Adaptation and behaviour of finishing pigs to *Vachellia tortilis* leaf meal inclusion

By

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DECLARATION

I, Fortune Thabethe, declare that this thesis entitled "Adaptation and behaviour of finishing pigs to *Vachellia tortilis* leaf meal inclusion" is my own work, apart from where stated through citations or quotes. This dissertation has not been submitted to any other University except to the University of KwaZulu-Natal and was conducted under the supervision of Professor Michael Chimonyo.

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F. Thabethe

General Abstract

The broad objective of the study was to determine the adaptation and behaviour of pigs fed on incremental levels of *Vachellia tortilis* leaf meal based diets. Forty-eight clinically healthy male pigs Large White × Landrace with a mean (± SD) body weight of 63.8 ± 3.28 kg were randomly assigned to individual pens in a completely randomized design. Eight pigs were fed on diets that contained 0, 30, 60, 90, 120 and 150 g/kg DM of *V. tortilis* leaf meal. Pigs were fed *ad libitum* and had free access to clean water throughout the experiment.

Average daily feed intake (ADFI), average daily gain (ADG) and gain: feed ratio (G: F) were determined weekly. The adaptation period was calculated at the end of the experiment for each individual pig. Coefficient of variation (CV) of feed intake across *V. tortilis* leaf meal diets was calculated. Number of visits to the feeder were calculated. The time spent eating, drinking, standing, lying down, sniffing, biting objects and licking objects were observed using six closed circuit television cameras (CCTV) once a week for three days.

There was a quadratic decrease ($P < 0.001$) in ADFI, while ADG increased linearly ($P < 0.001$) with incremental level of *V. tortilis* leaf meal. Incremental level of *V. tortilis* leaf meal increased G: F ratio quadratically ($P < 0.001$). A linear increase in adaptation period of pigs was observed ($P < 0.05$) with increasing inclusion level of *V. tortilis* leaf meal. The variation of feed intake, expressed as a coefficient of variation, increased linearly ($P < 0.05$) with increasing inclusion level of *V. tortilis* leaf meal. Increasing inclusion levels of *V. tortilis* leaf meal linearly decreased time spent eating, lying down and the number of visit to the feeder ($P < 0.05$). Time spent standing and biting objects increased linearly with increasing inclusion level of *V. tortilis* leaf meal ($P < 0.05$). There was no relationship ($P > 0.05$) between *V. tortilis* leaf meal inclusion and time spent drinking, sniffing and licking of objects. It can be concluded that pigs require a long time to adapt to *Vachellia* diets. Increasing levels of *V. tortilis* leaf meal also alters time spent on behavioural patterns of pigs.

Key words: finishing pigs, feed intake, polyphenolic compounds, behavioural activities.

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DEDICATION

This dissertation is devoted to my entire family, as it is the first Master's degree in the whole family. Devotion goes to my mother (Khulekile Jane Sambo), my father (Lawrence Thabethe) and my younger brother (Peaceful Thulasizwe Thabethe).

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List of abbreviations

ADF	Acid detergent fibre
ADFI	Average daily feed intake
ADG	Average daily gain
AOAC	Association of Official Analytical Chemists
BD	Bulk density
BW	Body weight
CCTV	Closed Circuit Television Cameras
CP	Crude protein
CT	Condensed tannins
DM	Dry matter
EE	Ether extract
GE	Gross energy
G: F ratio	Gain: feed ratio
NDF	Neutral detergent fibre
RSA	Republic of South Africa
SAS	Statistical Analysis System
SASAS	South African Society for Animal Science
S.D	Standard deviation
UKZN	University of KwaZulu-Natal
WHC	Water holding capacity

CHAPTER 1: General Introduction

1.1 Background

The demand for animal protein in human diets is increasing, particularly for pork (Martens *et al*., 2013). Pigs have a fast growth rate, high prolificacy and are good feed convectors (Adesehinwa, 2008). They are used for generating income and as a source of livelihood (Khanyile *et al*., 2014). The commonly used pigs are Large White, Landrace, Pietrian and Duroc (Visser, 2004). Currently, pork quality has become the main focus in pig production (Newcom *et al*., 2004). The use of crossbreed pigs has assisted in improving the carcass yield and composition of pigs. Crossbred pigs are recommended as they benefit from heterosis and are more vigorous than pure breeds (Latorre *et al*., 2003). They are also used to upturn the total efficiency in pig production.

In pig production enterprises, feed cost account for approximately 60 to 70 % of the total production costs (Niemi *et al*., 2010). Maize and soybeans are the most vital feedstuffs used in pig diets due to their high energy and protein contents, respectively. In addition, the nutrients in these feedstuffs are highly digestible. Continuous increases in prices of cereal grains and conventional legumes as well as rapid human population growth force pig enterprises to contemplate the incorporation of alternative feedstuffs. The incorporation of alternative non-conventional feedstuffs such as leguminous tree leaves can ease the competition between humans and livestock for food. Utilising these feedstuffs also broadens the range of available feed resources for pigs. Leaf meals have also been shown to improve pork quality.

The use of cost-effective feedstuffs in pig diets whose nutritional qualities are comparable to conventional feedstuffs should be considered (Woyengo *et al*., 2014). Nitrogen-rich leguminous trees are abundant and widely distributed. Leaves from *Vachellia* species such as

Vachellia tortilis can be used in pig feeding (Halimani *et al*., 2005). The use of *V. tortilis* leaf meals in pig diets has been reported earlier (Khanyile *et al.*, 2014). The period to which finishing pigs need to adapt to *V. tortilis* leaf meal is, however, unknown. Also, the behaviour of finishing pigs fed on *V. tortilis* leaf meals is unclear. Dose-response experiments on the effect of *V. tortilis* leaf meal inclusion on the growth performance of pigs have been conducted (Khanyile *et al*., 2014). It was argued that reduced performance of pigs could be due to a slow acclimatization to the diets. The reduction in feed intake could be caused by insufficient adaptation to diets. The period of adaptation in pigs is critical since intake of nutrients is depressed, which then reduces pig performance. Changing a diet can affect both feed intake and performance of pigs, particularly when the change is from one feed to another of poorer nutritional quality such as fibrous diets (Whittemore *et al*., 2003). Longer adaptation periods may increase the rearing time of pigs, making it impossible for farmers to bring new stocks, as a result more feed will be required which can decrease farm productivity and returns. The lack of such information makes it difficult for pig farmers to utilize non-conventional feedstuffs. It is, therefore, crucial to determine the adaptation period of pigs fed on increasing levels of *V. tortilis* leaf meal.

Consumption of *Vachellia* leaves in pigs is hampered by the abundance of fibre and secondary compounds such as polyphenolic compounds. Polyphenolic compounds have both toxic and antinutritional effects. These compounds depress growth rate and feed intake. The effects of *Vachellia* leaf meals, however, depend on the inclusion level. Inclusion level of up to 100 g/kg have been suggested to have positive effects (Makkar, 2003; Halimani *et al*., 2005), while higher inclusion levels have negative effects (Khanyile *et al*., 2014). Pigs that are exposed to tanniferous diets are expected to develop mechanisms that help them overcome the detrimental effects of the secondary compounds. They produce proline-rich proteins (PRPs) and histatins

(Salem *et al.*, 2005). These proteins have the ability to bind to polyphenolic compounds, thereby reducing their toxic effects.

To understand how pigs adapt to diets, it is crucial to investigate their behavioural patterns when fed on new diets. Pigs reared in an outdoor system spend a large part of their time feeding, compared to those reared in an indoor system which spent almost 5 % of their daily feeding time (Persson *et al.*, 2008). Pigs fed on fibrous diets have an increased motivation to eat because they do not easily meet their nutritional needs. *Vachellia tortilis* leaves are rich in proteins, amino acids and minerals. The amount of these nutrients, which vary with the inclusion level, may alter the time spent on behavioural activities of pigs. In addition to adaptation, behavioural activities of pigs assist in assessing the welfare of pigs fed on *V. tortilis* leaf meal diets. Understanding the time spent on different behavioural activities when pigs are fed on fibrous diets assist farmers to alter management practices.

1.2 Justification

Prolonged drought conditions instigating crop failures rises grain competition amongst humans and pig farmers. This requires pig nutritionists and feed compounders to use new techniques when formulating pig diets. Using *Vachellia tortilis* leaves as a feed ingredient can help solve feed challenges in pig production. Although optimum inclusion level of *V. tortilis* leaves in pig diets has been reported by Khanyile *et al*. (2014), information on the adaptation period of pigs fed on incremental levels of *V. tortilis* leaf meal diets and time spent on behavioural activities exhibited by pigs is lacking.

Pig nutritionists and feed compounders will be able to formulate diets and incorporate *V. tortilis* leaves based on the extent of the adaptation period and the nutritional needs of pigs. It is important to develop strategies that can reduce the adaptation period of pigs to leaf meals.

Poor welfare is an indication of stress or uncomforted zone in pigs. Time spent on behavioural activities of pigs with regards to *Vachellia tortilis* leaf meal inclusion can be used to assess the welfare of pigs and develop strategies that prevent stress in pigs. Researchers can then come up with simulation models that incorporate leaf meals as non-conventional feed resources to ensure that the antinutritional factors of *V. tortilis* leaf meal does not compromise the behavioural needs of pigs such as compromising their welfare. Improved pig welfare and nutritional needs of pigs due to *V. tortilis* leaf meal inclusion increases growth rate and pigs may attain slaughter weights earlier. Inclusion of leguminous leaf meals may also improve pork quality.

1.3 Objectives

The broad objective of the study was to determine the effect of feeding incremental levels of *Vachellia tortilis* leaf meals on the adaptation and behaviour of finishing pigs. The specific objectives were to:

1. Determine the effect of feeding incremental levels of *V. tortilis* leaf meal on the adaptation period of finishing pigs; and
2. Assess the relationship between incremental levels of *V. tortilis* leaf meal and time spent on behavioural activities of finishing pigs.

1.4 Hypothesis

The hypotheses tested were that:

1. There is a positive linear relationship between incremental levels of *Vachellia tortilis* leaf meal and adaptation period of pigs.
2. *Vachellia tortilis* leaf meal inclusion has no relationship with the time spent on behavioural activities of finishing pigs.

1.5 References

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CHAPTER 2: Review of Literature

2.1 Introduction

Leguminous grains and cereals are the main ingredients used in pig diets by feed compounders and farmers due to their high energy and protein content. The use of legume grains and cereals as feed sources for pigs is affected by drought and the increasing human population, thus leading to an increase demand for cereal and maize based diets. This review discusses pig production systems, nutritive value of *Vachellia tortilis*, effect of leaf meals on pig performance, deleterious effects of proanthocyanidins present in leguminous *Vachellia* leaf meals, adaptation of pigs to leaf meal diets, factors affecting adaptation and behaviour of pigs when fed on fibrous diets.

2.2 Pig production systems

Pig production systems comprises the extensive and intensive production systems. The pig breeds kept in these systems include the Large White, Landrace, Duroc, Koelbroek and the Windsnyer pig. The type of pig breed used depend on the production system. Adequate production of feed is required to ensure optimal production and management practices are determined by the production system of choice on the farm.

2.2.1 Intensive production systems

The intensive system requires skilled labour, capital and investment. Farmers usually keep many pigs requiring high capital, health facilities and expensive buildings. Pigs in this system greatly depend on maize and soya diets due to the large number of pigs kept by farmers. The use of maize-soya diets in this system intensify the competition for grains between feed compounders and food for human consumption. The high grain demand present a major impediment for pig farmers to constantly depend on maize-soya diets as a main feed source for pigs (McCalla, 2009; Nonhebel and Kastner, 2011). New models that incorporates non-conventional feed resources, such as leguminous leaf meals in pig rations need to be identified and characterised for the benefit of farmers.

2.2.3 Extensive production systems

The extensive production system is mostly used by small scale farmers. It can be divided into the backyard and free range system. Pigs reared in the backyard system rely on supplementary feeds that includes grasses, vegetables, kitchen waste and hominy chops (Chimonyo, 2012). Those in the free range systems are sometimes penned but allowed to roam around during the day in search for feed (Lekule and Kyvsgaard, 2003). Production in these systems is constrained by feed shortages, since most of the feed consumed does not meet all the nutritional requirements of pigs (Chiduwa *et al.*, 2008). Introducing the use of leaf meals under this system can increase efficiency and productivity of pigs. *Vachellia tortilis* trees are easily accessible and abundant in rural areas hence most of the small scale farmers have access to these trees. The amount of nutrients in feeds has major effects in the quality of pork (Dugan *et al*., 2004). Due to the good nutrient content of *V. tortilis* leaves, pork quality of pigs raised under extensive systems can be improved if pigs are fed on *V. tortilis* leaf meal diets.

2.3 Description of *Vachellia tortilis*

African *Acacia* species have now been grouped and named into two genera, *Vachellia* and *Senegallia*. *Vachellia* species have spinescent stipules and capitate inflorescences while the *Senegalia* species have spicate inflorescences with non spinescent stipules (Kyalangalilwa *et al*., 2013). *Vachellia tortilis* (umbrella Thorn*)* is a shrub or tree of small to medium size, having a height of about 4 to 8 m but it can grow up to a height of 20 m and can spread from 8 to 130 m, as shown in Figure 2.1 (Orwa *et al.*, 2009). *Vachellia* trees are widespread and abundantly distributed through the savannah biomes and the dry zone of the African continent.

The leaves of the tree contain high amount of fibre and polyphenolic compounds. The tree produces a large number of pods which are pale brown in colour, coiled in shape and fall unopened on the ground. Leaves are compound ranging from 6 to 12 pairs and the leaflets are very small, ranging from 1 to 4 mm × 0.6 to 1 mm wide, glabrous to pubescent (Confalonieri *et al*., 2013). It bears white thorns and hooked thorns which helps it protect its highly nutritious fruits (pods) from wild animals. It has a deep tap root system that enables it to survive under harsh environmental conditions with low amount of rainfall. Figure 2.2 is an image of *V. tortilis* tree leaf structure.

Figure 2.1: *Vachellia tortilis* tree. Source: Orwa *et al*. (2009)

Figure 2.2: *Vachellia tortilis* tree leaves. Source: Heuzé *et al*. (2015)

2.4 Nutritive value of *Vachellia tortilis* leaves

Fodders from *Vachellia* tree leaves are rich in crude protein, it ranges from 135-180 g/kg DM and it also contain moderate minerals and fibres. The use of *Vachellia* leaves as a protein supplement in pigs has been shown to enhance growth performance, digestibility and feed intake (Norton *et al*., 1994; Abdulrazak *et al*., 1996). Factors that determine the nutritive value of *V. tortilis* leaves include season, soil fertility and the stage of maturity of the leaf. Table 2.1 shows the chemical composition of *V. tortilis* leaves, while Table 2.2 gives the mineral composition of *V. tortilis* leaves. Other forage leaves used in non-ruminant feeding apart from *Vachellia tortilis* include cassava, groundnut leaves, Leucaena leaves, sweet potato vines, water spinach leaves and Trichantera leaves. These forages also contain considerable amounts of nutrients which are beneficial to livestock. The main disadvantage of these forages is that humans also use them as food for household consumption, resulting in competition. Table 2.3 shows the chemical composition of forage leaves used in monogastric feeding.

