EFFECTS OF SPROUTING OR ROASTING COWPEAS (*VIGNA UNGUICULATA (L.) WALP*) AS A PROTEIN SOURCE IN DIETS FOR SOUTHERN AFRICAN WINDSNYER TYPE, LARGE WHITE X LANDRACE AND THE THREE-WAY CROSSBRED GROWING PIGS

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A thesis submitted in fulfillment of the requirement for the degree of Doctor of Philosophy in the Department of Animal Science, Faculty of Science, Engineering and Agriculture

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DECLARATION

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I, Mfanuzile Welcome Lubisi student No. 11533732, declare that the thesis with the title: “Effects of sprouting or roasting cowpeas (Vigna *unguiculata (l.) Walp*) as a protein source in diets for Southern African Windsnyer type, Large White X Landrace and the three-way crossbred growing pigs” is my research and hereby submitted for the Doctor of Philosophy in Agriculture degree at the Department of Animal Science, Faculty of Science, Engineering and Agriculture, University of Venda. I have not previously submitted it for a degree at this or any other university. All reference material contained therein has been duly acknowledged and referenced accordingly.

Student .... Date 15 /02 / 2023

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LIST OF ABBREVIATIONS

AA - Amino Acid
ADF - Acid Detergent Fiber
ADG - Average Daily Gain
AID - Apparent Ileal Digestibility
ALP - Alkaline Phosphatase
ALT - Alanine Aminotransferase
AN - Absorbed nitrogen
ANFs - Antinutritional Factors
AOAC - Association of Official Analytical
AST - Aspartate Aminotransferase
ATTDC - Apparent Total Tract Digestibility Coefficient
BVFP - Biological value of feed protein
Ca - Calcium
CD - Chromium Concentration in Diets
CF - Chromium Concentration in Faeces
CP - Crude Protein
Cu - Copper
DE - Digestible Energy
DM - Dry Matter
EAL - Endogenous Amino Acid Losses
FAO - Food and Agriculture Organization
FCR - Feed conversion ratio
FI - Feed intake
FNO - Faecal nitrogen output
IDF - Insoluble Detergent Fiber
IVDMD - In vitro dry matter digestibility
K - Potassium
LR - Landrace
LW - Large White
ME - Metabolizable Energy
N - Nitrogen
Na - Sodium
ND - Nutrient concentration in diets

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NDF	- Neutral Detergent Fiber
NF	- Nutrient Concentration in Faeces
NI	- Nitrogen intake
NR	- Nitrogen retention
PBS	- Phosphate buffered saline
SDF	- Soluble Detergent Fiber
SID	- Standardized Ileal Digestibility
TIA	- Trypsin Inhibitor Activity
TNE	- Total nitrogen excretion
UNO	- Urinary nitrogen output
VFA	- Volatile Fatty Acid
W	- Windsnyer
Zn	- Zinc

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DEDICATION

This thesis is dedicated to my mother and father, S.S Ngomane and M.J Lubisi, my siblings, beloved children, nephews, and the late Dr Ronald Thomas (Co-promoter).

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ABSTRACT

In poorly resourced settings, livestock feeding options are often narrowed to the affordable, readily available legume grains, and to their most convenient biological or thermal processing methods. The tolerance of different pig genotypes to toxic legume anti-nutritional factors and to the negative impact on dietary dietary nutrient availability may differ. The study aimed to determine the capacity of different local pig genotypes to utilise differently processed cowpeas (*Vigna unguiculata (L.) Walp*) as a dietary protein source during growth. Preliminary studies were conducted to determine the effective sprouting or roasting of cowpeas for feeding to growing pigs. In a sprouting experiment 1, cowpeas were soaked for 12 hours, open-sprouted over 1, 2, 3 and 4 days, with daily sampling, and the samples rapidly sundried to terminate the sprouting. In a roasting experiment 2, cowpeas were placed in an empty, preheated (150°C interior temperature) cast-iron drum, and roasted for 10, 15, 20, or 30 minutes, coincident with 55°C, 95°C, 105°C, and 130°C respective sample grain temperatures. The effectiveness of both processing procedures was evaluated by determining the *in vitro* dry matter digestibility (*IVDMD*) of the processed, compared to raw cowpeas. The *IVDMD* was estimated using a standard three-step (gastric, small, large intestines) *in vitro* pig digestion procedure, in a setup modified for micro (0.5 g) sample digestion within Ankom® 57 filter bags. Both the sprouting and roasting *in vitro* digestion were completely randomised, with 14 replicates per sample. Cowpea samples obtained at key sprouting or roasting points were analised for proximate and detergent fibre components, and for trypsin inhibitor activity (TIA), as auxiliary variable determinants of *IVDMD*. The steps 1-2 (gastric-ileal) *IVDMD* coefficient dropped ($p<0.05$) in the 2-day (0.78 ± 0.00486) and 3-day (0.77 ± 0.00486) cowpea sprouts, with equally high ($p<0.05$) step 3 *IVDMD* for 2-day and 3-day sprouts (0.13 ± 0.00605) compared to 1-day (0.07 ± 0.00605) and 4-day (0.10 ± 0.00605) sprouts. The total (steps 1-3) *IVDMD* increased ($p<0.05$) in 2-day (0.91 ± 0.00336) and 4-day (0.90 ± 0.00336) sprouts. Roasting did not affect steps 1-2 *IVDMD.* The 20-minute (105° C) roasting resulted in high ($p<0.05$) step 3 (0.17 ± 0.00734) and the total (0.98 ± 0.00449) *IVDMD*. The compartmental and total *IVDMD,* and quantitative change in chemical components and TIA indicated 4-day sprouting, and 20-minute (105° C) roasting were respectively most effective for cowpea processing, procedures which were subsequently used to process cowpeas in bulk for a metabolic, and a performance feeding trial. For the metabolic trial, raw (control) and the differently processed cowpeas were used to constitute iso-nutrient (15% CP, 14.17 MJ ME kg^{-1}) maize-based grower pig diets. The metabolic trial used nine growing pigs, three each of Windsnyer (W), Large White (LW) X Landrace (LW) and the 3-way crossbred (W X LW X LR) genotypes. The

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pigs were inducted into the experiment at respective initial live weights 11.0 ± 1.15. 4 ± 1.15, and 12 ± 1.15 kg. Pigs were randomly allocated to diets for a factorial experiment within three balanced, 3 (genotype) x 3 (period) Latin squares with 12-day feeding periods split into seven-day adaptation, plus five days measurement of feed intake, and the total faecal and urine excretions. Metabolic size-scaled feed consumption was high (122.6 ± 3.01 g kg^{-1} Live weight (LW) $^{0.75}$) for the raw cowpeas, compared to the roasted cowpea diet (108.8 ± 3.01 g kg LW$^{0.75}$) ($p<0.05$). On scaled bases, feed consumption was higher on the sprouted cowpeas compared to the raw diet ($P<0.05$). Sprouting and roasting both reduced the ash digestibility. Ash digestibility was lower ($P<0.05$) for LW x LR compared to W x LW x LR pigs. Genotype x Diet interaction was observed for ash, fat, ADF and NDF, however the main mean showed non-significant ($P<0.05$) for fat, ADF and NDF. Pig responses to the differently processed cowpeas were further evaluated in a 60-day growth trial which used a total of 36 animals, twelve male, 28-day weaned pigs of each of the W, LW X LR, and W x LW x LR genotypes (respective initial live weights 15.0 ± 2.3, 39.0 ± 1.4 and 37.0 ± 1.2 Kg). To balance the initial weights across treatments, pigs were blocked by weight within genotype, and randomly allocated within the weight blocks to the experimental diets for a 3 (genotype) X 3 (diets) factorial experiment replicated four times. The experimental diets were a standard, maize-soybean commercial pig grower (control) feed, and iso-nutrient (14.26 ± 1.2 MJ ME kg, 160.0 ± 2.1 g CP kg), roasted or sprouted cowpea-maize grower pig diets. Pig growth and slaughter parameters, visceral organ sizes, and plasma biomarkers of protein, energy utilization, and organ function were measured. Pig growth was different by genotype in the order LW x LR ≥ LW x LR x W > W ($p<0.05$). Feed intake ranked LW x LR ≥ LW x LR x W > W ($p<0.05$). Dietary treatments ranked control > sprouted cowpeas > roasted cowpeas diet ($p<0.05$). The FCR was similar ($p>0.05$) across the genotypes, but differed by diet in the order control (3.41 ± 0.551) < sprouted cowpeas (4.94 ± 0.551) ≤ roasted cowpeas (5.93 ± 0.551) diet ($p<0.05$). Back fat was thickest on the control diet (9.91 ± 0.321) compared to the other diets ($p<0.05$). The W pigs dressed inferior ($p<0.05$) compared to the other genotypes. Scaled on the metabolic weight, the liver and kidneys were large ($p<0.05$) in W pigs, and the kidneys enlarged (0.567 ± 0.0105 %) ($p<0.05$) in pigs on the roasted cowpea diet. The W x L x LR had low alkaline phosphatase activity (99.50 ± 6.090 U/L), W had low creatinine (44.94 ± 2.32 µmol/L) and cholesterol. (1.23 ± 0.0833 mmol/L); and LW x LR had low plasma total protein (69.20 ± 0.915 g/L). Pigs on the sprouted cowpea diet had high ($p<0.05$) urea (5.30 ± 0.255 mmol/L) and cholesterol (1.82 ± 0.0833 mmol/L). Albumin was low in pigs on the sprouted diet (35.58 ± 0.902 g/L) and on the roasted diet (35.60 ± 0.902 g/L), while alkaline phosphatase activity was high on the sprouted (110.92 ± 6.090 U/L) and the roasted (130.13 ± 6.090 U/L) diet ($p<0.05$). In

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conclusion, compartmental and total *IVDMD,* and quantitative change in chemical components and TIA indicated 4-day sprouting, and 20-minute (105° C) roasting were respectively most effective for cowpea processing. The observed genotype x diet interactions on N balance parameters confirmed genotype influences on the digestive capacity, and intermediary nutrient metabolism in pigs fed the raw, and differently processed cowpeas, which suggested unique adaptive digestive and metabolic traits among the experimental pig genotypes. The underlying mechanisms need further investigation. Roasting and sprouting of dietary cowpeas produced diets which supported similar, slower growth compared to the soybean-based diet, with higher FCR by pigs on the sprouted, compared to the roasted cowpea diet. Further research is recommended to evaluate the cost-effectiveness and practicality of cowpea sprouting or roasting in large-scale pig production systems.

Keywords: *Antinutritional factors, Legumes, Pig breeds, Plasma metabolites*

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CHAPTER 1
INTRODUCTION

1.1. Background

In smallholder farming systems, pigs are often the primary source of meat protein (An *et al.,* 2004). They also provide manure for cropping (Sommer *et al.,* 2015). Most smallholder pig farmers depend on systems in which pigs scavenge for food, which is a health risk, given that they can carry worms with potential transfer to human beings who consume partially cooked pork (Lekule *et al.,* 2003; Fincham, 2005). Pig production provides these farmers with an extra source of income and dietary animal protein (Gcumisa, 2013; Chauhan *et al.*2016; Kimbi *et al.,* 2016). The commercial sector produces most of the slaughter pigs for the commercial market (Deka *et al., 2014*). The contribution of of the commercial pig production sector varies among countries. The commercial farms are well-resourced, well controlled, and have higher productivity (Berthouly-Salazar *et al.,* 2012). The Large White (Yorkshire) and Landrace pigs are popular exotic breeds in this system (Berthouly-Salazar *et al.,* 2012). Pigs produced under intensive systems are highly productive since they are genetically improved, consume high quality commercial feed, with strict diseases control (Albernaz-Gonçalves *et al.,* 2022).

There are many advantages to producing pigs in rural areas. Positive impacts of pig production on the livelihoods of people living in the rural areas are reported in diverse communities such as in Western Kenya (Mutua *et al.,* 2010), Tanzania (Kimbi *et al.,* 2016) and Madagascar (Costard *et al.,* 2009). In Southern Africa, pig farming plays a similarly important role for emerging small scale pig farmers in Limpopo (Mokoele, 2015) and Kwazulu-Natal province (Gcumisa, 2013). Small-scale farmers also benefit from farming with pigs be it for generating extra income or, for providing protein in the form of meat for household consumption (Mutua *et al.,* 2010; Duniya *et al.,* 2013; Gcumisa, 2013).

There are variations in farming systems and scale of pig production among different countries in the region. The contribution from medium scale farms is generally 15% of total pig production and they also house a husking mill, or a small feed-mixing facility (Phogole, 2017). The pigs are usually crossbreeds and exotic breeds. Feeds used in this system are home mixed concentrates or commercial feed (Dey *et al.,* 2022). However, because of the high cost for commercial feeds, only the very resourceful farms can high scale production in the long term (Kukuyi *et al.,* 2011).

Optimum pig performance in terms of growth, health and welfare requires a diet balanced in energy, protein, amino acids, vitamins and minerals (Crafter *et al.,* 2010). In modern pig

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farming, precision feeding systems are employed which meet these nutrient requirements using expensive commercial feeds and additives. However, for enterprise viability, pig producers can utilise cheaper feeds which may be readily available in their localities.

