ESTIMATING THE VOLUNTARY HERBAGE INTAKE AND DIGESTIBILITY OF GROWING PIGS FED A CONCENTRATE SUPPLEMENT ON A KIKUYU PASTURE BY THE N-ALKANE AND ACID-INSOLUBLE ASH MARKERS

by

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Submitted in accordance with the requirements

for the degree of

MASTER OF SCIENCE

in the subject

AGRICULTURE

at the

UNIVERSITY OF SOUTH AFRICA

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November 2010

DECLARATION

“I, JEAN SERGE KANGA declare that “Estimating the voluntary herbage intake and digestibility of growing pigs fed a concentrate supplement on a Kikuyu pasture by the n-alkane and acid-insoluble ash markers” is my own work and that all the sources that I have used or quoted have been indicated and acknowledged by means of complete references.

Signature: …….

J. S. KANGA

Date: ……..

ACKNOWLEDGEMENT

First and foremost thanks are due to the Eternal God for everything he helped me to achieve. I am also grateful to the following people:

- Dr J. J. Baloyi (University of Venda) for his expert advice. I wish to show my appreciation.
- Dr Arnold T. Kanengoni (Agricultural Research Council, Animal Production Institute), his constant motivation, support and criticisms kept me going and were essential to the completion of this project.
- Ronald Thomas, Makgothi “Gagi” Gogakgamatsamang, Mmaseala Seshoka and Eunice Bhila.
- The staff at the Agricultural Research Council, Nutrition division, Irene: Chris Van der Walt, Thomas Modungoane, Samuel Monesi, Sophie Khoza, Albert Mphuloane, Douglas Nkosi, without them this study would not have been possible.
- My family (Brigitte and Venance Kanga, my brothers Jacques and Carole) and A. B. Landangoye and others, for their invaluable support during these years.

PUBLICATION

Parts of the work described in this dissertation have been published in the following conference meeting.

J. S. Kanga, A. T. Kanengoni, J. J. Baloyi, and M. M. Masafu **(2010)**. Estimating intake and digestibility on growing pigs fed concentrate-forage diets by using n-alkane and acid-insoluble ash markers. Proceedings of the 45th Congress of the Grassland Society of Southern Africa (GSSA), Kimberley, South Africa, 19-23 July 2010.

ABSTRACT

Pigs can consume a wide range of feeds to meet their nutritional needs and there is a renewed interest in the use of cheaper nutrient resources for animal feeding. Forages have been proved to be a substantial source of nutrients for pigs, however, the bulk of the existing work has focused on sows and grower-finisher pigs above 50 kg. This study was conducted during May-June 2009 at the Agricultural Research Council (Irene, Pretoria) to determine the voluntary forage intake and nutrient digestibility in growing pigs fed a mixed diet (concentrate + Kikuyu grass). Twenty five 8 weeks old Large White x Landrace crossbred pigs (27 ± 3.8 kg) were blocked by weight into 5 groups of 5 pigs each. One of 5 treatments (A, B, C, D and E), corresponding to 100, 90, 80, 70 and 80 % of a basal concentrate ration, respectively, was randomly assigned to a pig within each block. Indoor treatments were either fed the concentrate only (A) or also received freshly cut Kikuyu grass (*Pennissetum clandestinum*) *ad libitum* (B, C and D). Only treatment E animals were housed outdoors in Kikuyu grass paddocks while all other treatments were housed indoors. Forage intake was recorded daily and also estimated using a pair of n-alkanes as markers. Nutrient and diet digestibility were calculated using acid-insoluble ash (AIA) and dotriacontane (C_{32}) as markers. The results showed that the concentrate intake (CI) in treatments A, B and C was significantly different from treatments C and E ($P < 0.05$) and there was positive correlation between the concentrate level and its intake ($P < 0.01$). The recorded intake of Kikuyu grass (RKI) and the animal's average daily gain (ADG) were similar between treatments ($P > 0.05$). The estimated (EKI) and recorded (RKI) Kikuyu grass intakes were not influenced by CI or the level of concentrate allowance (CL) and RKI was higher ($P < 0.05$) than EKI. Digestibility estimates with AIA were higher than C_{32} estimates ($P < 0.05$). It was concluded that Kikuyu grass intake was not affected by the reduction of the concentrate level allowance. It was proposed that forage intake in a mixed diet (forage + concentrate) was more dependant on its own characteristics than the concentrate's nutritional value.

Keywords: Dotriacontane; Kikuyu; growth performance; forage; monogastrics

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CHAPTER 1

INTRODUCTION

1.1 Background and motivation

Pigs can consume an extensive variety of feeds to meet their nutritional needs (Rodríguez-Estévez *et al.*, 2009). However, in commercial practice, they are fed concentrate diets to maximize their growth potential. Inevitably such an emphasis on efficiency entails the inclusion of high quality feed ingredients such as cereals and grains in livestock feed, which are also highly favoured by humans.

Concerns about the escalating feed costs, exacerbated by competition with human demand, together with consumers' perceptions that modern intensive farming practices do not promote animal welfare (Tovar & Giraldo, 2006; Lassen *et al.,* 2006) prompted the expansion of husbandry practices into extensive and outdoor systems aimed at making pig production more acceptable from an ethical perspective.

These extensive outdoor pig production systems today exist all over the world in different forms and follow various guidelines, with the stricter ones labelled as organic (Fernandez & Woodward, 1999; Ferre *et al.*, 2001; Hermanssen *et al.*, 2004); and they share some common features:

- They use larger areas than in commercial practice to ensure freedom of movement for the animal by means of permanent or time-controlled access to an outdoor run.
- They provide pasture so as to let the animals express their natural behaviour and to also contribute to their nutritional requirements.
- They position their products in a niche market which fetches a higher premium than the conventionally produced pigs.

The extent to which pigs can derive their daily dietary requirements from roughage has been investigated and depends on the intake as well as the properties of the ingested matter (Ferre *et al.,* 2001). Pastures have huge prospects as feedstuffs for pigs in South Africa given that they are relatively cheap to establish and maintain (Meeske *et al.*, 2006), with common pastures being diverse varieties of ryegrass (*Lolium perenne*), and also weeping lovegrass (*Eragrostis curvula*). There is however a scarcity of information about the quantities of pasture that pigs will voluntarily consume and to what extent they can contribute to the animals' needs and the different types of pastures best suited for South African pigs. The bulk of available information is either on ruminants (Mayes & Dove, 2000) or if on pigs it is from other countries based on lucerne, ryegrass, clover (Kelly *et al.*, 2001*;* Sehested *et al.,* 2004), while there is not much information on grasses common to South Africa like *Pennisetum Clandestinum* (Kikuyu) grass. This information is critical if decisions are to be taken of the pigs' grazing and supplementary needs.

1.2 Problem Statement

Pasture intake in pigs raised outdoors has been documented mainly in sows (Ferre *et al*., 2001; Santos Ricalde & Lean, 2002, 2006; Sehested *et al.,* 2004), but less so in growing pigs. The challenge has always been to determine the actual amount of pasture consumed by the pigs and to what extent this contributes to the animal's nutrient needs.

The methods often used to determine intake of pastures in grazing animals include the classical sward cutting (herbage disappearance method) and marker methods (Piasentier *et al*., 1995; Ferre *et al*., 2001; Gustafson & Stern, 2003). The methods that are used for the determination of herbage are not considered to be 100 % accurate and therefore the determined herbage intakes are accepted as estimates (Macoon *et al*., 2003). The n-alkane method was originally developed for estimating intake in ruminants (Mayes *et al.,* 1986) but has also been applied in non-ruminants studies including pigs (Ferre *et al.,* 2001; Mowat *et al.*, 2001), and horses (Stevens *et al.*, 2002), and was demonstrated to have many advantages over the other methods. More work

however still needs to be done before the method can be used effectively and accurately in estimating intake and digestibility of pastures in pigs.

Very little is known about how much commercial feed can be substituted with pasture for growing pigs, which makes it difficult to advise farmers raising pigs on pastures. Most of the available information deals mainly with sows (Ferre *et al.,* 2001; Santos Ricalde & Lean, 2006; Sehested *et al.*, 2004), or grower-finisher pigs above 50 kg (Mowat *et al.*, 2001; Kelly *et al.*, 2001), animals which tend to have large gastrointestinal capacities enabling them to ferment fibrous feeds. Since the use of commercial concentrates pushes pig production costs higher, it would therefore be economically wise to feed pasture to pigs as early as possible, before the finishing stage. It is common for resource poor South African pig farmers to have their herds on pasture for varying periods because of a lack of adequate housing facilities and/or to substitute some of the animal feed with fodder. The clear economic motivation of these measures should not demean the fact that some farmers might also be sensible to the ethical dimension of their business and value animal wellbeing. It was envisaged that the results from this investigation would assist these farmers in formulating inexpensive pig diets as early as the grower stage.

1.3 Objectives

The main objective of this study was to determine the voluntary intake of *Pennisetum Clandestinum* (Kikuyu) grass in growing pigs receiving a concentrate ration and to assess diet and nutrients digestibility using markers.

The specific objectives were to:

- Estimate the voluntary intake of Kikuyu grass for grower pigs in indoor and outdoor groups with dotriacontane (C_{32}) and tritriacontane (C_{33}) n-alkanes.
- Evaluate the digestibility of organic matter and specific nutrients in grower pigs in indoor and outdoor treatments using acid-insoluble ash (AIA) and C_{32} n-alkane as markers.

1.4 Hypotheses

The hypotheses tested were that:

- Decreasing the concentrate allowance in growing pigs will lead to an increase in consumption of Kikuyu grass and a decrease in digestibility of nutrients.
- There is no difference between the recorded forage intake and the n-alkane estimate.

CHAPTER 2

LITERATURE REVIEW

2.1 Introduction

Some objectives and rewards of outdoor farming systems are the reduction of production costs owing to an increase of low-cost quality feedstuff in the animal's diet. In recent years, this drive for the exploitation of cheaper nutrient and energy sources for pig diets has seen the inclusion of roughage either fresh or dried (Ferre *et al.,* 2001; Leterme *et al.*, 2006; Santos Ricalde & Lean, 2006), as hay (van Wieren, 2000; Hodgkinson *et al.*, 2008), silage (Hodgkinson *et al.*, 2008; Presto *et al.*, 2009) or ground and mixed into the concentrate (Reverter *et al.,* 1999; Hodgkinson *et al.*, 2008).

2.2 Main considerations in raising pigs on pasture

Producing pigs on pasture gives an opportunity to simplify management procedures seeing that it is less work intensive than confinement systems. It is also worth mentioning that land that is inappropriate for commercial crop exploitation can be converted into pastures and organic pig farms. In contrast, the increase of parasites, pathogens (Bach Knudsen, 2001b) and associated health expenditures are a major problem in outdoor pig production. In addition, pigs' rooting activities rapidly destroy the vegetation cover (Sehested *et al.,* 2004), thus affecting subsequent plants' organic matter yield (Van Oudtshoorn, 1992). This is one reason why an ideal type of pasture grass that is resilient needs to be identified and promoted. Finally the larger space requirement than in intensive production systems may be of particular concern in parts of the world where there are limited land resources.

2.3 Pasture plants and their potential

A survey of literature on outdoor pig farming suggests that the *Poaceae* and *Fabaceae* plant families contribute extensively to meeting pig nutritional

requirements, as natural plant communities or cultivated pastures (Tainton, 2000). The family of Poaceae commonly known as grasses is certainly one of the most economically important group of plants, as they have constituted a staple in human and animal feeding for many years. *Fabaceae* or legumes are particularly valued for their soil enrichment properties due to the presence of nitrogen-fixing bacteria in their roots. Legumes are rich in nitrogen-compounds and can be cultivated with or without supplement grasses, to improve the diet of the foraging animal (Cheeke, 2005; Baloyi *et al.,* 2006) and they are commonly used as protein concentrates in pig diets (Gatel, 1994). The higher cell content: cell wall ratio than in grasses makes them relatively more palatable and digestible (Tainton, 2000). It has been observed that sows select more clover than grasses and prefer leaves to stems (Sehested *et al.*, 2004). In contrast, legume fibre is more lignified than other plants (Baurhoo *et al.*, 2008). In addition, the utilisation of legumes by the pig is constrained due to their relative deficiency in sulphur amino acids (methionine and cysteine) and tryptophan (Seabra *et al.*, 2001; Mullan *et al.*, 2009). Legumes also contain a wide array of anti-nutritional factors (Baloyi *et al.*, 2007).

Pennisetum clandestinum (Kikuyu grass) together with *Lolium perenne* (ryegrass) have been preferred plants, for outdoor pig production. Kikuyu grass is a warm season perennial creeping grass, whose distinctive features include among others, an inflorescence that is generally not visible (Marais, 2001; Donaldson, 2001). The grass generally has high levels of non-protein nitrogen (Marais, 2001) and this can potentially lead to an over-estimation of the available protein, as common analytical procedures for feedstuff express the protein content as a function of its nitrogen concentration. Kikuyu grass has a low magnesium content in spring (Cheeke, 2005) and is most likely to present a phosphorus deficiency since grasses grown within the Southern African region are deficient in phosphorus (Tainton, 2000). The extent to which these nutritional deficiencies can affect outdoor pigs grazing Kikuyu grass is not currently fully established. Although pastures may be deficient in important minerals (vitamin D and B_{12}), intake of soil-contaminated pasture, soil ingestion from rooting activities and supplementation are expected to provide pigs with the required minerals (McDonald *et al.,* 1995; Lewis & Southern, 2001).

When selecting species with a view of establishing a pasture, special attention should be directed to the nutritional value. Challenges are mostly related to the issue of nutritional balance, notably amino-acids, and the relative presence and importance of anti-nutritional factors. Productivity and persistence of a pasture must be taken into account to ensure long term sustainability. Adaptation to environmental conditions (biotic and abiotic factors), in addition to the tolerance of the species to defoliation and grazing pressure should also be considered, and should ideally inform management procedures to ensure the profitability of the pasture based enterprise.

2.4 Factors affecting pasture intake and utilisation

Productivity levels of animals getting all or a fraction of their nutritional requirements from pasture are dependent upon a variety of factors ranging from the amount and quality of ingested forage, its palatability, to the type of the animal's digestive tract (Bach Knudsen, 2001b). Voluntary feed intake in farm animals generally follows a hierarchy of factors. A diet's palatability and the occurrence of anti-nutritional factors seem to be of prime importance. In effect, Fergusson *et al.* (2002) noted that pigs prefer an unbalanced feed to one that contains an anti-nutritional factor even when it is more balanced. Voluntary intake is also determined by the metabolic status of the animal and/or the size of its digestive tract. In fact, intake of low digestibility or bulky feedstuffs will mainly be limited by gastric distension whereas voluntary consumption of high nutrient density diets will begin and continue until the satisfaction of the requirements for one or more limiting nutrient(s) (Forbes, 1995; Tainton, 2000).

