive 98/58/EC (EC, 1998) concerning the protection of animals kept for farming purposes was the first to provide guidelines on freedom of movement of farm animals, including chickens. The hen directive 1999/74/EC of 19 July 1999 (EC, 1999) stipulated clear measurements of the space to be allowed around each hen in alternative housing systems for laying hens: 10 cm per hen for linear feeders or 4 cm per hen for circular

feeders; 2.5 cm per hen on continuous or 1 cm per hen on circular drinking troughs; 1 nest per 7 hens or 1 m^2 per 120 hens for group nests; 15 cm per hen on perches; 250 cm^2 per hen on litter and 9 hens per m^2 usable area. Laying hens must be protected from predators and unfavorable weather conditions. These are the basic guidelines for the modern day chicken aviary for laying hens classified as free range. Alternative pasture management techniques have been developed to offer the best nutrients for laying hens. This has included the use of mobile houses (Skřivan *et al.*, 2015).

2.2.3 Code of practice for laying hens in South Africa

The battery cage and free range systems are legally allowed in South Africa according to the South African Poultry Association (SAPA). In 2012, SAPA followed the European Union and laid down guidelines for producers intending to raise pullets or laying hens in the country (SAPA, 2012). However, den Hartigh (2016) highlighted that the implementation of these guidelines by farmers is less than ideal due to the high costs attached to the incorporation of such welfare standards into practice. Moreover, the SAPA code of practice for laying hens does not stipulate penalties for not adhering to the guidelines. One of SAPA's concerns is that farmers do not provide the required minimum space requirements for battery caged and free range laying hens, requirements which are more/less similar to the European standards (section 2.2.2). The latter are to ensure that hens get to express their natural behaviour and have access to feed. To qualify as a free range production system, 50% of the accessible outdoor area must be covered with green grass (SAPA, 2012).

2.3 Canola meal in laying hen feed

Canola (*Brassica napus*) is a registered name at the Western Canadian Oilseed Crushers Association (De Kock and Agenbag, 2009). Canola is a winter crop derived from rapeseed

varieties as a result of genetic selection and breeding (Moraes *et al.*, 2015). The term 'canola' can only be used for rapeseed species having less than 2% erucic acid in the oil and less than 30 µmol/g of aliphatic glucosinolates (Khajali and Slominski, 2012). Currently, canola is the second-most important oilseed crop in the world after soybean (Moraes *et al.*, 2015; USDA, 2017). The processing of whole canola seeds yields approximately 44% of one of the world's healthiest oils and by-products (canola meal), which is an excellent protein source for livestock (Canola Council of Canada, 2015; Adewole *et al.*, 2016).

Layer diet mainly comprises of energy and protein sources. The energy and protein ingredients contribute 70% and 20% respectively of the formulated rations (NRC, 1994; Bu *et al.*, 2015; Ding *et al.*, 2016). The energy ingredients usually consist of corn, wheat bran, oat bran, barley and oils, while protein is obtained from soybean meal (SBM), fishmeal (Moghaddam *et al.*, 2012; Hassan *et al.*, 2013), and rarely insect-meal (Charlton *et al.*, 2015; Al-Qazzaz *et al.*, 2016; Gunya *et al.*, 2016). The energy levels influence egg production and the protein content affects both egg production and quality (Gunawardana *et al.*, 2008; Bu *et al.*, 2015; Ding *et al.*, 2016). Other minor ingredients include essential amino acids (mainly lysine, threonine, tryptophan and methionine), limestone and a vitamin and mineral premix (NRC, 1994; Moghaddam *et al.*, 2012; Hassan *et al.*, 2013).

The poultry feed industry has relied on soybean as a reference plant-origin protein source for a while (Messerschmidt *et al.*, 2014; Wickramasuriya *et al.*, 2015; An *et al.*, 2016). Escalating soybean prices resulting from human competition has triggered poultry farmers to search for cost effective alternative protein sources (Khajali and Slominski, 2012; An *et al.*, 2016). The effects of using these alternative feedstuffs should be considered (Wickramasuriya *et al.*, 2015; Radfar *et al.*, 2017). Feedstuffs such as canola meal might not only alter the production performance of animals but also the quality of the final product

intend for human consumption. Recently, there has been an increased use of canola meal in laying hen diets. While keeping other factors constant, the low cost of canola meal has been the leading factor for its increased use as a laying hen feedstuff (An *et al.*, 2016). The incorporation of canola meal into layer diets has been scientifically studied for years (Summer *et al.*, 1989; Angelovicova and Angelovic, 2013). However, little is known about the impact of feeding canola meal to laying hens on their carcass and meat quality.

2.3.1 Chemical composition of canola meal

The chemical composition of canola meal (CM) is determined by a number of factors; these include the cultivar, soil profile, climate and processing conditions. Although the nutritional composition of CM is generally low, especially in crude protein when compared to SBM as seen in Table 2.1, the amino acid content of CM is fairly comparable to SBM (Table 2.2). Canola meal has a well-balanced amino acids profile, less lysine and more methionine and cysteine (the sulfur-containing amino acid) than SBM. The aforementioned point has been the basis of the inclusion of the two meals in poultry diets; in combination, they yield a complementary amino acids effect (Khajali and Slominski, 2012; Grageola *et al.*, 2013; Mejicanos *et al.*, 2016).

During the processing of canola seeds for oil, phospholipids, glycolipids, triglycerides and free FA contained in the gum are added to the meal; hence the high fat content (Khajali and Slominski, 2012; Grageola *et al.*, 2013; Canola Council of Canada, 2015). The high fat content of CM minimizes the energy difference between the two diets (Khajali and Slominski, 2012). The high fat content of CM is coupled with a good profile of FA (Table 2.3). Canola meal supplies the essential FA requirements of chicken so that there is no need for supplementary fat in the diet (Ayton, 2014).

Table 2.1: Chemical composition (%) of canola meal (CM) and soybean meal (SBM) (%)

	Dry Matter		Crude Protein		Crude Fat		Crude Fiber		NDF		ADF		Total Ash		Reference
	Highest	Lowest	Highest	Lowest	Highest	Lowest	Highest	Lowest	Highest	Lowest	Highest	Lowest	Highest	Lowest	
CM	90.10	90.00	41.70	41.50	2.90	2.80	-	-	27.00	17.60	21.70	11.80	8.30	7.90	Radfar *et al.*, 2017
CM	90.38	87.10	31.39	25.81	20.20	10.54	8.31	5.84	18.79	15.32	14.98	11.45	5.74	4.49	Grageola *et al.*, 2013
CM	88.00	82.90	37.30	37.00	11.10	3.40	15.00	6.90	-	-	18.20	17.20	7.30	6.30	Wickramasuriya *et al.*, 2015
CM	90.00	-	36.50	-	3.60	-	11.60	-	26.00	-	18.20	-	6.40	-	Khajali and Slominski, 2012
CM	93.20	-	39.90	-	2.16	-	10.80	-	-	-	-	-	6.80	-	An *et al.*, 2016
CM	90.00	-	42.90	40.20	4.30	2.70	-	-	33.90	26.90	-	-	7.90	7.10	Adewole *et al.*, 2016
CM	88.00	-	36.70	-	3.30	-	11.20	-	25.40	-	16.20	-	6.70	-	Canola Council of Canada, 2015
CM	90.00	-	45.90	34.80	-	-	-	-	45.70	24.50	32.00	19.30	10.70	6.70	Ayton, 2014
CM	90.05	-	35.58	-	1.81	-	9.13	-	30.50	-	-	-	6.44	-	Mikulski *et al.*, 2012
CM	90.00	-	42.30	36.90	3.80	3.40	11.60	-	23.60	15.90	17.00	9.70	7.90	6.60	Mejicanos *et al.*, 2016
CM	92.30	85.30	41.90	34.80	5.40	0.60	17.70	10.40	39.10	22.60	24.20	16.40	9.10	6.60	Heuzé *et al.*, 2016
SBM	90.00	-	45.60	-	1.30	-	5.40	-	12.00	-	7.50	-	-	-	Mejicanos *et al.*, 2016
SBM	92.10	85.00	56.10	45.20	4.40	0.60	10.10	3.50	18.10	10.70	6.00	1.50	9.40	6.10	Heuzé *et al.*, 2017
SBM	88.70	88.50	54.86	47.47	1.48	0.76	-	-	6.68	4.96	3.91	3.09	-	-	Baker *et al.*, 2011
SBM	90.00	-	45.60	-	1.30	-	5.40	-	12.00	-	7.50	-	-	-	Khajali and Slominski, 2012

NDF – neutral detergent fiber; ADF – acid detergent fiber.

Table 2.2: Amino acids profile of canola meal (CM) and soybean meal (SBM) (%)

	Essential amino acids																				
	Arg		**His**		**Ile**		**Leu**		**Lys**		**Met**		**Phe**		**Thr**		**Trp**		**Val**		**Reference**
	High	Low	High	Low	High	Low	High	Low	High	Low	High	Low	High	Low	High	Low	High	Low	High	Low	
CM	2.62	2.38	1.09	-	1.38	1.33	2.95	2.92	2.27	2.13	0.85	0.80	1.61	1.56	-	-	-	-	1.82	1.68	Radfar *et al.*, 2017
CM	2.04	-	-	-	-	-	-	-	2.00	-	0.74	-	-	-	1.57	-	0.48	-	-	-	Mejicanos *et al.*, 2016
CM	1.89	1.59	0.80	0.67	1.26	1.05	2.83	1.23	1.79	1.52	0.58	0.47	1.30	1.03	1.33	1.11	0.39	0.34	1.63	1.33	Grageola *et al.*, 2013
CM	2.15	2.10	0.96	0.95	1.50	1.39	2.43	2.52	1.98	1.96	0.71	0.70	1.43	1.39	1.51	1.50	0.49	0.44	1.88	1.79	Wickramasuriya *et al.*, 2015
CM	2.38	-	1.22	-	1.25	-	2.22	-	2.13	-	0.70	-	1.46	-	1.54	-	0.48	-	1.78	-	Canola Council of Canada, 2015
CM	2.39	2.21	1.23	1.13	1.29	1.19	2.67	2.53	2.11	2.00	0.72	0.64	1.50	1.41	1.62	1.49	-	-	1.80	1.60	Adewole *et al.*, 2016
CM	1.83	-	1.04	-	1.53	-	2.70	-	1.98	-	-	-	1.45	-	1.83	-	0.29	-	1.39	-	An *et al.*, 2016
CM	2.04	-	-	-	-	-	-	-	2.00	-	0.74	-	-	-	1.57	-	0.48	-	-	-	Khajali and Slominski, 2012
SBM	4.27	3.56	1.44	1.25	2.54	2.25	4.35	3.76	3.56	3.14	0.78	0.68	2.89	2.48	2.13	1.83	0.78	0.69	2.64	2.36	Baker *et al.*, 2011
SBM	3.23	-	-	-	-	-	-	-	2.86	-	0.65	-	-	-	1.74	-	0.64	-	-	-	Mejicanos *et al.*, 2016
SBM	3.56	3.48	1.25	1.21	2.24	2.17	3.76	3.60	3.04	2.89	0.66	0.63	2.43	2.37	1.84	1.82	0.68	0.63	2.36	2.30	Wickramasuriya *et al.*, 2015
SBM	3.23	-	-	-	-	-	-	-	2.86	-	0.65	-	-	-	1.74	-	0.64	-	-	-	Khajali and Slominski, 2012

High – Highest; Low – Lowest; Arg – Arginine; His – Histidine; Ile – Isoleucine; Leu – Leucine; Lys – Lysine; Met – Methionine; Phe – Phenylalanine; Thr – Threonine; Trp – Tryptophan; Val – Valine.

Table 2.3: Fatty acid profile of canola meal (CM) and soybean meal (SBM)

Common name	Abbreviation	CM	CM	CM	SBM	SBM
Myristic acid	C14:0	0.08	-	0.00	0.10	0.28
Palmitic acid	C16:0	5.17	-	4.00	10.30	10.62
Palmitoleic acid	C16:1	0.66	-	0.20	0.20	0.28
Stearic acid	C18:0	2.05	-	1.80	3.80	3.57
Oleic acid	C18:1n9c	58.81	-	56.10	22.80	21.81
Linoleic acid	C18:2n-6	21.61	20.10	20.30	51.00	49.79
α-Linolenic acid	C18:3n-3	9.66	9.60	9.30	6.80	6.67
Arachidic	C20:0	0.46	-	-	-	-
Eicosenoic	C20:1	0.82	-	1.70	0.20	-
Behenic	C22:0	0.24	-		-	-
Erucic	C22:1	0.12	0.20	0.60	-	-
Lignoceric	C24:0	0.19	-	-	-	-
Nervonic	C24:1	0.14	-	-	-	-
Total SFA		-	6.00	7.10	14.20	14.46
Total MUFA		-	61.90	-	-	22.09
Total PUFA		-	29.70	-	-	56.46
		Spragg and Mailer, 2007	Canola Council of Canada, 2015	NRC, 2012	NRC, 2012	Stein *et al.*, 2013

SFA – saturated fatty acids; MUFA – monounsaturated fatty acids; PUFA – polyunsaturated fatty acids.

2.3.2 Anti-nutritional factors in canola meal

A variety of cruciferous plants have secondary plant metabolites that are nontoxic to them. However, the breakdown of these products can adversely affect animal performance. Canola is one of these cruciferous plants. Canola has been bred for a long period to reduce the content of unfavorable components such as high dietary fiber, glucosinates, sinapine, phytic acid and phenolic compounds such as tannins. However, some of these anti-nutritional factors have been considered beneficial to humans and therefore contributing factors to functional foods (Nosenko *et al.*, 2014; Wanasundara *et al.*, 2016). The latter is limiting the reduction of these anti-nutritional factors through canola breeding to favorable levels that its meal can completely substitute SBM in poultry diets. Canola meal contains dietary fiber that is three times higher than SBM. This high fiber content of canola meal decreases the metabolizable energy, amino acids and protein digestibility of the diet (Moraes *et al.*, 2015). The main glucosinates identified in canola meal include gluconapin, glucobrassicanapin, progoitrin, gluconapoleiferin, glucobrassicin and 4-hydroxyglucobrassicin (Khajali and Slominski, 2012). Although glucosinates do not affect ruminants, the products of their breakdown (isothiocyanates, goitrin, nitriles and thiocyanates) have been reported to adversely affect the thyroid gland and kidney function and growth performance of non-ruminants (Mejicanos *et al.*, 2016). Through breeding and processing, the concentration of these anti-nutritional factors in canola meal has been reduced over the years. A recent study by Adewola *et al.* (2016) has reported glucosinates as low as 1.59 µmol/g DM in canola meal.

2.4 Factors that affect carcass characteristics and meat quality of laying hens

Carcass characteristics of chicken are defined by carcass yield and quality. Carcass yield includes the dressing percentage, edible portions, non-carcass portions and inedible parts. Carcass quality is defined by the amount of lean tissue on the economically valuable portions (breast and leg quarter) (Murawska, 2017). Meat quality of chicken is a complex set of physiochemical and sensory attributes, and carcass characteristics and meat quality are determined by both intrinsic and extrinsic factors (Figure 2.1). Although these intrinsic and extrinsic factors have been widely studied in broiler chicken meat, little is known about the influence of production and dietary systems on the carcass characteristics and meat quality of spent laying hens.

2.4.1 Production system

The system of production defines animal feeding, behavior and production performance. Laying hens are commonly reared in battery cages (caged) or free range (free range) systems. The two systems have three major differences; diet, hen movement and environmental conditions. Although the formulated dietary composition of caged and free range hens is normally the same in terms of feedstuff and inclusion levels, free range hens gain access to supplemental green pasture. Pasture supplies vitamins, minerals and trace elements to hens that might impact on the quality of the eggs and meat of the chicken (Holt *et al.*, 2011).

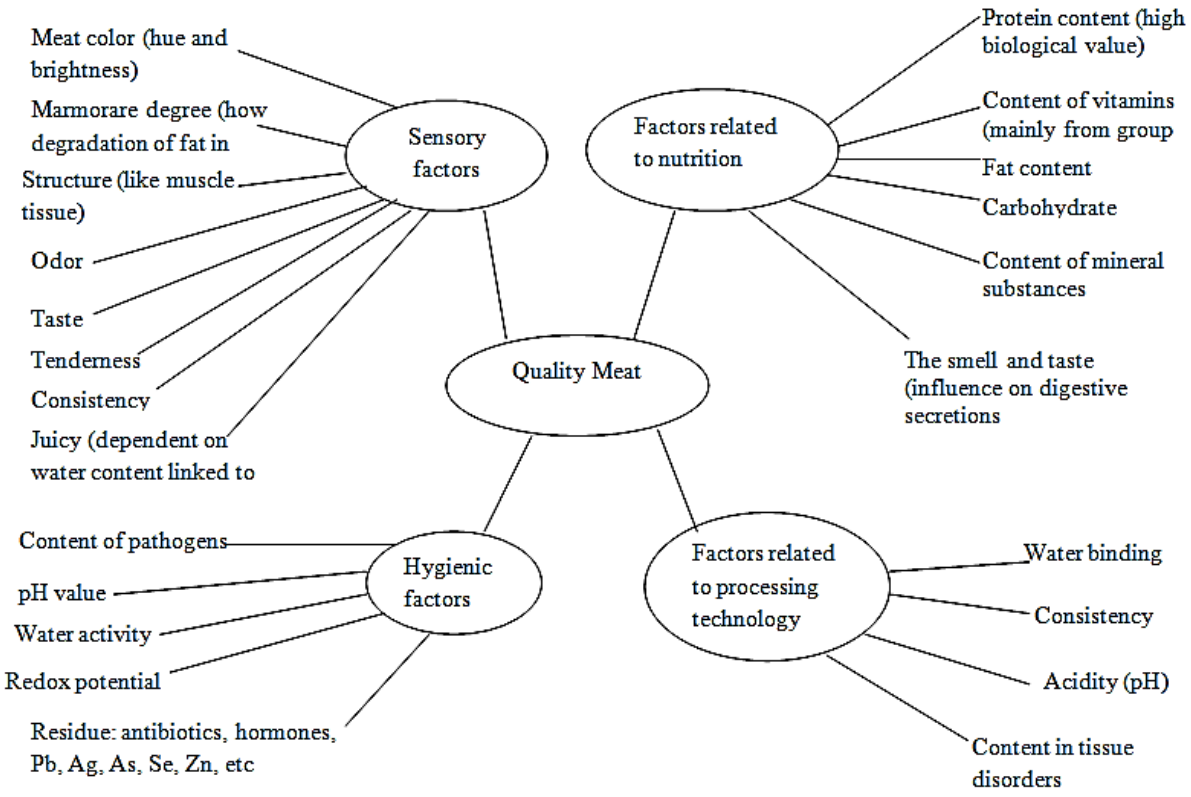

Figure 2.1: Factors defining the concept of quality of meat

(Adapted from Cristina, 2009)

2.4.1.1 Carcass characteristics

2.4.1.1.1 Carcass yield

To date, there have been contradictory findings on the effects of access to pasture on the production performance of poultry. An increase in the carcass yield of quails was reported when fed *ad libitum* with pasture access, although breast percentage was reduced (Inci *et al.*, 2016). However, most of the literature suggests that there is little or no significant differences between carcass yields of broiler chickens reared under free range and caged systems (Fanatico *et al.*, 2007; Wang *et al.*, 2009; Smith, 2012; Funaro *et al.*, 2014; Skřivan *et al.*, 2015). Where differences exist, a free range system decreases the carcass yield of broiler chickens. The cause of differences in the rearing systems has been attributed to the variations in slaughter age and genetics (fast- versus slow-growth rate) (Fanatico *et al.*, 2005a). Major differences between free range and caged systems have been highlighted with regards to carcass composition specifically in the breast and thigh percentages of broiler chickens. Free range has been reported to decrease breast percentage and increase thigh percentage of broiler chickens (Fanatico *et al.*, 2005a; Funaro *et al.*, 2014). The high thigh percentage has been explained by the increase in the locomotion of free range birds. The level of muscle exercise translates into muscle fiber diameter and collagen levels and hence into increased size and weight (Coggins, 2012).

