Response in Physicochemical Characteristics of Broiler Meat to Incremental Levels of *Vachellia tortilis* Leaf Meal

By

Andiswa N. Sithole

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Supervisor: **Professor Michael Chimonyo**

Declaration

I, Andiswa Ntonhle Sithole declare that:

- The work presented in this dissertation, except where indicated by the specific acknowledgment of the respective authors, is my own original research work.
- The thesis has not been submitted for any degree or examination at any other University but the University of KwaZulu-Natal.
- The thesis does not contain any other person's data, tables, pictures, graphs or other information, unless specifically sourced from those persons.

Adhering to the points listed above:

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Signed:…………………………………………… Date:……………….................

Name: Andiswa Ntonhle Sithole

As the Research Supervisor, I agree to the submission of this dissertation/thesis for examination.

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Name: Professor Michael Chimonyo

Dedication

This dissertation is dedicated to all the innovative minds that are passionate about research and my family.

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List of Abbreviations

a^*	redness in meat
ADF	acid detergent fibre
ATP	adenosine triphosphate
b^*	yellowness in meat
$CaCl_2$	calcium chloride
CP	crude protein
DDGS	distiller’s grains
EE	ether extract
ICP	International Poultry Counsel
L^*	lightness in meat
Mg	magnesium
Na^+	sodium ions
$NaCl_2$	sodium chloride
ND	not documented
NDF	neutral detergent fibre
pH_u	ultimate pH (pH at 24 hours post slaughter)
PM	post mortem
SAPA	South African Poultry Association
STPP	sodium phosphate
WHC	water holding capacity
Zn	zinc
$ZnCl_2$	zinc chloride
FAO	Food and Agriculture Organisation

General Abstract

The broad objective of the current study was to determine the relationship between inclusion levels of *Vachellia tortilis* and physicochemical attributes of broiler meat. A total of 120 Cobb 500 broilers were randomly allocated to 0, 30, 60, 90, 120, 150 g/kg *Vachellia tortilis* leaf meal diets at 14 days of age. At the age of 32 days the birds were humanely slaughtered.

Average feed intake and average daily gain had a positive linear relationship with *Vachellia tortilis* inclusion levels. Moisture, crude protein, fat, calcium, potassium, zinc, iron and magnesium were not significantly (P>0.05) related to *Vachellia tortilis* inclusion. The relationship between ash and *V. tortilis* was described by Y = 5.41 (± 2.56) – 50.23 (± 18.40) + 203 (± 27.79). The equation Y= 0.07 (± 0.20) x^2 + 1.79 (± 1.40) x + 0.36 (± 2.12) described the relationship between calcium and *V. tortilis*. The relationship between copper and *V. tortilis* inclusion levels was described by Y = 0.04 (± 0.01) x^2 – 0.35 (± 0.08) x + 0.78 (± 0.13). The equation Y = -0.80 (± 0.32) x^2 + 6.64 (± 2.29) x + 12.10 (± 3.47) described the relationship between sodium and *V. tortilis*. The increase in calcium may assist with diabetes management. It is, therefore, crucial to monitor mineral content of feeds when leaf meals are included.

Cooking loss, shear force, drip loss and redness were not significantly related to *V. tortilis* inclusion. The relationship between *V. tortilis* and lightness was described by Y = 0.484 (± 1.638) x -14.435 (± 43.904). The increase in 1 g/kg in *V. tortilis* let to a 2.15 unit increase in yellowness. The equation Y = 2.393 (± 2.180) x -23.782 (± 20.456) described the relationship between *V. tortilis* increase and chroma range. The relationship between pH at 24 hours and *V. tortilis* inclusion levels was described by Y = 29.467 (± 60.463) x -75.148 (± 183.795). The increase in yellowness is likely to increase consumer satisfaction on broiler meat.

Keywords meat quality, colour, cooking loss, minerals, drip loss, polyphenolic compounds.

Table of Contents

List of Tables

List of Figures

Chapter 1

General Introduction

1.1 Background

Chicken meat reduces health risks to humans (Kearney, 2010). The preference for chicken meat by consumers may be driven by the increased awareness about diseases caused by saturated fats which lead to obesity, heart disease and cancer (WHO, 2003; Charlton *et al*., 2008). There is, therefore, a need to develop appropriate broiler feeding strategies that are aimed at improving meat quality.

The rapid increase in chicken production increases the use of conventional feed resources such as maize and soybean. These feed resources are used by humans, feed compounders and farmers. The competition between humans and livestock increases the demand for these conventional feed resources. There is, therefore, a need to explore the utilization of non-conventional feed resources. Non-conventional feed resources are raw materials that are not used in the formulation of commercial feeds for livestock (Amata, 2014). Non-conventional feed resources such as agro-industrial by-products include maize cobs, palm kernel meal, brewer's grains. These non-conventional feed resources are neither used for feed nor food. They are available in large quantities and are discarded when they may be recycled to yield high quality feed.

The use of leaf meals in monogastric feeding has been investigated (Halimani *et al*., 2005; Khanyile *et al*., 2014; Ncube *et al.*, 2018). Inclusion of *Vachellia* leaf meals in poultry and pig diets at appropriate levels does not compromise growth performance due to the high amount of crude protein, essential amino acids, minerals and vitamins. Grass swards are invaded by Vachellia species that may be used in broiler feeding (Smit *et al*., 1999; Mahajana and Cronje, 2000; Ward, 2005). *Vachellia tortilis* leaf meal has protein levels greater than

200 g/kg, desirable amino acid composition, vitamins and minerals (Khanyile *et al*., 2014). Assessing the potential of *Vachellia tortilis* should not be confined to growth performance, but also on the quality of end products that consumers demand for. The potential of *V. tortilis* leaf meal in improving broiler meat quality is yet to be determined.

Vachellia tortilis, formerly known as *Acacia tortilis,* is an umbrella shaped legume tree with curled pods which is found in various arid and semi-arid regions. This legume could be included in poultry diets as a protein source, which would greatly reduce the proportion of soybean meal. *Vachellia tortilis,* however, contains a considerable amount of anti-nutritional polyphenolic compounds. These polyphenolic compounds reduce feed intake and nutrient utilization (Forbes, 1995). They also increase endogenous losses of protein (Barry *et al*., 1986). The increase in *V. tortilis* could, therefore, reduce the amount of fat in meat. Polyphenolic compounds act as antioxidants, minimize rancidity and reduce lipid peroxidation without damaging meat sensory and nutritional quality (Jang *et al*., 2008; Lahucky *et al*., 2010; Qwele *et al*., 2013). *Vachellia tortilis* could, thus, improve the chemical and physical properties of meat.

One measure of meat quality is the lean-to-fat ratio. Lean contains protein, ash, minerals and moisture (Cassar-Malek and Picard, 2016). Acceptability is influenced by appearance, firmness, juiciness, tenderness and the flavour of the meat. Colour, marbling and water holding capacity, in turn, influences the appearance of meat. Freshness, flavour, safety and tenderness are some of the most important meat attributes consumers look for (Schönfeldt and Jooste, 2015). Polyphenolic compounds present in most leaf meals are expected to alter, if not improve most of these meat quality characteristics (Priolo and Vasta, 2007; Ngambu *et al*., 2013; Moyo *et al*., 2014).

1.2 Justification

Consumer consciousness of meat quality awareness is increasing (Ncube *et al*., 2018). There is a need to explore the inclusion of non-conventional feed resources that improve meat physical and chemical attributes. There is also an increase in competition between humans and chickens for grains, restricting the growth of the poultry industry, mostly in developing countries (Mapiye *et al.*, 2010). Leaf meals could mitigate the feed shortages. Utilisation of leaf meals is also likely to contribute towards reducing bush encroachment (Smit *et al*., 1999). The limited understanding of the relationship between leaf meals and meat physicochemical properties, as well as human health restricts the exploitation of these technologies. Studies on the role of leaf meals have focused mostly on digestibility and carcass yield). The use of leaf meals also has the potential to contribute to the production of organic meat. Organic or free range chickens have been reported to have a better vitamin, mineral and fatty acid profile (omega 3 to omega 6 ratio) (Lorente-Cebrián *et al*., 2013). Utilization of plants that are rich in polyphenolic compounds, therefore, has a potential to enhance food and nutritional security of households in semi-arid areas (Mlambo and Mapiye, 2015). Due to the presence of polyphenolic compounds in *Vachellia tortilis* leaves, its inclusion should be restricted. Dose-response trials are, therefore, required to determine inclusion levels that optimize broiler meat quality. Such trials also allow prediction of meat quality at any given inclusion level. Meat colour is used primarily to predict meat freshness during purchase. Cooking loss and tenderness are related to meat juiciness (Michalczuk *et al*., 2014). For growing pigs, Vachellia leaf meals inclusions should range from 60 to 130 g/kg (Halimani *et al*., 2005; Khanyile *et al*., 2017). Based on growth performance, similar ranges were also suggested for broilers (Ncube *et al*., 2018).

1.3 Objectives

The broad objective of the study was to determine the relationship between feeding incremental levels of *Vachellia tortilis* leaf meal and broiler meat quality. The specific objectives were to:

1. Determine the relationship between *Vachellia tortilis* leaf meal inclusion and chemical composition of broiler meat; and
2. Assess the relationship between feeding incremental levels of *Vachellia tortilis* leaf meal and the physical attributes of broiler meat.

1.4 Hypotheses

The study tested the hypotheses that:

1. *Vachellia tortilis* leaf meal inclusion has a relationship with the chemical composition of broiler meal; and
2. *Vachellia tortilis* leaf meal has a relationship with the physical attributes of broiler meat.

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Chapter 2

Review of literature

2.1 Introduction

The increase in nutritional based health conditions has increased consumer consciousness of broiler meat quality (Ncube *et al.,* 2018). There has also been an increase in the human population which has driven the increase in meat consumption, particularly broiler meat (FAO, 2015). Both these factors have increased the demand for healthier chicken meat. There is no universal definition for meat quality. This is because meat quality is defined by consumers in various definitions and it also varies with culture (Borgaard and Anderson, 2004; Monin, 2004; Xazela *et al*., 2011). Meat quality describes the quality characteristics including physical and chemical characteristics of meat that can be measured using scientific methods for research purposes (Joo *et al*., 2013). It is affected by nutrition, genetics, environment, cooking, pre-and post-slaughter handling, lairage, husbandry, aging time and slaughter weight (Hedji *et al*., 2015; Moholisa *et al*., 2018; Nogalski *et al*., 2018). Pre- and post-slaughter conditions influence meat physical properties but no evidence supports any changes in meat chemical composition except for fat content (Hall *et al*., 2015). Meat fat content reduces with the reduction in dietary energy (Hall *et al.*, 2015; Moholisa *et al.*, 2018). The current review discusses chicken consumption and elaborate on how the need for healthier meat has driven the need for non-conventional feed resources. Leaf meals that improve meat quality and their characteristics are also reviewed. The effects of leaf meal diets along with *Vachellia tortilis* effects on the physical and chemical meat characteristics are also discussed.

2.2 Current global status of chicken meat consumption

Often, mixed portions consist of frozen wings, thighs, drumsticks and breasts. Breast quarters are often sold alone as fresh meat along with drumsticks. Wings and thighs are mostly sold as frozen or fresh mixed portions. Frozen chicken is injected with brine to improve water holding capacity. The reduction in potassium, magnesium and iron by brine solution results. There is, therefore, a need to produce more chicken that is appetizing and healthy. Non-conventional raw materials need to be explored. Utilization of non-conventional feeds is likely to reduce competition for raw materials with the growing human population.

Chicken consumption has increased by 25 % between 2008 and 2013 (FAO, 2015). Meat consumption in developing countries is growing three times faster than in developed countries (FAO, 2015). The reason could be the increase in household incomes and reduction in poverty levels in developing countries (Delgado, 2003). In South Africa, for example chicken consumption has increased by 0.5 kg per person between 2014 and 2015 (SAPA, 2015). Demonstrating that chicken consumption is increasing at an accelerating rate. The accelerated increase could be caused by the consumer perceptions of chicken meat, chicken having less ethical, religious and lack of traditional restrictions (Font-i-Furnols and Guerrero, 2014; Skunca *et al*., 2017). Such patterns suggest that development of non-conventional feed resources for chicken feeds that improve meat quality need to increase rapidly. Most non-conventional feeds have an acceptable amount of protein and amino acid profile along vitamins and minerals (Khanyile *et al*., 2014) making them viable options to replace conventional feed resources in feeding chickens. Figure 2.1 illustrates the increase in global chicken meat consumption at different year intervals.