2.4.1 Anti-nutritional factors

In addition to the nutrients that they provide to animals, plants also contain bio-molecules, elements and compounds bearing toxic and anti-nutritional properties which can adversely affect intake, due to a decreased palatability of the plant, or reduced protein digestibility and energy utilisation (Seabra *et al.*, 2001). A commonly reported case of pasture toxicity is the Kikuyu grass poisoning which is generally linked to environmental conditions favouring rapid growth (i.e. irrigation, fertilization), that promote an increased synthesis of toxins

and deleterious compounds such as nitrates, nitrites and oxalates (Cheeke, 1995; McDonald *et al.*, 1995; Frape, 2010). Animals grazing Kikuyu grass pastures that have recently received nitrogenous fertilizers are prone to nitrate poisoning. Nitrates are non-protein nitrogen compounds involved in protein synthesis and are not directly toxic (McDonald *et al.,* 1995). It is only when they are metabolised into nitrites, which interfere with the blood oxygen transportation system by oxidizing haemoglobin into methahaemoglobin causing methahaemoglobinaemia (Marais, 2001), that they become harmful. Nitrate poisoning has been generally associated with the consumption of waste water.

Pigs are more susceptible to nitrite poisoning than ruminants, which are able to convert nitrites into ammonia. Nitrite poisoning is more prevalent during wet conditions and good nitrogen fertilization (Wiese & Joubert, 2001). However, death is unusual and only occurs once the level of methaemoglobin makes up 80-90 % of the total haemoglobin. It thus appears that nitrate poisoning in foraging pigs could be avoided by adequate pasture management, i.e. controlling weeds in pastures and a strict control of irrigation and fertilisation practices (Marais, 2001).

Another anti-nutritional factor associated with pastures is oxalate toxicity. Oxalates cause toxicity when in excess of 5 g/kg dry matter in Kikuyu grass by inducing calcium deficiencies as a result of binding to it in the animal's gut to form calcium-oxalate complexes (McDonald *et al.*, 1995). Attempts to address the resultant calcium deficiencies will cause the animal to mobilize its bone calcium reserves causing all sorts of skeletal disorders. Frape (2010) reported that ruminants are less prone to oxalate poisoning, due to their ability to degrade oxalates in their rumen.

The legume species contain anti-nutritional factors such as lectins, phytates, tannins, trypsin inhibitors and mimosines which effects are relatively more detrimental than those in grasses. For instance, it has been reported that lectins impair nutrient absorption and damage the intestinal lining in pigs (McDonald *et al.,* 1995). Phytates and trypsin inhibitors on the other hand reduce the efficacy of proteolytic enzymes and the availability of a number of major minerals

through the formation of chelates and blocking the enzyme trypsin, preventing it from degrading ingested proteins (Whittemore, 1993). Although leguminous forages such as *Leucaena leucocephala* are high in protein, their utilization in non-ruminants is limited due to the presence of mimosines, tannins and other polyphenolic compounds (Echeverria *et al*., 2002). Besides their direct effect on digestibility and palatability, tannins are also known to make dietary protein unavailable by binding to them in the animal's digestive tract. The presence of glucosinolates, tannins and saponins has been reported to be responsible for the under-performance of livestock (Baloyi *et al*., 2006). Marais (2001) reported that Kikuyu grass is low in condensed tannins.

2.4.2 Protein content

It has been reported that animals are able to select food according to their protein content (Forbes, 1995), with pigs shown to pick feed ingredients that best matched their protein requirements (Fergusson *et al.*, 2002). However, the amino-acid profile of a diet is more important than its protein concentration and a clear preference for food with inadequate protein content over one that presents an amino-acid imbalance was observed in pigs (Forbes, 1995). This is because in mammals, excess dietary amino-acids not used for protein accretion cannot be stored in the body and must undergo the removal of their amino group by deamination (Ndindana *et al.*, 2002) which produces heat and an energy cost. Protein content is generally over-estimated in Kikuyu grass owing to the presence of other nitrogenous compounds, which inflates the crude protein (N * 6.25) content of the grass (Forbes, 1995). It is therefore not expected for pasture intake in pigs to be limited by a high protein content, but rather by other dietary components such as dietary fibre.

2.4.3 Dietary fibre content

From a functional perspective, dietary fibre is defined as all the dietary components of plant origin resistant to degradation by mammalian enzymes (Bach Knudsen, 2001a) and are chemically defined as cell wall non-starch polysaccharides (NSP) and lignin, which are closely associated with carbohydrates and constitute up to a third of plant cell wall contents and confer

mechanical strength to the plant (Baurhoo *et al.*, 2008). Dietary fibres are generally classified based on their solubility in neutral (NDF) or acid (ADF) detergents (Bach Knudsen, 2001a) or characterized by relative comparison of their digestibility with that of starch (Jung & Allen, 1995). However some starches are reported to resist enzyme degradation and are therefore comparable to NSP's (Bach Knudsen, 2001a).

It is acknowledged that an increase in the proportion of fibrous component in a diet is responsible for a depression in nutrients' digestibility. This effect is attributed to a reduction in the mean retention time (Ndindana *et al.*, 2002; Wilfart e*t al.,* 2007b) of the diet in the gastro-intestinal tract due to an increased mucus secretion and water holding capacity. The botanical origin, composition and content of a diet's fibre source will also affect its digestibility and that of other nutrients to various extents. Reverter *et al*. (1999) pointed out that pigs are able to utilise some fibre sources better than others. In their study, energy was more efficiently utilised from diets containing forage fibre than the ones containing cereal fibres. LeGoff *et al.* (2003) investigated the digestibility of two diets differing in their fibre content. Using the differential method they found that the nitrogen fraction of maize bran was poorly digested. This influence might also be exacerbated by processing; and it is of great significance since cereal by-products are being increasingly included in pig diets (LeGoff & Noblet, 2001).

It is well established that grazing animals prefer younger plant material. As the plant matures, the proportion of fibrous components increases due to an increase in the plant's cell wall content. Lower proportions of protein in the dry matter are also observed (Chaves *et al.,* 2006).

High fibre content in a diet depresses the digestibility of proteins also, because of an increment in endogenous losses in the pig (LeGoff & Noblet, 2001; LeGoff *et al*., 2003), resulting from an increase of bacterial nitrogen and mucus secretions. In fact, increasing the diet's fibre level causes a rise in the hindgut's population of fibre-fermenting microbiota, which will contribute to an increase of the faecal nitrogen content. Research in monogastrics has also shown that protein and amino-acid containing compounds from mucoproteins, desquamated cells from the gut lining and digestive secretions are not

reabsorbed during digestion (Ravindran *et al.,* 2004; Stein *et al.,* 2007). Moreover, endogenous secretions in pigs have relatively high levels of proline, threonine and serine (Hong *et al.,* 2002) and might considerably influence the digestibility of amino-acids and crude protein.

Alternatively, the fibre fraction of a feedstuff can directly prevent absorption of amino-acids, peptides and some energy yielding nutrients from the diet as happens with lignin, which is resistant to enzymatic and acid hydrolysis and consequently limits structural polysaccharide digestibility by forming bonds with carbohydrates (Robbins, 1983). High fibre content also limits the extent of energy availability because high fibre levels trigger an earlier satiety so that the animal's energy intake is incomplete (Wenk, 2001).

2.4.4 Physiological status

A diet's nutrients and energy are not digested to the same degree by pigs that are in different physiological stages. As the animal grows, its body weight and the size of its digestive tract increases and improved digestive ability and efficiency of nutrient utilisation are observed as a consequence (Jørgensen *et al.*, 2007). These are due to a longer retention time of digesta (LeGoff *et al*., 2003) and a greater contribution of hindgut fermentation to overall digestion (LeGoff & Noblet, 2001; LeGoff *et al*., 2003). Jørgensen *et al.* (2007) reported that sows can derive energy more efficiently from large intestine fibre fermentation than growing pigs.

The digestion of pasture largely takes place in the animal's hindgut through fermentation because of its insoluble and lignified fibre content. The relative contribution of hindgut's bacterial communities to an improvement in digestibility could certainly be influenced by the duration of exposure to the diets. Castillo *et al.* (2007) suggested that the adaptation period of the gut microflora "to express a maximum enzymatic potential" could last up to six weeks.

2.5 Estimation methods of pasture intake

Nutrient intake is a key predictor of animal performance (Mayes & Dove, 2000). The diverse methods available for estimating herbage intake, whether plant or animal based, were primarily developed for use with ruminants but are nevertheless applicable to any animals consuming forage to some extent.

2.5.1 Plant based herbage disappearance technique

This is a differential technique traditionally used in ruminants whereby the voluntary forage intake is estimated by the diminution of herbage mass over the period that an animal spends grazing (Mayes & Dove, 2000). A sward's herbage mass is quantified before allowing the animal to graze, by harvesting the totality of its vegetation to a specified height. The procedure is repeated in the grazed sward after removing the animal and the herbage mass difference between the adjacent sward and the grazed plot is assumed to correspond to the animal's voluntary forage intake.

The accuracy of the herbage disappearance method may however be questioned in light of some observations. As herbage in the plot grows continuously, a correction factor for regrowth of the pasture should be implemented (Mayes & Dove, 2000), unless intake estimation is conducted within a short period. The sward has to be sufficiently grazed to avoid selection by the animals and the topography of some terrains makes the estimation of residual herbage mass difficult. Moreover, hand-picked forage samples collected for analysis might not be representative of the grazed forage. In fact, Swainson *et al.* (2005) and Smith *et al.* (2001) pointed out that differences exist in the concentration patterns of substances between the plant parts. Therefore, visual observation of the animal's feeding behaviour may improve the sampling process. Alternatively, *in vivo* collection of the digesta from oesophagal-fistulated animals can be considered representative of the animal's diet.

2.5.2 Animal based techniques

There is a consensus that animal methods present more advantages over the plant based technique, because of the possibility to perform measurements on any individual animal (Mayes & Dove, 2000) and the recognition that animal factors greatly contribute to variability (Keli *et al.,* 2008).

2.5.2.1 Behavioural observations

This method was initially devised for ruminants and is based on the animal's ingestive behaviour and does not require expert knowledge to take measurements. Intake evaluation is either done by direct observation or assisted with an apparatus that estimates intake (I kg/day) as a function of how much feed the animal can accommodate in one bite (bite mass), the frequency and the amount of time spent on feeding according to the following formula:

I = Bite mass (kg/bite) x Bite frequency (bites per unit of time) x Time spent feeding (unit of time)

This method has several limitations, such as differences in the size and width of the animals' dental pads and the fluctuation of bite size over time (Oliveira & Silva, 2007). As a consequence such observations within short periods might not be reliable. In addition, the use of an apparatus is an invasive method likely to disrupt the animal's ingestive behaviour (Mayes & Dove, 2000). Furthermore, this technique is not suitable for group-housed animals (Macoon *et al.,* 2003).

2.5.2.2 Weighing of the animal

It is a differential technique in which the amount of feedstuff consumed by an animal is estimated after a short term intake, by determining the animal's initial and final live-weights, with the difference corresponding to its herbage intake. The animal is fitted with a harness so as to collect the excreta for a short period. Although this method gives accurate estimates, its consistency may be affected by the dampening of the animal's coat as well as daily fluctuation of bite size, which affect the quantity of the ingested feed. Furthermore, adjustments are to be made to take into account weight loss due to metabolic processes (Mayes &

Dove, 2000). This method is of practical importance in pigs because inaccuracies would be minimised due to their light hair coat.

2.5.2.3 Animal performance method

Dry matter intake can be derived in dairy cows from the animal's net energy requirements, the net energy content of the grass and concentrate offered (Smit *et al.,* 2005). A comparable technique has been developed in pigs whereby individual feed intake ($I_{Individual}$) can be estimated from intake estimated in group housed animals (I_{group}) as follows:

$$I_{Individual} = I_{maintenance} + I_{growth}$$

With $I_{growth} = [I_{group} - Sum\ I_{maintenance}] \times [BW\ Gain_{Individual} / sum\ BW\ Gain]$

and $I_{maintenance} = (106 * BW^{0.75} \times day) / (ME_{Feed})$.

(Lindemann & Kim, 2007).

Where $I_{maintenance}$ and I_{growth} are respectively the portion of the intake for maintenance and growth; BW is the body weight, in kilograms; Sum $I_{maintenance}$ is the sum of the maintenance feed intake for all pigs in the group, in kilograms; Sum BW gain is the sum of body weight gain for all pigs in the group, in kilograms. However, this method is only valid when no weight loss occurs during the period over which intake is estimated. Another shortfall is that equations used to calculate intake assume that no variations occur between the experimental animals (Macoon *et al.*, 2003).

2.5.2.4 Faecal collection technique

The faecal collection technique involves the systematic gathering of the animal's faecal output at set frequencies over a collection period. Total faecal collection can be carried out on caged or partially restrained animals, directly from the floor or using a collection apparatus attached to their back (bag or harness). A review of Lippke (2002) suggests that faecal collection is stressful and more likely to alter the animal's normal feeding behaviour, which in turn will

influence intake estimates. In some instances, grab sampling directly from the animal's rectum is implemented in situations when total collection is not possible.

2.5.2.5 Use of indigestible chemical indicators

Nutrient digestibility can alternatively be recorded by chemical indicators, known as markers. With markers, a diet's intake is determined from the ratio between the total faecal output and its indigestibility (Mayes & Dove, 2000) from the formula:

$$Intake = Faecal\ output \,/\, (1 - Digestibility)$$

While faecal output is estimated from the ratio between the external marker's dose rate and its faecal concentration (Fulkerson *et al.,* 2006), the digestibility can be derived from the use of an internal marker or an *in vitro* digestibility procedure. The use of markers in nutrition studies is based upon the fact that they are largely indigestible chemical compounds; should ideally be completely recovered from diets, and have no influence on the digestive tract physiology and their analysis must be simple and accurate (Kotb & Luckey, 1972, cited by Lippke, 2002). However, to date no marker fully complies with these requirements (Dove & Mayes, 2005).

Current nutritional studies with markers involve daily collection and pooling of faecal material (Vulich & Hanrahan, 1995) which is an advantage over total faecal collection that is a labour intensive method (Kavanagh *et al.*, 2001) and is either impractical or impossible in studies on free-ranging wild animals. Cost and labour associated with the processing of individual samples can thus be greatly reduced.

There are a number of concerns about the use of markers in determining intake and digestibility. Faecal output and digestibility values determination are two distinct procedures that are sources of bias in the determination of voluntary intake. The digestibility coefficient, which is typically derived from trials using a few animals or estimated *in vitro* is assumed to be similar in other groups or

classes of animals. In addition, the use of the *in vitro* technique is also precluded when animals are fed supplements (Dove & Mayes, 1996; Dove & Mayes, 2005). Furthermore, the extent to which the collected faecal sample is representative of the ingested diet is of prime importance when for practical reasons the frequency of faecal collection has to be reduced (Olivan *et al.,* 2007).