The edible legumes, unlike the energy-rich cereals, have a high protein content (Malomo, 2013), which is highest in soybean (Stein *et al.,* 2013). Legumes are also rich sources of energy, vitamins, dietary fibre and minerals (Arawande & Borokini, 2010). Cereal grains are classified as carbohydrate -rich sources since they contain roughly 75% carbohydrates (Tufail *et al.,* 2022). The nutrient content is highly variable. The protein content ranges from 6–15% (Tufail *et al.,* 2021). Cereals are deficient in lysine and tryptophan, but rich in methionine and cysteine, whereas legumes are deficient in sulphur-containing amino acids such as methionine and cysteine and rich in lysine (Sodipo & Fashakin, 2011). The nutrient profiles of legumes and cereals are therefore complementary. However, lysine is typically limiting in cereal based diets (Tufail *et al.,* 2021). Among the native legumes, the cowpea (*Vigna unguiculata*), an ancient crop grown throughout the tropics and subtropics, is an option to expensive soybean because of its high protein and quality, and is relatively inexpensive (Singh, 2006; Oyarekua, 2009). The cowpea protein content is in the range of 16-31%, in addition to significant starch, minerals and B-vitamins (Boukar *et al.,* 2011; Jayathilake *et al.,* 2018). Akande *et al.,* (2010), Chakam *et al.,* (2010) and Ragab *et al.* (2010) reported high (up to 24.8%) crude protein in cowpeas. Overall, cowpea protein quality is considered complementary to cereals (Jayathilake *et al.,* 2018), being rich in lysine and tryptophan (Sai-Ut *et al.,* 2009), but deficient in the sulphur-containing amino acids, methionine and cysteine (Khattab, 2009; Chakam *et al.,* 2010; Frota *et al.,* 2017).

Legumes should not be fed raw to monogastric livestock due to high levels of anti-nutritional factors, including protease inhibitors, lectins, anti-metals, saponins and other toxic substances (Thakur & Kumar, 2017). A wide range of processing techniques could improve legumes protein and starch digestibility (Alonso *et al.,* 1998). Doblado *et al.* (2007) reported that heat-labile factors such as trypsin inhibitors, which reduce protein digestibility, could be effectively destroyed by heat treatment. Lectins can directly bind to the intestinal mucosa, interacting with the enterocytes, and interfering with the absorption and transportation of nutrients (particularly carbohydrates) during digestion, causing epithelial lesions (Thakur & Kumar, 2017). Muralidhar, (2019) suggested lectins consumed in everyday foods function as chemical messengers that can bind to the sugars of cells in the gut and to blood cells, initiating inflammatory responses.

Cooking, roasting and sprouting legumes is known to increase nutrient bioavailability while germination, sprouting or malting involve biochemical processes that increase the nutritive value of foods/feeds for humans and animals (Oboh *et al.,* 2000). During germination, proteinases are activated which release amino acids and peptides from complex proteins and facilitate the synthesis or utilisation of these to form new, more digestible proteins (Gulewicz *et al.,* 2008). Sprouting of legumes increased protein and mineral bioavailability, which was attributed to the reduction of protease inhibitors, tannin, and phytic acid (Hussein *et al.*, 1999; Ghavidel *et al.*, 2007). El-Adawy *et al.* (2004) reported increased vitamin concentrations and trace elements and minerals bioavailability. Kaushik *et al.* (2010) observed improved calcium, copper, manganese, zinc, riboflavin, niacin and ascorbic acid content.

Sangronis *et al.* (2007) conducted a study on Trypsin Inhibitor Activity (TIA), phytic acid, tannins, ascorbic acid, thiamine, protein digestibility, and minerals in germinated black peas (*Phaseolus vulgaris L*.), white peas (*Phaseolus vulgaris L.)* and pigeon peas (*Cajanus cajaan L. Millsp).* The un-germinated legumes were analysed as a control. Their study revealed substantial reduction in TIA of pigeon peas (19.2%), white beans (52.5%) and black peas (25%). The decrease in phytic acid was above 40% in all three types of germinated peas. Tannins were reduced by 14.3% in pigeon peas. Sprouting increased protein digestibility by between 2% and 4%, and increased ascorbic acid by 30% in white peas, 33% in black peas and 28% in pigeon peas. The thiamine content increased by 12.8%, 26.5% and 7.4%, respectively, in white, black and pigeon peas. Overall, sprouting affected mineral content depending on grain factors, and the type of mineral. Variations in the content of nutrients and anti-nutrients in germinated grains were attributed to interaction of the germination and previous soaking to which the grains were subjected. Sprouting has also been reported to increase the availability of minerals such as copper, sodium, potassium, iron, phosphorus, calcium, manganese, and magnesium (Youssef *et al.,* 1987).

Phytic acid, tannins, ascorbic acid, thiamine, protein digestibility, and minerals in germinated black beans (*Phaseolus vulgaris L*.), white beans (*Phaseolus vulgaris L.*), and pigeon beans were studied by Sangronis *et al.,* in 2007. Their research showed that trypsin inhibitor activity (TIA) of pigeon beans (19.2%), white beans (52.5%), and black beans (25%), respectively, had significantly decreased. In all three varieties of germinated beans, the phytic acid reduction was greater than 40%. Pigeon beans had a 14.3% reduction in tannins. Between 2% and 4% more protein was digestible after sprouting, and ascorbic acid levels rose by 30% in white beans, 33% in black beans, and 28% in pigeon beans. The levels of thiamine rose by 12.8%, 26.5%, and white, black, and pigeon beans each had a 7.4% yield. Overall, depending on the grain characteristics and the type of mineral, sprouting had an impact on

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the mineral content. Differences in the amount of nutrients and anti-nutrients in germinated grains were thought to be caused by the combination of germination and earlier soaking. The availability of minerals like copper, sodium, potassium, iron, phosphorus, calcium, manganese, and magnesium has also been observed to rise with sprouting (Youssef *et al.,* 1987).

Depending on the intensity, thermal, hydrothermal, and bio-processing may alter the physicochemical properties of proteins, starch, and other components of legume seeds to limit their overall nutrient value (De Pilli *et al.,* 2011). Therefore, it is important to determine the best method by which legumes such as the cowpea can be optimally processed to reduce the anti-nutritional factors for incorporation into growing pig's diets. It is also important to consider genetic factors which may affect the growing pigs' performance. This is because pig growth may be influenced by interaction of genotype and dietary factors (Lopez *et al.,* 2001).

1.2. Problem statement

Commercial pig feeds are increasingly expensive, such that pig production is becoming uneconomic, particularly for poorly resourced smallholder producers (Yaduraji *et al.,* 2015; Fausto-Castro *et al.,* 2020; Herrera *et al.,* 2022). Soybean oil cake is the conventional plant protein source around the world. However, smallholder pig farmers cannot afford soybean oil cake (Mthiyane & Mhlanga, 2018). Therefore, there is a need to identify cheaper, readily available alternative protein sources to replace soybeans. Like most other legumes, cowpeas contain anti-nutritional factors (ANF) such as trypsin inhibitors, phytic acid, tannins and lectins which affect feed utilisation. The available evidence suggests anti-nutritional factors might be neutralised by using eco-friendly biological processing such as sprouting (Colletti *et al.,* 2020). Sprouting requires minimal energy input compared to alternative processing such as thermal or hydrothermal processing. The comparative efficacy of bio-processing relative to energy intensive thermal processing such as roasting needs to be established. The improved pig genotypes may not perform optimally on inefficiently processed cowpea-based diets due to the high dietary fibre (over sprouted cowpeas) and residual ANF (sub optimally sprouted or roasted cowpeas), Windsnyer type genotypes may be more tolerant to both the fibre and other inhibiting factors (Woyengo *et al.,* 2017). Therefore, the study sought to evaluate the capacity of diverse pig genotypes to utilise differently processed cowpeas when used as the primary plant dietary protein source in growing pig diets, to provide cowpea processing options in relation to the genotypes which are used in both small-scale and commercial pig production systems.

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1.3. Justification

Pig producers are choosing the exotic, over the native breeds which they believe to be more advanced (Munzhelele, 2015) due to their commercial attractiveness. However, the native pigs (Windsnyer) remain a source of food and revenue for people who farm in rural areas of African nations, including South Africa (Madzimure *et al.,* 2013; Kimbi *et al.,* 2016). Exotic breeds such as the Duroc, Landrace, and Large White outperform the native pigs in size, prolificacy, litter sizes, and carcass confirmation (Halimani *et al.,* 2012). Pig genotypes may respond differently to (e.g. inefficiently or differently processed) cowpea-based diets due to processing effects on the dietary fibre (e.g. sprouted cowpeas), nutrients and residual ANF's (e.g. sprouted or roasted cowpeas). Commercial pig feeds are increasingly expensive; (Herrera *et al.,* 2022). There is need for sustainable alternatives to the expensive conventional ingredients such as soybean. In the arid tropics, the cowpea is considered an agro-ecologically (Dabo *et al.,* 2019) suitable replacement for expensive dietary soybean, depending on how its processed to improve the protein quality, and remove anti-nutritional factors (ANF) (Colletti *et al.,* 2020). Practical processing options include thermal or bioprocessing, where the least cost, most efficient are most desirable (Maleki & Razavi, 2021). During sprouting or germination, proteinases are activated which release amino acids and peptides from complex proteins and facilitate the synthesis or utilisation of these to form new, more digestible proteins (Moaveni *et al.,* 2022). Sprouting of legumes increased protein and mineral bioavailability, which was attributed to the reduction of antinutritional factors ANFs (trypsin inhibitor, protease inhibitors, tannin, and phytic acid) (Samtiya *et al.,* 2020).
Several options of thermal treatment are available that can decrease the anti-nutritional factor activities in cowpeas (Sofi *et al.,* 2023). Of these methods, an effective, simple method that is easy and cost-effective for small-scale farmers, such as drum roasting, mud oven and steel plate roasting, is required.

Local pig producers prefer indigenous pig breeds (Windsnyer) or their crosses because of their hardiness and low management requirements, particularly the low feed and water consumption (Halimani *et al.,* 2012). Indigenous breeds ("Kolbroek" and "Windsnyer") require less protein, and are better able to digest fibre than improved pig breeds (Halimani *et al.,* 2010 & Madzimure *et al.,* 2012). Therefore, both the cowpea market and pig production can be dramatically improved for both small-scale and commercial farms if an ecologically versatile legume can be effectively utilised by the most adapted pig genotypes. It is envisaged that the low-cost, minimal energy and labour-intensive legume processing methods such as sprouting and roasting are best placed for adoption to process cowpeas to the quality acceptable for feeding to growing pigs, to replace expensive commercial feed partially or completely.

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1.4. Objectives

1.4.1. Main objective

The broad objective of the study was to evaluate the growth and developmental responses of different pig genotypes fed diets containing sprouted, compared to roasted cowpeas (*Vigna unguiculata (L.) Walp*) as sole plant protein complements to maize (*Zea mays*).

1.4.2. Specific objectives

The specific objectives were to determine the following;

1.4.2.1 Effective cowpea sprouting period using a rapid, micro (0.5g) *in-vitro* pig dry matter digestibility technique.

1.4.2.2 Effective cowpea roasting intensity using a rapid, micro (0.5g) in-*vitro* pig dry matter digestibility technique.

1.4.2.3 Nutrient digestibility and nitrogen balance in indigenous, improved and crossbred growing pigs fed raw, sprouted and roasted cowpea diets.

1.4.2.4 Growth rate, slaughter performance, visceral organ sizes and plasma metabolic marker profiles of indigenous, improved and crossbred growing pigs fed roasted and sprouted cowpea diets.

1.5. Hypotheses

1.5.1. The sprouting period (1, 2, 3 and 4 days) is not critical to the quality of cowpeas for pig feeding, as indicated by *in-vitro* pig dry matter digestibility.

1.5.2. The roasting intensity (10, 15, 20, or 30 minutes roasting, coincident with 55°C, 95°C, 105°C, and 130°C respective terminal grain temperatures) is not critical to the quality of cowpeas for pig feeding, as indicated by *in-vitro* pig dry matter digestibility.

1.5.3. Sprouting and roasting cowpeas do not affect nutrient digestibility and nitrogen balance in indigenous, improved and crossbred growing pigs.

1.5.4. Sprouting and roasting cowpea diets do not affect growth, slaughter performance, visceral organ sizes and plasma metabolic marker profiles of indigenous, improved and crossbred growing pigs.

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CHAPTER 2

LITERATURE REVIEW

2.1. General introduction

Pigs are economically important, especially among the poor communities in African countries. They provide food security, alleviate poverty, and contribute to human livelihood through creation of employment (Mergenthaler *et al.,* 2009; Antwi *et al.,* 2011; Dietze, 2011). In the smallholder sector, pig production is largely through traditional, low-input, semi-intensive scavenging systems. Indigenous pigs are typically left to scavenge freely for feed without expensive supplementary feeds. After the main crops have been harvested, the pigs are allowed to feed on residual feeds in the field during dry seasons. Supplementary feeds include small amounts of inferior feedstuffs such as rice or maize bran, cassava and weeds that occur naturally or on fallow lands, forests and along stream banks (Phengsavanh & Stür, 2006).

The contribution of emerging and small-scale pig farmers to the South African rural economy is unclear. Production is limited due by numerous constraints, including the pig genotype, reproduction management, health, housing and nutrition, and limited marketing opportunities (Chikazunga *et al.,* 2007; Antwi *et al.,* 2011). However, there are many opportunities for improving smallholder pig production. For example, locally grown pulses such as the cowpea can supply high protein at low cost. The nutrient composition of improved legumes, including cowpeas, is highly influenced by agronomic factors and seed genotype (O'Doherty *et al.,* 2001). Therefore, it is important to understand the nutrient content of locally grown legumes before incorporating them into pig diets. It is also essential to investigate the effects on pig performance of substituting expensive soybean meal in pig diets with locally grown legumes (Stein *et al.,* 2004).

Reviewed under this chapter are the cowpea production statistics and patterns, its ecological and nutritive value, the methods of processing, as well as its nutritive evaluation and feeding to pigs.

2.2. Production and importance of cowpeas

Cowpeas, also commonly known as the Blackeye pea, Southern pea or Crowder pea, is a legume crop which originated and was domesticated in Southern Africa (IITA, 2015). The crop has spread to the semi-arid tropical zones across Africa, Asia, Europe and the Americas (IITA, 2015). Globally, cowpea's annual production is about 3 million tonnes (Onyenekwe *et al.,*

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2000). In 2013, most production was from the African continent, amounting to 95% of the global cowpea production, on more than 11 million hectares. Minor producing regions include Asia (3.2%), the Americas (1.3%) and Europe (0.5%) (IITA, 2015). Cowpea is a source of income for small-scale farmers and provides food security during the dry season. Mishili *et al.,* (2009); (Abizari *et al.,* 2013) observe that production in West and Southern Africa has increased across several socio-economic areas and is rapidly expanding to urban populations.

