# Response in antioxidant activity and shelf life of meat from broilers fed on incremental levels of *Vachellia tortilis* leaf meal

**By**

**Mthethwa Nomalungelo**

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**Supervisor: Professor Michael Chimonyo**

**Declaration**

I, Nomalungelo Mthethwa, declare that the thesis entitled "**Response in antioxidant activity and shelf life of meat from broilers fed on incremental levels of *Vachellia tortilis* leaf meal**" is my own work. This dissertation has not been submitted to any University and my work was conducted under the supervision of Professor Michael Chimonyo. All assistance towards the production of this work and all references contained herein have been duly acknowledged.

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Nomalungelo Mthethwa Date

Approved as to style and content by:

_____

Prof M. Chimonyo

(Promoter)

**List of Abbreviations**

| Abbreviation | Definition |
|---|---|
| a* | Redness of meat |
| AA | Antioxidant activity |
| ANT | Antioxidant activity |
| ADF | Acid detergent fibre |
| AOAC | Association of Official Agricultural Chemists |
| b* | Yellowness of meat |
| BWG | Body weight gain |
| B-carotene | β-carotene-linoleic acid assay |
| BHT | Butylated hydroxytoluene |
| CF | Crude fibre |
| CL | Cooking loss |
| CP | Crude protein |
| DAFF | Department of Agriculture, Forestry and Fisheries |
| DL | Drip loss |
| DM | Dry matter |
| DPPH | 2, 2-diphenyl-1-picryhydrazyl |
| FRAP | Ferric reducing antioxidant |
| GLM | General Linear Procedure |

| | |
|---|---|
| L* | Lightness of meat |
| N | Nitrogen |
| NDF | Neutral detergent fibre |
| RNS | Reactive Nitrogen Species |
| ROS | Reactive oxygen species |
| RSA | Radical scavenging activity |
| SA | South Africa |
| SAS | Statistical Analysis Systems |
| SEM | Standard error of means |
| ORR | Oxidation rate ratio |
| UKZN | University of KwaZulu-Natal |
| VTLM | *Vachellia tortilis* leaf meal |
| OM | Organic matter |
| PUFA | Polyunsaturated fatty acids |
| WBSF | Warner-Bratzler shear force |

**Abstract**

**Response in antioxidant activity and shelf life of meat from broilers fed on incremental levels of *Vachellia tortilis* leaf meal**

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**N Mthethwa**

The broad objective of the study was to determine the antioxidant activity and shelf life of meat from boilers fed on incremental levels of *Vachellia tortilis* leaf meal. One hundred and twenty Cobb-500-day old unsexed broilers were assigned in a completely randomized block design to six experimental diets containing 0, 30, 60, 90, 120 and 150 g/kg DM of *Vachellia tortilis* leaf meal. Each diet was offered *ad libitum* to 10 broilers in each pen, with a total of 12 pens. Each diet had one replicate. After overnight fasting and slaughtering of chickens, skinless and boneless chicken samples (breast and thigh) were collected, vacuum sealed and packed at 4°C until analysis.

The determination of antioxidant properties in meat samples was measured using three assays; 2, 2-diphenyl-1-picryhydrazyl (DPPH), ferric reducing power (FRAP) and β-carotene-linoleic acid assay. The antioxidant activity using the DPPH model did not go beyond 50 % in all the inclusion levels. The FRAP assay exhibited a concentration-dependent linear response to the inclusion of *V. tortilis* leaf meal. The β-carotene-linoleic acid had the highest activity of 48.9 and 40.99 % recorded at 60 and 90 g/kg inclusion level respectively. The β-carotene-linoleic acid displayed a quadratic response, with the equation ($y = -0.0037x^2 + 0.7756x + 2.7811$) showing 104.8 g/kg to be useful in improving the potency of natural antioxidants.

Broiler breast and thigh samples were collected for the determination of shelf life. Drip loss (%) was measured after 24 hours of slaughter, meat pH, colour (L*, a* and b*), texture and

cooking loss (%) were measured at day 0, 7, 14 and 21 days. Broilers assigned to diets containing high levels of *Vachellia tortilis* leaf meal had tougher meat. High values of Warner-Bratzler Shear Force (WBSF) were recorded on day 21. Effect of leaf meal and days on texture had a linear response. High cooking loss results were obtained from the study, whilst there was no significant effect of leaf meal on the drip loss. The redness of meat had a quadratic response ($y = -0.0107x^2 + 0.2779x + 6.145$) over storage time. A period of not more than 13 days was found to be more ideal at retaining meat quality without development of lipid oxidation. An inclusion level of 94.5 g/kg of leaf meal improved the redness and yellowness of broiler breast and thigh meat samples respectively. In conclusion, *Vachellia tortilis* leaf meal can be incorporated in broiler diet up to 90 g/kg for improved storage time and antioxidant activity.

**Keywords:** Broilers, antioxidants, *Vachellia tortilis*, lipid oxidation, meat stability, texture and cooking loss

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## Dedication

This work is dedicated to my family: Mother (Nelisiwe Princess), Father (Mr Mthethwa), Sisters (Nomathemba and Nomfundo), Brother (Malusi) and Nephew (Sbongakonke).

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# CHAPTER 1: General Introduction

## 1.1 Background

Poultry plays a major role in ensuring food security, mitigation of malnutrition and provides employment for many households. With the commonness of malnutrition worldwide, poultry meat and meat products in people's daily diets significantly reduces cases of malnutrition. Red meat was a preferred food commodity until incidences of an outbreak of bovine spongiform encephalopathy (BSE) were reported in cattle (Nathanson *et al.,* 1997). The outbreak led to reduced consumer confidence in beef products resulting in a lower consumer demand for this product.

Consumers shifted their preference to chicken meat as a healthier and tasty option. Chicken meat contains essential amino acids, vitamins, minerals and the long chain of polyunsaturated fatty acids. In addition to its affordability and its promising nutrient composition, it also aids in reduction of incidences of cardiovascular diseases in human (Mothershaw *et al.,* 2009). The production of poultry meat had since then been increasing exponentially. The Department of Agriculture, Forestry and Fisheries (DAFF, 2010) reported broiler meat to contributing 17.5 % in 2010 while all animal products contributed 35 % in South Africa. Moreover, broiler production and management are convenient to farmers as they reach their target slaughter weights within four to five weeks compared to long production periods required for beef.

Poultry meat is, however, constrained by its high amounts of unsaturated fatty acid. High fat content exposes meat to lipid oxidation which affect the chemical and physical properties of meat leading to microbial spoilage (Al-Hijazeen *et al.,* 2016). Chicken meat products such as bacon, polony and sausages require processing before reaching the final product. These processing's of meat products for mincing, cooking and salt addition, enhances the occurrence of lipid oxidation due to formation of reactive oxygen species (ROS). The oxidation of lipids

causes changes in meat colour, texture, smell which later reduces the shelf-life of meat. Meat colour, texture, pH and cooking losses are among the measurements that are used to determine the quality of meat (Tshabalala *et al.,* 2003). Among these quality parameters, consumers, however, place special attention to colour and texture.

Synthetic antioxidants are chemically synthesized petroleum-based antioxidants which were used primarily to retard lipid oxidation. Their continued use, is however, considered harmful to humans. They produce carcinogenic effects. Because of this, consumers have since shifted their preference to meat produced naturally without the use of growth hormones and chemicals. The use of plant extracts of legume trees has been explored as an alternative route towards meeting consumer needs and increasing the quality of meat and meat products at a low cost (Tshabalala *et al.,* 2003; Reyes-Sanchez *et al.,* 2006).

Legume trees exhibit natural antioxidant activities and are likely to be safe for humans (Moyo *et al*., 2011). The presence of polyphenolic compounds and fibre, however, limits the level of usage of legume trees in livestock (Heuzé *et al*., 2011; Mokoboki *et al*., 2013). Tannins bind to proteins, making them unavailable to animals. Little, if any, information is available on the recommended level of legume inclusion in broiler diets to improve shelf-life of broiler meat with its antioxidant properties.

One promising legume leaf meal is *Vachellia tortilis,* formerly known as *Acacia tortilis.* The plant is a nitrogen-fixing legume with a crude protein range of between 140 and160 g/kg (Dube *et al*., 2001; Heuzé *et al.,* 2011; Khanyile *et al*., 2014). Other common species include *Vachellia tortilis, Vachellia nilotica, Vachellia robusta, Vachellia nigrescens* and *Vachellia xanthophloea*. The trees are readily available and easily accessible to farmers. Several studies have explored the use of *Vachellia* species in animal feed by assessing growth performance,

meat quality and nematode burden, where the presence of *Vachellia* have been successful (Xhomfulana *et al.,* 2009; Mapiye *et al.,* 2010; Khanyile *et al.,* 2014).

## 1.2 Justification

It is important to explore and investigate sustainable and safe methods that are effective, easily accessible and cost effective to improve the quality of meat over time. Policy-makers and decision makers can make appropriate policies on advancing the poultry industry, particularly for smallholder farmers. Responses in organ weights, growth performance and blood chemistry cannot give a precise inclusion level of *Vachellia tortilis* leaf meal to be used in broiler diets for improved shelf life. These measures tend to highlight the negative impacts of leaf meals. It is crucial that antioxidant and shelf life properties of the meat are also included when exploring optimum inclusion levels of these leaf meals.

It is important to ensure that consumer demands for natural produced meat and products are met, and cuts losses in market. Incorporating *Vachellia tortilis* in broiler diet is likely to benefit resource-poor farmers who have access to these leaf meals. Raising awareness and economic benefits to small-scale broiler production may not only improve food and nutrition security, but also household income. Such efforts may also lead to the conservation of *Vachellia tortilis* trees in the communities. The use of *Vachellia tortilis* with natural antioxidants properties will promote human health and develop sustainable strategies to conserve and increase production of local trees.

## 1.3 Objectives

The objectives of the study were to:

1. Determine the antioxidant activity of incremental levels of *Vachellia tortilis* leaf meal in broiler meat.
2. Determine the shelf life of broiler meat from broilers fed on *Vachellia tortilis* leaf meal at different inclusion levels.

## 1.4 Hypothesis

1. High inclusion levels of *V. tortilis* will increase antioxidant activity of broiler meat; and
2. *Vachellia tortilis* leaf meal at high inclusion levels improves the shelf life of broiler meat.

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# CHAPTER 2: Review of Literature

## 2.1 Introduction

The search for protein-rich legume leaf meals that can be used in diet formulations to reduce the use of conventional feed ingredients such as soybean and maize in poultry feed remains (Rubanza *et al.,* 2005). The limited availability and poor quality of raw materials raises the cost of poultry feeds. The low rainfall and the usage of raw materials among human and livestock consumption intensifies the situation. *Vachellia tortilis* is one legume tree of interest which has little, if any, available information on its potential to extend the shelf-life of broiler meat and possesses natural antioxidant properties. The review discusses the importance of incorporating *Vachellia tortilis* leaves in broiler diets to enhance the quality of meat. The antioxidants activity of *Vachellia tortilis* leaf meals in broiler meat will also be reviewed. In addition, optimum inclusion levels, benefits and limitations of using *Vachellia tortilis* in broiler feeds will be discussed.

## 2.2 Broiler meat production

The poultry sector is the largest meat producer industry worldwide followed by beef, pork and mutton respectively. The patterns of chicken meat consumption have been on the rise over the past years, reaching 1.658 million tonnes of poultry meat sold in 2017, with 3.86 % coming from subsistence farming (South African Poultry Association, 2017). Its production not only creates opportunities for employment but also ensures continuous supply of good quality meat and meat products to the growing human population. It has a desirable nutritional composition with low fat content, cholesterol, high polyunsaturated fatty acids and high levels of iron (Jaturasitha *et al.,* 2008; Mothershaw *et al.,* 2009). On these bases, consumers view it as a safety protein source for their daily diet.

Moreover, chickens are not linked with cultural beliefs, unlike goats and pigs (Dyubele *et al.,* 2010) and it minimises cases of food insecurity and malnutrition in financially needy households. However, growth of poultry production is hindered by high costs of raw material and its exposure to lipid oxidation (Allen *et al.,* 1998). Biochemical and microbial mechanisms are the leading causes of the rapid loss of quality and freshness of chicken meat.

All breeds of chicken come from the same genus, species, and subspecies of bird *Gallus domesticus* (Hascik *et al.*, 2010). Cobb 500, Ross 308/ 708 and Hubbard F15 are the commonly used broiler species in the meat industry. These are commercially reared species, in a deep litter system, unlike indigenous chickens, their survival solely depends on the farmer. Broilers reach its target slaughter weight within the period of five weeks.

### 2.3 Properties of *Vachellia tortilis*

*Vachellia tortilis* is a protein-rich legume tree found in the areas of Africa and Middle- East. It belongs to the family of *Fabaceae,* under the sub family of the *Mimosoideae.* There are 32 species studied to be under the *Vachellia* genus which some including; *Vachellia nilotica, Vachellia karroo, Vachellia angustissima, Vachellia galpinii* are shown in Table 2.3. These trees grow under various climatic conditions, and can grow up to 4 to 20 m, attaining its full growth for longer periods (Heuzé *et al.,* 2011). Its strong sloped roots allow it to grow under different soils including; sandy, high alkalinity and stony soils. The tree is beneficial to both mankind and animal. The tree can be used for medications and as a feed supplement to livestock. Moreover, it can survive heavy grazing during winter where the quality and quantity of feed declines (Abdulrazak *et al.,* 2000).

#### 2.3.1 Nutritional composition of *Vachellia tortilis*

Tables 2.1 and 2.2 show the chemical composition and mineral content of *Vachellia tortilis* leaves. Table 2.3 shows the chemical composition of other *Vachellia* species. The crude protein

of *Vachellia tortilis* leaves is within the range of 140 to 218 g/kg (Dube *et al*., 2001) which is equivalent to the broiler protein requirements. Broilers require a crude protein content between 210 and 225 g/kg in the starter phase and declines to 175 g/kg in the finisher phase, which makes *V. tortilis* a valuable protein source in broiler feed. Dietary protein is vital in broiler growth and development, it assists in the synthesis of body tissue. Furthermore, protein exists in the form of enzymes and hormones which play vital roles in the physiology of functioning of broilers. Acid detergent fibre (ADF) levels range between 125 and 180 g/kg, whilst neutral detergent fibre (NDF) lies within the range of 168 to 285 g/kg. The crude fibre content of fresh leaves is 182 g/kg DM and the crude protein for pods 188 g/kg DM (Dube *et al.,* 2001; Halimani *et al*., 2005).

The levels of minerals in V. tortilis leaves are also favourable, having a concentration of 6.1 g/kg DM of calcium, 1.8 g/kg DM phosphorus and 11.4 g/kg DM potassium (Heuzé et al., 2011). The nutrient composition of Vachellia tortilis depends on various factors such as the condition of soil, season and stage of leaf growth. The polyphenolic compounds contents for Vachellia species range between 11 and 90 g/kg DM (Mokoboki et al., 2013).

**Table 2.1: Chemical composition of *Vachellia tortilis* leaves**

| Chemical composition | Concentration (g/kg) |
|---|---|
| Dry matter | 944 |
| Ash | 65 |
| Crude protein | 189 |
| Crude fibre | 218 |
| Neutral detergent fibre | 452 |
| Acid detergent fibre | 145 |
| Acid detergent lignin | 82 |
| Acid detergent insoluble nitrogen | 18.3 |
| Crude fat | 40.1 |
| Condensed tannins | 51.5 |

Sources: Heuzé *et al*., (2011).

**Table 2.2: Mineral content of *Vachellia tortilis* leaves**

| Mineral | Concentration (g/kg DM) |
|---|---|
| Calcium | 9.60 |
| Phosphorus | 23.0 |
| Magnesium | 3.02 |
| Potassium | 17.3 |
| Sodium | 0.41 |
| Zinc (mg/kg DM) | 21.6 |
| Copper (mg/kg DM) | 2.01 |
| Manganese (mg/kg DM) | 12.3 |
| Total extractable phenolics | 241 |
| Total extractable tannins | 226 |
| Total condensed tannins | 51.5 |
| Soluble condensed tannins | 18.9 |

Sources: (Dube *et al.,* 2001; Rubanza *et al.,* 2005; Heuzé *et al*., 2011).

**Table 2.3: Chemical composition of leaves (g/kg DM) for common *Vachellia* species in Southern Africa**

| Species | DM | OM | CP | NDF | ADF |
|---|---|---|---|---|---|
| *Vachellia karroo* | 945.4 | 897.0 | 108.0 | 504.6 | 406.9 |
| *Vachellia nilotica* | 951.6 | 882.6 | 151.7 | 572.0 | 472.2 |
| *Vachellia galpinii* | 944.5 | 886.7 | 149.6 | 509.0 | 454.7 |
| *Vachellia sieberiana* | 970.2 | 887.8 | 183.2 | 561.3 | 414.9 |
| *Vachellia hebeclada* | 971.8 | 915.1 | 164.9 | 570.1 | 428.8 |
| *Vachellia rhemniana* | 957.4 | 874.0 | 102.7 | 487.9 | 441.6 |

DM: dry matter; OM: organic matter; CP: crude protein; NDF: neutral detergent fibre; ADF: acid detergent fibre.

Source: Mokoboki *et al*. (2005).

The parts of *Vachellia tortilis* apart from leaves are useable to both human and animal for consumption. Seeds, flowers, and pods are consumed by humans as vegetables, while farmers use it as feed for livestock (Tanner *et al.,* 1990). Moreover, *V. tortilis* seeds can be used to clean water containing hazardous chemicals (Ackacha *et al.,* 2014). *Vachellia tortilis* seeds, pods and fruits are a valuable source of energy and protein if harvested in the early dry season or in the wet season. Large amounts of these can be harvested and stored to be used in times of feed scarcity without losing its nutritional composition. The ability to use all parts of the tree ensures the continual availability of feed throughout the year, especially during dry season where the quality and quantity of raw materials decline.