2.4.1.1.2 Skin color

Whether sold as a whole carcass or portions, the color of the skin is the primary determinant of consumers' willingness to buy chicken meat (Barbut, 2015; Funaro *et al.*, 2015). Access to pasture has been reported to impart a yellow skin color due to the absorption and deposition of xanthophylls/carotenoids available in the grass (Fletcher, 1999; Funaro *et al.*, 2015). Yellow skin

color is an indicator of healthy birds in some parts of the world and is a desired trait in free range products with a distinct traditional appearance (Lyon *et al.*, 2010; Funaro *et al.*, 2015). As highlighted above, consumer selection of raw chicken is critically influenced by skin color and therefore establishing whether there are differences in skin color between free range and caged spent laying hens is paramount.

2.4.1.2 Meat quality

Consumers usually claim free range chicken meat is more nutritious than caged. Slow-growing broiler chickens (8 to10 weeks at slaughter age) have been reported to have more nutritive value than fast-growing broilers (6 to 7 weeks at slaughter age) when reared with access to pasture. Laying hens are reared on pasture for longer periods (40 to 60 weeks). However, little is known about the impact of this long period of access to pasture on the meat quality of laying hens.

2.4.1.2.1 Meat color

Myoglobin is the main determinant of meat color and is dependent on species, muscle and age of birds. The amount of myoglobin and the oxidation state of iron attached to it influences meat color (AMSA, 2012). Free range production increases muscle activity, which in turn increases oxygen demand, thus raising the amount of myoglobin in the muscle. However, the use of redness to measure meat color in chicken breast meat is limited by the fact that its myoglobin content is not readily detectable (Zhuang and Savage, 2012; Barbut, 2015). Hence the main variable for the color of free and caged chicken breast meat is determined by lightness (L* value) and yellowness (b* value) (Funaro *et al.*, 2015). Although there are contradictory reports about the impact of free range rearing on chicken meat lightness, several studies agree that yellowness

of breast meat is increased when birds are given access to pasture (Castellini *et al.*, 2002; Fanatico *et al.*, 2005b; 2007; Smith, 2012; Michalczuk *et al.*, 2017).

2.4.1.2.2 Meat pH

Muscle pH is a major determinant of the conversion process of muscle to meat. Among live animals, muscle pH is approximately 7.0. After slaughter, the anaerobic glycogenolytic pathway is initiated, converting muscle glycogen into lactic acid (Aberle *et al.*, 2001; Honikel, 2014). The final meat pH value also referred to as ultimate pH (pH_u) is reached after 24 h *post-mortem*. The ideal pH is 5.3 – 5.8. The rate of pH decline to pH_u is dependent on muscle glycogen reserves, which are controlled by species, age, diet, muscle type, muscle activity, production system and pre-slaughter stress factors. Pre-slaughter activities such as handling, transportation and immobilization predispose birds to stress and deplete glycogen reserves (Honikel, 2014). Reactions to these stress factors differs for free range and caged chickens. Free range chickens are often light and more active; light birds struggle more along the slaughter lines and during handling and transportation, thereby quickly depleting the glycogen reserves (Debut *et al.*, 2005; Michalczuk *et al.*, 2017). This results in higher pH_u values than normal. Moreover, muscle activity and environmental temperature have been reported to regulate glycogen reserves among poultry. Free range birds are exposed to cooler temperatures. Thus there is increased thermogenesis to regulate body temperature, thereby depleting adenosine triphosphate in muscles (Funaro *et al.*, 2014). Several studies consistently agree that access to pasture increases pH_u of chicken breast meat with slow-growing birds being more susceptible to higher pH_u (Alvarado *et al.*, 2005; Ponte *et al.*, 2008; Połtowicz and Doktor, 2011; Michalczuk *et al.*, 2014; 2017). However, contradictory results of lower pH_u among free range birds were reported by Castellini *et al.* (2002), Wang *et al.* (2009) and Fanatico *et al.* (2007).

2.4.1.2.3 Meat tenderness

Meat tenderness is simply defined as the force required to bite through a piece of meat. Tenderness is influenced by a series of complex factors, both intrinsic and extrinsic. Intrinsic determinants are connective tissue, collagen, pH_u, sarcomere length, proteolytic activity and intramuscular fat (An *et al.*, 2010; Purchas, 2014). Other factors such as age, genetics, diet and production system act upon these intrinsic determinants to influence meat tenderness. Free range production increases the muscle activity of chickens through motory activity and increases muscle fiber diameter, connective tissue and collagen content (An *et al.*, 2010; Fu *et al.*, 2014; Funaro *et al.*, 2014). However, muscle glycogen decreases with high muscle activity and the low glycogen leads to higher pH_u values which deter the proteolytic activity (Honikel, 2014). The low protein breakdown by proteolytic enzymes leads to tougher chicken meat derived from a free range production system. It has been postulated that an increase in the concentration of intramuscular fat makes the meat more tender; however the relationship is not linear (Purchas, 2014). Fu *et al.* (2014) observed higher intramuscular fat content in free range and caged Beijing-you chickens. However, the high intramuscular fat might not counteract the increase in muscle fiber diameter, connective tissue and collagen content; hence, free range chicken meat remains tougher. Similar results of tougher free range chicken meat have been reported by Funaro *et al.* (2014) and Michalczuk *et al.* (2014; 2017). Studies finding no differences between meat tenderness of free range and conventional cage broiler chickens have also been reported (Fanatico *et al.*, 2005a; 2005b)

2.4.1.2.4 Meat chemical composition

The chemical composition of meat is generally defined by the moisture, protein, fat, carbohydrates and ash content. However, the primary constituents are moisture, protein and fat. The concentration of moisture and protein is inversely proportional to fat in meat (Keeton *et al.*, 2014). The variations in the primary constituents of meat are determined by species, maturity (age), nutritional plane, anatomical location, amount of skin and bone and to a lesser extent, the production system. Although there are contradictory results on the effect of caged and free range production systems on the primary constituents of chicken meat, several studies have consistently reported a significant reduction in the fat content of meat when chickens are reared in free range conditions than battery cages (Castellini *et al.*, 2002; Cheng *et al.*, 2008; Fu *et al.*, 2014; Funaro *et al.*, 2014). The increase in the motory activity of free range chickens seems to promote myogenesis more than lipogenesis (Castellini *et al.*, 2002). Fanatico *et al.* (2005b; 2007), Wang *et al.* (2009) and Michalczuk *et al.* (2014; 2017) reported no differences in the intramuscular fat content of chicken meat from animals reared under free range and conventional cage systems.

2.4.2 Dietary inclusion of canola meal

The effect of dietary composition on carcass and meat quality of broilers has been widely studied. However, little is known about the effects of dietary manipulation used to increase the nutritional profile of table eggs on carcass characteristics and meat quality of spent laying hens. Canola meal is widely used in layer diets due to increasing demand for functional foods with high n-3 polyunsaturated fatty acids (PUFA). This section intends to highlight the impact of dietary inclusion of canola meal on the carcass characteristics and meat quality of chicken.

2.4.2.1 Carcass characteristics

The effects of incorporating canola meal into poultry diet on carcass characteristics have been well studied among broilers, quails, ducks and turkeys. Several reports agree that increasing the levels of canola meal in the poultry diet decreases the final body weight (Woyengo *et al.*, 2011; Khajali and Slominski, 2012; Moraes *et al.*, 2016). Canola meal's anti-nutritional factors negatively impair growth performance by inhibiting digestion and nutrient absorption. This occurs through phytic acids and dietary fiber increasing digesta viscosity and gastrointestinal retention time. Moreover, Tang *et al.* (2007) demonstrated that metabolizable energy also impacts on live body weight, and the metabolizable energy is low in canola meal. However, in studies where a balanced nutrient density (especially lysine) was used, there were no significant differences observed between broiler chickens and turkeys fed canola meal and SBM (Baloch *et al.*, 2003; Mikulski *et al.*, 2012; An *et al.*, 2016).

Carcass yield is not affected by the inclusion of canola meal in chicken diets except that the breast weight is significantly reduced (An *et al.*, 2016; Moraes *et al.*, 2016). Muscle development is controlled by protein synthesis; lysine directly influences chicken muscle growth (Tang *et al.*, 2007). The amount of lysine in canola meal is limited. Khajali and Slominski (2012), Grageola *et al.* (2013) and Mejicanos *et al.* (2016) recommended that a better balance of amino acids is available to the animal when canola meal is supplemented with SBM.

2.4.2.2 Meat quality

The effects of feeding chicken canola meal on meat color and pH have not been emphasized. However, Mack *et al.* (1999) and Lessire *et al.* (2013) demonstrated that the level of protein and the amino acids profile (specifically lysine) in the diet significantly impacts on broiler chicken

meat pH_u, and thus on meat color. The authors also highlighted the potential of low dietary amino acids and lysine to cause high pH_u and *vice versa*. Canola meal is deficient in lysine and the protein content is lower than that of SBM. Therefore the inclusion of canola meal in chicken diets could result in high pH_u; however, there is little data to conclude on this. An *et al.* (2016) reported no difference in the pH_u when broiler chickens were fed with graded levels of canola meal, up to 15% inclusion level. The authors also noted no differences in the meat color. Moraes *et al.* (2016) and Mikulski *et al.* (2012) reported similar color results in their studies, except that they found a significant increase in yellowness (b* value). Yellowness is associated with an increase in the fat content and PUFA of the meat. The increase in meat fat content can explain the significant decrease in shear force values of turkeys fed graded levels of canola meal (Mikulski *et al.*, 2012).

2.4.2.2.1 Meat chemical composition

Several studies consistently concur with regard to the effect of canola meal on the chemical composition of poultry meat, stating that it makes no difference to moisture, protein, and ash content but the fat and total PUFA content is increased (Mikulski *et al.*, 2012; Tuunainen *et al.*, 2016; Moraes *et al.*, 2016). Canola meal contains a high percentage fat and PUFA. Wood and Enser (1997), López-Ferrer *et al.* (2001), NRC (2012) and Tuunainen *et al.* (2016) noted that the dietary composition of fat and FA profile directly translates into chicken meat and eggs. For decades, the manipulation of chicken diets has applied this principle to incorporate more beneficial components (n-3 PUFA) into meat and eggs (Barbut, 2015). Fish meal, linseed oil, marine algae, fish oil and recently canola meal have all been used to enrich poultry meat and eggs with n-3 PUFA (López-Ferrer *et al.*, 2001; Wood *et al.*, 2003; Bhalerao *et al.*, 2014). However, precautions need to be taken when feeding chicken high n-3 PUFA-containing diets as

this may result in detrimental effects, such as low shelf-life, the formation of off flavors and very tender meat (Wood *et al.*, 2003; Moraes *et al.*, 2016). Long chain PUFA readily oxidizes which is an important consideration during flavor development, however, the intensity of oxidation needs to be regulated as it can cause rancidity and color deterioration (Woods and fearon, 2009; Mikulski *et al.*, 2012). More so, Wood *et al.* (2003) noted that due to differences in the melting points of FA, the FA impacts on intramuscular, intermuscular and subcutaneous fat, thus affecting the firmness and softness of meat. The high melting temperature FA appears whiter than liquid fat with low melting temperature. In this way, FA controls texture and color of meat.

2.4.2.3 Sensory attributes

Whether measured by people or instruments, sensory evaluation involves characterizing all meat attributes perceived by the human five senses; taste, sight, smell, touch and hearing (Figure 2.2). Human testers rely on training and/or experience to evaluate meat characteristics; instruments directly determine the physical and chemical characteristics that control the perceived human sensory stimulus (Lyon *et al.*, 2010). In poultry meat, a consumer's degree of liking is dependent on aroma, flavor and texture. Flavor is a combination of taste and aroma (as perceived in the mouth); flavor is defined by the amount and composition of the volatile compounds in the meat (Dawson and Spinelli, 2012). The flavor of raw chicken meat is generally low and characterized by a bloody, metallic, salty taste and no aroma (Ba *et al.*, 2012; Jayasena *et al.*, 2013a). Flavor is developed from volatile compounds produced by the Maillard reaction between lean and intramuscular fat, lipid oxidation and vitamin degradation during cooking. The amount and composition of volatile compounds and hence the flavor developed, relies on the fat and FA composition of the chicken meat (Rabe *et al.*, 2003; Dawson and Spinelli, 2012). Texture has

also been reported to fall under the influence of FA, as FA occupies the space between myofibrils initiating the tenderisation process (Wood *et al.,* 2003; Moraes *et al.,* 2016).

Meat flavor is influenced by species, breed, age, diet and *post-mortem* processes. However, a large dietary change is needed to manipulate chicken meat flavor (Land and Hobson-Frohock, 1977; Barbut, 2015). Enser (1999) noted that any dietary change of feed other than manipulation of FA profile (PUFA) is likely not to cause a change in meat flavor. Feeding animals with a supplement or graded levels of feedstuff such as fish meal and linseed oil with high PUFA content results in a change in meat flavor (Wood *et al.*, 2003; Ba *et al.*, 2012; Jayasena *et al.*, 2013b). Canola meal has a higher content of unsaturated FAs than SBM (Table 2.2). This could influence the meat FA profile and hence meat flavor. Moraes *et al.* (2016) substituted SBM with graded levels of canola meal in broiler chicken diet and found no differences in the PUFA content or meat flavor, although the overall degree of liking of the meat numerically increased with increased concentrations of canola meal in the diets. The latter could be associated with the significant reduction in meat hardness in the same study. Little is yet known about the sensory characteristics of the meat of spent laying hens when affected by canola meal in the diet.

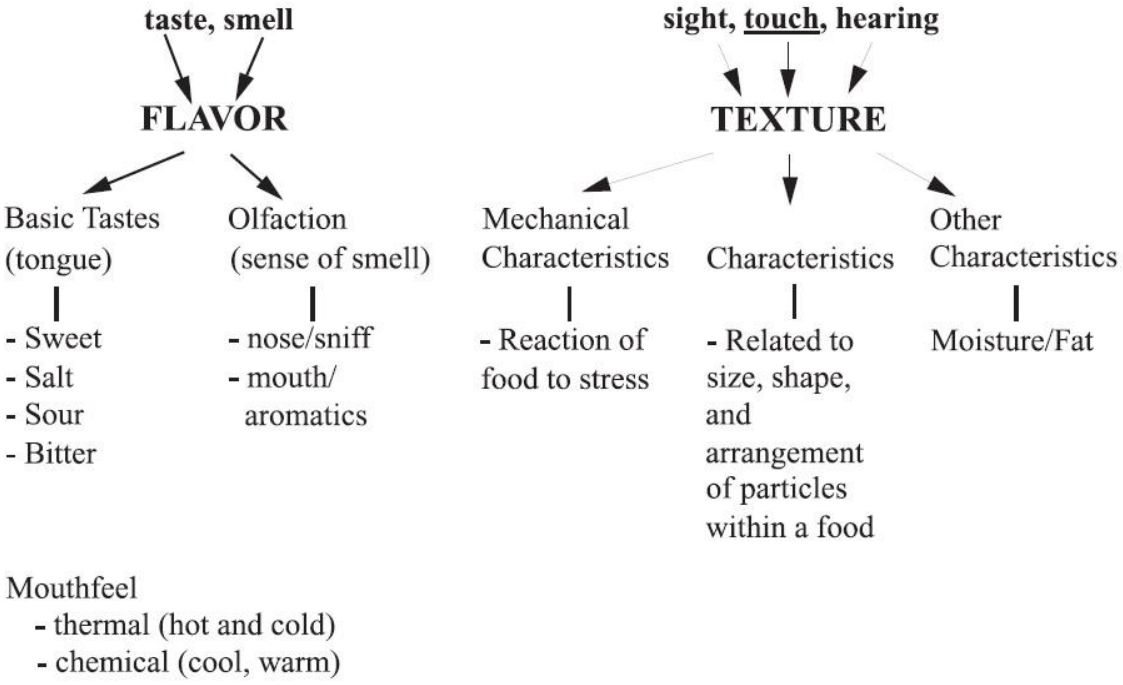

Figure 2.2: Components of basics senses

(Adapted from Lyon *et al.*, 2010)

2.5 Summary of the review

The carcass and meat quality of spent laying hens has been studied for the past decades. However, this review has highlighted that little or no emphasis has been placed on the effect of the production system and dietary composition (canola meal inclusion) on carcass and meat quality characteristics of spent laying hens. Spent laying hen carcass and meat quality are usually reported holistically. Production system can impact on carcass and meat quality of chicken especially when rearing is conducted for a longer period. Although free range production lowers egg production, it is also envisaged to lower feed intake, feed costs and improves behavior expression. Among broilers, the free range production system results in beneficial characteristics of the carcass and meat, such as yellow skin and meat color though this has not been investigated for spent laying hen meat. Consumers perceive the yellow color of skin and meat as natural and healthier. Nowadays, consumers are eagerly attentive to the quality of food they eat, especially when it comes to the amount of fat and the FA profile. Meat is receiving criticism for containing low levels of beneficial n-3 PUFA. However, this is not the case for chicken meat. Manipulation of the chicken dietary composition of n-3 PUFA has been confirmed as resulting in meat and eggs with the same n-3 PUFA profile. This has led to the inclusion of canola meal in poultry diets which contains high levels of n-3 PUFA. Due to genetic breeding, laying hens have been selected for assimilation of beneficial nutrients into eggs in the same way as broiler chickens are selected for breast yield. Although laying hens are able to incorporate dietary n-3 PUFA into eggs, little is known concerning how much of the dietary n-3 PUFA can be deposited into their meat.