2.6 The use of markers in nutritional studies

Markers are classified into natural constituents of an animal's diet (internal markers) or external markers, in which case they are exogenous to the diet and are provided separately as a dose (Lippke, 2002). Daily dosing is by far the most common method of administering markers to animals. It requires that the same quantity of a marker be given daily to the animal for an initial adaptation period not less than five days (Olivan *et al.*, 2007), following which representative faecal samples can be collected.

2.6.1 External markers

Markers are natural (internal) or exogenous (external) constituents of an animal's diet (Lippke, 2002), in the latter case, they are provided as a dose. Chromic oxide (Cr_2O_3) is the most common external marker in use in animal nutrition studies (Mayes & Dove, 2000; Kavanagh *et al.*, 2001; Lippke, 2002). More recently titanium oxide (Ti_2O_3) has increasingly been employed as an alternative to chromic oxide (Thompson & Wiseman, 1998) and there is some evidence that Ti_2O_3 gives better results than Cr_2O_3 in monogastric animals (Hatt *et al.,* 2001). The main concern over the use of these markers is their probable carcinogenic effects and incomplete faecal recovery that will affect the accuracy of intake estimation.

2.6.2 Internal markers

Internal markers commonly occur in the feed either as discrete chemical entities or analytical products. Over the years, a wide range of markers have been experimented with (Klason lignin, indigestible neutral detergent fibre, n-alkanes)

(Tamminga *et al.*, 1989). They have the advantage that their behaviour in the animal's gut is more predictable than that of external markers in the sense that they generally mimic that of the feed; however their utilization is limited due to the fact that they are not totally indigestible (Tamminga *et al.*, 1989).

2.6.2.1 Radioactive elements

Nutrient intake has been determined from the turnover rate of a radioactive isotope of (e.g. Sodium) following its single injection and assuming its total absorption from the animal's gut. The accuracy of this technique is not guaranteed since herbivores have diets poor in sodium and the use of radioactive elements poses safety issues (Mayes & Dove, 2000).

2.6.2.2 Acid-insoluble ash

Acid-insoluble ash (AIA) is a marker mainly composed of silica (McDonald *et al.,* 1995; Mayes & Dove, 2000). It is an analytical product obtained by a series of sample extractions by dissolution in a strong acid solution followed by filtering of the residue and further "ashing" at a high temperature. Its use has been advocated as an alternative to chromium oxide (external marker), which is subject to interference with other minerals in the ration and also because chromium (Cr) is a heavy metal, hence an environmental risk (Van Leeuwen *et al*., 1996). According to Kavanagh *et al.* (2001), AIA can be reliably detected in faeces as long as its concentration exceeds 2 g/kg. The concentration of AIA in the diet is therefore of great importance and these workers recommend the inclusion of celite in cases when ash levels are low.

An indicator of efficiency in markers is their recovery rate, i.e, the ratio of the quantity of the marker excreted over what was consumed. Although AIA, may be present in low quantities in feeds (2 %), McCarthy *et al.* (1974), cited by Kavanagh *et al.* (2001) determined that AIA was a superior marker over chromic oxide for used in pig nutritional studies. Their study with pigs reported a high recovery rate, in comparison to those of chromic and titanium oxides (99. 9 vs. 96 and 92.3 % respectively). According to the same workers, AIA recovery generally ranges from 97 % to an almost complete recovery.

2.6.2.3 N-alkanes

N-alkanes are saturated straight hydrocarbon chains contained in the cuticular wax found on the surface of higher plants (Dove & Mayes, 1996). They seem to play a role in water retention mechanisms in plants (Tulloch, 1976, cited by Smit, 2005). These hydrocarbons are useful as internal markers because they are available in most dietary plants and are relatively indigestible, although some plants and by-products such as legumes, grain and oilseed meals are deficient in n-alkanes, due to the absence of plant wax in their cuticular layers (Mayes & Dove, 2000). The distribution of alkanes is generally in favour of odd-numbered carbon chains especially in herbaceous and woody plants where they constitute more than 90 % of natural n-alkanes (Malossini *et al.,* 1994; Piasentier *et al.,* 2000) and molecules ranging between C_{27} and C_{33} are the most prevalent (Ali *et al.,* 2005). N-alkanes have also been used as external markers since they can still be dosed by applying synthetic n-alkanes or natural beeswax, which is also a good source of alkanes (Mayes & Dove, 2000).

The n-alkane technique is suitable for intake measurements as the handling of the animals is limited (Mayes & Dove, 2000; Ferre *et al.,* 2001) and it was reported superior to the faecal sampling and *in vitro* methods in sheep and cattle (Mayes & Dove, 2000). In fact, measurements can be performed on any individual animal and because dietary and dosed alkanes are analysed concurrently, it significantly greatly reduces analytical bias. Moreover, there seems to be no aversion when fed to supplemented animals through the concentrate. This technique has also been validated in non-ruminant animals (rabbit, elephant, horse, pig, deer, wombat, tortoise, pigeon) (Hatt *et al.,* 2001; Mayes & Dove, 2000; Swainson *et al.,* 2005).

N-alkanes are particular in the sense that they are dosed to determine intake but they can also be used as internal markers, for digestibility determination. There are two methods available for estimation of herbage intake with n-alkanes. For mixed concentrate-forage diets and using the n-alkane marker as an internal marker to determine digestibility, herbage intake (organic or dry matter basis) is deducted from the difference between the total nutrient intake and the proportion contributed by the concentrate, which amount and nutrient

composition are known (Elwert & Dove, 2005). This method is however, based on the assumption that the alkane's faecal recovery of a mixed diet is not affected by the amount of concentrate (Elwert *et al.,* 2004).

Alternatively, voluntary intake can be estimated using the double alkane technique, from the simultaneous use of a dietary natural odd chain and a dosed synthetic even chain alkane markers (Mayes & Dove, 2000). Intake will be derived using the formula:

$$I = C_j / (F_j/F_i) * H_i - H_j$$ (Mayes & Dove, 2000; Smit *et al*., 2005).

with F_i, H_i, F_j *and* H_j being respectively the faecal and herbage concentrations (dry matter) of the odd (natural) and even (dosed) chain alkanes in the faeces and herbage. C*j* is the daily dose rate (mg/day). N-alkanes are also useful in determining a diet's botanical composition (Mayes & Dove, 2000) and digestion kinetics (Lippke, 2002).

2.7 Factors affecting intake estimation using alkanes

2.7.1 Choice of alkanes for intake estimation

In most herbage species, large odd chain molecules (C_{31}, C_{33}, C_{35}) account for the bulk of n-alkanes present in the plant cuticular wax (Piasentier *et al*., 2000) and it appears that not all n-alkanes pairs are suitable for intake estimation (Dove & Mayes, 1996). In fact, this is because the low concentrations of the other alkanes prevent their successful dosing. Adjacent pairs of odd and even chain alkanes are commonly used for estimation of herbage intake (Dove & Mayes, 1996; Smith *et al*., 2007), based on the assumption that they have similar faecal recovery rates. In a review of the use of n-alkanes as nutritional markers, Oliveira & Silva (2007) suggested that incorrect estimates might arise as a result of using n-alkanes whose concentrations are below 50 mg/kg. N-alkane pairs selected for intake estimation should present the highest dietary concentration and faecal recovery rates (Peiretti *et al*., 2006). Thus C_{31}, C_{32} and C_{33} n-alkanes are largely preferred. The pair C_{32}:C_{33} has been used by several workers (Mayes *et al.,* 1986; Vulich *et al.,* 1991*;* Dove & Mayes, 1996)

and yielded reliable estimates while Peiretti *et al*. (2006), and also Ordakowski *et al.* (2001) preferred C_{31}:C_{32} alkanes, due to the relative abundance of C_{31} in the hay forage fed to horses. The choice of the most suitable n-alkane pair should therefore take into account the dietary n-alkane concentration, since plant materials may possess different n-alkane concentration profiles. Ordakowski *et al.* (2001) contend that any n-alkane pair can be used in monogastrics, provided that they have similar faecal recoveries.

2.7.2 Faecal recovery

A crucial point in the analysis of a marker is its recovery rate, i.e., the ratio of the excreted concentration of that marker over that of the ingested amount. Contrary to previous thinking, n-alkanes may not be totally inert in the digestive tract of animals, but are subjected to some modifications (Swainson *et al*., 2005; Ordakowski *et al*., 2001; Oliveira & Silva, 2007). Alkanes are not fully recovered in the faeces (Elwert *et al.,* 2004) and Dove & Mayes (1996) suggested that the error in intake estimation is proportional to the faecal recovery difference between the dosed and natural n-alkanes. Because of the evidence of incomplete faecal recovery, a common practice in nutrition research is to use a correction factor by assuming a faecal recovery value from available data in the literature 0.95 (Mayes *et al.,* 1986), 0.84, (Hatt *et al.,* 2001), 0.96 (Gedir & Hudson, 2000, cited by Oliveira & Silva, 2007) in case total faecal collection is not possible. Intake and digestibility are often under-estimated when no correction factor for faecal recovery is applied (Mayes *et al.,* 1986; Hatt *et al*., 2001; Ordakowski *et al.,* 2001). An analysis of the literature suggests that the faecal recovery of a marker is under the control of numerous factors.

2.7.2.1 Feeding management

The influence of diet composition on faecal recovery is possibly linked to the change in the ratio of alkanes contributed by the different dietary components (Elwert *et al*., 2004). Swainson *et al.* (2005) and Olivan *et al.* (2007) suggested that feeding levels affect the recovery rate of alkanes by modulating the passage rate of the digesta in the animal's digestive tract, with a higher rate of passage leading to a higher alkane recovery rate. However, the n-alkane faecal

concentration in pigs does not seem to be affected by the dietary lipid content (Wilson *et al.,* 1999). In addition, in their study with sheep, Elwert *et al*. (2004) did not find an influence of varying dry matter on faecal alkane recovery when diet composition was constant.

2.7.2.2 Digestive tract physiology

Various studies report a relationship between the faecal recovery of n-alkanes and their carbon chain length in ruminants (Elwert *et al.*, 2004; Olivan *et al.,* 2007; Ferreira *et al.,* 2009), that follows a curvilinear pattern. In fact, faecal recovery of alkanes increase with chain length and similar recovery rates can only be assumed for longer molecules, i.e. possessing more than 31 carbon atoms. On the contrary, the recovery of alkanes in monogastrics seems to be independent of the n-alkane chain length and is higher than in ruminants (Dove & Mayes, 1996; Mayes & Dove, 2000; Ordakowski *et al.,* 2001). However, Hatt *et al.* (2001) observed that faecal recovery in pigeons appears to increase with alkane chain length.

2.7.2.3 Dosing and sampling procedures

In order to ensure effective ingestion and minimise wastage, n-alkanes are generally absorbed into carrier substances/matrices because of the minute amounts the animals are dosed with. It has become apparent that the choice of the carrier matrix used to dose the synthetic alkanes seems to influence their faecal excretion pattern (Mayes *et al*., 1986; Swainson *et al.*, 2005; Olivan *et al.*, 2007) because it regulates the likelihood that these elements react in the digestive tract. In fact there are instances of differences in faecal recovery between natural and the dosed (synthetic) n-alkanes (Hatt *et al.,* 2001). A review of literature shows that paper (Mayes *et al.,* 1986; Sibbald *et al.*, 2000*)*, beeswax (Elwert & Dove, 2005), cake (Mowat *et al.,* 1999), gelatine capsule (Piasentier *et al*., 1995), xantham gum (Fushai, 2006), a mixture of maize flour and sugar cane syrup (Oliveira *et al.,* 2007)*,* have been used with varying results to dose n-alkanes. The use of carrier is intended to reduce the incomplete mixing of dosed alkanes with the gut content and, although both natural and dosed markers' kinetics in the animal's digestive tract should be

similar to that of natural n-alkanes (Sibbald *et al.*, 2000), incomplete marker recovery is a common issue (Smith *et al.,* 2007). Another explanation could be the difference of the bond between the dosed alkane and the carrier matrix and that linking natural alkanes to the plant's cuticular waxes (Elwert & Dove, 2005).

The method used during sample collection is very critical for n-alkane analysis (Newman *et al.,* 1998). The challenge with markers is to collect faecal samples that are not only representative of the daily total output, but which also reflect the dietary n-alkane proportions (Lippke, 2002). The faecal concentration ratio of dosed and herbage alkanes might be influenced by the dosing frequency of the even chain of alkanes (Mayes & Dove, 2000). Whether the provision of the daily dose rate to animals, in one or more occurrences, affects the external alkane excretion pattern needs more examination.

Dove & Mayes (1991) contended that the dosing schedule (once daily vs. twice daily) may affect the diurnal excretion pattern of the dosed n-alkanes. According to Ferraz de Oliveira *et al.* (2007), once or twice daily dosing did not influence the dosed n-alkane's excretion pattern. Mayes *et al.* (1986) also deemed a single dose adequate. In a study with pigs, diurnal variation was not a concern in the work of Wilson *et al*. (1999). In addition, Sibbald *et al.* (2000) found that more accurate intake estimation figures were obtained when sheep were dosed once a day. Mann & Stewart (2003) prescribed that, when using a xantham gum suspension as a carrier matrix, twice-a day dosing regimen should be adopted. The use of controlled-release capsules to address diurnal variation is now widespread in ruminants. They typically ensure a steady delivery rate of markers in the animal's rumen.

Representative samples of the daily faecal output are difficult to obtain; the faecal sampling frequency compounds further the issue of diurnal variation. As Dove & Mayes (1991) point out, it mostly involve changes in the faecal concentration of the dosed n-alkane. This is often due to incomplete mixing of the dosed marker in the animal's gut, caused by the choice of the carrier matrix and/or the diet's part. The challenge of the faecal collection, which is laborious and sometimes impossible in field conditions, was addressed when Vulich & Hanrahan (1995) found that the pooling of faecal samples, by forming

composites of faeces (by constant volume or mass) obtained over several days, provided reliable intake estimates. Olivan *et al*. (2007) suggested that when total collection cannot be done, grab samples obtained once a day at the time of dosing could allow an accurate intake estimation since they represent the n-alkane concentration of the total faecal production.

2.8 Conclusion

The extent of pasture utilisation in monogastrics is subject to its quality and the quantity ingested. Of all the methods of estimation of intake, total faecal collection would prove the most accurate. However, it is labour intensive and its potential to disturb normal feeding behaviour in test animals outweighs the benefits. N-alkanes, among other markers are thus more appropriate for the reason that they allow concurrent estimation of digestibility and faecal output and reduce the extent of inherent biases and disturbances to the animals. However, the suitability of this method for intake estimation is challenged for the reason that one of the principles is that the faecal recoveries of n-alkanes of adjacent carbon length are assumed to be similar. Moreover, challenges relative to the dosing method and the carrier matrix need to be addressed and low concentrations of some n-alkanes in the plants often make them difficult to quantify. In this regard, long chain alcohols abundant in plants cuticular waxes, in conjunction with n-alkanes can be useful to estimate diet composition. In addition, benefits could be gained from extending this to intake estimation and research should be orientated towards that goal.