*Vachellia tortilis* tree has the potential to improve nutrition, boost food security and foster rural development (Abdulrazak *et al.,* 2005). Table 2.4 shows the chemical composition of *Vachellia tortilis* parts of the tree. Araya *et al.* (2003) reported that feeding *V. tortilis* pods to goats at up to 75 % of the diet increases digestibility of both dry and organic matter, which results in an increased in body weight gain (BWG). Abdulrazak *et al.,* (2005) found fruits to be more nutritious when ground. However, Bwire *et al.* (2004) found milk of dairy cows fed *Vachellia tortilis* pods to have an unpleasant odour.

**Table 2.4: Chemical composition of pods, seeds and fruits of *Vachellia tortilis***

| | | (g/kg) | | | |
|---|---|---|---|---|---|
| **Item** | **Ash** | **Nitrogen** | **NDF** | **ADF** | **ADL** |
| Whole fruits | 46.6 | 21.7 | 324 | 242 | 48 |
| Pods | 68.9 | 12.5 | 394 | 264 | 75 |
| Seeds | 45.9 | 30.5 | 337 | 239 | 19 |

NDF: neutral detergent fibre; ADF: acid detergent fibre; ADL: acid detergent lignin

Source: (Tanner *et al.,* 1990)

Lipid oxidation is the biggest economic problem in the meat industry, it affects the quality of meat and the selling price of the product (Allen *et al.,* 1998). The upscaling production of broilers depends on the nutrition they acquire. Feed constitutes of 80 % of poultry production, however, the increasing costs of raw materials reduces the economic efficiency and viability of poultry enterprises. Seasonal changes affect the quantity and quality of raw materials produced all year round. This challenge has motivated animal nutritionists, researchers and other stakeholders in the poultry industry to search for possible replacements to conventional feed sources.

Researchers, nutritionist and other stakeholders in the poultry industry found *Vachellia tortilis* as a legume tree to use in broiler feed to reduce costs. It is easily accessible, available and easy to use as it can be used without being processed (Abdulrazak *et al*., 2000). Conventional feeds

require extensive processing including thermal and mechanical processing which affects the nutritional composition of ingredient before being used in broiler diets. Processing destructs the amino acid profile causing a reduction of microorganisms in the final product (Al-juhaimi *et al.,* 2018).

### 2.3.2 Antioxidant properties

Plant extracts of legume trees are referred to as ‘‘natural’’ alternatives to chemical preservatives and their use in foods meets the demand of consumers for natural meat products (Jiang *et al.,* 2016; Jaroszewska *et al.,* 2017). Antioxidants are molecules which can safely interact with free radicals and terminate the chain reaction before vital molecules are damaged (Alajmi *et al.,* 2017). The two classes of antioxidants include; synthetic and natural antioxidants, with a role of maintaining the freshness of meat (Arshad *et al.,* 2011). Common synthetic antioxidants are; butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), tertiary butyl hydroquinone (TBHQ) and propyl gallate (PG) (Fasseas *et al.,* 2007).

Many of the natural antioxidants of interest are of plant origin and they belong to the polyphenolic class of compounds. Current consumer preference restricts the use of synthetic antioxidants as feed additives due to their potential toxicological effects, replacing their use with natural antioxidants (Valesco *et al.,* 2011; Jaroszewska *et al.,* 2017). Brenes *et al.,* (2008) reported that, plants and their products are potential sources of phytochemicals that counteract free radicals due to their antioxidant activity.

Leguminous trees exhibit antioxidant properties which improve meat quality, animal performance and is used as medicine to human health (Nkukwana *et al*., 2014; Jiang *et al*., 2016; Alajmi *et al.,* 2017). Flavonoids are one of the major compound classes of plant phenolic. Their antioxidant activity is superior to the synthetic antioxidants. The capacity of antioxidant of flavonoids increases with increasing numbers of free hydroxy groups on the aromatic rings

(Muhaisen *et al*., 2002). Naturally occurring antioxidant compounds can be divided into groups based on their chemical structure (Rice-Evans *et al.,* 1996). The structural variations between groups of these compounds include the number and arrangement of hydroxy moieties, the varying degrees of double bonds in the rings, as well as the nature and extent of alkylation and/or glycosylation (Rice-Evans *et al.,* 1996). These physical variations ultimately determine the efficacy of phenolic compounds as functional antioxidants.

The meat industry uses antioxidants, with the aim of preserving its quality (Valesco *et al.,* 2001). Antioxidants are either synthetic or natural, they are organic molecules which can scavenge the active forms of oxygen involved in the initiation phase or propagation of oxidation in meat and meat products (Valenzuela, 1995). The naturally occurring compounds prolong the quality of meat with their antioxidant properties. These include polyphenols, tocopherols, ascorbic acid, flavonoids, and carotenoids (Mokoboki *et al.,* 2013; Alajmi *et al.,* 2017). Leaf meals exert the antimicrobial, anti-carcinogenic, anti-inflammatory and antioxidant effects (Valesco *et al*., 2001; Cui *et al.,* 2018). However, the interest is more on the quantity rather than the quality of natural antioxidant.

Arshad *et al.,* (2011) highlighted that antioxidative compounds that can contribute to the antioxidant protection of meat products including carotenoids, hydroxycinnamic acids, flavonoids, terpenes, and vitamins. Oxidation in meat manifests when conversion of the red muscle pigment myoglobin turns to brown metmyoglobin. Dietary supplementation with vitamin E increases its deposition in the muscle and fat so that the oxidation is retarded, and shelf-life of the meat is enhanced (Bou *et al.,* 2009). Minimal amounts of antioxidants maintain gizzard activity and gastro-intestinal tract functionality of broilers. Natural antioxidants reduce the occurrence of lipid oxidation and microbial growth (Moyo *et al.,* 2011; Wapi *et al.,* 2013). The usage of antioxidants can be applied directly in feed or during meat packaging (Barbosa-Pereira *et al.,* 2014). During production, processing, distribution, and storage, meat and meat

products undergo chemical and physical changes in quality (Barbosa-Pereira *et al.,* 2014). Processing methods including thermal processing, mincing or ionizing exposes meat and meat products to quality deterioration (Jongberg *et al.*, 2017). In addition, the deterioration of meat is enhanced by vast amounts of unsaturated lipids resulting to rancid meat, development of off-favour, nutrient and drip losses and reduced storage time (Fletcher, 2002; Sampaio *et al.,* 2012; Al-Hijazeen *et al*., 2016). The process of oxidation of lipids involves a three-step radical chain reaction which consist of initiation, propagation and termination with the production of free radicals.

The process takes place when free radicals react with reactive oxygen species (ROS) and reactive nitrogen species (RNS) which triggers oxidative stress and damage of macromolecules including the lipid and protein fractions. Reducing light and heat are physical methods of reducing the occurrence of oxidation. The proportion of PUFA in lipid bilayers, the amount of ROS produced and the level of endogenous or nutritional antioxidants determines the susceptibility of meat to the oxidation (Brenes *et al.,* 2008). Oxidation in meat is usually assessed by measuring the amount of peroxide value (PV), Thiobarbituric acid-reactive substances (TBARS), sulphyryl and carbonyl group generated during the process.

### 2.4 Limitations to using *Vachellia tortilis* in animal feed

Leaf meals contain polyphenolic compounds and fibre which constrain their utilisation in chickens.

#### 2.4.1 Presence of polyphenolic compounds

Plants generally contain chemical compounds such as saponins, tannins, oxalates, phytates, trypsin inhibitors and cyanogenic glycosides. These compounds are generally known as secondary metabolites, and are biologically active (Soetan *et al.,* 2009). *Vachellia tortilis* contains high levels of phenolic compounds that causes nutritional limitations in broiler health

(Dube *et al.,* 2001; Rubanza *et al.,* 2005). The tannin concentration of legume trees varies with species and stage of maturity. Polyphenolic compounds form complexes with nutrients which are difficult to digest and utilize. Tannins are polyphenolic substances with various molecular weights and a variable complexity, they can bind to proteins in aqueous solution (Makkar, 2003). Tannins are classified into two classes: hydrolysable and condensed tannins, tannins that can be hydrolysed are found in smaller amounts in plants.

Tannin-rich diet reduces feed intake, protein degradation and the fractional absorption of amino acids reaching the small intestine. Polyphenolic compounds cause astringent or a bitter taste which reduces the intake of food (Phengsavanh, 2013, Martens *et al.,* 2014). However, tannins also possess antioxidant properties; anticarcinogenic, antimicrobial and antiparasitic and acts as a defence mechanism against insects and pests. The presence of tannins reduces gastrointestinal parasite and slows the distribution of intestinal parasites and viruses that cause diarrhoea in piglets (Gxasheka *et al.,* 2015).

Tannins are also responsible for clearing of poisonous substances in the body of animals (Halimani *et al.,* 2005; Gxasheka *et al.,* 2015). The leaf meals from extracts of plant origin are rich in tannins and dietary fibre but if consumed at optimum levels, they do not compromise growth performance of animals. Additionally, tannin-rich plants act as natural alternative anthelmintic for controlling haemonchosis in goats (Min *et al*., 2003).

### 2.4.2 High fibre content

Fibre is naturally present in plant-based feed ingredients and is an important component in poultry diets. *Vachellia tortilis* leaves have high fibre content (see Table 2.1). High fibre diets are more appropriate in ruminants because of their accommodating four-chamber stomach unlike in monogastric and avian. Fibre content of the feed have the energy dilution effect which causes decreased metabolizable energy concentrations that leads to increased feed intakes with

increasing levels for the leaf meals (Olugbemi *et al.,* 2010). Fibre provides energy to the bacteria in the lower gastrointestinal tract where the bacteria uses nitrogen that would otherwise be excreted as uric acid for bacterial protein synthesis. The excessive use of fibre sources in the diet may increase viscosity of the intestinal content, which adversely affects body weight gain and carcass quality (Min *et al.,* 2003). Excess feeding of fibre sources may lead to enlargement of the intestinal villi.

### 2.4.3 Presence of thorns

The presence of thorns in most *Vachellia* species is one of the factors restricting livestock animals from direct feeding (Dube *et al.,* 2001; Mapiye *et al.,* 2011). Thorns reduce the surface area for leaf-biting by animals thus lowering nutrient intake (Mapiye *et al.,* 2011). Thorns can be avoided by cutting the small branches and drying it thereby collecting only leaves which can be stored and used in animal feeding (Halimani *et al.,* 2005). The binding of these nutrients with proanthocyanidins, decreases their palatability, digestibility, absorption and availability.

## 2.5 Meat quality characteristics

The common meat quality exist in meat are colour, texture, drip loss and cook loss. These depend on pH of meat at slaughter

### 2.5.1 Meat pH

Meat quality is defined by the combination of many factors including; colour, pH, tenderness and flavour (Tshabalala *et al.,* 2003). Meat quality is dependent on various factors before slaughter, at slaughter, type of packaging and storage temperature. Extremely high temperatures, overcrowding, and restriction to water can cause stress to broiler and affect meat pH (Quiao *et al.,* 2001). Under normal conditions, the pH of the chicken breast is about 7.2 (before slaughter) and after six hours post mortem decrease between 5.7 and 6.0. Stressing animals before slaughter causes high depletion levels of glycogen, which results to high pH

meat. A low meat pH is often associated with low water holding capacity (WHC) and a pale meat colour which results in increased cooking and drip loss (Northcutt *et al.,* 1994).

Water-holding capacity is defined as the ability of meat to retain its water during application of external forces, such as cutting, heating, grinding or pressing. Broilers limited to adequate good quality of feed, experiences dehydration which aggravates electrolyte imbalance and glycogen depletion thus increasing the pH of meat (Quiao *et al.,* 2001). Animals that experience malnutrition, often has a high ultimate pH meat (Fletcher *et al.,* 2000) and such meat is normally darker in colour than meat with a normal pH (Ledward *et al*., 1986). Antioxidants are used to lower pH of meat during lipid peroxidation. A very low ultimate pH (< 5.4) results in a very pale and soft (PSE) muscle colour (Allen *et al.,* 1997). If the pH does not decline much post mortem, the meat turns dark with dry surface.

### 2.5.2 Meat colour

Colour is the most used indicator by consumers when assessing the freshness of meat. In other words, consumer's use meat colour to make decisions on whether to buy or not to buy the product. Colour is expressed in three categories: brightness (L*), redness (a*) and yellowness (b*). These categories are used in the laboratories on behalf of testing whether colour meets consumer preferences and standards. Pink or red appearance of cooked poultry meat is generally associated with undercooking and is highly undesirable (Fletcher, 2002). Dark or black bones are also considered to be a defect in fully cooked products. Bone darkening is primarily associated with frozen products prior to cooking.

Meat colour is affected by several factors including; age of animal, myoglobin content, storage, cooking temperature and method, feed and conditions at slaughter (Qwele, 2012). Older animals tend to have darker meat in colour because the myoglobin level increases with age. In addition, exercised muscles are always darker in colour, which causes variations of colour in

muscles of the same animal. Ngambu *et al*. (2013) reported supplementing goat feed with *Vachellia karroo* produced higher b* (yellowness) values. Moreover, Mapiye *et al.* (2009) reported that *Vachellia karroo* improved the quality of meat from Nguni cattle.

### 2.5.3 Texture

Texture is the second parameter used by consumers when assessing the acceptability of cooked meat. Meat texture sensation is dictated by the presence of several factors including the amount of intramuscular fat, water holding capacity and actomyosin complex (Grashorn *et al.,* 2010). Texture of meat relies on the amount of connective tissue, type of muscle fibre and cooking method. Texture assessment can be categorised based on; hardness, cohesiveness, elasticity, dryness, fattiness, roughness and chewiness. The texture of meat can be measured by instruments such as the Warner Bratzler shear device (Zhuang *et al.,* 2010). After slaughter, muscles become stiff due to no blood circulation, absence of oxygen or nutrients to the muscles. Soon after rigor mortis, muscles become soft again.

It is, however, the quality of collagen, which gives toughness to meat (Saláková *et al.,* 2009). Collagen is the major component of the intramuscular connective tissue and plays a key role in determining meat toughness (Liu *et al.* 1996). Meat tenderness refers to the ability of meat to resist fragmentation when being chewed. It includes initial bite by the teeth, the breakdown of meat into fragments and the amount of residue remaining after chewing (Lawrie, 1998). Pre-slaughter handling, freezing, thawing and aging have a significant effect on tenderness (Liu *et al.* 1996). The rate and extent of the chemical and physical changes in conversion of muscle to meat also has an impact on the tenderness of meat (Sentandreu *et al.,* 2002). The level of condensed tannins in *Vachellia* species, positively influences meat fatty acids composition (Vasta *et al.,* 2009) which improves meat texture. Ngambu *et al.,* (2012) reported that supplementing goat feed with *Acacia karroo* improved meat tenderness and juiciness.

### 2.5.4 Cooking loss

Cooking loss is the degree of shrinkage of meat during cooking. The total loss that occurs during the cooking of meat includes the losses known as drippings and the volatile losses (Al-Hijazeen *et al.,* 2016). The greater part of the volatile loss is from evaporation of water. It may include volatile substances from the decomposition of fat and volatile aromatic substances. Losses of meat are normally due to cooking methods and thawing. Frying, boiling, grilling and roasting are the common methods used for cooking meat. These methods affect the texture of meat and consumers' decision on assessing the final product of meat. Dyubele *et al.* (2010) reported a significant effect of thermal preparation on sensory scores of chickens where the roasted meat had higher sensory scores than the cooked meat.

### 2.5.5 Drip loss

Drip loss is the loss in weight of food products owing to extruding and dripping away of tissue juices, such as meat juices lost during the thawing of frozen meat. The drippings include fat, water, salts, and both nitrogenous and non-nitrogenous extracts (Fletcher *et al.,* 2000). Protein denaturation leads to greater drip loss and pale meat.

**Table 2.5: Meat quality indicators in broilers during storage**

| Attributes | Time (Days) 0 | 6 | 12 | 18 |
|---|---|---|---|---|
| Colour: L* | 54.9 | 48.42 | 43.5 | 40.2 |
| a* | 4.8 | 3.02 | 5.8 | 6.2 |
| b* | 12.9 | 14.1 | 8.5 | 13.2 |
| pH | 5.5 | 5.86 | 6.9 | 7.5 |
| Texture (N) | 4.32 | 4.94 | 5.98 | 6.42 |
| Cooking loss (%) | 5.98 | 8.68 | 12.6 | 15.4 |
| Drip loss (%) | 1.1 | 2.8 | 3.6 | 4.2 |

Sources: Allen *et al.,* 1997; Fletcher *et al*., 2000; Quiao *et al.,* 2001; Ristic *et al.,* 2010; Karre *et al.,* 2013; Wapi *et al.,* 2013

## 2.8 Summary of review

It is crucial for the poultry industry to improve the quality of broiler meat and products to reduce market loss and not to endanger human health. The industry of poultry is a huge supplier of proteins from meat and meat products and has a major market compared to red meat. It supplies good quality of proteins, minerals and amino acids which improves the well-being of consumers in their daily diet. The shortages of good quality feed and competition among growing human population remains a challenge. Therefore, *V. tortilis* leaf meal is expected to enhance and preserve the quality of broiler meat with its natural antioxidant properties. This will reduce the use of conventional feed sources and shift to easily accessible and readily available feed sources. The broad objective of the study was to determine the antioxidant activity and enhancing shelf life of broiler meat by feeding different levels of *V. tortilis* leaf meal.