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Chapter 3

Effects of production systems on the carcass and meat quality characteristics of spent laying hens

Abstract

This study evaluated the carcass characteristics and meat quality attributes of spent laying hens raised under conventional battery cage and free range systems. Thirty free range and 30 conventional battery-caged Lohmann Brown-Elite spent laying hens of 53 and 40 weeks of age, respectively were selected in a completely randomized design from a commercial egg farm. Carcass, portion and organ weights and percentages were determined. Physicochemical analyzes were performed on thigh and breast meat samples. Caged hens had heavier ($P \leq 0.05$) warm and cold carcasses, thigh, wing and feet. The percentages of the breast (26.1 ± 0.51 vs. 28.3 ± 0.28), drum, breast bones, breast thaw and cooking loss and thigh cooking loss were higher ($P \leq 0.05$) for free range than for caged hens. Free range hens had heavy ($P \leq 0.05$) gizzards (33.9 ± 1.04 vs. 30.5 ± 0.73) and bones and a lower ($P \leq 0.05$) breast meat percentage (47.3 ± 0.94 vs. 51.7 ± 1.35). Meat redness (a*) (0.54 ± 0.222 vs. 1.40 ± 0.135) and hue angle, skin redness (a*), breast (12.37 ± 0.411 vs. 17.10 ± 0.751) and thigh (29.68 ± 0.306 vs. 39.75 ± 0.826) Warner-Bratzler shear force values (N), breast moisture and thigh ash content were higher ($P \leq 0.05$) for free range hens. Caged hens had a higher ($P \leq 0.05$) thigh thaw loss percentage and breast ash content. Production system significantly influenced the carcass characteristics and meat quality of spent laying hens. The results of this study supply baseline information for the utilization of spent laying hens by the poultry industry and consumers.

Key words: rearing system, off layer, carcass yield, meat quality

3.1 Introduction

The nutrition, freedom from diseases, behavioral expression and ability to adapt to environmental stress of laying hens is defined by the production system, which impacts on hens' welfare and egg productivity. Modern laying hen strains are offspring of wild fowl. Although genetic breeding is aimed at eradicating some of the traits of wild fowl from laying hens, the modern laying hen strains still preserve a number of ancestral behaviors (e.g. building nests, foraging and night perching) (Kjaer and Mench, 2003; Bingham, 2013). The conventional battery cage system (caged) has been criticized (Mugnai *et al.*, 2011) and banned in some countries (most of Europe) for restraining laying hens from exposing their natural behavioral patterns (EC, 1999; Keeling and Svedberg, 1999).

Under free range systems, laying hens are provided with sufficient space (250 cm^2 per hen indoors, 2 m^2 per hen outdoors), perches, laying nests and access to natural pasture (EC, 1999; SAPA, 2012). This stocking density allows laying hens under free range systems to express their natural behavioral patterns, foraging abilities and also results in the manifestations of fewer diseases (Moe *et al.*, 2010; Janczak and Riber, 2015). Emphasising good welfare is scientifically proven to improve a chicken's immune system and the quality of the products produced (Miao *et al.*, 2005; Janczak and Riber, 2015).

To date, the majority of consumers are well informed about the issue of animal welfare and how it relates to final products. Rodić *et al.* (2010) and Napolitano *et al.* (2010) highlighted that consumers are willing to pay 20% more for eggs produced under free range than caged systems. Also, foraging on pasture imparts specific quality characteristics such as n-3 fatty acids into the meat and eggs; hence many consumers perceive free range eggs and meat as a functional food (Sossidou *et al.*, 2015; Perić *et al.*, 2016).

For broiler chickens, several studies have reported significant differences in body weight, meat and skin color, abdominal and meat fat content, vitamins, tenderness and fatty acid profiles between free range and cage reared birds (Wang *et al.*, 2009; Funaro *et al.*, 2014). Sales, (2014) provides a thorough meta-analyzes of the effect of the two production system on the meat quality of broilers. Free range systems impart desired meat traits such as yellowness of the skin and meat, chewiness, vitamins, low fat content, a high content of polyunsaturated fatty acids (PUFA) and a low n-6:n-3 PUFA ratio (Castellini *et al.*, 2002; Michalczuk *et al.*, 2014; 2017).

The influence of a free range production system on meat quality has been extensively researched for broiler chickens, turkeys and ducks. However, for laying hens, studies on the influence of production system are limited to table eggs. There is little or no literature on carcass and meat quality characteristics of free range and cage reared spent laying hen. Therefore, the aim of this study was to evaluate the carcass characteristics and meat quality attributes of spent laying hens (Lohmann Brown-Elite) raised under conventional battery cage and free range systems.

3.2 Materials and methods

3.2.1 Experimental design

A total of 60 Lohmann Brown-Elite spent laying hens were obtained from a commercial egg producer (ethical clearance number MUC441SSEM01). From a large commercial flock reared in battery cages (caged), a group of 30 hens was selected; one hen was randomly selected per cage (each cage contained 10 hens). Thirty free range raised hens were also randomly selected. All hens were fed the same commercial layer diet. Caged hens were 40 weeks of age while free range hens were 53 weeks of age. Age after sexual maturity (18 weeks of age) has little effect on meat quality (Barbut, 2015), hence age was not considered for this study.

3.2.2 Slaughtering

All hens were transported in certified chicken crates from the farm to a certified commercial poultry abattoir. The hens were held for 12 h in a free range facility and provided with feed and water *ad libitum* before being transported to the abattoir for slaughter (Anon., 2000): Electrical stunning with 50 – 70 V for 3 – 5 s; immediately followed by exsanguination through severing of the carotid arteries and jugular veins. After bleeding for 5 min, the carcasses were submerged in a water bath at 60°C for 2 min, mechanically defeathered in a rotating drum for 30 s and washed.

3.2.3 Carcass yield, portioning and deboning

The weights of the warm and cold carcasses (with neck skin), eight intact portions, gizzard, liver, heart, feet and head were recorded using a sensitive weighing scale (Carcass: DIGI, Model: DS-673; Japan. Organs: RADWAG, Model: PS 750/C/2, Poland) 30 min *post-mortem*. After chilling at 4°C (±1°C) for 24 h, the carcasses were portioned into eight pieces: breasts, thighs, drums and wings (NAMP, 2007; DAFF, 2012).

All breast portions were deboned after physical analyzes (pH and color): skin (with fat); bones and meat were weighed individually. The right breast meat (*M. Pectoralis major* and *M. Pectoralis minor*) and right thigh (intact portion) were individually vacuum packed and stored (6 weeks) at -20°C for further analyzes. The left breast meat (*M. Pectoralis major* and *M. Pectoralis minor*) and left thigh meat (deboned, skin on) were individually vacuum packed and stored (24 h) at 4°C (±1°C) for proximate analysis. The portions and organs were presented as average weights as well as percentages of the cold carcass weights. The breast meat, bones and skin were presented as individual weights and as a percentage of the intact breast portion weight.

3.2.4 Physical measurements

The pH and color readings were measured after 24 h of chilling at 4°C (±1°C). The pH was measured using a calibrated handheld portable pH meter (CRISON pH 25+, CRISON instruments, Spain) before portioning. A spectro-guide 45/0 gloss colorimeter (Cat no: 6801; BYK-Gardner GmbH, Germany) was standardized against a white calibration tile (D65/10°: L* = 95.73; a* = -0.83; b* = 1.31) and used to measure skin and meat color according to CIE (1976). Skin color was measured on both breasts (before portioning) and meat color was measured on the right deboned breast meat. The hue angle (h_{ab}) (°) and chroma values (C*) were calculated using the a* and b* values (AMSA, 2012):

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

3.2.4.1 Thaw and cooking loss

Thaw and cooking losses were measured on the right breast meat (*M. Pectoralis major* and *M. Pectoralis minor*) and thigh (intact portion) according to Honikel (1998) and AMSA (2015). The breasts and thighs reached an internal temperature of 80°C within 7 min and 35 min of cooking, respectively. All cooking times, endpoint temperatures and cooling times were determined in a pre-trial to suit the meat samples in the study.

3.2.4.2 Tenderness

The Warner-Bratzler shear force (WBSF) test was used to measure the instrumental shear force (N) of the cooked meat samples (Lyon and Lyon, 1997). For the thighs, the *M. Iliotibialis* and *M. Biceps femoris* were excised and two adjacent strips of 1 cm width x 1 cm breadth x 4 cm length (parallel to the muscle fiber) were sampled, with both muscles sheared at the same time. An Instron Universal Testing Machine (Instron UTM, Model 2519-107) attached to a Warner-

Bratzler fitting was used to determine the force required to shear the cooked rectangular (1 x 1 cm) meat strips perpendicular to the muscle fiber direction. The Warner-Bratzler fitting was a 1 mm thick triangular (V-notch) blade with a semi-circular cutting edge (radius of 0.508 mm). The Instron was driven with a 2 kN compression load cell recording in Newton (N). The shear test was executed at a speed of 200 mm/min.

3.2.5 Chemical analysis

Chemical analyzes were performed on deboned breast meat (*M. Pectoralis major* and *M. Pectoralis minor*) and thigh (skin on) samples.

3.2.5.1 Sample preparation

The breast and thigh meat (skin on) samples were chilled at 4°C (±1°C) for 24 h after deboning, homogenized (DAMPA CT-35N Bowl cutter, Golasecca (VA) Italy) for 20 s, vacuum packed and stored at -20°C until chemical analyzes were executed. Prior to each analysis, meat samples were defrosted at 4°C (±1°C) for 12 h.

3.2.5.2 Proximate analyzes

The moisture and ash content (%) of the meat samples were determined by using a 2.5 g homogenized meat sample according to the official methods of analysis 934.01 and 942.01, respectively (AOAC, 2002a; 2002b). A 5 g homogenized meat sample was used for the chloroform/methanol (1:2 v/v for breasts; 2:1 v/v for thighs) extraction technique as described by Lee *et al.* (1996) to determine the total lipid content (%). The defatted meat sample was dried, finely ground and 0.15 g was weighed for analysis by a Leco Nitrogen/Protein Analyzer (FP – 528, Leco corporation). Prior to each session, EDTA samples (Leco corporation, 3000 Lakeview Avenue, St. Joseph, MI 49085-2396, USA, Part no. 502-092, Lot no. 1061) were used to

standardize the Leco. The results were articulated in % nitrogen (N) which was multiplied by a conversion factor (6.25) in order to determine the crude protein content (%) in the meat sample. The precision of all the proximate analyzes in the laboratory was established by a national inter-laboratory scheme (AgriLASA: Agricultural Laboratory Association of South Africa) where blind samples are analyzed once each three months to manage and ensure the accuracy and repeatability of the procedures used.

3.2.6 Statistical analysis

All data collected in the study was subjected to the General Linear Model (GLM) procedure of SAS (SAS, 2003) and an univariate analysis of variance (ANOVA) was generated. A Shapiro-Wilk test was executed for a non-normality of residuals test (Shapiro and Wilk, 1965). Outliers were identified and removed from the data when non-normality was significant ($P \leq 0.05$). Differences between treatment means were tested according to Fisher's least significant difference (LSD) test of SAS. Means with a standard error (SE) of the mean were used to present the data. A significant level of $P \leq 0.05$ was used to conclude differences between means. For all the variables measured in duplicate or more, means were calculated and used in statistical analysis.

The statistical model used was:

$Y_{ij} = \mu + \alpha_i + e_{ij}$

Where Y_{ij} = response variable, μ = the common mean, α_i = the effect of production system ($_i$=2: P1 and P2; where P1 is the battery cage system and P2 is the free range system) and e_{ij} = the random error associated with response to the j^{th} observation in i^{th} production system.

3.3 Results

3.3.1 Carcass characteristics

Table 3.1 shows carcass characteristics of spent laying hens from different production systems. Caged hens had heavier ($P \leq 0.05$) warm carcass, cold carcass, thigh and wing weights, as well as higher ($P < 0.001$) thigh percentages than free range hens. The breast and drum percentages were higher ($P \leq 0.05$) for free range than caged hens. The gizzard weight and percentage were higher ($P \leq 0.05$) for the free range than caged hens. The non-carcass components, neck and head, did not differ ($P > 0.05$) between production systems, while caged hens had heavier ($P \leq 0.001$) feet. Free range hens had heavier ($P < 0.001$) breast bones weights, higher ($P < 0.001$) bones percentages and lower ($P \leq 0.05$) meat percentages (Table 3.1).

3.3.2 Physical characteristics

The effects of production systems on the physical attributes of spent laying hen meat are shown in Table 3.2. Free range hens had higher ($P \leq 0.05$) breast thaw and cooking loss percentages, thigh cooking loss percentages, meat redness (a*), hue angle value, skin redness (a*), and breast and thigh shear force values. Caged hens had higher ($P < 0.001$) thigh thaw loss percentages.

3.3.3 Chemical composition

The proximate composition of the breast (skinless) and thigh (skin on) meat of caged and free range spent laying hens is shown in Table 3.3. Free range hens showed higher ($P \leq 0.05$) breast meat moisture content and lower thigh meat ash content. However, the breast ash content of the caged hens was higher ($P < 0.001$). Crude protein and fat of the breast and thigh meat, as well as thigh meat moisture content, did not differ ($P > 0.05$) between caged and free range hens.

Table 3.1: Means (± SE) of carcass characteristics of caged and free range spent laying hens

	Caged	Free range	P-value
Carcass composition[1]			
Warm carcass (g)	1207.9 ± 22.91	1127.1 ± 24.16	0.018
Cold carcass (g)	1202.0 ± 22.73	1119.3 ± 23.75	0.015
Breast (g)	156.6 ± 3.89	158.7 ± 3.87	0.711
Thigh (g)	233.9 ± 5.70	206.5 ± 5.20	<0.001
Drum (g)	80.3 ± 1.88	79.2 ± 1.59	0.677
Wing (g)	99.7 ± 2.60	89.3 ± 1.48	0.001
Breast (%)[3]	26.1 ± 0.51	28.3 ± 0.28	<0.001
Thigh (%)[3]	38.9 ± 0.37	36.8 ± 0.27	<0.001
Drum (%)[3]	13.4 ± 0.20	14.2 ± 0.20	0.004
Wing (%)[3]	16.6 ± 0.30	16.0 ± 0.21	0.132
Organs[1]			
Heart (g)	8.4 ± 0.26	8.1 ± 0.19	0.356
Liver (g)	29.6 ± 1.16	26.0 ± 1.58	0.067
Gizzard (g)	30.5 ± 0.73	33.9 ± 1.04	0.009
Heart (%)[4]	0.7 ± 0.02	0.7 ± 0.02	0.264
Liver (%)[4]	2.5 ± 0.11	2.3 ± 0.13	0.325
Gizzard (%)[4]	2.5 ± 0.07	3.0 ± 0.02	<0.001
Non-carcass parts[1]			
Neck (g)	50.3 ± 0.96	49.9 ± 1.06	0.786
Head (g)	55.7 ± 1.00	55.1 ± 0.98	0.642
Feet (g)	58.1 ± 1.38	51.7 ± 1.06	<0.001
Breast composition[2]			
Meat (g)	75.0 ± 3.83	76.0 ± 3.22	0.833
Bones (g)	48.1 ± 3.37	64.3 ± 2.33	<0.001
Skin (g)	18.8 ± 1.39	18.9 ± 0.93	0.949
Meat (%)[5]	51.7 ± 1.35	47.3 ± 0.94	0.012
Bones (%)[5]	33.0 ± 1.49	40.1 ± 0.97	<0.001
Skin (%)[5]	13.2 ± 1.02	11.7 ± 0.39	0.204

[1]Means with n = 30 per treatment.
[2]Means with n = 15 per treatment.
[3]Calculated as a percentage of the cold carcass weight.
[4]Calculated as a percentage of the warm carcass weight.
[5]Calculated as a percentage of the right breast portion.

Table 3.2: Means (± SE) of physical attributes of breast and thigh meat of caged and free range spent laying hens

	Caged	Free range	P-value
Thaw loss (%)[1]			
Breast	6.1± 0.48	8.0 ± 0.77	0.043
Thigh	8.5 ± 1.62	5.5 ± 0.54	<0.001
Cooking loss (%)[1]			
Breast	12.1 ± 0.69	15.0 ± 0.96	0.023
Thigh	19.4 ± 0.93	23.5 ± 0.94	0.005
Meat pH_{24}[1]			
Breast	6.15 ± 0.016	6.18 ± 0.022	0.322
Thigh	6.32 ± 0.022	6.31 ± 0.026	0.832
Meat color[1]			
L*	54.51 ± 0.398	54.24 ± 0.532	0.682
a*	0.54 ± 0.222	1.40 ± 0.135	0.003
b*	8.45 ± 0.215	8.25 ± 0.171	0.468
Hue angle (°)	0.36 ± 0.229	1.30 ± 0.172	<0.001
Chroma	8.57 ± 0.213	8.41 ± 0.172	0.566
Skin color[2]			
L*	71.74 ± 0.421	72.09 ± 0.325	0.512
a*	0.01 ± 0.089	0.53 ± 0.144	0.003
b*	7.44 ± 0.401	6.58 ± 0.330	0.102
Hue angle (°)	0.02 ± 0.130	0.36 ± 0.130	0.071
Chroma	7.52 ± 0.398	6.75 ± 0.329	0.138
Shear force[1] (N)			
Breast	12.37 ± 0.411	17.10 ± 0.751	<0.001
Thigh	29.68 ± 0.306	39.75 ± 0.826	<0.001

[1]Means with n = 12 replicates per treatment.
[2]Means with n = 30 replicates per treatment.
Meat and skin color were measured on breast.

Table 3.3: Means[1] (± SE) of proximate composition of breast and thigh meat of caged and free range spent laying hens

	Caged	Free range	P-value
Breast			
Moisture content	73.9 ± 0.12	74.5 ± 0.19	0.036
Crude protein	21.7 ± 0.24	21.5 ± 0.31	0.699
Fat	3.8 ± 0.20	3.3 ± 0.24	0.107
Ash	1.3 ± 0.04	1.1 ± 0.02	<0.001
Thigh			
Moisture content	67.3 ± 0.67	69.0 ± 0.81	0.116
Crude protein	10.0 ± 0.91	10.1 ± 0.58	0.905
Fat	21.5 ± 1.24	20.2 ± 0.77	0.389
Ash	2.0 ± 0.02	1.0 ± 0.03	0.026

[1]Means with n = 12 per treatment

3.4 Discussion

3.4.1 Carcass characteristics

Carcass characteristics are important in determining the economic value of chickens at slaughter. The proportion of the highly valued portions (breast and thigh) of the carcass is key. Studies have shown a reduction in carcass and portion yields when broiler chickens have been granted access to free range (Castellini *et al.*, 2002; Fu *et al.*, 2014). However, there are also a number of studies indicating no significant differences between carcass and portion yields of caged and free range broiler chickens (Fanatico *et al.*, 2005a; Cheng *et al.*, 2008). Ponte *et al.* (2008) and Inci *et al.* (2016) reported contradictory results of improved carcass and portion yields when birds were reared under free range systems.