Table 2.1: Chemical composition of *Vachellia tortilis* leaves (g/kg DM)

Component	Mean	Source
Dry matter	948	Mokoboki *et al*. (2005)
Organic matter	865	Mokoboki *et al.* (2005)
Crude protein	150	Mokoboki *et al*. (2005)
Neutral detergent fibre	494	Khanyile *et al*. (2014)
Acid detergent fibre	298	Khanyile *et al.* (2014)
Hemicellulose	110	Mokoboki *et al*. (2005)
Ether extract	40	Khanyile *et al*. (2014)
Condensed tannins	77.8	Rubanza *et al*. (2006)
Gross energy	17.23	Khanyile *et al*. (2014)

Table 2.2: Mineral concentration of *Vachellia tortilis* leaves (g/kg DM)

Component	Mean	Source
Magnesium	7.9	Abdulrazak *et al*. (1996)
Manganese	1.0	Abdulrazak *et al.* (1996)
Phosphorus	1.4	Abdulrazak *et al*. (1996)
Potassium	9.1	Tefera *et al*. (2008)
Iron	178	Khanyile *et al*. (2014)
Copper	2.0	Khanyile *et al*. (2014)
Sodium	0.5	Khanyile *et al*. (2014)
Potassium	11.4	Abdulrazak *et al*. (1996)
Zinc	0.2	Tefera *et al*. (2008)

Table 2.3: Chemical composition of other forage legume leaves (g/kg DM)

Forage leaves	DM	CP	EE	Ash	CF	NDF	Source
cassava	-	167- 399	38 - 105	57 - 125	48 - 290	-	Ravindran, (1993)
	260	239 - 347	113 - 156	5 - 81	97 - 165	32 - 335	Phuc *et al*. (2000)
Groundnut	269	175	22	86	201	-	Phuc et al. (2001)
	245	223	23	81	-	387	Phuc *et al*. (2001)
Leucaena	255	283 - 302	54-107	80-86	157	344 - 375	Phuc *et al*. (2000)
Sweet potato vines	142	185	-	125	235	-	Phuc *et al*. (2001)
Trichantera	200 - 260	151 - 225	-	167-199	167- 183	297	Rosales (1997)
	-	209	40	264	-	330	Phuc *et al*. (2001)
Water spinach	106	264	26	112	-	229	Phuc *et al*. (2001)

Cassava leaves (*Manihot esculanta Crantz*), Groundnut foliage (*Arachis hypogaea*), Leucaena leaves (*Leuceana leucocephala*), Sweet potato vines (*Ipomoea batatas*), Triachanthera leaves (*Trichanthera gigantean*), Water spinach (*Ipomoea aquatic* Forsk)

2.5 Effects of leaf meal inclusion on pig performance

Measuring pig performance fed on leaf meal diets gives understanding on the utilization of leaf meals by pigs. Average daily feed intake, average daily gain and gain: feed ratio are the main variables used when measuring and assessing growth performance in pigs.

2.5.1 Average daily feed intake

Feed intake indicates the edibility and acceptability of a feed (McSweeney *et al*., 2008). Nutrient level can be determined by the amount of feed consumed by pigs, thus feed intake has serious influence on pig production (Nyachoti *et al*., 2004). Khanyile *et al*. (2014) reported an initial increase in the overall average daily feed intake (ADFI) of pigs fed on inclusion levels of *Vachellia tortilis* leaf meal. The initial increase explain that pigs increase the ingestion of leaf meal feeds to complement unavailable nutrients that are constrained by polyphenolic compounds (Lee *et al.*, 2010). Halimani *et al*. (2005) also reported a decrease in feed intake of pigs fed on *V. karroo* leaf meal inclusion. Leaf meal diets should be included up to 100 g/kg DM since they do not depress feed intake at such level (Cappai *et al*., 2013). Incremental levels of forages in pig diets increase the antinutritional factors of forages in leaf meal diets. Such factors are associated with a negative effect in pig production such as lowering feed intake due to the astringent taste of soluble phenols which reduce palatability (Kaitho *et al*., 1997).

2.5.2 Average daily gain

Average daily gain (ADG) is the ratio of overall growth and overall number of days of feed consumption. Increasing levels of forages in pig diets is associated with a decline in average daily gain while low levels may increase growth rate. Barry *et al*. (1986) reported an increase in growth rate of pigs fed on diets containing low levels of phenolic compounds. Khanyile *et al*. (2014) reported a decrease in average daily gain of pigs fed high levels of *Vachellia tortilis* leaf meal. Other studies also reported a general decline in growth rate of pigs fed on high levels of leaf meals (Laswai *et al*., 1997; Halimani *et al*., 2005). The decline in growth rate can be explained by low nitrogen retention since pigs fed on leaf meal diets are reported to have low nitrogen retention (Echeverria *et al*., 2002). Leaf meals can be incorporated in pig diets up to 100 g/kg without decreasing average daily gain of pigs (Phuc *et al*., 2000; Halimani *et al*., 2005).

2.5.3 Gain: feed ratio

Gain: feed ratio (G: F) can be defined as a measure of efficiency at which pigs utilise the feed they consume. A higher G: F ratio implies that pigs are less efficient at converting feed into body mass gain while a lower G: F ratio implies that pigs are more efficient at converting feed into body mass gain. Mukumbo *et al*. (2014) reported a low gain: feed ratio of pigs fed on leaf meal diets containing 25 g/kg DM and 50 g/kg DM *Moringa oleifera* leaves and a high gain: feed ratio in pigs fed 75 g/kg DM *Moringa oleifera* leaf meal. Khanyile *et al*. (2014) also reported a decline in G: F ratio of pigs fed on increasing levels of *Vachellia tortilis* leaf meal diets. It started to decrease beyond 129 g/kg DM. Higher inclusion levels of leaf meals decrease G: f ratio of pigs. The difference between the studies may be due to the vast differences in the age of the pigs used in the experiments (pre-starter and finishers). Hence further research is required to investigate the effect of age on pigs fed on increasing levels of leguminous leaf meals. A decline in G: F ratio of pigs with increasing inclusion levels of leaf meals diets was also reported by (Laswai *et al*., 1997). The presence of polyphenolic compounds in leaf meal diets has the potential of reducing feed efficiency, nutrient digestion and absorption (Mikkelsen *et al*., 2004; Acamovic, 2005). Table 2.4 shows the effect of *V. tortilis* leaf meal inclusion on pig growth performance.

Table 2.2: Effects of *Vachellia tortilis* leaf meal inclusion on pig performance

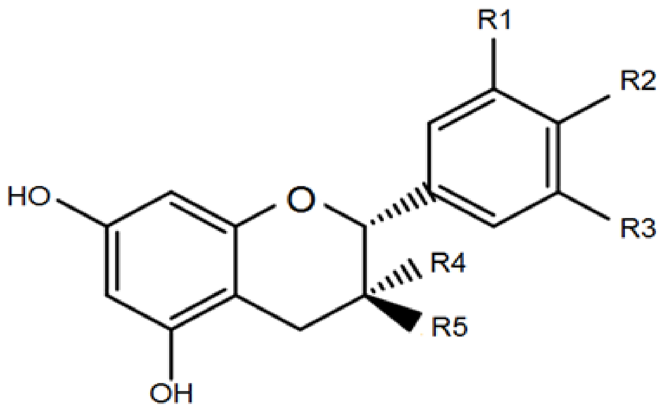
Variable	Week	Inclusion level of leaf meal (g/kg DM)							
		0	50	100	150	200	250	SE	P value
ADFI	1	2.02	3.18	2.54	2.64	1.85	1.35	0.12	<0.006
	2	2.91	3.22	3.06	2.99	2.42	2.22	0.21	<0.0119
	3	3.22	2.84	2.73	3.45	2.78	2.52	0.21	NS
Overall		2.72	3.08	2.78	3.02	2.35	2.03	0.15	0.0007
ADG	1	0.626	0.709	0.737	0.523	0.406	0.336	0.06	<0.0279
	2	0.72	0.844	0.826	0.623	0.429	0.387	0.06	<0.0001
	3	0.845	0.921	0.903	0.708	0.497	0.414	0.06	<0.0001
Overall		0.714	0.860	0.853	0.559	0.442	0.387	0.05	<0.0001
G:F	1	0.461	0.342	0.336	0.383	0.266	0.362	0.039	<0.0001
	2	0.415	0.333	0.307	0.288	0.334	0.286	0.039	<0.0213
	3	0.452	0.430	0.381	0.315	0.334	0.323	0.039	<0.001
Overall		0.443	0.368	0.341	0.329	0.308	0.324	0.023	<0.0007

ADFI = Average daily feed intake (kg/d DM): ADG = Average daily gain (g BW/d): G: F = Gain: feed ratio: NS = P > 0.05: SE = Standard error: Source: Khanyile *et al*. (2014)

2.6 Description of proanthocyanidins

Proanthocyanidins are defined as naturally occurring water-soluble polyphenolic compounds of high molecular weight ranging from 500 to 3000 Daltons. They are found mostly in leguminous tree plant leaves and herbaceous legumes (de Jesus *et al.,* 2012). They are chemically reactive and have the ability to form inter- and intra-molecular hydrogen bonds that interact with and precipitate macromolecules such as polysaccharides and proteins (de Jesus *et al*., 2012). Low levels of pholyphenolic compounds may reduce physiological effects of alkaloids by preventing their absorption into the blood stream and be excreted without influencing the pig's physiology. They can also reduce the detrimental effects of saponins (Bernays *et al*., 1989). Proanthocyanidins are classified into two groups, hydrolysable tannins and condensed proanthocyanidins. Hydrolysable tannins are harmful relative to condensed proanthocyanidins.

Hydrolysable tannins are referred to as catechin, they contain an internal primary carbohydrate whose hydroxyl groups are esterified to phenolic carboxylic acids like allagic and gallic acid. They are readily hydrolyzed by weak acids such as alkali, acids or some enzymes and are decomposed by high temperatures to produce pyrogallol (Jiménez *et al*., 2014). Condensed proanthocyanidins, are oligemers of flavan-3-o1 (catechin monomers) and flavan-3,4-diol are the most abundant plant derived polyphenols. They exist in feeds like browse plants and fodder legumes. They are connected by C-O-C bonds with a wide structural variety (de Jesus *et al*., 2012; Lamy *et al*., 2016). These compounds are easily hydrolyzed and decompose in acidic alcoholic conditions yielding to phlobaphens. They have been recognized as the main factors affecting pig productivity. Figure 2.3 and Figure 2.4 are structures of hydrolisable tannin and condensed proanthocyanidins.



Flavanols	R1	R2	R3	R4	R5
Afzelechin	H	OH	H	H	OH
Epiafzelechin	H	OH	H	OH	H
Catechin	H	OH	OH	H	OH
Epicatechin	H	OH	OH	OH	H
Gallocatechin	OH	OH	OH	H	OH
Epigallocatechin	OH	OH	OH	OH	H

Figure 2.3: A hydrolisable tannin (flavonol structures)

Source: Währn, (2017)

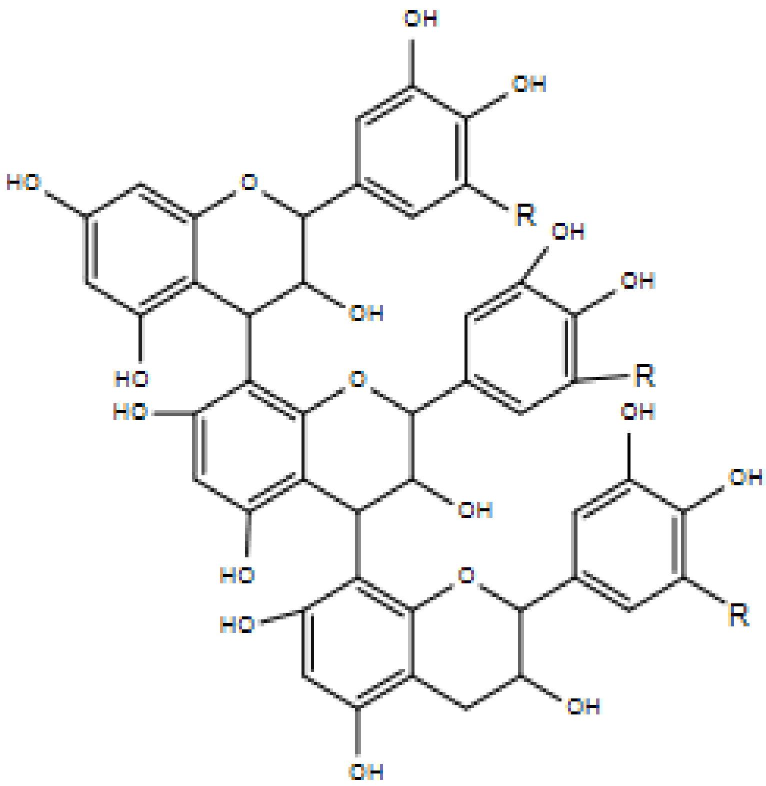


Figure 2.4: A condensed proanthocyanidins trimer

R = H: procyanidins, R = OH: prodelphinidins. Source: Wahrn, (2017)

Table 2.3: Content of total extractable phenolics (TEP), total extractable proanthocyanidins (TEP) and total proanthocyanidins (TP) (mg/g DM), soluble and bound condensed proanthocyanidins (BCP) (mg/g DM) in *Vachellia tortilis* leaves

Phenolics	Amount	SEM
Total extractable phenolics	241	8.8
Total extractable	226	8.7
Total proanthocyanidins	77.8	1.46
Soluble condensed proanthocyanidins	18.9	1.45
Bound condensed proanthocyanidins	37.5	1.60
Fibre bound condensed proanthocyanidins	21.5	1.55

Source: Rubanza *et al*. (2005), SEM; Standard error mean

2.7 Deleterious effect of proanthocyanidins present in leguminous *Vachellia* leaf meals

The use of *Vachellia* leaf meals as a potential feed ingredient in pigs is constrained by the presence of polyphenolics, however, the influence of polyphenolic compounds depend on the inclusion level of the leaf meal and the nature of polyphenolic compounds that are present (Van, 2006). Deleterious effect of proanthocyanidins include decreasing voluntary feed intake and nutrient digestion (Frutos *et al*., 2004). A reduction in feed intake and nutrient digestion is due to the fact that polyphenolic compounds reduce protein digestibility

Silanikove *et al*. (1996) reported an inverse relationship between high condensed proanthocyanidins (CP) level in leguminous forages (more than 50 g CP/kg DM), feed intake and palatability. Lee *et al*. (2016) also reported a reduction in feed intake of pigs fed on tannin-rich chestnut meal of 150 g/kg DM (Table 2.6). Poor nutrient digestibility causes retarded growth and lowers animal performance (D'Mello 1995; Van, 2006). Tannic acid in livestock feeds not only reduce digestibility and nutrient absorption, but also affects different parts of internal organs. Karim *et al*. (1978) reported necrosis of the liver and kidneys of chicks that were fed on diets containing between 10 and 30 g/kg DM tannic acid. Chang and Fuller (1964) observed fatty livers in chicks fed diets that had tannic acid in their diets. Bilić-Šobot *et al*. (2016) reported an in increase in the villus height of the duodenum of pigs supplemented with 30 g/kg DM of proanthocyanidins. The size of the intestinal villi is important for digestion and absorption of nutrients, longer villus has larger surface area for nutrient absorption (Zhang *et al*., 2013; Han *et al*., 2014). The effect of supplementing proanthocyanidins in boar diets is shown in Table 2.7.