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## Chapter 3: Antioxidant activity of meat extracts from birds fed on incremental levels of *Vachellia tortilis* leaf meal


### Abstract

The study investigated the antioxidant potency of different levels of *Vachellia tortilis* leaf meal on broiler meat extracts using the 2, 2-diphenyl-1-picryhydrazyl (DPPH), ferric reducing power (FRAP) and β-carotene-linoleic acid assays. Broiler diets contained 0, 30, 60, 90, 120 and 150 g/kg DM of *Vachellia tortilis* leaf meal. Breast meat samples were collected at slaughter, vacuum sealed and kept at 4˚C for antioxidant activity analysis. The antioxidant activity did not go beyond 50 % in the DPPH assay on all the concentration levels. The FRAP assay exhibited a concentration-dependent linear response among the inclusion levels. The linear regression equation was ($y = 0.0004x + 0.1366$). The activity of antioxidants exhibited a quadratic response using the β-carotene-linoleic acid model ($y = -0.0037x^2 + 0.7756x + 2.7811$). An inclusion level of 104.81 g/kg had the highest antioxidant activity. An activity of antioxidant of 48.98 and 40.99 % had the highest activity, making an inclusion level of 60 and 90 g/kg appropriate for broiler feeding respectively. The findings not only suggest the importance of knowing the appropriate leaf meal inclusion level but also provides a guide on preventing the process of lipid oxidation in meat.



**Keywords**: Antioxidant activity; β-carotene-linoleic acid; meat extracts; Ferric reducing power; 2, 2-diphenyl-1-picryhydrazyl

## 3.1 Introduction

Antioxidants are compounds that are capable of donating hydrogen (H·) radicals for pairing with available free radicals (Shahidi, 2000). These are included in animal diets to prevent the propagation reaction during oxidation, with the aim of preventing the oxidation of lipids in meat and meat products. Descalzo *et al.* (2008) reported that antioxidants get incorporated within cell membranes and protect tissues against oxidation from reactive oxygen species, thus maintaining the overall quality of meat. Synthetic antioxidants are chemically synthesized petroleum-based antioxidants which are primarily used to retard lipid oxidation, preserve and stabilize the refined oils and fats within food products (Karakaya *et al.,* 2011).

The most common synthetic antioxidants in foods are butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), tertiary butyl hydroquinone (TBHQ) and propyl gallate (PG) (Fasseas *et al.,* 2008). The safety of using synthetic antioxidants in animal feed is, however, questioned and viewed as harmful to humans (Descalzo *et al.,* 2008). Their effect has been found to be detrimental to major organs like lungs, kidney and liver and aids in suppression of humoral immunity. The complications over human health triggered by synthetic antioxidants resulted to consumers shifting their interest to foods that are produced from extracts of plant origin. To ensure consumer safety was met and their choice of meat and meat products was sold to them, the poultry industry had to eliminate the use of synthetic antioxidants in feed. This led to a search for alternative feed sources which resulted to viewing nitrogen-rich leguminous leaf meals as the alternative protein sources and are safer to use (Mapiye *et al.,* 2010; Moyo *et al*., 2011; Ndou *et al*., 2015).

During meat processing and storage, intrinsic antioxidants, including tocopherols, carnosine, and antioxidant enzymes, are depleted making meat susceptible to lipid oxidation (Karre *et al.,* 2013). Lipid oxidation is responsible for the development of primary and secondary oxidation

products, reduction in nutritional quality, as well as changes in flavour (Maqsood *et al.,* 2011), which causes health issues and major economic losses. Incorporating plants that contain natural antioxidants in animal diets has the potential to retard lipid oxidation, microbial growth (Djenane *et al*., 2003; Qwele *et al.,* 2013; Shah *et al.,* 2014) and improve meat colour, thus, extending the shelf life of meat. Karre *et al*. (2013) highlighted that natural antioxidants activity delays the oxidative degradation of lipids in meat. In plants, phenolic compounds are secondary plant metabolites that are involved in the normal growth, development, act as defence mechanisms of plants against pathogenic, parasites infection and free radicals' generation (Maisuthisakul *et al.,* 2007).

Nitrogen-rich leguminous leaf meals are a good source of protein and contain natural antioxidants with high levels of polyphenolic compounds (Dube *et al.,* 2001; Ndou *et al.,* 2015). Leguminous leaf meals contain a range of different compounds which possess antioxidant properties including Vitamin E, flavonoids, phenolics, carotenoids and ascorbic acid (Makkar *et al.,* 1996; Muhaisen *et al.,* 2002; Camo *et al*., 2008). These properties of compounds are present in considerable amounts in *Vachellia* species (Dube *et al.,* 2001; Muhaisen *et al.,* 2002). Extracts from nitrogen-rich legume leaf meals also improves growth performance and reproductive performance of livestock (Mapiye *et al*., 2010; Hlatini *et al.,* 2016).

*Vachellia tortilis* is a rich protein source, with high contents of polyphenolic compounds (Dube *et al.,* 2001). Polyphenolic compounds reduce protein and amino acid digestibility in pigs and chickens (Hlatini *et al*., 2016), however, they possess a wide range of positive biological effects. These include antioxidant, antimicrobial, anti-inflammatory effects (Funatogawa *et al.,* 2004; Khanyile *et al.,* 2014). These plant extracts protect the biological cellular components, such as deoxyribonucleic acid, proteins, and membrane lipids, from reactive oxygen species (ROS) attacks (Sharma *et al*., 2012).

Using plant extracts that display the natural antioxidant properties in broiler feeds reduces microbial spoilage of meat and improves its quality, aiding in extended shelf life (Djenane *et al.,* 2003). The antioxidant properties of plant extracts have been also documented to reduce cases of coccidiosis in broilers (Naidoo *et al.,* 2008). To further ensure the delay of lipid oxidation in meat, natural antioxidants can be applied directly in meat, administered orally or added in packaging (Djenane *et al.,* 2002). The quantity of antioxidants is, however, of great interest in maintaining the quality of broiler meat. The objective of the study was to determine the antioxidant activity of meat produced from broilers fed on different inclusion levels of *Vachellia tortilis* leaf meal. It was hypothesized that broilers assigned to diets with high *V. tortilis* leaf meal, will have meat samples with high antioxidant activity.

## 3.2 Material and Methods

### 3.2.1 Study site

The rearing and slaughtering of broilers were done at Ukulinga Research Farm, University of KwaZulu-Natal, and Pietermaritzburg, South Africa. It is a flat open area with GPS coordinates 29°39'48.3"S 30°24'19.4"E. The trial was conducted from August to September 2017. The area experiences rainy season from October to February and dry season between March to September. The study of antioxidant activity of *Vachellia tortilis* leaf meals in meat was conducted at the Agricultural Research Council, Roodeplaat, South Africa. It lies in the Gauteng province, South Africa at $25^0$59"S $28^0$35"E.

### 3.2.2 Leaf collection, processing and diet formulation

The harvesting of *Vachellia tortilis* leaves was done at Makhathini Research Station, Jozini Municipality, South Africa. The leaves were hand harvested during the post rainy season at an advanced stage of maturity. The harvest was done in March 2017, on harvest, the leaves were stored in plastics and transported to Ukulinga Research Farm. The leaves were air dried in an

open house and grinded through a 2mm sieve for removal of thorn, twigs and pods. After drying, the leaves were kept in sealed feed bags under the shade to avoid exposure to light before used for feed formulation. Diets of six dietary treatments were formulated using Winfeed diet feed formulation software.

The six dietary treatments contained 0, 30, 60, 90, 120 and 150 g/kg inclusions of *Vachellia tortilis* leaf meal to meet the nutrient requirements for growth. Broilers assigned to a 0 g/kg dietary treatment were used as a control. All experimental diets were supplemented with vitamins and minerals to meet the National Research Council (NRC, 2012) recommended specifications for broilers. The ingredient composition of the diets for the finisher broilers is shown in Table 3.1. After feed formulation, a feed sample on each of the six diets was collected for proximate analysis.

### 3.2.3 Experimental design and bird management

The experimental procedures were performed according to the ethical guidelines specified by the Certification of Authorization to Experiment on Living Animals provided by the UKZN Animal Ethics Committee (Reference No: 004/13/Animal). A total of 120 Cobb-500-day-old healthy unsexed broilers were purchased at National chicks, Camperdown, South Africa, transported to Ukulinga Research Farm. Birds were allocated to single pen on arrival, which after 14 days, they were distributed in two houses of similar environments where they were allocated to their dietary treatments. The six dietary treatments were displayed in a completely randomised block design.

Each treatment had one replicate, with 10 birds randomly assigned in one pen. Straws were used for bedding. From day 0-14, broilers were kept in a single pen. During this growth phase, they were assigned to broiler mash, after which they were assigned to dietary treatments. Fresh water and feed were provided *ad libitum* throughout the trial. The trial was conducted for 35

days, the feeding of *Vachellia tortilis* leaf meal-based diets commenced on day 15 up to day before slaughter. No vaccinations were performed on chick arrival and throughout the trial. Chicks were kept at a temperature-controlled room and the brooding lights were provided during brooding period.

### 3.2.4 Chemical analyses of diets

After milling of *Vachellia tortilis* leaves on a 2 mm sieve, the experimental diets were formulated at Ukulinga Research Farm, Feed Formulation Unit, which after a sample of each treatment was taken to the laboratory for chemical analyses. The analysis of feed samples was analysed at the Animal and Poultry Science Laboratory which is located at the University of KwaZulu-Natal (UKZN), Pietermaritzburg, South Africa, the results are presented in Table 3.2. Experimental diets were analysed in triplicates for their chemical composition to minimise errors. The dry matter, ash, crude protein and ether extract were determined according to method described by AOAC (1995). Crude protein was calculated using N x 6.25, where the nitrogen contents were analysed using the Leco Truspec Nitrogen Analyser following Dumas Combustion method. The neutral detergent fibre (NDF) and acid detergent fibre (ADF) were analysed using ANKOM Fibre Analyser (Ankom Macedon, NY, USA) as described by Van Soest *et al.,* (1991). Butanol-HCL method was used for the determination of condensed tannins (Reed *et al.,* 1982). Ash samples were burnt at $550^0$C and kept for the determination of minerals. Minerals content were detected using Varian 720 Inductively Coupled Plasma Emission Spectrometer (ICP-OES, Frankfurt, Germany) using atomic absorption. Table 3.3 shows the mineral analysis for dietary samples and *V. tortilis* leaves.

### 3.2.5 Slaughter of birds

A night before slaughter, feed was removed from the feed troughs, but water was given *ad libitum.* On the day of slaughter, 120 broilers were slaughtered at the Ukulinga Research Farm Abattoir. All birds were electrically stunted (50-70 volts) for five seconds to make them unconscious and exsanguinated left to bleed for 10 minutes. Left breasts and thigh muscles were collected on eight birds per treatment and four birds per replicate. Skinless and boneless samples were collected, vacuum packed and kept at temperature of 4°C for analysis. All samples were labelled by dietary treatments for easily identification.

### 3.2.6 Meat sample extraction

Twelve breast meat samples were collected at slaughter for the determination of antioxidant properties. About 5 g of meat sample was obtained from each of the vacuum-sealed samples for meat extraction. The samples were homogenized in 50 mL of 50 % aqueous methanol (v/v) and placed in a sonication bath for 1 hour. Methanol and ethanol have been proven as effective solvents to extract phenolic compounds (Siddhuraju *et al.,* 2003). The extracts were filtered under vacuum using Whitman's No. 1 filter paper and concentrated under vacuum using a rotary evaporator at 35 ºC. The samples were then kept at room temperature for them to completely dry for the determination of antioxidant activities.

**Table 3.1: Ingredient composition (g/kg) of the finisher diets containing *Vachellia tortilis* leaf meal-based diets**

| Ingredient | Inclusion levels (g/kg DM) | | | | | |
|---|---|---|---|---|---|---|
| | 0 | 30 | 60 | 90 | 120 | 150 |
| Maize | 545 | 519 | 493 | 469 | 445 | 421 |
| Soybean 46 | 392 | 388 | 393 | 376 | 370 | 363 |
| Vachellia tortilis | 0 | 30 | 60 | 90 | 120 | 150 |
| Oil-Sunflower | 45 | 46 | 47 | 47.9 | 48.8 | 49.8 |
| Limestone | 1.38 | 1.37 | 1.28 | 1.23 | 1.2 | 1.2 |
| Monocalcium-phosphate | 1.45 | 1.44 | 1.38 | 1.3 | 1.28 | 1.23 |
| Salt | 5 | 5 | 5 | 5 | 5 | 5 |
| Vitamin- mineral premix | 5 | 5 | 5 | 5 | 5 | 5 |
| Lysine-HCL | 1.2 | 1 | 0.98 | 0.96 | 0.95 | 0.94 |
| Threonine | 0.97 | 0.86 | 0.75 | 0.64 | 0.52 | 0.4 |
| Methionine | 3 | 2.66 | 2.61 | 2.65 | 2.6 | 2.54 |

**Table 3.2: Physicochemical properties of the experimental diets**

| Item | *V. tortilis* inclusion level (g/kg) DM | | | | | |
|---|---|---|---|---|---|---|
| | 0 | 30 | 60 | 90 | 120 | 150 |
| DM | 993 | 987 | 988 | 973 | 974 | 963 |
| ME(MJ/kg) | 138.9 | 138.7 | 140.2 | 141.8 | 140.9 | 143.7 |
| Ash | 41 | 39 | 42 | 43 | 46 | 47 |
| Crude Protein | 194 | 203 | 204 | 194 | 221 | 201 |
| 2Ether Extract | 68.9 | 67.9 | 75.1 | 79.5 | 75.7 | 85.9 |
| ADF | 20.1 | 29.2 | 28.4 | 32.6 | 41.1 | 42.0 |
| NDF | 80.9 | 106.7 | 100.4 | 115 | 130 | 129 |
| CT | 0.1 | 0.6 | 0.7 | 2.2 | 5.9 | 9.2 |

ME- Metabolizable Energy; ADF –acid detergent fibre (g/kg DM); NDF – neutral detergent fibre (g/kg DM); CT- condensed tannins (mg/kg DM)

**Table 3.3: Mineral content of *Vachellia tortilis* leaf meal diets**

| Item | *Vachellia tortilis* inclusion level (mg/L) | | | | | | |
|---|---|---|---|---|---|---|---|
| | 0 | 30 | 60 | 90 | 120 | 150 | *V. tortilis* |
| Calcium | 78.1 | 132.6 | 139.8 | 332 | 100.7 | 6.8 | 8.74 |
| Potassium | 422.7 | 283.3 | 308 | 476 | 234.3 | 467 | 518 |
| Magnesium | 26.3 | 37.6 | 40.4 | 25.6 | 33.0 | 15.6 | 13.8 |
| Sodium | 47.1 | 57.7 | 61.6 | 46.0 | 17.6 | 21.9 | 20.9 |
| Potassium | 96.6 | 78.5 | 81.2 | 105.3 | 67.4 | 195 | 268 |
| Copper | 0.9 | 0.7 | 0.7 | 0.7 | 1.0 | 1.8 | 0.8 |
| Iron | 1.1 | 1.9 | 2.6 | 9.8 | 9.9 | 0.5 | 0.6 |
| Manganese | 1.6 | 1.5 | 1.3 | 1.6 | 0.4 | ND | ND |
| Zinc | 1.4 | 1.6 | 1.5 | 1.8 | 0.2 | 0.3 | 0.1 |

ND: not determined

### 3.2.7 Determination of antioxidant activity in meat samples

The determination of antioxidant activities was measured using three assays, the radical scavenging (DPPH), ferric reducing antioxidant power (FRAP) and the β-carotene-linoleic acid assays.

#### 3.2.7.1 DPPH (2, 2-diphenyl-1-picryhydrazyl) radical scavenging activity

Free radical scavenging activity (DPPH) was measured using the method described by Karioti *et al*. (2004). The stock solution of each meat extract consisted of a range of concentrations (0.625, 1.25, 2.5, 5, 10 and 20 mg/ml). Twenty microliters of each meat extract (stock solution) were diluted using methanol (130 µL) and then added to a mixture of DPPH solution to give a final volume of 1500 µL. The reactions were done under dim light and incubated at room temperature. The decrease in purple colouration was measured at 517 nm using a spectrophotometer with ascorbic acid used as a standard.

The free radical scavenging activity (RSA) was determined by the decolouration of the DPPH solution using the equation:

% RSA= (1- AE/ AD) x 100 where;

AE is the absorbance of the reaction mixture containing the standard antioxidant or extract, and AD is the absorbance of the DPPH solution only. Radical scavenging activity (%) was then plotted against the extract concentration. The EC50 values, representing the amount of extract required to decrease the absorbance of DPPH by 50 %, are calculated from the logarithmic non-linear regression curve derived from the plotted data.

#### 3.2.7.2 Ferric-reducing power assay

The ferric-reducing power (FRAP) assay was used to determine the activity of antioxidants in meat extracts derived from a diet with different levels of *Vachellia tortilis* leaf meal. The method used was described by Kuda *et al.* (2005). The assays were performed in a microtiter

plate containing 96 wells with each treatment done in triplicate. Methanol (30 µl) was mixed with sample (30 µl) and two-fold serially diluted to all the wells. With pH 7.2, a potassium phosphate buffer 40 µL was prepared and added to all the wells. Potassium ferricyanide was prepared in a dark room, its 40 µl, 1% (w/v) was added to the wells, after which the microtiter plate was wrapped with aluminium foil for 20 min at 50˚C.