Cowpea is a drought-tolerant plant which can extract deep underground water using its deep root system to maintain its growth (Dabat *et al.,* 2012). It is adapted to various climates and soils (Gondwe *et al.,* 2019). In the advent of climate change, cowpea might be increasingly adopted by communities in countries which face the most adverse effects of climate change. As a leguminous plant, it reloads nitrogen-depleted from soils. Cowpea is intercropped chiefly with maize, millet, sorghum and sugar cane (Singh *et al.,* 2003). In smallholder farming systems, cowpea is traditionally used as a rotational cover crop for soil nitrogen fixation, to control erosion, and to improve soil properties (Valenzuela & Smith, 2002). (Valenzuela & Smith 2002) stated that, when used as a cover crop, cowpea destroys weeds in the field when grown with cash crops and can boost populations of beneficial insects to defend cash crops from insect pests. Cowpea is considered a drought tolerant, largely rain-fed crop requiring at least 600 mm of annual rainfall (Valenzuela & Smith 2002).

In arid small-holder farming systems, native legumes such as the cowpea remain largely outside the commercial value chain, thanks to disproportionate breeding and nutrition research (Ragab *et al.,* 2010; Carvalho *et al.,* 2017). Promoting traditional drought-tolerant crops such as cowpea should be considered to mitigate the impact of water shortages and climate-change effects on vulnerable tropical small-scale food production. Hence there is growing consensus to promote these typically drought-tolerant, commercially excluded traditional food legumes, whose surplus can be diverted to stock feeding. However, native, drought-tolerant crops are among the least researched crops (Ragab *et al.,* 2010). In Southern Africa, domestic utilisation of the cowpea is minimal, given the largely subsistent production. This scenario can be turned into an advantage for pig farmers who are facing challenges in cost effective, nutritionally balanced pig feeding. The biology of the cowpea and its genetic diversity was previously reviewed in relation to nutritional and agroecological factors (Boukar *et al.,* 2011; Boukar *et al.,* 2015; OECD, 2015), and to drought tolerance (Carvalho *et al.,* 2017). The cowpea is considered highly agroecologically adaptable (Boukar *et al.,* 2011; Smýkal *et al.,* 2015; Carvalho *et al.,* 2017). Given characteristics such as nitrogen fixation and adaptation in poorly resourced arid tropical small-holder farming systems, the cowpea is versatile for different agronomic applications, such as rotational or inter-cropping with resilient

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popular small grains such as sorghum and millet (Boukar *et al.,* 2011), or, in higher rainfall areas, with maize (Boukar *et al.,* 2011; Smýkal *et al.,* 2015).

2.3. Nutritive value of cowpeas

Leguminous crop plants are an essential source of protein for animals. The pig diet's protein content can be enhanced by including readily available and accessible plant protein sources, especially legumes, to improve the overall nutritional status of the low-income groups (Khattab & Arntfield, 2009). The variable chemical composition of cowpeas is illustrated in Table 2.1. The chemical composition of cowpea grain is similar to that of other pulses (e.g. mung bean, kidney bean, and pinto bean) with a relatively low-fat content and total protein content that is two- to fourfold higher than cereal (da Silva *et al.,* 2018). Cereal proteins are deficient in some essential amino acids, mostly in lysine (Anjum *et al.,* 2005), and are qualitatively complemented by the legumes. The cowpea protein content is in the range of 16-31%, in addition to significant starch, minerals and B-vitamins (Boukar *et al.,* 2011; Jayathilake *et al.,* 2018). Akande *et al.,* (2010), Chakam *et al.,* (2010) and Ragab *et al.,* (2010) reported high (up to 24.8%) crude protein in cowpeas. The dominant proteins are the globulins, with fewer albumins, glutelin, and prolamins (Gupta *et al.,* 2010; Vasconcelos *et al.,* 2010; Gonçalves *et al.,* 2016; Carvalho *et al.,* 2017). Detailed nutritional chemistry of vicilin, the main cowpea storage protein, was previously described by (Kimura *et al.,* 2008 & Oliveira *et al.,* 2012). Overall, cowpea protein quality is considered complementary to cereals (Jayathilake *et al.,* 2018), being rich in lysine and tryptophan (Sai-Ut *et al.,* 2009), but deficient in the sulphur-containing amino acids, methionine, and cysteine (Khattab, 2009; Chakam *et al.,* 2010; Frota *et al.,* 2017).

Table 2.1 Chemical composition (%) of cowpeas

Components	Martin *et al.,* 2013	Arawande *et al.,* 2010	Frota *et al.,* 2008
Moisture	5.77	9.20	10.9
Crude protein	21.95	24.13	25.9
Crude fat	1.21	4.37	2.0
Crude fibre	-	0.97	-
Dietary fibre	-	-	15.2
Ash	4.67	4.73	3.6
Minerals (mg/100 g)			
Iron	0.19	0.80	-
Ca	-	0.44	-
Na	-	1.20	-
P	-	29.40	-
Amino Acids	Martin *et al.,* 2013	Rivas-Vega *et al.,* 2006	Alexandre *et al.,* 2016
Lysine	6.81	4.9	7.9
Threonine	4.77	2.9	4.0
Tryptophan	0.97	-	1.3
Methionine	0.49	1.1	3.0
Cysteine	1.61	-	2.4

2.3.1. Anti-nutritional factors in legume seeds

In their raw state, the seeds of legumes typically contain a wide range of antinutrients (Table 2.2), many of which are potentially toxic (D'Mello, 2000). Welch & Graham (2004) suggested the ANFs in plants may be a way of storing excess nutrients or a means of defence from destruction by insect pests and grazing animals. Common anti-nutritional factors (ANFs) in legumes include trypsin inhibitors (Barth *et al.,* 1993; Marty, 1993; Schulze, 1994), lectins (Schulze 1994) and tannins (Jansman *et al.,* 1995). Akande *et al.,* (2010) identified the major ANFs in legumes as the protease inhibitors, lectins, gossypol, goitrogens, antivitamins, phytates, saponins, estrogens, flatulence factors, chlorogenic acid, amylase inhibitors, allergens and lysino-alanine. Collins *et al.,* (2006) reported that phytic acid, condensed tannins, lectins, and protease and α-amylase inhibitors limit legume nutritional value. Effects are largely limited to interference with gastrointestinal tract physiology (Salgado *et al.,* 2002).

The ANF have harmful effects on the gastrointestinal system sufficiently to affect growth performance (Grant *et al.,* 1995; Makinde *et al.,* 1996). Digestibility is strongly affected by the content of dietary fibre and by plant-secondary compounds with toxic or antinutritive activities

(Martens *et al.,* 2012). In swine, tannins depress growth rates and protein utilisation, damage the digestive tract's mucosal lining, alter certain cations' excretion, and increase excretion of proteins and essential amino acids (Cannas, 2008). Legume alkaloids such the quinolizidine in lupins, which has a bitter taste, lower the amount of feed consumed (Acamovic *et al.,* 2004). The availability of minerals including zinc, calcium, and magnesium is significantly decreased by phytate (Rimbach *et al.,* 2008). The trypsin inhibitor, an ANF with a significant impact on protein digestion, is arguably the most significant (Kumar *et al.,* 2006). Monogastric animals are affected by trypsin inhibitors, whereas ruminants are not affected since the rumen bacteria break them down (Afiukwa *et al.,* 2012). Trypsin and chymotrypsin, two protease inhibitors found in soybeans, slow growth and can result in pancreatic hypertrophy and hyperplasia (Swiech *et al.,* 2004). Overall, it has been demonstrated that the ANFs increase the quantity of nitrogen and endogenous amino acids that pass through the terminal ileum (Nyachoti *et al.,* 1997).

Dietary inclusion of unprocessed cowpea seeds for feeding to growing animals leads to impaired growth due physiological and biochemical effects, including toxicity (Belal *et al.,* 2013). Trypsin inhibitors activate pancreatic juice secretion which causes pancreatic hypertrophy (Kayembe, 2013). Protein inhibitors of the wide spectrum of hydrolases present in pulses are active against proteases, amylases, lipases, glycosidases, and phosphatases, those inhibiting proteases being the most well-known (Ritchie, 2020). The inhibitors of serine proteases, trypsin and chymotrypsin are of utmost importance (Shi *et al.,* 2017). Trypsin inhibitors and the serine protease inhibitors, are low molecular weight proteins found in a wide range of food sources, including pulses (Wang & Daun, 2004). They are bind lysine and arginine residues in trypsin, a proteolytic enzyme secreted by the pancreas (Mondor *et al.,* 2009). This results in a reduction of protein digestion, which leads to insufficient available amino acids (Savage, 1989) cited by Shi *et al.,* (2017).

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Table 2.2 Anti-nutritional Factors in legume seeds and their metabolic/physiologic effects on different livestock species.

Legume seed	Antinutritional Factors	Content (mg)	Physiological Effects	Species	Reference
Raw kidney bean	Trypsin inhibitors	1.6	Physiological anomaly of liver, pancreas and gizzard	Broiler	Adewale, 2007
Cowpea	Tannin	0.76	Reduction in feed intake and lower body weight gain	Broiler	Nasara *et al.,* 2011
	Trypsin inhibitors	6.1 1.6	hypersecretion of pancreatic enzymes and hypertrophy of the pancreas	Rats	Corring *et al.,* 1986; Sauer *et al.,* 1999 and Erlwanger *et al.,* 2011
Processed soya-bean	Trypsin inhibitors	-	Pancreatic hypertrophy	Piglets	Qin *et al.,* 2002
Cooked beans	Amylase inhibitor	-	Poor digestion	Piglets	Lajolo *et al.,* 1984
Heated cowpea	Trypsin inhibitor	1.6	No effects	Piglets	Ekambaram *et al.,* 2008
	Phytate	0.8			
	Lectins	6.4			
	Tannins	1.0			
Raw pigeon pea	Trypsin inhibitor	-	Growth depression	Piglets	Le Guen *et al.,* 1995
Sprouted barley and wheat	-	-	Increased ADG	Weanling and finishing pigs	Greg, 2013

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2.3.2. Methods, and benefits of legume processing

Soaking, sprouting, roasting or cooking, are among the traditional legume processing methods. They have variable efficacy in eliminating anti-nutritional factors (Table 2.3). Protaineceous ANFs are heat-labile, while heat-stable ANFs (e.g., phytate and polyphenols) are not eliminated by simple soaking and heating, but through germination or fermentation (Sridhar & Seena, 2006). Bioprocessing can affect certain functional properties (Yusuf *et al.,* 2008). In addition to decreasing or eliminating some anti-nutritional factors, germination may also increase functional compounds (Doblado *et al.,* 2007). Gharachorloo *et al.,* (2013) reported that bioprocesses such as germination significantly enhanced antioxidant properties in pulses. Fernandez-Orozco *et al.,* (2009) reported vitamins C and E, phenolic compounds, and glutathione as the main antioxidant compounds in pulses. An increase in the antioxidant activity of beans and peas during germination was observed for lupines (Duenas *et al.,* 2009) and oat, (Tain *et al.,* 2010). Pallavi & Kanika, (2016) observed increased antioxidant activity after germinating soybean and mung beans.

Hong *et al.,* (2004) showed that bio-processed legumes contained more small peptides than unprocessed legumes. This is because during biological processing, the large protein, lipid and carbohydrate molecules are broken down into smaller molecules such as peptides, amino acids (AA), fatty acids and sugars (Kwon *et al.,* 2010). The rate and extent of absorption of small peptides is greater than free AA (Gilbert *et al.,* 2008). Therefore, an increased concentration of small peptides should improve digestibility. The presence of both TIA and oligosaccharides in soybean products reduces AA digestibility (Smiricky *et al.,* 2002). Removal of the oligosaccharides may also be an advantage because of reduction in diarrhoea associated with feeding unprocessed legumes (Liener, 2000; Liying *et al.,* 2003).

Wang *et al.,* (2008) observed increased levels of trypsin inhibitors after soaking. Leaching trypsin may be lower than other chemical constituents, resulting in increased concentration in the soaked product (Shi *et al.,* 2017). El-Hag *et al.,* (1987) reported a 50 percent reduction in TIA in kidney beans during 10 days of germination. Ramakrishna *et al.,* (2006) reported decrease in TIA to 51 per cent during 12-h soaking, which progressively decreased to 17 per cent after germination. The reduction is mainly a catabolic process, as the reserved substances present in the cotyledons are used for the development of an embryo (Ramakrishna *et al.*, 2006). Rasha *et al.,* (2011) reported reduction in the phytic acid in processed legumes.

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Table 2. 3 Effects of processing on the bioactive compounds in legume seeds

Legume seed	**Processing Method**	**Effect on Antinutritional Factors**	**Dietary inclusion level (%)**	**Monogastric livestock responses**	**Reference**
Kidney bean	Raw	-	30	--	Adewale, 2007
Cowpea	Raw	-	100	--	Makinde *et al.,* 1996
	Heat-treated	+	15 - 30	++	Ekambaram *et al.,* 2008
Soya-bean	Cleaved	+	30	++	Ekambaram *et al.,* 2008
Pigeon pea	Cooked	-	45 - 75	--	Etuk *et al.,* 2005;
	Raw	-	25	--	Grimaudi, 1988,
		-	5 - 10	--	Batterham *et al., 1990*
Raw lupine seed	Ground, Expanded and Flaked	+	20 - 37	++	Petterson, 2000
			15 - 30		Kwak *et al.,* 2000

(+) Legume seed had a positive effect on the analytical bioactive compound, (-) legume seed had a negative effect on the analytical bioactive compound, (++) Legume seed had a positive effect on monogastric livestock responses, and (--) legume seed had a negative effect on monogastric livestock responses.