Table 2.4: **Effect of increasing levels of chestnut meal on feed intake and digestibility of fattening pigs**

Variable	Chesnut leaf meal inclusion (g/kg DM)					Relationship	
	0	50	100	150	SEM	Linear	Quadratic
Intake (g/day)							
Dry matter	2760	2807	3237	2674	152.2	> 0.05	0.046
Crude protein	543.0	530.8	551.2	4589	36.34	> 0.05	> 0.05
Ether extract	144.5	144.0	148.4	126.2	9.83	> 0.05	> 0.05
Crude ash	107.5	114.1	118.4	107.7	7.92	> 0.05	> 0.05
Proanthocyanidins	4.33	4.79	5.92	5.12	0.216	> 0.05	0.046
Digestibility (%)							
Dry matter	87.1	85.0	83.7	81.3	0.01	0.010	> 0.05
Crude protein	86.6	82.6	78.7	77.6	0.01	<0.001	> 0.05
Ether extract	65.8	58.3	49.8	43.8	0.02	<0.001	> 0.05
Crude ash	58.5	56.7	41.5	40.3	0.04	0.031	> 0.05
proanthocyanidins	67.7	68.8	61.0	59.1	0.04	0.016	> 0.05

Source: Lee *et al*. (2016), SEM: Standard error mean

Table 2.5: Sections of small intestine in boars fed on diets with different tannin levels

Variable (μm)	Tannin supplementation (%)				
	0	10	20	30	P value
Duodenum					
Villus height	377 ± 19[a]	442 ± 12[ab]	404 ± 22[ab]	462 ± 24[b]	0.025
Villus width	182 ± 9	209 ± 4	193 ± 7	200 ± 11	0.150
Villus perimeter	953 ± 47[a]	1112 ± 27[ab]	1017 ± 51[ab]	1144 ± 63[b]	0.040
Jejunum					
Villus height	363 ± 18	268 ± 42	327 ± 20	303 ± 29	0.204
Villus width	156 ± 7	125 ± 16	147 ± 8	139 ± 14	0.390
Villus perimeter	890 ± 41	673 ± 103	810 ± 48	757 ± 74	0.260
Ileum					
Villus height	404 ± 38	408 ± 32	370 ± 9	372 ± 14	0.653
Villus width	192 ± 15	189 ± 19	189 ± 3	183 ± 3	0.963
Villus perimeter	1009 ± 87	1016 ± 82	945 ± 22	941 ± 29	0.763

[ab]Values with different superscripts are significantly different at $p < 0.05$; n = 6

Source: Bilić-Šobot *et al*. (2016)

2.8 Adaptation of pigs to leaf meal diets

Adaptation period refers to the time from introduction of a new diet when feed intake is still low because animals have not yet adapted to the diet (Brunsgaard *et al*., 1995). Adaptation can be categorized into enzymatic and physical adaptation. Enzymatic adaptation involves the secretion of enzymes while physical adaptation is associated with the elongation of the internal organs such large intestine.

2.8.1 Enzymatic adaptation

Another form of adaptation of pigs to high phenolic ingestion of leaf meals is based on parotid gland hypertrophy and the secretion of salivary proteins that bind and neutralise the toxic effects of polyphenolic compounds (Dearing and Cork, 1999; Cappai e*t al*., 2010, 2012). However, knowledge about how polyphenolic compounds interact with the digestion process of pigs is lacking, and it requires further research. Two classes of salivary proteins secreted by non-ruminants consuming tanniferous diets are proline-rich proteins (PRPs) and histatins (Shimada, 2006). Proline-rich proteins are compounds that comprises approximately 20 to 40 % of amino acids present in PRPs, they have high affinity to phenolic compounds (Mehansho *et al*., 1987). Proline-rich proteins have a molecular weight that range from 5000 to 25000 with proline being the main constituent of PRPs (Bennick, 1982). An increase in the level of polyphenolic compounds in the diet stimulate the parotid gland to produce salivary rich proteins, these proteins contain non-essential amino acids (proline, glycine and glutamic acid). The secreted PRPs act as binding agents to polyphenolics in the gastro intestinal tract, they also prevents other harmful and antinutritional effects of polyphenolic compounds (Butler *et al*., 1986).

Histatins are a type of salivary proteins produced by animals consuming feeds containing polyphenolics. Histatins are the main constituents of the salivary proteins, they have less molecular weight (less than 5000) compared to proline-rich proteins. They also have high affinity to polyphenolics. Less proteins will be required to bind to available polyphenolics, thus resulting in a qualitative saving of nitrogen for the pig (Yan and Bennick, 1995; Makkar, 2003). The implications of histatins in adaptation of pigs to tanniferous diets is still not clear, hence further research is required to investigate the role of histatins in adaptation of pigs (Makkar, 2003).

2.8.2 Physical adaptation of pigs to leaf meal-based diets

Pigs adapt to leaf meal diets by increasing endogenous fluid. The increase in secretion of endogenous fluids is enhanced by high amount of fibres (Wenk, 2001). Zebrowska *et al*. (1983) reported that dietary fibres ranging from 50 to 180 g /kg caused a double increase in saliva and gastric juice secretion in pigs. He also reported an increase in pancreatic juice and bile contents, increasing amounts of the secreted fluids means more metabolic demand for the pig. Effective feed digestibility can be expected due to the increased secretion of endogenous fluids.

Pigs also adapt to leguminous leaf meal diets by increasing or decreasing feed intake, adaptation of pigs to leaf meals can depend on the inclusion level of the leaf meal since it affects feed intake. Feed intake of non-ruminants animals fed leafy forage legumes is generally higher than that of grass meal, this is caused by the difference in fibre contents, digestibility of the feeds and retention time (Martens *et al.,* 2013). Adaptation of growing-finishing pigs includes the accommodation of the gastrointestinal tract to the increased involvement of some of the part that are associated with fibre digestion such as the large intestines (Whittemore *et al.*, 2003).

Volume and weight of the gastrointestinal track also increases more especially those of the large intestine (caecum and colon). Jørgensen *et al*. (1996) reported a significantly heavier stomach, caecum, colon and also longer colon in growing-finishing pigs fed leaf meal diets having fibre fractions of approximately 260 g/kg DM when compared with pigs which were fed leaf meal diets containing 59 g/kg DM dietary fibre.

2.8.3 Factors affecting the adaptation of pigs

The adaptation period differs considerable amongst researchers, some use 18 days (Nyman and Nils-Georg, 1985), 14 days (Whittemore *et al.,* 2003) and as far as up to 6 weeks (Lajvardi *et al*., 1993) depending on the type animal species and feed used. Adaptation period can be measured by collecting feed residues, determining the number of days the pigs where on a specific diet and collecting fecal samples to determine the feed or dry matter intake of the pigs. Evaluating the period of adaption by feed or dry matter intake requires calculating the feed intake and taking into account the number of days when pigs were at equilibrium with the diets (Figure 2.4; Kyriazakis & Emmans, 1995).

Possible changes in feed intake and body weight are always confounded with the effect of the adaptation period. Brunsgaard *et al*. (1995) reported that body weight of rats on different diets containing different indigestible polysaccharides increased progressively over period of eight weeks. Systemic exploration of the adaptation period is important in livestock animals (Brunsgaard *et al*., 1995). Apart from the adaptation period, behaviour of pigs can be influenced by incremental levels of *Vachellia tortilis* leaf meals. The presence of polyphenolic compounds my influence growth performance and alter the pig's behaviour. It can be determined by assessing the time spent on behavioural activities displayed by each pig.

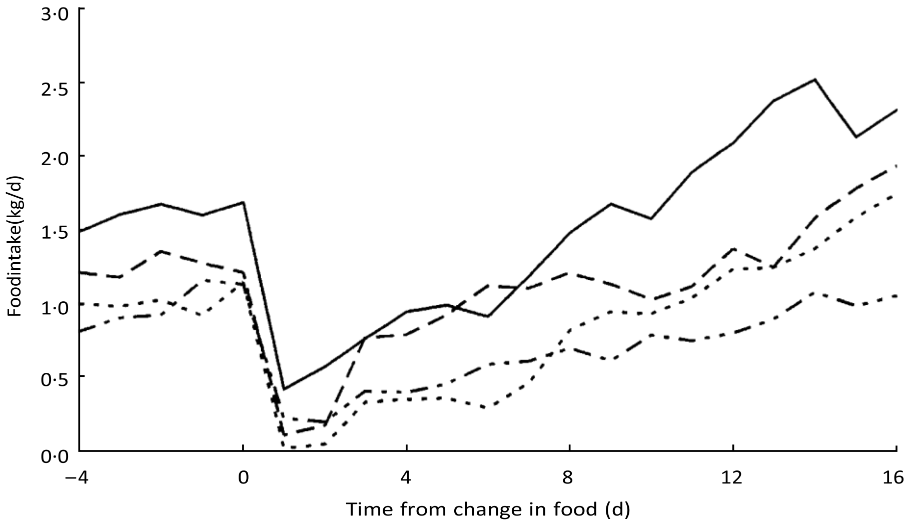


Figure 2.5: Adaptation of pigs when changed from a basal diet to a diet containing 97% wheat bran feed

Source: Kyriazakis & Emmans (1995)

2.9 Behaviour of pigs fed on fibrous diets

All features of pig production such as management practices and environmental factors relate to the way how pigs behave and it also influence their well-being (Broom *et al*., 2007). Pig behaviour can be categorized into feeding (eating, drinking feeder visit), postural (lying down, sitting/standing and walking) and stereotypic behaviours (object biting, licking and constant sniffing of objects). Behavioural data are obtained using video cameras with time used as a measurement unit. It can be recorded in hours, minutes or seconds depending on the observer. Using video cameras when collecting behavioural data aid to prevent disturbances that may be caused by the observer. Parameters used when colleting behavioural data are number or frequency of feeder visits, duration of feeding, drinking, lying down and standing (Nielsen, 1999). Sufficient consumption of feed ensures maximal productivity and survival of pigs hence information about the behaviour and factors that affects it are very important in pig production.

2.8.1 Influence of feeding leaf meal diets on feeding behaviour

Feeding behaviour refers to actions that are concerned with obtaining nutrients, it also reflects feeding patterns of pigs (Bakare *et al*., 2014). Diet type (mash, pellet or meal) and presentation (dry, wet or liquid) can alter the feeding behaviour of pigs (time spent eating) (Gonyou and Lou, 2000). In commercial pig production systems, feed include cereal based diets which can be presented in the form of mash, pellets or meal and balanced in terms of nutrients. The daily time spent eating is very short and it can range from 15 to 20 minutes (Nihlstrand, 2016). The short daily time spent eating indicate that pigs quickly meet their nutritional requirements while prolonged daily time spent eating shows that pigs slowly meet their nutritional requirement or it may be an indicative of poor nutrients in a particular source of feed. Bakare *et al*. (2014) showed that pigs spent greater proportion of their time eating to meet nutritional needs on fibrous diets. Fibrous diets are lacking in terms of energy, they are mostly used for maintenance purposes in sows units. Apart from diet, other factors that can influence feeding behaviour of pigs are health, environment, and housing system (Maselyne *et al*., 2015).

2.8.2 Influence of leaf meal diets on postural behaviour

Increasing inclusion level of leguminous leaf meal diets also increases indigestible components of the feed (neutral detergent fibers and lignin). As a result, pigs are expected to spent greater proportion of their time lying down so as to eradicate some of the undigested components in the gastro-intestinal tract. Postural behaviours (standing, sitting and lying down) can be used to indicate discomfort in pigs. Pigs show their discomfort level by standing or sitting (Pearce and Paterson, 1993). Another factor that affect postural activity is the type of flooring used in pig production. Slatted floors offers minimal support to the pig and high pressure is located on the shoulder as related to floors that are hard. Pigs on hard floors decrease the risk of shoulder lesions by exhibiting postural behaviours (Rolandsdotter *et al*., 2010).

2.8.3 Influence of leaf meal diets on stereotypic behaviours

Stereotypic behaviours are fixed repetitive action with no purpose, occurring when pigs are frustrated (Meunier-Salaün *et al*., 2001). Stereotypic behaviours have been widely used as welfare and stress indicators in pregnant sow. Some of the factors that actuate stereotypic behaviours are environmental conditions, type of diet used and medications (Bakare *et al*., 2014). Examples are pacing, bar or object chewing or licking, chain chewing or licking and vacuum chewing. *Vachellia tortilis* leaves are rich in polyphenolic compounds and fibre, higher levels may induce stereotypic behaviours therefore, impairing their welfare. But low inclusion levels of *V. tortilis* leaves may decrease the incidence of these behaviours.

Table 2.6: Behaviour of gilts fed on diets containing 176 DF kg $^{-1}$(control), 446 DF kg $^{-1}$ DF (sugar beet pulp (SBP)) and 344 g DF kg $^{-1}$ (mixed fibre sources, dry grass meal, wheat bran and oat hulls kg $^{-1}$(MFS))

Behaviours	Gilts treatment group diets			
	Control	SBP	MFS	S.E.M
Postures				
Lying down	0.423^{a}	0.631^{b}	0.623^{b}	0.050
Standing	0.477^{a}	0.300^{b}	0.281^{b}	0.040
walking	0.059	0.040	0.041	0.001
Activities				
Eating	0.055^{a}	0.135^{b}	0.113^{b}	0.011
foraging	0.297^{a}	0.162^{b}	0.192^{b}	0.029
Sham chewing	0.114	0.046	0.084	<0.001
Aggression	0.002^{a}	0.004^{b}	0.012^{a}	<0.001
Resting	0.494^{a}	0.698^{b}	0.627^{b}	0.034

Proportion of observed time (180 min). S.E.M: standard error of means.

a,b,c: Values within row with different letters differ significantly, $p < 0.05$.