After 20 min incubation, 40 µl of 10 % (w/v) trichloroacetic acid was added to each well with 120 µL of distilled water as well as 30 µl of 0.1 % w/v FeCl3 which was also prepared in a dark room. The reaction mixtures were kept at room temperature for 30 min after which the blue coloration was observed. The absorbance was measured at 630 nm using an ELISA plate reader (Jonfia-Essien *et al.,* 2008). Ascorbic acid was used as the standard. The results of absorbance were plotted against concentrations.

**3.2.7.3 β-carotene-linoleic acid assay**

The β-carotene-linoleic acid assay was done to determine the activity of antioxidants on broiler meat. The procedure was conducted as described by Amarowicz *et al.* (2004). One milligram of β-carotene was dissolved in 1 mL of chloroform which was prepared in a brown Schott bottle. Excess chloroform was removed under vacuum in the fume hood. Linoleic acid (20 µL), Tween 20 (200 µL) and distilled water (50 mL) were mixed thoroughly to give an orange emulsion. The tests were done in triplicate, and butylated hydroxyanisole was used as the standard. Twenty microliters (20 µL) of sample extract was added to the wells.

Absorbance values were then obtained every 30-minute interval for 3 h, with incubation in a water bath at 50 ºC. The rate of β-carotene bleaching was calculated using the following formula:

Rate of β-carotene bleaching = In (At= 0 / At =t) x1/t; where;

At=0 is the absorbance of the emulsion at 0 min; and At = t is the absorbance at time, t (30, 60, 90 min). The average rate of β-carotene bleaching was then calculated based on time intervals at 30, 60 and 90 min. The calculated average rates were used to determine the antioxidant activity (ANT) of the sample extracts, and expressed as percentage inhibition of the rate of β -carotene bleaching using the formula:

% ANT = (R control – R sample) / R sample x100

Where R control and R sample represent the respective average β -carotene bleaching rates for the negative control and plant extracts. Antioxidant activity was further expressed as the oxidation rate ratio (ORR) based on the equation:

ORR = R sample /R control

Antioxidant activity (AA) was calculated based on the inhibition of coupled oxidation of β-carotene-linoleic acid against the negative control at t = 90 min using the following equation:

% AA= [1- (A0 – At)]/ (A00 –A0t) x 100

### 3.2.8 Statistical analyses

Regression (PROC REG) procedure of SAS (2008) was used to measure the amount of antioxidant activities on each of the broiler samples assigned to different *V. tortilis* inclusion level. The assays were used to compare the activity of antioxidants among all inclusion levels. A completely randomized block design with six treatments was used in this study.

## 3.3 Results

### 3.3.1 Influence of *Vachellia tortilis* leaf meal inclusion on antioxidant activity of broiler meat

The DPPH free radical scavenging activity of *V. tortilis* leaf meal in meat is shown in Table 3.4. There were no significant (P>0.05) differences on the antioxidant activity of the meat samples across all the levels of *V. tortilis* leaf meal.

### 3.3.2 Ferric-reducing power assay

The concentration (mg/ ml) of meat extracts is plotted against the absorbed in the assay for each treatment. Figure 3.1 depicts the ferric reducing power of meat extracts from *V. tortilis* leaf meal, it shows a concentration-dependent response. All inclusion levels had similar patterns at low concentration including ascorbic acid as the standard solution. Higher reducing power was demonstrated by the 120 g/kg followed by ascorbic acid at high concentrations, 30 g/kg had a low reducing power and 0 g/kg respectively. A linear response pattern was observed on the FRAP assay obtained from the least square means ($y = 0.0004x + 0.1366$) as shown in Figure 3.2.

### 3.3.3 β-carotene-linoleic acid assay

A range between 4.26 and 48.98 % of the antioxidant activity calculated based on the average rate of β-carotene bleaching is presented in Table 3.5. The highest activity in the assay recorded was 48.9 and 40.9 % for inclusion level of 60 g/kg followed by 90 g/kg, while the lowest antioxidant activity was recorded for 0 g/kg (4.6 %) followed by 30 g/kg (13.8 %) respectively. The ORR of the extracts ranged from 0.510 ± 0.023 to 0.946 ± 1.54. The highest ORR was recorded for 0 g/kg inclusion level while the lowest ORR was recorded for 60 g/kg. The lower the oxidation rate ratio (ORR) the higher the antioxidant activity of the sample. The least square means showed a quadratic relationship between meat samples and graded levels of leaf meal

as indicated in Figure 3.3. The equation $y = -0.0037x^2 + 0.7756x + 2.7811$ indicated an inclusion level of 104.8 g/kg to be useful in improving the potency of natural antioxidants.

**Table 3.4: Activity of antioxidants on broiler meat extracts using DPPH assay**

| Inclusion level (g/kg) | Antioxidant activity |
|---|---|
| 0 | 33.9 |
| 30 | 32.2 |
| 60 | 30.6 |
| 90 | 32.6 |
| 120 | 31.4 |
| 150 | 34.5 |
| Standard error | 1.4 |

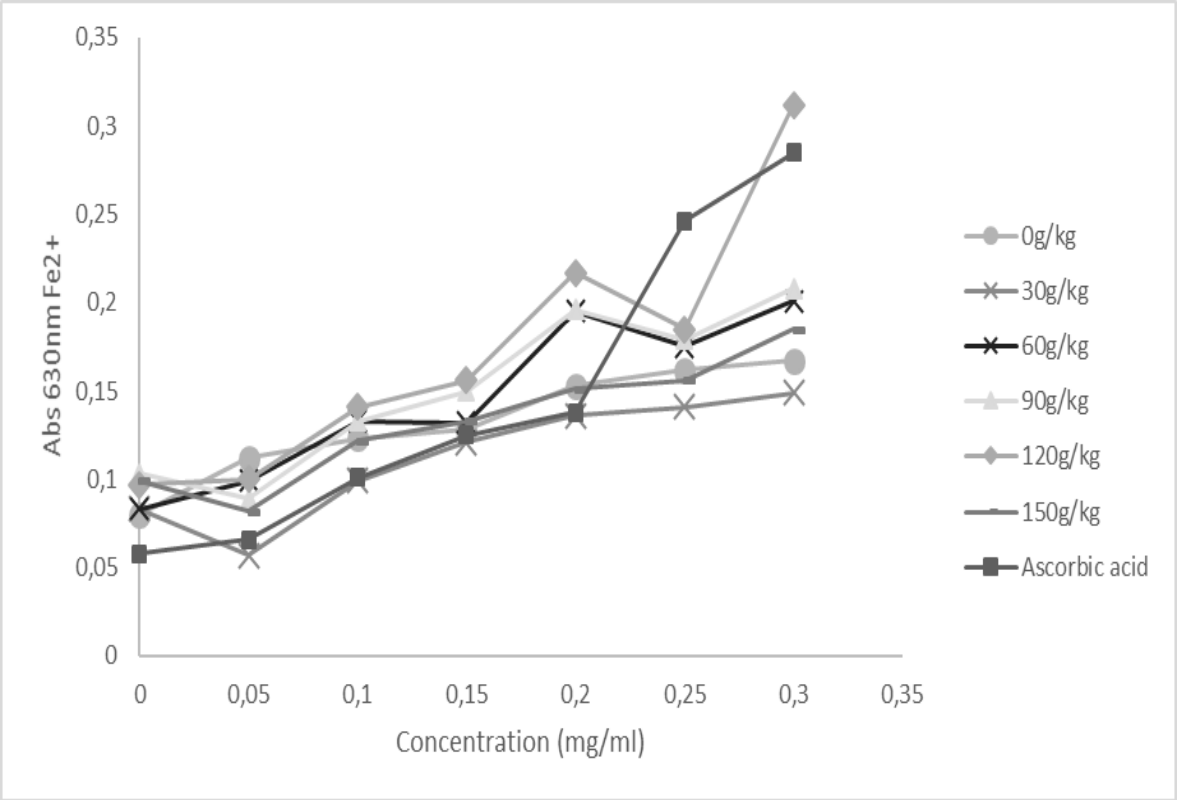


**Figure 3.1: Antioxidant activity of *Vachellia tortilis* leaf meals as assessed by ferric ion reducing antioxidant power**

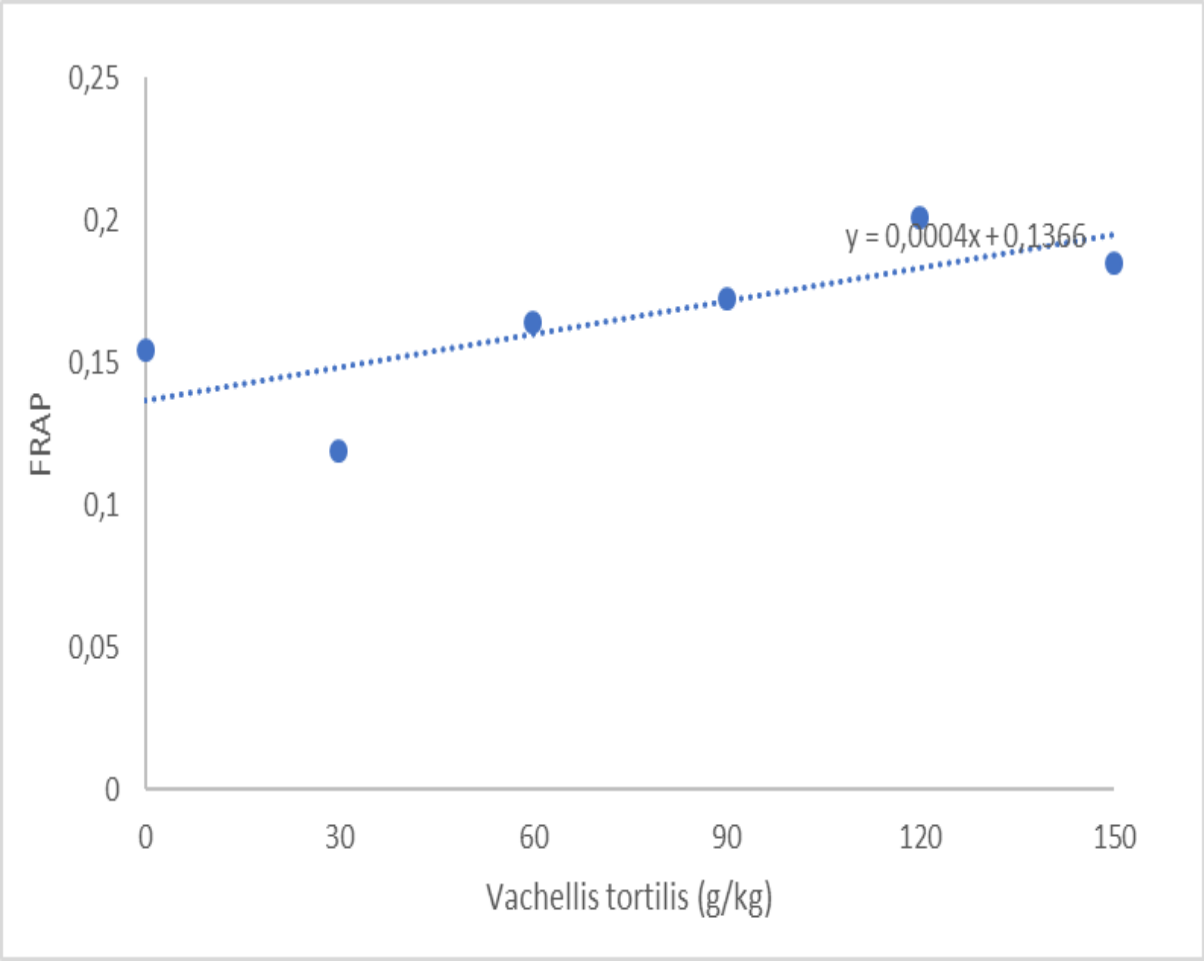


**Figure 3. 2: Response on the antioxidant activity of meat extracts using least square means.**

**Table 3.5: Antioxidant activities of *Vachellia tortilis* leaf meal measured using β-carotene-linoleic acid assay**

| Inclusion level (g/kg) | β-carotene-linoleic acid | |
|---|---|---|
| | Oxidation rate ratio | Antioxidant capacity (%) |
| 0 | 0.95 | 4.26 |
| 30 | 0.84 | 13.88 |
| 60 | 0.51 | 48.98 |
| 90 | 0.54 | 40.99 |
| 120 | 0.67 | 32.98 |
| 150 | 0.60 | 39.35 |
| Butylated hydroxytoluene | 0.37 | 62.50 |
| Standard error | 0.02 | 1.54 |

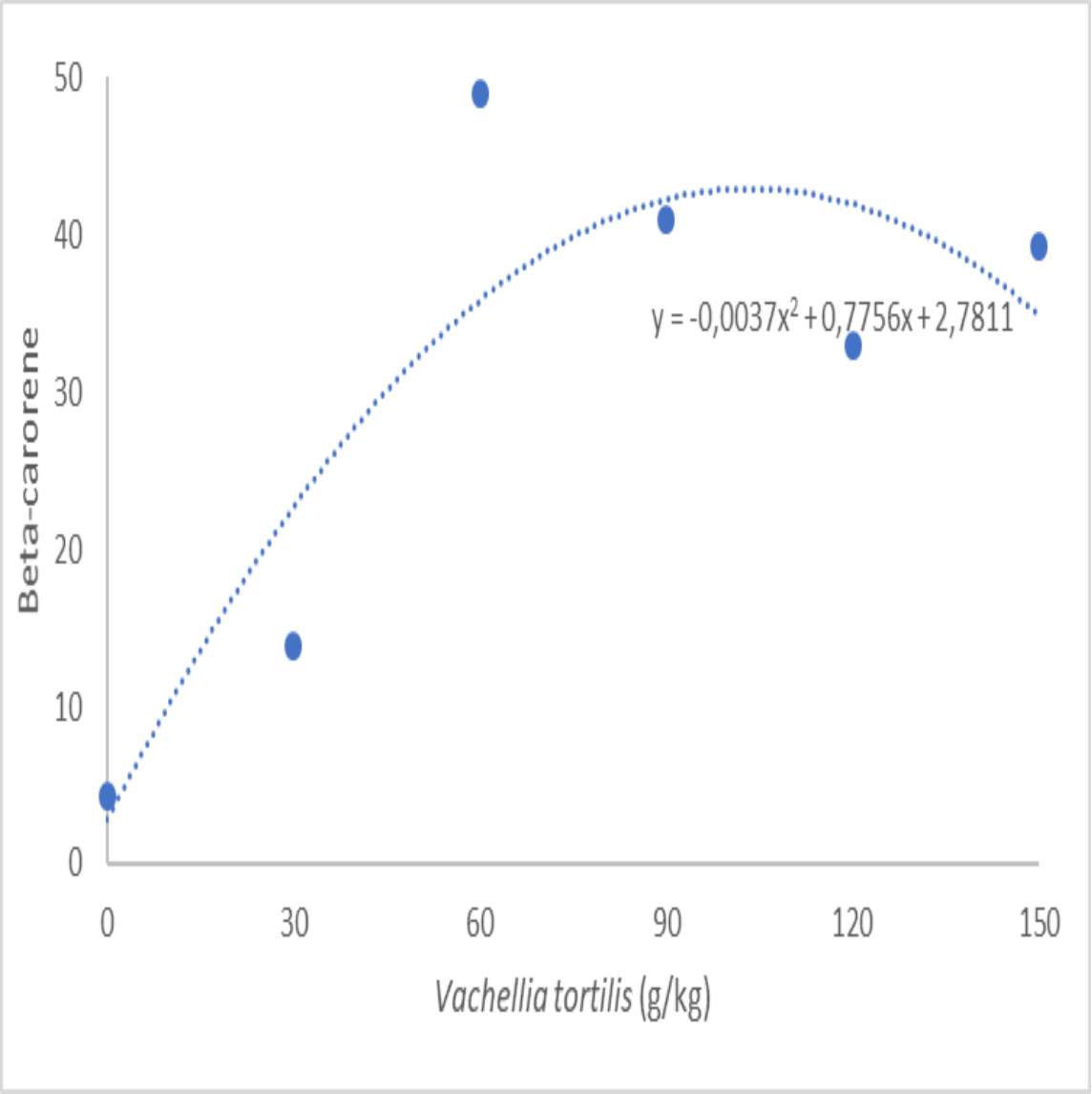


**Figure 3.3: Response of antioxidant activity on breast meat samples at different *V. tortilis* inclusion levels based on β-carotene-linoleic acid assay**

### 3.4 Discussion

Plant extracts of legume trees are the recent strategy in retarding the occurrence of lipid oxidation in broiler meat, they contain polyphenols which are beneficial in the meat industry (Lahucky *et al*., 2010; Kumar *et al*., 2015). Polyphenols are a broad class of compounds which are present in plants, these include: vitamins (e.g. ascorbic acid, tocopherols and β-carotene), flavonoids (e.g. catechin and kaempferol), natural antioxidants (e.g. flavonoids and vitamins) and tannins (e.g. condensed tannins) (Camo *et al.,* 2008). Plant extracts of legume trees are considered safe as they exhibit anti-carcinogenic, anti-atherogenic, anti-inflammatory, anti-microbial properties (Khanyile *et al*., 2014). However, compounds like tannins and crude fibre at high concentrations negatively affect the performance of broilers.

All broilers used in the trial were healthy throughout the trial, this might have been caused by balanced-nutrients in feed which contained natural antioxidant properties. Natural antioxidant properties can combat pathological disorders generated by reactive oxygen species (Mishra *et al.,* 2012), and any mortalities were removed. The objective of the study was to determine the antioxidant activity of different levels of *V. tortilis* leaf meal on broiler meat. The hypothesis tested was, broilers assigned to high inclusion levels of *V. tortilis* leaf meal will exhibit meat samples with high antioxidant activity.