CHAPTER 3

MATERIALS AND METHODS

3.1 Study area

The experiment was conducted during the dry season of winter 2009 (May and June) in the pig herd facilities of the Agricultural Research Council (ARC) - Irene (S: $25^0$34'0'', E: $28^0$22'0'', altitude 1523 m), South Africa.

3.2 Animals, Housing and Treatments

The use of animals conformed to the guidelines on the welfare and use of animals in research and was approved by the Animal Ethics Committee of the Animal Production Institute of the ARC.

Twenty five crosses of Large White and Landrace male grower pigs (27 ± 3.8 kg), aged 8 weeks, were selected from the ARC Animal Production Institute herd. The pigs were blocked in 5 groups balanced for weight (see Appendix), which randomly contributed only one animal to each one of the five treatment groups (A, B, C, D and E). The procedure was repeated until each treatment received one animal from the 5 different weight groups. The treatments were as follows:

- Indoor treatment (A): Animals from this group received 1500 g (100 %) of the basal diet (3 % of BW).
- Indoor treatment (B): Animals from this group received 1350 g (90 %) of the basal diet together with freshly cut Kikuyu grass from a pasture.
- Indoor treatment (C): Animals from this group received 1200 g (80 %) of the basal ration and freshly cut Kikuyu grass from a pasture.
- Indoor treatment (D): Animals from this group received 1050 g (70 %) of the normal ration together with freshly cut Kikuyu grass from a pasture.

- Outdoor treatment (E): Animals in this group were restrictively fed 1200 g (80 %) of the basal diet and were kept on planted Kikuyu grass paddocks.

The pigs were treated against internal and external parasites using Ivotan® before being moved to the experimental sites. Animals in treatments A, B, C and D were individually housed indoors in 2 m by 1 m pens. Pigs in treatment E were housed individually in 5 m by 4 m paddocks implanted on a Kikuyu grass pasture established the year before. The pasture was irrigated weekly and cut twice before transferring the animals. Drinking water was available through nipples drinkers for both indoor and outdoor animals. This design was chosen to establish two contrasts: firstly by comparing all indoor treatments to assess the influence of decreasing the basal concentrate ration on Kikuyu pasture intake and digestibility and secondly to compare intake and digestibility in treatments C and E, with respect to housing.

3.3 Feeding management and preparation of feeds

The concentrate and Kikuyu grass were provided to the pigs once daily at 09.00 in separate feeding troughs. The respective feed allowances were calculated based on *ad libitum* feeding of the concentrate during the first 14 days of the adaptation period. For each treatment, the pigs were given as much as they could eat and the quantities recorded daily by weighing and recording the amount offered and subtracting leftovers in the morning. If a pig finished all the offered feed then incremental levels of 10 % of the quantity offered the previous day was added until it stabilised. Kikuyu grass was cut from areas adjacent to the outdoor paddocks and stored in a cold room (5 ^{0}C) during the 24 days adaptation period. All the grass fed during the collection period was cut and stored at once, so as to minimize variations in nutritional composition. The basal diet was formulated to exceed the recommended maintenance requirements for young pigs of the National Research Council (NRC, 1998), such that it contained 14.5MJ/kg DM of digestible energy and 18 % of crude protein. The chemical composition of the concentrate ration and the Kikuyu grass are provided below.

Table 3.1: Concentrate and Kikuyu grass nutritional composition (g/kg), C_{32} and C_{33} n-alkane (mg/kg), and energy (MJ/kg) content of the experimental diet (DM)

Parameter	Concentrate	Kikuyu grass
Dry Matter (g/kg)	88.32	93.89
Organic Matter (g/kg)	82.65	83.21
Ash (g/kg)	5.67	10.68
Crude Protein (g/kg)	15.73	19.81
Ether Extract (g/kg)	9.42	1.17
Crude Fibre (g/kg)	3.28	28.3
Acid Detergent Fibre (g/kg)	2.43	28.74
Gross energy (MJ/kg)	18.6	14.9
AIA (g/kg)	0.39	1.22
C_{32} (mg/kg)	9.06	14.9
C_{33} (mg/kg)	5.66	236.44

DM: Dry matter; OM: Organic matter; CP, Crude protein (N x 6.25); AIA: Acid-insoluble ash; EE; Ether extracts; CF: Crude fibre; ADF: Acid detergent fibre; GE, Gross Energy estimated in MJ/kg. N-alkane concentration in mg/kg. Units expressed on wet basis; CP: Crude protein content expressed as Nitrogen x 6.25.

3.4 Alkane marker preparation

Herbage intake was estimated from the ratio of concentrations of a pair of dosed and natural alkanes in the diet, the faeces and the daily alkane dose rate. Adjacent n-alkanes (C_{32} and C_{33}) were chosen to ensure that their faecal recoveries were similar in order to minimise errors (Mayes & Dove, 2000). Animals on treatments B, C and D received decreasing levels of a basal diet together with Kikuyu grass, that contained naturally occurring n-alkanes (notably tritriacontane, C_{33}), and were dosed with a synthetic n-alkane (dotriacontane, C_{32}) using pellets. The dosed n-alkane chains are generally even-numbered as they are in minute quantities in grasses, and thus of negligible effect, in comparison to the large amounts of their odd-numbered

counterparts, which constitute more than 90 % of total n-alkanes (Malossini *et al.*, 1994).

The procedure to produce n-dotriacontane marker doses was a modification of that by Byrd (2003), in which water and wheat flour were used to produce "granola bars". In this trial, wheat flour was replaced with maize meal. Forty five grams (45 g) of dotriacontane (Sigma-Aldrich, RSA) were thoroughly mixed with 963 g of maize meal (IWISA, Premier Foods, RSA). Water (1500 mL) was then added to the mixture and the resulting porridge was heated for 9 minutes in a microwave oven (Kelvinator, 100 % power, 1350 watts) and allowed to cool overnight at room temperature. The porridge was further dried for 15 minutes in the microwave oven (Kelvinator, 100 % power, 1350 watts) the following day. Approximately seven point twenty five grams (7.26) of the porridge were weighed on a Sartorius analytical balance (LE series) and compacted into pellets by hand and kept in a fridge/freezer at -5 ^{0}C until there were fed to the pigs. Subsequently, laboratory analyses showed that the C_{32} content of pellets was in average 167.06 mg.

3.5 Experimental design

A 29 day trial was conducted as a completely randomized design experiment consisting of four treatments of five pigs each fed different levels of concentrate (A,B,C,D) and kept indoors. A fifth group also containing five pigs and fed the same level of concentrate as group C was kept outdoors. A 24-day adaptation period allowing the animals to adjust to the experimental conditions was followed by 5 days of faecal collection. From the 19th day till the end of the experiment, each pig was hand-fed with one maize meal porridge pellet each morning at the same time the concentrate and forage were added to the troughs. This allowed faecal concentration of the markers to stabilise after five days as reported in earlier studies (Ferraz de Oliveira *et al.*, 2007; Olivan *et al.*, 2007). Thereafter, a 5-day faecal collection period was carried out. Faeces were collected once daily every morning within one hour of the feeding time in 300 mL plastic containers and stored in a freezer (-5 ^{0}C). Freshly voided faeces were collected first, followed by rectal samples or alternatively from the floor while taking care to avoid foreign contamination. The faecal samples were

frozen and pooled for each animal at the end of the collection period and a composite sample taken for laboratory analysis.

3.6 Measurements

The concentrate offered, herbage allowances and refusals were recorded daily in indoor pigs just before feeding time and feed intake for the previous day was deducted. Concentrate intake was similarly estimated for the outdoor treatment group. In the outdoor animals, the grass was sampled in areas of the paddock showing evidence of grazing activity, placed in 10 L sterile plastic bags and stored in a freezer (-5 ^{0}C). The pigs were weighed at the beginning and at the end of the trial.

3.7 Chemical analysis

Prior to analysis; concentrate, herbage and pellets samples were pooled for each animal, then thawed and dried in an oven at 60 ^{0}C for 48h, as done previously by Ferre *et al*. (2001) and Kavanagh *et al*. (2001). The concentrate and herbage samples were then ground to a particle size of 1mm before being analysed for dry matter (DM), ash, organic matter (OM), energy (GE), crude protein (CP), crude fibre (CF), neutral detergent fibre (NDF), acid detergent fibre (ADF), and ether extracts (EE) according to the Methods of AOAC (1990).

The DM was determined by oven drying to a constant mass at 100 ^{0}C for 6 hours. Organic matter was determined by the difference between the dry matter and the ash. Ash was determined by ignition of a known amount of the sample (feed and faeces) in a muffle furnace and weighing the remainder. Energy was determined using an adiabatic bomb calorimeter (Parr Instrument Company, IL, USA). The crude protein content was obtained from the Nitrogen (N) content (CP = N *6.25) as estimated from the Kjeldahl analysis according to the standard method (AOAC, 1990). Acid-insoluble ash (AIA) content of the samples was determined by boiling samples (20 g for feed and 10 g for the faeces) in 200 ml HCL for 30 minutes (min), the residue was then filtered through an ashless Whatmann no. 41 filter paper with boiling water to free it from acid and finally ashed in an oven for 6 hours at 650 ^{0}C. NDF and ADF

were determined according to the method developed by Goering & Van Soest (1970).

N -alkane gas chromatograph analysis for intake estimation was performed on dried herbage, concentrate and faecal samples and ten pellets randomly chosen, according to the method outlined by Olivan *et al.* (2001). Samples were saponified by treatment with 1 mol/l Potassium hydroxide (KOH) at 90 ^{0}C for 3 hours and then extracted in a Soxhlet extractor using n-heptane as a solvent at 65 ^{0}C. Aliquots of the solution obtained were purified through a silica gel column, evaporated and re-diluted in n-heptane before gas chromatograph analysis. The extracts obtained were injected (0.5 µL) onto a 15-m DB-1 megabore column (J and W Scientific, USA) of 0.530 mm internal diameter and 1.5 µm film thickness. Helium was used as carrier gas at a constant flow of 15 mL/min. Gradients of temperature were used for the injector (80 ^{0}C for 0.2 min; 200 K/min to 380 ^{0}C) and the column (200 ^{0}C for 1 min; 6 K/min to 300 ^{0}C; 6 min at 300 ^{0}C). The detector oven was maintained at 350 ^{0}C. Quantification of C_{32} and C_{33} was performed using a HP-G1800A GCD equipped with a mass spectrophotometer detector. The chromatograph was calibrated using 5 mL of a commercial standard n-alkane mixture solution in n-undecane (C_{21}-C_{40}; > 99 % pure, Sigma Aldrich, Midrand, RSA). The mixture was processed as a sample and injected with each run in order to monitor GC response. Alkane peaks were identified by reference to known standards. Peak areas were converted to alkane concentrations using the peak area, the known weight of the internal standard, the sample weight and its DM content.

3.8 Calculations

3.8.1 Intake estimation

The concentrate and herbage intake (I) were determined using two procedures:

- Using records of each animal's daily feed allowances and refusals of feed.
- Using the double n-alkane technique with the following formula:

$$I = C_j / (F_j/F_i) * H_i – H_j$$

(Mayes & Dove, 2000; Smit *et al*., 2005)

Where Hi and Fi are the concentrations of the natural odd-chain n-alkane (C_{33}) in the herbage and faeces (mg/kg DM), respectively. Likewise, Hj and Fj are the respective concentrations of the even-chain n-alkane (C_{32}) in the herbage and faeces (mg/kg DM); Dj is the daily dose of the even chain alkane (C_{32}) (mg/day). The calculations of herbage intake and digestibility were made on the basis of OM, so as to minimize errors due to contamination from soil ash (Ferre *et al.*, 2001). The value for the intake of AIA in the treatment E (outdoor pigs) was adjusted using the mean ratio (% AIA $_{diet}$ / % AIA $_{faeces}$) obtained from treatments A, B and C.

3.8.2 Digestibility determination

The diet‘s nutrients digestibility D_N (%) was calculated using AIA and dotriacontane (C_{32}) as internal and external markers, as follows:

$$D_N = 100 [(1- ((\%\ Marker_D /\%\ Marker_F) * (\%\ Nutrients_F / \%\ Nutrient_D))]$$

(Hatt *et al.*, 2001).

Where Marker $_D$ and Marker $_F$ were the concentrations of the internal markers (C_{32} or AIA) in the diet (Concentrate + herbage) and the faeces, respectively. Likewise, Nutrient $_D$ and Nutrient $_F$ were the respective nutrients’ concentrations in the diet (Concentrate + herbage) and the faeces.

3.9 Statistical analysis

A one way, Analysis of Variance (ANOVA) and Analysis of Covariance (ANCOVA) using the Generalised Linear Model procedures of SAS software (SAS, 2008) was performed to compare concentrate and pasture intake and nutrient and the diets digestibility between treatments A, B, C and D. A t-test was carried out to compare treatments C and E. The model used included the concentrate level as the main effect and the initial weight of the animal as a

covariate and one way ANOVA was used to compare concentrate and pasture intake and digestibility of the various nutrients.

$$Y_{ijk} = \mu + C + W + e_{ijk}$$

Where Y_{ijk} is the dependant variable, μ is the overall mean, C is the effect of the concentrate level, W is the effect of the animal's weight and e_{ijk} is the experimental error variable, assumed to follow a normal distribution. The comparison of treatment means was done using Fisher's Least Significant Difference (LSD) test.

A regression analysis was performed using PROCREG in SAS (SAS, 2008) to test for relationships between variables, according to the following model.

$$Y = \alpha_o + \alpha_1 X_1 + \alpha_2 X_2 + \alpha_3 X_3 + \alpha_n X_n + \varepsilon$$

Where y is the dependant variable of interest, α_o represents the intercept, $\alpha_{1;}$ α_2 and α_n are the respective coefficients associated to the independent variables to be tested X_1 … X_n and ε is the error. All data were tested for normality and homogeneity and comparisons were made at the 95 % significance level ($P < 0.05$).

CHAPTER 4

RESULTS

4.1 Growth performance

The initial and final weights and average daily gain of the pigs in the trial are shown in Table 4.1. The mean starting weight (SW) at the beginning of the intake estimation period and the final (FW) weight for the trial were 46.9 and 50.2 kg, respectively. There were no significant differences in SW and FW among the treatments ($P > 0.05$). Although the average daily gain (ADG) means were not different between treatments, there was a highly significant ($P < 0.01$) correlation between ADG and the animal's weight (SW and FW).