Source: Danielsen and Vestergaard. (2001)

2.9 Summary

Prolonged drought conditions, high demand for grain product and price variations are the main factors that are challenging researches to discover new alternative ingredient sources that can only be used in livestock diets. *Vachellia tortilis* leaves can be used as an alternative source of ingredient, its inclusion in pig diets can help reduce the quantity of grain used when formulating pig diets. There is, however, a need to explore the behaviour of pigs fed on incremental levels of *Vachellia* leaf meal diets. This can be achieved by determining the adaptation period and time spent on behavioural activities of pigs fed on *Vachellia* diets. Knowing the time it takes for pigs to adapt on different inclusion levels of *V. tortilis* leaf meal diets can assist farmers when using *V. tortilis* leaf meal as a feed to pigs. They can feed or formulate rations for pigs with the required amount of *V. tortilis* leaves that has the least period of adaptation in order to reduce the finishing period of pigs and maximize returns. The aim of the study is, therefore, to determine the adaptation and behaviour of finishing pigs fed on incremental level of *V. tortilis* leaf meal diets.

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CHAPTER 3: Adaptation of finishing pigs to *Vachellia tortilis* leaf meal inclusion

(Submitted to Livestock Science)

Abstract

The current study was conducted to determine whether *Vachellia tortilis* leaf meal inclusion affect the adaptation periods in finishing pigs. A total of 48 clinically healthy male Large White × Landrace male pigs with a mean (± SD) body weight of 63.8 ± 3.28 kg aged 14 weeks were used. Pigs were assigned to individual pens in a completely randomized design and allotted to each of six experimental diets which contained 0, 30, 60, 90, 120 and 150 g/kg DM of *V. tortilis* leaf meal inclusion. Average daily feed intake (ADFI), average daily gain (ADG) were measured to calculate, gain: feed ratio (G: F) and the adaptation period of pigs. Regression analyses was used to analyse the data. There was a quadratic decrease ($P < 0.001$) in ADFI, while ADG increased linearly ($P < 0.001$) with incremental level of *V. tortilis* leaf meal. Incremental level of *V. tortilis* leaf meal increased G: F ratio quadratically ($P < 0.001$). A linear increase in adaptation period of pigs was observed ($P < 0.05$) with increasing inclusion level of *V. tortilis* leaf meal. Variation of feed intake, expressed as a coefficient of variation of feed intake, increased linearly ($P < 0.05$) with increasing inclusion level of *V. tortilis* leaf meal. Adaptation period of finishing pigs was influenced by the inclusion level of *V. tortilis* leaf meal diets, by causing a positive linear relationship, therefore pigs fed on increasing levels of *V. tortilis* leaf meal diets require a long time to adapt.

*Key words***:** finishing pigs, feed intake, variation, polyphenolic compounds

3.1 Introduction

Pork is the most commonly consumed meat, but it is also a cause of saturated fatty acids, which can affect human health negatively. The quality of pork can be altered and improved by feeding diets containing varying concentration of fatty acids (Fontanillas *et al*., 1998). Nitrogenous leguminous leaf meal are the main cause of saturated fatty acids in pork meat. The use of low levels of leguminous leaf meal diets can decrease the harmful effects of saturated fatty acids of pork meat due to the presence of phenolic compounds and natural antioxidants. Natural antioxidants from leguminous plant have gained popularity due to their safety as compared to synthetic antioxidants (Moyo *et al*., 2011).

Leguminous tree forages, such as *Vachellia*, formerly subgenus *Acacia* (Kyalangalilwa *et al*., 2013) are important in livestock production since they can be used as protein sources (Makkar, 2003). These leguminous tree leaves contain high amounts of crude protein of almost 218 g/kg DM (Mlambo *et al*., 2007; Khanyile *et al*., 2014) and favourable mineral concentration (Dube *et al*., 2001). The trees are abundant in the dry zones of the African continent. The use of *Vachellia* leaves in livestock feeding is, however, constrained by the excessive amounts of fibre and polyphenolic compounds. Although the use of *Vachellia* leaves as feed sources for pigs has been reported earlier (Halimani *et al.*, 2007; Khanyile *et al.*, 2014), the adaptation of pigs to these diets has not been assessed.

In all systems of production, feed is changed for weaners to growers and for growers to finishers. When tanniferous diets are fed to pigs, the pigs have to adapt to the proanthocyanidins. The amount of proanthocyanidins and fibre present in *Vachellia* leaf meals can prolonged the adaptation phase (Mapiye *et al*., 2009). Most researchers and farmers allow an adaptation period when conducting experiments and when changing diets. Adaptation period is the time from introduction of a new diet, at which feed consumption is still low because pigs are not yet accustomed to the feed (Brunsgaard *et al*., 1995). For any new feed offered, pigs are expected to develop mechanisms to cope with that feed. Pigs may adapt to *Vachellia tortilis* leaf meal diets by either decreasing or increasing feed intake. A reduction in feed intake limits nutrient intake, resulting in low growth rates (Tsaras *et al.*, 1998). The low growth rates increases the finishing period of pigs by increasing the time taken to reach market weight hence reducing turnovers. In addition, pigs also adapt to leaf meal diets by producing a unique salivary proline - rich proteins (PRPs) which then specifically bind proanthocyanidins (Halimani *et al.*, 2007). Khanyile *et al*. (2017) reported that gut capacity of pigs increases in size with response to *V. tortilis* meal diet. Feeding an increased amount of dietary fibre increases the volume of digesta in the stomach, decrease transit time and increases satiety (Kerr and Shurson, 2013).

Knowledge about the adaptation of pigs to *Vachellia tortilis* leaf meal inclusion is vital. It may take up to 14 days for a pig to become adapted to fibrous diet (Kyriazakis and Emmans, 1995). Information about the adaptation period of pigs fed on diets that are rich in polyphenolic compounds is scarce. The objective of the study was, therefore, to determine the effect of feeding increasing levels of *V. tortilis* leaf meal on the adaptation period of finishing pigs. It was hypothesized that inclusion level of *V. tortilis* leaf meal has a positive linear relationship on the adaptation period of pigs.

3.2 Materials and methods

3.2.1 Study site

The study was conducted at Ukulinga Research Farm, University of KwaZulu-Natal (UKZN), Pietermaritzburg, South Africa. The farm is positioned 29º24’E and 30º24’S having an altitude of 775 m above sea level. The daytime mean temperatures in the hot wet season reach highs of around 29°C with variation that can range from 28 to 43°C. The vegetation at the farm consist of several types of trees and grass species. The dominant tree species being *V. karroo, V. nilotica*, *V. sieberiana*, while the grass species are dominated by *Themeda triandra*. The average rainfall is 735 mm, which occurs mostly in the hot wet season with light to moderate frost falling occasionally in winter (Devereux *et al*., 2000).

3.2.2 Collection of *Vachellia* leaves

Vachellia tortilis leaves were hand harvested at Makhathini Research Station, Jozini, South Africa. The leaves were harvested between April and May 2016 during post rainy season at an advanced stage of maturity according to the method described by Khanyile *et al*. (2014). Briefly, leaves were harvested green, air-dried in under shade by spreading on polyethylene sheets at room temperature for 72h.

3.2.3 Pigs and housing

The care and use of pigs was done according to the ethical guidelines stated by the certification of Authorization to Experiment on Living Animals given by the UKZN Animal Ethics Committee: (Reference Number: AREC/101/015D).

A total of 48 clinically healthy male F1 hybrid (Large White × Landrace) pigs of 14 weeks of age, were used in the study. The pigs remained healthy throughout the trial those that were not active, showing symptoms of illness were treated with Terramycin. The mean (±SD) body weight of the pigs was 63.8 ± 3.28 kg. The pigs were bought at Kanhym farm in KwaZulu-Natal, South Africa. A truck was used for transporting the pigs to Ukulinga Research Farm (UKZN) in June 2016.

Prior to arrival, the pig house was disinfected with a green solution natural disinfectant. A footbath was placed at the entrance of the pig house for biosecurity control. On arrival, all pigs were ear tagged for easy identification. The pigs were reared in individual pens having slatted floors, measuring 2.1 × 1.1 m^2. The pig house was arranged in three rows of 16 pens each. The space of the pens was sufficient for a pig to move, rest. Natural light was used during the day and artificial light was only used at night. The house had raising curtains on both sides that were opened at 0845h and closed at 1630h. All pigs had free access to clean fresh water provided through low pressure nipple drinkers and they had *ad libitum* access to feed which was offered through pre-weighed plastic self-feeder troughs (Big Dutchman Lean Machine®, Postfach). Empty feeders were refilled daily at 0800h.

3.2.4 Experimental design, diets and pig management

Eight pigs were allocated individually using a completely randomized design into each of the six experimental diets and each pig was used an experimental unit. Six experimental diets were used. The basal diet did not contain *Vachellia tortilis* leaf meal. The other five diets contained 30, 60, 90, 120 and 150 g/kg (DM basis) of the leaf meal (Table 3.1). Maximum inclusion level of the leaf meal was determined by the digestibility estimates of energy and amino acids. Each diet contained equal amounts of vitamins, minerals, supplementary vitamins and trace minerals. The diets were formulated using Winfeed® diet formulation software to be isoproteinic and isocaloric. Vitamins and mineral were supplemented to meet National Research Council recommended specification for finishing pigs (NRC, 2012). Pigs were not given any growth promoters. The ingredient composition of experimental diets are shown in Table 3.1.

3.2.5 Chemical composition of diets

Before analyzing the experimental diets, samples from each diet were ground through a 2 mm sieve at Ukulinga Research Farm, UKZN, Pietermaritzburg. After milling, the samples were analyzed in triplicate, at the Animal and Poultry Science Laboratory at UKZN, Pietermaritzburg. Briefly, dry matter (DM) content was determined by the oven drying method, samples were dried for four days at 65 ^{0}C. Dry samples were incinerated at 550 ^{0}C overnight for ash content determination according to method 990.05 (AOAC, 1990). The dried samples were subjected to bomb calorimetry to determine gross energy (GE). Ether extract (EE) was determined using the Soxhlet apparatus following methods described by AOAC (1990); 920.39. Crude protein (CP) content was calculated using the formula: N × 6.25, were nitrogen content was determined following the Dumas Combustion method in a Leco Truspec Nitrogen Analyser, St. Joseph, MI, USA by method 990.3 of AOAC (1990). Neutral detergent fibre (NDF) and acid detergent fibre (ADF) were determined using the Ankom Fiber Analyzer (Ankom Macedon, NY, USA) according to Van Soest *et al.* (1991). The NDF was analysed using heat stable α-amylase (sigma A3306; Sigma Chemical Co., St. Louis, MO, USA).

The water holding capacity (WHC) was determined following methods described by Whittemore *et al*. (2003). Bulk density was determined using the water displacement method as described by Kyriazakis and Emmans (1995). For mineral analyses, ground samples were ashed at 550 ^{0}C overnight and dissolved in a 1 M HCL (Abdou *et al.*, 2011), then analysed using the Varian 720 Inductively Coupled Plasma Emision Spectrometer (ICP- OES, Frankfurt, Germany) with an atomic absorption (UKZN Westville campus). Proanthocyanidins content were detected calorimetrically by the butanol-HCL process (Reed *et al.*, 1982). For amino acids, acid hydrolysis was used (AOAC, 990; method 982.30). Before analyses via amino acid analyser (SY-KAM, Erising, Germany) following modifications by Mills *et al.* (1989). The chemical composition of the diets is given in Table 3.2.

3.2.6 Growth performance of pigs

At the beginning of the trial, all pigs were fed on the control diet purchased from Meadow Feeds, Pietermaritzburg South Africa (RSA). Average daily feed intake (ADFI) was measured for four days prior to introducing the experimental diets. Daily feed intake for each pig was determined by measuring the difference between the feed supplied on the previous day and feed left in the feeder every day between 0830h to 0930h and feed refusals were also considered (Nyman and Nils-Georg, 1985). A hanging scale was used to lift the feeders (bin feeders). Pigs were weighed every week at 0700h. Weight of pigs was recorded weekly. The average daily gain (ADG) was determined by dividing the differences at the beginning and at the end of each week by seven. The gain: feed ratio (G: F) was determined by dividing ADG by ADFI for each pig.

Table 3.1: Ingredient composition of the diets

Ingredient (g/kg)	Inclusion levels of *Vachellia tortilis* leaves (g/kg DM)					
	0	30	60	90	120	150
Yellow maize	341	322	302	284	266	247
Wheat bran	265	258	251	243	234	226
Soybean 46	64	68	70	75	79	83
Oil-sunflower	45	45.5	46	47	47	48
Limestone	15.6	15.2	14.8	14.5	14.1	13.5
Monocalcium phosphate	7.2	7.4	7.6	7.8	8	8.3
Salt	3.6	3.7	3.7	3.7	3.7	3.8
Vitamin + mineral premix[#]	1.5	1.5	1.5	1.5	1.5	1.5
L-lysine-HCL	1.2	1.1	0.9	0.8	0.6	0.5
Threonine	0.722	0.64	0.558	0.476	0.387	0.298
Methionine	0.283	0.312	0.342	0.372	0.402	0.432

[#]Supplemented (/kg of diet): vitamin A, 4.8 mg; vitamin $_{D3}$, 0.09 mg; vitamin $_{E}$, 50 mg; vitamin $_{K3}$ (43%), 1.0 mg; vitamin B1, 1.6 mg; vitamin B2, 2.6 mg; niacin (99.5%), 33.6 mg; vitamin B_{12} , 0.01 mg; vitamin B_6 98%, 2.0 mg; choline (chloride 60%), 121 mg; folic acid (96% pure), 0.48 mg; biotin, 0.18 mg; calcium pantothenate (98%), 5.2 mg; zinc balitracin, 90.0 mg; manganese sulphate, 120.0 mg; zinc, 100 mg; copper, 8 mg; potassium iodide (Iodine 76.45 %), 0.4 mg; cobalt sulphate, 0.2 mg; ferrous sulphate, 100.0 mg and selenium, 0.32 mg.