According to Di Masso *et al.* (1998) and Galeano-Vasco *et al.* (2014), the 53 week-old laying hens should be heavier than those which were 40 weeks old. However, the reverse was observed in this study as the free range laying hens (53 weeks) had lower carcass weights than the battery caged hens (40 weeks). The lower carcass and portion yields for free range spent laying hens in the present study could be ascribed to a number of factors: environmental temperatures, light intensity, diet, exercise and pasture. All the above-mentioned factors could have interfered with growth performance of the laying hens, and hence carcass and portion yields. Moreover, the increase in the digestive tract weight of the free range birds to adapt to high fiber in natural pastures could also negatively impact on carcass weight and composition (Ponte *et al.*, 2008; Mateos *et al.*, 2012). To qualify as free range production system in South Africa, 50% of the accessible outdoor area must be covered with green grass (SAPA, 2012). The variation in the percentage yield of the portions could be attributed to the differences in the portion weights. For instance, the caged hens had heavier thighs than free range hens, which would have led to the

higher thigh and lower breast percentage. Free range systems favor breast muscle development due to the motory behavior of the birds (Castellini *et al.*, 2002).

Access to pasture in the free range system could be the reason for the increased gizzard weight and percentage for the free range hens; it was noted that the hens had access to pastures that had some vegetative growth. However, it was not determined whether they had consumed some of this plant material. High dietary fiber diets stimulate gizzard muscle development in order to grind and digest feed effectively (Mateos *et al.*, 2012). For non-carcass parts, caged hens had heavier feet than free range hens. In some countries, chicken feet are ranked third among the prime economic portions of the chicken after breast and wings (Chen *et al.*, 2015). The heavier feet for caged hens could be a result of high fat and less connective tissue in the caged hen's feet. Free range bird feet go through intensive movement and exercise, which could increase the amount of connective tissue and lower the fat and muscle content. Additionally, caged birds are prone to foot lesions due to high stocking density and the nature of the cage floors (Kiyma *et al.*, 2016; Farhadi and Hosseini, 2016). Foot lesions could have contributed to the heavy feet of the caged hens as they do cause foot swelling.

Breast bones weight was noted to be the only attribute of breast composition that significantly varied between the production systems. The heavy breast bones for free range hens could be attributed to the development of bone and cartilage of the breast in order to support breast muscles (Lewis *et al.*, 1997; Sales, 2014). Although free range hens are not flight birds, most of the times they do attempt to fly for a short distance. For instance, hens under free range systems fly from litter to perches as avoidance behavior and to escape capture.

3.4.2 Physical characteristics

Meat physical traits are paramount to the quality of wholesome meat and for its processing into other products. Most studies have observed no significant difference in the physical attributes of broiler chickens when granted access to free range (Wang *et al.*, 2009; Fu *et al.*, 2014; Michalczuk *et al.*, 2014). The high thigh thaw loss of caged hens could be ascribed to the numerically high intramuscular fat content of the caged hen thighs coupled with the state of the protein. Moreover, caged hen thighs in the study had a high level of abdominal fat attached. Although water holding capacity is more related to meat protein functional properties and pH (Bowker and Zhuang, 2015), Colmenero (2014) noted that thaw and cooling losses which are a function of the water holding capacity of meat, can also be influenced by meat fat content when stored and cooked.

The fluctuation of environmental temperatures coupled with high average temperatures, reduces the water holding capacity of muscles (Wang *et al.*, 2009). Free range hens are exposed to uncontrolled environments (as discussed previously). The aforementioned aspects could be the cause of the high thaw and cooking losses of the free range hen meat. Castellini *et al.* (2002) also reported an increase in the cooking loss of the breast (caged: 31.1% and 30.3%; free range: 34.0% and 33.5%) and thigh (caged: 32.7% and 31.0%; free range: 35.2% and 34.0%) when broiler chickens were given access to free range at 51 and 81 days of age, respectively. The cooking loss results in this study are similar to those observed by Castellini *et al.* (2002). However, these findings contradict those of Funaro *et al.* (2014). Thaw and cooking loss results in this study also pose a challenge literature on the relationship between muscle physical as well as chemical properties and water holding capacity. Fu *et al.* (2014) noted that free range birds had larger muscle fiber diameter. A positive association exists between muscle fiber diameter

and plasma creatine kinase activity which may be revealed in protein turnover and hence in muscle growth (Funaro *et al.*, 2014). Furthermore, free range hens are bound to have higher collagen thickness and cross-linking owing to their higher level of motory activity (Astruc, 2014). The latter factors are expected to result in a higher water holding capacity, hence, low thaw and cooking losses; however, this is not the case in this study as higher thaw and cooking loss percentages were recorded for free range hens than for caged hens (Table 3.2). The high thaw and cooking loss observed in the current study is detrimental to meat quality, as it may result in drier and tougher meat.

The effect of production systems was not observed in the meat pH. The pH values (6.15 – 6.32) in this study were higher than 5.8 expected, which could be attributed to the light weight of the birds and pre-slaughter handling. Michalczuk *et al.* (2017) explained that light birds are highly predisposed to pre-slaughter stress as they tend to struggle a lot along the slaughter lines *ante-mortem*, since they are accustomed to being active. The prolonged struggling depletes the glycogen reserves resulting into higher ultimate pH values of the meat (Honikel, 2014). Moreover, these birds were transported the previous afternoon and held overnight in a free range holding facility (with *ad lib* access to water and feed; although the feed was different from what they had been fed during their production life) prior to being transported to the abattoir. If the birds had not consumed any feed during this period, their muscle glycogen reserves may have become depleted and this would have resulted in a higher muscle pH *post-mortem*. Nonetheless, the pH values recorded are in acceptable ranges as observed in other studies (Cheng *et al.*, 2008; Funaro *et al.*, 2014; Michalczuk *et al.*, 2017).

Skin and meat color are key determinants of consumer's acceptance of chicken meat (Barbut, 2015). According to CIE (1976), redness (a*) spans from +60 (red) to -60 (green). The use of

redness (a*) to measure chicken meat color is limited as myoglobin (the protein that determines redness of meat) is not readily detectable in chicken meat (Zhuang and Savage, 2012; Barbut, 2015). The increase in the redness (a*) of the skin of free range compared to caged birds (Table 3.2) indicates undesirable pink and red tones (Ponte *et al.*, 2008). Although most of the literature agrees that pasture imparts a desirable yellow characteristics to the skin (Fanatico *et al.*, 2007; Michalczuk *et al.*, 2017), Barbut (2015) noted that submerging in warm water (as in this study) results in a loss of this desirable, traditional yellowness and may even lead to the skin becoming more red. Thus, the influence of scalding could have resulted in the skin color differences between caged and free range hens in this study. The higher redness of the muscle from the free range hens could be ascribed to the increased motor activity, as noted by Castellini *et al.* (2002). The redness values of the current study show a similar trend to that as reported for the skin by Fanatico *et al.* (2007) (caged: -0.17; free range: 0.44; for slow-growth genotype) and for meat by Skřivan *et al.* (2015) (caged: 0.3; free range: 1.9).

Although perceived only after the biting and chewing of meat, the toughness (or tenderness) of meat is the third aspect that influences consumers' perceptions and acceptance of meat (Coggins, 2012). An *et al.* (2010) and Purchas (2014) highlighted connective tissue, collagen, ultimate pH, muscle fiber diameter, proteolytic activity and intramuscular fat as the major determinants of meat tenderness. Moreover, Aalhus *et al.* (2009) noted that a strong relationship exists between muscle fiber diameter and meat tenderness with meat containing small muscle fiber diameters being more tender. Free range systems have been reported to increase muscle development which translates into larger fiber diameters (Fu *et al.*, 2014; Funaro *et al.*, 2014). Although muscle fiber diameter was not analyzed in the current study, an increase in muscle fiber diameter could be the reason for the higher shear force values recorded for both the breast and thigh meat

of free range hens. Furthermore, the motor activity of free range birds is known to increase the amount of connective tissue and collagen cross-linkages (Astruc, 2014), which could lead to higher shear force values. Castellini *et al.* (2002) (breast: 20.59 N vs. 26.58 N; thigh: 28.15 N vs. 34.13 N at 81 days of age), Cheng *et al.* (2008) (breast: 60.80 N vs. 102.96 N) and Michalczuk *et al.* (2014; 2017) (breast: 26.64 N vs. 28.95 N; breast: 28.66 N vs. 30.39 N) results also showed significant higher shear force values for broiler chickens reared under free range than caged systems as in this study. However, there are studies where no significant differences in shear force values of meat were recorded between free range and caged chickens (Fanatico *et al.*, 2005b; Wang *et al.*, 2009).

The breast shear force values (caged: 12.37 N and free range: 17.10 N) in the current study are within range with those of broiler chickens recorded by Chen *et al.* (2007) (11.9 N to 17.36 N) and Hashim *et al.* (2013) (16.96 N to 18.63 N). Chuaynukool *et al.* (2007) reported spent laying hen breast meat as being tougher (30.79N) than indigenous (22.36 N) and broiler (15.59 N) meat. The lower than expected shear force values of spent laying hen breast meat in this study could be characteristic of the lower breast weights compared to those of broiler chickens, as Lyon *et al.* (2010) concluded that breast weight is correlated to tenderness, with lower weights being more tender.

3.4.3 Chemical composition

The nutrient composition (moisture, protein, fat and ash) of spent laying hens meat recorded (Table 3.3) in this study are in range with those of chicken in literature by Funaro *et al.* (2014) (breast: moisture 73.4%; protein 23.3%; fat 1.0%; ash 1.2% and thigh (skin on): moisture 67.9%; protein 18.6%; fat 10.8%; ash 1.0%) and Keeton *et al.*(2014) (meat: moisture 75.5%; protein 21.4%; fat 3.1%; ash 1.0%). However, the breast meat moisture content results in the current

study contradict those of Fanatico *et al.* (2005b) and Funaro *et al.* (2014). These authors found caged broiler chicken breast meat to have higher moisture content (72.2% and 73.4%) than free range reared broilers (71.1% and 72.5%). Keeton *et al.* (2014) noted that the relationship between moisture, protein and ash is inversely proportional to the fat content of the meat. The literature generally agrees that free range production decreases the intramuscular fat content of meat (Cheng *et al.*, 2008; Fu *et al.*, 2014; Funaro *et al.*, 2014). The reduction in intramuscular fat of the breast and thigh was also noted in the current study, although not significant. Based on Keeton *et al.* (2014), the low fat content of the breast meat of free range hens could have resulted in the higher moisture content observed in this study. Nonetheless, other studies have reported no significant differences between the moisture, protein, fat or ash content for free range and caged birds (Skřivan *et al.*, 2015; Michalczuk *et al.*, 2014; 2017).

3.5 Conclusions

Production systems had an effect on carcass characteristics, physical and chemical attributes of the meat derived from spent laying hens. The carcass and portion weights of the spent laying hens were also lower than the minimal market weights (carcass weight: 1.5 kg) of broiler chickens. This constitutes a further reason for the lower economic value of spent laying hens. The free range production system increased the weight of the prime economic portion (breast); however, the meat percentage of the breast portion was reduced. As expected, the selected physical attributes of free range hen meat were higher than those of caged hens, which could be attributed to increased motor activities and the uncontrolled environmental conditions experienced by the former. The skinless breast meat fat content of spent laying hens in this study was lower than that of broiler chicken breasts reported in the literature. Thus we might recommend spent laying hen breast meat to consumers concerned about high fat content in

chicken. Further studies are recommended to evaluate the fatty acids and sensory profile of the meat of spent laying hens as influenced by production systems.

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Chapter 4

Meat quality, fatty acid profile and sensory attributes of spent laying hens fed canola meal and conventional diets

Abstract

This study evaluated the effects of feeding a canola meal supplement (20%) on the carcass, meat and sensory quality characteristics of spent laying hens. Thirty canola meal based and 30 conventionally fed Lohmann Brown-Elite spent laying hens were selected in a completely randomized design from a commercial egg farm. Carcass, portions, physical, proximate, fatty acids and sensory quality were determined. Canola-fed hens had higher ($P \leq 0.05$) drum percentages, breast bone weights and percentages, but lower ($P \leq 0.05$) thigh and breast meat percentages. Conventionally fed hens had higher ($P \leq 0.05$) thaw losses, skin yellowness (b*), Chroma values and breast fat content with lower ($P \leq 0.05$) cooking losses, skin redness (a*) and hue angle values, as well as breast Warner-Bratzler shear force values (N) (15.43 ± 0.600 vs. 12.37 ± 0.411). Palmitic acid, stearic acid, heneicosanoic acid, palmitoleic acid, saturated fatty acids (SFA) (34.0 ± 0.56 vs. 38.7 ± 0.71), n-6:n-3 polyunsaturated fatty acids (PUFA) ratio (5.5 ± 0.13 vs. 7.2 ± 0.28), atherogenic index (IA), thrombogenic index (IT), delta-5 desaturase (D5D), elongase index and thiosterase index were lower ($P \leq 0.05$) for canola-fed hen breast meat. Myristic acid, lignoceric acid, nervonic acid, alpha-linolenic acid (ALA), docosahexaenoic acid (DHA), PUFA:SFA ratio (0.7 ± 0.05 vs. 0.9 ± 0.02), n-3 PUFA (3.4 ± 0.31 vs. 5.1 ± 0.17), hypocholesterolemic:Hypercholesterolaemic (h/H), stearoyl-CoA desaturase 16 (SCD16) and stearoyl-CoA desaturase 18 (SCD18) were higher ($P \leq 0.05$) for canola-fed hen breast meat. Metallic flavor was decreased ($P \leq 0.05$) for canola-fed hen breast meat. Generally, effects of

canola meal supplementation were observed on the carcass, physical, proximate, fatty acids and health indices. The sensory profiles did not differ between canola and conventionally fed spent laying hen breast meat. Canola meal improved the nutritional profile of spent laying hen meat with low intramuscular fat, low n-6:n-3 PUFA ratio and favorable lipid health indices.

Key words: off layer, lipid health index, sensory profile, meat quality

4.1 Introduction

Chicken meat has gained popularity among consumers in the past few decades. The popularity has been triggered by the rising number of consumers who have developed healthy eating habits. The preference of chicken meat among other types of meat is attributed to its low amount of fat, high n-3 polyunsaturated fatty acids (n-3 PUFA), high levels of vitamins and minerals; hence referred to as a 'functional food' (Lyon *et al.*, 2010; Funaro *et al.*, 2014). The increasing demand for chicken meat has overwhelmed the production of, especially, broiler chickens (6 - 8 weeks old) which contribute 87% of the total slaughter of poultry meat globally (Kokoszynski *et al.*, 2016). Hence there is a need to look into other chicken meat sources. Laying hens at the end of their production cycle are termed as 'spent'. Spent laying hen meat can be one of the alternative chicken meats to supplement broiler meat supply.

Spent laying hen meat is usually disregarded as it is considered tough. Commercial abattoirs in South Africa slaughter only broiler chickens because of the lack of market value for older chicken meat. Spent laying hens end up in the informal market at giveaway prices (SAPA, 2016). However, in France, older chickens are regarded as a delicacy and have been part of special recipes like traditional chicken *bourride* ('boiled chicken') for decades. Chicken *bourride* specifically utilizes spent laying hen meat and the recipe is enjoyed by natives as well as

foreigners/tourists (Child and Beck, 1970; Hill, 2009). In Thailand, tom yum soup is another spent laying hen delicacy (Chuaynukool *et al.*, 2007). Considering the status quo in food security and increasing population in South Africa, spent laying hen meat could play a crucial role. However, it is imperative to evaluate the carcass, meat and sensory quality attributes of spent laying hens as a way of establishing this meat's food functionality, especially, with the recent increased use of canola meal in layer diets.

Canola meal is an expelled by-product of canola seed oil processing that is gaining popularity among livestock feeds as a substitute for soybean meal. Canola meal has high crude protein, fat, n-3 PUFA and a well-balanced amino acids content (Khajali and Slominski, 2012; Mejicanos *et al.*, 2016). The inclusion of canola meal in laying hen diets impacts on the nutrient composition of eggs, specifically with regard to the egg fatty acid (FA) profile and PUFA content. In South Africa, canola enhanced eggs have become a high priced commodity and are readily consumed. In monogastric animals, the dietary FA composition is directly correlated to the FA profile of the products (meat and eggs) (López-Ferrer *et al.*, 2001; NRC, 2012; Tuunainen *et al.*, 2016). Similar results have been reported in turkey and broiler chicken meat (Khajali and Slominski, 2012; Mikulski *et al.*, 2012; Moraes *et al.*, 2016).

The FA profile has been highlighted as a major determinant of the sensory profile. Meat flavor and texture are influenced by the FA profile. The reaction between FAs and carbohydrates yields the cooked meat flavor through the production of volatiles. The amount and composition of volatiles determine the variations of flavor within the cooked meat (Rabe *et al.*, 2003; Dawson and Spinelli, 2012). In addition, the amount and variation in melting points of FAs result in differences in meat color, pH and texture. The high melting point FAs occur as solid yellow fat, impacting on meat yellowness, while the low melting point FAs are in a liquid state between

intramuscular and intermuscular spaces. The low melting point FAs also occupy spaces between muscles and connective tissues initiating the tenderizing process (Wood *et al.*, 2003; Moraes *et al.*, 2016). There is limited literature on the effect of canola meal in laying hen diet on meat quality, lipid healthy profile and sensory attributes. Therefore, the aim of this study is to evaluate the effect of canola meal inclusion in laying hen diets on carcass characteristics, meat quality and fatty acid composition, as well as the sensory attributes of spent laying hens.

4.2 Materials and methods

4.2.1 Experimental design

A total of 60 Lohmann Brown-Elite spent laying hens were obtained from a commercial egg producer. The layer flocks were housed in large sheds and maintained in battery cages; each cage contained 10 hens and a single hen was randomly selected from a cage; the latter were also randomly selected for sampling. Of the hens sampled, 30 were fed a conventional diet containing soybean as a protein source and 30 hens were fed a conventional diet with 20% of the soybean being replaced with canola meal as a protein source. The chemical composition of the canola meal and conventional diet is presented in Table 4.1 and Table 4.2. The hens on canola supplemented diet hens were 48 weeks of age while the hens on a conventional diet were 40 weeks of age. According to Barbut (2015), once chickens have reached sexual maturity (18 weeks of age), age has little or no effect on carcass and meat quality, hence it was not considered for this study.