Table 4.1 Weight changes and average daily gain (LS Mean, kg) in pigs fed 100, 90, 80 and 70 % of voluntary concentrate levels and Kikuyu grass *ad libitum* over 29 days

	Parameter		
	Starting weight (kg)	Final weight (kg)	Average Daily Gain (Kg DM/ day)
Treatments			
A	46.45	50.08	0.62
B	46.32	54.50	0.81
C	47.53 (47.53)	48.48 (48.48)	0.65 (5.73)
D	49.12	49.66	0.67
E	(45.33)	(48.50)	(0.63)
SEM	1.70	3.99	0.10
P-value	0.58	0.23 (0.995)	0.13 (0.654)
Significance	ns	ns (ns)	ns (ns)
r^2	-	0.757	0.088
CV	-	9.064	5.148

Treatments A, B, C, D and E received 100, 90, 80, 70 and 80 % of the basal concentrate ration. Values in brackets were from a t-test between treatment C and E. Means on the same row with different superscripts differ significantly P< 0.05. LS mean - Least Squares Mean; SE - Standard error of the mean; ns: non-significant; * P< 0.05; ** P < 0.01.

4.2 Concentrate and forage intake

The concentrate, Kikuyu grass and acid-insoluble ash intake (LS Mean ± SEM kg OM) of pigs fed 100, 90, 80 and 70 percent of voluntary concentrate levels and Kikuyu *ad libitum* are shown in Table 4.2. Mean concentrate intakes were similar ($P > 0.05$) between treatments A, B, and C but higher ($P < 0.01$) than in treatment D. In addition, the concentrate intake (CI) was not different in treatments C and E. Acid-insoluble ash intake was significantly ($P < 0.05$) higher in outdoor animals in comparison to indoor pigs. There was no linear correlation between the mean recorded Kikuyu grass intake (RKI) in treatments B, C and D (0.298; 0.307 and 0.304 kg OM /day respectively) and CI ($P < 0.05$) (Table 4.3). In addition, there was no linear correlation between the animals' starting weight (SW) and RKI ($P < 0.05$) (Table 4.3). The mean n-alkane intake estimates of Kikuyu grass (EKI) (0.190; 0.205 kg OM /day for the treatments B and C) were also not significantly different ($P > 0.05$) and were poorly correlated to the concentrate intake (CI) (Table 4.2; Figure 3.1). In contrast, EKI was lower in treatment E than in treatment C. In treatments B and C, RKI was higher than EKI ($P < 0.05$). In general, except for two pigs, n-alkane intake estimates were lower than the recorded intake. The difference between recorded and estimated intake was 0.076 ± 0.032 (Table 4.2).

Table 4.2 Concentrate (CI), recorded (RKI) and estimated (EKI) Kikuyu grass intake (LS Mean kg OM) for pigs fed 100, 90, 80 and 70 % of voluntary concentrate levels and Kikuyu grass *ad libitum*

	Parameter						
	Concentrate level (%)	Concentrate intake (kg OM)	Recorded Kikuyu intake	Estimated Kikuyu intake	Difference RKI-EKI (kg	Correlation CL-RKI	Acid-insoluble ash intake (%)
Treatment							
A	100	0.912^{ab}			0.076	-	0.42^{a}
B	90	0.921^{ab}	0.297	0.190			0.66^{a}
C	80	0.818^{b} (0.989^{a})	0.307	0.205 (0.301^{a})			0.68^{a}
D	70	0.649^{d}	0.304	-			0.73^{a}
E	80	(0.992^{a})	-	(0.131^{b})			^{1}8.11^{b}
SEM		0.07 (0.002)	0.016	0.102	0.032		-
P-value		< 0.001 (0.169)	0.636	0.402 (0.001)	0.042	0.487	0.007
Significance		** (ns)	ns	ns (**)	*	ns	**
r^2		0.757	0.088	0.153			
CV		9.064	5.148	51.416			
R						0.192	

Treatments A, B, C, D and E received 100, 90, 80, 70 and 80 % of the basal concentrate ration respectively. Values in brackets are from a t-test analysis between treatment C and E. Means on the same column with different superscripts differ significantly P < 0.01. CV- coefficient of variation; [1] Adjusted values using mean (% AIA $_{Diet}$ / % AIA $_{Faeces)}$ ratio from treatments A, B and C. SEM - Standard error of the mean. ns: non-significant; * P< 0.05; ** P < 0.01.

Table 4.3 Simple correlation coefficients (with p-value) of concentrate intake (CI), starting weight (SW), estimated Kikuyu grass intake (EKI) and recorded Kikuyu grass intake (RKI) of grower pigs

	CI	**SW**	**EKI**	**RKI**
CI	-			
SW	-0.388 (0.915)	-		
EKI	-0.616 (0.104)	-0.300 (0.470)	-	
RKI	-0.266 (0.458)	-0.305 (0.458)	0.232 (0.580)	-

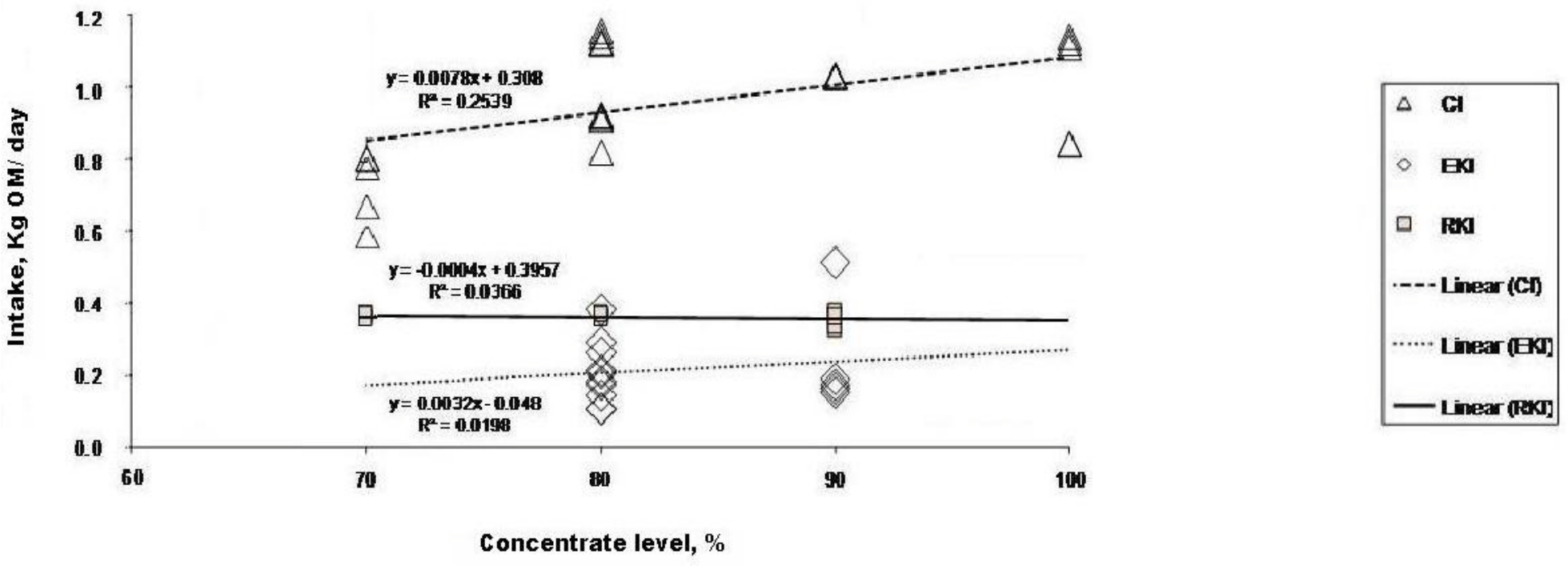

Figure 4.1 Graph of the concentrate intake (CI), recorded (RKI) and estimated (EKI) Kikuyu grass intakes in grower pigs fed 100, 90, 80 and 70 % of the concentrate ration.

4.3 Nutrients and total diet digestibility

Organic matter digestibility data for the diets, obtained using AIA and C_{32} are reported in Table 4.4. Pigs in treatment D (70 % concentrate + *ad lib* Kikuyu) group were not dosed with n-alkanes. Consequently that treatment was not included in analyses of OMD estimates using C_{32} or AIA. The AIA OMD digestibility estimates were higher than C_{32} estimates ($P < 0.01$). There were no differences ($P > 0.05$) for all other estimates AIA OMD, C_{32} (OMD). Nutrient digestibility data for the diet using AIA marker are reported in Table 4.5.

Digestibility coefficients of CP were not different ($P > 0.05$) between A, D and E but were lower ($P < 0.05$) than treatment B. On the other hand, treatment B mean was higher than in treatments A, D and E and not different from treatment that of C. Energy and EE digestibility values were not affected by the different dietary treatments ($P > 0.05$). Digestibility values for fibrous components (CF, ADF and NDF) were similar among treatments B, C and D, but higher than those of treatment A. Values in treatments C were significantly higher than those of treatment E.

Table 4.4 Organic matter (OMD) digestibility (%) of diets calculated using acid-insoluble ash (AIA) and dotriacontane C_{32} markers

	Organic matter digestibility AIA (%)	**Organic matter digestibility C_{32} (%)**	**Difference OMD $_{AIA}$ - OMD C_{32}**
Treatments			
A	78.546	68.635	17.231
B	83.814	64.248	
C	82.618 (82.083)	63.467 (63.467)	
E	(80.413)	(60.267)	
SEM	3.401 (2.775)	10.148 (3.398)	-
P-value	0.114 (0.265)	0.638 (0.518)	< 0.0001
Significance	ns	ns	**
r^2	0.301	0.097	-
CV	4.182	15.818	2.533

Treatment A received only the basal concentrate ration; B, C and D received 90, 80 and 70 % of the basal ration plus *ad libitum* Kikuyu grass. Treatment E received 80 % of the basal ration on a Kikuyu pasture. Values in brackets are from a t-test analysis between treatment C and E. P-value obtained from a t-test comparison between mean AIA and C_{32} digestibility estimates for treatments A, B, C and E. SEM, standard error of the mean determined as the square root of the MSE. ns: non-significant; * P< 0.05; ** P < 0.01.

Table 4.5 Digestibility coefficients (LS Means, OM) of nutrients and energy of the experimental diet using acid-insoluble ash as a marker, OM basis

	Parameter					
	Crude Protein	Energy	Ether extract	Crude fibre	Neutral Detergent Fibre	Acid Detergent Fibre
Treatments						
A	69.213[a]	73.985	65.617	29.406[a]	49.464[a]	-
B	80.309[b]	80.730	77.952	75.413[b]	73.001[b]	68.358[a]
C	76.901[ab]	79.323	77.586	74.655[b] (73.373[a])	73.984[b] (73.310 [a])	66.662[a] (64.333 [a])
D	69.786[a]	74.128	70.421	71.742[b]	68.729[b]	64.487[a]
E	(71.626[b])	(77.968)	(77.603)	(53.876[b])	(58.320 [b])	(35.321 [b])
SEM	5.617 (1.581)	4.715 (1.547)	9.076 (2.817)	8.873 (4.311)	7.355 (2.304)	17.808 (8.87)
P-value	0.027 (0.03)	0.082 (0.407)	0.195 (0.805)	< 0.0001 (0.005)	0.0004 (0.001)	< 0.0001 (0.015)
Significance	*	ns	ns	**	**	**
r^2	0.425	0.347	0.267	0.822	0.663	0.834
CV	7.623	6.105	12.227	14.553	11.385	42.514

Treatment A received only the basal concentrate ration; B, C and D received 90, 80 and 70 % of the basal ration plus *ad libitum* Kikuyu grass. Values in brackets are from a t-test analysis between treatment C and E. Treatment E received 80 % of the basal ration on a Kikuyu grass pasture. Means with different superscripts significantly differ; SEM - Standard error of the mean. ns: non-significant; * P< 0.05; ** P < 0.01.

4.4 Estimate of digestibility and intake parameters through regression technique

Regression equations in which selected independent variables explained at least half the variation of the digestibility parameters ($R^2 \geq 0.5$), are reported in Table 4.6. Generally, nutrients and organic matter digestibility coefficients were all affected by the diet's fibre content (CF or NDF) and the animal's starting weight. Digestibility of CP was found to be affected by NDF, SW, the diet energy content (E) and its total intake (TI). The digestibility of fibrous components (CF, NDF and ADF) was affected by the animal's weight (SW) and the dietary crude fibre level (CF). Energy digestibility (DE) was influenced by CF, FW and CP. The best correlation was found for the organic matter digestibility (0.99), which was dependent on SW, NDF, CP. TI and E.

Table 4.6 Relationships between digestibility parameters and selected independent variables in treatments B, C D and E

Parameter	[1]Equation	R^2	P-value	Significance
PD	0.1626*NDF + 0.034*SW + 1.011*E + 0.2929*TI – 12.7911	0.77	< 0.0001	**
CFD	- 0.40696*SW + 5.82301*CF + 22.6875	0.76	< 0.0001	**
NDFD	- 0.1641*SW + 2.881*CF + 44.7427	0.52	0.0003	**
ADFD	- 0.6821*SW + 11.8177*CF – 39.6777	0.76	< 0.0001	**
DE	- 0.33225*CF + 0.243414*SW + 0.606601*PD + 29.5333	0.84	< 0.0001	**
OMD	- 0.0082*SW – 0.0447*NDF + 0.9978*CP -2.2768*TI + 0.8311*E + 5.8333	0.99	< 0.0001	**

R^2- fraction of the dependant variable's variance predicted by the independent variables; [1]Follows linear regression model $y = \alpha_0 + \alpha_1 X_1 + \alpha_2 X_2 + \alpha_3 X_3 + \alpha_4 X_4 \ldots$ with X_1 = CF, X_2 = SW and α_0 = intercept; CF: dietary crude fibre percentage; NDF: neutral detergent fibre percentage; E: energy (MJ/kg); TI: total diet intake; PD: protein digestibility (%); CL: concentrate level; RKI: recorded Kikuyu grass intake (g) ; CI: concentrate intake (g); SW: starting weight (kg). ** P < 0.01.

CHAPTER 5

DISCUSSION

5.1 Pasture nutritional value

The experiment was conducted in winter, when Kikuyu grass which is a summer growing species becomes dormant (Rautenbach *et al.,* 2008). Nonetheless the Kikuyu grass sampled was atypical of a warm-season forage sampled during winter. In fact, the forage CP content (19.8 %) in the current study compared well with those reported by Miles *et al.* (2000) (21.06 %), for fertilized Kikuyu grass pastures sampled during summer. It was also similar to the value by Meeske *et al.* (2006) observations, (21.8 %) in an experiment conducted during winter. The forage CP values were higher than the 11.9 % reported in Rautenbach *et al*. (2008) (11.9 %).