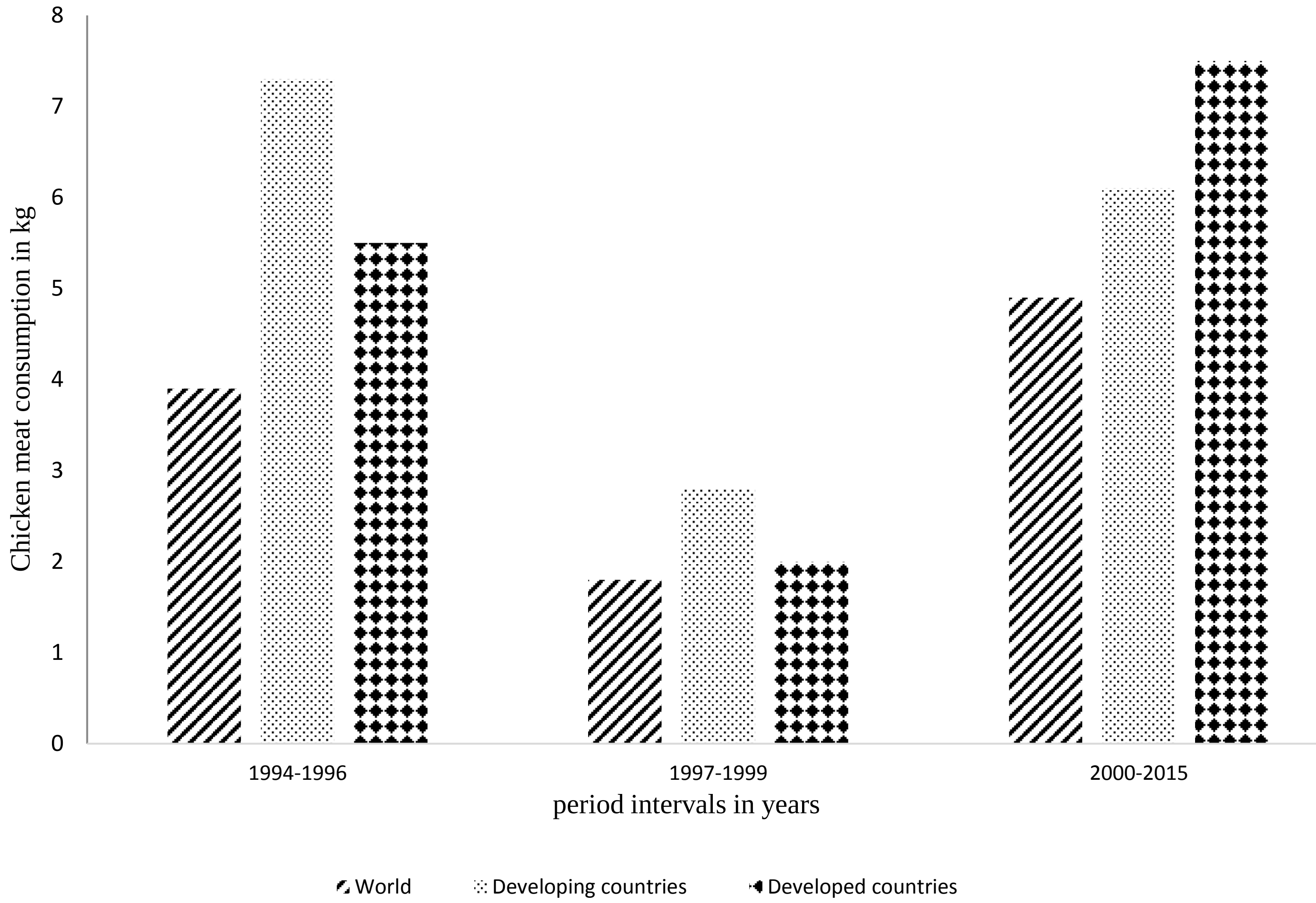

Figure 2.1: Chicken meat consumption over years in developing and developed countries

2.3 Use of non-conventional feed resources in broiler diets

Examples of non-conventional feed resources include agro-industry by-products, tropical crop residues and leaf meals. Non-conventional feed resources are usually characterized by low energy and protein levels, high concentrations of lignin and anti-nutritional factors. These resources are usually found in abundance and are readily available (Sogbesan and Ugwumba, 2008). Non-conventional feed resources also include earthworms, snails, termites and tadpoles which have a good amino acid profile and may replace fishmeal.

Agro-industrial by-products include brewers dried grains, palm kernel meal, winery mash and maize cobs. Brewers dried grains, palm kernel meal and maize cobs are important in West Africa (Sogbesan and Ugwumba, 2008). Palm kernel meal does not affect juiciness, tenderness, and flavour in broiler chickens' meat (Okeudo *et al*., 2006). There is, however, minimal understanding of how palm kernel meal, maize offal and brewers dried grains affect acceptability of broiler meat. There is abundant understanding of their digestibility, growth performance and carcass yield (Onifade and Babatunde, 1998; Ironkwe and Bamgbose, 2012; Diarra *et al*., 2015).

Kitchen waste is recycled organic waste is mostly used as pig feed, commonly practised in Asia and the United States of America (FAO, 2015). It is also known as garbage. These wastes also include bakery products, cheese, eggs, yoghurt, and ice cream; left over and expired food along with mislabelled food (FAO, 2015). If such wastes contain meat it could predispose chickens to salmonella infection. Waste has a low dry matter content, thus, reduces nutrient intake thereby limiting growth performance and increasing soft fat deposition consequently reducing meat quality. The nutrient composition of garbage varies greatly, making it difficult to use it in formulating consistent diets that optimise nutrient intake. Waste has, otherwise, a great potential in mitigating the increased conventional raw materials needed without negatively affecting meat quality. Sensory, chemical and physical attributes of indigenous chicken meat in Taiwan was not affected by garbage feeding (Chen *et al*., 2007). Waste consisting mostly of fruits and vegetables; contains phytochemicals, antioxidants/ polyphenolic compounds, antimicrobials and vitamins (FAO, 2015). Polyphenolic compounds and antimicrobials improve shelf life and meat quality. Table 2.1 shows the chemical composition of some agro-industrial by-products that have been tested on chicken performance. The availability of these nutrients has not been determined, making it difficult to predict meat quality.

2.4 Use and nutritional attributes of leaf meals in broiler diets

Leaf meals have been reported to improve meat quality in goats, cattle and poultry. *Vachellia karroo*, *Moringa oleifera*, *Gliricidia sepium*, *Vachellia angustissima* and *Manihot esculenta,* are some of the leaf meals that have been reported to improve broiler meat quality (Moyo *et al.*, 2014; Nkukwana *et al*., 2014; Ncube *et al.*, 2018).

Both *Vachellia karroo* and *Gliricidia sepium* are from the Fabaceae family. These trees are used for fencing, feed, and medicine. Moringa is used as food, feed, dietary supplements and medicine. Leaf meal from these plants have varying amounts of protein, fat, ash, fibre and polyphenolic compounds. Regardless of their varying nutrient composition they all have protein levels higher than 200 g/kg, thus may be used as a supplementary protein sources and may, in turn, improve meat quality. Table 2.2 shows the chemical composition of the common leaf meals.

Table 2.1: Chemical composition of agro-industrial by-products and kitchen waste

Component	Agro-industrial by-products (g/kg)			Kitchen waste
	Palm kernel meal[a]	Maize cobs[a]	Brewers dried grains[a]	
Max inclusion	450[b]	200	200	ND
Dry matter	906	913	925	954
Crude protein	183	124	216	234
Crude fibre	163	349	197	45
Ether extract	80	21	34	97
Ash	45	34	36	ND
ADF	427	285	382	ND
NDF	668	509	712	ND

Sources: [a] Onifade & Babatunde (1998); [b] Okeudo *et al*., 2006; ND- Not documented

ADF: Acid detergent fibre; NDF: Neutral detergent fibre.

2.5 Leaf meals anti-nutritional factors on broiler diet utilization

Leaf meals contain polyphenolic compounds and high amounts of fibre that reduce nutrient digestibility. Some fibre sources reduce egg yolk and plasma cholesterol, minimizing health hazards associated with consumption of chicken meat and eggs (Esmail, 2012). In sheep, polyphenolic compounds were also found to reduce plasma cholesterol (Silanikove *et al*., 2006). Polyphenolic compounds also bind to proteins, lipids, carbohydrates and fibres making then unavailable to enzyme digestion.

Embedded in fibre are high levels of bioactive anti-nutritive factors such as saponins. Those compounds have hypocholesterolemic properties which cause fibre to bind to nutrients (Silanikove *et al.*, 2006). Fibre binds to the bile acids in the intestines and causes more acids to be excreted with the faeces. Appropriate fibre inclusion could also reduce the amount of abdominal fat in broilers (Safaa *et al*., 2014). In turn, this reduces the chances of cardiovascular diseases that are usually associated with high cholesterol levels in meat consumers.

Nitrogen balance has been reported to be improved by incorporating maize distillers' grains (DDGS) and wheat middling in hen diets (Esmail, 2012). Khanyile *et al*. (2017) reported that *V. tortilis* had a similar effect. Amino acids are less digestible in fibrous feed than in low-fibre feed. More amino acids are needed to satisfy the requirements for digestible amino acids (Duodu *et al*., 2003). An improved nitrogen balance would, therefore, lead to lean tissue as there would be adequate nitrogen for energy provided thus less excess energy to be stored as fat.

Table 2.2: Chemical composition of leaf meals

Component (g/kg)	Leaf meal				
	Vachellia karroo[a]	*Moringa oleifera*[b]	*Gliricidia sepium*[c]	*Manihot esculenta*[e]	*Acaciella angustissima*
Optimum inclusion	12.0	25.0	50.0[c, h]	50.0[d]	50.0[f]
Dry matter	919.0	922.0	925.0	253.0[d]	900.0[f]
Crude protein	232.0	267.6	244.0	251.0[d]	234.0[f]
Crude fibre	ND	157.2	125.0	114.0[d]	130.0[f]
Ether extract	39.5	56.3	16.2	127.0[d]	27.8[g]
Ash	51.0	108.1	86.2	91.0[d]	40.1[g]
Acid detergent fibre	289.0	137.9	ND	303.0[e]	153.0[g]
Neutral detergent fibre	502.0	137.9	ND	414.0[e]	287.0[g]
Polyphenolics	5.0	12.0	13.0	ND	10.6[g]

a- Ngambu *et al*., 2013; b- Nkukwana *et al*., 2014; c- Olorunola *et al*., 2016; d- Iheukwumere *et al*., 2007 e- Ravindran *et al*., 1986 ; f- Ncube *et al*., 2018; g- Gudiso, 2016 ND-Not documented; Max inclusion-maximum/optimal inclusion levels

2.5.1 Distribution and uses of *Vachellia tortilis*

Vachellia tortilis is an umbrella shaped legume thorn tree with curled pods. It is one of the most important species from the genus *Acacia* in the Leguminaceae family. Usually, it is found in arid and semi-arid regions of the world like, North Africa, Arabian Peninsula and Asian countries. *Vachellia tortilis* is resistant to drought, high temperatures, low temperatures and defoliation. It is, therefore, an even more valuable legume for non-conventional raw materials in the Southern region. Where these trees are distributed, soil erosion is not likely, and they also keep soils fertile.

Vachellia tortilis protein content is not comparable to that of soybean meal. The amount of fibres and lignin in *V. tortilis*, which is the cause for restricting its inclusion in broiler diets, is higher than the reported quantity in soybean meal. Due to its nutritious attributes when compared with other non-conventional feeds and its potential in improving meat quality, it could be used as a supplement in conventional feeds. Tables 2.3 and 2.4 show the differences in nutritional attributes between soybean meal and *V. tortilis*.

Vachellia tortilis is used as medicine, food, feed and commercial products. Its leaves are high in fibre, which is regularly higher than its crude protein content, polyphenolic compounds with some being condensed, and lignin. These characteristics in *V. tortilis* imply its inclusion should be restricted especially in chicks. For example, when included to mitigate fibre content, fibrous raw materials should be restricted. In small ruminants, polyphenolic compound-rich raw materials have been reported to have a negative effect on flavour, tenderness and juiciness (Lanza *et al*., 2001).

2.5.2 Anti-nutritional factors in *Vachellia tortilis*

Vachellia tortilis inclusion levels should be restricted because, like most leguminous leaf meals it is high in polyphenolic compounds which restrict nutrient utilization. Fibre and polyphenolic compounds dilute nutrients especially with increased inclusion in a diet. Fibre may have a positive effect on mineral absorption when included at accurate levels. *Vachellia tortilis* fibre content does not cause reason for concern unless there are other fibre sources which may increase the overall fibre content of the diet. Increased fibre content results in a reduced amino acid availability, thus, the reduced meat quality due to the increased fat deposition expected under these circumstances.

2.5.2.1 Polyphenolic compounds

Polyphenolic compounds are a distinctive group of polymers which have the capacity to form complexes with carbohydrates and proteins rendering them unavailable (Theodoridou, 2010). These interactions depend on temperature and ionic strength (Hagerman *et al*., 1998). Polyphenolic compounds have been reported to improve meat quality and shelf life of the meat. The type of polyphenolic compounds present in *V. tortilis* leaves are proanthocyanidins and hydrolysable polyphenolic compounds, and 1, 3-di-O-4, 6- (-) hexahydroxydiphenoyl-β glycopyranose. proanthocyanidins are flavonoids which when hydrolytically cleaved yield anthocyanidins (Davis, 2005). Hydrolysable polyphenolic compounds have a low molecular weight, cleaved to a monosaccharide and Gallic acid or ellagic acid. These are highly toxic to monogastrics as they may lead to gastro-enteritis and increase endogenous losses caused by the damaged gut wall. The damage also reduces the reabsorption or cause hyper-secretion of protein (Brooker, 2000). These effects may be increased in chickens as they have a lower amount of taste buds, thus more tolerant to diets with polyphenolic compounds compared to pigs (Nakalebe, 2010). Meat protein and fat may, therefore, be reduced. Figure 2.2 shows hydrolysable polyphenolic compounds which include gallic, ellagic and cynammic acid.

High levels of polyphenolic compounds are characterized by low carcass weights and increased abdominal fat caused by imbalances in the protein to energy ratio (Kumar *et al*., 2005). Theodoridou (2010) highlighted that polyphenolic compounds bind to both carbohydrates and proteins. Further, Kumar *et al*. (2005) reported that broilers fed on red sorghum containing 8.58 g/kg polyphenolic compounds exhibited a reduced energy efficiency. A similar effect was also reported for crude protein (CP) efficiency. Crude protein efficiency only reduced at 750 g/kg red sorghum inclusion, implying that crude protein efficiency is more resistant to the effect of polyphenolic compounds. The abdominal fat yield is thus, reduced feed high in polyphenolic compounds is fed. Contradicting results on cholesterol levels have been reported in meat infested with polyphenolic compounds from varying sources (Kamboh and Zhu, 2013; Przywitowski *et al*., 2016). Ahmed (2015) reported that broilers ingesting sorghum polyphenolic compounds had an adaptive effect at higher protein levels. Further, ash was reduced in diets with 2.35g/kg of polyphenolic compounds from faba beans (Przywitowski *et al.*, 2016). As expected with reduction in ash, the increase in polyphenolic compounds decreases mineral bioavailability (Ahmed, 2015). Since polyphenolic compounds vary in chemical composition their effect may also vary with the different plant species. The nutritional and metabolic consequences of proanthocyanidins are still poorly understood. Proanthocyanidin variation in chemical structure and composition is also not fully understood making it difficult to predict responses in meat quality parameters (Brooker, 2000).