Table 3.2: Chemical composition of *Vachellia tortilis* leaf meal diets

	Vachellia tortilis inclusion level (g/kg DM)					
Component	0	30	60	90	120	150
Dry matter (g/kg)	946	935	899	940	897	896
Gross energy (MJ/kg)	17.6	17.5	17.3	17.3	17.4	17.2
Ash (g/kg DM)	90	90	90	89	89	86
Crude protein (g/kg DM)	144	140	142	146	146	148
Ether extract (g/kg DM)	114	104	109	114	113	116
Starch (g/kg DM)	318	321	305	274	239	225
Acid detergent fibre (g/kg DM)	137	136	139	125	125	127
Neutral detergent fibre (g/kg DM)	303	313	321	339	348	356
Lysine (g/100g DM)	10.3	10.1	10.1	10.5	11.0	10.7
Threonine (g/100g DM)	7.2	7.2	7.5	8.0	7.5	8.0
Methionine (g/100g DM)	5.0	4.3	4.5	4.3	4.8	5.0
Proanthocyanidins content (mg/kg DM)	ND	2.3	3.1	5.1	6.6	7.9
Bulk density (ml/g DM)	1.59	1.47	1.51	1.53	1.47	1.47
Water holding capacity (g_{water}/g_{feed} DM)	1.36	1.34	1.62	1.59	1.52	1.63
Calcium (g/kg DM)	10.1	15.4	16.8	17.3	17.2	19.8
Phosphorus (g/kg DM)	7.3	9.7	10.1	10.7	10.3	11.9
Magnesium (g/kg)	9.8	8.9	10.1	10.1	9.3	10.1
Potassium (g/kg)	8.8	9.0	9.3	10.2	10.4	11.1
Sodium (g/kg)	2.3	5.3	10.2	10.1	9.8	12.3
Zinc (mg/kg)	90.8	91.1	88.3	81.2	82.3	78.2
Copper (mg/kg)	8.9	7.8	9.14	8.15	7.23	7.31
Manganese (mg/kg)	124	111	109	124	123	128
Iron (mg/kg)	146	201	304	338	344	359

ND = not detected

3.2.7 Measurement of adaptation period of pigs

Data for adaptation of each pig were generated in Excel by plotting a graph of daily feed intake for each pig, the feed intake pattern of each pig was observed over time. A pig was considered to have adapted to a diet when its intake was starting to increase, recovering from a reduction until equilibrium feed intake was reached (Whittemore *et al*., 2003). The number of days when feed intake started to stabilize were then measured as the number of days at which pigs were adapted to their diets. As an illustration, Figure 3.1 shows the adaptation period of Pig Number 35 fed on a diet containing 30 g/kg DM of *Vachellia tortilis* leaf meal. It took four days for the pig to adapt to the diet. After four days, feed intake remained more or less the same throughout the observation period.

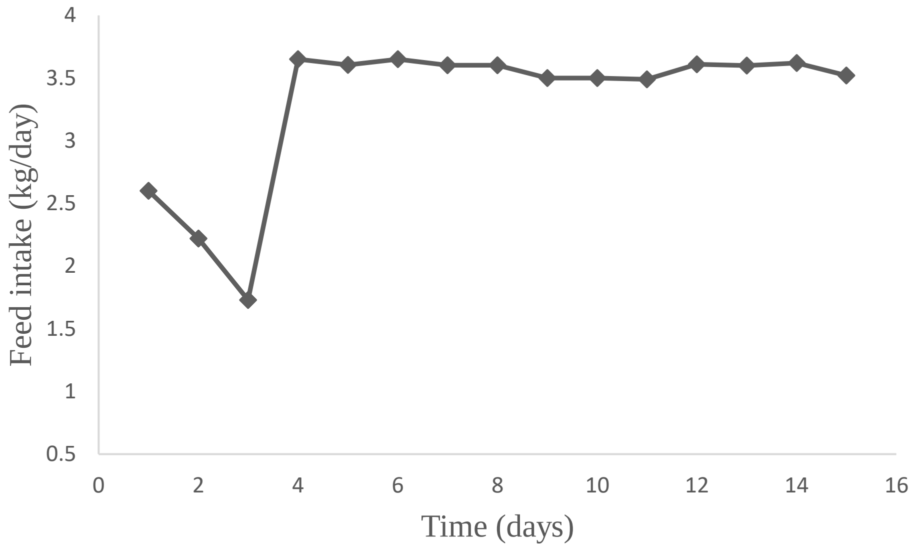

Figure 3.1: Adaptation period of Pig Number 35 consuming a diet that was containing 30 g/kg DM *Vachellia tortilis* leaf meal

3.2.8 Statistical analyses

Relationships between *Vachellia tortilis* leaf meal inclusion level and average daily feed intake, average daily gain, gain: feed ratio, adaptation period and coefficient of variation of feed intake were determined using regression analyses (SAS, 2009). The PROC RSREG procedure of SAS (2009) was used to determine the types of relationships (linear or quadratic) between *V. tortilis* leaf meal inclusion level with the adaptation period, ADFI, ADG, G: F ratio and coefficient of variation (CV) of feed intake. Variation of feed intake was estimated as the CV for each inclusion level of *V. tortilis* leaf meal.

The model used was: $Y = \beta_0 + \beta_1 V + \beta_2 V^2 + E$

Where: Y is the response variables (ADFI, ADG, G: F ratio, adaptation period and CV)

β_0, β_1, β_2 regression coefficients

V is the inclusion level of *Vachellia tortilis* leaf meal

E is the residual error

3.3 Results

3.3.1 Influence of *Vachellia tortilis* leaf meal inclusion level on pig performance

The performance of pigs fed on increasing levels of *Vachellia tortilis* leaf meal is shown in Table 3.3. The relationship between increasing levels of *V. tortilis* leaf meal with average daily feed intake (ADFI), average daily gain (ADG) and gain: feed ratio (G: F) is presented in Table 3.3. Average daily feed intake decrease quadratically ($P < 0.001$) with increasing inclusion of *V. tortilis* leaf meal. There was a positive linear relationship in ADG with increasing inclusion level of *V. tortilis* leaf meal ($P < 0.001$). Increasing inclusion levels of *V. tortilis* leaf meal displayed a quadratic increase ($P < 0.001$) in in G: F ratio of pigs.

Table 3.3: Relationship between *Vachellia tortilis* leaf meal inclusion level and pig performance

	Inclusion level of *Vachellia tortilis* leaf meal (g/kg DM)							Regression coefficient		
Parameter	0	30	60	90	120	150	SEM	Linear	Quadratic	Significance
ADFI	2.970	2.960	3.080	2.820	2.730	2.760	0.290	0.031 ± 0.330	-0.002 ± 0.022	***
ADG	1.012	1.005	1.026	0.961	0.908	1.024	0.289	-0.012 ± 0.030	0.063 ± 0.018	***
G: F	0.359	0.349	0.344	0.332	0.341	0.355	0.327	-0.056 ± 0.010	0.034 ± 0.067	***

SEM= standard error mean; *** $P < 0.001$; ADFI = daily feed intake (kg DM/ d); ADG = average daily gain (kg BW/d); G: F ratio = feed to gain ratio (kg/kg); n= 8

3.3.2 Influence of *Vachellia tortilis* leaf meal inclusion levels on adaptation period of pigs

The effect of increasing inclusion levels of *Vachellia tortilis* leaf meal diets on adaptation period is shown in Figure 3.2. Inclusion levels of *V. tortilis* leaf meal had a linear ($P < 0.05$) increase on the adaptation period of pigs. Inclusion level of *V. tortilis* leaf meal diets increases the adaptation period of pigs. The coefficient of determination (R^2) value was 0.39.

Figure 3.3 depicts feed intake of pigs fed on increasing levels of *Vachellia tortilis* leaf meal diets during the adaptation period. There was a quadratic ($P < 0.05$) decrease in the observed daily feed intake of pigs as the inclusion levels of *V. tortilis* leaf meal increased.

3.3.3 Influence of *Vachellia tortilis* leaf meal inclusion level on variation of feed intake

Figure 3.4 depicts the coefficients of variation of feed intake of pigs across *Vachellia tortilis* leaf meal diets. The variation of feed intake increased linearly ($P < 0.05$) with inclusion level of *V. tortilis* leaf meal across the different diets during the adaptation period. As *V. tortilis* leaf meal increased, the variation of feed intake became larger.

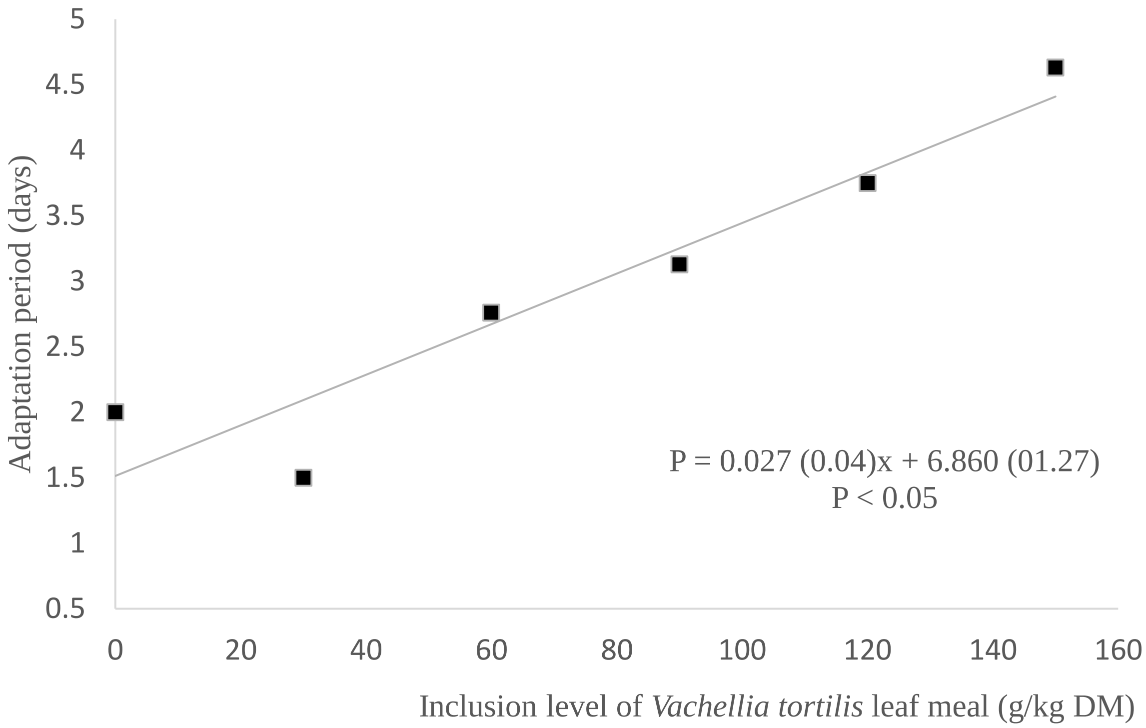

Figure 3.2: Adaptation period of finishing pigs fed on incremental levels of *Vachellia tortilis* leaf meal. Values in parentheses are standard errors of the estimates.

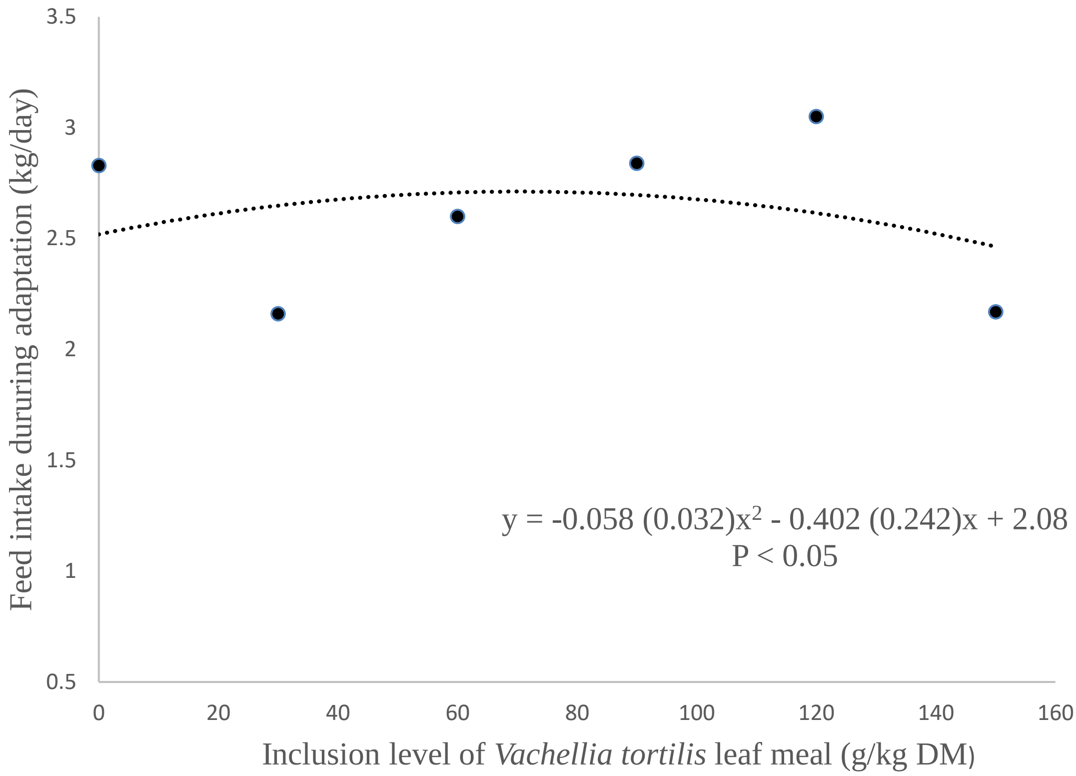

Figure 3.3: Observed feed intake of pigs during the adaptation period to *Vachellia tortilis* leaf meal inclusion. Values in parentheses are standard errors of the estimates.