In comparison with other experiments, NDF and ADF contents in the present study (59.6 and 28.7 %, respectively) were lower than values from Rautenbach *et al*. (2008) (75.6 and 36.1 %) and Fushai (2006) (66.7 and 33.2 %), but comparable with those of Meeske *et al*. (2006) (59.2 and 29.1 %). As reported by Marais (2001), this favourable nutritional profile is most likely related to the environmental and climatic conditions on site. In fact it is not unusual to find fairly green Kikuyu if grown under relatively warm winter conditions (Rautenbach *et al.*, 2008).

5.2 Dotriacontane and tritriacontane profile of Kikuyu grass

The n-alkane profile of the Kikuyu grass in this experiment was different from that in the studies of Stevens *et al.* (2002) and Marais (2001), who reported a forage with a lower C_{32} and higher C_{33} contents (6 vs.14.9 and 272 vs. 236.9 mg/kg, respectively). N-alkanes, like other leaf wax constituents play a role in water retention mechanisms (Smit, 2005). Consequently, the difference between n-alkanes patterns is likely to be due to environmental factors

influencing the plant's hydric balance such as soil properties and climate (Zhang *et al.,* 2004) and also by the plant's age or physiological stage (Dove & Mayes, 1996; Mayes & Dove, 2000; Ali *et al.*, 2005). Nevertheless, these findings confirm the observation that grasses contain very low levels of even-chain alkanes, in comparison to their odd-chain adjacent counterparts (Piasentier *et al*., 2000).

5.3 Concentrate intake

Mean concentrate intake (CI) values differences were not significant between treatments A, B and C, which received 100 %, 90 % and 80 % of the basal concentrate ration, respectively, but differed from treatment D. However correlation analysis indicated a highly significant relationship between the concentrate level provided (CL) and CI; as a result, CI tended to decrease from treatment B (90 %) to treatment D (70 %). From this, it is apparent that a concentrate allowance decrease up to a maximum of 80 % of requirements does not significantly affect concentrate intake but a 30 % decrease will have a negative impact on concentrate intake. Intake of concentrate differed ($P < 0.05$) between treatments C and E, which both received 80 % of the concentrate. In general, treatment E animals on outdoor camps consumed all of their daily concentrate allowance (0.99 kg/day OM) whereas pigs from the indoor group (treatment C) consumed 0.82 kg/day OM. The analysis model confirmed that concentrate intake differences were not caused by the animal's weight. It is suggested that concentrate intake increase in the outdoor treatment is a response to an increased energetic demand caused by environmental conditions.

Outdoor pigs are stimulated by their surroundings and for the most part engage in exploratory and rooting activities (Edwards, 2003; Presto *et al.,* 2009) that are known to increase their daily energy requirements (Stern & Andresen, 2003; Millet *et al.,* 2004). Additionally, nutrient intake generally increases to fuel body heat production when environmental temperature drops below the animal's thermo-neutral zone (Forbes, 1995). The pig's thermo-neutral zone lies within the 19-25 ^{0}C range. During this trial, outdoor animals were exposed to cold weather especially in the early morning; minimum temperature fluctuated in the

range 0-10 ^{0}C. They were thus likely to improve their feed intake to achieve thermoneutrality. Furthermore, outdoor pigs experienced longer exposure to day light periods than indoor animals; giving them the opportunity to increase their feeding time (Forbes, 1995). These results are consistent with the findings from Sather *et al.* (1997) that pigs reared outdoors during winter have an increased feed consumption.

5.4 Pasture intake

One assumption in this investigation was that decreasing the concentrate level in the different treatment groups would drive higher the intake of forage. However results showed that differences were not significant between treatments B and C for both recorded (RKI) and estimated (EKI) Kikuyu grass intakes. They were also independent from CL or CI. Comparable observations were reported by Ferre *et al*. (2001) during their investigation. In contrast, Stern & Andresen (2003) reported an increase in forage intake in outdoor pigs, when the basal concentrate allowance was decreased by 20 %. Also, a review by Edwards (2003), [citing Danielsen *et al.,* 1999] reported an increase in forage intake in growing pigs when concentrate intake was restricted to 70 % of the normal allowance. In addition, despite a lower CI in treatments D, RKI was similar among treatments B, C and D. The lower EKI for treatment E than in treatment C is likely the effect of ash ingestion (see discussion on the effect of AIA on digestibility below)

In the work of Ferre *et al.* (2001), when forage quality declined in summer, there was a substantial increase in forage intake. In that report, as well as in this study, the forage provided to the animals was in excess of their daily intake. It thus seems that the range of concentrate levels provided was not wide to such an extent that it would show significantly differences in terms of forage intake. This suggests that the intake of forage is partially dependant on its nutritive value.

Another explanation is that there is an upper limit to forage intake. In this experiment, the increasing fibre level could have diluted the diet's nutrient content and it is recognized that pigs are not efficient at increasing their intake

in response to reduced dietary nutrient concentration (Edwards, 2003). Still it would be difficult to explain the constant concentrate intake decrease from treatment B to D.

In their experiment, Carlson *et al.* (1999) estimated forage intake in gilts (30 kg) to be 18-19 % of the total daily DM intake, roughly 22 ± 1 % of the concentrate intake (DM). A similar contribution of freshly cut herbage 22 % DM was also reported by other workers (García-Valverde *et al*., 2007) in heavy-weight Iberian pigs fed cut grass and acorns. The present study is in general agreement with these figures; EKI constituted 20, 25 and 18 % of the concentrate intake, in treatments B, C and E, respectively, on an OM basis. Roughage intake was however limited in a study where concentrate was provided *ad libitum* (0.1 kg OM, 4 % of daily OM intake, Mowat, *et al.,* 2001).

5.5 Growth performance

The average daily gain (ADG) was similar in all groups and not correlated to CI, EKI or RKI. The animals' initial weight (IW) did not significantly differ between these treatments. Despite that, ADG in treatment B was observed to be marginally higher than other treatments. Despite consuming the same amount of concentrate as in treatment A, animals in treatment B also received forage, such that they had the highest mean daily nutrient intake among all the indoor treatments. Treatments C and D had similar ADG. That is remarkable since ADG was found to be influenced by the dietary CF level, which increased in treatment D, due to the larger contribution of forage to the total nutrient intake. In fact, an increase in dietary fibre content is recognised to negatively affect growth performance (Pluske *et al.,* 2003).

This abnormality could be partially explained by the forage's good nutritional value, which was able to sustain growth, despite the deleterious effect of dietary fibre. Similar growth rate between treatment C and E contradict results of Sather *et al.* (1997) and Hoffman *et al.* (2003) who reported a lower ADG in animals reared outdoors when compared to confined animals. Except for treatment B, the ADG in the present study was comparable to findings by Stern

& Andresen (2003) in 50 kg pigs foraging on a mixed pasture and receiving 80 % of the indoor concentrate requirements.

5.6 Energy and nutrients digestibility

The difference between digestibility values in treatments A and B attests to a notable improvement of nutrient digestibility when forage was provided to the animals. Analyses showed that this effect is related to the fibre intake. In fact, a steady supply of forage to an animal leads to an increase of the fibrous substrates in its diet, which would therefore promote the growth of fibre-fermenting microflora in that animal's hindgut (Varel, 1987; Varel & Yen, 1997; Yen *et al.,* 2004; Castillo *et al*., 2007). However, it is also well understood that as dietary fibre intake increases, digestive function is impaired. In fact, increasing a diet's fibre content is associated with a decrease of nutrient digestibility (LeGoff & Noblet, 2001; Ndindana *et al*., 2002; Wilfart *et al.,* 2007a).

In the present study, Kikuyu grass intake was not different between treatments, but CI diminished from treatment B to treatment D. This must be attributable to an increase of the forage contribution to the total diet, which hampered nutrients digestibility and explains the declining trend observed for the crude protein, crude fibre, neutral and acid detergent fibre in this investigation.

High herbage intake is regarded as responsible for a decline of a diet's digestibility (Leterme *et al.,* 2006; Santos Ricalde & Lean, 2006; Lindberg & Andersson, 1998). The effect of fibre on CP digestibility could either be due to increased endogenous nitrogen losses in the animal's gastrointestinal tract (LeGoff *et al.*, 2003) or a relative increase of the less digestible nitrogen bound to the forage's cell wall (An *et al.,* 2004). The lack of effect of dietary fibre on ether extract digestibility in the present study is in contrast with the expected reduction in fat (ether extract) digestibility described in the review by Degen *et al.* (2007).

Dietary fibre (CF, NDF and ADF) digestibility was generally lower in treatment E (outdoor pigs) than treatment C, in which animals received the same amount of concentrate. It is suggested that this is due to the intake of ash (soil). In fact,

AIA intake in outdoor pigs was higher than in treatment C. It was not possible to determine ash intake in outdoor pigs per se, however the amount of faecal AIA provides a good indication of the ash level in the animals' diet.

Observations as well as the AIA analysis of faecal samples collected from outdoors pigs and their darker appearance also attested of a high intake of soil. Silica is the major constituent of AIA (McDonald *et al.,* 1995; Mayes & Dove, 2000). The silica content of forage has been linked to a decreased nutrient utilisation, by reducing the mechanical breakdown of the cell wall (Hunt *et al.,* 2008), thus making the cell content inaccessible to further degradation. Although a good source of essential elements, soil ingestion negatively affects availability of nutrients in the digestive tract of the animal (Miller *et al.,* 1977). In this study the consumption of soil by the animals might have influenced digestibility in that manner or alternatively, by increasing the digesta bulk such that the transit time was shortened. Nutrient digestibility values for CF, NDF and ADF in indoors treatments receiving forage were not significantly different; largely because fibre intake between these treatments did not differ to such an extent that it would affect significantly digestibility.

Energy digestibility was similar across treatments. Due to the presence of grains, the concentrate fraction is the main energy source in pig diet (McDonald *et al.,* 1995). A decrease in the concentrate intake would therefore influence energy intake in the animal. However animals were also offered forage, which is known to contribute to their energy balance (Edwards, 2003), by the fermentation of dietary fibre in the animal's hindgut, which produces volatile fatty acids (Jørgensen *et al*., 2007). Nevertheless, intake of forage did not increase as expected, so as to compensate for the lower energy intake from the concentrate. This explains the marginally lower energy digestibility value for treatment A and the tendency to decrease observed from treatment B to D. The trend observed in this study is consistent with the decline of energy digestibility with the increasing inclusion of forage in the diet (Lindberg & Andersson, 1998). However, as for digestibility of nutrients, the extent and significance of such a decline could depend on the type, chemistry and origin of the dietary fibre (Reverter *et al*., 1999; Len *et al.*, 2007).

5.7 Use of n-alkanes to estimate voluntary intake

Another objective of the present investigation was to test the suitability of the n-alkane technique for estimating forage intake. There is a scarcity of studies comparing the n-alkane method to the actual intake (Mann & Stewart, 2003)**.** The recorded forage intake was estimated by recording the daily forage disappearance from the pen feeding troughs. Unfortunately, forage was usually scattered and would fall down the pens' perforated floor. This is because, unlike in normal conditions where forage is firmly attached to the soil, pigs had difficulties biting and cutting loose forage. This was remedied by cutting the grass offered to smaller portions and putting wasted forage back in the troughs. However there was a possibility that forage lost in that manner was incorrectly recorded as consumed by the animal. Kikuyu grass intake estimated by n-alkane was lower than the RKI. This difference corresponded to an underestimation of intake by 25.7 %. For the reasons explained above, it cannot be stated beyond doubt that RKI accurately reflected the animal's actual intake and it is more likely that it was lower than RKI. In that respect, the 25 % underestimation should be taken very cautiously. It is plausible that the difference between the real forage intake and EKI could be less than the estimated value, making the n-alkane a reliable method of measuring the intake of growing pigs receiving a mixed diet.

5.8 Comparison of markers to estimate digestibility

As explained above, digestibility improves after an initial adaptation period when animals are exposed to fibrous diets (Lindberg & Andersson, 1998) and would justify the lowest diet digestibility value obtained in treatment A using AIA as a marker. In contrast, C_{32} digestibility estimates were not different from other treatments. This contradicts the concept that prolonged exposure to fibrous diet improves digestibility. This is not a true reflection of reality, but rather suggests that n-alkane digestibility estimates were sensitive to the diet nutritional composition, more specifically to the inclusion of forage in the diet. In this study, the effect of the mixed diet on faecal n-alkanes concentration may have been different from when the concentrate is fed alone. Indeed, Ordakowski *et al*. (2001) observed that certain faecal n-alkane's concentrations in horses were

affected by the diet. In addition, the influence of diet composition on faecal recovery has been frequently reported in the literature, for ruminants and monogastrics (Piasentier *et al.,* 1995; Mayes & Dove, 2000; Elwert *et al*., 2004; Olivan *et al.,* 2007)

Recovery rate is an excellent measure of a marker's efficacy and is generally useful to establish relative comparisons between markers. However in the present study it was not possible to suitably estimate faecal recovery of AIA and n-alkanes. Thus it is only adequate to establish a partial comparison of these two markers. Moreover, a given marker is not necessarily appropriate for the concurrent estimation of both intake and digestibility. In fact, in the investigation of Olivan *et al.* (2007), the marker pairs giving the best intake estimation were the least suitable for the assessment of digestibility. From the above, it is concluded that AIA was a superior marker to C_{32}, as far as the estimation of digestibility was concerned.

CHAPTER 6

CONCLUSIONS AND RECOMMENDATIONS

Various objectives were set out in this investigation, of which the estimation of Kikuyu grass intake was central. In that perspective, it was hypothesized that forage intake estimation would provide similar figures, irrespective of the determination method. On the contrary, the recorded forage intake and the n-alkane estimate were found to be different. It is however necessary to understand that in the context of the present investigation, this difference should not be considered as a measure of the effectiveness of the alkane technique; since the faecal recovery was not estimated and because of uncertainties regarding the recorded forage intake. In contradiction with one of our hypothesis, but nevertheless in accord with some reports in the literature, it was found that decreasing the concentrate allowance had no effect on the consumption of forage in growing pigs, irrespective of the method employed to determine intake.

The determination of diet and nutrient digestibility was another objective of this trial. The higher digestibility values obtained for acid-insoluble ash than dotriacontane refuted our supposition that the both markers would be give similar digestibility figures. Tritriacontane (C_{33}) was not suitable for digestibility determination and thus was omitted from the present report. In agreement with our hypothesis, decreasing the proportion of concentrate in the diet caused a reduction in the digestibility of nutrients, although in the case of energy, the extent of concentrate decline did not significantly affect digestibility.

An important finding was that the average daily gain seemed to improve in pigs fed *ad libitum* Kikuyu grass when the basal concentrate ration is decreased to 90 %. Based on these preliminary results, it is suggested that a decrease between 10 and 20 % of the concentrate ration would enable pork producers to strike a balance between growth performance and financial return. This tendency needs to be further investigated.