Table 2.3: Chemical composition of *Vachellia tortilis* and soybean meal

Nutrients (g/kg)	Raw materials	
	Soybean meal	*Vachellia tortilis*
DE (MJ/kg DM)	17	9
Energy digestibility (%)	85	49
Gross energy (MJ/kg DM)	20	18
Dry matter	879	910
Crude protein	518	140
Ether extract	20	83
Crude fibre	67	94
Neutral detergent fibre	137	551
Acid detergent fibre	83	321
Ash	71	117
Lignin	8	137
Polyphenolic compounds	69	602

Table 2.4: Mineral composition of *Vachellia tortilis* and soybean meal

Minerals (g/kg)	Raw materials	
	Soybean meal	*Vachellia tortilis*
Calcium	3.90	34.10
Phosphorus	6.90	1.50
Sodium	0.10	0.60
Magnesium	3.10	3.90
Zinc	0.05	0.02
Copper	0.02	0.04
Iron	0.35	0.23

2.5.2.2 Fibre

Vachellia tortilis fibre content is good for gut health. Fibre reduces nutrient digestibility when inclusion levels are higher than 100 g/kg in broilers (Esmail, 2016). This means the fibre in *V. tortilis* may not affect digestion negatively but is subject to other fibrous raw materials that are in the diet and inclusion levels.

Long term feeding of moderately fibrous diets may lead to improved mineral utilization varying with various fibre sources. Fibrous diets containing oat hulls improves the retention of sodium (Na) and potassium (K). Sodium, potassium and chloride are essential for acid base balance and fluid balance in tissues (Henry, 1995). Increased sodium and/ or potassium in relation to chloride may lead to alkalosis which may reduce meat physical and chemical quality attributes (Henry, 1995).

Excess chloride in relation to sodium and/ or potassium leads to acidosis. This could lead to lower meat pH values possibly reducing meat quality. Soybean hulls improved Copper retention and iron retention was improved by Lucerne meal, soybean hulls and oat hulls (Esmail, 2016). The improved mineral retention would lead to improved meat quality with an adequate mineral balance. The influence of *Vachellia tortilis* on mineral content of meat needs to be investigated. *Vachellia tortilis* has been shown to increase the amounts of sodium in the blood, suggesting it could lead to higher pH values in the meat improving tenderness.

2.6 Meat quality measures and composition

Meat quality is composed of two characteristics, compositional and nutritional characteristics. Compositional meat quality is the lean-to fat ratio factors (Font-i-Furnols and Guerrero, 2014). Nutritional meat quality traits refer to the nutritional composition of the meat; protein, minerals and fat. Nutritional quality as perceived by the consumers is thought-out and most of the physical characteristics are greatly biased (Font-i-Furnols and Guerrero, 2014). As such, consumers need to be considered when assessing meat quality. Behavioural factors of consumers in determining meat quality also needs to be understood. Font-i-Furnols and Guerrero (2014) have reported that the factors that affect consumer behavioural patterns the most in chicken consumption are sensory characteristics. These include appearance, texture, flavour and odour respectively. For consumers, appearance is mostly influenced by the colour of the meat. Unfortunately, appearance is also influenced by the consumers' expectations of the meat by visually assessing the quality at the point of purchase.

Meat quality traits include sensory, chemical and physical properties. Chemical properties include crude protein, ash, fat or ether extract, moisture, minerals and fatty acids. Physical properties include colour, tenderness, pH, temperature and water holding capacity. Minerals that affect meat quality are dominantly calcium (Kerth *et al.*, 1995), phosphorus, magnesium, zinc and sodium (Koohmaraie, 1992). Some meat quality traits may be evaluated using surveys and/ or using laboratory testing.

In lean muscles, there is approximately 750 g/kg water, 20 g/kg protein, 3 g/kg lipids, 2 g/kg non-protein substances (carbohydrates, organic compounds and ash which is minerals) (Cömert *et al.*, 2016). When there is a higher plain of nutrition there is higher fat and less moisture in muscle fibres and may also indicate glycogen stores (Woods, 1999). These nutrients are essential for health and for biochemical processes which include homeostasis in the broiler and human body.

2.7 Factors affecting chemical properties of broiler meat

Water is held mostly in muscles and muscle structures; within myofibrils, between myofibrils and between myofibril and cell membrane (Huff-Lonergan and Lonergan, 2005). Most of the water is held in the myofibrils by capillary forces arising from arrangement of thick and thin filaments (Offer and Cousins, 1992). The location of the water can be changed by the change in muscle volume during rigor (Offer and Cousins, 1992). Water holding capacity and juiciness of the meat could, therefore, be reduced by the movement of water to extra-myofibrillar space. Sarcomeres remain iso-volumetric during contraction and relaxation, thus, the amount of water in the living muscle does not change in the filamentous structure of the cell. When rigor takes place, muscle cell diameter reduces and may lead to the transmittal of lateral shrinkage of myofibrils to the whole cell. When sarcomeres are shortened, they reduce the space available for water retention within the myofibrils. In some cases, this change could be linear. Further, water may be bound; water that is found in close vicinity with non-aqueous elements, protein for example, because water is dipolar (Offer and Cousins, 1992). This water has reduced mobility, resistant to freezing and being driven off by translation heating. Cooking loss is reduced if there is an increased amount of bound water. It could also be entrapped, which means it is attracted by bound water and is most affected by conversion of muscle to meat during rigor.

Polyphenolic compounds reduce the utilization of energy, thus lipogenesis. Lipids are also important in human health (Essen-Gustavsson *et al*., 1994; Kouba *et al*., 2003). White muscles have lower marbling than red muscle types (Wood *et al*., 2004). Excess energy in feed is converted to fat which enhances flavour. When there is too much fat, overconsumption of that meat by humans may lead to obesity. Wood *et al*. (2004b) demonstrated that feed intake in animals is determined by the need to satisfy their requirements for protein. Should polyphenolic compounds affect energy efficiency more than protein efficiency, there would be less marbling. Demonstrating polyphenolic compounds reduction in energy efficiency. Diarra *et al*. (2014) reported a reduced fat deposition with the increase in *Manihot esculenta* leaf meal inclusion. Marbling enhances juiciness and tenderness. In contrast, Renand *et al*. (2003) and Thompson (2014) reported marbling reduces these characteristics. Plausibly, this could be due to the amount of charged fatty acids or hydrophilic carboxylic acid groups deposited in the muscle. The charged fatty acids attract water molecules leading to the meat being juicy through retaining its water binding ability. If there are charged proteins attracting water molecules, it could also lead to juicy meat (Huff-Lonergan and Lonergan, 2005). Fat presence also assists with ensuring fibres do not contract too fast too soon pre-rigor leading to juicy meat.

Table 2.5 shows broiler meat composition for various body parts. Water, protein and fat contents are greatly related to muscle content and are muscle dependent. Khanyile *et al*. (2016) reported that *V. tortilis* increased crude protein and reduced fat in feed, and thus absorbed nutrients will be altered. In contrast there are reports that Moringa and *V. angustissima* inclusion did not alter crude protein and moisture content (Qwele *et al*., 2013; Ncube *et al.*, 2018). Ash and fat contents were altered by the inclusion of leaf meals.

Minerals are naturally occurring inorganic solid substances. They are separated into macro and trace minerals. Trace minerals are found in smaller quantities and macro minerals are needed in greater quantities. Minerals play an essential role in meat quality and the functioning of the live chicken. Doyle (1980) reported that minerals are absorbed in a different manner for different meat types and differs with gender. There is minimal understanding on mineral absorption and factors affecting their composition. Table 2.6 shows broiler meat mineral composition (Zapata *et al*., 1998).

2.8 Factors affecting physical properties of broiler meat

Vachellia tortilis inclusion has been reported to increase phosphorus, magnesium, potassium, sodium and iron and reduces zinc and copper in the diet (Khanyile *et al*., 2016). These findings suggest that *V. tortilis* as a supplement to soybean meal may improve meat quality. There is evidence that Calcium chloride ($CaCl_2$) improves the rate of proteolysis post-mortem by accelerating inactivation of calpastatin, given the pH levels and temperatures are conducive (Koohmaraie, 1992). Kerth *et al*. (1995) report implies that 0.2 or 0.25 ml $CaCl_2$ increases tenderness and purge and has no effect on meat colour. It also does not accelerate proteolysis because ionic strength increase does not alter proteolysis. Koohmaraie (1992) reported that calcium inhibits Z-disk disappearance and myofibrillar fragmentation when magnesium in present.

Vachellia tortilis and soybean have similar magnesium content, so this effect might not be obvious with partial replacement of the one by the other. Calcium needed to activate μ-calpains in the muscle is 0.01 ml and 0.2 to 0.3 ml to activate m-calpains which is much higher than calcium in living muscles (Koohmaraie, 1992). The calcium in *Vachellia tortilis* as a supplement possibly, could lead to the activation of μ-calpains leading to tender meat. Fat presence assists with ensuring fibres do not contract too fast too soon pre-rigor for this effect.

During proteolysis, the calpain proteases depend on calcium to proteolyze the myofibrillar proteins for tenderization of the meat (Koohmaraie, 1992). Post-mortem calcium may reach up to half the minimum required to activate m-calpains which is adequate to activate μ-calpains. Slower proteolysis may be halted before complete proteolysis has taken place due to proteolytic enzyme denaturing at pH of less than 6. *Vachellia tortilis* may lead to a higher pHu, ensuring that proteolytic enzymes do not denature. Supplementation with vitamin D_3 between 0.06 and 0.19 ml improves plasma and muscle calcium thus tenderness (Swanek *et al*., 1999).

Table 2.5: Broiler meat chemical composition

Component(g/kg)	Meat		
	Whole Chicken	Breast	Thigh
Weight (g)	GD	350[b]	338[b]
Moisture	746[a]	749[b]	753[b]
Ash	10[a]	ND	ND
Protein	121[a]	215[b]	181[b]
Lipid	111[a]	10[b]	51[b]
Fibre	0	0	0
Carbohydrates	12[a]	ND	ND

Sources: a- USDA; b- Sevcikova *et al*. (2006) (Muscle) feed with DM-898.4; CP-215.3; Fat-60.0; Fibre-35.2; Ash-51.0; Calcium-8.20; Phosphorus-5.74; ME (MJ/Kg)-12.55; GD- Genotype Dependent.

Table 2.6: Broiler meat mineral composition

Minerals	Mineral composition (g/kg)	Mineral composition (g/kg) (breast)	
		Females	Males
Phosphorus	0.33	2.74	1.77
Potassium	0.32	3.73	2.86
Sodium	0.15	0.43	0.25
Magnesium	0.04	0.02	0.02
Calcium	0.008	0.05	0.07
Zinc	0.004	0.01	0.008
Iron	0.004	0.007	0.004
Manganese	0.0002	0.28	0.27

Sources: Zapata *et al*. (1998); Karakök *et al*. (2010)

Vitamin D_3 stimulates calcium mobilization from previously formed bone mineral and through 1, 25-dihydroxyvitamin D_3. Renal reabsorption of calcium, thus plasma calcium concentration is increased by 1, 25-dihydroxyvitamin D_3 (Kerth *et al.*, 1995). Supplementing with vitamin D_3 reduces magnesium concentrations but, glucose, phosphorus, sodium and potassium remains the same. Tender meat may result as magnesium concentrations are reduced optimizing Z-disk disappearance.

Vachellia tortilis has a lower zinc content than soybean meal which may be advantageous as zinc chloride ($ZnCl_2$) has been reported to inhibit post-mortem proteolysis. Koohmaraie (1992) has attributed this to the failure of calpastatin inactivation postmortem. Fast twitching white muscles have been reported to have this effect as they are naturally higher in zinc. Sodium chloride ($NaCl_2$), post-mortem solubilizes proteins resulting in calpain activation or non-enzymatic weakening of structural proteins that stabilizes Z-disk protein (Koohmaraie, 1992). These effects result in tender meat. *Vachellia tortilis* inclusions may also have similar effects, increasing tenderness as it has greater sodium content. Jerez *et al*. (2003) reported that with different sodium sources, tenderness, colour and WHC varies due to inhibition of glycolysis effect.

Handling the birds pre-and post-slaughter should be monitored as this could affect meat quality. When chickens are handled in a manner that induces stress before slaughter muscle glycogen stores are depleted. In turn, this results in a higher pH owing to insufficient glycogen to convert to lactic acid (Pollard *et al*., 2002). Long-term stress also reduces glycogen stores leading to pale, soft and exudative (PSE) meat. Increased drip loss owing to the PSE state of meat results. Short term stress leads to rapid glycolysis post slaughter, pre-rigor. Excessive pH reduction over a short period of time denatures muscle proteins and proteases especially in high temperatures is usually observed under these conditions. Broilers need to be slaughtered quickly and bled as much as possible to avoid any redox reactions which could result in darker meat.