4.2.2 Slaughtering

The details of this section are as described in Chapter 3.

4.2.3 Carcass yield, portioning and deboning

The details of this section are as described in Chapter 3; except that, after physical analyzes (color and pH), 12 right breast meat (*M. Pectoralis major* and *M. Pectoralis minor*) were also individually vacuum packaged and stored (6 weeks) at -20°C for shear force analysis. Twelve left breast meat (*M. Pectoralis major* and *M. Pectoralis minor*) were individually vacuum packaged and stored (24h) at 4°C (±1°C) for proximate analysis. Both the right and left breast meat samples (*M. Pectoralis major*) from 18 birds (six breast meat samples for training and 12 for testing) were vacuum packaged immediately after deboning and stored (4 weeks) at -20°C for descriptive sensory analysis.

4.2.4 Physical measurements

The details of this section are as described in Chapter 3.

4.2.4.1 Thaw and cooking loss

The details of this section are as described in Chapter 3; except that, thaw and cooking loss was measured on breast meat only.

4.2.4.2 Tenderness

The details of this section are as described in Chapter 3; except that, tenderness was measured on breast meat only.

4.2.5 Chemical analysis

4.2.5.1 Sample preparation

The details of this section are as described in Chapter 3; except that, only breast meat samples were prepared and FA samples that were stored (6 weeks) at -80°C.

4.2.5.2 Proximate analyzes

The details of this section are as described in Chapter 3.

4.2.5.3 Fatty acid analysis

The FA profile was determined using a 1 g meat sample according to the technique described by Folch *et al.* (1957). A chloroform:methanol (2:1; v/v) solution containing an antioxidant of 0.01% butylated hydroxytoluene (BHT) was used for extraction. An internal standard (Heptadecenoic acid) (Cat no. H3500, Sigma–Aldrich Inc., 3050 Spruce Street, St. Louis, MO 63103, USA) was added to the mixture with the purpose of quantifying the single FAs present in the meat sample. Using a Polytron mixer (WiggenHauser Homogenizer, D-500 fitted with a standard shaft 1; speed setting A, Germany), the meat sample was mixed uniformly for 30 s with the extraction solvent. A 250 µL sub-sample of the lipid extract was dried under nitrogen for 5 min at 45°C. Using a transmethylating agent of methanol/sulphuric acid (19:1; v/v) solution (2 mL), the dried sub-sample was then transmethylated for 2 h at 70°C. Room temperature (25°C) was used to cool the mixture. The extraction of the fatty acid methyl esters (FAME) from the mixture was executed with water and hexane. The top hexane layer was transferred using a spotting tube and then dried at 45°C under nitrogen. The dried FAME sample was then mixed with hexane (100 µL). A ThermoFinnigan Focus gas-chromatograph (Thermo-Electron

Corporation, Rodano, Milan, Italy) fitted 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) was used to analyze the FAME. The hydrogen gas flow rate was 30 mL/min. The following temperature settings were applied: initial temperature of 60°C; injector and detector 220°C and 260°C, respectively and a final temperature of 160°C. The 1 μL GC injection volume was used with approximately 45 min of run time. The FAME levels in the meat sample were categorized in comparison 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 results were recorded as a percentage (%) of the total FAs. Lipid health indices were calculated as follows:

Atherogenic index (IA) = $\frac{(4\ X\ C14{:}0+C16{:}0)}{[\sum MUFA+\sum n6+\sum n3]}$ (Ulbricht and Southgate, 1991);

Thrombogenic index (IT) = $\frac{(C14{:}0+C16{:}0+C18{:}0)}{[0.5\ X\ \sum MUFA+0.5\ X\ \sum n6+3\ X\ \sum n3+\sum n3/\sum n6}$ (Ulbricht and Southgate, 1991);

Hypocholesterolaemic:Hypercholesterolaemic ratio (h/H) = $\frac{(C14{:}1+C16{:}1+C18{:}1+C20{:}1+C22{:}1+C18{:}2+C18{:}3+C20{:}3+C20{:}4+C20{:}5+C22{:}4+C22{:}5+C22{:}6)}{(C14{:}0+C16{:}0)}$ (Fernández *et al.*, 2006);

Stearoyl-CoA 16 (SCD16) = $\frac{(C16{:}1)}{(C16{:}0)}$ (Haug *et al.*, 2014);

Stearoyl-CoA 18 (SCD18) = $\frac{(C18{:}1)}{(C18{:}0)}$ (Haug *et al.*, 2014);

Delta-5 Desaturase (D5D) = $\frac{(C20{:}4n6)}{(C20{:}3n6)}$ (Haug *et al.*, 2014);

Delta-6 Desaturase (D6D) = $\frac{(C18{:}3n6)}{(C18{:}2n6)}$ (Haug *et al.*, 2014);

Elongase index = $\frac{(C18{:}0)}{(C16{:}0)}$ (Haug *et al.*, 2014);

Thioesterase index = $\frac{(C16{:}0)}{(C14{:}0)}$ (Haug *et al.*, 2014).

4.2.6 Descriptive sensory analysis

4.2.6.1 Preparation of samples

The right and left breasts (*M. Pectoralis major* and *M. Pectoralis minor*) were deboned from randomly selected 18 birds per replicate. The meat samples were defrosted for 24 h at 4°C (± 1°C) prior to the scheduled descriptive sensory analysis (DSA) training or testing sessions; the *M. Pectoralis major* was separated from the *M. Pectoralis minor*. The *M. Pectoralis major* from the left and right sides were blotted dry, weighed individually (RADWAG®, Model: AS 220/C/2, Poland) and placed on a metal grid wrapped in aluminum foil (shiny surface outside towards the meat). The metal grid was then inserted into a medium roasting bag (Checkers house brand, South Africa) and closed with a twist tie. No salt or seasoning was added to the meat samples during DSA. Two roasting bags were placed onto an oven roasting pan. The roasting pan was placed in an industrial forced-convection oven (Hobart 10 Grid Combi Oven, UK), preheated to 163°C (AMSA, 2015). The meat samples were roasted for 14 min. The latter cooking time was determined in a series of pre-trials as the breast muscles were too thin, consequently the use of a thermocouple probe affixed to the handheld digital temperature monitor (Hanna Instruments, South Africa) and inserted into the center of each breast muscle to determine the internal temperature was ineffective. Additionally, the temperature monitors indicated that the internal temperature to be 80°C; however, the meat was not cooked sufficiently for evaluation. As a result, the cooking time was standardized at 14 min for all samples and an average core temperature of 85°C (± 5°C) was attained during this time (AMSA, 2015). The core temperature was determined by thermocouple probe inserted immediately after removing the cooked meat samples from the oven. The cooked samples were allowed to cool for 20 min, blotted dry and weighed. Cubes of 1 cm x 1 cm x 1 cm were cut from the middle of the cooked

meat samples. The meat cubes were wrapped individually in aluminum foil (shiny surface towards the meat) and three wrapped meat cubes were placed into a glass ramekin (one per panelist). The ramekins were coded using randomized three-digit codes. The ramekins with wrapped meat samples were reheated for 4 min in the same oven preheated to 70°C. To ensure that each panelist tests each sample at a constant temperature, the ramekins with the wrapped meat samples were placed near the panelists in water baths set at 70°C.

4.2.6.2 Panel selection and training

A panel of 10, one male and nine female, were selected. All the panelists had previous experience in the sensory profile analysis of meat. The panel was trained with the protocol described by AMSA (2015). The panelists were trained for six sessions within three days, one hour per session and two sessions per day. During each session, the panelist received three1 cm x 1 cm x 1 cm meat cubes from each of the two treatments (diets) as well as reference standards. All the reference standards were pre-selected to attempt to simulate the aroma, flavor and texture attributes associated with chicken meat. Some additional reference standards were suggested during the training sessions. The preparation, cooking times, endpoint temperatures, codes and final panel scores of the reference standards are shown in Table 4.3. Using a consensus procedure described by Murray *et al.* (2001), a 23 attribute lexicon was agreed on and developed: overall aroma intensity; chicken, metallic, fishy, brothy aroma and flavor; wet feather/sweaty flavor; sour, sweet and salty taste; initial and sustained juiciness; tenderness; chewiness; mealiness; residue and fatty mouthfeel. The sensory attributes, scales and descriptors are illustrated in Table 4.4. Aroma and flavor were analyzed orthonasally and retronasally, respectively.

4.2.6.3 Descriptive sensory analysis

Descriptive sensory analysis (DSA) was performed with a test re-test method with 12 replicates per two treatments. The meat samples for the two treatments were presented to each panelist in a completely randomized order and each sample was analyzed for the intensity of the sensory attributes (Table 4.4). Individual tasting booths fitted with a computer with Compusense® *five* software program (Compusense, Guelph, Canada) were provided to each panelist. An unstructured line scale was used for scoring, where zero indicated 'low intensity' and 100 indicated 'high intensity', the line scale had four marks indicating 0, 25, 50 and 100 (AMSA, 2015). The sensory panel booth was designed according to guidelines provided by ASTM MNL60-2nd (2008). The sensory room had artificial daylight and was temperature-controlled (21°C) (AMSA, 2015). Each panelist was provided with distilled water, apple segments (Top Red, Woolworth, South Africa) and water biscuits (Original Plain, Woolworths, South Africa) to refresh their palate between samples.

4.2.7 Statistical analysis

All the data collected for the variables was subjected to a general linear model (GLM) procedure of SAS (2003) using the model of the completely randomized design to generate an univariate analysis of variance (ANOVA). A Shapiro-Wilk test was executed for non-normality of residuals test (Shapiro and Wilk, 1965). Outliers were identified and removed from the data when the non-normality was significant ($P \leq 0.05$). Where applicable, correlation coefficients were calculated for data by means of the Pearson's correlation coefficient (r) (Snedecor and Cochran, 1980). According to Næs *et al.* (2010), the relationships between data were tested using the correlation matrix and discriminant matrix (DA) for principal component analysis (PCA).

The statistical model used was;

$Y_{ij} = \mu + \alpha_i + e_{ij}$

Where Y_{ij} = response variable e.g. warm carcass, μ = the common mean, α_i = the effect of diet ($_i$=2; D1 and D2, where D1 is the canola meal diet and D2 is the conventional diet) and e_{ij} = the random error associated with a response to the j^{th} observation in the i^{th} diet. Differences between treatment means were tested according to Fisher's least significant difference test of SAS (2003). Means with a standard error (SE) of the mean were used to present the data. A significant level of $P \leq 0.05$ was used to conclude differences.

Table 4.1: Chemical composition of the canola meal and conventional diet

Diet	Canola	Conventional
Moisture content	9.9	9.6
Crude protein	14.3	14.7
Fat	7.2	6.1
Ash	14.3	12.1
Acid detergent fiber	6.0	6.4
Neutral detergent fiber	15.7	14.0
Crude fiber	5.6	3.1

Table 4.2: Fatty acid methyl esters (%) (FAME) of conventional and canola meal diet

FAME	Common name	Conventional	Canola
Saturated fatty acids (SFA)			
C14:0	Myristic acid	0.4	0.4
C15:0	Pentadecylic acid	0.1	0.1
C16:0	Palmitic acid	11.6	14.0
C18:0	Stearic acid	3.2	4.6
C20:0	Arachidic acid	0.5	0.6
C21:0	Heneicosanoic acid	0.3	0.5
C22:0	Behenic acid	0.2	0.2
C23:0	Tricosylic acid	0.6	0.8
C24:0	Lignoceric acid	0.3	0.4
Monounsaturated fatty acids (MUFA)			
C14:1		0.1	0.1
C15:1		ND	ND
C16:1		0.4	0.4
C18:1n9c	Oleic acid	38.2	31.7
C18:1n9t	Elaidic acid	ND	ND
C20:1		1.1	0.7
C22:1n9	Erucic acid	ND	ND
C24:1		ND	ND
Polyunsaturated fatty acids (PUFA)			
C18:2n-6c	Linoleic acid	34.3	42.6
C18:2n-6t	Linolelaidic acid	ND	ND
C18:3n-6	Gamma-linolenic acid	ND	ND
C18:3n-3	Alpha-linolenic acid	4.7	1.5
C20:2n-6	Eicosadienoic acid	0.3	ND
C20:3n-6	Dihomo-gamma-linolenic acid	0.4	ND
C20:3n-3	Eicosatrienoic acid	0.3	ND
C20:4n-6	Arachidic acid	0.4	0.6
C20:5n-3	Eicosapentaenoic acid, EPA	ND	0.4
C22:2n-6	Docosadienoic acid	ND	0.4
C22:5n-3			
C22:6n-3	Docosahexaenoic acid, DHA	0.5	ND
SFA		19.2	21.5
MUFA		39.8	33.1
PUFA		40.9	45.4
PUFA:SFA		2.1	2.1
n-6		35.4	43.5
n-3		5.5	1.9
(n-6)/(n-3)		6.4	22.7

ND – not detectable for spent laying hen breast meat; ∑ - Summation; PUFA:SFA ratio = ∑PUFA/∑SFA; ∑n-6 PUFA = C18:2n-6c, C18:2n-6t, C18:3n-6, C20:2n-6, C20:3n-6 and C20:4n-6; ∑n-3 PUFA = C18:3n-3, C20:3n-3, C20:5n-3 and C22:6n-3; n-6:n-3 PUFA ratio = ∑n-6 PUFA/∑n-3 PUFA.

Table 4.3: Reference standards used during training for descriptive sensory analysis of spent laying hen breast meat fed conventional and canola meal diets

	Sensory attributes	Cooking time (min)	Endpoint temperature (°C)	Scores
Aroma				
Chicken breast[a]	Chicken aroma	28	74.3	70
Hake[b]	Fishy aroma	29	73.0	100
Broth[c]	Brothy	-	-	60
Flavor				
Chicken breast[d]	Chicken flavor	32	80.0	CF 50; MF 40
Hake[e]	Fishy flavor	29	73.0	80
Broiler breast broth[f]	Brothy flavor	-	-	Brothy flavor 60; Salty taste 30
Sour solution[g]	Sour taste	-	-	-
Sweet solution[h]	Sweet taste	-	-	-
Salty solution[i]	Salty taste	-	-	-
Bitter solution[j]	Bitter taste	-	-	-
Texture				
Hake[k]	Texture attributes	29	73.0	T 100
Chicken breast[l]	Texture attributes	30	75.0	IJ 50; SJ 30; T 80; M 20
Chicken breast[m]	Texture attributes	32	80.0	IJ 30; SJ 10; T 70; M 20; R 10
Duck breast[n]	Texture attributes	16	74.0	SJ 30; T 70; C 10; R 10; F 0
Indigenous chicken breast[o]	Texture attributes	12	74.5	SJ 30; T 60; C 20; M 20; R 20

[a, d, l, m]Skinless broiler chicken breast (Woolworth, South Africa); [b, e, k]Hake fillet (Woolworth, South Africa); [c, f]Broth from cooked skinless broiler chicken breast; [g]0.07% Citric acid solution; [h]2.0% Sucrose solution; [i]0.2% Sodium chloride solution; [j]0.07% Caffeine solution; [n]Skinless duck breast (The duck farm, South Africa); [o]Skinless indigenous chicken breast (Mariendahl farm, South Africa); CF – Chicken flavor; MF – Metallic flavor; T – Tenderness; IJ – Initial juiciness; SJ – Sustained juiciness; C– Chewiness; M – Mealiness; R – Residue; F – Fatty mouthfeel; Scores refer to a score on a line scale marked from 0 (low intensity) to 100 (high intensity).

Table 4.4: Sensory attributes, descriptors and scale for descriptive sensory analysis of spent laying hen breast meat fed conventional and canola meal diets

Sensory Characteristics	Description of Attributes	Scale
Overall aroma intensity	Intensity of the aroma in the first few sniffs as the foil is removed	0 = Extremely bland; 100 = Extremely intense
Chicken aroma	Aroma associated with typical cooked chicken (Broiler) as the foil is removed	0 = Extremely bland; 100 = Extremely intense
Metallic aroma	Aroma associated with slightly oxidized metal, such as iron, copper and silver spoon as the foil is removed	0 = Extremely bland; 100 = Extremely intense
Fishy aroma	Aroma associated with fish (Hake) as the foil is removed	0 = Extremely bland; 100 = Extremely intense
Fatty aroma (Chicken-like)	Aroma associated with rendered chicken (Broiler) fat as the foil is removed	0 = Extremely bland; 100 = Extremely intense
Brothy aroma	Aroma associated with juices of cooked chicken meat (roasted) as the foil is removed	0 = Extremely bland; 100 = Extremely intense
Overall flavor intensity	Overall intensity of flavor upon chewing	0 = Extremely bland; 100 = Extremely intense
Chicken flavor	Flavor associated with typical cooked chicken (Broiler)	0 = Extremely bland; 100 = Extremely intense
Metallic flavor	Flavor associated with metal/liver	0 = Extremely bland; 100 = Extremely intense
Fishy flavor	Flavor associated with fish (Hake)	0 = Extremely bland; 100 = Extremely intense
Fatty flavor (Chicken-like)	Flavor associated with rendered chicken (Broiler) fat	0 = Extremely bland; 100 = Extremely intense
Brothy flavor	Flavor associated with the juice of cooked chicken (roasted)	0 = Extremely bland; 100 = Extremely intense
Wet feather/sweaty flavor	Flavor associated with wet feathers and/or a sweat-like characteristic	0 = Extremely bland; 100 = Extremely intense
Sour taste	Sour taste on the tongue	0 = Extremely bland; 100 = Extremely intense
Sweet taste	Sweetness on the tongue	0 = Extremely bland; 100 = Extremely intense
Salty taste	Salty taste on the tongue	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	Perceived juiciness after the first 5 chews using the molar teeth	0 = Extremely dry; 100 = Extremely juicy
Tenderness	Perceived tenderness after the first 5 chews using the molar teeth	0 = Extremely tough; 100 = Extremely tender
Chewiness	Ease of chewing to a point of swallowing	0 = Extremely easy; 100 = Extremely chewy
Mealiness	Disintegration of muscle fibers into very small particles (perception within the first few chews)	0 = None; 100 = Abundant
Residue	Amount of residue left in mouth after 10 chews	0 = None; 100 = Abundant
Fatty mouthfeel	Fatty coating left in the mouth after swallowing	0 = None; 100 = Abundant

4.3 Results

4.3.1 Carcass characteristics

The carcass characteristics of spent laying hens fed canola meal and conventional diets are shown in Table 4.5. The effect of canola meal on carcass characteristics of spent laying hens was significant for the thigh, drum, breast meat and bone percentages, as well as for breast bone weight. Hens fed on canola meal had a higher ($P \leq 0.05$) drum and breast bone percentages, as well as breast bone weight, while conventionally fed hens had higher ($P \leq 0.05$) thigh and breast meat percentages.