2.3.2. Effects of roasting cowpeas

Most anti-nutritional factors are heat-labile (Emiola *et al.,* 2006), so heat treatment could be suitable for their denaturation. Several options of thermal treatment are available that can decrease the anti-nutritional factor activities in cowpeas (Bene, 2007). Of these methods, an effective, simple method that is easy and cost-effective for small-scale farmers, such as drum roasting, mud oven and steel plate roasting, is required. Residual activities of trypsin inhibitors and lectins were observed in some mucung and kidney bean varieties after dry heat treatment (Udedibie, 2000). Despite intensive heat treatment, soybean might still contain 20% residual trypsin inhibitors (Friedmann, 1992). According to Simoongwe (1998), roasting cowpea seeds can decrease the content of trypsin inhibitors from 57.0 - 31.92 %. On the other hand, autoclaving of cowpea seeds at 120°C for 15 minutes improved digestible energy when estimated in pigs and poultry, respectively (Nell, 1992).

2.3.4. Effects of boiling cowpea

Boiling is an inexpensive heat treatment method and can be easily executed by small-scale farmers (Ragab *et al.,* 2010). Furthermore, boiling is the heat treatment that small-scale farmers commonly use, whereby they use firewood which is readily available as an energy source. Boiling increases carbohydrate digestibility since heat treatment gelatinizes starch, which improves its digestion (Emiola *et al.,* 2006). Excessive boiling time is a major factor limiting the utilisation of grain legume protein (Ragab *et al.,* 2010).

2.3.5. Effects of sprouting cowpeas

Sprouting is a natural or bio-processing method that does not require heat energy and should therefore be suitable for small-scale farmers with limited resources. Sprouting as a technology is widely applied to reduce levels of antinutritional factors present in legume seeds and improve the concentration and availability of their nutrients (Urbano *et al.,* 2005b).

Sprouting enhances the nutritive value of legumes by initiating the production of amylolytic enzymes that decrease the anti-nutritional factors and indigestible factors such as stachyose, raffinose and verbascose in legumes (Uriyo, 2001). These enzymes are not found in significant amounts in dormant seeds and become active at the onset of sprouting (Urbano *et al.,* 2005a). These enzymes assist in degrading starch during sprouting and use it as a source of energy for sprouting, while oligosaccharides are also degraded to improve carbohydrate digestibility (Urbano *et al.,* 2005). Thiamine and mineral bioavailability as well as protein and starch digestibility, increased, while phytic acid and tannin concentrations reduced during sprouting (Uriyo, 2001). Moreover, sprouting has the benefits of simplicity, reproducibility, and lower costs (Urbano *et al.,* 2005), which makes it appropriate for implementation by small-scale farmers. Most tannins are located in the testa of cowpea seeds (Ghavidel *et al.,* 2006). Bene (2007) reported that the sprouting of un-soaked seeds increased tannin content. The increases were due to the solubilisation of insoluble tannins due to sprouting, which causes soluble tannins to transfer from the seed coat to the cotyledons of the seed (Ahmed *et al.,* 2006).

The period of sprouting plays a significant role in the palatability and digestibility of the seeds. Sprouting of 12 h pre-soaked cowpea seeds to 60 h improved the digestibility of starch and protein (Negi *et al.,* 2001). The α-galactosidase activity reaches maximum values within three days of sprouting in cowpea (Ghavidel *et al.,* 2006), but the amylolytic enzymes remained active after three days, as verified by the decreased α-galactosidase content from day three until day six of sprouting, causing a simultaneous increase in levels of reducing sugars (Urbano *et al.,* 2005). These mono- (glucose, galactose and fructose) and disaccharides (sucrose, lactose and maltose) are more simply absorbed by the small intestine of monogastric (Urbano *et al.,* 2005).

However, sprouting might be accompanied by toxic fungal growth (Azeke *et al.,* 2011). Therefore, the addition of a feed mycotoxin binder or inexpensive, or sterilisation with non-toxic household disinfectors may be essential to prevent mycotoxicosis (Azeke *et al.,* 2011).

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2.4. Pig responses to cowpea processing and level of dietary inclusion

Pig responses to cowpea processing are outlined in Table 2.4. The ANFs contained in cowpeas are known to have various effects, including morphological changes and enzyme inhibition along the intestinal mucosa (Erickson *et al.,* 1985; Kik, 1991). Cowpea ANFs cause poor digestibility, diarrhoea, poor growth performance and sometimes death (Huisman *et al.,* 1990; Li *et al.,* 1991). When fed to weanling pigs, raw cowpea affect growth and cause haematological and systemic changes (Umapathy *et al.,* 1995). Makinde *et al.,* (1996) observed that cowpea-fed pigs experienced hyper-regenerative villus atrophy, which was severe at seven days, and persisted up to 21 days.

According to Umapathu (1999), cowpea feeding increased exocrine pancreatic secretions in pigs, with increased trypsin activity. However, pancreatic homogenates from pigs fed cowpea did not show any variances in protein content and trypsin activity (Erlwanger *et al.,* 1999). Umapathy *et al.*(2008) observed negative effects of 30% replacement of soybean meal with heat-treated cowpeas on protein utilisation of growing pigs. Factors contributing to poor utilisation of protein include poor digestibility, deficiency of sulphur amino acids and the presence of anti-nutritional factors such as phytate and polyphenols, and enzyme (trypsin and chymotrypsin) inhibitors in the feed.

Table 2. 4 Responses in weaned pigs fed diets containing cowpeas processed by different methods at variable inclusion rate

Processing	**Dietary inclusion (%)**	**Effects**				**Reference**
		Digestibility	Feeding intake	Ileal histology	Blood parameters	
Raw	100	+	+	-	-	Makinde *et al.,* 1996
Raw	75	+	+	-	-	
	50	+	+	-	-	
	25	+	+	-	-	
Heat treated cowpea	15	+	+	+	+	Ekambaram *et al.,* (2008).
	30	+	+	+	+	

(+) Cowpea substitution had positive effect and (-) Cowpea substitution had negative effect.

2.5. Protein evaluation in pigs

The nutritive quality of a food protein source is defined as the capacity to supply the quantity and proportion of essential amino acids to meet the requirements of the species that consumes it (Barrón-Hoyos *et al.,* 2013). Święch (2017) states that, feed protein evaluation is presently based on the determination of the ileal digestibility of essential amino acids in cannulated pigs. Therefore, such a procedure can be replaced by measurement performed *in vivo* or assayed by the two-step to three-step *in vitro* method simulating the digestion of protein up to the end of the small intestine (Święch, 2017).

2.5.1. *In vitro* evaluation of digestibility

A commonly used model to estimate the *in vitro* digestibility of dry matter and protein was developed by (Boisen, 1991). Variants of this approach typically use pepsin and pancreatic proteases to mimic digestive functions *in vivo*. Simulation of the *in vivo* absorption process is applied based on the solubility or molecular sizes of digested peptides. The underlying assumption is that insoluble peptides or greater than a critical size (molecular weight cut-off) are not absorbed *in vivo* (Qiao *et al.,* 2004). The reported digestibility coefficients, however, are normally lower by as much as 40% than those measured *in vivo* (Brule *et al.,* 1988; Savoie *et al.,* 1989). Incomplete digestion resulting from short-time incubation and/or suboptimal enzyme concentration might be the reasons for the underestimation since *in vitro* digestibility was not influenced by endogenous losses.

In vitro bioavailability techniques are valuable in that they provide knowledge on possible interactions between nutrients and/or food components, the effects of luminal factors (including pH and enzymes), food preparation and processing practices, nature of the food matrix etc., on either micronutrient absorbability (a component of bioavailability) or on the potential for a nutrient to be absorbed (Etcheverry *et al.,* 2012). *In vitro* methods are less expensive, faster, and offer better controls of experimental variables than human or animal studies (Sandberg, 2005). However, *in vitro* studies cannot be substituted for *in vivo* studies and should be therefore regarded as a screening, ranking, or categorizing tool. Etcheverry *et al.,* (2012). In each of these methods, an *in vitro* digestion is conducted to simulate the digestive system via a two-step (sometimes a three-step) digestion that includes gastric and intestinal digestion. For gastric digestion, pepsin (from the porcine stomach) is added prior to the acidification of the samples to pH 2.

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2.5.2. Measurement of apparent digestibility and nitrogen balance

Efficient feed utilisation depends on understanding the optimum levels that meet protein requirements for growth, reproduction, and maintenance (Paul *et al.,* 2007). Incorrect supply of protein is not ideal for optimal protein deposition and can be harmful to pig performance (Whittemore *et al.,* 2001; Noblet *et al.,* 2004; Norgaard *et al.,* 2014). For instance, additional dietary protein increases nitrogen excretion through the urine (Carpenter *et al.,* 2004).

Adequate protein intake reduces N losses to the environment. An imbalance in the amino acids provided for protein synthesis for growth and other functions leads to the catabolism of surplus amino acids and the conversion of excess nitrogen to urea, which increases the outflow of urea in urine (Ball *et al.,* 2013). Nevertheless, gastrointestinal protein indigestion diverts N to colon fermentation (Bindelle *et al.,* 2009). When provided a suitable source of fermentable energy, colon bacteria use both endogenous and dietary N to lock it and switch excretion from microbial protein in faeces to urea in urine (Bindelle *et al.,* 2009). Increased colon protein fermentation could lead to the creation of toxic substances if energy is insufficient (Tunio *et al.,* 2017). Aquilani *et al.* (2019) observed slow growth rates in pigs. Lower protein requirements as compared to lean genotypes, whose recommended protein intake for growing pigs is 16% CP (National Research Council, 2012). Aquilani *et al.* (2019) reported that, opportunity for further lowering the dietary CP for growing pigs cannot be excluded, but the linear increase in fat gain might indicate that it has reached the lower limit of protein to energy supply below which, lacking the AAs required for protein deposition, the energy in excess is used for fat deposition. Indeed, diets excessively poor in protein enhance the genetic predisposition to fat deposition in local breeds, leading to carcasses that are extremely fat (Wang *et al.,* 2018), with a worse slaughtering yield and a lower consumer acceptability of meat when compared with those of leaner pigs.

A lack of some AAs or an unbalanced intake of some AAs could result from reducing the dietary CP content alone. Protein synthesis stops when an AA is initially limited, and the excess AAs are expelled, increasing N excretions (Aquilani *et al.,* 2019). According to Hlongwana *et al.,* (2021), a high nitrogen excretion through faeces indicates that more nitrogen is being utilised for microbial protein synthesis, increasing microbial biomass in the hindgut. Because it degrades more slowly than urine N, organic N can be excreted through faeces with less volatility and benefit (Patrá *et al.,* 2012; Jha and Berrocoso, 2016). The difficulty is the extensive quantitative and in terms of protein content (Gilani *et al.,* 2005; Gilani *et al.,* 2011) and anti-nutritional secondary chemicals, there is qualitative difference among legume species and their cultivars (Garry *et al.,* 2007; Jezierny *et al.,* 2010). According to Sauvant *et al.,*

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(2004) and the National Research Council (2012), antinutritional factors (ANFs) can worsen the low protein quality of native legume-based diets by impairing digestion, causing endogenous wastage through damaged gut epithelial cells, and inhibiting the secretion of protective mucoproteins (Brenes *et al.,* 2004).

2.6. Pig adaptation to legume antinutrients

In addition to dietary factors, the pig genotype determines the distribution of lean in relation to fat tissue though controls on growth and differentiation (Cline *et al.,* 2001). These effects influence the qualitative and quantitative protein requirement (Cline *et al.,* 2001). Rapid lean accretion increases requirements for amino acids and energy for growth (Schinckel *et al.,* 1996). Therefore, both feed quantity and quality determine growth and slaughter performance (Ellis *et al.,* 2001).

For proper livestock breeding and feeding, it is crucial to have a good awareness of breed traits (FAO, 2007). The range of alleles and genotypes existing in a population, which is represented in physical, physiological, and behavioural variances between diverse species, contributes to genetic diversity among pig breeds (Frankham *et al*., 2002). According to Hendrick (2001), genetic diversity can be beneficial, harmful, or adaptive. Pig breeds in South Africa come in a wide range and are genetically diverse, which is important for the socio-economic welfare of the nation. About 1.6 million pigs are kept for pork production to feed South Africa's growing population (Szűcs & Vida, 2017). The most common pig breeds in South Africa are the Large White, Landrace and Duroc (Halimani *et al.,* 2010). Windsnyer pig production is largely confined to non-commercial, low-scale free-range production due to slow growth rates, smaller carcasses' tendency for early fat deposition on commercial, typically high-energy diets (Bester, 2010; Chimonyo *et al.,* 2010). Windsnyer pigs are an indigenous genotype to Southern Africa, and, due to slow growth rates, does not contribute significantly to commercial pork production because they have smaller carcasses and tend to deposit fat early when fed high-energy diets (Bester, 2010; Chimonyo *et al.,* 2010). Most of these indigenous pigs are reared in rural areas of Southern Africa, so they rely on scavenging, eating grasses and insects, and digging out roots. Therefore, this may produce less fat and provide an opportunity to supply inorganically produced pork.

Genotype effects on the tolerance of the ANF’s are not fully understood. For example, relatively higher sensitivity of the Landrace piglet to the ANFs of soybeans is attributed to a relatively small intestine (Qin *et al.,* 2002). The high and negative correlation of small intestine weight and/or length to crude protein and crude fat digestibility indicate that animals with heavier small intestines do not digest or absorb dietary nutrients as well as those with lighter small intestines (Qin *et al.,* 2002). Therefore, heavier small intestine of Landrace piglets may be the pathological effect soybean ANFs (Qin *et al.,* 2002). Potential differences in dietary responses among indigenous exotic, or crossbred genotypes need investigation.