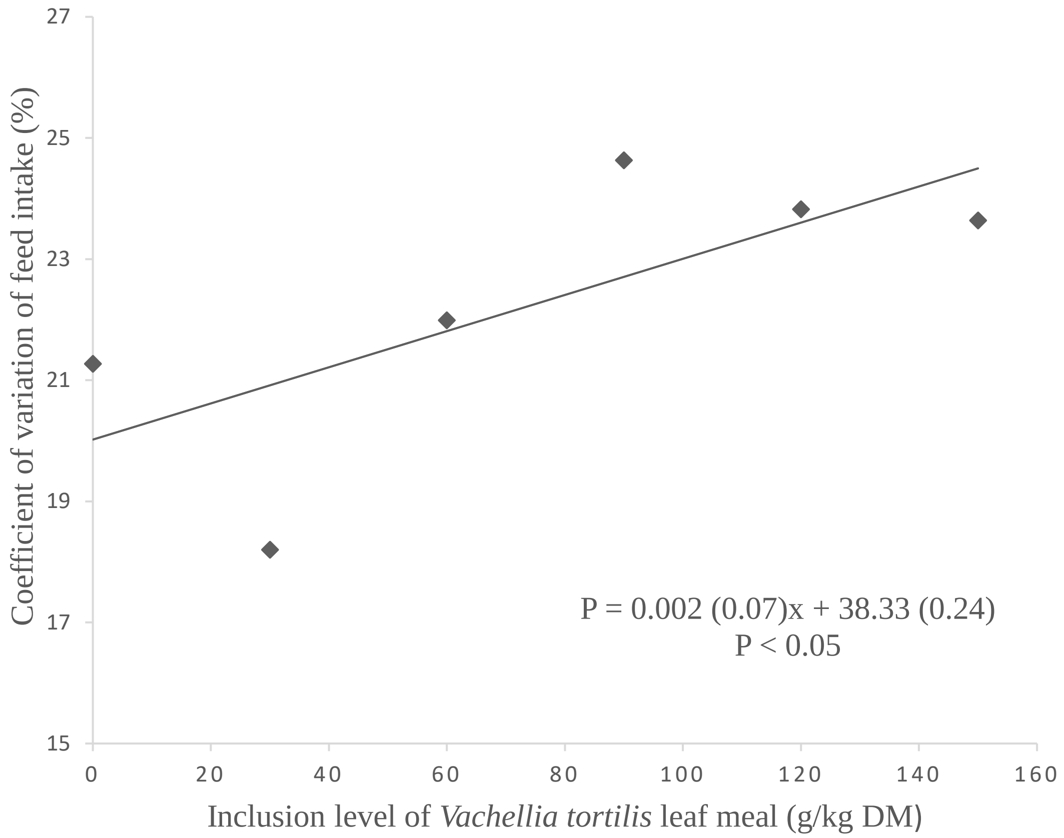

Figure 3.4: Coefficient of variation of feed intake across *Vachellia tortilis* leaf meal inclusion. Values in parentheses are standard errors of the estimates.

3.5 Discussion

The use of leguminous leaf meals in livestock feeding is valued for its nutritional, natural antioxidant and phenolic properties (Foidl *et al.,* 2001). They contain high amount of crude protein, Fatty acids, minerals and vitamins. The high phenolic content and influential antioxidant properties have been reported to affect oxidative properties and meat quality positively (Verma *et al.,* 2009). High inclusion levels of *Vachellia tortilis* leaf meal can have detrimental effects on the quality of pork by altering fatty acid profile (Miller *et al*., 1990). Phenolic contents bind with nutrients thereby reducing their availability and absorption (Ritcher *et al*., 2003; Olugbemi *et al*., 2010). Adaptation to new diets can have systematic effects on pigs. If pigs are not adapted to a particular feed or diet, the gastrointestinal tract of the pig will have difficulties in digesting the feed therefore, affecting digestibility of nutrients. Poor nutrient digestibility or absorption is reported to influence the quality of pork (Pettigrew & Esnaola, 2001; Mukumbo *et al*., 2014). Regardless of the presence of polyphonic compounds in leaf meals, pigs have to adapt to the diets. It is important to precisely estimate the adaptation period of pigs fed on increasing levels of *V. tortilis* leaf meal diets.

The initial increase in average daily feed intake before it started to decrease can be attributed to the high fibre and complexing effect of proanthocyanidins thereby causing pigs to eat more feed to compensate nutrient for deficiency. The results of the current study are in agreement with Halimani *et al*. (2007) and Khanyile *et al*. (2014) who reported an increase in average daily feed intake of pigs at low levels of leaf meal inclusion. Increasing inclusion levels of *Vachellia tortilis* leaf meals in the diet also increases the concentration of polyphenolic compounds and fibre content. Anguita *et al*. (2006) reported that dietary fibre lowers the energy content of the diet, thus pigs have to consume more of the diet to encounter the energy and amino acid required for growth and maintenance. These polyphenolic compounds and fibre reduce nutrient availability in the diet. Nutrient unavailability in the diet might be the reason for the increasing adaptation period with increasing inclusion levels of *V. tortilis* leaf meal.

A quadratic decrease in average daily gain with increasing inclusion levels of *Vachellia tortilis* leaf meal was expected in current study. A linear increase in ADG of pigs fed on increasing levels of *V. tortilis* leaf meals was observed. The results contradict Khanyile *et al*. (2014) who reported a reduction in ADG of pigs fed on incremental levels of *V. tortilis* leaf meal diets. Other studies have also reported a general decline in growth rate of pigs fed low to high levels of leaf meals (Halimani *et al*., 2005). A linear increase in ADG might be attributed to increasing weight of pigs since pigs used in the current study were still growing. The duration at witch pigs were exposed to *V. tortilis* leaf meal diets can also have effects on ADG and the utilization of *V. tortilis* leaf meal diets by pigs improves with time. Leaf meals can be incorporated in pig diets up to 100 g/kg without decreasing average daily gain pigs (Halimani *et al*., 2007).

The quadratic increase in gain: feed ratio shows that pigs are able to tolerate the deleterious effects of *Vachellia tortilis* leaf meal at low inclusion level. Pigs are more efficient at low inclusion levels of *V. tortilis* leaf meal. The findings attained in the current study are in line with those of (Khanyile *et al*. 2014; Mukumbo *et al*., 2014) who reported that higher inclusion levels of leguminous leaf meals diets reduce G: F ratio of pigs implying that pigs are less efficient at converting nutrients in to body mass gain.

The linear increase in the adaptation period with increasing levels of *Vachellia tortilis* leaf meal could be attributed to the presence of fibre and polyphenolic compounds, such as proanthocyanidins. These compounds are associated with a negative impact in pig production, such as retarding pig growth and reducing productivity, which can increase the finishing period of pigs, making it impossible for pigs to reach slaughter weight early and also making it difficult for farmers to purchase new stock which can result in negative returns. These compounds also hamper the utilization of nitrogen, carbohydrates and minerals (D'Mello, 1995). Provenza and Ropp (2001) reported that polyphenolic compounds cause an astringent impact in the mouth of the host animal which consequently reduce feed intake. This can be supported by the short extent of the adaptation period at low inclusion level of *V. tortilis* leaf meal and the longer adaptation period observed at high inclusion level of *V. tortilis* leaf meal. High levels of proanthocyanidin intake can produce toxicity (Garg *et al*., 1992). The longer adaptation period at higher inclusion levels of *V. tortilis* leaf meal can also be explained by the assumption, that proanthocyanins, which are resistant to hydrolysis and are not detoxify completely by the liver enzymes (uridine diphosphate glucoronyl transferase and glutathione s-transferase).

Fourteen days is sufficient for the gastrointestinal tract of a pig to adapt to a fibrous rich diet (Owen and Ridgman, 1967). The length of the adaptation period in the current study increased with the level of *Vachellia tortilis* leaf meals in the diets. At low levels it became lower but at higher levels it was higher, suggesting that pigs require more time to adapt to higher levels of *Vachellia* leaf meal diets. Increasing inclusion level of *V. tortilis* leaves increases the neutral detergent fibre content in the diet. Increasing the neutral detergent fibre, reduces the nutrient density of the feed which then lengthens the adaptation period. Mapiye *et al*. (2009) reported a longer adaptation period of 21 to 35 days in Nguni steers fed on *Vachellia karroo* leaf meal. It is possible that the presence of antinutritional factors of *Vachellia* species may be the reason why the adaptation period was prolonged at higher inclusion level, though the different species of animals adapt in different ways to the diets that are rich in polyphenolic compounds. The effect of polyphenolic compounds in both ruminants and non-ruminants may be similar but their adaptation periods with regards to *Vachellia* diets differs.

The linear increase in the adaptation period could be also attributed to the weight or the size of the pigs (Whittemore *et al.*, 2003). As the weight of the pig increases, the size of the large intestines also increases (Whittemore *et al*., 2003) and daily nutrient requirements increase thus pigs eat more feed to meet nutrient requirements for growth potential. Fully grown pigs utilize leaf meal diets more efficiently than young pigs. Partly because they have a complete developed gastrointestinal tract that can easily ferment fibres compered to growers (Kyriazakis and Emmans, 1995). As pigs consume *Vachellia tortilis* diets, the large bowel particularly the large intestines expand to accommodate the feed in the gut (Khanyile *et al*., 2017). Whittemore *et al*. (2003) also reported a prolonged adaptation of pigs to fibrous diets and ascribed that to increase in the size of the gut. Brunsgaard *et al*. (1995) showed that body weight of rats on different diets containing different indigestible polysaccharides increased progressively over eight weeks, rats on cellulose diets had lower weights compared to rats on fibre free diets and maize starch diets.

A quadratic decrease in feed intake was observed with increasing levels of *Vachellia tortilis* leaf meal during the adaptation period. The decrease indicates a stage where the minimum and maximum feed intake has been reached. Feed intake is the main factor influencing absorption of nutrients (Nyachoti *et al*., 2004). The findings that the feed intake increased at low inclusion levels of *V. tortilis* leaf meal during the adaptation period can also be ascribed to the increasing neutral detergent fibre and proanthocyanidins binding with amino acids (Halimani *et al.*, 2007). Requiring pigs to consume more of the diet to meet the unavailable nutrients (Anguita *et al*., 2006). Nutrients unavailability is reported to increase feed intake. Palatability of *Vachellia* leaf meal species is relatively low because of its detrimental effects (Kaitho *et al.*, 1997). It could vary with the inclusion level of the leaf meal hence affecting feed intake. If nutrient absorption is depressed because less feed is consumed by pigs, the adaptation period is more likely to increase. Polyphenolic compounds have several negative post -ingestive effects in animals (Lee *et al*., 2010). This might be another factor which might have reduce feed intake therefore influencing the adaptation period of pigs. Increase in concentration of polyphenolic compounds in diets decreases digestibility, nutrient assimilation and palatability by causing a bitter taste to pigs (Martens *et al.*, 2013).

The length of the adaptation period varies among individual pigs. Some pigs may have longer adaptation which may last for three weeks, some may have shorter adaptations lasting for two weeks (Owen and Ridgman, 1967). Feed intake varies between groups of pigs and it is affected by the composition of the diet (Nyachoti *et al.*, 2004). In the current study, feed intake was observed to increase with the inclusion level of *Vachellia tortilis* leaf meal. The variation of feed intake becomes larger as the level of the leaf meal increase across the diets. Whittemore *et al*. (2003) also reported a large variation in feed intake of pigs when changed from basal diet to a diet containing 97 % wheat bran. Increasing levels of *V. tortilis* leaf meal increased the coefficients of variation of feed intake. The linear increase in coefficients of variation of feed intake in the current study could also be attributed to proanthocyanidins and high fibre levels present in *V. tortilis* leaf meal. The high variation of feed intake across *V. tortilis* leaf meal diet indicate that pigs can tolerate low levels of the leaf meal easily compared to higher levels of *V. tortilis* leaf meal, this can also be supported by the positive relationship between *V. tortilis* leaf meal inclusion and the adaptation period. Thus, pigs respond to detrimental factors of *V. tortilis* leaf meal diets by either increasing or decreasing feed intake, this may be due to the toxic nature of polyphenolic compounds in the gastrointestinal tract of pigs.

3.6 Conclusions

Performance characteristics of pigs were influenced by increasing inclusions of *Vachellia tortilis* leaf meal diets. *Vachellia tortilis* inclusion resulted in a negative quadratic relationship in average daily feed intake, a positive linear relationship in average daily gain and a positive quadratic relationship in gain: feed ratio of pigs. Increasing inclusion level of *V. tortilis* leaf meal diets caused a positive linear relationship in the adaptation period of pigs. Pigs fed on *Vachellia* leaf meal diets require a long time to adapt. In addition, *V. tortilis* leaf meal inclusion also increased the variation of feed intake across the diets. It is, however, necessary to investigate the behaviour of pigs fed on *V. tortilis* leaf meal inclusion and the extent of the adaptation period even in sows so that *V. tortilis* leaf meals can also be used in both sows and boar diets.

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CHAPTER 4: Behaviour of finishing pigs to *Vachellia tortilis* leaf meal inclusion

(Submitted to Applied Behaviour Science)

Abstract

The objective of the current study was to establish the relationship between *Vachellia tortilis* leaf meal inclusion and time spent on different behavioural activities displayed by finishing pigs. A total of forty-eight male Large White × Landrace finishing pigs with a mean (± SD) body weight of 63.8 ± 3.28 kg aged 14 weeks were assigned to individual pens in a completely randomized design. Pigs were fed on diets that contained 0, 30, 60, 90, 120 and 150 g/kg DM of *V. tortilis* leaf meal *ad libitum* with fresh water provided throughout the trial. There were eight pigs in each dietary treatment. The behaviour of pigs was observed for three weeks once a day using six closed circuit television cameras. Regression analyses was used to analyse the data. Increasing inclusion levels of *V. tortilis* leaf meal caused a linear decrease ($P < 0.05$) in time spent eating, lying down and the number of visits to the feeder. Time spent standing and biting objects increased linearly ($P < 0.05$) with increasing inclusion level of *V. tortilis* leaf meal. There was no relationship ($P > 0.05$) between *V. tortilis* leaf meal inclusion and time spent drinking, sniffing and licking of object. It can be concluded that inclusion level of *V. tortilis* leaf meal alters the behaviour of pigs by causing in a negative linear relationship in time spent eating, lying down and the number of feeder visits and a positive linear relationship in time spent standing and biting of objects.

Key words: time spent, behavioural activities, linear relationship, pronthocynadins

4.1 Introduction

Feed is a critical component in pig production and it affects pork quality. One of the reasons behind the use of *Vachellia* leaf meals in monogastric diets is the high amount of crude proteins that range from 160 to 218 g/kg DM (Khanyile *et al*., 2014). Regardless of this advantage, inclusion level of leaf meal diets can also have negative effects in pig performance and production due to the presence of secondary compounds such as polyphenolic compounds. These compounds can alter the composition of fatty acids in pork which can have detrimental effects in human health (Hugo & Roodt, 2007).

The negative effects of leaf meal caused by presence of secondary compounds and high fibre content can change the behavioural patterns of pigs. To maximize the use of *Vachellia tortilis* leaf meal, it is indispensable to prioritise the behavioural activities of pigs fed on inclusion levels of *Vachellia* leaf meal. The observation of behavioural patterns of pigs fed on varying levels of *V. tortilis* leaf meal diets aids in providing information about the internal state and welfare status of pigs to producers for better management (Mench, 1998). *Vachellia tortilis* leaves contain high levels of fibres and polyphenolic compounds (Khanyile *et al*., 2014), therefore, leaf meal produced could also contain varying levels of the secondary compounds such as proanthocyanidins. These compounds can reduce the incidence of stereotypic behaviours by reducing time spent in stereotypic patterns that impairs the welfare of grow - finishing pigs. Information on the effect of leaf meals on stereotypic behaviors in finishing pigs is still not reported. Stereotypic behaviours can be defined as behavioral patterns that are done continually in a fixed order and have no actual function (Meunier-Salaün *et al*., 2001). Stereotypic behaviours such as bar biting, bar chewing and vacuum chewing (chewing nothing) are reduced if neutral detergent fibre content ranges from 250 to 400 g/kg DM (Ramonet *et al*., 1999). This changes the time pigs spent on other behavioural activities. D'Eath *et al*. (2009) reported an increase in feeding motivation of pigs fed on diets rich in fibre due to higher satiation.