4.3.2 Physical characteristics

The physical attributes of the meat of spent laying hens fed canola meal and conventional diets are shown in Table 4.6. The cooking loss, pH and meat color were not significantly influenced by the diet. The conventionally fed spent laying hens had higher ($P \leq 0.05$) thaw loss and lower ($P < 0.001$) shear force values. For skin color, higher ($P \leq 0.05$) yellowness (b*) and Chroma were observed among conventionally fed spent laying hens. Additionally, the canola meal fed spent laying hens had higher ($P \leq 0.05$) redness (a*) and hue angle values of the skin.

4.3.3 Chemical composition

Table 4.7 shows the proximate composition of breast meat derived from spent laying hens fed canola meal and conventional rations. The conventionally fed hens had a higher ($P \leq 0.05$) breast meat fat content than canola meal fed spent laying hens. The moisture, protein and ash contents did not differ between the two dietary treatments, although the ash content tended ($P = 0.064$) to be higher for the breast meat from conventionally fed spent laying hens.

Table 4.8 shows the fatty acid methylated ester percentages of breast meat derived from spent laying hens fed canola meal and conventional diets. The breast meat of spent laying hens fed on

canola meal diet had higher ($P \leq 0.05$) myristic acid (C14:0), lignoceric acid (C24:0), nervonic acid (C24:1n9), alpha-linolenic acid, ALA (C18:3n-3), docosahexaenoic acid, DHA (C22:6n-3), PUFA:SFA ratio and n-3 PUFA than conventionally fed hens. The breast meat of spent laying hens fed conventional diet had higher ($P \leq 0.05$) palmitic acid (C16:0), stearic acid (C18:0), heneicosanoic acid acid (C21:0), palmitoleic acid (C16:1n7), SFA and n-6:n-3 PUFA ratio than canola meal fed hens. Arachidic acid (C20:0), behenic acid (C22:0), myristoleic acid (C14:1n9c), pentadecenoic acid (C15:1n9t), docosadienoic acid (C22:2n-6) and docosapentaenoic acid (C22:5n-3) were not detectable in breast meat derived from spent laying hens. Palmitic acid, oleic acid (C18:1n9c), linoleic acid (C18:2n-6c) and DHA were the most abundant SFA, MUFA, n-6 PUFA and n-3 PUFA, respectively.

Table 4.9 shows the lipid health indices of breast meat derived from spent laying hens fed canola meal and conventional diets. The breast meat of spent laying hens fed canola meal diet had lower ($P \leq 0.05$) antherogenic index (AI), thrombogenic index (IT), Delta-5 Desaturase (D5D), elongase index and thiosterase index than conventional diet fed hens. The breast meat of spent laying hens fed the conventional diet had a lower ($P \leq 0.05$) Hypocholesterolaemic:Hypercholesterolaemic (h/H) ratio as well as Stearoyl-CoA 16 (SCD16) and Stearoyl-CoA 18 (SCD18) indices.

4.3.4 Sensory attributes

The statistical means of the majority of the sensory scores for the meat samples from spent laying hens fed canola meal and conventional diets did not differ significantly (Table 4.10). The exception was the breast meat from conventionally fed spent laying hen which had a higher ($P \leq 0.05$) metallic flavor than those fed on canola meal. Sour taste, fishy aroma and flavor were not detected in the meat derived from spent laying hens fed conventional and canola meal diets. The

relationships between sensory attributes of meat derived from spent laying hens fed canola meal and conventional diets are displayed in Figure 4.1 and Table 4.11.

Most of the sensory attributes of the meat from the two diets were correlated with each other (Table 4.11). The overall aroma intensity was found to be strongly positively correlated to chicken ($r = 0.965$, $P < 0.001$) and brothy ($r = 0.827$, $P < 0.001$) as well as overall ($r = 0.680$, $P < 0.001$), chicken ($r = 0.668$, $P < 0.001$) and brothy ($r = 0.548$, $P = 0.006$) flavor. Overall flavor was observed to be strongly positively correlated to chicken ($r = 0.948$, $P < 0.001$) and brothy ($r = 0.700$, $P < 0.001$) flavor. Initial juiciness was strongly positively correlated to sustained juiciness ($r = 0.771$, $P < 0.001$) and tenderness ($r = 0.537$, $P = 0.007$) and moderately negatively correlated to chewiness ($r = -0.415$, $P = 0.044$) and residue ($r = -0.476$, $P = 0.019$). Sustained juiciness was strongly positively correlated to tenderness ($r = 0.790$, $P < 0.001$) and negatively correlated to chewiness ($r = -0.655$, $P = 0.001$) and residue ($r = -0.783$, $P < 0.001$). As expected, tenderness was strongly negatively correlated to chewiness ($r = -0.845$, $P < 0.001$) and residue (r, $= -0.855$, $P < 0.001$), while chewiness was strongly positively correlated to residue ($r = 0.783$, $P < 0.001$).

Table 4.5: Means (± SE) of carcass characteristics of spent laying hens fed conventional and canola meal diets

	Conventional	Canola	P-value
Carcass composition[1]			
Warm carcass (g)	1207.9 ± 22.91	1196.2 ± 27.50	0.746
Cold carcass (g)	1202.0 ± 22.73	1187.2 ± 27.45	0.679
Breast (g)	156.6 ± 3.88	158.4 ± 4.58	0.771
Thigh (g)	233.9 ± 5.70	219.7 ± 5.31	0.074
Drum (g)	80.3 ± 1.88	85.2 ± 1.69	0.058
Wing (g)	99.7 ± 2.60	96.3 ± 2.08	0.318
Breast (%)[3]	26.1 ± 0.51	26.6 ± 0.28	0.391
Thigh (%)[3]	38.8 ± 0.37	37.0 ± 0.29	<0.001
Drum (%)[3]	13.4 ± 0.20	14.4 ± 0.17	<0.001
Wing (%)[3]	16.6 ± 0.30	16.3 ± 0.20	0.366
Organs[1]			
Heart (g)	8.4 ± 0.26	8.6 ± 0.24	0.722
Liver (g)	29.6 ± 1.16	26.5 ± 1.19	0.066
Gizzard (g)	30.5 ± 0.73	31.6 ± 0.67	0.242
Heart (%)[4]	0.7 ± 0.02	0.7 ± 0.02	0.418
Liver (%)[4]	2.5 ± 0.11	2.2 ± 0.11	0.127
Gizzard (%)[4]	2.5 ± 0.07	2.7 ± 0.09	0.195
Non-carcass parts[1]			
Neck (g)	50.3 ± 0.96	50.5 ± 1.13	0.879
Head (g)	55.7 ± 1.00	57.0 ± 1.41	0.465
Feet (g)	58.1 ± 1.38	59.1 ± 1.17	0.578
Breast composition[2]			
Meat (g)	75.0 ± 3.83	73.0 ± 3.89	0.729
Bone (g)	48.1 ± 3.37	62.0 ± 3.33	0.008
Skin (g)	18.8 ± 1.39	21.2 ± 1.51	0.247
Meat (%)[5]	51.7 ± 1.35	46.5 ± 1.54	0.017
Bone (%)[5]	33.0 ± 1.49	39.4 ± 1.22	0.003
Skin (%)[5]	13.2 ± 1.02	13.5 ± 0.90	0.785

[1]Means with n = 30 per treatment.
[2]Means with n = 15 per treatment.
[3]Calculated as a percentage of the cold carcass weight.
[4]Calculated as a percentage of the warm carcass weight.
[5]Calculated as a percentage of the right breast portion.

Table 4.6: Means (± SE) of physical characteristics of spent laying hens fed conventional and canola meal diets

	Conventional	**Canola**	**P-value**
Thaw loss (%)[1]	6.1 ± 0.48	4.7 ± 0.39	0.045
Cooking loss (%)[1]	12.1 ± 0.69	14.1 ± 0.91	0.088
pH_{24}[2]	6.2 ± 0.02	6.2 ± 0.03	0.556
Meat color[1]			
L*	54.51 ± 0.398	55.15 ± 0.449	0.302
a*	0.54 ± 0.222	0.85 ± 0.269	0.383
b*	8.45 ± 0.215	8.25 ± 0.364	0.636
Hue angle (°)	0.36 ± 0.229	0.73 ± 0.227	0.258
Chroma	8.57 ± 0.213	8.37 ± 0.360	0.633
Skin color[2]			
L*	71.74 ± 0.421	71.69 ± 0.315	0.920
a*	0.01 ± 0.089	0.56 ± 0.131	<0.001
b*	7.44 ± 0.401	6.03 ± 0.357	0.011
Hue angle (°)	0.02 ± 0.130	0.43 ± 0.133	0.033
Chroma	7.52 ± 0.398	6.20 ± 0.358	0.004
Shear force (N)[1]	12.37 ± 0.411	15.43 ± 0.600	<0.001

[1]Means with n = 12 replicates per treatment.
[2]Means with n = 30 replicates per treatment.

Table 4.7: Means[1] (± SE) of proximate composition (%) of breast meat derived from spent laying hens fed conventional and canola meal diets

	Conventional	**Canola**	**P–value**
Moisture content	73.9 ± 0.12	73.6 ± 0.19	0.164
Crude protein	21.7 ± 0.24	22.2 ± 0.31	0.176
Fat	3.8 ± 0.20	3.1 ± 0.21	0.037
Ash	1.3 ± 0.04	1.2 ± 0.05	0.064

[1]Means with n = 12 per treatment

Table 4.8: Means[1] (± SE) of fatty acid methyl esters (%) (FAME) of breast meat derived from spent laying hens fed conventional and canola meal diets

FAME	Common name	Conventional	Canola	P - value
Saturated fatty acids (SFA)				
C14:0	Myristic acid	0.4 ± 0.06	0.6 ± 0.05	0.006
C15:0	Pentadecylic acid	0.3 ± 0.02	0.3 ± 0.01	0.517
C16:0	Palmitic acid	22.7 ± 0.63	19.0 ± 0.33	<0.001
C18:0	Stearic acid	8.8 ± 0.19	7.0 ± 0.19	<0.001
C20:0	Arachidic acid	ND	ND	ND
C21:0	Heneicosanoic acid acid	0.02 ± 0.00	0.004 ± 0.00	0.003
C22:0	Behenic acid	ND	ND	ND
C23:0	Tricosylic acid	0.1 ± 0.05	0.4 ± 0.30	0.467
C24:0	Lignoceric acid	0.06 ± 0.01	0.07 ± 0.00	0.026
Monounsaturated fatty acids (MUFA)				
C14:1n9c	Myristoleic acid	ND	ND	ND
C15:1n9t	Pentadecanoic acid	ND	ND	ND
C16:1n7	Palmitoleic acid	1.6 ± 0.02	1.5 ± 0.02	0.010
C18:1n9c	Oleic acid	28.8 ± 1.36	29.6 ± 0.73	0.606
C18:1n9t	Elaidic acid	1.0 ± 0.57	0.8 ± 0.15	0.735
C20:1n9	Gondoic acid	0.4 ± 0.04	0.3 ± 0.02	0.167
C22:1n9	Erucic acid	0.1 ± 0.01	0.1 ± 0.01	0.075
C24:1n9	Nervonic acid	0.3 ± 0.03	0.5 ± 0.03	<0.001
Polyunsaturated fatty acids (PUFA)				
C18:2n-6c	Linoleic acid	17.8 ± 0.38	18.5 ± 0.36	0.310
C18:2n-6t	Linolelaidic acid	0.0 ± 0.00	0.1 ± 0.07	0.194
C18:3n-6	Gamma-linolenic acid	1.3 ± 0.14	1.4 ± 0.08	0.256
C18:3n-3	Alpha-linolenic acid	0.8 ± 0.06	1.6 ± 0.05	<0.001
C20:2n-6	Eicosadienoic acid	0.4 ± 0.01	0.3 ± 0.01	0.084
C20:3n-6	Dihomo-gamma-linolenic acid	1.6 ± 0.16	1.8 ± 0.09	0.170
C20:3n-3	Eicosatrienoic acid	0.1 ± 0.02	0.1 ± 0.03	0.066
C20:4n-6	Arachidonic acid	5.3 ± 0.57	5.6 ± 0.33	0.609
C20:5n-3	Eicosapentaenoic acid, EPA	1.0 ± 0.11	1.2 ± 0.07	0.128
C22:2n-6	Docosadienoic acid	ND	ND	ND
C22:5n-3	Docosapentaenoic acid	ND	ND	ND
C22:6n-3	Docosahexaenoic acid, DHA	1.1 ± 0.11	2.1 ± 0.11	<0.001
∑SFA		38.7 ± 0.71	34.0 ± 0.56	<0.001
∑MUFA		33.0 ± 1.18	32.8 ± 0.72	0.930
∑PUFA		29.1 ± 1.49	32.4 ± 0.81	0.069
PUFA:SFA ratio		0.7 ± 0.05	0.9 ± 0.02	<0.001
∑n-6 PUFA		25.6 ± 1.24	27.9 ± 0.31	0.09
∑n-3 PUFA		3.4 ± 0.31	5.1 ± 0.19	<0.001
n-6:n-3 PUFA ratio		7.2 ± 0.28	5.5 ± 0.13	<0.001

[1]Means with n = 12 per treatment; ND – not detectable for spent laying hen breast meat; ∑ - Summation; PUFA:SFA ratio = ∑PUFA/∑SFA; ∑n-6 PUFA = C18:2n-6c, C18:2n-6t, C18:3n-6, C20:2n-6, C20:3n-6 and C20:4n-6; ∑n-3 PUFA = C18:3n-3, C20:3n-3, C20:5n-3 and C22:6n-3; n-6:n-3 PUFA ratio = ∑n-6 PUFA/∑n-3 PUFA.

Table 4.9: Means[1] (± SE) of health indices of breast meat derived from spent laying hens fed conventional and canola meal diets

Index	Conventional	Canola	P - value
Atherogenic index (IA)	0.5 ± 0.01	0.4 ± 0.01	0.005
Thrombogenic index (IT)	0.8 ± 0.03	0.6 ± 0.01	<0.001
h/H	2.4 ± 0.09	3.0 ± 0.08	<0.001
SCD16	0.07 ± 0.001	0.08 ± 0.001	<0.001
SCD18	3.1 ± 0.11	4.6 ± 0.12	<0.001
D5D	3.6 ± 0.18	3.1 ± 0.11	0.016
D6D	0.1 ± 0.01	0.1 ± 0.11	0.615
Elongase index	0.43 ± 0.012	0.39 ± 0.018	0.047
Thiosterase index	48.7 ± 3.40	33.5 ± 2.84	0.005

[1]Means with n = 12 per treatment; h/H - hypocholesterolaemic:Hypercholesterolaemic ratio; SCD16 - Stearoyl-CoA 16; SCD18 - Stearoyl-CoA 18; D5D - Delta-5 desaturase; D6D - Delta-6 desaturase.

Table 4.10: Means (± SE) of cooking loss and sensory attributes of spent laying hen breast meat fed conventional and canola meal diets

	Conventional	Canola	P-value
Cooking loss (%)	20.4 ± 1.16	23.0 ± 1.59	0.194
Sensory attributes			
Aroma			
Overall aroma intensity	64.0 ± 0.73	63.8 ± 0.62	0.888
Chicken aroma	63.4 ± 0.72	63.1 ± 0.55	0.727
Metallic aroma	4.8 ± 0.57	4.2 ± 0.36	0.341
Fishy aroma	ND	ND	ND
Fatty aroma (chicken-like)	8.7 ± 0.36	9.1 ± 0.31	0.392
Brothy aroma	11.8 ± 0.59	10.9 ± 0.5	0.291
Flavor			
Overall flavor intensity	63.9 ± 0.61	63.4 ± 0.71	0.590
Chicken flavor	63.4 ± 0.60	62.7 ± 0.57	0.391
Metallic flavor	6.7 ± 0.37	5.3 ± 0.48	0.032
Fishy flavor	ND	ND	ND
Fatty flavor (chicken-like)	8.6 ± 0.22	8.8 ± 0.21	0.587
Brothy flavor	11.7 ± 0.53	10.9 ± 0.41	0.236
Feather	1.3 ± 0.29	0.8 ± 0.24	0.201
Sour taste	ND	ND	ND
Sweet taste	9.4 ± 0.20	9.7 ± 0.17	0.175
Salty taste	9.6 ± 0.10	9.8 ± 0.07	0.119
Texture			
Initial juiciness	35.8 ± 1.09	35.5 ± 0.97	0.837
Sustained juiciness	38.4 ± 1.24	39.1 ± 1.19	0.703
Tenderness	67.0 ± 1.15	66.8 ± 0.86	0.899
Chewiness	7.6 ± 1.00	7.6 ± 0.84	0.996
Mealiness	10.0 ± 0.55	8.6 ± 0.57	0.094
Residue	7.0 ± 0.87	6.3 ± 0.53	0.491
Fatty mouthfeel	1.0 ± 0.01	1.1 ± 0.03	0.142

[1]n = 12. ND – not detectable for spent laying hen breast meat.
Cooking loss is for oven grilled meat samples