2.8.1 Storage temperature

Temperature is heat intensity. Post-mortem room temperatures from 20 to 40^{o}C have been shown to lead to 30 % muscle shortening, 0 to 10^{o}C shortening of 50 % from its original size but minimal muscle shortening occurs at 14 to 20^{o}C pre-rigor (Lonergan *et al*., 2010). At higher temperatures, rapid post-mortem glycolysis leads to rapid drop in pH (Rathgeber *et al*., 1999a; Sandercock *et al*., 2001). The rapid drop in pH in turn, leads to a speeded post-mortem rigor onset. Subsequently, reduced WHC and high lightness L^* values and pale meat in appearance (Rathgeber *et al*., 1999). Rapid cooling leads to slower glycolysis which in turn results in tough meat (Wakefield *et al*., 1989).

Low temperatures lead to cold shortening and reduction in calcium sequestering ability of the sarcoplasmic reticulum. Best lightness and tenderness results have been reported at 10℃ with a pH of 6.2 (Dransfield and Sosnicki, 1999). Table 2.8 shows the effect of various environmental temperatures pre-rigor, post-mortem affects the sarcomere shortening pre-rigor. There have not been any reports of muscle temperature or on muscle temperature as affected by diet. A correlation between pork meat temperature and meat quality parameters such as drip loss, cooking loss, moisture, fat, protein and colour parameters (Kim *et al*., 2016). Temperature is, therefore, an important parameter to assist explain variations.

2.8.2 pH

The alkalinity or acidity of meat on a logarithmic scale is expressed as pH. Glycogen is a multi-branched polysaccharide of glucose that serves as a form of energy storage in animals, humans, fungi and bacteria. Glucose is a type of sugar found in food and feed which serves as an energy source. Muscle weight consists of 5 to 15 g/kg of glycogen (Przybylski *et al*., 2006). Glycogen also serves as an important source of energy for muscle contraction; as it breaks down post mortem it affects meat quality (Bendall, 1973).

Glycogen content in meat determines the ultimate meat pH (pHu), if there is greater glycogen then there will be a lower pHu. Treatment diets are formulated to have similar energy, so if the treatment does not alter the use of energy by the chickens, pHu would be the same. *Vachellia tortilis* may reduce protein and energy utilization thus, a reduction in glycogen would be expected with increased inclusion of *V. tortilis*. Meat colour, tenderness and WHC in meat muscles may be determined by pH (Aberle, 2001). Greater pH levels caused by diet composition have been reported to improve WHC and tenderness with poor meat colour (Jerez *et al*., 2003). Rapid pH drop leads to the inactivation of calpain systems which are responsible for meat tenderization, therefore, reducing post-mortem tenderization (Dransfield, 1994).

The drop in pH is affected by nutrition and muscle type (Sañudo *et al*., 2004). White muscles have a higher buffering capacity, so pH is higher compared to red muscles. Red muscles have initial glycogen stores, more active glycolytic metabolism, shorter sarcomeres and higher NAD^+ content (Lawrie, 1998; Jerez *et al*., 2003).

Table 2.8: Effects of temperature on pre-rigor shortening

Temperature Pre-Rigor (^{0}C)	Pre-rigor shortening (less than 30 minutes in broilers)
0-10	Sarcomeres shorten to half their size pre-rigor
14-20	Minimal pre-rigor sarcomere shortening
15-20	Shortest pre-rigor sarcomere shortening
20-40	30 % pre-rigor shortening

Source: Lonergan *et al*. (2010)

Higher pH_u which is 5.78 as compared to 5.56 leads to improved WHC and L^* lightness (Le Bihan-Duval *et al*., 1999). The higher pH would be expected with inclusion of *Vachellia tortilis* as it may reduce glycogen.

Vachellia tortilis reduces fat content in broiler meat, leading to reduced energy stored for post-mortem glycolysis, therefore, less pH reduction. As a result, meat may maintain its protein binding ability, therefore, more protein bound water. Rapid pH decline could lead to protein denaturation leading to the loss in protein binding ability (Hambrecht *et al*., 2004). There hasn't been any rapid pH decline associated with *Vachellia tortilis* inclusion levels. Water bound in protein is bound to protein because most proteins are charged. Protein binding to water molecules improves meat juiciness and acceptability.

2.8.3 Drip loss

Drip loss is the fluid lost, mainly consisting of water and protein expelled from a piece of meat without any mechanical force (Fischer, 2007). It is also defined as the weight lost in meat due to the extruding and dripping away of tissue juices or liquid. Diet has not been reported to directly affect drip loss. Low glycogen levels lead to the increase in drip loss (Berri *et al*., 2005). Increased drip loss is also reported in low pHu (Berri *et al*., 2005). Pre-slaughter acute stress also increases drip and cooking loss (Berri *et al*., 2005). *Vachellia tortilis* would be expected to reduce drip loss as its inclusion may lead to a greater pHu.

2.8.4 Cooking loss

Cooking loss is the amount of water lost during cooking (Al-Owaimer *et al*., 2014). Increased cooking loss reduces juiciness in meat which is not desired by consumers (Abu *et al*., 2015).

Cooking loss cannot be determined at purchase. Pre-slaughter, acute stress increases cooking loss (Berri *et al*., 2005). Ogungbesan *et al*. (2014) reported a reduced cooking loss on broilers fed *Gliricida sepium* leaf meal. Similarly, Moyo *et al*. (2014) reported a reduced cooking loss on goats fed on *Moringa oleifera* leaf meal diets. On the contrary, Ncube *et al*. (2018) reported that *V. angustissima* increased cooking loss. Different cooking methods used may have caused the variation. The expected reduction in pHu with inclusion of *Vachellia tortilis* could also result in higher cooking loss.

2.8.5 Colour

Colour is measured by 5 parameters which are L^* (lightness), a^* (redness), b^* (yellowness), chroma range and hue angle. The hue angle is described by the dominant wavelength; it describes colour dimension and saturation. Chroma range indicates the colourfulness of the meat and may be considered as saturation. Saturation indicates colour intensity and hue angle dominance.

Meat colour is related to different forms of sarcoplasmic proteins and depends on housing, diet and genetics ante-mortem (Mancini, 2009). It is determined by intramuscular fat, level and state of myoglobin and moisture content (Adam *et al*., 2010). Meat colour is also attributed to by the myoglobin type and the type of iron attached to a myoglobin type. The increase in iron increases redness values (Moyo *et al*., 2014). If glycolysis is inhibited, meat colour is affected due to the altered redox reactions (Jerez *et al*., 2003). Glycolysis is, therefore, also essential for the stability of meat colour. The diet also influences meat colour. Meat with *V. tortilis* may be darker with lower intramuscular fat (Bruce *et al*., 2004; Webb and Erasmus, 2013). The polyphenolic compounds reported in *V. tortilis* act as antioxidants that reduce myoglobin oxidation and activate mechanisms that modify pigment distribution, enhancing meat colour (Moyo *et al*., 2014). Moyo *et al*. (2014) reported that *Moringa oleifera* increased meat lightness and redness. Broiler meat may be considered as pale the L^* value should be greater than 54 (Woelfel *et al*., 2002).

Consumers perceive meat with colour ranging between red and purple chicken meat as fresh and brown as spoilt (Carpenter *et al*., 2001). They also believe eating satisfaction comes from the colour or is related to the colour. Table 2.7 shows the colour parameters without *V. tortilis.* There are no published papers reporting the colour of broiler meat when fed on *V. tortilis* leaf meal diet.

2.8.6 Tenderness

Tenderness is a multi-parameter sensory attribute (Szczesniak, 2002). It is experienced in first bite, chew down and residual. Tenderness is one of the eating quality attributes that influences most consumers' preferences (Polkinghorne and Thompson, 2010). It is positively correlated with juiciness (Pannier *et al*., 2014). Juiciness and tenderness are correlated with the consumers overall experienced quality, intention and willingness to pay (Banović *et al*., 2009). Texture is affected by refrigeration post-mortem, high temperature carcass hanging, aging period, and cooking procedure (Szczesniak, 2002). It is determined by collagen content and heat stability when cooking (Muchenje *et al*., 2009). After rigor mortis proteolysis takes place. Proteolysis is responsible for the tendering of the meat. Proteolysis is the breakdown of proteins in the muscle by enzymes. It is dependent on pH, temperature, glycogen and skeletal muscle protein composition. Within some breeds, muscle tenderness may vary owing to low protein diets (Wood *et al*., 2004a). *Vachellia Karroo* and *Moringa oleifera* leaf meals have been reported to increase tenderness in goat meat (Ngambu *et al*., 2012; Moyo *et al*., 2014).

Contrary, Ogungbesan *et al*. (2014) reported *Gliricidia sepium* and *Manihot esculenta* leaf meals having reduced meat tenderness.

2.9 Summary

There is minimal understanding of how meat quality is affected by *Vachellia tortilis*. A few outcomes may be deduced from theory work done on the factors that affect meat quality. Using characteristics of *V. tortilis* and what is known on factors that affect meat quality predictions may be made. The inclusion of *V. tortilis* could reduce broiler intermuscular fat leading to a reduced energy content in meat, thus, low glycogen stores. Low glycogen stores lead to a low pH decline. Greater lightness and tenderness values may result from lower glycogen stores. Protein will not be denatured. There would, therefore, be more protein to be degraded during proteolysis increasing tenderness. Increased muscle protein content increases the chances of an increased charged protein molecules. An increased amount of water may be held within the muscles ensuing a reduced drip loss and a greater water holding capacity. Cooking loss could also be reduced leading to an increase in juiciness. The objective of the study was to assess the response in physical and chemical characteristics of broiler breast meat to *Vachellia tortilis* leaf meal inclusion.

Table 2.7: Colour parameters in broiler breast meat

Components	Breast Meat	
L* (Lightness)	51.78	51.90
a* (Redness)	0.35	0.20
b* (Yellowness)	10.79	9.10
Hue	0.60	1.02
Chrome	10.80	9.10

Sources: Debut *et al*. (2003); Berri *et al*. (2005)

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Chapter 3

Response in chemical characteristics of broiler breast meat to *Vachellia tortilis* leaf meal

Abstract

The objective of the current study was to assess the relationship between inclusion levels of *Vachellia tortilis* and chemical attributes of broiler meat. A total of 120 Cobb 500 broilers were randomly allocated to 0, 30, 60, 90, 120, 150 g/kg *Vachellia tortilis* leaf meal diets at 14 days of age. At the age of 32 days the birds were humanely slaughtered. The average daily gain and feed intake were increased by *V. tortilis* inclusion, in the second week they were reduced. Moisture, crude protein, fat, potassium, zinc, iron and magnesium concentrations in meat were not related to *Vachellia tortilis* inclusion. The relationship between ash and *V. tortilis* was described by Y = 5.41 (± 2.56) – 50.23 (± 18.40) + 203 (± 27.79). The equation Y= 0.07 (± 0.20) x^2 + 1.79 (± 1.40) x + 0.36 (± 2.12) described the relationship between calcium and *V. tortilis*. The relationship between copper and *V. tortilis* inclusion levels was described by Y = 0.04 (± 0.01) x^2 – 0.35 (± 0.08) x + 0.78 (± 0.13). The equation Y = -0.80 (± 0.32) x^2 + 6.64 (± 2.29) x + 12.10 (± 3.47) described the relationship between sodium and *V. tortilis*. The increase in calcium is likely to reduce the effect of diabetes. It is, therefore, crucial to monitor mineral content of feeds when leaf meals are included.

Keywords: Calcium, Potassium, Protein, Polyphenolic compounds, Sodium

3.1 Introduction

Meat is a valuable source of highly digestible protein, vitamins and minerals for humans. It could, therefore, alleviate various nutritional deficiencies (Bender, 1992). White meat is deemed healthier than red meat. Chicken meat has fewer religious prohibitions. Perception of meat on human health influences consumers purchase patterns. Chemical composition of meat is, therefore, of great importance to consumers (Jimenez-Colmenero *et al*., 2001). Meat chemical composition is composed of moisture, protein, fat, ash and minerals.

The amount of protein and minerals that broiler meat contributes to the daily human requirement is low for a main digestible nutrient source. Minerals play an essential role in reducing the likelihood of hypertension, diabetes and cancer. There is, therefore, a need for non-conventional feed resources that will assist improve meat chemical composition. Exploring non-conventional feed resources such as leaf meals could aid in improving meat chemical and physical attributes. There is, however, minimal understanding on non-conventional feed resources such as leaf meals that may aid in improving meat chemical composition.

Vachellia species are important in livestock production and have a favourable available mineral composition (Dube *et al*., 2001). The use of Vachellia leaf meals could improve consumer health by improving meat chemical composition. They also have polyphenolic compound that act as antioxidants protecting cells from damage. These desirable polyphenolic compounds reduce the digestibility of feed nutrients in chicken. It is, therefore, important to conduct dose response trial. Dose-response trials assist with determining the optimum inclusions levels of the leaf meal and estimate response at any given inclusion. Vachellia species vary in chemical composition, to account for the variation inclusion levels started lower and ended higher than the suggested inclusions. For broilers, *Vachellia* leaf meal inclusions should range between 10 and 130 g/kg to achieve optimal growth performance (Ngambu *et al*., 2013; Gudiso, 2016; Ncube *et al*., 2018). It is not clear whether chemical properties of meat are realised within the same range.

Vachellia tortilis has high quality amino acids, vitamins and minerals, thus, would be a great supplement (Khanyile *et al*., 2014). A greater nutrient profile increases the chances of greater nutrient absorption. These nutrients could reduce the likelihood of disorders such as obesity, hypertension and cancer (Charlton *et al.*, 2008). The incorporation of *Vachellia tortilis* in broiler feed assist low-income farmers increase food and nutritional productivity. *Vachellia tortilis* incorporation into feed would also reduce bush encroachment and likely mitigate feed and food shortage challenges. The objective of the current study was, therefore, to determine the response in chemical composition of broiler breast meat to *V. tortilis* leaf meal inclusion. It was hypothesized that chemical composition will have a quadratic relationship with *Vachellia tortilis* inclusion.