Little, if any, scientific information has been gathered regarding the concentration of natural antioxidants required to reduce oxidation of lipids in meat. The antioxidant activity results using the DPPH assay did not go beyond 50 % on all the concentration levels. The free radical scavenging activity (DPPH) method determines the antioxidant activity by measuring the degree of discoloration which indicates the scavenging potential of the antioxidant extract, which is due to the hydrogen donating or radical scavenging ability (Karioti *et al*.,2004).

The FRAP assay exhibited a concentration-dependent linear response among the inclusion levels. Higher reducing power was demonstrated at high concentrations, and a low reducing power at low concentrations. The ferric-reducing antioxidant power assay was used to evaluate the antioxidant potential of meat extracts which had different levels of leaf meal on its ability to reduce the $Fe^{3+}$/ferricyanide complex to the ferrous $Fe^{2+}$ form. According to Kuda *et al.* (2005), the chemical mechanism behind antioxidant potency using the FRAP assay is based on the electron-transfer reactions, in which potassium ferricyanide (ferric salt) is the oxidant and antioxidant compounds act as reducing agents by donating a hydrogen atom to this ferric complex, thereby breaking the radical chain reaction.

Using the β-carotene-linoleic acid model, 48.98 and 40.99 % were the highest activity of antioxidants. The assay measures the ability of an extract to inhibit the oxidation of linoleic acid in an aqueous emulsion system with β-carotene. The mechanism involves the bleaching of carotenoids such as β-carotene in a heat-induced oxidation process, and the resultant discolouration being inhibited by antioxidants that donate hydrogen atoms to quench radicals (Huang *et al.,* 2005). The results also demonstrated a pattern of a quadratic-response, revealing and inclusion level of 104.8 g/kg ideal for reducing lipid oxidation in meat.

The overall results indicate high antioxidant activity of meat extracts at 60 and 90 g/kg inclusion levels of *V. tortilis* leaf meal, whereas 120 and 150 g/kg inclusion levels had the medium activity and 0 and 30 g/kg inclusion levels had the least antioxidant activity. The differences in the results of the study could be due to different levels of phenolic compounds present in each diet. High phenolics has high fibre (Kidd, 2009) which increases rate of passage, causing less antioxidants being absorbed, (Makkar, 2003) proanthocyanidins form complexes with plant proteins thereby making them unavailable for digestion and absorption in livestock. High levels of antinutritional factors present in leaf meal lead to impaired gizzard function and

other organs. Nonetheless, Hlatini *et al.,* (2016) added on the detrimental effects caused by the antinutritional factors and stated they can be treated using polyethylene glycol.

The results of the study contradict earlier reports (Bucklley *et al*., 1992; Razali *et al.,* 2008; Krishnaiah, *et al.,* 2009; Moyo *et al*., 2013). These reports showed that, plants with high levels of polyphenolic compounds exhibit high antioxidant capacities, thereby delaying the process of lipid oxidation. This could be caused by the stage of harvest; phenolic compounds increase as plant matures. Robards *et al.* (1999) stated that, depending on their concentration, phenolic compounds have a dual bioactive role in plants, acting as both antioxidant and pro-oxidant agents at low and high concentrations respectively. The dosage application of legume plant extracts in animal diets is vital as the concentration of antioxidant-compounds in plant materials varies considerably (Nkukwana *et al.*, 2014). Varying amounts of *V. tortilis* caused a variation in diet composition, *V. tortilis* antioxidant or protein content might have overpowered nutrients from other raw materials.

Polyphenolic compounds have been reported to retard lipid oxidation in meat (Mapiye *et al.,* 2010; Qwele *et al.,* 2013). The presence and level of concentration of different phytochemical compounds such as polyphenolics, flavonoid, alkaloids, saponins, tannins, carvacrol, terpenes, and thymol among others, have been recognised as the potential source of antimicrobial activities in plant materials (Sharma *et al*., 2012). The activity of antioxidants exhibited by presence of *Vachellia tortilis* leaf in broiler diet suggest it can reduce lipid oxidation and that it can serve as a potential source of antioxidant (Jonfia-Essien *et al.,* 2008). Guo *et al.* (2006) reported, supplementing high concentrations of vitamin E in pig diet during the growing and finishing periods significantly improved pork meat quality and carcass characteristics. Methionine and cystine are powerful antioxidants that help in the detoxification of harmful compounds and protect the body from radiation (Shahidi, 2000; Brisibe *et al.,* 2009).

Zheng *et al.* (2001) also indicated that polyphenolic compounds have a high antioxidant activity through three mechanisms: free-radical scavenging activity, transition-metal-chelating activity and/or singlet-oxygen quenching capacity. Different properties of antioxidants exhibit different antioxidant capacity. According to Podsędek, (2007), vitamin E is responsible for up to 20% of total antioxidant activity. Feeding of animals with plants containing these compounds can serve as a route to pass antioxidant activity to their body (Middleton *et al.,* 2000; Kuli-sic *et al*., 2004; Lahucky *et al.,* 2010). The study concludes the antioxidant potency present in broiler meat samples of *V. tortilis* leaf meal can reduce lipid oxidation in meat (Fasseas *et al.,* 2008; Falowo *et al.,* 2014).

### 3.5 Conclusions

The objective tested in this chapter was the activity of antioxidants in meat samples of broilers fed on different inclusion levels of *Vachellia tortilis* leaf meal. The meat extracts of all *Vachellia tortilis* dietary treatments had different concentrations of antioxidant activity. A leaf meal inclusion level of 60 g/kg exhibited high concentration of antioxidants followed by a 90 g/kg leaf meal. *Vachellia tortilis* leaf meal could be explored and exploited as a natural source of antioxidant in meat and retarding lipid oxidation. The findings show the significance of assessing the graded levels of leaf meal to be used in broiler diets for improved natural antioxidant effect. Feed compounders can use the information when formulating feed with the aim of retarding the oxidation of lipids in meat.

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## CHAPTER 4: Enhancing the shelf life of broiler meat by feeding broilers different incremental levels of *Vachellia tortilis* leaf meal


### Abstract

The objective of the current study was to determine the effect of different levels *Vachellia tortilis* leaf meal on the shelf life of broiler meat. Hundred and twenty Cobb-500-day old unsexed broilers were randomly allocated to six different dietary treatments containing 0, 30, 60, 90, 120, and 150 g/kg DM inclusion levels of *Vachellia tortilis* leaf meal. Each treatment diet was offered *ad libitum* to 10 broilers in each pen, with a total of 12 pens. The feeding phases were starter (0-14) days and finisher from 15 to 35 days. After feeding of dietary treatments, broilers were slaughtered, breast and thigh muscle were collected, vacuum sealed and kept at 4˚C for shelf-life analysis. The drip loss was measured after 24 hours of slaughter, while colour, pH, cooking loss and texture were measured once per week for 21 days.

An inclusion level of 115.75 g/kg was optimum for the redness of meat based on the quadratic equation y= $-0.0002x^2 + 0.0463x + 5.6107$. The incremental levels of *Vachellia tortilis* leaf meal had a quadratic response, giving 94.5 g/kg recommended for yellowness in thigh meat $y = -0.0002x^2 + 0.0378x + 15.75$. There was no significant effect of leaf meal on the drip loss ($p>0.05$). A linear response on the effect of leaf meal and storage time, displaying $y = 0.0253x + 17.9$. *Vachellia tortilis* leaf meal can be effective in preserving broiler meat in a linear response. A period of not more than 13 days was found to be more ideal at retaining meat quality without development of lipid oxidation.



Keywords**: colour, cooking loss, pH, texture**

## 4.1 Introduction

Hunger and malnutrition remain amongst the most common problems facing most South African households (DAFF, 2013). Lack of job opportunities and the high cost of living contributes to cases of malnutrition and food insecurity among financially struggling households. This leads to a limited variety of foods available for human consumption. Chicken meat and other chicken products becomes an easier food source to acquire (Mwale *et al.,* 2009) due to its desirable characteristics. Poultry meat is affordable, low in fat, high in protein making it a very popular food commodity in most households (Jaturasitha, 2008; Mothershaw *et al.,* 2009; Wapi *et al.,* 2013). Consumers view broiler meat as a healthy product that contains less fat, most predominantly unsaturated fatty acids (especially polyunsaturated fatty acids) as comparable to beef or pork products has contributed to the success in broiler meat industry (Bonoli *et al.,* 2007). In addition, broiler farming requires low costs and its reach its target slaughter weight within a period of five weeks, making it easier to resource-poor farmers to keep.

This had led to a huge demand of white meat in South Africa and worldwide. Mathews *et al.* (2014) reported an increase in broiler meat from 73.1 million tons in 2008 to 83.1 million tons in 2012. Biswas *et al.* (2010) reported the consumption of poultry meat is 90% compared to red meat worldwide, these statistics displays the importance of chicken production towards feeding the ever- growing human population. The industry ensures the continuous production of nutritious meat products from chickens. However, chicken meat is liable to spoilage due to its high levels of unsaturated fats which causes lipid oxidation (Allen *et al.,* 1998; Lorenzo *et al.,* 2012).

The lipid oxidation process starts immediately after slaughter when blood circulation stops, and anaerobic metabolism starts. Changes caused by lipid oxidation in meat are seen in sensory

attributes (colour, smell), nutritional value (drip loss, rancid) which affects the shell-life and consumer acceptability. These parameters including texture are used by consumers when selecting meat quality. Therefore, the control of lipid oxidation in meat is vital to meat quality.

Previously, the reduction of lipid oxidation in meat is largely corrected using synthetic antioxidants. Most common synthetic antioxidants in food are butylated hydroxytoluene (BHT), butylated hydroxyanisole (BHA), propyl gallate PG and tertiary butyl hydroquinone (TBHQ) (Karpinska *et al.,* 2001). These were however, lately observed to cause issues over human health including allergy, asthma and dermatitis. The complications led to consumers demanding meat that is free of chemicals and hormones but produced from natural products. The search for feed manipulation has since been placed on protein-rich legumes which are highly abundant in the tropics (D'Mello, 1995; Abdulrazak *et al.,* 2000).

Legume leaf meals such as *Vachellia* species are high in protein, contain natural antioxidants and are believed to be safer than antioxidants (Dube *et al.,* 2001; Moyo *et al.,* 2011). Moreover, leaf meals possess antimicrobial, anti-carcinogenic, anti-inflammatory and antioxidant effects (Alajmi, *et al.,* 2017). The use of leguminous trees reduces the use of conventional feed sources and feed costs in poultry industry. In addition, incorporation of *Vachellia* species in broiler feed improves growth performance, meat quality and reduces nematode burden (Mapiye *et al*., 2009; Xhomfulana *et al.,* 2009; Ncube *et al.,* 2018). Little research, however, has been conducted on improving shelf life of broiler meat. The incremental levels of *Vachellia tortilis* leaf meal on different types of muscles of broiler meat that consumers purchase requires investigation. The objective of the study was, therefore, to determine the changes in meat quality over time using *Vachellia tortilis* leaf meal at different inclusion levels. It was hypothesized that high inclusion levels of *Vachellia tortilis* leaf meal maintains the shelf life of broiler meat for long.

## 4.2. Materials and Methods

### 4.2.1 Study site, leaf meal collection and experimental design

All experimental procedures have been fully described in chapter 3.

### 4.2.2 Meat quality characteristics

For shelf life analysis, skinless and boneless breast and thigh samples were collected at slaughter, vacuum sealed and kept at 4℃.

#### 4.2.2.1 Drip loss

After slaughter, 2.5 g of samples were placed in plastics and sealed with rope. After, they were kept at 4$^{o}$C for 24 hours. Drip loss was calculated using the equation:

% Drip loss = [(Weight before – Weight after) / (Weight before)] × 100

#### 4.2.2.2 pH measurements

The pH of breast and thigh was determined using pH meter (CRISON pH, 2000) calibrated before each measurement at pH 4 and pH 9 standard solution. Each pH reading was taken by immersing the electrode into the mixture. Each sample on each treatment was weighed as 10g and homogenized in 50mL of deionized water for a minute. The pH meter electrode was rinsed using distilled water between each measurement to avoid treatment contamination. The measurements of pH were done at room temperature.

#### 4.2.2.3 Colour

Colour of thigh and breast muscles was observed using colour spectrophotometer in colorimetric parameters CIELabs. The measurements taken were lightness (L*), redness (a*) and yellowness (b*) (Commission Internationale de l'Eclairage, 1976). Before taking colour readings, the meat samples were left to bloom for 30 min. A 30 g portion of meat was measured in triplicate per sample.

#### 4.2.2.4 Cooking loss

On the day of data collection, samples were removed from the 4°C environment for the determination of cooking loss. Breast and thigh muscles were weighed using analytical balance, placed in polyethylene bags. For cooking of meat, water bath was used and set at 80°C for an hour. After cooking, samples were removed in the water bath including excess water and final weight was recorded. The calculations were done using the following equation:

% cooking loss = [(Weight before – Weight after) / (Weight before)] × 100

#### 4.2.2.5 Texture

Soon after determination of cooking loss, the cooked samples were used for texture measurements. For texture measurements, all breasts and thigh muscles were made identical. For breasts, a cylindrical of 14mm was used to cut similar pieces and for thighs, a scissor and the ruler were used to make samples of 10mm in length. The texture of meat samples was determined using the Warner-Bratzler shear force (Honikel, 1998).

### 4.3 Statistical analyses

The PROC REG procedure of SAS (2008) was used to determine the relationships between shelf life indicators with inclusion level of *Vachellia tortilis* leaf meal weekly. Levels of significance were considered with 95 % confidence ($P < 0.05$).

### 4.4 Results

#### 4.4.1 Meat colour

Table 4.1 shows the least square means for meat colour of breast and thighs. It shows the changes in meat colour as *Vachellia tortilis* leaf meal changes. The lightness of breast and thigh and the redness of thigh had no significant effect over incremental levels of leaf meal. Table 4.2 shows the changes in meat colour and cooking loss during storage. There was no significant

effect on the L*, a*, b* and cooking loss (P>0.05) of breasts and b* of thigh muscle. Figure 4.1 shows the changes in the a* of breast muscle. The redness of broiler breast samples had a quadratic pattern, the redness of meat improved until the optimum inclusion of leaf meal. Based on the quadratic equation; y= $-0.0002x^2 + 0.0463x + 5.6107$ , the optimum inclusion level of *Vachellia tortilis* leaf meal to improve the redness of broiler meat was 115.7 g/kg.

Figure 4.2 shows the changes in yellowness of breasts meat samples on different inclusion levels of *V. tortilis*. There was a linear response on the yellowness of breast $y = 0.0209x + 16.474$. The incremental levels of *Vachellia tortilis* in Figure 4.3 had a quadratic response on the colour, displaying an equation; $y = -0.0002x^2 + 0.0378x + 15.75$ giving an optimum inclusion level of 94.5 g/kg to improve the yellowness of thigh muscle. There was a week effect ($p<0.05$) on the lightness and redness of thigh muscles There was a significant effect ($p<0.05$) on the L* of meat samples over time, displaying a linear equation $y = 0.0586x + 47.14$. (Figure 4.4).

Figure 4.5 shows a quadratic response on the redness during storage. The regression model obtained an equation of $y = -0.0107x^2 + 0.2779x + 6.145$, showing a period of 13 days ideal for keeping meat in shelves. Figures 4.6 and 4.8 shows the effect leaf meal on cooking loss and the changes during storage time. Linear equations were obtained through regression; $y = 0.0253 + 17.9$ and $y = 0.1471 + 18.28$, respectively.

### 4.4.2 Texture, cooking loss and drip loss

Table 4.3 shows the least square means for the cooking loss, Warner-Bratzler shear force and drip loss. There was a significant effect ($p<0.05$) on the cooking loss and texture of breast meat as *V. Tortilis* levels increased. There was a linear response on thigh muscle ($p<0.05$). The increasing amount of leaf meal influenced texture of thigh. However, there was a quadratic decrease on the WBSF values of thigh meat. No week effect on the cooking loss of breast.

There was a non-significant effect on the drip loss of meat ($p>0.05$). The changes in the WBSF due to leaf meal inclusion levels and storage time are presented in Figures 4.7 and 4.9, with linear response equations $y = 0.0024x + 6.6205$ and $y = 0.2591x + 4.169$, respectively.

### 4.4.3 pH

Figures 4.10 and 4.11 shows the pH response on incremental levels of *Vachellia tortilis* leaf meal and over a period of 21 days. The effect of inclusion level over storage time was significant ($p<0.01$). The least square means of pH values ranged within a range of (5.7-6.5) in all treatments. The inclusion level of 0 g/kg had a much improving pH starting from (6.1-6.5), while other inclusion levels had a pH dropping as inclusion levels increased.