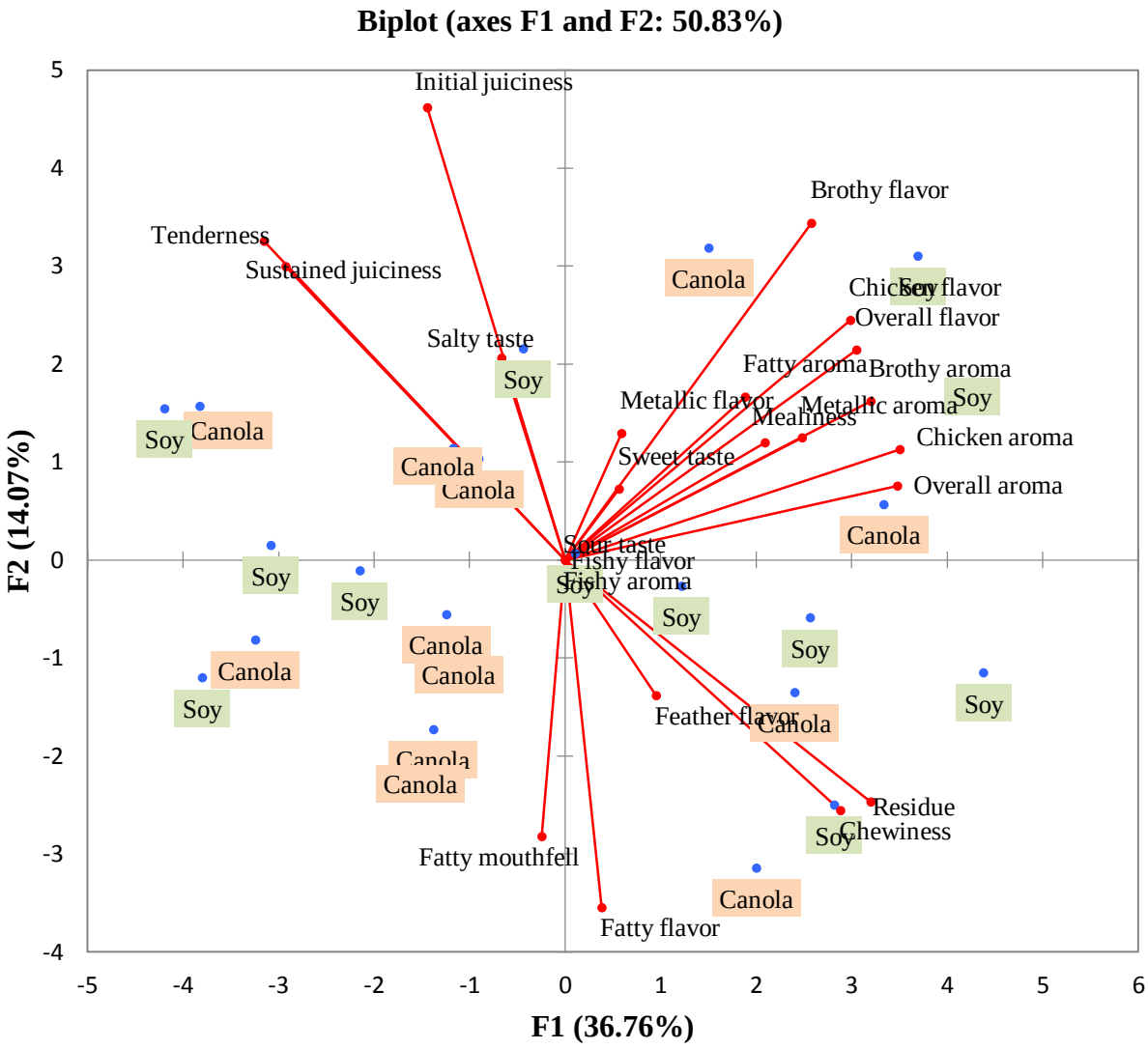

Figure 4.1: Principal component analysis (PCA) biplot of the sensory attributes of spent laying hen breast meat fed conventional and canola meal diets. Canola – canola meal diet; Soy – conventional diet

Table 4.11: Pearson's correlation matrix coefficients (r) and P-values for sensory attributes of spent laying hen breast meat fed conventional and canola meal diets

Variables	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
1. Overall A	**1**	**< 0.001**	**0.040**	**0.032**	**< 0.001**	0.358	**< 0.001**	**< 0.001**	0.696	0.724	**0.006**	0.795	0.333	0.834	**0.004**	**0.001**	**0.002**	0.073	**< 0.001**	0.947
2. Chicken A	**0.965**	**1**	**0.011**	**0.028**	**< 0.001**	0.391	**0.001**	**< 0.001**	0.852	0.763	**0.001**	0.928	0.313	0.829	**0.006**	**0.003**	**0.011**	**0.037**	**< 0.001**	0.831
3. Metallic A	**0.423**	**0.509**	**1**	**0.019**	**0.015**	0.623	**0.020**	**0.012**	0.101	0.864	**0.022**	0.591	0.889	0.779	**0.026**	**0.042**	0.198	0.221	**0.030**	0.589
4. Fatty A	**0.439**	**0.449**	**0.475**	**1**	**0.026**	0.841	0.182	0.237	0.568	0.498	**0.009**	0.596	0.161	0.677	0.451	0.064	0.472	0.895	0.249	0.450
5. Brothy A	**0.827**	**0.860**	**0.489**	**0.454**	**1**	0.768	**0.006**	**0.006**	0.622	0.405	**0.001**	0.218	0.842	0.579	**0.015**	**0.011**	0.095	**0.047**	**0.001**	0.458
6. Initial J	-0.196	-0.184	-0.106	0.043	-0.063	**1**	0.517	0.597	0.337	**0.004**	0.729	0.783	0.465	0.735	**< 0.001**	**0.007**	**0.044**	0.707	**0.019**	0.491
7. Overall F	**0.680**	**0.655**	**0.471**	0.282	**0.542**	-0.139	**1**	**< 0.001**	0.618	0.953	**< 0.001**	0.713	0.756	0.937	**0.047**	0.061	**0.009**	**0.012**	0.071	0.255
8. Chicken F	**0.668**	**0.680**	**0.504**	0.251	**0.541**	-0.114	**0.948**	**1**	0.456	0.797	**< 0.001**	0.945	0.563	0.797	0.050	0.112	**0.022**	**0.008**	0.080	0.385
9. Metallic F	-0.084	-0.040	0.343	0.123	0.106	0.205	0.107	0.160	**1**	0.791	0.357	**0.046**	0.199	0.560	0.830	0.889	0.631	0.366	0.494	0.764
10. Fatty F	-0.076	-0.065	-0.037	0.145	-0.178	**-0.561**	-0.013	-0.056	-0.057	**1**	0.716	0.555	0.621	0.716	0.280	0.110	0.534	0.679	0.529	0.402
11. Brothy F	**0.548**	**0.650**	**0.464**	**0.520**	**0.649**	0.075	**0.700**	**0.681**	0.197	-0.078	**1**	0.631	0.895	0.582	0.368	0.281	0.390	**0.032**	0.212	0.159
12. Feather F	0.056	0.020	0.115	0.114	0.261	-0.059	0.079	-0.015	**0.411**	0.127	0.103	**1**	0.597	**0.004**	0.391	0.079	0.348	0.570	0.337	0.895
13. Sweet T	0.207	0.215	0.030	0.296	0.043	0.157	0.067	0.124	0.272	0.106	0.029	0.114	**1**	0.298	0.898	0.579	0.846	0.840	0.637	0.310
14. Salty T	-0.045	-0.047	-0.060	0.090	-0.119	0.073	-0.017	0.055	-0.125	-0.078	-0.118	**-0.569**	0.221	**1**	0.648	0.077	0.168	0.650	0.337	0.993
15. Sustained J	**-0.564**	**-0.545**	**-0.453**	-0.161	**-0.489**	**0.771**	**-0.409**	-0.404	0.046	-0.230	-0.192	-0.184	0.028	0.098	**1**	**< 0.001**	**0.001**	0.244	**< 0.001**	0.838
16. Tenderness	**-0.621**	**-0.582**	**-0.418**	-0.384	**-0.511**	**0.537**	-0.387	-0.333	-0.030	-0.335	-0.229	-0.366	-0.119	0.368	**0.790**	**1**	**< 0.001**	0.149	**< 0.001**	0.550
17. Chewiness	**0.600**	**0.508**	0.272	0.154	0.349	**-0.415**	**0.523**	**0.466**	0.103	0.133	0.184	0.200	0.042	-0.291	**-0.655**	**-0.845**	**1**	0.085	**< 0.001**	0.515
18. Mealiness	0.373	**0.428**	0.259	0.029	**0.410**	-0.081	**0.506**	**0.527**	0.193	0.089	**0.440**	0.122	0.044	0.098	-0.247	-0.303	0.359	**1**	0.085	0.514
19. Residue	**0.680**	**0.678**	**0.443**	0.245	**0.632**	**-0.476**	0.375	0.364	0.147	0.135	0.264	0.205	0.101	-0.205	**-0.763**	**-0.855**	**0.783**	0.359	**1**	0.330
20. Fatty M	0.014	0.046	-0.116	-0.162	-0.159	-0.148	-0.242	-0.186	-0.065	0.179	-0.297	-0.028	0.216	0.002	0.044	-0.128	0.140	-0.140	0.208	**1**

Numbers and attributes in the first column match with the numbers in the first row and represent the attributes; Non-shaded regions represent Pearson correlation coefficients (r); Shaded region represents matching P-values for the Pearson correlation coefficients (r); Bold values show Pearson correlation coefficients that are significant at $P < 0.05$ as well as matching P-values in the non-shaded and shaded regions, respectively; A – aroma; F – flavor; J – juiciness; T – taste; M – mouthfeel.

4.4 Discussion

4.4.1 Carcass characteristics

The carcass characteristics of meat animals (including chicken) are critically controlled by stage of growth, plane of nutritional and age of slaughter (Keeton *et al.*, 2014). Feeding broiler chickens canola meal has been reported to significantly reduce final body and carcass weights (Khajali and Slominski, 2012; Moraes *et al.*, 2016). In the current study, a numerical reduction was observed in carcass weights. The negative impacts of canola meal on carcass weights are attributed to high dietary fiber content, anti-nutritional factors, low metabolizable energy and protein quality compared to soybean meal (Mushtaq *et al.*, 2007; Radfar *et al.*, 2017). The high dietary fiber content hinders and slows down nutrient digestion, increasing gastro-intestine retention time (Khajali and Slominski, 2012; Gopinger *et al.*, 2014; Radfar *et al.*, 2017). The latter negatively impacts on feed intake, nutrient absorption and growth performance (Tuunainen *et al.*, 2016; Radfar *et al.*, 2017). However, there are studies that show non-significant carcass weights between canola meal and conventional diet fed chicken (Mikulski *et al.*, 2012; Gopinger *et al.*, 2014; An *et al.*, 2016). The higher thigh percentage of the conventionally fed spent laying hens in the current study could be attributed to the amount of abdominal fat attached to the thigh portions (Table 4.5). Tuunainen *et al.* (2016) noted a decrease in the amount of abdominal fat of broiler chickens when fed canola meal in the diet. The reduction in the percentage of thigh could be the reason for the increase in the drum percentage of the canola meal fed spent laying hen carcasses.

The canola meal fed spent laying hens had a high breast bone weight and percentage (Table 4.5). A high carcass bone percentage is regarded as economically detrimental to producers as bones are not consumed by humans (Astruc, 2014). Additionally, a canola meal diet also reduced the breast meat percentage of the spent laying hens (Table 4.5). This is in agreement

with the results of Gopinger *et al.* (2014), An *et al.* (2016), Tuunainen *et al.* (2016) and Moraes *et al.* (2016), as these authors also reported that feeding canola meal to broiler chickens negatively impacts on breast yield. Tang *et al.* (2007) described lysine as the major amino acid that controls breast muscle development through protein synthesis. However, lysine is the major limiting amino acid in canola meal (Slominski *et al.*, 1999; Radfar *et al.*, 2017). Mushtaq *et al.* (2007) conducted a study using graded levels of canola meal and digestible lysine in which they showed that feeding broiler chickens 30% canola meal with increasing levels (0.9% and 1.0%) of digestible lysine enhances breast yield. Therefore, the low lysine content of canola meal could be the cause of the low breast meat percentage in this study.

4.4.2 Physical characteristics

The physical traits of meat are paramount as they determine the functional properties of meat, which are key during meat processing. Moreover, physical traits are the primary determinants of consumers' willingness to purchase the meat. For instance, color is ranked as number one and tenderness is third in the ranking of the determinants of the purchasing power of consumers (Coggins, 2012). Feeding of canola meal to hens increased the expression of undesirable characteristics in the carcass and meat; increased redness (a*) and reduction of yellowness (b*) of the skin and higher shear force values of the breast meat (Table 4.6). Yellowness of the skin is preferred by some consumers as it is considered a natural skin color for chickens (Fanatico *et al.*, 2007). The high redness and low yellowness of the skin result in an undesirable pinkish color (Ponte *et al.*, 2008). The color variation in this study could be attributed to alterations in the diet formulation or the effect of the water temperature during the defeathering process (as discussed in detail in Chapter 3). Moraes *et al.* (2016) noted that the inclusion of canola meal may result in a decrease of corn in the diet. Corn has been

reported to contain the carotenoid pigment xanthophyll which is responsible for imparting yellow skin color to chickens (Brown *et al.*, 2008; Sales, 2014); it is common for the South African animal industry to feed yellow maize rather than white maize to chicken. The increase in the hue angle with decreased Chroma can be interpreted to mean that feeding laying hens canola meal produced carcasses with a more reddish hue (color) and less vivid skin color. Moreover, since hue angle and Chroma are derivatives of a* and b*, the reasons (diet and water temperature during defeathering process) for changes in the a* and b* could be the same for the less and more Chroma and hue angle, respectively.

The tenderness of the breast meat was significantly reduced by feeding the hens canola meal (Table 4.6). The shear force values of breast meat this study differ from those of Mikulski *et al.* (2012); as these authors reported an increase in breast meat tenderness of turkey when the diet included 18% canola meal. However, Gopinger *et al.* (2014) and Moraes *et al.* (2016) recorded a non-significant decrease of breast meat tenderness of broiler chickens fed graded levels (0, 25, 50 and 100%) of canola meal as a protein source. The decrease of breast meat tenderness observed in this study (Table 4.6) could be attributed to the significant reduction of the intramuscular fat (IMF) with the addition of canola meal in the diet (Table 4.7). The relationship between IMF and meat tenderness was clearly highlighted as a positive correlation by O'Quinn *et al.* (2012), Purchas (2014) and Corbin *et al.* (2015). However, Purchas (2014) emphasized that the relationship between IMF and meat tenderness is not linear. The shear force values observed in this study (12.37 N and 15.43 N) are similar to the range reported for broiler chicken meat in the literature: 11.9 N, 16.0 N and 16.7 N by Chen *et al.* (2007), Schilling *et al.* (2008) and Hashim *et al.* (2013), respectively. However, the current shear force values are lower than those reported for spent laying hen meat: 30.79 N and 25.49 N by Chuaynukool *et al.* (2007) and Chueachuaychoo *et al.* (2011), respectively. Canola meal diet also decreased the thaw loss of the breast portion, taking it close to

acceptable limits of 4.7%, which is recommended for the better visual appearance of the packaged portions (Chambaz *et al.*, 2001). The decrease in the thaw loss could be ascribed to the diet and the nature of the protein of the muscles.

4.4.3 Chemical composition

Numerous studies have reported no significant difference in moisture, protein and ash content for the breast meat of broiler chickens (Gopinger *et al.*, 2014; Moraes *et al.*, 2016), turkeys (Mikulski *et al.*, 2012) and rabbits (El-Medany and El-Reffaei, 2015) when fed diets containing canola meal. Canola meal has been reported to contain high levels of fat due to the addition of the seed gum during processing (Khajali and Slominski, 2012; Grageola *et al.*, 2013; Canola Council of Canada, 2015). Hence, the high fat content of canola meal translates into increased dietary fat when included in the rations. López-Ferrer *et al.* (2001), NRC (2012) and Tuunainen *et al.* (2016) noted that dietary fat content is positively correlated with the fat content of meat among monogastric animals (including chicken). The latter has been clearly reported by Moraes *et al.* (2016), who reported a quadratic increase in the fat content for breast meat when broiler chickens were fed with graded levels (0, 25, 50 and 100%) canola meal as a protein source. Additionally, the inclusion of 30% of the canola meal decreased breast meat fat content while 100% canola meal in the diet increased the fat content. For the current study, the inclusion of 20% canola meal in the layer diet significantly reduced the fat content of the breast meat from 3.8 % to 3.1 % (Table 4.7). The reduction of the fat content of the breast meat could be ascribed to both major and minor feedstuffs included in the rations. The increase of canola meal in poultry diets is typically followed with a decrease of soybean oil to achieve an isoenergetic ration. The lower fat content of breast meat from canola meal fed spent laying hen could also be ascribed to the low soybean oil in the diet (Moraes *et al.*, 2016).

Chicken (monogastric) have the capacity to directly incorporate dietary FA profiles into the meat (NRC, 2012; Tuunainen *et al.*, 2016). In the current study, feeding canola meal to laying hens significantly reduced the percentage of palmitic acid, stearic acid and palmitoleic acid, while increasing lignoceric acid, nervonic acid and ALA in the breast meat of spent laying hens. Canola meal has been reported to contain lower percentages of myristic acid, palmitic acid, stearic acid, palmitoleic acid and higher percentages of lignoceric acid, nervonic acid and ALA than soybean meal (NRC, 2012; Jokić *et al.*, 2013). This could be the reason for the respective lower and higher percentages of the latter FAs in the breast meat of canola meal than conventional (soybean meal) fed spent laying hens. Myristic acid has been reported to be higher in soybean than canola meal (NRC, 2012), therefore it can be anticipated that the aforementioned principle would apply and its composition would reflect in the meat FA profile. However, the opposite was observed in this study as the myristic acid percentage was found to be higher in the breast meat of canola-fed (0.6 ± 0.05) than in soybean meal fed (0.4 ± 0.06) spent laying hens (Table 4.8). This is of great importance as Wood *et al.* (2008) explained that myristic acid is mainly derived from the diet and the dietary composition is expected to be strongly reflected in the meat. The possible explanation for this could be the interconversion of myristic acid into other FAs such as palmitic acid, stearic acid and myristoleic acid (Wang *et al.*, 1991).

Since palmitic acid was the main FA in the SFA, its significant decrease in the breast meat of spent laying hens when fed canola meal could be the reason for the reduction of SFA. The reduction of SFA is important to the human dietary composition as these FAs have hypercholesterolemic properties, which can lead to coronary heart diseases (Ahmed *et al.*, 2015). The increase of ALA cannot be ignored as this FA is a precursor for the synthesis of all n-3 PUFAs. Humans lack the enzymes for *de novo* synthesis of ALA; hence they strictly rely on the content of this FA in their dietary composition (Bradbury, 2011). This makes

ALA a major constituent of functional foods. Since DHA can be synthesized from ALA, the higher percentage of DHA in the breast meat of canola than soybean meal fed spent laying hen could be ascribed to the increased percentage of ALA. The significant increase in n-3 PUFA could be ascribed to the higher percentages of ALA and DHA in the breast meat of canola than soybean meal fed spent laying hens (Table 4.8). Additionally, n-3 PUFA in the tissue is more dependent on the n-3 PUFA content in the diet (López-Ferrer *et al.*, 2001) and canola meal has been reported to contain a higher percentage of n-3 PUFA than soybean meal.