3.2 Materials and methods

3.2.1 Study site

This study was conducted with full ethical approval from the University of KwaZulu-Natal Animal Research Ethics committee (Protocol reference no. AREC/014/017). The study was conducted at Ukulinga research farm at the livestock section. The farm is positioned at 30° 24′ S, 29° 24′ E with altitude that differs between 700 to 775 m above sea level. It has mean annual rainfall of 735 mm that normally falls mostly between October and April. Temperatures range between a mean minimum of 8.9°C and maximum of 25.7°C. There is also an occurrence of frost mostly during winter which ranges from light to moderate.

3.2.2 Leaf meal collection

Vachellia tortilis leaves were collected in South Africa, KwaZulu-Natal at the Jozini Makhathini Research station. Jozini is positioned at 27^0 25′45.72″ S, $32^0$3′54.39″ E at North-eastern KwaZulu-Natal. Temperatures at Jozini range from 22.3 to 30.3°C, higher temperatures being around January, February and November, lowest being in June. The leaf meal was collected at advanced maturity stage during the rainy season. Tree branches were clipped from growing point; air dried for 3 days by placement under a shade to prevent nutrient heat damage of heat sensitive nutrients and turned regularly to prevent build-up of moulds. Polyethylene sheets were used to prevent sand from contaminating the leaves. Leaf meal was created by beating the branches carefully to drop the leaves using a stick, to eliminate thorns and pods, a 2mm sieve was used. The grounded leaf meal was then stored at Ukulinga research farm in a ventilated dry room.

3.2.3 Experimental design

A total of 120 cobb 500 were randomly allocated to 6 experimental diets; 0, 30, 60, 90, 120 and 150 g/kg of *Vachellia tortilis* inclusion levels, respectively. The experimental units were individual broilers. The treatments were replicated twice, and the birds were fed for 17 days.

3.2.4 Broiler management

One hundred and twenty Cobb 500 broilers were placed in an environment-controlled house. During the first 14 days the chicks were brooded with infrared lights. They were fed on commercial broiler starter feed. Ventilation was also provided throughout the trial for air circulation. During the four weeks, temperature, broiler activity and feed intake was monitored. The broilers were reared on the floor to try mimic deep litter system. One fountain drinker and 1 plastic feeder for each pen were provided. Wood shavings were used as bedding. Stocking density was $0.21m^2$ per bird. Temperature ranged from 31^0C to 27^0C for Week 1 and 2. The final temperature ranged from 15^0C to 27^0C for the final two weeks depending on weather as the houses are environment controlled. Lighting was 12hL: 12hD during the trial. The average daily gains for the first week of treatment were 0.06, 0.08, 0.08, 0.08, 0.09 and 0.07 g/day for treatments 0, 30, 60, 90, 90, 120, 150 g/kg *V. tortilis* inclusion respectively. On the second week broilers gained 0.09, 0.06, 0.07, 0.07, 0.06 and 0.06 g/d for 0, 30, 60, 90, 90, 120, 150 g/kg inclusions, respectively.

3.2.5 Chemical composition of feeds

The experimental feed was formulated using Winfeed 3, to ensure that the diets had similar amounts of energy and protein. The experimental diets were supplemented with vitamins and minerals to meet the National Research Council (NRC, 2012) recommended specifications for broilers. The raw materials were mixed as specified by Table 3.1 at Ukulinga research farm.

Moisture content was analysed then calculated as described by the Association of Official Analytical Chemists (AOAC Official Methods 934.01). Nitrogen was analysed using the LECO TruSpec Nitrogen Analyzer. The nitrogen was multiplied by a factor of 6.25 under the assumption that 10 percent of protein is Nitrogen as described by the AOAC Official Methods 990.03. Ash was analysed then calculated and organic matter from ash as described by the AOAC Official Methods 942.05. Crude fat was analysed using Soxhlet method on Soxhlet Buchi 810 fat extractor (Soxhlet Buchi, Switzerland).

Calcium, sodium, iron, manganese, copper, zinc, potassium, magnesium and phosphorus concentrations were determined from ash content by taking the feed ash samples using

AOAC, 1990 Official Methods of Analysis. For acid digestion, the ash samples were placed in a 100ml conical flask. Hydrochloric acid (HCl) was then added. The mixture was boiled to evaporate. Nitric acid was added and boiled to dissolve the HCl and ash mixture. The nitric acid, ash and HCl solution was filtered with filter paper into 100 ml beaker and made up to 100 ml with deionized water. Mineral Analyser (ICP) was used to analyse the minerals using respective standards.

Neutral and acid detergent fibre were analysed separately using ANKOM Technology Methods 7-07-06 and ANKOM Technology Methods 08-26-05 respectively. The feed was also analysed for proanthocyanins using the butanol- HCl assay (Makkar and Goodchild, 1996). Three grams of feed was homogenized and extracted for pigmentation and fat using acetic acid and petroleum ether. Seventy percent acetone was used to dilute the sample to allow absorbance readings below 0.6 mg/l. One millimetre of the diluted sample was taken, then six millimetres of Butanol-HCl reagent was added to the diluted sample. Ferric reagent of 0.2 ml volume was added to the tubes, the mixtures were then vortexed. The mixture was exposed to 100°C of heat from a water bath for a period of one hour then placed in ice for cooling. The absorbance was recorded at 550 nm after 20 min. The amount of proanthocyanins were calculated using the equation by (Porter *et al*., 1985). Crude fibre was calculated using equation by Agabriel *et al*. (2007). Metabolizable energy was calculated using the equations:

Nitrogen Free Extracts= 100- (% ash + % moisture + % crude fat + % crude fibre + % crude protein)

ME (Kcal/kg) = 10 ((3.5*crude protein) + (8.5*crude fat) + (3.5*NFE))

3.2.6 Broiler slaughter management

Twelve hours before slaughter broiler feed was removed. After 12 hours six broilers per diet were randomly picked (three per replicate) and were electrically stunned. The slaughter weights were 1.6, 1.5, 1.6, 1.6, 1.6, 1.5 kg for 0, 30, 60, 90, 90, 120, 150 g/kg diets, respectively. Their throats were slit and allowed to bleed for 3 minutes. The birds were then dipped in warm water and had their feathers removed. The heads and feet were removed. The carcass was split then allowed to set for 30 minutes at temperatures around 20°C.

3.2.7 Meat chemical measurements

After freeze drying the meat moisture content was calculated as described by the Association of Official Analytical Chemists (AOAC Official Methods 934.01). The dry matter was then calculated from moisture. Ether extract was evaluated using an extractor. The meat was dried for an hour before being analysed for petroleum ether. Aliquots of an average of 5 grams were packed in cartridges covered in filter paper and underwent 4 hours of extraction at a rate of 6 drops per second. The cups were dried for 24 hours in a fumigator and weighed. The weight gain after extraction and solvent recovery was the ether extract as described by the AOAC methods 920.39. Nitrogen was analysed using the LECO TruSpec Nitrogen Analyser. The nitrogen was multiplied by a factor of 6.25 under the assumption that 10 % of protein is Nitrogen as described by the AOAC Official Methods 990.03. Crucibles and meat were weighed and placed into a furnace overnight at 550°C. Ash was then recorded as the final weight less crucibles. Organic matter was calculated from ash as described by the AOAC Official Methods 942.05.

Table 3.1: Inclusion rates of various raw materials as formulated using Winfeed®3

Component	*Vachellia tortilis* inclusion level (g/kg)					
	0	30	60	90	120	150
Maize	545.0	519	493	469	445	421
Soybean 46	392	388	383	376	370	363
Vachellia tortilis	0.00	30.0	60.0	90.0	120.0	150.0
Sunflower oil	45.0	46.1	47.0	47.9	47.9	49.8
Limestone	1.38	1.37	1.28	1.23	1.23	1.20
Monocalcium-phosphate	1.45	1.44	1.38	1.30	1.28	1.23
Salt	5.00	5.00	5.00	5.00	5.00	5.00
Vitamin-mineral premix	5.00	5.00	5.00	5.00	5.00	5.00
Lysine-HCL	1.20	1.00	0.80	0.96	0.95	0.94
Threonine	0.97	0.86	0.75	0.64	0.52	0.40
Methionine	3.00	2.66	2.61	2.65	2.60	2.54

Table 3.2: Experimental diets analysed chemical composition

Component	*Vachellia tortilis* inclusion levels (g/kg)					
	0	30	60	90	120	150
Dry matter	90.07	90.10	89.91	90.25	90.25	90.37
ME (MJ/Kg)	44.17	43.98	44.07	44.09	43.79	43.97
Crude protein	19.46	20.39	20.39	19.35	22.08	20.01
+9Crude fat	6.89	6.81	7.51	7.94	7.56	8.60
Ash	4.11	4.00	4.23	4.38	4.57	4.72
Neutral detergent fibre	8.09	10.70	10.04	11.50	13.00	13.00
Acid detergent fibre	2.00	3.00	2.84	3.26	4.11	4.20
Proanthocyanidins	0.05	0.62	1.70	2.15	5.87	9.16

AME-Apparent Metabolisable Energy

Table 3.3: Experimental diets analysed mineral composition

	Vachellia tortilis inclusion levels (g/kg)					
	0	30	60	90	120	150
Mineral (mg/kg)						
Calcium	78.10	132.6	139.8	332.0	100.7	6.79
Phosphorus	96.63	78.50	81.20	105.33	67.40	195.0
Potassium	422.7	283.3	308.0	476.0	234.3	467.0
Sodium	47.13	57.70	61.57	46.03	17.60	21.97
Zinc	1.41	1.56	1.46	1.82	0.21	0.20
Copper	0.88	0.65	0.70	0.73	0.95	1.81
Manganese	1.14	1.45	1.28	1.61	0.42	0.00
Magnesium	26.27	37.63	40.37	25.57	33.03	12.57
Iron	1.61	1.94	2.61	9.75	9.89	0.50

Calcium, sodium, iron, manganese, copper, zinc, and phosphorus were determined from ash content by taking the ashed meat and placing in a conical flask for acid digestion as indicated by AgriLASA Method 6.5.1 using flame spectrometry. For acid digestion, the ashed samples were placed into the 100ml conical flask, hydrochloric acid (HCl) added and boil to evaporate, nitric acid was added and boiled to dissolve the HCl and ash mixture. The Nitric acid, ash and HCl solution was filtered with filter paper into 100ml beaker with deionized water. An ICP-OES (Inductively Coupled Plasma Optical Emission spectrometers) mineral analysis method was used to analyse the minerals using respective standards.

3.2.8 Statistical analyses

Regression procedure (PROC RSREG) was used to assess the relationship between *Vachellia tortilis* inclusion and meat quality traits. The response variables analysed were colour, pH, temperature, WBSF, cooking and drip loss.

3.3 Results

3.3.1 Response in chemical composition to leaf meal inclusion

Tables 3.4 and 3.5 summarise the response in chemical characteristics of broiler meat to *V. tortilis* inclusion. They also include means and relationships between *V. tortilis* and meat chemical components. Moisture, crude protein, fat and ash content did not have a significant relationship with *V. tortilis* inclusion. Phosphorus, potassium, magnesium, manganese, iron and zinc did not have a significant (P>0.05) relationship with *V. tortilis* inclusion. Calcium slightly increased with the inclusion of *V. tortilis*, it was higher in meat from *V. tortilis* fed broilers. Meat phosphorus, zinc and manganese fluctuated in between treatments, they were also higher in meat from broilers fed *V. tortilis*. In general ash, sodium, calcium, iron, potassium and magnesium had a relationship with *V. tortilis* inclusion levels. These relationships were visible in an excel graph and not significant. Copper had a quadratic ($P<0.05$) relationship with *V. tortilis* inclusion levels. The relationship between copper and *V. tortilis* was described by equation: $Y = 0.04 (\pm 0.01) x^2 – 0.35 (\pm 0.08) x + 0.78 (\pm 0.13)$ (P= 0.003). Ash and *V. tortilis* was described by $Y = – 50.23 (\pm 18.40) x + 203 (\pm 27.79)$ (P=0.001). The equation $Y = -0.80 (\pm 0.32) x^2 + 6.64 (\pm 2.29) x + 12.10 (\pm 3.47)$ (P=0.001) described relationship between *V. tortilis* and sodium. The relationship calcium had with *V. tortilis* was described by $Y= 0.07 (\pm 0.20) x^2 + 1.79 (\pm 1.40) x + 0.36 (\pm 2.12)$ (P=0.04). Figures 3.1, 3.2, 3.3 and 3.4 show ash, potassium, sodium, magnesium, calcium and irons' relationship with *V. tortilis* inclusion levels, respectively.

3.4 Discussion

Meat quality may be done by evaluating the meat chemical composition. The chemical composition of meat and their ratio are, thus, important for the health of consumers. Improving the chemical composition of meat using organic methods like the use of *Vachellia tortilis* improves consumer health. Chances of disorders such as, obesity, hypertension and cancer may be reduced through the provision of organic protein, essential minerals and vitamins by lean chicken meat (Charlton *et al.*, 2008). Muscle has a finite absorption of these nutrients, thus, the inclusion of various raw materials to improve their content may ensure that the full absorption potential of these nutrients is reached.

In this study, broiler breast meat was analysed for protein, moisture, fat, ash and minerals to distinguish the relationship between these parameters and *V. tortilis* inclusion levels. Through a statistical software it was determined that ash, copper, sodium and *V. tortilis* inclusion had a relationship.