High concentrations of polyphenolic compounds are reported to cause an astringent taste in the animal's mouth (Huisman and Tolman, 1992), thereby reducing feed intake. Feeding *Vachellia tortilis* leaf meal may change the behaviour of pigs by changing the time spent feeding. Information on the relationship between inclusion level of *V. tortilis* leaf meal and time spent on behavioural activities enhances understanding of how pigs behave when fed on *V. tortilis* leaf meal diets. The relationship also improves our knowledge of the correct inclusion level of the leaf meal without compromising the behaviour and welfare of pigs. There is a paucity of information on the relationship between increasing inclusion levels of *V. tortilis* leaf meal diets with time spent on behavioural activities of pigs. In chapter 3, it was reported that *V. tortilis* leaf meal inclusion result in a linear relationship in adaptation period of pigs. This makes it necessary to determine the relationship between *V. tortilis* leaf meal inclusion and time spent on behavioural activities of pigs. The objective of the study was to assess the relationship between incremental levels of *V. tortilis* leaf meal and time spent on behavioural activities of finishing pigs. The hypothesis tested was that *V. tortilis* leaf meal inclusion has no relationship with time spent on behavioural activities of pigs.

4.2 Materials and methods

4.2.1 Study site

The study site has been described in detail in section 3.2.1

4.2.2 Collection of *Vachellia tortilis* leaves

Collection of *Vachellia tortilis* leaves has also been described in section 3.2.2

4.2.3 Pigs and housing

Details of pig management are given in section 3.2.3

4.2.4 Experimental design and pig management

Experimental design and feeding management were described in section 3.2.4

4.2.5 Measuring behavioural activities

Pigs were housed individually in separate pens and randomly assigned to experimental diets. They were adapted to the diets for one week. Behavioural activities were then observed on individual pigs for three days, from 0600 to 1800h, once a week using six motorized indoor closed circuit television (CCTV) cameras. Cameras were installed in different locations in the housing pen such that each camera appropriately captured eight pigs, rear, front views of other cameras could also be observed. All cameras were attached to a digital video recorder control system and transcend one terabyte external hard drive. The use of video cameras was adopted to avoid disturbances during behavioural data collection. Behavioural activities that were recorded were time spent eating, drinking, standing, lying down, sniffing, biting objects, licking objects and the number of visits to the feeder. The description of behavioural activities are given in Table 4.1.

Table 4.1: Ethogram of recorded behavioural activities

Behaviour	Description of behaviour
Eating	Feed consumption from the feeder / snout in contact with the feeder
Drinking	Manipulating the nipple / snout in contact with the nipple drinker
Feeder visit	Number of visits to the feeder/ When a pig goes to the feeder
Standing	Body supported by four legs without stamping
Lying down	Lying ventral or lateral with sternum in contact with the floor
Sniffing	Time spent sniffing on the floor and other objects
Biting	When a pig is biting objects (chain and sides of the pen)
Licking	When a pig is licking objects

4.2.7 Statistical analyses

The PROC UNIVARIATE procedure of SAS, (2009) was used to determine the normality of the data for time spent on each behavioral activity (eating, feeder visit, drinking, lying down, standing, sniffing, object biting and licking). Then, the data for time spent on each behavioural activity were normalized using logarithmic transformation since it was not normally distributed. Relationships between *Vachellia tortilis* leaf meal inclusion level and time spent on each behavioural activity were determined using regression analyses (SAS, 2009). The PROC RSREG procedure was used to determine relationship between inclusion level of *V. tortilis* leaf meal and time spent on each behavioral activity and also the relationship between behavioural activities and chemical components of *V. tortilis* leaf meal. The PROC CORR procedure of (SAS, 2009) was used to determine the correlation between each behavioral activity and the chemical components of *V. tortilis* leaf meal inclusion.

The regression used model used was: $Y = \beta_0 + \beta_1 V + \beta_2 V^2 + E$

Where: Y is the response variables (time spent eating, drinking, lying down, standing, sniffing, licking objects, biting objects and the number of feeder visits)

β_0, β_1, β_2 regression coefficients

V is the inclusion level of *Vachellia tortilis* leaf meal

E is the residual error

4.3 Results

4.3.1 Influence of *Vachellia tortilis* leaf meal inclusion level on time spent on behavioural activities of pigs

Proportion of time spent by pigs fed on increasing inclusion level of *Vachellia tortilis* leaf meal is shown in Figure 4.1. Pigs spent most of their time lying down, eating, drinking, sniffing, standing, visiting the feeders, biting and licking of objects inside their pens, in that order. However, time spent drinking, sniffing the walls and licking objects was not affected ($P > 0.05$) by *V. tortilis* leaf meal inclusion level.

4.3.2 Correlation coefficients between behavioural activities of pigs and chemical components of the feeds

The correlation coefficients among each behavioural activity are shown in Table 4.2. Time spent eating was positively correlated ($P<0.05$) with the number of visits to the feeders. Other activities were not correlated ($P > 0.05$) to time spent eating. A negative correlation ($P<0.05$) was observed between the number of feeder visits and time spent standing and sniffing walls in the pens. Time spent standing was positively correlated ($P<0.05$) to time spent sniffing the walls, biting and licking of objects. Time spent lying down was not correlated ($P>0.05$) to any behavioural pattern. Time spent sniffing the walls was positively correlated ($P<0.05$) to time spent biting objects. A positive correlation ($P<0.05$) was also observed in time spent biting and licking objects. Table 4.3 shows the correlation coefficients of chemical components of *Vachellia tortilis* leaf meal and behavioural activities of pigs. Acid detergent fibre content was negatively correlated ($P < 0.05$) with time spent standing, sniffing the walls and biting objects. Neutral detergent fibre content was negatively correlated ($P < 0.05$) with time spent eating and the number of feeder visits and positively correlated ($P < 0.05$) with time spent standing, sniffing the walls and biting objects. Proanthocyanidins content was positively correlated ($P < 0.001$) with time spent sniffing walls, biting and licking objects and negatively correlated with time spent eating, drinking, number of feeder visits ($P < 0.001$), standing and lying down ($P < 0.05$).

4.3.3 Relationship between increasing level of *Vachellia tortilis* leaf meal and time spent on behavioural activities of pigs

Table 4.4 shows the relationship on time spent on behavioural activities with increasing levels of *Vachellia tortilis* leaf meal. A linear decrease ($P < 0.05$) in time spent time eating, lying down and the number of visits to the feeder was observed with increasing level of *V. tortilis* leaf meal ($P < 0.05$). As *V. tortilis* leaf meal inclusion increased, time spent on eating, lying down and the number of feeder visits decreased linearly ($P<0.05$). The time spent standing and biting objects increased linearly with increasing level of *V. tortilis* leaf meal ($P < 0.05$). Increasing levels of *V. tortilis* produced a positive relationship with time spent standing and biting objects. There was no relationship ($P > 0.05$) in time spent on drinking, sniffing walls and licking of objects with increasing level of *V. tortilis* leaf meal ($P > 0.05$). The regression equations of behavioural activities are shown in Table 4.5. The relationship between chemical components of *V. tortilis* leaf meal are shown in Table 4.6. Condensed tannins and acid detergent fibre content produced a linear relationship in time spent eating, number of feeder visit, standing, sniffing walls and biting objects. The neutral detergent fibre content also produced a linear relationship in time spent eating, number of feeder visit, standing and biting objects but produced a quadratic relationship on time spent sniffing objects inside the pen.

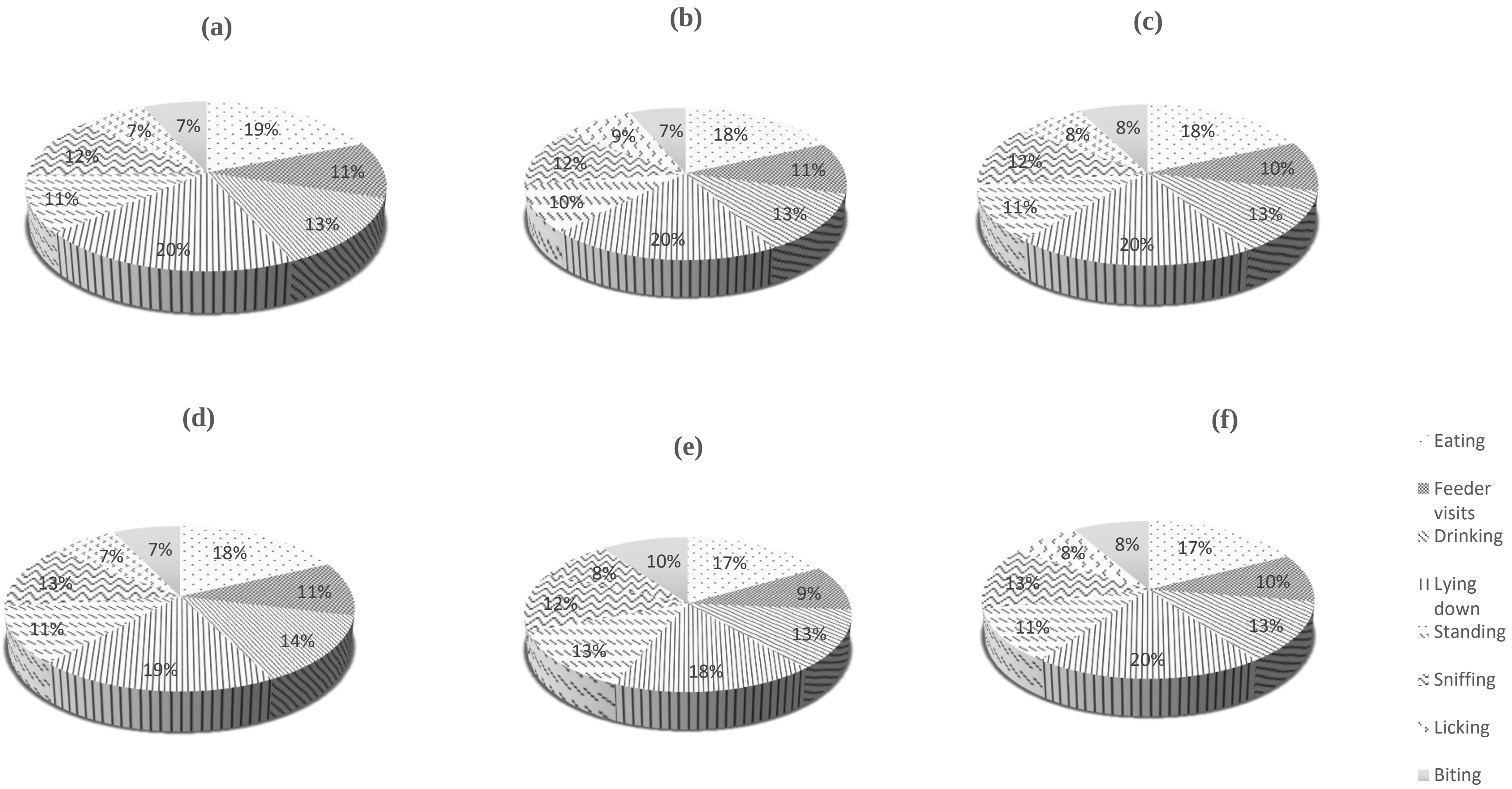

Figure 4.1: Proportion of time spent on behavioural activities of pigs fed on 0 (a), 30 (b), 60 (c), 90 (d), 120 (e) and 150 (f) g/kg DM *Vachellia tortilis* leaf meal

Table 4.2: Pearson's correlation coefficients among behavioural activities of pigs

Parameter	Eating	Drinking	Feeder visit	Standing	Lying down	Sniffing walls	Object biting	Object licking
Eating		1.18NS	0.24*	0.08NS	-0.02NS	0.09NS	-0.04NS	-0.00NS
Drinking			0.37**	0.03NS	0.84NS	0.32NS	0.27NS	0.1NS
Feeder visit				-0.21*	0.09NS	-0.23*	0.11NS	-0.01NS
Standing					-0.07NS	0.47**	0.62**	0.43**
Lying down						0.02NS	0.01NS	-0.12NS
Sniffing walls							0.29*	0.01NS
Object biting								0.36*
Objecting licking								

**$P < 0.001$; *$P < 0.05$; NS - not significant ($P > 0.05$)

Table 4.3: Pearson's correlation coefficients among chemical attributes of *Vachellia tortilis* leaf meal with behavioural activities of finishing pigs

Components	Eating	Drinking	Feeder visit	Standing	Lying down	Sniffing walls	Object biting	Object licking
Acid detergent fibre (g/kg DM)	0.18NS	-0.16NS	0.09NS	-0.27*	0.16NS	-0.28*	-0.30*	0.33NS
Neutral detergent fibre (g/kg DM)	-0.29*	0.19NS	-0.21*	0.26*	-0.13NS	0.28*	0.35*	0.35NS
Condensed tannins (mg/kg DM)	-0.50**	-0.44	-0.47**	0.37*	-0.45NS	0.49**	0.38*	0.42*

**P < 0.001; *P < 0.05; NS: not significant (P > 0.05)

Table 4.4: Relationship between time spent on behavioral activities with increasing level of *Vachellia tortilis* leaf meal

Activity (min)	Inclusion level (g/kg DM)							Regression	
	0	30	60	90	120	150	SEM	Linear	Quadratic
ET	2.31	2.22	2.21	2.21	2.21	2.17	0.01	*	NS
DK	1.64	1.58	1.53	1.67	1.66	1.58	0.02	NS	NS
FV	1.30	1.32	1.22	1.29	1.28	1.22	0.01	*	NS
ST	1.31	1.25	1.37	1.38	1.71	1.39	0.01	*	NS
LY	2.47	2.47	2.41	2.37	2.34	2.44	0.03	*	NS
SN	1.42	1.52	1.48	1.52	1.62	1.57	0.06	NS	NS
OB	0.08	0.74	0.92	0.87	1.31	1.04	0.12	*	NS
OL	0.97	0.75	0.92	0.87	1.31	1.04	0.11	NS	NS

ET: Eating; FV: Feeder visit; DK: Drinking; LY: Lying down; ST: Standing; SN: Sniffing; OB: Object biting; OL: Object licking; SEM – Standard error of means; NS – Not significant ($P > 0.05$); *$P < 0.05$.