2.7. Dietary effects on blood metabolites, digestive viscera and pig performance

In the pig production industry, pigs are bred to perform and grow as cost-efficiently as possible (Ewing & Tucker, 2008). Factors with a negative impact on the health of the animals are often related to the dietary composition, and to intestinal microbiota which assist in nutrient digestion (Fairbrother *et al.,* 2005). Animal feed and environmental factors interact to determine voluntary feed intake in growing pigs (Cline *et al.,* 2001; Ellis *et al.,* 2001; NRC, 1998).

Plasma proteins have long been favoured as surrogate metabolic markers by the biochemist and the clinician because they are the most readily available protein group with a wide variety of natural functions and biological properties (Frank, 1960). Blood plasma contains three major protein fractions: albumin, globulin, and fibrinogen. In horses, pigs and cattle, the ratio of albumins and globulins is almost equal, or globulins prevail (Swenson, 1993). Hollywood *et al.,* (2006) defined metabolomics as an omics approach to characterise the 'metabolome', the catalogue of the metabolites present in a biological fluid or system. Metabolites are biochemical intermediates or final products of metabolic pathways that constitute the basic biological mechanisms that directly or indirectly affect phenotypic traits, like production traits in farm animals (Bovo *et al.,* 2016). Therefore, metabolites can represent intermediate links between gene activities and complex phenotypes (Fiehn, 2002). For this reason, they can be considered the targets for identifying biomarkers for specific endpoint nutritional or clinical phenotypes (Monteiro *et al.,* 2013). According to Esonu *et al.,* (2001), the physiological reactivity of the animals to their internal and external environments, which includes diet and feeding regimens, is reflected in the haematological contents (Ekenyem & Madubuike, 2007).

The functions of the blood, which is made up of blood cells and plasma, include transport, regulation, protection, and homeostasis (Eze *et al.,* 2010). According to a study by Madubuike and Ekenyem from 2006, knowing an animal's haematological and biochemical parameters can be used as a gauge to gauge how well it will respond to any ration given to it. The use of hematological profiles in the diagnosis and treatment of numerous diseases has made them significant indicators of animal health and disease (Mbanasor *et al.,* 2003). Counting erythrocytes, measuring packed cell volume, and measuring hemoglobin can provide information about of the severity of disease circumstances. Moreover, animal haematological

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and plasma biochemistry tests provide evidence of the animals' physiological sensitivity to food (Madubuike & Ekenyem, 2006). The quantity and quality of a ration supplied to an animal impacts its physiological state, according to Machebe *et al.,* (2010).

2.8. Pig breeds in South African

Due to their commercial attractiveness, pig producers are choosing the exotic, over the native breeds which they believe to be more advanced (Munzhelele, 2015). However, the native pigs remain a source of food and revenue for people who farm in rural areas of African nations, including South Africa (Madzimure *et al.,* 2013; Kimbi *et al.,* 2016). Exotic breeds such as the Duroc, Landrace, and Large White outperform the native pigs in size, prolificacy, litter sizes, and carcass confirmation (Halimani *et al.,* 2012). The indigenous breeds common in South African pigs such as the Windsnyer are a hardy and versatile breed, able to survive on very little, and have been shown to have greater immunity than improved breeds (Visser, 2014; Munzhelele, 2015). Local breeds typically have higher piglet mortality rates and slower growth rates than exotic breeds (Halimani *et al.,* 2012).

The Landrace, Large White, and Duroc breeds, and hybrids of their breeds, are the most commonly used breeds by South African pig producers. Using the hybrid vigour present in the offspring, farmers have also mixed native breeds with exotic breeds to combine the hardiness of the former with the productivity advantages of the latter (Visser, 2014). Breeds such as the Duroc, Landrace, and Large White have been crossed with the Windsnyer to produce offspring who have improved grading, growth rates, feed conversion efficiency, and carcass quality (Visser, 2014).

According to Madzimure *et al.* (2012), there is potential for employing indigenous pigs in production systems that are focused on subsistence, and for crosses between the indigenous and imported breeds in systems that are focused on the market. Small-scale farmers in South Africa's provinces of Mpumalanga (Munzhelele, 2015), KZN (Gcumisa, 2013), and Limpopo (Mokoele, 2015) have all reported seeing a trend away from domesticated varieties toward exotic or commercial types. Madec *et al.* (2010) reported that small-scale farmers typically farm using crossbreeds and improved breeds like the Large White or Landrace. Similar observations were reported from studies conducted in Mpumalanga (Munzhelele, 2015). However, in the Limpopo (Mokoele, 2015), Eastern Cape (Madzimure *et al.,* 2012), and KZN (Gcumisa, 2013) provinces in in South Africa, small-scale rural farmers continue to raise indigenous pigs. This might be because native breeds continue to produce even when living in unsanitary surroundings. Local breeds may also be fed inferior feed due to their reduced

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nutrient needs (Madec *et al.,* 2010). In the rural areas of KZN, indigenous pigs are preferred because of their small size (Gcumisa, 2013). The Large White, Landrace, Duroc, Large Black and Pietrain are among the popular exotic breeds Gcumisa, (2013).

2.9. Summary

Factors contributing to poor utilization of legume protein by animals include poor digestibility, deficient sulfur amino acids and the presence of anti-nutritional factors such as phytate and polyphenols, and enzyme (trypsin and chymotrypsin) inhibitors in the feed. High levels of anti-nutritional factors in legume seeds, including protease inhibitors, lectins, anti-metals, saponins and other toxic substances affect exclude the feeding of raw legume grains to monogastric animals. Physical treatments include dehulling soaking, sprouting, germination, cooking, thermal treatments, irradiation and protein fractionation. A variety of processing methods could improve the digestibility legume protein and starch (Alonso *et al.,* 1998). Doblado *et al.,* (2007) reported that heat-labile factors such as trypsin inhibitors could be most effectively destroyed by heat treatment. The pig genotype influences growth, so factors such as feed intake level and diet composition should match the potential growth, which is a function of the genetic potential of an animal (Lopez *et al.,* 2001). Indigenous breeds are known to have a better ability to digest fibre and lower protein requirements than improved pig breeds (Halimani *et al.,* 2010; Madzimure *et al.,* 2012). Therefore, the cowpea market and pig production can be dramatically improved for both small scale and commercial farms if the legume can be effectively utilised in pig diets. The contribution of emerging small-scale pig farmers to the rural economy is unclear. Production is limited due to constraints, including the pig genotype, reproduction management, health, housing and nutrition, and marketing opportunities (Antwi *et al.,* 2011; Chikazunga *et al.,* 2007). According to FAO (2007), a thorough comprehension of the characteristics of a breed is a necessary guide for proper livestock development and breed production. Before including locally grown legumes in pig diets, it is critical to comprehend their nutrient content. Additionally, it is essential to investigate the effects on pig performance of substituting locally grown field legumes for expensive soybean meal in pig diets (Stein *et al.,* 2004). The pig diet's protein content can be enhanced by including readily available and accessible plant protein sources, especially legumes, and cowpea, to improve the overall nutritional status of the low-income groups (Khattab & Arntfield, 2009). It is essential to ensure effective cowpea processing by convenient methods such roasting or sprouting cowpea for optimal utilisation (Farran *et al.,* 2001).

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CHAPTER 3

IN-VITRO DRY MATTER DIGESTIBILITY EVALUATION OF THE EFFECTIVE COWPEA (*VIGNA UNGUICULATA (L.) WALP*) SPROUTING OR ROASTING FOR PIG FEEDING

Abstract

The study investigated the effective roasting or sprouting of cowpeas (*Vigna unguiculata*) based on effects on *in vitro* digestibility (*IVDMD*), supported by measurement of key chemical components, and trypsin inhibitor activity (TIA). In experiment 1, cowpeas were sterilised, soaked in water for 12 hours and allowed to to sprout (SCP) over four days under open air irrigation, with daily sampling and rapid sample sun-drying to terminate the sprouting. In experiment 2, cowpeas were roasted (RSCP) in a procedure in which raw cowpeas were placed in an empty, 150^{o} preheated, manually roasted cast-iron drum, and roasted for 10, 15, 20 or 30 minutes, coincident to 55° C, 95° C, 105° C and 130° C respective terminal sample grain temperatures. The *in vitro* dry matter digestibility (*IVDMD*)of raw and all processed cowpeas were evaluated using standard, 3-step (gastric-ileal-colon) simulation of porcine digestion, modified for micro (0.5g) sample digestion. Standard methods were employed to track processing effects on Ash, CP, Fat, ADF, NDF and trypsin inhibitor activity (TIA) at key processing points. Sprouting for 2 and 3 days significantly decreased ($p<0.05$) gastric-ileal *IVDMD* while increasing ($p<0.05$) colon *IVDMD*. Total (steps 1-3) *IVDMD* increased ($p<0.05$) in 2-day (0.911) and 4-day (0.902) sprouts. The 20-minute cowpea roasting to 105°C terminal grain temperature resulted in high ($p<0.05$) step 3 and total *IVDMD* coefficients. The lowest ($p>0.05$) total *IVDMD* was recorded in 15-minute (0.883) (95°C terminal grain temperature) roasts. In both experiments 1 and 2, quantitative changes in *IVDMD* were consistent with the changes in the chemical components (ADF, NDF, fat, CP, minerals), and trypsin inhibitor activity. In conclusion, the compartmental and total *IVDMD,* and quantitative change in chemical components and TIA indicated 4 days sprouting, and 20-minute (105° C) roasting were respectively most effective for cowpea processing.

Key words: Antinutrtional factors, digestibility, plant protein

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3.1 Introduction

Globally, legume grain ranks second to cereals in providing human and animal dietary plant protein. Depending on species and cultivars, the seed endosperm contains 20–40% protein, with substantial carbon and energy stored in lipids (soybean, peanut) and substantial starch (cowpea) (Smykal *et al.,* 2014). Promoting traditional, drought-tolerant legume crops for food and stock-feed should be considered to mitigate the adverse effects of climate change, particularly in arid regions. However, native pulses which are currently outside the commercial value chain are still largely excluded from main-stream animal nutrition research (Ragab *et al.,* 2010).

The cowpea is a highly agroecologically adaptable leguminous crop (Boukar *et al.,* 2011), with whole grain protein content in the range 16% to 31% (Boukar *et al.,* 2011). Cowpeas are also rich in starch, minerals and functional compounds, including the B group vitamins (Boukar *et al.,* 2011). Subject to varietal differences, the globulins are the dominant protein, with low levels of albumins, glutelins, and prolamins (Vasconcelos *et al.,* 2010; Gupta *et al.,* 2010; Bell *et al.,* 2011; Goncalves *et al.,* 2016). Details of the structure of the main storage protein (vicilin) were previously described by Macedo *et al.,* (1995), Kimura *et al.,* (2008); Oliveira *et al.,* (2012); Rocha *et al.,* (2014). As a dietary complement to cereals, typical of the legumes, cowpea protein is rich in lysine and tryptophan, though relative to animal protein, it is deficient in the sulphur containing amino acids (methionine, cysteine) (Khattab, 2009; Frota *et al.,* 2017).

For monogastric stock feed, cowpea protein value may be limited in digestibility and gut absorption of amino acids, due to antinutritional factors (Khattab, 2009; Frota *et al.,* 2017). Among key cowpea antinutrients are protease inhibitors and lectins (Boukar *et al.,* 2015). Trypsin inhibitors are considered the most important of the protease inhibitors (Kochhar *et al.,* 1988). Lectins are glycoproteins which interfere with carbohydrates absorption (Zhang *et al.,* 2009). Processing techniques to mitigate antinutrient effects include soaking, autoclaving, pelleting, dry roasting, dehulling, germination/ sprouting or fermentation (Amaefule *et al.,* 2004; Amaefule *et al.,* 2006; Babiker *et al.,* 2006; Saeed *et al.,* 2007; Udensi *et al.,* 2007). Thermal processing is traditionally preferred, given the protease inhibitors and lectins are particularly known to be heat labile (Boukar *et al.,* 2015). Thermal processing also improves starch digestion through gelatinization (Boukar *et al.,* 2015). Among bioprocessing methods, germination and sprouting are advantageous in being non-energy consumptive, and are known to substantially improve the nutritive value of legume seeds through stored nutrient

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mobilization coupled to biosynthesis, and antinutrient detoxifying germination metabolism (Boukar *et al.,* 2015).

Subject to genotypic variances and environmental factors, effective legume seed biochemical modification through processing demands process-optimization. The processing should be calibrated to chemically preserve the nutrients, and to maximize pig nutrient extraction. *In vivo* evaluation of digestibility is time-consuming and expensive, therefore not always practical. Instead, *in vitro digestion* methods which conveniently simulate pig digestion are commonly employed either to screen feeds (Regmi *et al.,* 2009; Cervantes-Pahm *et al.,* 2013), or to test the efficacy of processing (Kong *et al.,* 2014). The standard *in vitro* assay generically involves stepwise incubation of feed samples in media which contain different enzymes, to sequentially simulate digestion in the stomach, the small and the large intestines (Huang *et al.,* 2003).

This study involved two experiments designed to separately evaluate the effective sprouting or roasting of cowpeas (*Vigna unguiculata (L.) Walp.)* for feeding to growing pigs. In both experiments, the evaluation adopted standard *in vitro* porcine digestion, modified for rapid, micro (5g) sample dry matter digestibility measurement, supported by spot analyses for trypsin inhibitor activity and key chemical components.

3.2 Materials and methods

The Ethics Committees of the University of Venda (SARDF/17/ANS/07/0412) approved the experimental protocols used in the experiment.

3.2.1 Cowpea grain sampling and pre-processing

Cowpeas (*Vigna unguiculata (L.) Walp*.) (Southern cowpea) were sourced from the local market (Thusano grain Products, Makhdo, Limpopo Province, South Africa). Standard procedures were followed to obtain representative analytical samples of cowpeas from the bulk supply. Damaged grains (cracked and weevil-bored) and all debris were manually removed.