Table 3.4: Chemical meat quality parameters of broilers fed incremental levels of *Vachellia tortilis* diet

Parameters (g/kg)	*Vachellia tortilis* inclusion levels (g/kg)						SEM	Significance		Regression coefficient	
	0	30	60	90	120	150		Linear	Quadratic	Linear	Quadratic
Moisture	735.3	716.4	738.1	739.5	736.3	736.1	26.18	NS	NS	-	-
Crude protein	241.3	258.4	238.3	235.5	239.2	241.4	24.74	NS	NS	-	-
Crude fat	32.65	52.37	47.53	33.88	48.35	49.58	7.64	NS	NS	-	-
Ash	151.0	115.0	75.05	92.30	114.8	82.92	14.59	**	NS	-	204 ± 27.8

SEM: standard error of means; NS: not significant ** ($P < 0.001$) ($n = 6$)

Table 3.5: Mineral meat quality parameters of broilers fed incremental levels of *Vachellia tortilis* diet

Minerals (mg/kg)	*Vachellia tortilis* inclusion levels (g/kg)						SEM	Significance		Regression coefficient	
	0	30	60	90	120	150		Linear	Quadratic	Linear	Quadratic
Calcium	1.76	2.18	3.77	5.84	7.82	10.4	1.57	NS	*	-	0.36 ± 2.12
Phosphorus	225	236	231	253	218	242	36.33	NS	NS	-	-
Potassium	417	344	299	386	443	348	52.07	NS	NS	-	-
Sodium	20.1	15.0	23.9	28.3	25.0	21.3	2.38	NS	**	-	12.1 ± 3.47
Zinc	0.17	0.37	0.13	0.27	0.29	0.11	0.22	NS	NS	-	-
Copper	0.83	0.29	0.32	0.15	0.06	0.04	0.07	NS	**	-	0.04 ± 0.01
Manganese	0.01	0.01	0.01	0.01	0.01	0.01	0.01	NS	NS	-	-
Magnesium	12.7	11.4	14.1	16.3	15.7	13.5	1.68	NS	NS	-	-
Iron	0.45	0.55	0.27	0.23	0.26	0.15	0.16	NS	NS	-	-

SEM: standard error of means; NS: not significant; (** $P < 0.001$; * $P < 0.01$) ($n = 6$)

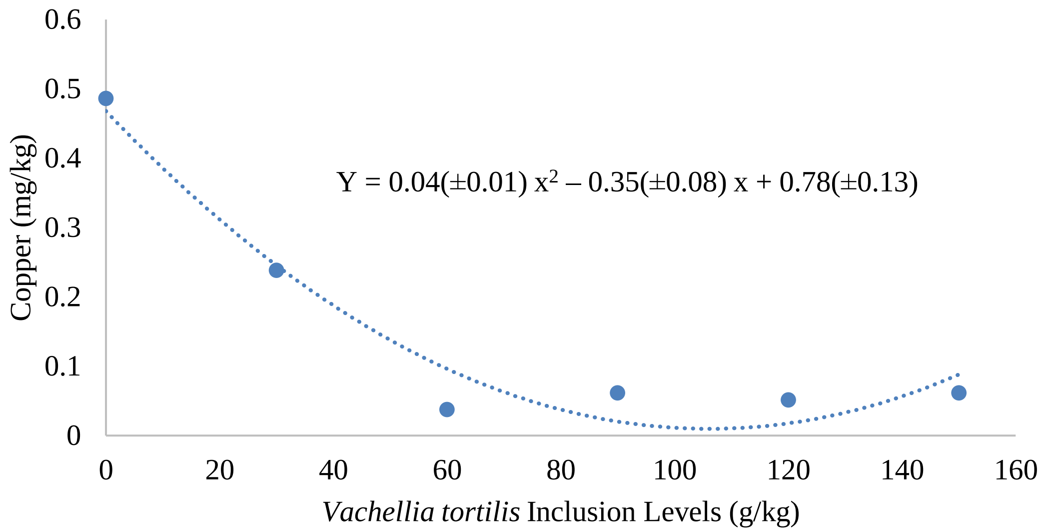

Figure 3.1: Response in broiler breast meast copper to *Vachellia tortilis* inclusion levels

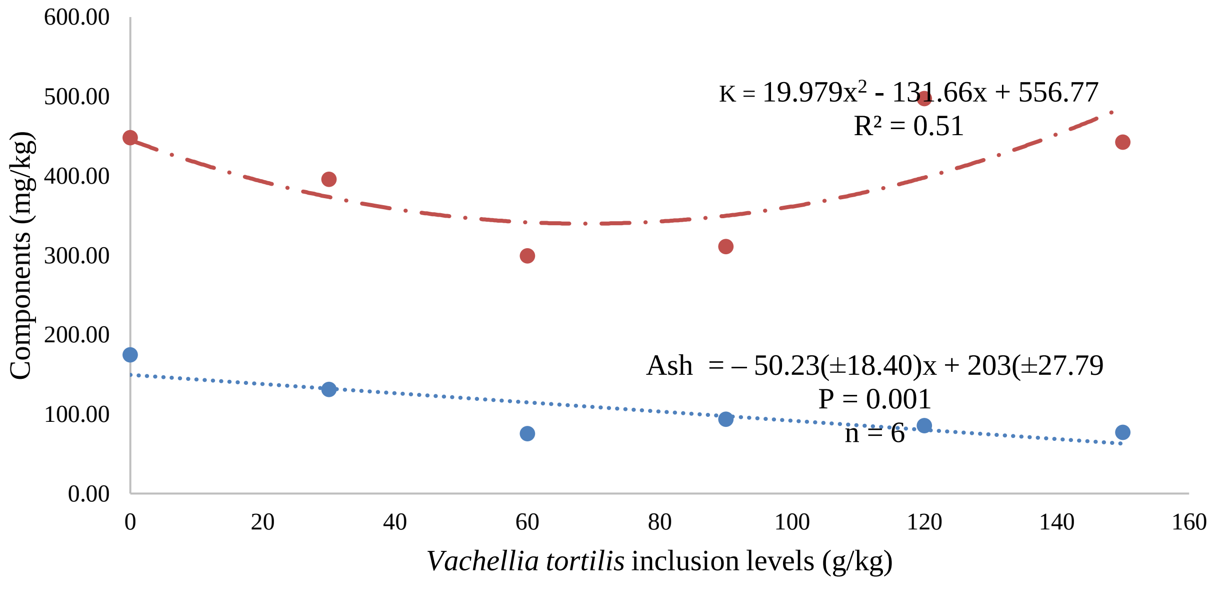

Figure 3.2: Response in broiler breast meat ash and potassium to *Vachellia tortilis* inclusion levels

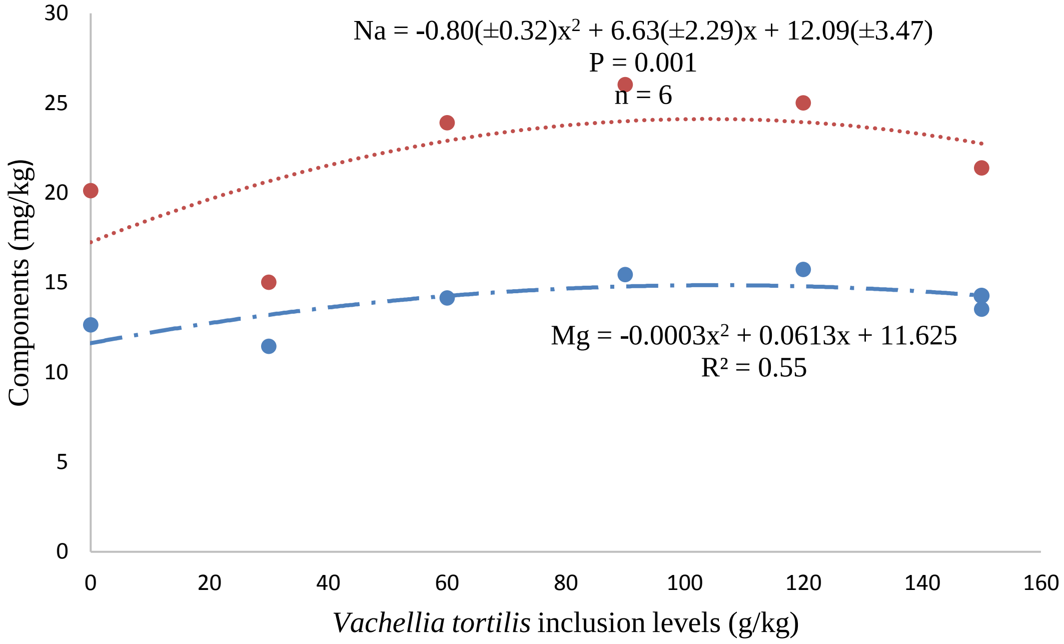

Figure 3.3: Response in broiler breast meat sodium and magnesium to *Vachellia tortilis* inclusion levels

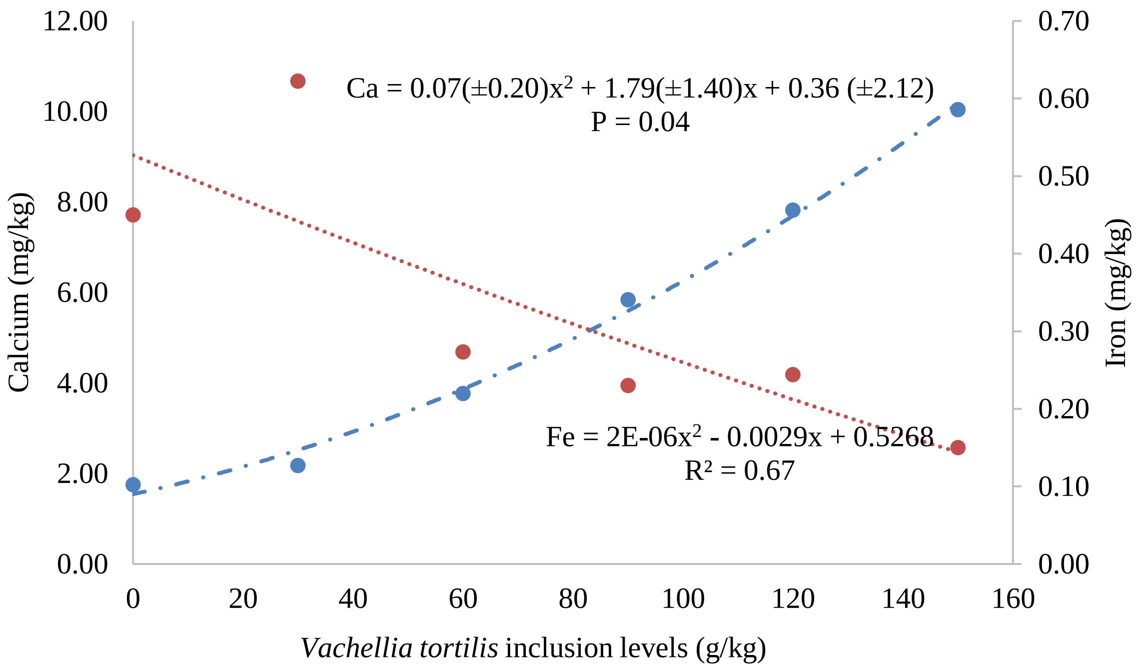

Figure 3.4: Response in broiler breast meat calcium and iron to *Vachellia tortilis* inclusion levels

Magnesium, calcium, iron and potassium also appeared to have a relationship with *V. tortilis* inclusion levels that. Unexpectedly, zinc, manganese and phosphorus did not have a relationship with the inclusion levels. Moisture is regulated by sodium and potassium.

As expected, Moisture contents were similar across all treatments. These findings were comparable to findings by Qwele *et al*. (2013) when feeding *Moringa oleifera* to goats. Karak *et al*. (2010) also reported similar moisture in commercially fed broilers. Increase in meat moisture content is not desired because that would imply that there is an imbalance in the minerals.

Protein in the current study was comparable to the protein of commercially fed broilers. Crude protein being similar regardless of the treatments demonstrates that supplemented meat compares favourably with the control diet. These findings imply that proanthocyanidins in *V. tortilis* did not have a negative effect on protein availability as the crude protein was not reduced (Barry *et al*., 1986; McDonald, 2002). The building and maintenance of muscles; organs and blood will, therefore, not be affected negatively by the inclusion of *V. tortilis*. Comparable to the current study, Mapiye *et al*. (2010) and Qwele *et al*. (2013) reported similar protein across treatments when *Moringa oleifera* and *V. Karroo*, respectively, were fed. These findings also imply that there was enough energy in form of carbohydrates and fats to incorporate these proteins.

Fat was unexpectedly increased by the inclusion levels of *V. tortilis*. These findings were not expected as polyphenolic compounds have been reported to inhibit lipase (Al-Mamary *et al*., 2001). The cause of these unexpected results could be *V. tortilis* proanthocyanidins were not high enough to reduce lipase activity and fat absorption. The high fat content reported in *V. tortilis* also increased the amount of fat available to deposited (Khanyile *et al*., 2014). Fat in the current study was 24 g/kg higher than the fat reported by Karak *et al*. (2010) on commercially fed broilers. On the contrary, ash was reduced by *V. tortilis* inclusion.

Ash was reduced by the inclusion of *V. tortilis* implying that proanthocyanidins prohibited the absorption of some minerals. Similarly, Przywitowski *et al.* (2016), reported a reduction in ash owing to the polyphenolic compounds. Some of the minerals may, therefore, be reduced by the increased inclusion levels of *V. tortilis*. The reduction in ash with the inclusion of *V. tortilis* opposes Qwele *et al*. (2013) report of an increase in ash content with moringa leaf meal inclusion. The species variation could have played a great role in the differences.

The increase in calcium was expected owing to *V. tortilis*' greater mineral content. The relationship between calcium and *V. tortilis* inclusion levels may also be due to calcium and phosphorus from *V. tortilis* being more available. The consistency of phosphorus with the inclusion of *V. tortilis* was favourable as it did not interfere with the absorption of calcium. Supporting this, Nkukwana *et al*. (2014) reported that moringa leaf meal improves the Ca:P ratio. They also reported a relationship between phosphorus and moringa. Phosphorus and calcium are necessary for bone and teeth formation. Phosphorus also takes part in the metabolism of fat, carbohydrates, and protein and b group vitamins. Its deficiency may, therefore, lead to reduced immunity leading to a deteriorated health.