Table 4.5: Regression equations of the relationship of behavioural activities of pigs

Activity	R^2	Type of relationship	Equation	P value
Time spent eating	0.16	Linear	Y = - 0.051(0.032)x - 2.349(0.048)	< 0.001
Number of feeder visits	0.05	Linear	Y = - 0.013(0.038)x +1.32(0.059)	< 0.05
Time spent standing	0.07	Linear	Y = 0.107(0.103)x + 1.152 (0.156)	< 0.05
Time spent lying down	0.05	Linear	Y = - 0.101(0.058)x +2.58(0.089)	< 0.05
Time spent biting objects	0.13	Linear	Y = 0.0822(0.113)x + 0.67(0.172)	< 0.05

Values in parentheses are standard errors of the estimates

Table 4.6: Relationship between chemical components of *Vachellia tortilis* and behavioural activities

Activity	Component	Regression	P value
Eating	Proanthocyanidins	Linear	0.0015
Feeder visit	Proanthocyanidins	Linear	0.0414
Standing	Proanthocyanidins	Linear	0.0101
Sniffing	Proanthocyanidins	Linear	0.0193
Object biting	Proanthocyanidins	Linear	0.0114
Eating	Neutral detergent fibre	Linear	0.0022
Feeder visit	Neutral detergent fibre	Linear	0.0402
Standing	Neutral detergent fibre	Linear	0.0056
Biting object in side pens	Neutral detergent fibre	Linear	0.0091
Eating	Acid detergent fibre	Linear	0.0506
Standing	Acid detergent fibre	Linear	0.0092
Sniffing	Acid detergent fibre	Linear	0.0135
Biting objects in pens	Acid detergent fibre	Linear	0.0401

4.4 Discussion

Vachellia tortilis leaf meals are a rich source of proanthocyanidins, fibres and protein content (Khanyile *et al*., 2014). Their use in pig production, depending on the inclusion level in a diet could improve the welfare of pigs by decreasing stereotypic behaviours (sham chewing, bar biting, chain pulling and biting objects), and improve the health by acting as anthelmintics in gastrointestinal tract of pigs (Day *et al*., 1996). Low levels of proanthocyanidins intake enhance digestibility by acting as antioxidants (Makkar *et al*., 2007). Due to its good nutrient content, it is more likely that growth performance of pigs fed on low levels of *V. tortilis* leaf meal diets be improved. Measuring time spent on behavioural activities of pigs fed on varying levels of *V. tortilis* leaf meal diets (feeding, drinking, standing, and lying down) enhances our understanding about the combined effect of proanthocyanidins and fibre on the welfare of pigs.

A quadratic increase in time spent eating was expected because increasing levels of *Vachellia tortilis* leaf meal also increases the availability of secondary compounds that restrict nutrient availability and its digestion. Previous report by Khanyile *et al*. 2014 showed that feeding activity of pigs particularly feed intake decrease quadratically due to incremental levels of *V. tortilis* leaf meals, therefore it could affect time spent eating. In addition, the NDF content also increases with the inclusion level of *V. tortilis* leaf meal, therefore reducing the energy density of the feed. Pigs, therefore, spent more time consuming the diets at lower levels to balance the nutrients that are inhibited by the polyphonic compounds and NDF contents. McGlone and Fullwood, (2001) reported an increase in time spent eating of sows fed on diets that contain increasing fibre levels. The discrepancies between the results could be a result of age. Sows are fed on fibrous rations for maintenance purpose during farrowing while boars are fed on high energy rations for fattening purpose.

The reduction in time spent feeding could be attributed to fibre and proanthocynadins levels. High fibre levels cause early satiety while pronthocynadins are known to reduce palatability due to astringent taste which consequently reduce feed intake or time spent feeding (Whittemore *et al*., 2003; Rubanza *et al*., 2005).

Another factor that could have resulted in a decrease in time spent eating might be the palatability of *Vachellia tortilis* leaf meal. The palatability of *Vachellia* of species is relatively low (Kaitho *et al*., 1997). Therefore, it can be assumed that the palatability of *V. tortilis* leaf meal which is altered by proanthocyanidins (Ngwa *et al*., 2003) decreased with increasing levels of *V. tortilis* leaf meal resulting in pigs to reduce time spent eating. If pigs are irritated while eating due to the bitter taste of the leaf meal, they become less motivated to eat, thus spending less time eating. Palatability of the diets in the current study was, however, not measured and it warrants further examination.

The decrease in number of visits to the feeders with increasing inclusion level of *Vachellia tortilis* leaf meal could be attributed to the type of the diet. Diet type or composition alter the behaviour of pigs. Pigs consuming coarse diets spend more time eating or chewing leading to an increased mastication time (Wenk, 2001) while those consuming finer nutrient rich diets spend less time. *Vachellia tortilis* leaf meal diet is a finer diet, therefore, pigs spent time chewing while eating resulting in a reduction in number of feeder visits. The duration of eating has an effect of on the number of feeder visits. Pigs spend less time in feeders when their satiety levels or gut fill has been met, if not, the number of feeder visits will increase because pigs will be constantly be seen in feeders.

Inclusion level of *Vachellia tortilis* leaf meal diets produced a linear decrease in time spent lying down. These findings are in line with those of (Bakare *et al*. 2014) who also reported a decline in time spent lying down of growing pigs fed on increasing levels fibrous diets. The decrease in time spent lying down resulted from the decrease in time spent eating which also increases the occurrence of other behaviours. If pigs spend more time eating and lying down, it prevents them from performing other behavioural activities which may be harmful.

Time spent eating was positively correlated with the number of visits to the feeder, suggesting that as pigs eat, the number of feeder visits increase. The number of feeder visits was positively correlated to time spent drinking. This suggests that inclusion level of *Vachellia tortilis* leaf meal increases the number of visits to the feeders. An increase in time spent drinking with the number of feeder visit might be a result of the positive correlation in eating time which makes pigs to drink water after eating. The number of feeder visits, time spent standing and sniffing walls were negatively correlated to each other. This suggest that pigs reduce time spent standing and sniffing walls while eating. The positive correlation in time spent standing and sniffing of wall material, biting and licking of objects suggest that pigs increases the chance of sniffing, biting and licking of walls when in standing position.

Acid detergent fibre was negatively correlated to time spent standing, sniffing walls and biting objects. This suggest that increasing inclusion levels of *Vachellia tortilis* leaf meal also decrease the occurrence of such behaviours. Neutral detergent fibre was negatively correlated with time spent eating and the number of visits to the feeder. This suggest that increasing inclusion levels of *V. tortilis* leaf meal decreases the duration of time spent eating and the number of feeder visits. Increasing content of fibrous material in the diet decreases nutrient density (Ndou *et al*., 2013) and energy, requiring pigs to eat more, but that was not the case in the current study because a reduction in time spent eating was observe. Finishing pigs have a complete developed gut system that permit fibre fermentation, therefore, it can be assumed that the decrease in time spent eating and number of feeder visits could be attributed to retention time in the gut. The positive correlation between time spent standing, sniffing walls and biting objects suggest that increasing *V. tortilis* leaf meal could increase the occurrence of stereotypic behaviours which may be harmful to pigs (Ramonet *et al*., 1999). Proanthocyanidins showed a negative correlation with time spent eating and feeder visits and a positive correlation between time spent standing, sniffing walls, biting and licking objects. This suggest that proanthocyanidins decrease time spent eating and number of feeder visits while resulting in an increase in time spent standing, sniffing wall, biting and licking of objects. Consumption of proanthocyanidins that are above 50 g/kg DM reduces feed intake, therefore time spent eating (Frutos *et al*., 2004).

A linear increase in time spent standing and biting objects was observed with increasing inclusion of *V. tortilis* leaf meal. Time spent standing and biting objects may be a sign of stress or discomfort in pens since pens were not designed to allow pigs to walk enough. Pigs also stand to find comfortable positions of resting (Pearce and Paterson, 1993). Ramonet *et al*. (1999) observed a reduction of stereotyped behaviours in sows fed on fibrous diets containing NDF component that ranged from 250 to 400 g/kg DM. The fibrous components were higher than 300 g/kg DM particularly for the NDF component. The higher level of NDF content in the leaf meal may cause discomfort in pigs resulting in stereotypic behaviours.

4.5 Conclusions

Increasing inclusion level of *Vachellia tortilis* leaf meal diets alters the behaviour of finishing pigs. *Vachellia* diets provides linear relationships in behaviour of pigs by reducing time spent eating, lying down, number of feeder visits and increasing time spent standing and biting objects.

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Chapter 5: General discussion, Conclusions and Recommendations

5.1 General discussion

Prolonged dietary adaptation periods may have significant effects in pigs by increasing the finishing period and causing pigs not to reach slaughter weight early. The main hypothesis tested in the study was that increasing inclusion of *Vachellia tortilis* leaf meal diets will produce a linear relationship in the adaptation period and no relationship on time spent on behavioral activities of pigs. The use of different inclusion levels of *V. tortilis* leaf meal diets was expected to provide a positive relationship in adaptation of pigs. In relation to the adaptation period of pigs to *V. tortilis* leaf meal inclusion, time spent on behavioural activities of pigs fed on *V. tortilis* diets was expected to differ with the inclusion level of the leaf meal. The relationships between inclusion level of *V. tortilis* leaf meal, adaptation period and time spent on behavioural activities of pigs improves our knowledge on the utilization of *Vachellia* diets by pigs.

In Chapter 3, the hypothesis tested was that incremental levels of *Vachellia tortilis* leaf meal inclusion linearly increase the adaptation period of pigs. In every phase of production, pig producers change feeds according to the nutrient requirement of pigs. Diets are changed from weaners to growers and from growers to finishers. The adaptation of pigs to new diets is very importance because it can have detrimental effects on the performance and productivity of pigs. When *Vachellia tortilis* diets are used in pig production, pigs have to adapt to such diets. The utilization of *V. tortilis* leaves by pigs is constrained by the presence of secondary compounds that results in low performance of pigs at higher levels. *Vachellia tortilis* leaf meal inclusion level was expected to produce a positive relationship with the adaptation period of pigs because increasing inclusion of the leaf meal also increases the level of secondary compounds (polyphenolic compounds). Information about the relationship improves our knowledge on the appropriate inclusion level of the leaf meal based on the length of the adaptation period of pigs.

Increasing levels of *Vachellia tortilis* leaf meal produced a positive linear relationship in the adaptation period of pigs. The observed relationship between *V. tortilis* leaf meal inclusion and adaptation period, suggest that increasing levels of *Vachellia* leaves in pig diets increase the adaptation period of pigs. The linear increase may be attributed to the fact that increasing inclusion levels of *V. tortilis* leaf meal induces the effect of antinutritional factors such as polyphenolic compounds which also increase with the inclusion level of the leaf meal. Incremental levels of *V. tortilis* leaf meal also produced a positive relationship in the coefficient of variation of feed intake. This suggest that *V. tortilis* leaf meal inclusion result in a large variation of feed intake across the diets. Since increasing inclusion levels of *V. tortilis* leaf meal diets influenced the adaptation period of pigs as ascribed by the presence of antinutritional factors of *Vachellia* leaf meal, it is, however, necessary to predict the time spent on behavioural activities of pigs to understand the behaviour of pigs and also explain the results obtained in the third chapter.

The hypothesis tested in Chapter 4 was that increasing inclusion of *Vachellia tortilis* leaf meal diets has no relationship on time spent on behavioural activities of pigs. *Vachellia tortilis* inclusion altered the behavioral activities of pigs in the current study. It produced a negative linear relationship with time spent eating, lying down and the number of feeder visit. The decrease in time spent eating, lying down and the number of feeder visits may be attributed to the deleterious effects of the antinutritional factors of *Vachellia* diets which in turn reduces the palatability of the diets and increase the antinutritional effects of the leaf meal.

Increasing inclusion of *Vachellia tortilis* leaf meal produced a positive linear relationship in time spent standing and biting objects, suggesting that *V. tortilis* inclusion in pig diets increase the time spent standing and biting object. The increase in time spent standing and biting objects may be as a result of the decrease in time spent lying down. If pigs spent more time lying down due to gut fill or satiety, the occurrence of other behaviours which might be harmful to the pigs are expected to be reduced because pigs will be resting or lying down after eating. That was not the case in the current study since time spent standing and biting of objects increased. This also suggest that pigs are not comfortable with higher levels of *V. tortilis* leaf meal diets and it may be an indication of poor welfare of pigs.

5.2 Conclusions

Increasing levels of *Vachellia tortilis* leaf meal caused a positive linear relationship in the adaptation period of pigs, therefore, making pigs to require more time to adapt to *Vachellia* leaf meal diets. Increasing inclusion level of *V. tortilis* leaf meal alters the behaviour of pigs by causing linear relationships on time spent eating time, lying down, number of feeder visits, standing and biting objects.

5.3 Recommendations and further research

Low levels of *Vachellia tortilis* (0 – 60 g/kg DM) leaf meal diets should be used when feeding pigs, since the adaptation period of pigs linearly increase with increasing inclusion of the leaf meal and altering time spent in behavioural activities. The use of *Vachellia tortilis* leaf meal in pig production requires farmers to be advocated on the processing of the leaves before use.

Aspects that require further research include:

- Assessing the adaptation and behaviour of sows fed on incremental levels of *V. tortilis* leaf meal diets.
- Assessing the behaviour of group housed pigs fed incremental levels of *V. tortilis* leaf meal diets.
- Determining nutrient digestibility and gut health of pigs fed on incremental levels of *V. tortilis* leaf meals.
- Assessing the welfare of pigs by measuring the amount of cortisol hormone level in pigs which may indicate the stress level of pigs subjected to high inclusion of polyphenolic rich diets.
- Assessing the haematological parameters of pigs fed on incremental levels of *V. tortilis* leaf meal.

Appendix 1: Ethical Approval of Research Project on Animals

UNIVERSITY OF KWAZULU-NATAL™
INYUVESI YAKWAZULU-NATALI

10 November 2015

Mr Mbongeni Khanyile
School of Agricultural, Earth & Environmental Sciences
Pietermaritzburg Campus

Dear Mr Khanyile,

Protocol reference number: AREC/101/015D
Project title: Fatty acid profile, oxidative stability and quality of pork from pigs fed on *Acacia tortilis* leaf meal-based diets

Full Approval – Research Application

With regards to your revised application received on 03 November 2015. The documents submitted have been accepted by the Animal Research Ethics Committee and **FULL APPROVAL** for the protocol has been granted.

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

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

The ethical clearance certificate is only valid for a period of one year from the date of issue. Renewal for the study must be applied for before 10 November 2016.

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

Yours faithfully

....

Dr Shahidul Islam
Chair: Animal Research Ethics Committee

/ms

Cc Superivsor: Dr M Chimonyo
Cc Registrar: Mr Simon Mokoena
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