3.2.2 Experiment 1: Evaluation of effective cowpea sprouting

Cowpeas were sterilized by soaking for 30 minutes in 20% sodium hypochlorite solution. Sterile grain was soaked overnight (approximately 12 hours) in tap water within sterilized soaking drums, after which the grains were subjected to 4-day open-air sprouting at ambient

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conditions during April 2019 (mean and diurnal temperature range ± 30 oC and 24 ^{o}C – 35 ^{o}C, respectively), with daily sampling. Daily sampled sprouts were rinsed in distilled water and the growth and differentiation terminated by sun-drying on an open concrete surface over 4 days.

3.2.2 Experiment 2: Evaluation of effective cowpea roasting

Screened and cleaned cowpeas were roasted in 20 kg batches within a cylindrical (Length =1.5 m; Diameter= 0.50 m) manually rotating, cast-iron, gas heated drum. Pre-roasting, the rotating empty roasting drum was heated to a maximum initial constant maximal interior temperature of 150°C, after which cowpeas were subjected to 10, 15, 20 and 30-minute roasting, which culminated in respective internal drum temperatures at sampling of 55°C, 105°C and 130°C. The highest or terminal roasting temperature was the point at which cowpeas turned golden brown, to avoid over-roasting.

3.2.3 *In vitro* digestion

Sprouted (experiment 1) or roasted (experiment 2) cowpea samples were subjected to 3-step *in vitro* porcine digestion (Boisen and Fernández, 1997), with modifications for micro-sample (0.5 g) dry matter digestibility evaluation (Fushai *et al.,* 2019). Samples milled to pass through a 1 mm sieve were oven-dried to a constant weight in a 105 ^{o}C, forced air oven, after which they were dry-cooled and stored in a desiccator. Approximately 0.5g samples were weighed into similarly dried Ankom® F57 filter bags which had been pre-rinsed in pure acetone (Acetone for HPLC, ≥99.8% (Sigma-Aldrich® product) 34850). Empty, and the sample filter bags were pre-weighed and suspended in digestive media within 250 ml glass digestion bottles immersed in a shaking water bath (CNW Model, WBS 450-B) set at 39 ^{o}C. Samples were digested in two parallel experiments, both arranged in a completely randomised design with 14 replicate bags per sample. For each treatment, seven sample + 1 empty (blank) filter bags were placed within one x 250 ml digestion bottle. The setup accommodated a shared (raw cowpeas) control, and four sprouting or roasting treatments for concurrent, experiments 1 (raw cowpea, 1, 2, 3 and 4 day sprouts) and experiment 2 (raw cowpea, 10, 15, 20 and 30 minute roasts). The digestion was conducted in two runs, to obtain 14 replicates per treatment. Given the small micro substrate samples and the relatively large filter bag surface, the empty bags were considered necessary to correct for potential treatment-dependent exchange of fine, non-digestible particulate matter, including particle attachment to the filter bag matrix, to effectively account for the net flux of the fine particles.

Stepwise simulation of pig digestion was conducted concurrently for experiments 1 & 2 as follows; as follows:

Step one (gastric digestion): 87.5 ml phosphate buffer (pH 7.2, 0.1 M, pH 6.0), 35 ml 0.2 M HCl, pH adjusted to 2.0 using 1 M HCl/M NaOH solutions, 3.5 ml aliquot freshly primed pepsin solution [10 mg/ml pepsin (Pepsin from porcine gastric mucosa powder, ≥250 units/mg solid) (Sigma-Aldrich® product P7000)], 1.7 ml of a chloramphenicol solution (0.5 g Chloramphenicol ≥98% (HPLC) (Sigma-Aldrich® product C0378, per 100 ml ethanol), two hours digestion.

Step two *(Small intestine digestion);* After the pepsin digestion, pH was accustomed to 6.8 by adding 35 ml of sodium phosphate buffer solution (0.2 M, pH 6.8), 17.5 ml NaOH (0.6 M, pH 13.8), 3.5 ml aliquot freshly prepared pancreatin solution containing 50 mg pancreatin [(Pancreatin from the porcine pancreas) (Sigma-Aldrich® product P3292)], five h digestion. Filter bags were successively gently rinsed in warm tap water, 95% ethanol, and 99% acetone, forced air oven-drying to constant weight at 105 ^{o} C over 24 hours to calculate digestibility.

Step three (Large intestine digestion): Step two media discarded, 218.75 ml freshly prepared phosphate buffer (0.1 M, pH 4.8), 1.75 ml Viscozyme [(Viscozyme® L, mixture of beta-glucanase, pectinase, hemicellulase and xylanase enzymes) (Sigma-Aldrich® product V2010)], 18 hours' digestion. Filter bags were similarly rinsed and dried for digestibility calculation.

3.2.4 Chemical analyses

Raw cowpeas and the two and 4-day sprouts (experiment 1) and for 10, 20 and 30 roasts (experiment 2) were analysed for key nutrients, fibre components and protease inhibitor activity (Table 3.1). Oven-drying two grams samples determined dry matter at 105°C for 48 hours (AOAC, 2000 method 976.050); Ash by heating two-gram samples at 550^{o} C overnight in an electric furnace (AOAC, 2000 method 923.03); Nitrogen using the micro-Kjeldahl method (AOAC, 2000 method 976.05). Ether extract (EE) by Soxlet extraction (AOAC 2000 method 920.39); Neutral (NDF) and acid (ADF) detergent fibre, according to (Goering & Van Soest, 1991).

To determine minerals, samples were subjected to acid digestion, followed by the determination of calcium by atomic absorption spectrophotometry (Brand GBC, Mod. Avanta

PM) (AOAC, (2000; method 968.08) and phosphorous by colourimetry (Clesceri *et al.,* 1989, method 4500-P).

Samples for amino acid analyses (AOAC, 2000; method 923.03) were hydrolyzed in 6 N HCl for 24 h at 110°C for determination using an Automatic Analyser (L-8800 Hitachi Automatic Amino Acid Analyzer, Tokyo, Japan). After cold performic acid oxidation overnight and hydrolysis with 7.5 N HCl for 24 h at 110 °C methionine was analyzed as methionine sulfone (Method 999.13) and tryptophan after LiOH hydrolysis for 22 h at 110 °C by High-Performance Liquid Chromatography (Agilent 1200 Series, Santa Clara, CA, USA) (Method 988.15).

Trypsin inhibitor activity (TIA) was assayed according to the American Oil Chemists' Society procedures (AOCS; 1998; method Ba 12-75). One-gram samples were incubated in 50 µl with 20 µl of commercial bovine trypsin (1 mg mL^{-1}) at 37 °C for 15 min. Thereafter, a 40 µl of stock solution of 10 mg mL^{-1} in (Dimethyl Sulfoxide) BApNA was added to the solution, and the mixture was further incubated at 37 °C for 30 minutes. The reaction was terminated by adding 500 µl of 10% glacial acetic acid, and the absorbance was measured at 410 nm against a reference to correct for the absorbance from the yellow pigment of the crude extract. Trypsin inhibitor activity was estimated by the difference between the activity recorded with and without inhibitors (Nair *et al.,* 2013).

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Table 3. 1 Trypsin inhibitor activity and chemical components of raw, sprouted or roasted cowpeas

Parameters	Raw cowpeas	Experiment 1		Experiment 2		
		[1]Sprouting period (days)		[2]Extent of Roasting		
				Duration (minutes)		
				10	20	30
		2	4	Endpoint grain temperature (° C)		
				55	105	130
Trypsin Inhibitor Activity (Units mg^{-1} DM)	5.63	8.7	4.63	5.25	2.26	1.67
Chemical components (% DM)						
Crude protein	25.9	28.2	29.1	25.7	26	26
Ether extract	1.6	1.2	0.9	1.12	1.31	1.2
NDF	37.3	23.6	36.7	19	24	25.4
ADF	12.5	12.9	18.7	12.1	13.4	10.1
Ca	0.07	0.09	0.10	0.09	0.09	0.09
P	0.4	0.53	0.55	0.51	0.51	0.52
Essential amino acids (g/100 g DM)						
Arginine	1.38	1.98	1.97	1.92	1.84	1.87
Histidine	0.62	1.11	1.11	1.29	1.32	1.26
Isoleucine	0.73	1.01	1.03	0.99	1.03	1.03
Leucine	1.25	1.77	1.82	1.73	1.76	1.78
Lysine	1.38	1.7	1.68	1.84	1.93	1.75
Methionine	0.22	0.32	0.32	0.32	0.32	0.33
Phenylalanine	0.94	1.34	1.37	1.31	1.3	1.32
Threonine	0.53	0.83	0.87	0.9	0.86	0.9
Tryptophan	0.29	0.24	0.23	0.31	0.3	0.31
Tyrosine	0.27	0.59	0.61	0.66	0.9	0.72
Valine	0.8	1.13	1.18	1.1	1.12	1.14

[1] Sprouting; 12-hour pre-soaking in water in water, four-day open-air sprouting at ambient conditions

[2] Roasting: cylindrical (L =1.5 m; Diameter= 0.50 m) manually rotating, cast-iron, gas heated drum, 20 kg cowpeas, initial maximal constant interior drum temperature 150°C.

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3.2.5 Statistical analysis

The estimated of *IVDMD* coefficients were analised separately for the sprouted (experiment 1) or roasted (experiment 2) cowpeas. For each experiment, parameters were subjected to One-Way ANOVA using the GLM of MINITAB software (Version 17.0) using the model:

$$\boldsymbol{Y_{ij}} = \boldsymbol{\mu} + \boldsymbol{T_i} + \mathcal{E}_{\boldsymbol{ij}}$$

Where Y_{ij} is the observation (*IVDMD*) parameter value on the ith processing level, μ *the* overall mean, T_i the ith processing level and $\mathcal{E}_{ij}{}^{th}$ erandom error. Tukey's test was used to compare means where significant differences occurred.

3.3 Results

Experiment 1: The *IVDMD* coefficients of raw (control) and sprouted cowpeas, the measured key-spot chemical components and the inhibitor activity auxiliary variables are presented in Table 3.1. Quantitative effects on the main auxiliary explanatory variables (TIA, crude protein, acid and neutral detergent fibre) are plotted in Figure 3.1 (Plate 1-4, respectively). Sprouting influenced the compartmental and total digestion ($p<0.001$). The steps 1-2 (gastric-ileal) *IVDMD* dropped ($p<0.05$) in the two and three-day sprouts, the sprouts which had highest ($p<0.05$) step three *IVDMD*. The total (steps 1-3) *IVDMD* coefficient increased ($p<0.05$) in 2-4 day sprouts ($p<0.05$).

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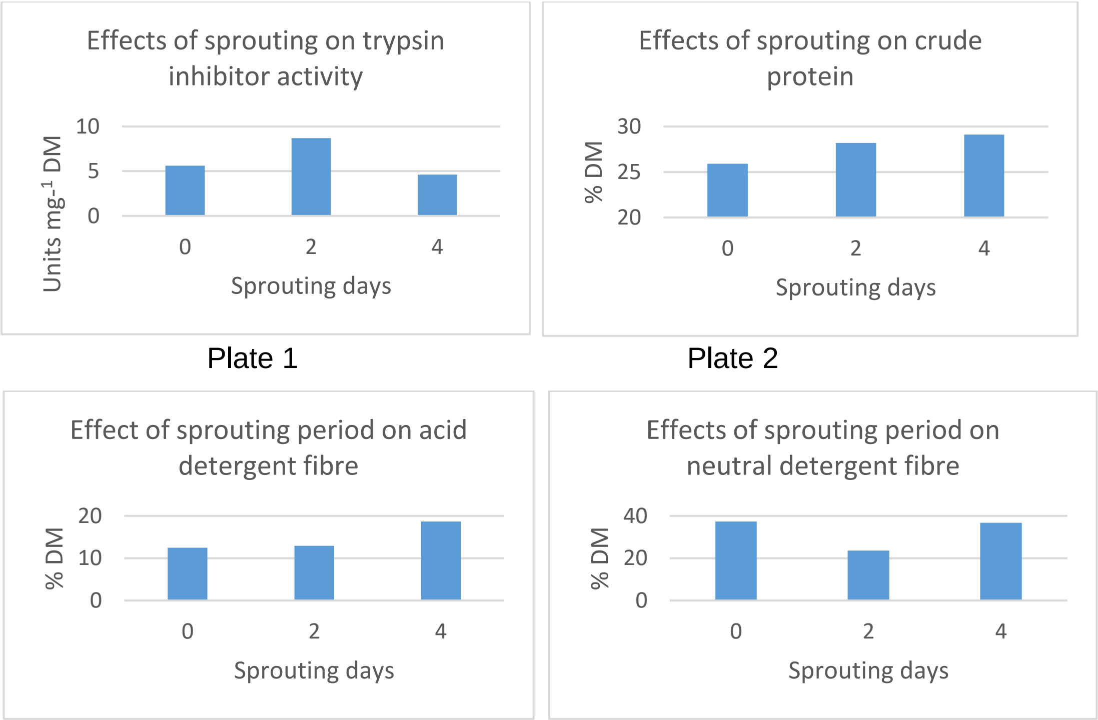

Plate 1 Plate 2

Plate 2 Plate 3

Figure 3. 1 Quantitative effects of cowpea sprouting period (days) on trypsin inhibitor activity (Plate 1), crude protein (Plate 2), acid detergent fibre (Plate 3) and neutral detergent fibre (Plate 4). Sprouting; 12-hour pre-soaking in water in water, four-day open-air sprouting at ambient conditions

Table 3.3 Effects of cowpea sprouting period on *in vitro* dry matter digestibility

[1]*In vitro digestion*	N	Raw cowpeas	Sprouted cowpeas [2]Sprouting period (days) 1	2	3	4	SEM	Significance
		Digestibility						
Step 1-2	14	0.818^{ab}	0.832^{a}	0.786^{b}	0.777^{b}	0.805^{ab}	0.00486	***
Step 3	14	0.063^{b}	0.068^{b}	0.128^{a}	0.131^{a}	0.103^{ab}	0.00605	***
Total	14	0.881^{b}	0.900^{ab}	0.914^{a}	0.908^{ab}	0.908^{a}	0.00336	***

^{abcd}*In vitro* digestibility means within a row with different letter superscripts are significantly different at (*p<0.05*); SEM - Standard error of the mean; *** - Significant at p<0.001

[1] *Step 1 (gastric digestion)*: 87.5 ml phosphate buffer (pH 7.2, 0.1 M, pH 6.0), 35 ml 0.2 M HCl, pH adjusted to 2.0 using 1 M HCl/M NaOH solutions, 3.5 ml pepsin solution (10 mg/ml pepsin (Pepsin from porcine gastric mucosa powder, ≥250 units/mg solid (Sigma-Aldrich® product P7000), 1.7 ml of a chloramphenicol solution (0.5 g Chloramphenicol ≥98% (HPLC) (Sigma-Aldrich® product C0378, per 100 ml ethanol), 2 hours digestion. *Step 2 (Small intestine digestion);* After the pepsin digestion, pH adjusted to 6.8 by adding 35 ml of sodium phosphate buffer solution (0.2 M, pH 6.8), 17.5 ml NaOH (0.6 M, pH 13.8), 3.5 ml aliquot freshly prepared pancreatin solution containing 50 mg pancreatin (Pancreatin from porcine pancreas (Sigma-Aldrich® product P3292), 5 h digestion. *Step 3 (Colon or large intestine digestion):* step 2 media discarded, 218.75 ml freshly prepared phosphate buffer (0.1 M, pH 4.8), 1.75 ml Viscozyme (Viscozyme® L, mixture of beta-glucanase, pectinase, hemicellulose and xylanase enzymes (Sigma-Aldrich® product V2010), 18 hours digestion.