**Table 4.1: Least square means and standard errors for L*, a*, b* of chicken breast and thigh samples on incremental levels during storage**

| Parameter | Inclusion level (g/kg) | | | | | | SEM | Regression co-efficient | | Sig |
|---|---|---|---|---|---|---|---|---|---|---|
| | 0 | 30 | 60 | 90 | 120 | 150 | | linear | Quad | |
| **Breast** | | | | | | | | | | |
| L* | 48.4 | 49.8 | 47.3 | 46.0 | 49.9 | 49.4 | 0.5 | 0.662 | 0.191 | NS |
| **Thigh** | | | | | | | | | | |
| L* | 47.6 | 44.6 | 45.8 | 45.1 | 47.9 | 47.7 | 0.80 | 0.44 | 0.51 | NS |
| a* | 6.8 | 7.9 | 7.5 | 7.8 | 7.0 | 6.2 | 0.37 | 0.19 | 0.71 | NS |

Level of significance (*=p<0.05; **= p<0.01; ***= p<0.001) NS: not significant

**Table 4.2: Least square means and standard errors for L*, a*, b* and cooking loss of chicken breast and thigh samples on incremental levels during storage**

| Parameter | Days | | | | SEM | | | Sig |
|---|---|---|---|---|---|---|---|---|
| | 0 | 7 | 14 | 21 | | Linear | Quadratic | |
| Breast | | | | | | | | |
| L* | 50.3 | 44.6 | 48.3 | 50.9 | 0.45 | 0.63 | 0.27 | NS |
| a* | 6.9 | 7.9 | 6.9 | 6.8 | 0.26 | 0.39 | 0.57 | NS |
| b* | 8.2 | 17.6 | 18.3 | 18.2 | 0.33 | 0.71 | 0.69 | NS |
| CL (%) | 23.9 | 25.7 | 20.01 | 22.5 | 1.19 | 0.08 | 0.67 | NS |
| Thigh | | | | | | | | |
| b* | 17.7 | 15.5 | 18.6 | 16.1 | 0.36 | 0.44 | 0.18 | NS |

CL: cooking loss; Level of significance (*=p<0.05; **= p<0.01; ***= p<0.001) NS: not significant

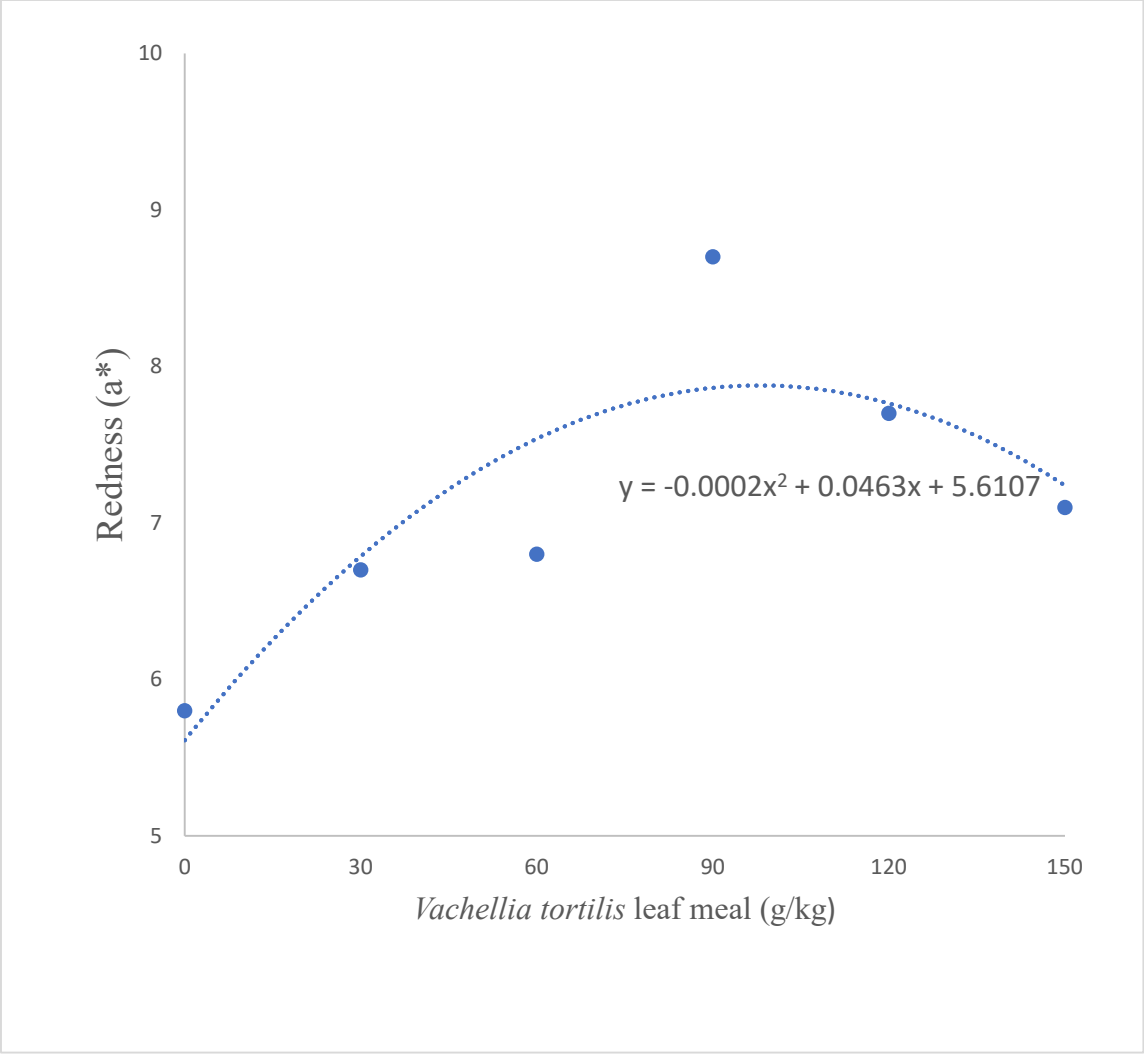


**Figure 4.1: Changes in the redness of breast meat on different inclusion levels of *Vachellia tortilis* leaf meal**

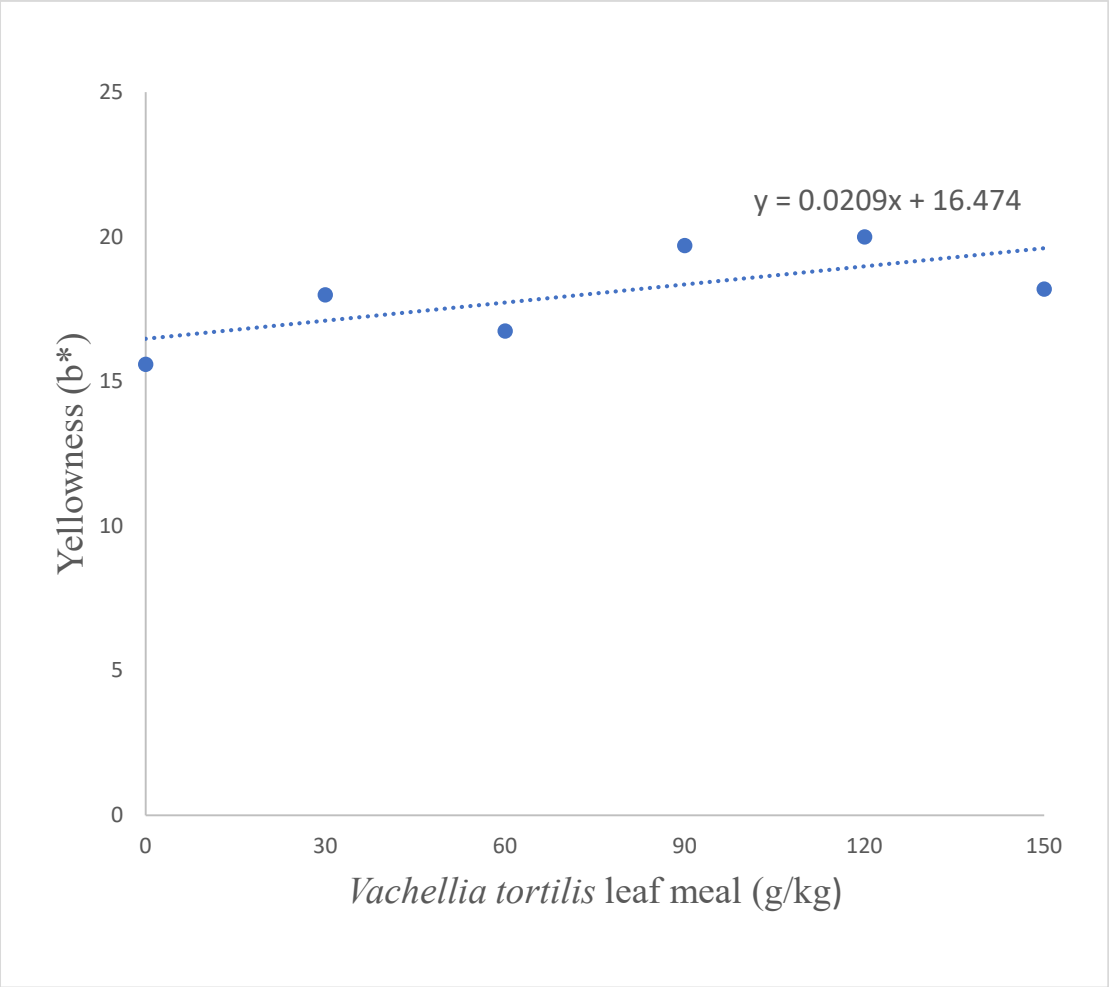


**Figure 4.2: Changes in the yellowness of breasts meat samples on different inclusion levels of *Vachellia tortilis* leaf meal**

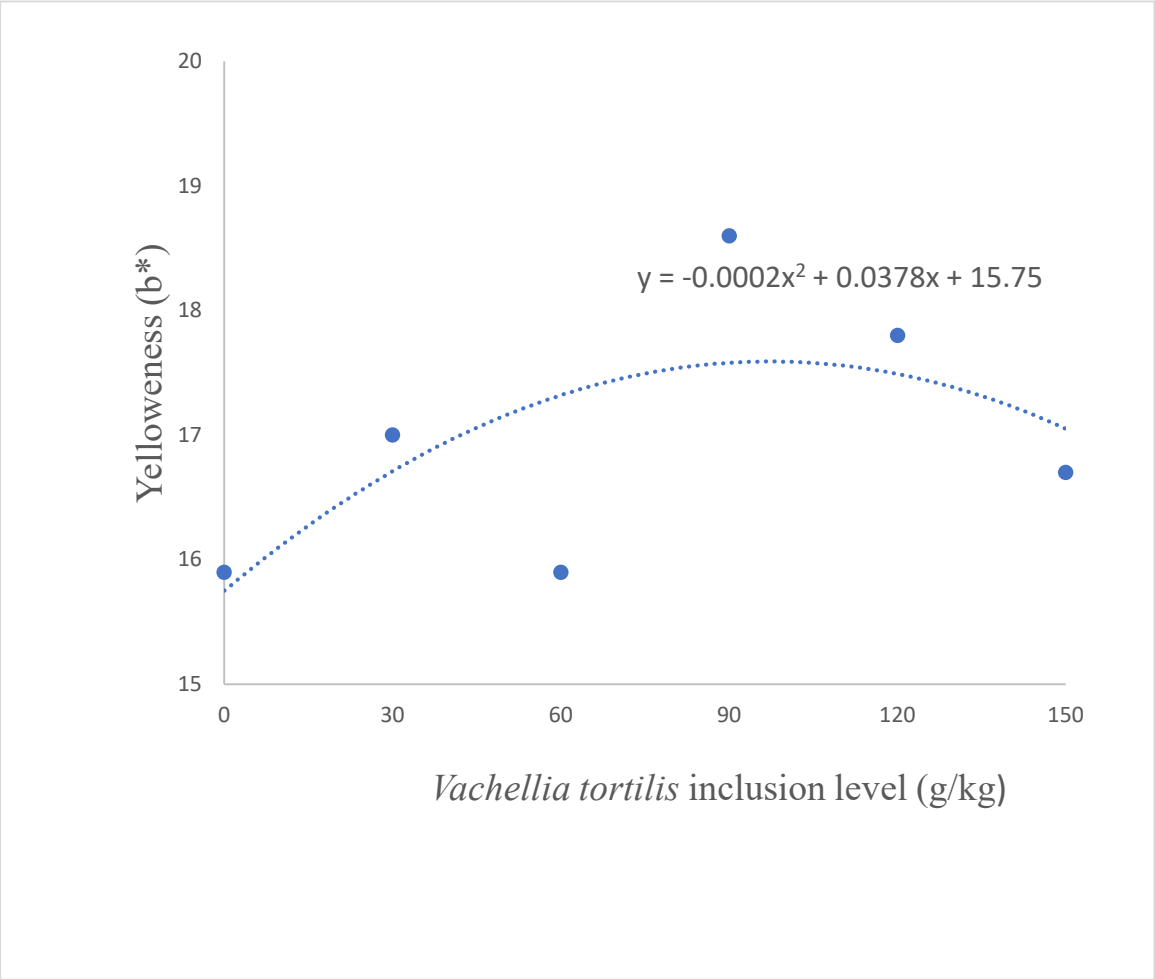


**Figure 4.3: Changes in the yellowness of thigh meat samples on different inclusion levels of *Vachellia tortilis* leaf meal**

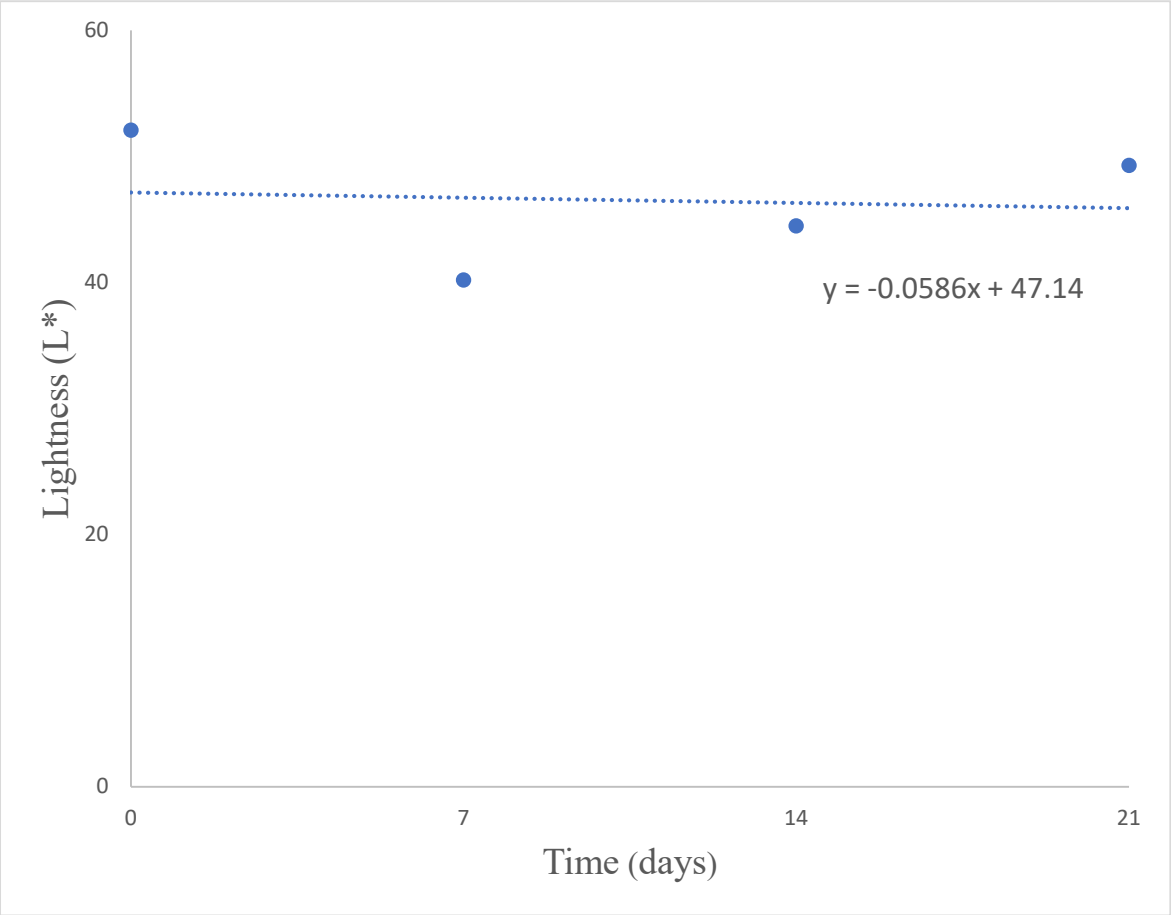


**Figure 4.4: Response on the lightness of broiler thigh over time**

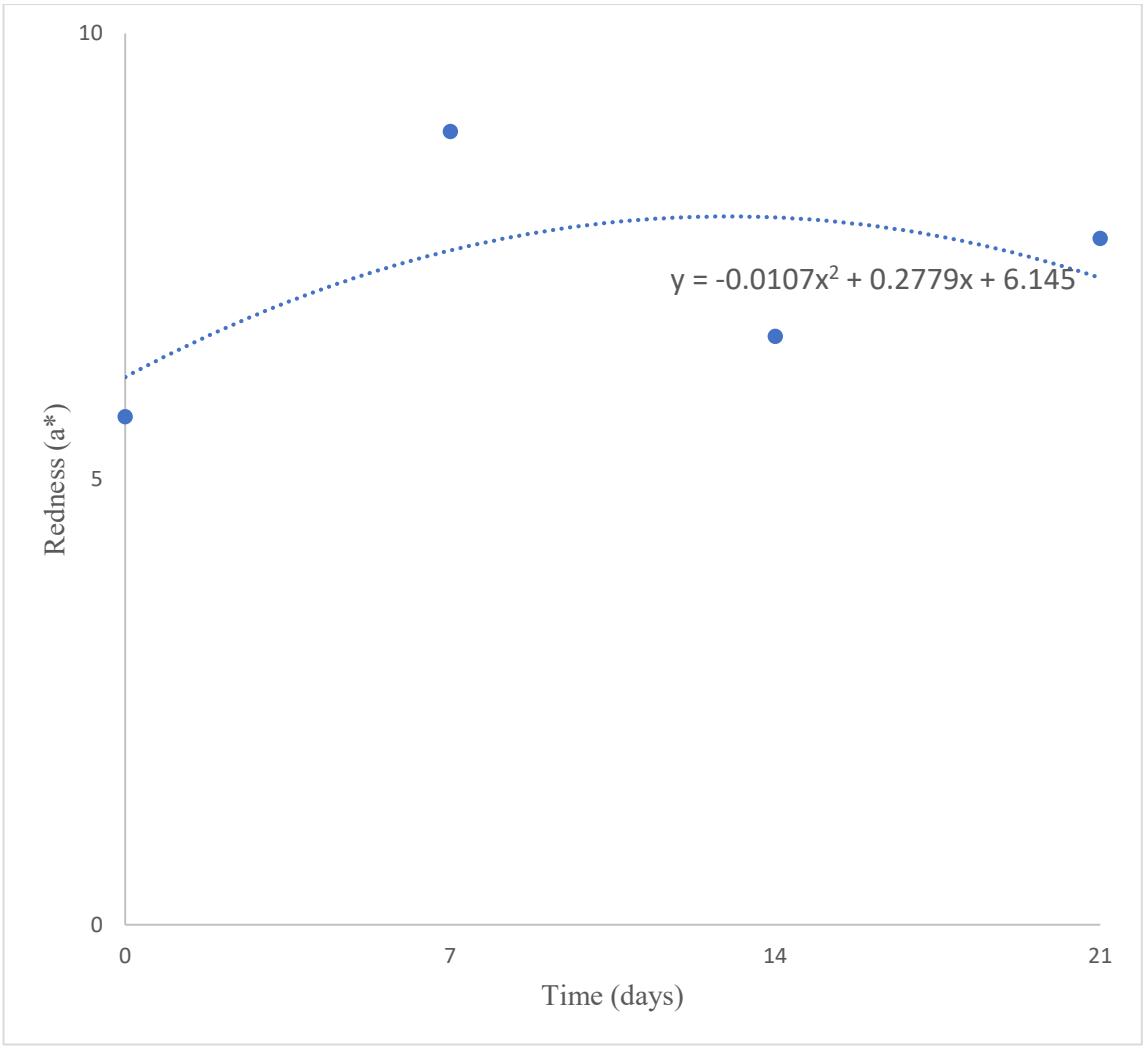


**Figure 4.5: Changes in redness of thigh meat over storage time**

**Table 4.3: Least square means and standard errors for cooking loss, texture and drip loss of broiler meat affected by incremental levels during storage**