The reported amount was 95 % of the required daily amount per kilogram of meat consumed. These amounts are desired as potassium is essential for nerve functioning, muscle control and the reduction in hypertension chances in people who are sensitive to sodium (Coruzzi *et al*., 2001). Thus, its stability is crucial to avoid heart attacks and fluctuations in blood pressure.

The lack of a relationship between zinc, manganese and *V. tortilis* supplementation was not expected. The unexpected lack of a relationship is not clear because Afsana *et al*. (2004)

reported that tannic acid lower than 10 g/kg did not affect zinc. Magnesium shadowed the expected relationship trend. Contradictorily, Hassan *et al*. (2003) reported high levels of polyphenolic compounds reduced magnesium.

Sodium having a relationship with *V. tortilis* was expected. According to the current study polyphenolic compounds improved the absorption of magnesium. These findings are desired and mean the supplementation with *V. tortilis* may reduce the chances of hypertension because high sodium diets contribute to hypertension in human. There are also various sodium sources in the human diet.

Copper and iron were affected drastically by *V. tortilis* inclusion. The reduction in iron with inclusion levels as low as 30 g/kg could be due to their increased susceptibility to binding with proanthocyanidins. Mineral antagonistic effects may also be the cause in the case of copper. Afsana *et al*. (2004) reported copper was affected by tannic acid higher than 10 g/kg. Qwele *et al*. (2013) reported copper values which were comparable to the copper values observed in the current study when feeding moringa leaf meal.

The reduction in iron when *V. tortilis* is supplementing the diet is not desired as iron assists with normal growth and development and carries oxygen around the body. Legumes have been reported to have phytate and iron-binding polyphenols which may lead to the reduction in iron availability (Sandberg, 2002). Iron-binding polyphenols are more plausible because zinc and phosphorus was not affected. Karak *et al*. (2010) also reported 3.65 mg/kg higher iron in broilers which had been commercially fed. These results imply that polyethylene glycol needs to be explored to reduce polyphenolic compound effects.

The increased mineral may be used by retailers to sell their product, more especially it may lead to healthier consumers. The increase in feed intake and growth performance with the inclusion levels in the first week of the treatment was a positive outcome, meaning *V. tortilis* may be used to improve meat quality during the last week of production. Fat content being higher than the expected amount might lead to obesity, especially if these fats are saturated. There is, therefore, a need to assess fatty acid profile in meat from broilers fed *V. tortilis*. The increase in most of the minerals when *V. tortilis* is incorporated into the diets brings the muscles closer to reaching their full potential. *Vachellia tortilis* may, therefore, improve mineral consumption in humans.

3.5 Conclusions

Ash content increased with the increase in *Vachellia tortilis* inclusion levels. Copper and sodium levels in meat increased with the increase in *V. tortilis*. *Vachellia tortilis* inclusion did not affect moisture, protein, zinc, phosphorus and manganese concentrations in meat. Apart from chemical properties, there is a need to investigate physical characteristics that consumers consider when making decisions to purchase meat.

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Chapter 4

Response in physical characteristics of broiler breast meat to *Vachellia tortilis* leaf meal

Abstract

The objective of the current study was to evaluate the relationship between *Vachellia tortilis* inclusion levels and broiler meat physical attributes. A total of 120 Cobb 500 broilers were randomly allocated to 0, 30, 60, 90, 120 and 150 g/kg *Vachellia tortilis* leaf meal diets from 14 days of age. At 32 days of age, the birds were humanely slaughtered. The average daily gain and feed intake were increased on the first week and reduced in the second week. Cooking loss, shear force, drip loss and redness were not significantly related to *V. tortilis* inclusion. The relationship between *V. tortilis* and lightness was described by Y = 0.484 (± 1.638) x -14.435 (± 43.904). The increase in 1 g/kg in *V. tortilis* let to a 2.15 unit increase in yellowness. The equation Y = 2.393 (± 2.180) x -23.782 (± 20.456) described the relationship between *V. tortilis* increase and chroma range. The relationship between pH at 24 hours and *V. tortilis* inclusion levels was described by Y = 29.467 (± 60.463) x -75.148 (± 183.795). The increase in yellowness is likely to increase consumer satisfaction on broiler meat.

Keywords: Colour, Cooking loss, Drip loss, Polyphenolic compounds, Shear force

4.1 Introduction

Broiler meat colour affects how consumers perceive meat quality at purchase. The relationship between minerals and *Vachellia tortilis* had to be sustained with the evaluation of broiler meat physical attributes. Chicken meat, deemed healthier than red meat, has less religious and health restrictions. There is also an increase in fresh meat purchasing; meaning meat physical attributes like colour are becoming more important.

Meat physical properties include colour, pH, tenderness, water holding capacity (WHC), drip and cooking loss. Colour is the main physical characteristic that influences consumers' willingness to purchase meat (Ngambu *et al.,* 2013). Tenderness and cooking loss depend on previous experience. These parameters are affected by exercise thus breast meat is used to minimise error in the results due to walking. The introduction of non-conventional raw materials, such as, leaf meals could improve meat physical properties without compromising the nutritional composition of the meat. Based on growth performance, Vachellia leaf meal inclusion levels for broilers should range between 10 and 130 g/kg (Gudiso, 2016; Khanyile *et al*., 2017; Ncube *et al*., 2018). There is insufficient information on inclusion levels of non-conventional feed resources such as *Vachellia* leaf meals that may aid in improving meat physical attributes. The current study, however, had a greater inclusion to account for species variation.

Vachellia tortilis is a legume tree widely distributed around the whole world. Khanyile *et al*. (2014) reported that *Vachellia tortilis* leaf meal had high quality amino acids, vitamins and minerals content, thus, would be a great supplement. The increased nutrient composition in leaf increases nutrients that may be absorbed. *Vachellia tortilis* also has polyphenolic compounds which aid in improving meat physical attributes. Polyphenolic compounds reduce the availability of nutrients to the chicken. Dose response trials could, therefore assist estimate optimum inclusion levels and response in boiler meat physical attributes at any given inclusion. The incorporation of *Vachellia tortilis* in broiler feed may assist low input farmers in semi-arid areas increase food and nutritional productivity. The utilization of *Vachellia tortilis* could also reduce bush encroachment and likely mitigate feed shortage challenges. The objective of this study was to assess the relationship between physical characteristics of broiler meat and incremental levels of *V. tortilis*. It was hypothesized that leaf meal inclusion would have a relationship with meat physical characteristics.

4.2 Materials and methods

4.2.1 Study site

Study site was as described in section 3.2.1.

4.2.2 Leaf meal collection

Leaf meal collection was as described in section 3.2.2.

4.2.3 Experimental design

Experimental design was as described in section 3.2.3.

4.2.4 Broiler management

Broiler management was as described in section 3.2.4.

4.2.5 Chemical composition of feeds

Chemical composition of feeds was as described in section 3.2.5.

4.2.6 Broiler slaughter management

Broiler slaughter management was as described in section 3.2.6.

4.2.7 Meat measurements and analyses

The pH was determined using a pH meter probe at 24 hours post mortem. Three areas of the meat were poked with the probe. Thermometer pointer was pointed into the meat after being cleaned with distilled water and cotton towel. Temperature was measured at seven hours post mortem.

Twenty-four hours after the meat was allowed to cool down after cutting. Meat was placed on a colorimeter randomly. The meat samples were similar in size. They were turned 3 times for 3 different readings per sample. Symbols a^*, b^* and L^* were used to assess L^*-lightness, b^*-yellowness and a^*-redness. The chroma or saturation was calculated using (Hunter and Harold, 1987) method using the equation by $(a^2+b^2)^{0.5}$. Hue angle was also calculated using Hunter and Harold (1987) method ($\text{Tan}^{-1} = \frac{b}{a}$).

Portions of approximately 5 g were cut and weighed. They were then hung in a plastic by a string. The plastic was inflated so the meat did not touch the plastic. Meat was then placed at 4°C for 24 hours. After 24 hour the samples were weighed again. The weight lost was considered as the drip loss. The equation below was used to determine drip loss percentage.

$$\%\ \text{drip loss} = \frac{Weight\ before\ 24hr - weight\ after\ 24hr}{Weight\ before\ 24hr} \times 100$$

The meat was placed into an 80°C water bath for 60 minutes to reach and internal temperature of 75°C. Cooking loss was determined by weighing the chicken cuts before cooking and after cooking. The weight that was lost during the cooking was the cooking loss. Cooking loss was determined using methods described by Ding *et al*. (2010)

$$\% \text{ cooking loss} = \frac{Weight\ before\ cooking - weight\ after\ cooking}{Weight\ before\ cooking} \times 100$$

The samples that were used for the analysis of shear force were the same samples used to analyse cooking loss. The samples were cut into approximately 14 mm diameter. The Warner Bratzler shear force test was used to measure texture. For the shear force on myofibrillar protein to be measured the meat samples were perpendicular to the longitudinal axis of muscle fibres. An average maximum shear force value was calculated based on shear force (g/mm^2) required to shear 14 millimetres. The diameter was of the cylindrical core perpendicular to the grain at the crosshead speed of 200 mm per minute.

4.2.6 Statistical analyses

Regression procedure (PROC RSReg) was used to assess the relationship between *V. tortilis* inclusion and the meat quality traits. Both linear and quadratic relationships were analysed to predict response in meat colour, pH, temperature, WBSF, cooking and drip loss.

4.3 Results

4.3.1 Response in physical characteristics to leaf meal inclusion

Table 4.1 and 4.2 summarises the relationship between *Vachellia tortilis* inclusion and broiler meat physical characteristics. Lightness had a positive linear ($P<0.05$) relationship with inclusion levels of *V. tortilis*. Redness did not have a significant relationship with the inclusion of *V. tortilis*. It was higher in broiler meat that had *V. tortilis* than the control diet.

Yellowness had a significant (P<0.05) positive linear relationship with *V. tortilis* inclusion. Chroma had a significant (P<0.05) positive linear relationship with *V. tortilis* inclusion.

Meat pH at 24 hours post mortem had a significant (P<0.05) negative linear relationship with the increase in *V. tortilis* inclusion. Muscle temperature and cooking loss were not related to the inclusion levels of *V. tortilis*. Figure 4.1 shows relationship between shear force and *V. tortilis* inclusion levels.

4.4 Discussion

Meat physical properties are some of the major properties that affect consumers' perception of meat quality. Low cooking loss and increased tenderness keep the consumers satisfied. Reduction in drip and cooking loss leads to nutritious meat, therefore, healthy consumers. These parameters depend on one another and they are dominantly and indirectly influenced by pH reduction. The rate of pH reduction is influenced by temperature. Colour is the primary and most utilised characteristic by consumers to predict meat quality, thus may be the most important meat physical attribute to consumers at purchase (Resurreccion, 2004). Chicken meat is known as white meat, therefore, normally looks more white than red, especially the breast. Although lightness should be higher than the other colour parameters in broiler meat, pale meat is not desired. The letters L*, a* and b* are chroma perceptions of different colours. Chroma is the objects saturation ratio to the brightness of the meat.

The current study measured meat physical characteristics. A statistical software was used to evaluate relationships between the physical characteristics and the increased inclusion levels of *Vachellia tortilis*. Through the statistical software it was determined that meat lightness, yellowness and chroma had a positive linear relationship.

Table 4.1: Physical meat quality parameters of broilers fed incremental levels of *Vachellia tortilis* diets

	Inclusion Levels							Significance		Regression coefficient	
Parameters	0	30	60	90	120	150	SEM	Linear	Quadratic	Linear	Quadratic
L^*	48.96	51.58	51.57	50.15	56.74	51.18	0.95	*	NS	0.48 ± 1.64	-
a^*	5.30	6.79	7.71	7.06	5.43	6.05	0.59	NS	NS	-	-
b^*	15.89	16.20	18.63	18.85	20.82	17.70	0.52	**	NS	2.15 ± 2.03	-
Chroma	16.77	17.60	20.19	20.25	21.56	18.78	0.43	**	NS	-2.39 ± 2.18	-
Hue angle	4.59	-1.17	-1.01	-1.73	0.15	-1.62	2.55	NS	NS	-	-
pH24	6.18	5.87	5.82	5.90	5.84	5.92	0.06	*	NS	29.46 ± 60.46	-
Cook loss %	17.94	16.42	18.35	20.19	17.37	22.60	1.99	NS	NS	-	-
WBSF (N)	4.86	5.39	4.70	2.66	3.80	4.46	0.28	NS	NS	-	-
Drip loss %	39.70	25.98	35.98	36.13	21.18	31.55	8.31	NS	NS	-	-
Temperature	26.62	25.68	27.50	27.22	27.45	28.42	0.46	NS	NS	-	-

L^* –Lightness; a^* – Redness; b^* – Yellowness; pH24- pH 24 hours after slaughter; Cook Loss- Cooking Loss; WBSF N- Warner Bratzler in Newtons Shear Force; temperature- muscle temperature at 12-hour post mortem, n=6

Table 4.2: Meat physical parameters that showed significant relationships with *Vachellia tortilis* inclusion levels