[2] 12-hour soaking, 1, 2, 3 and 4-day open air sprouting, sun-dried

Experiment 2: The *IVDMD* coefficients of raw (control) and roasted cowpeas are presented in Table 3.2. Roasting influenced the partial step 3, and the total *IVDMD* (*p<0.05*), with no effect on steps 1-2 *IVDMD* (p>0.05). The roasting increased (*p<0.05*) both step 3 and the total *IVDMD* to peak in the 20-minute (105° C) roasts (*p<0.05*). The lowest (*p<0.05*) total *IVDMD* was recorded in 15-minute (95° C) roasts (*p<0.05*). Quantitative effects on the main auxillary explanarory variables (TIA, crude protein, acid and neutral detergent fibre) are plotted in Figure 3.2 (Plate 1-4, respectively).

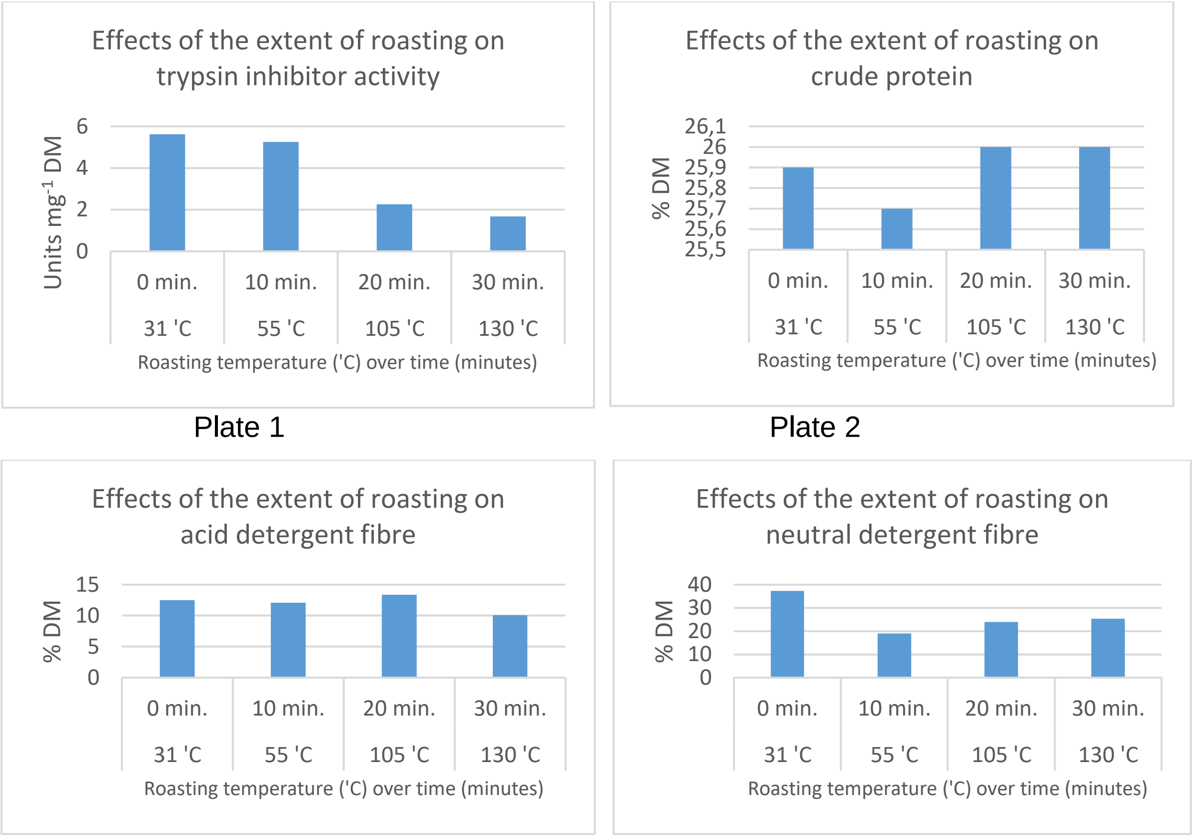

Plate 1

Plate 2

Plate 3

Plate 4

Figure 3. 2 Effects of roasting intensity on trypsin inhibitor activity (Plate 1), crude protein (Plate 2), acid detergent fibre (Plate 3) and neutral detergent fibre (Plate 4). Roasting intensity - grain temperature changes over time after placement in roasting drum heated to 150 ^{o}C initial empty temperature.

Table 3.4 Effects of the extent of roasting cowpeas on *in vitro* dry matter digestibility

[1]*In vitro digestion*	N	Raw cowpeas	Roasted cowpeas				SEM	Significance
			[2]Extent of Roasting					
			Duration (minutes)					
			10	15	20	30		
			Terminal grain temperature (° C)					
			55	95	105	130		
		DM digestibility						
Step 1-2	14	0.818	0.816	0.823	0.810	0.821	0.00586	ns
Step 3	14	0.063^{b}	0.102^{b}	0.054^{b}	0.171^{a}	0.111^{ab}	0.00734	***
Total	14	0.881^{cd}	0.918^{bc}	0.877^{d}	0.981^{a}	0.934^{b}	0.00449	***

[abcd] *In vitro* digestibility means within a row with different letter superscripts are significantly different at ($p<0.05$); SEM - Standard error of the mean; *** - Significant at p<0.001

[1] *Step 1 (gastric digestion)*: 87.5 ml phosphate buffer (pH 7.2, 0.1 M, pH 6.0), 35 ml 0.2 M HCl, pH adjusted to 2.0 using 1 M HCl/M NaOH solutions, 3.5 ml pepsin solution (10 mg/ml pepsin (Pepsin from porcine gastric mucosa powder, ≥250 units/mg solid (Sigma-Aldrich® product P7000), 1.7 ml of a chloramphenicol solution (0.5 g Chloramphenicol ≥98% (HPLC) (Sigma-Aldrich® product C0378, per 100 ml ethanol), 2 hours digestion. *Step 2 (Small intestine digestion);* After the pepsin digestion, pH adjusted to 6.8 by adding 35 ml of sodium phosphate buffer solution (0.2 M, pH 6.8), 17.5 ml NaOH (0.6 M, pH 13.8), 3.5 ml aliquot freshly prepared pancreatin solution containing 50 mg pancreatin (Pancreatin from porcine pancreas (Sigma-Aldrich® product P3292), 5 h digestion. *Step 3 (Colon or large intestine digestion):* step 2 media discarded, 218.75 ml freshly prepared phosphate buffer (0.1 M, pH 4.8), 1.75 ml Viscozyme (Viscozyme® L, mixture of beta-glucanase, pectinase, hemicellulose and xylanase enzymes (Sigma-Aldrich® product V2010), 18 hours digestion.

[2] Roasting: cylindrical (L =1.5 m; Diameter= 0.50 m) manually rotating, cast-iron, gas heated drum, 20 kg cowpeas, initial maximal constant interior drum temperature 150°C.

3.4 Discussion

Experiment 1: Sprouting affected the compartmental *IVDMD* in unpredictable fashion, with the high total digestibility observed in 2-4-day sprouts (Table 3.1). The pattern of total *IVDMD* digestibility was largely quantitatively consistent with confounding effects of the increasing fibre content, and reduction in TIA (Figure 3.1). In previous studies, soaking and sprouting similarly altered the compositional quality of cowpea sprouts (Devi *et al.,* 2015). The positive trend in CP in cowpea sprouts (Figure 3.3) was quantitatively similar to the trend reported in 2-5 day sprouts (Malomo *et al.,* 2013a; Malomo *et al.,* 2013b), though without effect on the chemical score, essential amino acid index, biological value and requirement index (Malomo *et al.,* 2013a). Crude protein and embryonic structural carbohydrate constituents also increased in 4-day sprouts (Nonogaki *et al.,* 2010). Similar to the present study, sprouting cowpeas for 4 days increased CP (Mehta *et al.,* 2007; Uppal and Bains, 2012), in addition to crude fibre (Uppal & Bains, 2012; Devi *et al.,* 2015), calcium (Dave *et al.,* 2008; Devi *et al.,* 2015) and Vitamin C (Uppal & Bains, 2012; Devi *et al.,* 2015). In previous studies, *IVDMD* also depended on the sprouting period and roasting intensity Recharla *et al.* (2019). Soaking (8-10 hours) and sprouting (1-3 hours) increased cowpea *in vitro* protein digestibility from 6 to 17% (Uppal & Bains, 2012). However, in the present study, given small indigestible residue sample recovery from the *in vitro* digestion, computation of DM digestibility and nutrient and anti-nutrient content expression on DM basis should be carefully interpreted. Depletion of organic substrates inevitably occurs via respiratory (sprouts) or evaporative and combustible carbon wastage, such that nutrient or antinutrient accumulation could be largely partially apparent, and not absolute. Substrates subject to rapid initial disappearance upon seed soaking are the soluble sugars and proteins, which then progressively deplete during continued growth and development of the increasingly respiratory developing embryo. Compared to cereals, cowpea seed, typical of food legumes is richer in free oligosaccharides and amino acids, and soluble proteins (Agume *et al.,* 2016) such that substantial initial germination utilization is expected to reflect in buffer only extraction, and not as pig enzymatic digestive DM disappearance. Starch digestibility of legume sprouts was improved by pressure and microwave cooking (Uppal and Bains, 2012), which suggested suboptimal sprouting beneficial effect on starch digestion. Starch (Uppal and Bains, 2012), oligosaccharides Sampath *et al.* (2008) and fat (Onimawo & Asugo 2004) significantly depleted during initial germination. Egounlety & Aworh, (2003) reported decreased oligosaccharides during 12–14 h soaking of soybean, cowpea and ground bean seeds. Notwithstanding the respiratory, evaporative or oxidative DM removal, the observed pattern of nutrient/antinutrient DM densities, and of DM

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digestibility, were attributed to confounded, cumulative effects of germination metabolism, (sprouts) Egounlety & Aworh, (2003) and of thermal macromolecular modification (roasts) Egounlety & Aworh, (2003). The net flux of solubilized organic compounds and minerals reflects the interchange between nutrient mobilization and new embryonic tissue biosynthesis (sprouts), alteration of spontaneous macromolecular acidic hydrolysis (sprouts), destruction of inhibitory ANF's, which synergistically increased pig enzymatic action (sprouts). In sprouts, the initial metabolic depletion of soluble substrates, nutrient mobilization for biosynthesis of highly digestible substrates are followed by incremental accumulation of less digestible complexes, including complex structural and nutrient-encapsulating carbohydrates which, in the *in vitro* digestion model, depending on the sprouting period (growth stage) and growing conditions, become subject to variable, partial viscozyme or colon digestibility. In the *in vitro* digestion model, resistant starch is fully recovered in pepsin-pancreatin digesta, which seemed poorly digested by viscozyme (Fushai *et al.,* 2019).

Understanding cowpea germination and the time-lapse progressive sprouting molecular biology is important to customize bioprocessing to optimize nutritional outcomes. Upon activation of germination, nutrient mobilization, gene transcription and biosynthesis of embryonic tissue growth with differentiation (Bamadad *et al.,* 2009; Masood *et al.,* 2014) commence and progress with significant nutritional implications. Detailed nutritional implications of the physiologic-biochemical activity associated with legume seed germination were previously discussed elsewhere (Benincasa *et al.,* 2019). Specialized endosperm protein bodies (Derbyshire *et al.,* 1976) contain endopeptidases, and a milieu of other hydrolases which are activated to drive germination metabolism (Müntz *et al.,* 2001) which includes protein, starch and lipids. In food legumes including cowpeas, physiologic activation occurs upon water imbibition, denaturation of either storage proteins or antinutritional factors (Stein & Bohlke, 2007), through endosperm protein control Ski *et al.,* 2018). The metabolic rate is mediated by phytohormones, mainly abscisic acid and gibberellin (Toh *et al.,* 2008; Akman, 2009). Seed germination involves mobilization of protein reserves in the cotyledons, coupled with the synthesis of new proteins necessary for the sprout's growth (Rodriguez *et al.,* 2008). Complex genetic and environmental influences interact to influence germination metabolism (Smykal, 2014; Fayyaz *et al.,* 2018; Tayade *et al.,* 2019). Insoluble storage proteins are initially reduced to soluble peptides, which undergo further depolymerisation to smaller peptides and amino acids (Rocha *et al.,* 2014). Starch is reduced to free sugars (Rocha *et al.,* 2014). Coupled to embryonic biosynthesis, germination macromolecular degradation are key determinants of mammalian enzymatic digestibility and nutrient absorption

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(Singh *et al.,* 2012; Vera, 2012). Therefore, time lapse, in relation to temperature and other conditions, are critical to manipulate germination for optimal product quality. Alpha-amylase activity, indicating starch hydrolysis (Liu *et al.,* 2004; Uppal & Bains, 2012; Zou, 2017), Kubicka *et al.,* (2000) increased with increasing temperature and germination time. Singh *et al.* (2001), cited by (Fayyaz *et al.,* 2018), reported greater effect of temperature on plant enzyme activity compared to the germination time. Germinated and sprouted cowpeas had low trypsin inhibitors but retained similar levels of chymotrypsin inhibitors compared to unprocessed cowpea (Aguilera *et al.,* 2013; Devi *et al.,* 2015).