| Parameter | Inclusion level (g/kg) | | | | | | SEM | Regression co-efficient | | Sig |
|---|---|---|---|---|---|---|---|---|---|---|
| | 0 | 30 | 60 | 90 | 120 | 150 | | Linear | Quad | |
| **Breast** | | | | | | | | | | |
| CL (%) | 23.1 | 17.6 | 17.3 | 22.8 | 22.5 | 24.6 | 1.45 | 0.69 | 0.75 | NS |
| WBSF (N) | 6.97 | 5.89 | 6.28 | 6.38 | 7.16 | 7.46 | 0.29 | 5.09 | 9.61 | NS |
| DL (%) | 0.55 | 0.71 | 0.43 | 0.61 | 0.36 | 0.59 | 0.11 | 0.56 | 0.79 | NS |
| **Thigh** | | | | | | | | | | |
| DL (%) | 0.27 | 0.19 | 0.29 | 0.21 | 0.20 | 0.19 | 0.05 | 0.29 | 0.89 | NS |

CL: cooking loss; DL: drip loss; WBSF: Warner-Bratzler shear force; SEM: standard error of means; Level of significance (*=p<0.05; **= p<0.01; ***= p<0.001) NS: not significant

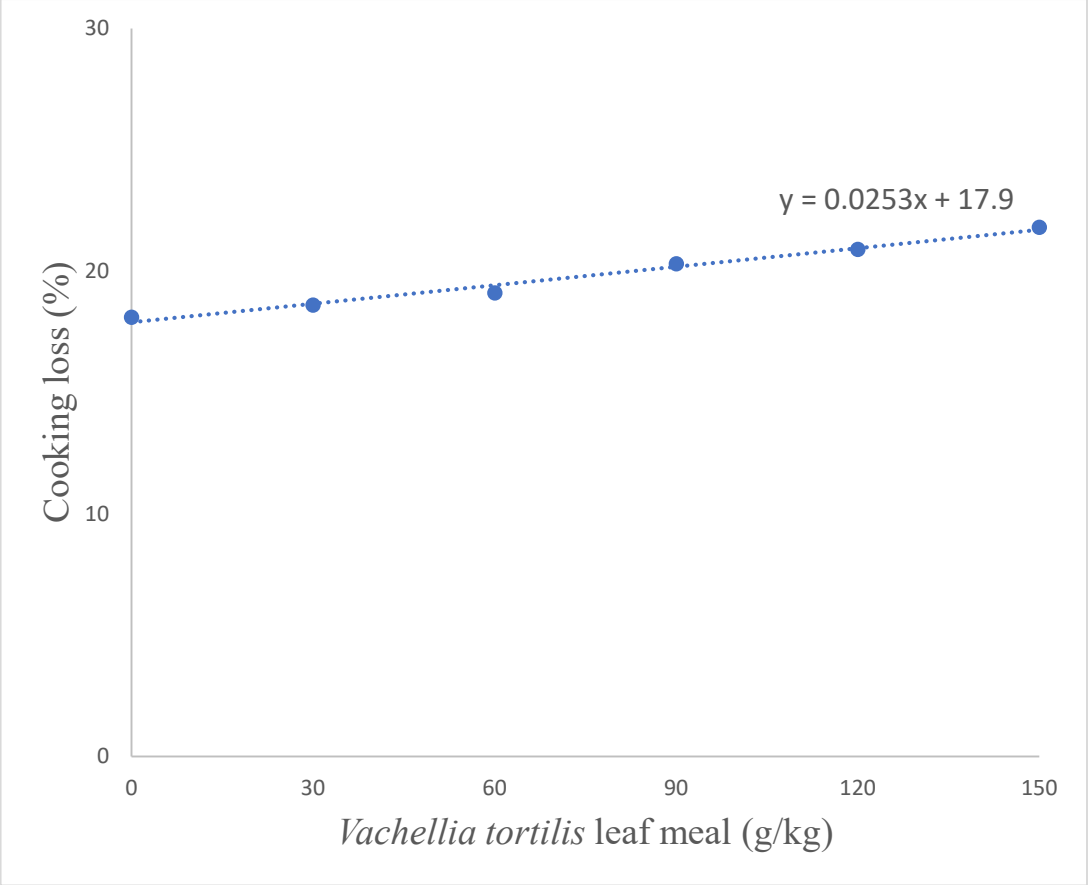


**Figure 4.6: Changes in cooking loss of thigh meat from broilers fed various inclusion levels of *Vachellia tortilis* leaf meal**

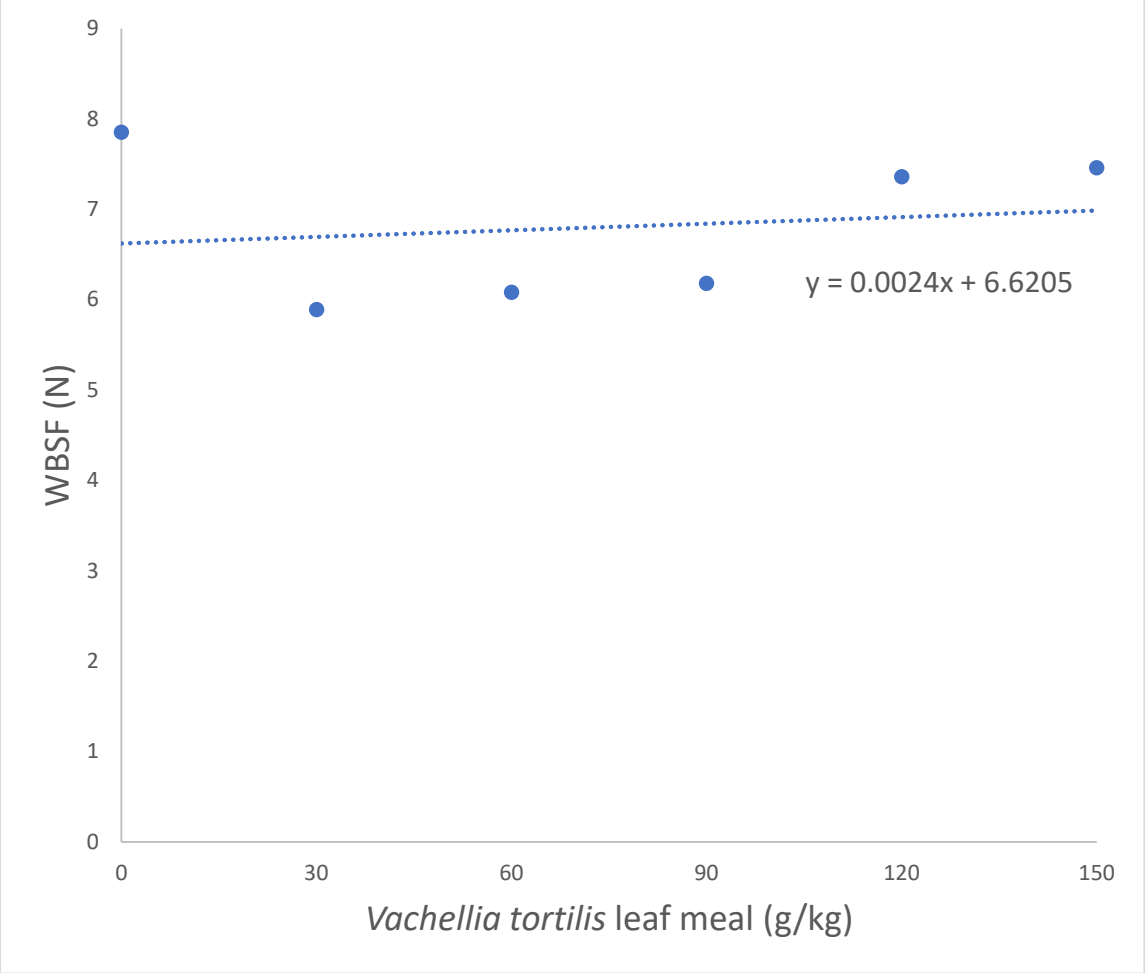


**Figure 4.7: Changes in texture of thigh from broilers fed different inclusion levels of *Vachellia tortilis* leaf meal**