Parameter	Equation	P value
Lightness	Y = 0.484 (± 1.638) x -14.435 (± 43.904)	0.0244
Yellowness	Y = 2.147 (± 2.028) x -19.796 (± 18.085)	0.0005
Chroma	Y = 2.393 (± 2.180) x -23.782 (± 20.456)	0.0004
pH24	Y = 29.467 (± 60.463) x -75.148 (± 183.795)	0.0249

pH24 - pH 24 hours after slaughter

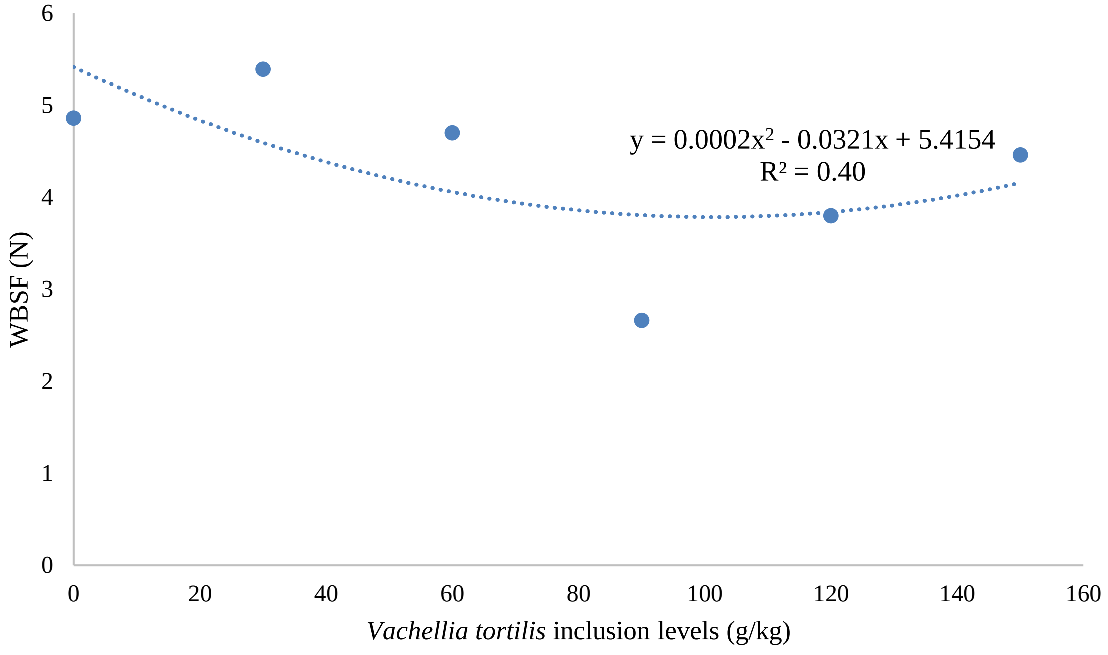

Figure 4.1: Response in broiler breast meat shear force to *Vachellia tortilis* inclusion levels

These relationships were not expected as optimal Vachellia inclusion levels have been reported as 10 to 130 g/kg (Ngambu *et al*., 2013; Khanyile *et al.,* 2014; Ncube *et al*., 2018). The increase in meat lightness (L*) with *Vachellia tortilis* inclusion levels was still within the normal lightness range which is 50 to 56 (Wapi *et al*., 2013). These lightness findings agree with earlier reports (Moyo *et al*., 2014; Mukumbo *et al*., 2014) on animals fed on *Moringa oleifera* leaf meal. Priolo and Vasta (2007) reported that meat with polyphenolic compounds from feed was lighter due to the reduced microbial biosynthesis of vitamin B12. This vitamin is a precursor for the synthesis of haem pigment (Priolo and Vasta, 2007). High temperatures also lead to rapid glycolysis which results in faster rigor onset and higher lightness values (Rathgeber *et al.,* 1999b; Sandercock *et al*., 2001).

As expected, meat yellowness had a relationship with *V. tortilis* inclusion levels. Yellowness (b*) in this study was increased as expected owing to the beta-carotene. The brighter colour is attributed to the presence of pigments referred to as oxy-carotenoids (D'Mello *et al*., 1987). These pigments also enhance yellowness. Unlike *M. oleifera*, *V. tortilis* has not been documented for its beta-carotene content which a few studies have found to be the reason for higher yellowness values (Richter *et al*., 2003; Moyo *et al*., 2011). Polyphenolic compounds ensure beta-carotenes are not destroyed ensuring the meats yellowness is not lost (Amarowicz *et al*., 2004). A positive linear relationship between yellowness and *V. tortilis* inclusion levels would, therefore, be expected.

The colour chromas having a positive relationship with *V. tortilis* inclusion meant meat was brighter with the inclusion of *V. tortilis*. *Vachellia tortilis* antioxidants reduced the meats ability to oxidise deoxymyoglobin and oxymyoglobin to metmyoglobin. Metmyoglobin is associated with meat discoloration to a brownish appearance which consumers perceive as meat that is not good enough to be consumed, thus not desired. It is also reported to activate even pigment distribution in animal tissue (Simitzis *et al*., 2008). Antioxidants presence in *V.*

tortilis increase colour stability, thus, colour distribution (Wapi *et al*., 2013). Khanyile *et al*. (2014) also reported that *Vachellia* leaves can reduce ferricynide (F^{3+}) to ferrous (F^{2+}). Meat is therefore more yellow than red due to this reaction.

Greater yellowness values have also been associated with the presence of polyphenolic compounds with anti-oxidant properties (Moyo *et al*., 2014). The mechanism used is thought to be that antioxidants minimize rancidity and reduce lipid peroxidation without damaging nutritional meat properties sparing proteins (Jang *et al*., 2008; Lahucky *et al*., 2010; Qwele *et al*., 2013). The high pH and low temperature values also assist with ensuring haem proteins are not degraded.

The relationship between pH values and *V. tortilis* was expected as *V. tortilis* has greater fat content, thus, increased energy stores. These expectations would need further analysis of the rate of pH and glycogen decline to increase certainty. The pH values were higher than the values reported by Mukumbo *et al*. (2014) when feeding pigs on Moringa leaf meal. Pigs store more fat than chickens which leads to greater energy stores, thus, lower pH. *Vachellia tortilis* has been documented to have greater fat values Khanyile *et al*. (2014), which would explain the lower pH values with the inclusion of *V. tortilis*. Relatively, the pH values are higher than pH values that were reported as being the normal final pH (Fischer, 2007). Polyphenolic compounds have not been documented to bind to fats. Fats are higher in energy than carbohydrates leading to higher energy stored as fat in the muscles. Lower energy stores would, therefore, lead to short lived glycolysis and higher pH. In turn, the higher pH could have promoted proteolysis which would also reduce shear force.

The lack of a relationship between shear force and *V. tortilis* was not expected as pH was higher. Higher pH values observed may lead to the increase in tenderness (Jerez *et al.*, 2003). The calpain system which is responsible for tenderizing the meat is activated at high calcium and pH, thus, leading to greater tenderness (Lian *et al*., 2013). Consumers can only evaluate the meat by using previous experience through observing meat cut, shape and colour (McGill, 1981). Its importance comes from the willingness of consumers to pay for guaranteed tenderness as it contributes greatly to eating quality (Lusk *et al*., 1999). Improving shear force may, therefore, benefit retailers as an advertising strategy. Similarly, Ogungbesan *et al*. (2014) reported shear force reduction in shear force when feeding *G*. *sepium*. The reduction in shear force is an indication of meat tenderness which is a desired consumers attribute (Platter *et al*., 2005). Tender meat may also be related to lower cooking and drip losses as it indicates less muscle contracted post mortem leaving more room for water retention.

Meat redness, drip and cooking loss not having a relationship with inclusion levels was expected as *V*. *tortilis* is not related these parameters (Lawrie and Ledward, 1998). The fluctuations in the results may be because each bird responds to different stress factors, such as, temperature differently. Room temperatures fluctuated rapidly especially when it was hot during the day then temperatures dropped rapidly in the afternoons and nights. The fluctuation in temperatures at different times of the day could have also continuously stressed the birds. Stocking density being low would also make the effect of temperatures more pronounced.

Vachellia tortilis improved meat physical attributes, especially colour which is the main purchasing influencer in fresh meat. Shear force were reduced by *V. tortilis*, which may, therefore, lead to tender meat. These attributes would be improved without compromising the chemical composition of the meat. Given the observed growth performance outcomes, *V. tortilis* may be added on the last week of production.

4.5 Conclusions

Vachellia tortilis increased meat yellowness and chroma linearly is desired and likely to increase broiler meat consumer satisfaction. Cooking and drip loss were not related to the increase in *V. tortilis* inclusion levels, suggesting that *V. tortilis* inclusion is likely not to change consumers recurring purchasing of the broiler meat.

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Chapter 5

General discussion, Conclusions and Recommendations

5.1 General discussion

The main hypothesis tested was that *Vachellia tortilis* leaf meal inclusion into a diet increased meat yellowness, chroma, whiteness, shear force, sodium; reduced ash, copper, iron, potassium, pH_{24} ranges and has no effect on meat moisture, protein, fat, zinc, phosphorus, manganese, redness, drip and cooking loss. The relationship between *V. tortilis* inclusion levels and meat chemical and physical characteristics measurements is likely to improve consumer satisfaction, health and reduce competition between human food and broiler feed.

In chapter 3, chemical analyses of the meat was conducted to test the hypothesis that chemical composition had a relationship with the increased *V. tortilis* inclusion. The most variation was observed in fat and minerals, the least, on protein and moisture. Ash, magnesium and calcium were reduced by *V. tortilis* inclusion levels, whilst, iron and potassium were reduced quadratically by the inclusion levels. Iron was reduced by the inclusion levels owing to the iron-binding polyphenols. Ash, sodium and copper had a relationship owing to the increase in polyphenolic compounds. Copper and iron could be more susceptible to binding to polyphenolic compounds rendering them unavailable for absorption. Consumers look at colour at the point of purchase. It is, therefore, important to evaluate meat physical properties.

Meat physical analyses were conducted in chapter 4 to test the hypothesis that broiler meat physical properties had a relationship with the increased *V. tortilis* inclusion levels. Meat lightness, yellowness, chroma and pH_{24} had a linear relationship with the leaf meal owing to polyphenolic compounds in the leaf meal. Colour brightness was attributed to myoglobin oxidative state. Antioxidants led to the stability of colour by minimizing rancidity and lipid peroxidation without damaging meat haem proteins. The pH_{24} being reduced with *V. tortilis* inclusion was due to the increased fat content in *V. tortilis*. In turn, the higher pH than the normal pH led to an improved tenderness and lower drip loss.

There is still a huge gap in the study of non-conventional feed resources, their recommended inclusion levels and the effect they have on various meat types. The incorporation of *Vachellia tortilis* is likely to improve mineral and meat colour. These are desired and is likely to increase consumer satisfaction. Retailers may also benefit from using these findings on their labels.

5.2 Conclusions

The inclusion of *Vachellia tortilis* is likely to reduce chances of hypertension and diabetes in broiler chicken consumers. It may also increase broiler meat consumer satisfaction. Retailers may benefit from using *V. tortilis* based meat by advertising their meat. Inclusion of *V. tortilis* improved meat colour parameters whilst reducing ash along with some essential minerals. The inclusion of *V. tortilis* is restricted by the presence of proanthocyanins as they restrict the optimum absorption of some minerals. Proanthocyanins reduce the quantity of ash and some minerals leading to the need for polyethylene glycerol exploration.

5.3 Recommendations

It may be advised that *V. tortilis* be used to improve meat quality if a few more parameters were addressed, such as, the feasibility of including *V. tortilis* leaf meal at a larger scale. The use of this raw material in feed mills to assist with meat quality also needs to be evaluated.

Further research is needed on the following:

1. Evaluate broiler meat physical attributes with inclusion levels higher than 150 g/kg.
2. Evaluate *V. tortilis* treated broiler meat sensory attributes.
3. Evaluate broiler meat fatty acid profile when *V. tortilis* is included.

4. Determine cooking loss, drip loss, glycogen and pH at different intervals post slaughter when there is *V. tortilis* in feed.
5. Determine the relationship between *V. tortilis* and meat quality when there is enzyme polyethylene glycerol is in the feed.
6. Determine meat fat quantity when there is *V. tortilis* and a higher protein quantity.

Appendix 1: Animal Research Project Ethical Approval

UNIVERSITY OF
KWAZULU-NATAL
INYUVESI
YAKWAZULU-NATALI

26 May 2016

Professor Michael Chimonyo (28007)
School of Agricultural, Earth & Environmental Sciences
Pietermaritzburg Campus

Dear Professor Chimonyo,

Protocol reference number: AREC/014/017
Project title: Response in growth performance, nematode counts and meat quality of broilers fed on incremental levels of *Vachellia tortilis*

Full Approval – Research Application

With regards to your revised application received on 21 May 2017. The documents submitted have been accepted by the Animal Research Ethics Committee and **FULL APPROVAL** for the protocol has been granted.

Any alteration/s to the approved research protocol, i.e Title of Project, Location of the Study, Research Approach and Methods must be reviewed and approved through the amendment/modification prior to its implementation. In case you have further queries, please quote the above reference number.

Please note: Research data should be securely stored in the discipline/department for a period of 5 years.

The ethical clearance certificate is only valid for a period of one year from the date of issue. Renewal for the study must be applied for before 26 May 2018.

Attached to the Approval letter is a template of the Progress Report that is required at the end of the study, or when applying for Renewal (whichever comes first). An Adverse Event Reporting form has also been attached in the event of any unanticipated event involving the animals' health / wellbeing.

I take this opportunity of wishing you everything of the best with your study.

Yours faithfully

....

Prof S Islam, PhD
Chair: Animal Research Ethics Committee

/ms

Cc Academic Leader Research: Professor O Mutanga
Cc Registrar: Mr Simon Mokoena
Cc NSPCA: Ms Stephanie Keuder
Cc Ukulinga Research Farm

Animal Research Ethics Committee (AREC)
Ms Mariette Snyman (Administrator)
Westville Campus, Govan Mbeki Building
Postal Address: Private Bag X54001, Durban 4000
Telephone: +27 (0) 31 260 8350 Facsimile: +27 (0) 31 260 4609 Email: animalethics@ukzn.ac.za
Website: http://research.ukzn.ac.za/Research-Ethics/Animal-Ethics.aspx

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