A decrease of down to 70 % of the basal concentrate ration did not significantly stimulate Kikuyu grass intake. Further examination is also required to assess the extent and range of concentrate decrease that would stimulate forage intake. Special attention should be paid to the nutritional content of the forage used since this study seems to suggest that forage intake is more dependant on its nutritional properties than the concentrate’s nutritional value.

REFERENCES

Ali, H. A. M., Mayes, R. W., Hector, B. L. & Orskov, E. R. 2005. Assessment of n-alkanes, long-chain fatty alcohols and long-chain fatty acids as diet composition markers: The concentrations of these compounds in rangeland species from Sudan. *Animal Feed Science and Technology*. 121: 257-271.

An, L. V., Thu Hong, T. T. & Lindberg, J. E. 2004. Ileal and total tract digestibility in growing pigs fed cassava root meal diets with inclusion of fresh, dry and ensiled sweet potato (*Ipomoea batatas* L. (Lam.)) leaves. *Animal Feed Science and Technology*. 114: 127-139.

Association of Official Analytical Chemists (AOAC). 1990. *Official methods of analysis, 15th Edition*. Washington DC: AOAC.

Bach Knudsen, K. E. 2001a. The nutritional significance of 'Dietary fibre" analysis. *Animal Feed Science and Technology*. 90: 3-20.

Bach Knudsen, K. E. 2001b. Influence of feed and feed structure on disease and welfare of pigs. In: Hovi, M., Baars, T. 2001. *Breeding and feeding for animal health and welfare in organic livestock systems*. Proceedings of the Fourth Network for Animal Health and Welfare in Organic Agriculture workshop. NAHWOA, Wageningen, 24-27 March 2001.

Baloyi, J. J., Ngongoni, N. T. & Hamudikuwanda, H. 2006. Voluntary intake, nitrogen metabolism and rumen fermentation patterns in sheep given cowpea, silverleaf desmodium and fine-stem stylo legume hays as supplementary feeds to natural pasture hay. *African Journal of Range and Forage Science.* 23: 191-195.

Baloyi, J. J., Acamovic, T., Odoardi. M., Berardo, N., Ngongoni, N. T. & Hamudikuwanda, H. 2007. Condensed tannins and saponin content in different plant parts of *Stylosanthes scabra* (Fitzroy) plants harvested from agro-ecologically different sites in Zimbabwe. *African Journal of Range and Forage Science.* 24: 149-154.

Baurhoo, B., Ruiz-Feria, C. A. & Zhao X. 2008. Purified lignin; Nutritional and health impacts on farm animals: A review. *Animal Feed Science and Technology*. 144: 175-184.

Byrd, B. M. 2003. Alkanes as internal and external markers in horses and the digestibility of a high fat cereal by-product. Msc dissertation, Virginia Polytechnic Institute and State University. Virginia, USA.

Carlson, D., Laerke, H.N., Poulsen, H.D. & Jørgensen, H. 1999. Roughages for growing pigs, with emphasis on chemical composition, ingestion and faecal digestibility. *Acta Agricultura Scandinavica, Section A.* 49: 129-136.

Castillo, M., Martín-Orúe S. M., Anguita, M., Pérez, J. F. & Gasa, J. 2007. Adaptation of gut microbiota to corn physical structure and different types of dietary fibre. *Livestock Science*. 109: 149-152.

Chaves A.V., Waghorn G. C., Brookes I. M. & Woodfield D. R. 2006. Effect of maturation and initial harvest dates on the nutritive characteristics of ryegrass (*Lolium perenne* L.). *Animal Feed Science and Technology*. 127: 293-318.

Cheeke, R.P. 1995. Endogenous toxins and mycotoxins in forage grasses and their effects on livestock. *Journal of Animal Science*. 73: 909-918.

Cheeke, R. P. 2005. *Applied Animal nutrition: Feeds and feeding.* (3rd ed). New Jersey: Pearson Education.

Degen, L., Halas, V. & Babinszky, L. 2007. Effect of dietary fibre on protein and fat digestibility and its consequences on diet formulation for growing and fattening pigs: A review. *Acta Agriculturae Scandinavica, Section A*. 57: 1-9.

Donaldson, C. H. 2001. *A practical guide to planted pasture*. Cape Town: Kalbas publishers.

Dove, H. & Mayes, R. W. 1991. The use of plant wax alkane as marker substances in studies of the nutrition of herbivores: a Review. *Australian Journal of Agricultural Research.* 42: 913-952.

Dove, H. & Mayes, R. W. 1996. Plant wax component: A new approach to estimating intake and diet composition in herbivores. *Journal of Nutrition*. 126: 13-24.

Dove, H. & Mayes, R. W. 2005. Using *n*-alkanes and other plant wax components to estimate intake, digestibility and diet composition of grazing/browsing sheep and goats. *Small Ruminant Research.* 59: 123-139.

Echeverria, V., Belmar, R., Ly, J. & Santos-Ricalde, R. H. 2002. Effect of *Leucaena leucocephala* leaf meal treated with acetic acid or sodium hydroxide on apparent digestibility and nitrogen retention in pig diets. *Animal Feed Science and Technology.* 101: 151-159.

Edwards. S. A. 2003. Intake of nutrients from pasture by pigs. *Proceedings of the Nutrition Society*. 62: 257-265.

Elwert, C. & Dove, H. 2005. Estimation of roughage intake in sheep using a known daily intake of a labelled supplement. *Animal Science*. 81: 47-56.

Elwert, C., Kluth, H. & Rodehutscord, M. 2004. Effect of variable intake of alfafa and wheat on faecal alkane recoveries and estimates of roughage intake in sheep. *Journal of Agricultural Science*. 142: 213-223.

Fergusson, N. S., Bradford, N. M. V. & Gous, R. M. 2002. Diet selection priorities in growing pigs offered a choice of feeds. *South African Journal of Animal Science*. 32: 136-143.

Fernandez, M. I. & Woodward, B. W. 1999. Comparison of conventional and organic beef production systems I. Feedlot performance and production costs. *Livestock Production Science*. 61: 213-223.

Ferraz de Oliveira, M. I., Mendes, C. A., Ribeiro, T. & Cancela d'Abreu, M. 2007. Study for the validation of the n-alkane technique to estimate intake and digestibility of acorns and pasture by "Alentejano pigs". In: *Proceedings of the British Society of Animal Science,* April 2007, Southport, p 127*.* Penicuik: British Society of Animal Science.

Ferre, M. G., Edward, S. A., Mayes, R. W., Riddoch, L. & Hovell, F. D. DeB. 2001. The effect of the season and the level of concentrate on the voluntary intake and digestibility of herbage by outdoor sows. *Animal Science*.72: 501-510.

Ferreira, L. M. M., Celaya, R., Garcia, U., Rodrigues, M. A. M. & Osoro, R. 2009. Differences between domestic herbivore species in alkane faecal recoveries and the accuracy of subsequent estimates of diet composition. *Animal Feed Science and Technology*.151: 128-142.

Forbes, J. M. 1995. *Voluntary intake and diet selection in farm animals.* Oxon, UK: CAB International.

Frape, D. 2010. Equine Nutrition and Feeding. (4th Ed). John Wiley and Sons. http://books.google.co.za/books?id=qTtgmCp5izQC&dq=Kikuyu+%2B+toxins&source=gbs_navlinks_s. [Accessed: 25 september 2010].

Fulkerson, W. J., Nandra, K.S., Clark, C.F. & Barchia, I. 2006. Effect of cereal-based concentrates on productivity of Holstein-Friesian cows grazing short-rotation ryegrass (*Lolium multiflorum*) or Kikuyu (*Pennisetum clandestinum)* pastures. *Livestock Science*. 103: 85-94.

Fushai, F.M. 2006. Estimates of intake and digestibility using *n*-alkanes in yearling Holstein-Friesian and Hereford heifers grazing on Kikuyu (*Pennisetum clandestinum*) pasture. *Animal Feed Science and Technology*. 128: 331-336.

García-Valverde, R., Nieto, R., Lachica, M. & Aguilera, J. F. 2007. Effect of herbage ingestion on the digestion site and nitrogen balance of heavy Iberian pigs fed an acorn-based diet. *Livestock Science*. 112: 63-77.

Gatel, F. 1994. Protein quality of legumes seeds for ruminants animals: a literature review. *Animal Feed and Science Technology*. 45: 317-348.

Goering, H.K. & Van Soest, P.J. 1970. *Forage fibre analysis*, Agricultural handbook no.379., Washington DC: United States Department of Agriculture.

Gustafson, G.M. & Stern, S. 2003. Two strategies for meeting energy demands of growing pigs at pasture. *Livestock Production Science.* 80: 167-174.

Hatt, J. M., Mayes, W., Clauss, M. & Lechner-Doll, M. 2001. Use of artificially applied N-alkane as markers for the estimation of digestibility, food selection and intake in pigeons (*Columbia livia*). *Animal Feed Science and Technology.* 94: 65-76.

Hermanssen, J. E., Strudsholm, K. & Horsted, K. 2004. Integration of organic animal production into land use with special reference to swine and poultry. *Livestock Production Science.* 90: 11-26.

Hodgkinson, S. M., Schmidt, M. & Ulloa, N. 2008. Comparison of the digestible energy content of maize, oats and alfalfa between the European wild boar (*Sus scrofa* L.) and Landrace × Large. *Animal Feed Science and Technology*. 144: 167-173.

Hoffman L. C., Styger E., Muller, M. & Brand, T. S. 2003. The growth and carcass and meat characteristics of pigs raised in a free-range or conventional housing system*. South African Journal of Animal Science.* 33: 166-175.

Hong, D., Raglan, D. & Adeola, O. 2002. Additivity and associative effects of metabolisable energy and amino acids digestibility of corn, soybean meal and wheat red dog for white Pekin duck. *Journal of Animal Science*. 80: 3222-3229.

Hunt, J. W., Dean, A. P., Webster, R. E., Johnson, G. N. & Ennos, A. R. 2008. A novel mechanism by which silica defends grasses against herbivory. *Annals of Botany.* 102: 653-656.

Jørgensen, H., Serena, A., Hedemann, S. M. & Bach Knudsen, K. E. 2007. The fermentative capacity of growing pigs and adult sows fed diets with contrasting type and level of dietary fibre. *Livestock Science.* 109: 111-114.

Jung, H. G. & Allen. M. S. 1995. Characteristics of plant cell walls affecting intake and digestibility of forages by ruminants. *Journal of Animal Science.*73: 2774-2790.

Kavanagh, S., Lynch, P. B., O'Mara, F. & Caffrey, P. J. 2001. A comparison of total collection and marker technique for the measurement of apparent digestibility of diets for growing pigs. *Animal Feed Science and Technology*. 89: 49-58.

Keli, A, Andueza, D., de Vega, A. & Guada, J. A. 2008. Validation of the n-alkane and NIRS techniques to estimate intake, digestibility and diet composition in sheep fed mixed lucerne: ryegrass diets. *Livestock Science*. 119: 42-54.

Kelly, H. R. C., Browning, H. M., Martins, A. P., Pearce, G. P., Stopes, C. & Edwards, S. A. 2001. Breeding and feeding pigs for organic production In: Hovi M., Baars, T. 2001 (Eds). *Breeding and feeding for animal health and welfare in organic livestock systems (4th NAHWOA Workshop).* Proceedings of NAHWOA: Network for Animal Health and Welfare in Organic Agriculture, Wageningen, Netherlands, 24-27 March 2001, Wageningen Network for Animal Health and Welfare in Organic Agriculture. http://www.veeru.reading.ac.uk/organic/FINALProceedingsEdited.pdf [accessed: 10 March 2009].

Lassen, J., Sandøe, P. & Forkman, B. 2006. Happy pigs are dirty! – Conflicting perspectives on animal welfare. *Livestock Science*. 103: 221- 230.

LeGoff, G. & Noblet, J. 2001. Comparative total tract digestibility of dietary energy and nutrients in growing pigs and adult sows. *Journal of Animal Science.* 79: 2418-2427.

LeGoff, G., Noblet, J. & Cherbut, C. 2003. Intrinsic ability of the faecal microbial flora to ferment dietary fibre at different growth stages of pigs*. Livestock Production Science*. 81: 75-87.

Len. N. T., Lindberg, J. E. & Ogle, B. 2007. Digestibility and nitrogen retention of diets containing different levels of fibre in local (Mong Cai), F1 (Mong Cai x Yorkshire) and exotic (Landrace x Yorkshire) growing pigs in Vietnam. *Journal of Animal Physiology and Animal Nutrition*. 91: 297-303.

Leterme, P., Botero, M., Londoño, A. M., Bindelle, M. & Buldgen, A. 2006. Nutritive value of tropical leaf meals in adult sows. *Animal science*. 82: 175-182.

Lewis, A.J. & Southern, L. L. *Swine Nutrition*. (2nd Ed). 2001. CRC Press. http://books.google.co.za/books?id=NWSo6OdeCoAC&pg=PA147&dq=Lewis,+A.J.+%26+Southern,+L.+L&hl=en&ei=Oq-cTJO4Edy4jAeQ7Iz-DA&sa=X&oi=book_result&ct=book-humbnail&resnum=3&ved=0CDcQ6wEwAg [accessed: 12 March 2009].

Lindberg, J. E. & Andersson, C. 1998. The nutritive value of barley-based diets with forage meal inclusion for growing pigs based on total tract digestibility and nitrogen utilization. *Livestock Production Science*. 56: 43-52.

Lindemann, M. D. & Kim, B. G. 2007. Technical note: A method to estimate individual feed intake of group-fed pigs. *Journal of Animal Science.*85: 972-975.

Lippke, H. 2002. Estimation of forage intake by ruminants on pasture. *Crop Science*. 42: 869-872.

Macoon, B., Sollenberger, L. E., Moore, J. E., Staples, C. R., Fike, J. H. & Portier, K. M. 2003. Comparison of three techniques for estimating the forage intake of lactating dairy cows on pasture**.** *Journal of Animal Science*. 81: 2357-2366.

Malossini, F. Boloventa. S., Piasentier, E. & Valentinotti, M. 1994. Variability of N-alkane content in a natural pasture and in faeces of grazing dairy cows. *Animal Feed Science and Technology*. 50: 113-122.

Mann, J. & Stewart, P. G. 2003. Kikuyu (*Pennisetum clandestinum*) intake determined by alkanes administered in a xantham gum suspension. *South African Journal of Animal Science*. 33: 27-31.

Marais, J.P. 2001. Factors affecting the nutritive value of Kikuyu grass (*Pennisetum clandestinum*) - A review. *Tropical Grasslands*. 35: 65-84.

Mayes, R. W. & Dove, H. 2000. Measurement of dietary nutrient intake in free ranging mammalian herbivores. *Nutrition Research Reviews*. 13: 107-138.