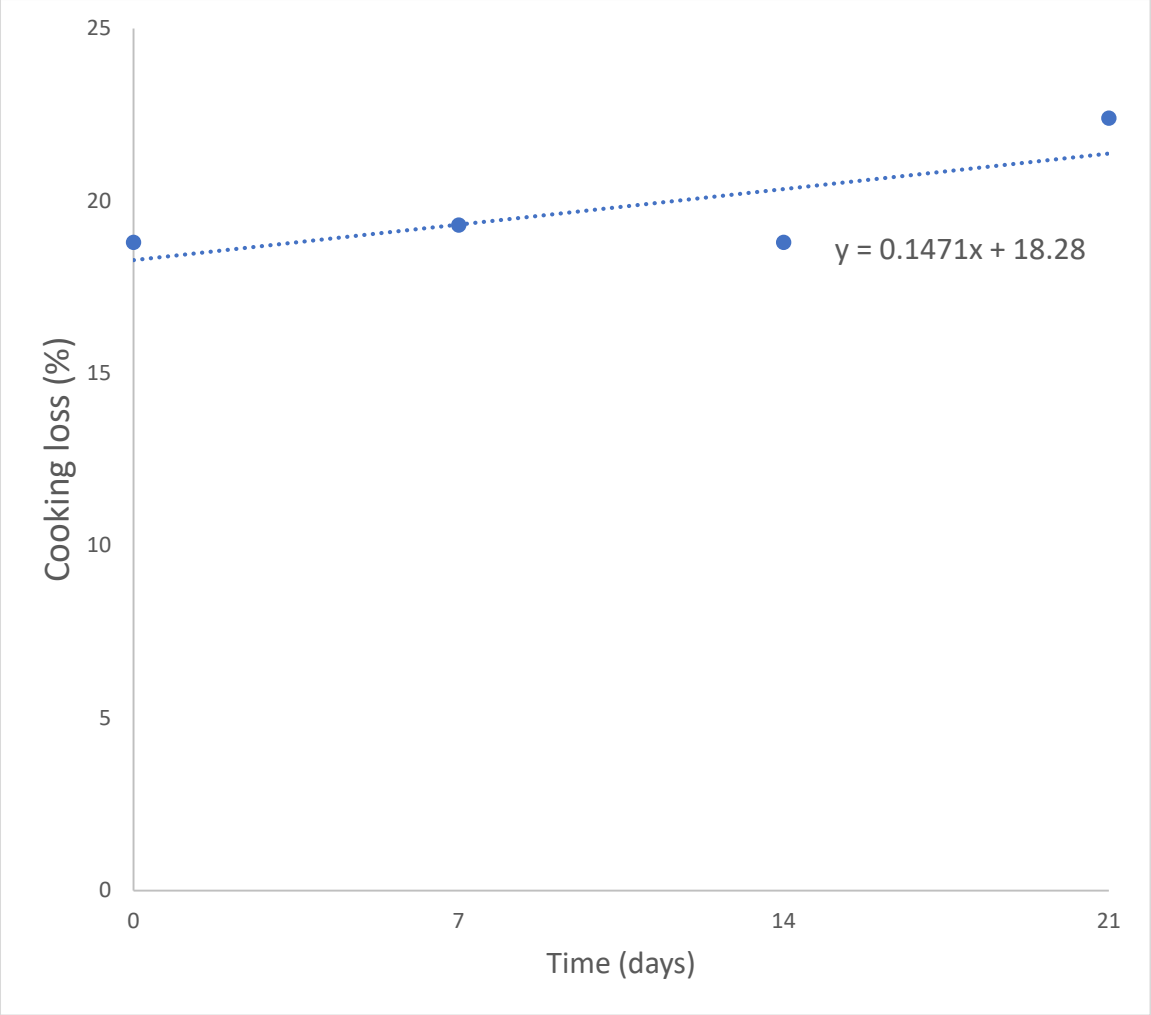


**Figure 4.8: Response in cooking loss of broiler thigh meat kept over time**

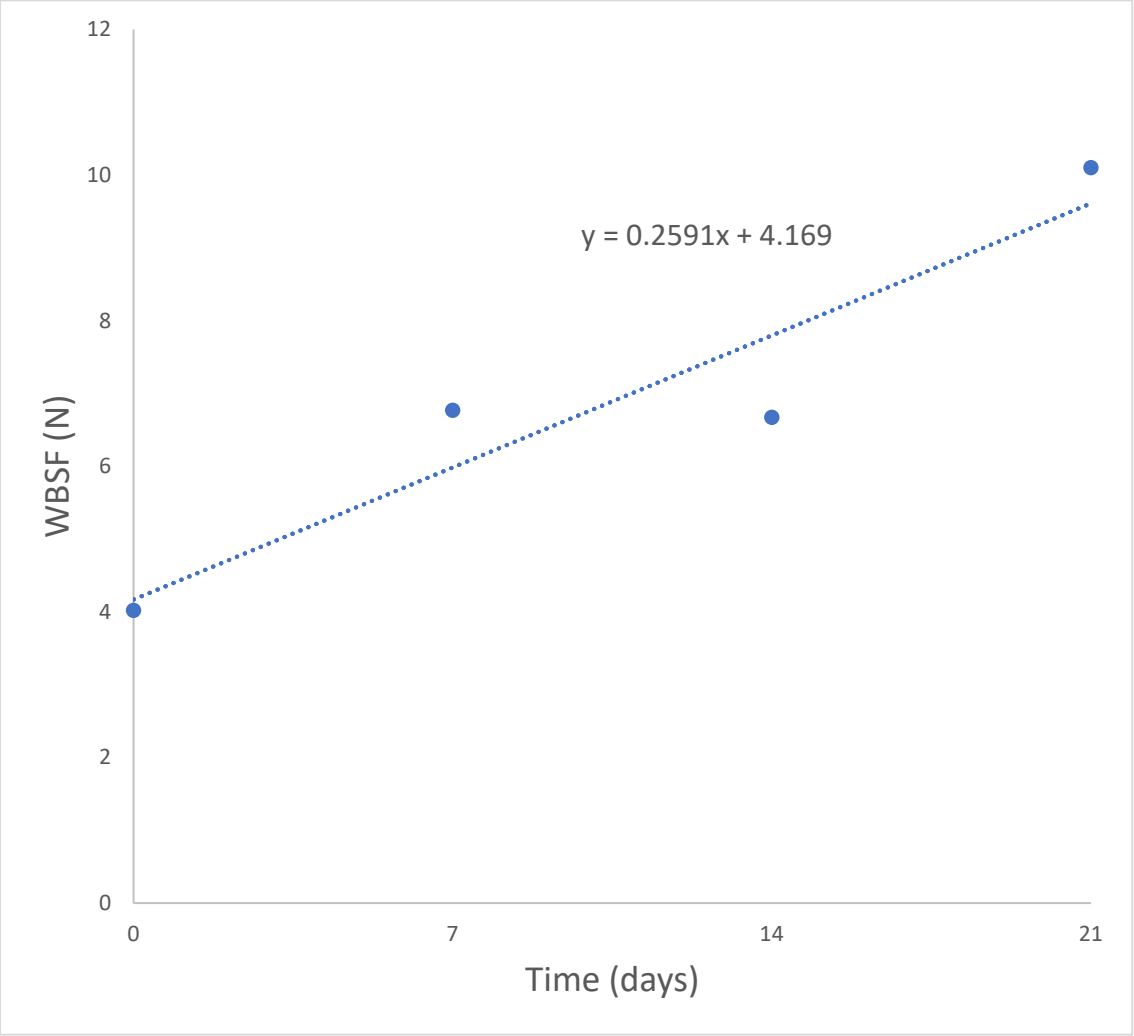


**Figure 4.9: Changes in texture of broiler thigh meat during storage**

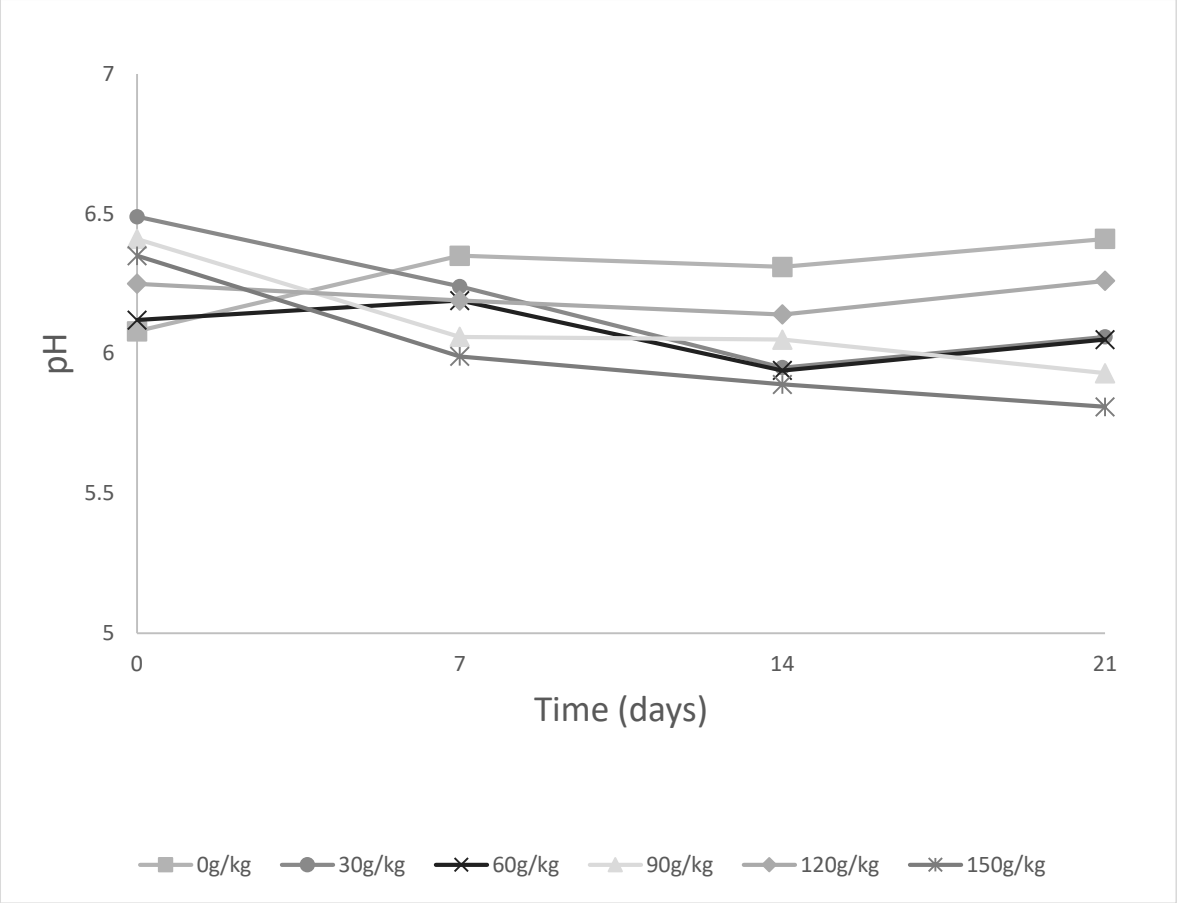


**Figure 4.10: Effect of incremental levels of *Vachellia tortilis* leaf meal over time (days) on pH levels of breast**

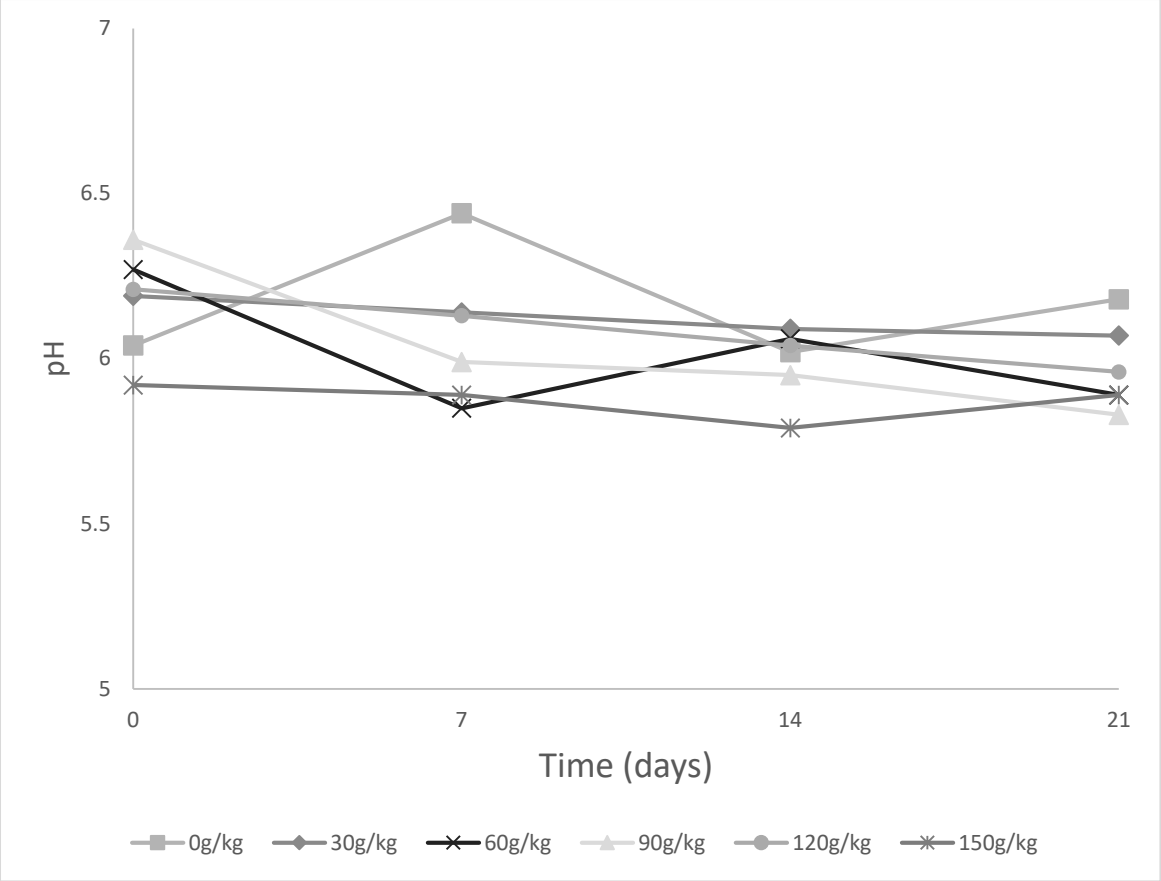


**Figure 4.11: Effect of incremental levels of Vachellia tortilis leaf meal over time (days) on pH levels of thigh**

### 4.5 Discussion

It is crucial to maintain external factors in meat like light and packaging, however, feed plays a major role in retaining the quality of meat. The focus of searching for alternative feed sources in poultry feed remains the strong point towards reducing feed costs and improving the shelf life of meat. Their use in animal diet has been documented as a useful protein source (Ngambu *et al.,* 2013; Wapi *et al.,* 2013; Ndou *et al.,* 2015).

The results of colour of the study corroborates to those reported by Ncube *et al.,* (2018) who reported that L* values ranged from 53.66 to 49.23 and the b* values ranged from 12.93 to 19.97. Similar results were also reported by Wapi *et al.* (2013) who made colour measurements on broiler breast meat at different inclusion levels of *Moringa oleifera* leaf meal. (Allen *et al.,*

1998) also obtained similar values of 43.1 to 48.8 on lightness breast samples. The redness values increased as leaf meal and storage days increased.

The highest L* values were reported on 120 g/kg inclusion level on day 21. High lightness (L*) values at day 0 indicate broilers were not stressed at slaughter, they were all handled the same way while transported to the slaughter house. Birds stressed at slaughter, releases high amounts of glycogen causing meat to become darker (Lacourt *et al.,* 1985) leading to reduced lightness values. The values of colour changes of breast and thigh meat were almost similar, the results disagree with Luna *et al.* (2010), who found thigh meat was more susceptible to lipid oxidation compared to breast meat. Alasnier *et al.* (2000) also reported, thigh muscles contained more pro-oxidant agents such as iron and vitamin E and the antioxidant system which slowed down the rate of lipid oxidation.

The redness of breast values increased as *V. tortilis* leaf meal increased. This might have been caused by high levels of antioxidants in leaf meal. These studies show that consumers generally prefer broiler skin colours ranging from white, to pale yellow. *Vachellia tortilis* leaf meal had no significant effect on drip loss. The results of the study support the findings by Lawrie (1998) that diet does not seem to affect the drip loss. Nonetheless, Cornale *et al*. (2011) reported that drip and cooking losses are not globally influenced using the phytotherapic compound in the diets. The drip loss results of the study contradict those reported Wapi *et al.,* (2013), the difference in results might be due to packaging. Vacuum packaging results in higher drip loss than other modified atmosphere techniques.

High levels of cooking loss were obtained on all inclusion levels and during all days of storage. The results of the study indicate very high levels of fats, salts and other volatile fluids were lost during cooking which results in dryer meat. This shows meat had a low water holding capacity. The cook loss results of the study are in total disagreement to those reported by Ncube *et al.*

(2018) who obtained cook loss results which ranged within 5.95 to 7.64 % after feeding *Acacia angustissima* leaf meal to broilers.

During cooking, meat losses fat and moisture and muscles become firmer. Connecting tissue becomes tenderer and overcooking makes the muscle strands tough. More fluids lost during cooking, results to meat of poor quality, as the losses are essential nutrients. High losses were reported in 150 g/kg leaf meal inclusion level. Fluid losses may cause an increase in the concentration of the solutes, which results in pH decrease where fast decline in pH or low pH leads to high drip loss (Qiao *et al.,* 2001). Castellini *et al.* (1998) reported that vitamin E supplementation reduces drip loss, however, results of the study showed no effect of leaf meal.

Tender meat symbolises good quality as it is easy to chew. The texture of meat depends on the muscle composition, cooking time and temperature. The breast meat became tougher as inclusion levels increased. The differences in results of breasts and thigh over leaf meal and storage time could be caused by type of muscle and the level of activity. The breast muscles are well rested whilst thigh muscles are involved in walking. An inclusion level of 30-90 g/kg had a tender meat, while 0, 120 and 150 g/kg had tougher meat samples. This is caused by moderate levels of vitamin E which improves the tenderness of meat (Dirinck *et al.,* 1996). Similar shear force results were obtained by Ncube *et al.* (2018), where texture of chicken values ranged within 14.14 N to 14.54 N. Less force was used in cutting meat, which symbolises tender meat. Whereas, day 21 had an insignificant effect, more force was used in cutting meat. Liu *et al*. (2003) mentioned that, meat becomes tender as storage days increase. This is caused by the level of absorbance of vitamins and antioxidants in meat.

The pH range of meat represents the acidity, neutrality and basic state of meat. Normal poultry uncooked meat has a pH of between 6.2 and 6.5. The results of the study show pH values were within a range of 5.5 to 6.5 during storage. The pH values from the study showed both breast

and thigh muscle had a pH from 5.5 to 6.5 which is an acidic state of meat. In chickens where no leaf meal was incorporated, there was a drastic increase in pH from day 0 to 7. Qiao *et al.* (2001) reported an accumulation of ammonia and amines by psychotropic bacteria can lead to a high pH increase. Moreover, high pH meat is characterized as being dark, firm, and dry (DFD-like) which is undesirable (Zhang *et al.,* 2005; Ristić *et al.,* 2010). A decrease in pH fall is enhanced by the glycogen break down into glucose during slaughter. Glucose undergoes glycolysis but in the absence of oxygen, lactic acid is formed which causes pH in muscles to drop (Vimiso *et al*., 2013).

Inclusion level of 150 g/kg had a lower pH, which decreases meat tenderness (Froning *et al.,* 1978; Barbut, 1993). The results agree to those reported by Sahoo *et al.,* (1997); Brenesselová *et al.* (2015), where vacuum-packed samples had lower pH value, due to the predominance of acid-producing bacteria. At lower pH, myoglobin becomes more prone to the oxidation and metmyoglobin formation increases. In relation to storage time, day 13 had a significant response in texture.

The presence of antioxidants resulted in a significant decrease in the amounts of metmyoglobin developed during storage which was attributed to the strong antioxidant activity. The decrease in the pH values could be attributed to the microbial growth during thawing (by consuming sugar and producing organic acids). Slight decrease in pH values at sometimes during storage may be attributed to the dissolution of carbon dioxide ($CO_2$) in the chicken muscle. Antioxidants were able to retard lipid oxidation and reduce bacterial growth for a period of 13 days, the results agree to those reported by Kubmarawa *et al.* (2007), who stated *V. tortilis* inhibits bacteria like Escherichia coli.

### 4.6 Conclusions

There was a quadratic increase in the redness of meat as the inclusion levels of leaf meal increased. Meat texture increased as inclusion levels increased in feed. Drip loss was not affected by the leaf meal at different inclusion levels. pH remained at a stable range of good meat quality. The meat quality parameters over storage time indicate the effectiveness of *Vachellia tortilis* leaf meal at an inclusion levels of not more than 115.7 g/kg within a period of 13 days. *Vachellia tortilis* leaf meal can be used in broiler feed for extended storage time.

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# Chapter 5: General Discussion, Conclusion and Recommendations

## 5.1 General Discussion

The objectives of the current study were to determine the changes in broiler meat over time on broilers fed different inclusion levels of *Vachellia tortilis* leaf meal by taking measurements in meat attributes: colour, pH, texture, drip loss and cooking loss. As well determining the activity of antioxidant of *V. tortilis* leaf meal on broiler meat by measuring the activity using Ferric-reducing power, β-carotene-linoleic acid and Radical scavenging activity assays. The hypotheses of the study were high inclusion levels of *Vachellia tortilis* leaf meal will retain good meat quality for extended storage time and that high inclusion levels of *Vachellia tortilis* leaf meal will results in meat with greater antioxidant activity. Incorporating *Vachellia tortilis* leaves in broiler diets favours consumer preference of producing meat from natural plants. Not only does *Vachellia tortilis* leaf meal reduces feed costs in broiler industry but it also reduces the use of conventional feed sources which are hard to acquire, and they require complex methods of processing.

Before commencement of the trial, the quality of feed for broilers was tested to avoid detrimental health issues. Experimental diets contained graded levels of *Vachellia tortilis* leaf meal 0, 30, 60, 90, 120 and 150 g/kg DM respectively. A total of 200-day-old Cobb 500 broilers were reared for a period of 35 days before data collection. The determination of antioxidant

activity varied among the diets. It was hypothesised, leaf meals containing high amounts of *Vachellia tortilis* will exhibit high activity of antioxidants in meat. The FRAP assay exhibited a concentration-dependent linear response for all the inclusion levels, while the β-carotene-linoleic acid had the highest activity (48.9 %) recorded at 60 g/kg inclusion level. The study revealed, 60 to 90 g/kg of *V. tortilis* leaf meal results to high activity of antioxidants in meat, which improves the quality of meat. The hypothesis was therefore rejected.

The objective in chapter 4 was to determine the effect of incremental levels of leaf meal in meat stability in 21 days. Breast and thigh muscles were used for shelf life data collection, measuring colour (L*, a* and b*), WBSF, pH, drip loss and cooking loss of meat. It was hypothesised that broilers assigned to diets containing high levels of *V. tortilis* leaf meal will retain meat for longer shelf life. An inclusion level of not more than 115.7 g/kg of leaf meal was able to retain the quality of meat for a period of 13 days. All experimental diets-maintained meat pH to be normal as stated in the literature. The study therefore revealed, incorporating *V. tortilis* leaf meal at 94.5 to 115.7 g/kg in broiler diet, improves colour of meat and shelf life. The findings on the shelf life of meat agree with the hypothesis, therefore, it was accepted. Inclusion of *V. tortilis* leaf meal above 120 g/kg can cause negative effect on broiler health and production.

## 5.2 Conclusions

*Vachellia tortilis* improves broiler meat quality and extends storage time with its natural antioxidant properties. *V. tortilis* above 120 g/kg can cause negative effect on broiler health and meat quality. The poultry industry will make market improvements, healthy meat and meat products will be sold to consumers. It can be concluded that feed costs can be reduced through using *V. tortilis* leaf meal in broiler feed.

## 5.3 Recommendations

*Vachellia tortilis* can be included in broiler diet in the starter feeding phase and presented in a mash form. This can be expanded to layers and broiler breeders changing the shape of feed. Further research can check for breed effect and explore the explore the microbial growth in meat samples during storage.