The PUFA:SFA and n-6:n-3 PUFA ratios are the main determinants of the nutritional status of fat in meat (Ahmed *et al.*, 2015). A PUFA:SFA and n-6:n-3 PUFA ratio of ≥ 0.4 and ≤ 0.4, respectively are recommended for health conscious consumers as this reduces the incidence of cholesterolaemia (Santos-Silva *et al.*, 2002). The PUFA:SFA and n-6:n-3 PUFA ratios were both higher than 0.4 for the breast meat of canola and soybean meal fed spent laying hens, even though the canola meal fed spent laying hens breast meat had more favorable ratios (Table 4.8). The significant difference in the PUFA:SFA and n-6:n-3 PUFA ratio could be ascribed to the higher SFA and lower n-3 PUFA percentages of breast meat from conventional than canola meal fed spent laying hens which are dependent on the diet. A decrease in n-6:n-3 PUFA ratio was reported in studies when broiler chickens were fed canola meal as a substitute for soybean meal (Moraes *et al.*, 2016; Tuunainen *et al.*, 2016). The n-6:n-3 PUFA ratios (7.2 ± 0.28 vs. 5.5 ± 0.13) of spent laying hens observed in this study (Table 4.8) are lower than those (10.00 vs 7.99) of broiler chicken reported by Moraes *et al.* (2016) for soybean and canola meal fed, respectively.

For a better understanding and nutritional evaluation of fat, use of health indices based on the functional effects of the FAs is essential (Ahmed *et al.*, 2015). The atherogenic (IA) and

thrombogenic (IT) indices should be maintained as low as possible and the opposite for hypocholesterolemic:Hypercholesterolaemic (h/H) ratio in a healthy heart diet (del Puerto *et al.*, 2017). Feeding canola meal to laying hens decreased the IA and IT indices in the breast meat from 0.5 ± 0.01 to 0.4 ± 0.01 and 0.8 ± 0.03 to 0.6 ± 0.01, respectively (Table 4.9). Moreover, the h/H ratio was increased from 2.4 ± 0.09 to 3.0 ± 0.11 for breast meat from soybean and canola meal fed spent laying hens, respectively (Table 4.9). Similar results for IA (0.5 – 0.4) and higher for IT (1.2 – 1.0) were observed for broiler chicken (del Puerto *et al.*, 2017). The decrease of IA and IT with an increase of h/H indices illustrates the reduction of inducer (C14:0, C16:0, C18:0) and the increase of inhibitor (C16:1n7, C24:1n9, C18:3n-3, C22:6n-3) FAs of hypercholesterolaemia (Table 4.8). Hence, breast meat from canola meal fed spent laying hens would be recommended for consumers' heart health.

Substrate and products can also be used in calculus to compute the enzyme activity indices of respective enzymes. The indices can be an alternate determinant of actual enzyme activities. The enzymes stearoyl-CoA desaturase 16 (SCD16) and stearoyl-CoA desaturase 18 (SCD18), delta-5 desaturase (D5D) and delta-6 desaturase (D6D) are prime in the synthesis of MUFA and PUFA, respectively, as they insert double bonds into saturated FA (Haug *et al.*, 2014). The SCD16 and SCD18 indices were higher for the breast meat from canola than soybean meal fed spent laying hens. This could be attributed to the higher substrate FA for the two enzymes in the breast meat of the canola meal fed spent laying hens. Higher activities of SCD16 and SCD18 are recommended as it increases the percentage of unsaturated FAs in the diet. The lower D5D could be ascribed to the lower n-6:n-3 PUFA ratio of the diet containing canola meal. Haug *et al.* (2014) also observed a decrease in desaturase enzymes when broiler chickens were fed diets with low n-6:n-3 PUFA ratios. Elongase and thiosterase are responsible for synthesis and termination/release of FA (del Puerto *et al.*, 2017). The activities of the aforementioned enzymes are reflected on the selective cleavage of C14 and

C16, hence the release of C14:0 and C16:0. The higher the cleavage, the lower the index (Popova *et al.*, 2016; del Puerto *et al.*, 2017). In this study, breast meat from canola meal fed spent laying hens had low elongase (0.39 ± 0.018 vs. 0.4 ± 0.012) and thiosterase (33.5 ± 2.84 vs. 48.7 ± 3.40) indices (Table 4.9). The elongase and thiosterase indices reported in this study are in line with those reported by Popova *et al.* (2016)

4.4.4 Sensory attributes

Flavor which is a combination of taste and aroma, as well as texture forms the core of the sensory profile of meat and meat products. The aforementioned attributes are strongly correlated to the physicochemical characteristics of meat and meat products. Fat content and FA profile have been identified as the primary determinant of chicken meat flavor and texture (Wood *et al.*, 2003; Rabe *et al.*, 2003; Dawson and Spinelli, 2012; Moraes *et al.*, 2016). In this study, although the instrumental texture (shear force) and chemical analyzes (fat content and FA profile) differed between breast meat derived from spent laying hens fed conventional and canola meal diets (Tables 4.4, 4.5 and 4.6), these differences could not warrant significant differences in the sensory profile except for metallic flavor. Chicken meat flavor has been reported to be influenced by dietary composition. However, Barbut (2015) highlighted that chicken meat flavor is less influenced by diet, since a large change in the dietary composition is needed to effect a minute change in the flavor. Land and Hobson-Frohock, (1977) and Barbut (2015) also stated age as the major determinant of chicken meat flavor, with flavor increasing with age until sexual maturity. Hence the lack of differences between the breast meat sensory profile (except for metallic flavor) of conventional and canola meal fed spent laying hens could be ascribed to the fact that both groups were sexually mature hens and within the same age range of 40 and 48 weeks of age, respectively. The higher metallic flavor of breast meat from soybean meal fed spent laying hens than canola

meal fed could be ascribed to the dietary iron content. The NRC (2012) noted that soybean meal has higher iron content (173 ppm) than canola meal (163 ppm). The iron content of food has been reported to be responsible for the metallic flavor (Ömür-Özbek *et al.*, 2012).

The PCA biplot (Figure 4.1) grouped most of the sensory attributes in the right top quadrant which was associated with meat derived from both canola meal and conventional fed spent laying hens; hence the lack of variance between the meat samples. The separation along PC 1 (F1) and PC 2 (F2) was principally ascribed to the association between texture attributes (initial juiciness, tenderness, residue and chewiness). The PCA biplot explained only 50.83% of the association between the sensory attributes and the dietary treatment, with 36.76% and 14.07% ascribed to PC 1 and PC 2, respectively, which is considered low. The strong correlation observed (Table 4.11) between sensory attributes could be used to accurately predict other with minimum errors. The overall flavor was very strongly correlated to chicken aroma which means that the sensory panel was able to perceive chicken aroma as the major contributor to the total flavor of chicken meat derived from spent laying hens. The aforementioned aspects also apply to overall, chicken and brothy flavor. The amount of moisture in the meat is mainly linked to the initial and sustained juiciness which in turn control meat tenderness (Barbut, 2015). The initial and sustained juiciness were both found to be strongly positively correlated to tenderness. The perceived increase of meat tenderness as moisture increases could also be attributed to the reduction in the chewiness, which both lead to a reduction of residues left in the mouth. The tenderness, initial and sustained juiciness were all found to be negatively correlated to chewiness and residue of breast meat derived from spent laying hens.

4.5 Conclusions

The effects of feeding canola meal to laying hens were observed to be more positive for the carcass characteristics and for the breast meat physical, proximate, fatty acid profile and lipid health indices, but not affecting for the sensory profile when compared to hens fed on conventional diets. The carcass weights of spent laying hens in this study were lower than the minimum standard market weight for broiler chickens, irrespective of the dietary regime. This emphasizes the lower economic value of spent laying hens, which discourages abattoirs from slaughtering them for meat production purposes. Feeding canola meal to laying hens decreased the percentage of the breast meat; this was ascribed to the low lysine content of canola meal. Hence, the lysine content of the diets should be given attention when formulating diets containing canola meal. The sensory profile of breast meat from spent laying hens was similar for canola and soybean diets. Spent laying hen breast meat (with more focus on canola meal fed) can be marketed as a functional food as this study highlight low fat and better lipid health indices.

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Chapter 5

General discussion, conclusions and recommendations

5.1 General discussion

The inadequate utilization of spent laying hen meat by the poultry industry is aggravated by limited scientific information on the carcass and meat quality of these hens. Scientific research has concentrated on broiler chickens, as they contribute 96% of the total poultry meat produced and consumed in South Africa (SAPA, 2016). Although broiler chicken production has increased in South Africa, the chicken meat market is still overwhelmed by the ever-increasing demand by consumers (DAFF, 2014). The high demand for chicken meat is ascribed to a number of beneficial components of this meat: a low fat content, low n-6:n-3 PUFA ratio, tenderness and the low cost per kg. In some countries where little research has been executed on spent laying hens, their meat is used for special delicacy recipes which are highly valued, and helping to curb the heavy demand for broiler chicken meat (Hill, 2009). Additionally, spent laying hens are regarded as a byproduct of the egg producing farms/industry as they have little economic value since their meat is considered tough.

For the past decade, there has been an increasing use of canola meal in laying hen diets in order to produce eggs with high nutritive value, also referred to as 'functional eggs'. Monogastric animals have been proven to directly assimilate dietary fatty acid profiles into the final products (eggs and meat) (NRC, 2012; Tuunainen *et al.*, 2016). The aforementioned is the basic principle to the recent incorporation of canola meal and oil into poultry diets to achieve functional foods. Although the impact of canola meal inclusion in poultry diets has been well studied among broilers and turkeys (Coetzee and Hoffman, 2000; Mikulski *et al.*, 2012; Moraes *et al.*, 2016), scientific research on laying hens is limited to its effects on eggs. Providing consumers with vital information on the carcass, meat and sensory quality of spent

laying hens overturn current negative perceptions of this meat source; hence increasing the consumption of spent laying hens to help meet the high demand for chicken meat.

Through genetic breeding, the current laying hen strains have been modified to suit modern housing conditions for improved egg production and quality. However, these current laying hen strains are still showing some behavioral traits (perching, nesting, dust bathing, etc) of their wildfowl ancestors (Kjaer and Mench, 2003; Bingham, 2013). The conventional battery cages restrict laying hens from expressing their natural behaviors which impacts on egg production and quality (Mugnai *et al.*, 2011). Modern production systems such as free ranging improve the quality of eggs. The free range systems have also been reported to impart beneficial aspects to the meat of broilers such as low fat content, skin and meat yellowness and n-3 polyunsaturated fatty acids (n-3 PUFA). However, for the latter to be achieved, broiler chickens must be reared for a longer period of 8 – 10 weeks than the normal 5 – 6 weeks (Fanatico *et al.*, 2005). Also, free range systems have gained popularity among egg producing farmers and consumers. Although laying hens spend most of their entire lifespan free ranging, little is known about the effect of foraging on the carcass and meat quality of these hens when their production cycle is terminated.

In this thesis, two separate studies were conducted to establish the effects of production systems and canola meal supplementation on carcass characteristics and meat quality of spent laying hens (Chapters 3 and 4). In Chapter 3, the effect of free range and conventional battery cage production systems on the carcass characteristics, physical attributes and proximate composition of spent laying hens was evaluated. Production systems were observed to have an effect on all the aforementioned attributes of spent laying hens. It was observed that granting access to free range decreased carcass, thigh, wing and foot weights as well as breast meat percentages, and increased breast and thigh Warner-Bratzler shear force (WBSF) values

plus gizzard and breast bone weights. The observed significant differences could be ascribed to the increased motory activity, uncontrolled environmental conditions and diet in the free range vs. battery cage systems. Free range hens are given more space indoors and outdoors to exercise which impacts negatively on the growth rate, carcass characteristics and meat physical attributes, results similar to those reported by Castellini *et al.* (2002). Moreover, the increased dietary fiber in the pasture increases the digestive tract weight in order to effectively break down the feed, which also reduces the weights and percentages of the prime portions of the carcass (Ponte *et al.*, 2008; Mateos *et al.*, 2012). Free range spent laying hens also had higher skin and meat redness. This could be ascribed to the increased motor activity as well, which leads to accumulation of myoglobin in muscles to provide oxygen and support for the increased activities. Myoglobin is the main determinant of meat redness; however, it is not readily detectable in chicken meat (Zhuang and Savage, 2012; Barbut, 2015), hence the redness values in this study were less than one.

Chapter 4 evaluated the effect of diet (canola supplementation of 20% vs. conventional feed) on carcass characteristics, meat quality, fatty acid profile, lipid health indices and sensory attributes of spent laying hens. Diet had an effect on all the aforementioned attributes, but less effect on sensory traits of spent laying hens. The low thigh percentages of the canola meal fed spent laying hen could be ascribed to the reduction of abdominal fat (usually included in the thigh portion), as canola meal has been reported to decrease abdominal fat of broiler chickens (Tuunainen *et al.*, 2016). Furthermore, the numerical reduction in the muscle weight could be the reason for an increase in bone weight and percentage. Lysine is the main determinant of muscle development for chicken breast (Tang *et al.*, 2007). However, lysine is the most limiting amino acid in canola meal. Several studies have reported a decrease in breast meat weight and percentage when broiler chickens were fed canola meal (Tuunainen *et al.*, 2016; Moraes *et al.*, 2016). Spent laying hens fed canola meal had undesirable skin color

and tenderness: lower b* and Chroma with higher a* values, hue angle and WBSF values than conventionally fed hens. The changes in the aforementioned color values resulted in a more reddish/pink skin color that could lead to product rejection by some consumers (Ponte *et al.*, 2008). The changes in the skin color values could be ascribed to the reduction of corn in the canola meal supplemented diet. Corn provides the carotenoid pigment xanthophyll which imparts the desired yellow skin color to chickens (Brown *et al.*, 2008; Sales, 2014). The lower intramuscular fat (IMF) content of the breast meat of spent laying hens fed canola meal than a conventional diet could be the explanation for the higher WBSF values of the breasts. The relationship between IMF and meat tenderness was clearly highlighted as a positive correlation by O'Quinn *et al.* (2012), Purchas (2014) and Corbin *et al.* (2015).

Feeding a 30% canola meal supplementation has been reported to decrease the IMF of broiler chicken breasts (Moraes *et al.*, 2016), as observed in Chapter 4 with a 20% canola meal supplementation fed spent laying hens' breast meat. The lower fat content of breast meat from canola meal fed spent laying hens could also be ascribed to the minor (low soybean oil) and major (canola meal vs. soybean meal) feedstuffs in the diet. Moraes *et al.* (2016) observed that substituting soybean meal with 100% canola meal increased the IMF of broiler chickens. These authors explained that an increase of canola meal in poultry diets is followed with the increased inclusion of soybean oil to achieve isoenergetic rations which could be the reason for the high IMF of the breast meat. Canola meal has been reported to contain lower percentages of myristic acid (C14:0), palmitic acid (C16:0), stearic acid (C18:0), palmitoleic acid (C16:1n7) and total saturated fatty acids (SFA), with higher percentages of lignoceric acid (C24:0), nervonic acid (C24:1n7), alpha-linolenic acid (ALA, C18:3n-3) and n-3 PUFA than soybean meal (NRC, 2012; Jokić *et al.*, 2013). Chickens similar to other monogastric animals have the ability to directly incorporate dietary fatty acids (FA) into their muscle. This

principle was observed with the FA profile of breast meat from spent laying hens fed a canola meal supplementation of 20% as well as a 100% soybean diet (Chapter 4).

A PUFA:SFA and n-6:n-3 PUFA ratio of ≥ 0.4 and ≤ 0.4, respectively, is recommended for health conscious consumers as this ratio reduces the incidence of cholesterolaemia (high blood cholesterol) (Santos-Silva *et al.*, 2002). Spent laying hen breast meat in this study was healthy and had higher than recommended PUFA:SFA ratio (≥0.7), though canola meal fed spent laying breast had a better ratio (0.9 ± 0.02) and was thus even healthier (Chapter 4). Canola meal fed spent laying breasts also had a better n-6:n-3 PUFA ratio (5.5 ± 0.13) since it was closer to the recommended value than soybean fed (7.2 ± 0.28). However, the n-6:n-3 PUFA ratios recorded in the study were above the recommended value of 0.4. Canola meal fed spent laying hen breast meat had lower a antherogenic index (AI), thrombogenic index (IT), delta-5 desaturase (D5D), elongase and thiosterase with a higher hypocholesterolemic:Hypercholesterolaemic (h/H), stearoyl-CoA desaturase 16 (SCD16) and stearoyl-CoA desaturase 18 (SCD18) than soybean meal fed; the objective being to keep AI and IT as low as possible, and h/H as high as possible for a healthy heart of the consumer (del Puerto *et al.,* 2017). The enzymes SCD16 and SCD18, D5D and delta-6 desaturase (D6D) are prime in the synthesis of MUFA and PUFA (Haug *et al.*, 2014). Since all the aforementioned ratios and indices are calculus derivatives of individual FA percentages, the reasons for the changes in the individual FA percentages could be the same as for the lower and higher ratios and indices observed in Chapter 4. Similar results for IA and higher for IT were observed for broiler chicken (del Puerto *et al.,* 2017).

Metallic flavor was observed to be lower for breast meat from canola meal than soybean fed spent laying hens (Chapter 4). Metallic flavor was the only significant different attribute in the sensory analysis of the meat from the two diets. The iron content of food has been

reported to be responsible for a metallic flavor (Ömür-Özbek *et al.*, 2012). The higher metallic flavor of breast meat from soybean meal fed spent laying hens than canola meal supplemented could be ascribed to the dietary content of iron. The level of iron in canola meal (173 ppm) has been reported to be higher than that of soybean meal (163 ppm) (NRC, 2012). However, the metallic flavor score (6.7 ± 0.37 vs. 5.3 ± 0.48) recorded in this study could be low enough not to be picked up by a normal consumer.

5.2 Conclusions and recommendations

Production systems and canola meal inclusion (20%) in layer diets had an effect on selected carcass characteristics, physical attributes, chemical composition and the sensory profile of meat from spent laying hens. The carcass weights observed in the study were lower than the minimal market target weight of broilers, which partly explains the lower economic value ascribed to spent laying hens. The breast fat content of the spent laying hen meat was observed to be close to the recommended fat content (< 3%) of meat, and could therefore be recommended for health conscious consumers. Canola meal incorporated beneficial components in the breast meat of spent laying by lowering the IMF, SFA and n-6:n-3 PUFA ratio as well as having more favorable healthy indices. This would indicate that canola meal fed spent laying hens can be marketed as a food for a healthy heart (a functional food). Further research is recommended on:

- The shelf life of the meat derived from spent laying hens as influenced by canola meal inclusion (20%) in the diet and production systems.
- The functional and technological properties (protein fractions and properties, water retention capacity, electrical conductivity) of spent laying hen meat as influenced by production systems and canola meal inclusion (20%) in the diet as the properties affect further processing of the meat and meat products.

- The fatty acid profile, lipid health indices and sensory attributes of meat from spent laying hens reared under the free range and conventional battery cage systems. This will give a better understanding of the eating quality and lipid related benefits of meat derived from spent laying hens reared under these production systems.

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Appendices

Appendix 1: Laying hens in the conventional battery cage production system

Appendix 2: Laying hens in the deep litter house of the free range production system

Appendix 3: Outdoor access area of the free range production system