Mayes, R. W., Lamb, C. S. & Colgrove, P. M. 1986. The use of dosed and herbage n-alkanes as markers for the determination of herbage intake. *Journal of Agricultural Science.* 107: 161-170.

McDonald, P., Edwards, R. A., Greenhalgh, J. F. D. & Morgan, C. A. 1995. *Animal nutrition*. Essex: Longman.

Meeske, R., Rothauge, A., van der Merwe, G. D. & Greyling, J. F. 2006. The effect of concentrate supplementation on the productivity of grazing Jersey cows on a pasture based system. *South African Journal of Animal Science*. 36: 105-110.

Miles, N., Thurtell, L. & Riekert, S. 2000. Quality of Kikuyu herbage from pastures in the Eastern Cape coastal belt of South Africa. *South African Journal of Animal Science*. 30: 85-86.

Miller, J. K., Madsen, F. C. & Swanson, E. W. 1977. Effects of ingested soil on ration utilization by dairy cows. *Journal of Dairy Science.* 60: 618-622.

Millet, S., Hesta, M. Seynaeve, M., Ongen, E., De Smet, S., Debraekeleer, J. & Janssens, G.P.J. 2004. Performance, meat and carcass traits of fattening pigs with organic versus conventional housing and nutrition. *Livestock Production Science.* 87: 109-119.

Mowat, D., Watson, C. A., Mayes, R. W., Kelly, H., Browning, H. & Edwards S A. 2001. Herbage intake of growing pigs in an outdoor organic production system. In: *Proceedings of the British Society of Animal Science*, March 2000, Scarborough, p 169. Penicuik: British Society of Animal Science*.*

Mullan, B. P., Pluske, J. R., Trezona, M., Harris, D. J., Allen, J. G., Siddique, K. H. M., Hanbury, C. D., van Barneveld, R. J. & Kim, J. C. 2009. Chemical composition and standardised ileal digestible amino acid contents of *Lathyrus*

(*Lathyrus cicera*) as an ingredient in pig diets. *Animal Feed Science and Technology*. 150: 139-143.

National Research Council. 1998. *Nutrients requirements of Swine.* Washington, DC: National Academy Press.

Ndindana, W., Dzama, K., Ndiweni, P. N. B., Maswaure, S. M. & Chimonyo, M. 2002. Digestibility of high fibre diets and performance of growing Zimbabwean indigenous Mukota pigs and exotic Large White pigs fed maize based diets with graded levels of maize cobs. *Animal Feed Science and Technology*. 97: 199-208.

Newman, J. A., Cribaro-Neto, F. & Jensen, M. J. 1998. The sensitivity of n-alkane analysis to measurement error: implications for use in the study of diet composition. *Journal of Agricultural Science*. 131: 465-476.

Olivan, M., Ferreira, L.M.M., Celaya, R. & Osoro, K. 2007. Accuracy of the N-alkane technique for intake estimates in beef cattle using different sampling procedures. *Livestock Science.* 106: 28-40.

Oliveira, D. E. & Silva, S. C. 2007. Alkanes as markers in nutritional studies with wild ruminant and non-ruminant animals. *Scientia Agricola*. 64: 657-662.

Ordakowski, A. L. , Kronfeld, D. S, Holland, J. L., Hargreaves, B. J., Gay L. S., Harris ,P. A., Dove, H. & Sklan, D. 2001. Alkanes as internal markers to estimate digestibility of hay or hay plus concentrate diets in horses. *Journal of Animal Science*. 79: 1516-1522.

Peiretti, P. G. Meineri, G., Miraglia, N., Mucciarelli, M. & Bergero, D. 2006. Intake and apparent digestibility of hay or hay plus concentrate diets determined in horses by the total collection of feces and n-alkanes as internal markers. *Livestock Science*. 100: 189-194.

Piasentier, E., Boloventa, S. & Malossini, F. 2000. The n-alkane concentrations in buds and leaves of browsed broadleaf trees. *Journal of Agricultural Science.* 135: 311-320.

Piasentier, E., Boloventa, S., Malossini, F. & Susmel, P. 1995. Comparison of n-alkanes or chromium oxide methods for estimation of herbage intake by sheep. *Small Ruminant Research.* 18: 27-32.

Pluske, J. R., Black, B., Pethick, D. W., Mullan, B. P. & Hampson, D.J. 2003. Effects of different sources and levels of dietary fibre in diets on performance, digesta characteristics and antibiotic treatment of pigs after weaning. *Animal Feed Science and Technology*. 107: 129-142.

Presto, M. H., Algers, B., Persson, E. & Andersson, H. K. 2009. Different roughages to organic growing/finishing pigs - Influence on activity behaviour and social interactions. *Livestock Science.* 123: 55-62.

Rautenbach, E., van Ryssen, J. B .J. & van Niekerk, W. A. 2008. Changes in nutrient composition of Kikuyu foggage as winter progressed. *South African Journal of Animal Science*. 38: 342-349.

Ravindran, V., Hew, L. I., Ravindran, G. & Bryden, W. L. 2004. Endogenous amino acid flow in the avian ileum: quantification using three techniques. *British Journal of Nutrition*. 92: 217-223.

Reverter, M., Lundh, T. & Lindberg, J. E. 1999. Ileal amino acid digestibilities in pigs of barley-based diets with inclusion of Lucerne (*Medicago sativa*), white clover (*Trifolium repens*), red clover (*Trifolium pratense*), or perennial ryegrass (*Lolium perenne*). *British Journal of Nutrition*. 82: 139-147.

Rodríguez-Estévez, V., García, A., Peña, F. & Gómez, A. G. 2009. Foraging of Iberian fattening pigs grazing natural pasture in the dehesa. *Livestock Science.* 120: 135-143.

Robbins, C. T. 1983. *Wildlife feeding and nutrition*. New York: Academic Press.

Santos Ricalde, R.H. & Lean, I. J. 2002. Effect of feed intake during pregnancy on productive performance and grazing behaviour of primiparous sows kept in an outdoor system under tropical conditions. *Livestock Production Science.* 77: 13-21.

Santos Ricalde, R. H. & Lean, I. J. 2006. Digestibility of dried star grass (*Cynodon nlemfuensis*) and consumption of fresh star grass in primiparous pregnant sows. *Animal Feed Science and Technology*. 129: 12-22.

SAS. 2008. SAS/STAT *User's Guide. Version 9.2*. North Carolina: SAS institute Inc.

Sather, A. P., Jones, S. D. M., Schaefer, A. L., Colyn, J. & Robertson, W. M. 1997. Feedlot performance, carcass composition and meat quality of free range reared pigs. *Canadian Journal of Animal Science.* 77: 225-232.

Seabra, M., Carvalho, S., Freire, J., Ferreira, R., Mourato, M., Cunha, L., Cabral, F., Teixeira, A. & Aumaitre, A. 2001. *Lupinus luteus*, *Vicia sativa* and *Lathyrus cicera* as protein sources for piglets: Ileal and total tract apparent digestibility of amino acids and antigenic effects. *Animal Feed Science and Technology*. 89: 1-16.

Sehested, J., Søegaard, K., Danielsen, V., Roepstorff, A. & Monrad, J. 2004. Grazing with heifers and sows alone or mixed: Herbage quality, sward structure and animal weight gain. *Livestock Production Science*. 88: 223-238.

Sibbald, A.M., Davidson, G. C. & Mayes, R. W. 2000. Effect of dosing regime on intake estimation using the *n*-alkane technique in sheep fed pelleted grass meal. *Journal of the Science of Food and Agriculture.* 80: 1206-1210.

Smit, H. J., 2005. Perennial ryegrass for dairy cows: effects of cultivar on herbage intake during grazing. PhD Thesis, Wageningen University. Wageningen, the Netherlands.

Smit, H. J., Taweel, H. Z., Tas, B. M., Tamminga, S. & Elgersma, A. 2005. Comparison of techniques for estimating herbage intake of grazing dairy Cows. *Journal of Dairy Science*. 88: 1827-1836.

Smith, D. G., Mayes, R. W., Hollands, T., Cuddeford, D., Yule, H. H., Malo Ladrero, C. M. & Gillen, E. 2007. Validating the alkane pair technique to

estimate dry matter intake in equids. *Journal of Agricultural Science*. 145: 273-281.

Smith, D. G., Mayes, R. W. & Raats, J. G. 2001. Effects of species, plants part, and season of harvest on N-alkane concentrations in the cuticular wax of common rangeland grasses from Southern Africa. *Australian Journal of Agricultural Research.* 52: 875-882.

Stein, H. H., Fuller, M. F., Moughan, P. J., Sève, B., Mosenthin, R., Jansman, A. J .M., Fernández, J. A. & de Lange, C.F.M. 2007. Definition of apparent, true, and standardized ileal digestibility of amino acids in pigs. *Livestock Science*. 109: 282-285.

Stern, S. & Andresen, N. 2003. Performance, site preferences, foraging and excretory behavior in relation to feed allowance of growing pigs on pasture. *Livestock Production Science*. 79: 257-265.

Stevens, D. M., van Ryssen, J. B. J. & Marais, J. P. 2002. The use of N-alkane markers to estimate the intake and apparent digestibility of ryegrass and Kikuyu by horses. *South African Journal of Animal Science*. 32: 50-56.

Swainson, N., Hoskin, M., Clark, S. O. & Krause, H. 2005. Validation of the double N-alkane technique to estimate the dry matter intake of red deer fed fresh ryegrass-based pasture or plantain. *Proceedings of the New Zealand society of animal production*. 65: 23-28.

Tainton, N. M. 2000. *Pasture management in South Africa*. Scottsville: University of Natal Press.

Tamminga, S. Robinson, P. H., Meijs, S. & Boer, H. 1989. Feed components as internal markers in digestion studies with dairy cows. *Animal Feed Science and Technology*. 27: 49-57.

Thompson, J. E. & Wiseman, J. 1998. Comparison between titanium dioxide as an inert marker and total collection in the determination of digestibility of

components in diets fed to pigs. In: *Proceedings of the British Society of Animal Science*, 1998, p 171. Penicuik: British Society of Animal Science.

Tovar, A., M. A., & Giraldo, V. J. D. 2006. Considerations on ethics and animal welfare in extensive pig production: Breeding and fattening Iberian pigs. *Livestock Science*. 103: 237-242.

Van Leeuwen, P., Veldman, A., Boisem, S., Deuring, K., Van Kempen, G. J. M., Derksen, G. B., Verstegen, M. W. A. & Schaafsma, G. 1996. Apparent ileal dry matter and crude protein digestibility of rations fed to pigs and determined with the use of chromic oxide (Cr_2O) and acid-insoluble ash as digestive markers. *British Journal of Nutrition.* 76: 551-562

Van Oudtshoorn, F. 1992. *Guide to Grasses in South Africa*. Cape Town: Briza Publishers.

Van Wieren, S. E. 2000. Digestibility and voluntary intake of roughages by wild boar and Meishan pigs. *Animal Science.* 71: 149-156.

Varel, V.H. 1987. Activity of fiber-degrading micro-organisms in the pig large intestine. *Journal of Animal Science*. 65: 488-496.

Varel, V. H. & Yen, J. T. 1997. Microbial perspective on fiber utilization by swine. *Journal of Animal Science*. 75: 2715-2722.

Vulich, S. A., O'riordan, E. G. & Hanrahan, W. J. P. 1991. Use of N-alkane for the estimation of herbage intake in sheep: accuracy and precision of the estimates. *Journal of Agricultural Science.*116: 319-323.

Vulich, S. A. & Hanrahan, J. P. 1995. Faecal sampling for the estimation of herbage intake using n-alkanes: evaluation of sample pooling and the use of rectal grab samples. *Journal of Agricultural Science*. 124: 79-86.

Wenk, C. 2001. The role of dietary fibre in the digestive physiology of the pig. *Animal Feed Science and Technology.* 90: 21-33.

Whittemore, C. 1993. *The Science and Practice of Pig Production*. Essex: Longman Group.

Wiese, W. J. & Joubert, J. P. J. 2001. Suspected nitrite poisoning in pigs caused by *Capsella bursa-pastoris (L.) Medik.* ('herderstassie', shepherd's purse). *Journal of the South African Veterinary Association*. 72: 170-171.

Wilfart, A., Montagne, L., Simmins, P. H., Milgen, J. van & Noblet, J. 2007a. Sites of nutrient digestion in growing pigs: Effect of dietary fiber. *Journal of Animal Science*. 85: 976-983.

Wilfart, A., Montagne, L., Simmins, P. H., Noblet, J. & Milgen, J. van. 2007b. Effect of fibre content in the diet on the mean retention time in different segments of the digestive tract in growing pigs. *Livestock Science*. 109: 27-29.

Wilson, H., Sinclair, A. G., DeB. Hovell., F. D., Mayes, R. W. & Edwards, S. A. 1999. Validation of the n-alkane technique for measuring herbage intake in sows. In: *Proceedings of the British Society of Animal Science*, March 1999, Scarborough, p 177. Penicuik: British Society of Animal Science.

Yen, J. T., Varel, V. H., & Nienaber, J. A. 2004. Metabolic and microbial responses in western crossbred and Meishan growing pigs fed a high-fiber diet. *Journal of Animal Science*. 82: 1740-1755.

Zhang, Y., Togamura, Y. & Otsuki, K. 2004. Study on the n-alkane patterns in some grasses and factors affecting the n-alkane patterns. *Journal of Agricultural Science*. 142: 469-475.

APPENDIX

Experimental units and treatments and markers faecal concentration

Pen number	Initial Weight (kg)	Final weight (kg)	Group	AIA (g/kg)	C_{32} (mg/kg)	C_{33} mg/kg)
9	29.9	44.2	A	1.28	665	38
10	24.1	53.7	A	1.78	325	40
11	31.4	46.5	A	2.04	519	N/A
18	29.1	42.9	A	1.15	339	31
20	25.9	57.6	A	1.51	274	12
5	28.7	50.7	B	2.88	318	68
7	25.2	57	B	3.48	476	108
14	29.6	46.7	B	2.33	278	69
16	25.2	44.3	B	3.94	163	108
17	29.5	48.6	B	2.72	411	82
3	20.9	52	C	2.39	438	123
4	30.6	46.4	C	2.6	268	135
8	23.3	53.3	C	2.88	328	89
15	23.1	56	C	2.74	253	58
19	30.6	48.2	C	3.72	258	99
1	30.8	52.4	D	2.15	84	56
2	31.6	49.5	D	3.39	312	134
6	21.4	48.2	D	2.24	326	67
12	21.4	58.1	D	2.14	12	25
13	22.1	57.3	D	3.03	294	93
21	29.4	46.6	E	8.67	336	63
22	20.9	45.5	E	10.71	327	112
23	23.8	46.3	E	13.69	297	41
24	30.2	54.6	E	30.82	175	24
25	27.6	49.5	E	42.63	164	39

AIA: Acid-insoluble ash.