Abdelatief, (2011) reported higher chymotrypsin inhibitor (15.02 TIU/mg protein) in unprocessed cowpeas compared to those previously (Sumathi & Pattabiraman, 1976) reported for cowpeas (7.2 TIU/mg protein), and for and soybeans (6.6 TIU/mg protein). The reasons for higher levels of these enzyme inhibitors in the experimental local cowpea seeds could be attributed to adverse environmental conditions as well as varietal differences (Abdelatief, 2011). In different cowpea cultivars, Devi *et al.* (2015) reported 29-56% decrease in TIA with soaking. Khattab & Arntfield (2009) reported similar TIA reduction in soaked cowpeas. Though protease inhibitor activity tends to decrease as germination proceeds (Adil Shah *et al.,* 2011; Kayembe & van Rensburg, 2013), in the present study, the lowest level TIA was obtained after day 4 sprouting, and after 20-minute roasting. In sprouts, initial TIA (day 2) was recorded before gradual depletion (figure 3.2). Antinutritional factors may be endogenous, partially as byproducts during the processing of proteins (Gilani *et al.,* 2011) in legume seeds. Sprouting increases the permeability of cell membrane, increasing the amount of antinutrient leaching (Pusztai, 1991; Pallavi & Kanika 2016). On a residual DM basis, similar to the nutrient, such change in TIA could merely reflect depletion of readily soluble and, or metabolized organic matter, in as much as soluble inhibitors might also not be recovered in solid digesta.

Of importance to monogastric mineral nutrition from the legumes is phytate. Generally, legumes contain phytate at levels which significantly limit mineral absorption Shah *et al.,* 2011. Depending on legume cultivar/species, and the sprouting period, decreases in phytic acid within a wide approximate range of 5-43 % were reported previously (Chopra & Sankhalla, 2004; Modgil *et al.,* 2009; Uppal & Bains, 2012; Devi *et al.,* 2015). However, such phytate depletion might be largely partially apparent, since, upon soaking in water, phytate ions can be leached out following the ion gradient (Modgil *et al.,* 2009; Shah *et al.,* 2011).

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Experiment 2: Roasting influenced the partial step 3, and the total *IVDMD,* without effect on steps 1-2 *IVDMD*. The roasting increased both step 3 and the total *IVDMD* to peak in the 20-minute (105^{o} C) roasts ($p<0.05$), which was considered most effective processing. Compared to roasting, the quantitative effect of roasting was less pronounced on fibre components, in contrast to greater reduction of the TIA (Figure 3.1). Roasting affects DM digestibility by gelatinizing resistant starch (Uppal & Bains, 2012), protein denaturation and breaking of cross-linkages (Khatoon and Prakash 2005), and through complex mallard oxidation reactions involving carbohydrates, lipids and nitrogenous compounds (Khatoon & Prakash 2005), processes which when excessive, may negatively affect digestibility. Overall, the major legume antinutrients are heat labile (Khatoon and Prakash 2005). Heat inactivated cowpea enzyme inhibitors (Khatoon & Prakash 2005). Udensi *et al.* (2007) reported heat lability phytic acid. Trypsin inhibitor thermal stability persisted to 80 ^{o}C, after which activity decreased to detectable low levels at 100 ^{o}C (Kansal *et al.,* 2008), which is consistent with the apparent deactivation in the present study (figure 3.2). Trypsin inhibitor thermal stability is attributed to the rigid, compact protein structure which is stabilized by several disulfide linkages (Sierra *et al.,* 1999). Roasting increased the lipid and protein content of cereal seeds such as millet (Sade & Proximate, 2009), maize (Oboh *et al.,* 2010), and sesame (Makinde and Akinoso, 2014), an effect attributed to the destruction of cell structure which enables efficient release of the oil reserve (Cuevas-Rodriguez *et al.,* 2004). In the current study, roasting had insignificant quantitative effect on cowpea lipid content.

In legumes, perhaps of equal importance to macromolecular depolymerization facilitated nutrient release is antinutrient detoxification to expose biomolecules to plant endogenous and animal enzymatic degradation. The main proteinaceous anti-nutritional factors in cowpeas are trypsin inhibitors and lectins (Mihailović *et al.,* 2005). The molecular architecture (Rocha *et al.,* 2014; Kimura *et al.,* 2008; Oliveira *et al.,* 2012) of these key proteins is relevant to optimal seed processing. The trypsin inhibition assay is the standard test for the efficacy of thermal, hydrothermal and methods used to process grain legumes for food (Mikić *et al.,* 2009). Nutritionally, TIA is classified (Mikić *et al.,* 2009) into very low (2-4 TIU mg-^{1} DM), low (4-7 TIU mg-^{1} DM), medium (7-10 TIU mg-^{1} DM) and high (10-13 TIU mg-^{1} DM). A 2 TIU level is considered the minimal threshold enzyme-inhibiting TIA (Mikić *et al.,* 2009).

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3.5 Conclusion

*Experiment 1***:** The study concluded that cowpea sprouting is a potent tool to improve DM digestibility, only if optimally calibrated to maximize the nutritional benefit. Under the conditions of the experiment, the *IVDMD,* supported by chemical components and TIA suggested the most effecting processing in 4 day-sprouts, though the trend of effects suggested sprouting beyond four days could reveal threshold *IVDMD*.

*Experiment 2***:** Similar to sprouting, study concluded that cowpea roasting is a potent tool to improve DM digestibility, only if optimally calibrated to maximize the nutritional benefit. Under the conditions of the experiment, *IVDMD,* supported by chemical components and TIA suggested the most effective processing occurred with the 20 minute (150 ^{o} C terminal grain temperature) roasting.

For both experiments 1 and 2, further studies are recommended which include chemically specific residual digesta and solute analyses, to track the nutritional significance of the observed alterations in gut compartmental, and the total *IVDMD*, with validatory *in vivo* studies.

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CHAPTER 4

NUTRIENT DIGESTIBILITY AND NITROGEN BALANCE IN DIFFERENT PIG GENOTYPES FED RAW, SPROUTED OR ROASTED COWPEA *(Vigna unguiculata)* DIETS

Abstract

Legume grains are important feed protein sources, whose utilisation efficiency may depend on animal genotypic influences in terms of legume ANFs' tolerance and the essential nutrient requirements. The study investigated effects on nutrient digestion and metabolism of sprouting vs roasting cowpeas for feeding to Windsnyer (W), Large White (LW) x Landrace (LR) and the 3-way (W x LW x LR) crossbred pig genotypes. Iso-nutrient (15% CP, 17.3 MJ ME kg-1) grower diets were formulated using raw, sprouted, and roasted cowpeas. To provide insight into the *in vivo* nutrient digestibility and metabolic evaluation, the *IVDMD* of diets formulated using raw, and the most effectively processed (4 day sprouted, 20-minute roasted) cowpea diets were evaluated in a completely randomized design replicated 14 times. Sprouting cowpeas resulted in lower (0.35) step 3 dietary *IVDMD* compared to roasting (0.39) ($P<0.05$), with no effect on the steps 1-2, and the total dietary IVDMM ($P>0.05$). The three diets were assigned to nine weaned pigs, three each of W, LW x LR and W x LW x LR genotypes for feeding in a 3 X 3 factorial arrangement within three balanced, 3 X 3 Latin squares in a cross-over design. Feeding periods consisted of 7 days' adaption + 5 days' measurement of feed intake, faecal and urine excretions. On scaled bases, feed consumption was higher on the sprouted cowpeas compared to the raw diet ($P<0.05$). Sprouting and roasting both reduced the ash digestibility. Ash digestibility was lower (P<0.05) for LW x LR compared to W x LW x LR pigs. Period 3 had lower ash digestibility as compared to period 1 ($P<0.05$). TheADF was highly digestible by W x LW x LR and highly digestible on period 1 as compared to LW x LR. In conclusion, different *IVDMD* between the sprouted and roasted cowpea diets, the different dietary *in vivo* ash, DM and ADF digestibility, and the genotype x diet interaction on pig N balance responses were attributed to different digestive, and intermediary nutrient metabolism, including primarily dietary amino acids, effects which suggested unique digestive and metabolic adaptive traits among the experimental pig genotypes.

Keywords: *Cowpea processing, Large White, Landrace, Nitrogen utilisation, Windsnyer*

4.1 Introduction

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In the advent of climate change, in the arid and semi-arid tropical farming systems, ecologically adaptable native legumes could provide cost-effective, sustainable solutions to increasingly restricted, and erratic local commercial plant protein feed supply chains (Ameen *et al*., 2005; Adino *et al*., 2018). There is wide quantitative and qualitative variation among legume species and their cultivars in protein content (Gilani *et al*., 2005; Gilani *et al*., 2011) and in anti-nutritional secondary compounds (Garry *et al*., 2007; Jezierny *et al*., 2010). Typical of the legumes, cowpea protein is rich in lysine and tryptophan, though relative to animal protein, it is deficient in the sulphur containing amino acids such as methionine and cysteine (Khattab, 2009; Frota *et al*., 2017). Structural details relevant to the utilization of the main cowpea storage protein, vicilin, were previously described by Kimura *et al*., (2008), Oliveira *et al*., (2012) and Rocha *et al*., (2014). Through impaired digestion (Garry *et al*., 2007), and by endogenous wastage via damaged gut epithelial cells and secretory protective mucoprotein (Brenes *et al*., 2004), antinutritional factors (ANFs) may aggravate the inferior protein quality of native legume-based diets (Sauvant *et al*., 2004; NRC, 2012). Therefore, for maximal dietary efficacy, native legumes require tailored thermal (Farran *et al*., 2001) or biological (Malomo *et al*., 2013) processing, optimally calibrated for effective deactivation of ANFs, without deleterious impact on protein quality.

Nutrigenetic studies suggest the pig genotype may be important in its capacity to digest and metabolize nutrients from characteristically chemically complex novel diets. Previous studies suggested different gut morphology (Barea *et al*., 2011) and profiles of gut microbiota (Fairbrother *et al*., 2005; van der Meulen *et al*., 2010; Rist *et al*., 2013). The genotype also determines the nutrient requirement (Fontanesi *et al*., 2015). Such genetic differentiation in nutritional traits should be expected from prolonged native or exotic pig's selection in different production systems.

In the experiments under Chapter 3 and 4, the trends in compartmental and the total *IVDMD* coefficients suggested that, under the experimental conditions, sprouting to at least or beyond four -days was necessary to determine threshold *IVDMD* for the test cowpeas, while quantitative trends of TIA and chemical components indicated the 20-minute roasting to be most effective for roasting cowpeas. Therefore, these findings were then tested by evaluating the effects on nutrient digestibility, and N utilisation of processing cowpeas by either sprouting or roasting cowpeas for feeding as the primary plant protein source in maize-based diets for Windsnyer (W), Large White x Landrace (LW), and Windsnyer x Large White x Landrace (W x LW x LR) crossbred growing pigs.

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4.2 Materials and methods

The Ethics Committees of the University of Venda (SARDF/17/ANS/07/0412) approved the experimental protocols used in the experiment.

4.2.1 Cowpea processing and preparation of experimental diets

In the experiments 1 and 2 in Chapter 3, 4 days sprouting, and 20-minute ((105° C) roasting were respectively the most effective for cowpea processing. These findings were then used in formulating sprouted and roasted cowpea growing pig diets. Maize grain and the raw and sprouted and roasted cowpeas which were used in constituting the dietary treatments were hammer-milled (Jacobson hammer mill, model P160 Teordrop 10HP, China) through a 3mm screen and mixed (MORHLANG VERTA MIX, 1200VM, United States) for 20 minutes in one-tonne lots including amino acid, mineral and vitamin supplements. The balanced diets are presented in Table 5.2.

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Table 4.1 Chemical composition and trypsin inhibitor activity of raw, sprouted and roasted cowpeas (dry matter basis)

Components	Cowpeas		
	Raw	Sprouted[1]	Roasted[2]
Trypsin inhibitor activity (mg $^{-1}$)	5.6	4.6	2.3
Dry matter (g kg $^{-1}$)	934	933	934
Organic matter (g kg $^{-1}$)	879	886	893
Ash (g kg $_{-1}$)	55	47	41
Crude Protein (g kg $^{-1}$)	258	291	260
Ether Extract (g kg $^{-1}$)	157	92	131
ADF (g kg $^{-1}$)	125	187	134
NDF (g kg $^{-1}$)	373	367	240
Amino Acids (g/100g)			
Tryptophan	0.29	0.23	0.30
Arginine	1.38	1.97	1.84
Serine	0.80	1.27	1.19
Aspartic acid	1.34	2.67	2.59
Glutamic acid	2.48	3.77	3.81
Glycine	0.65	0.93	0.91
Threonine	0.53	0.87	0.86
Alanine	0.65	1.13	0.97
Tyrosine	0.27	0.61	0.9
Proline	0.69	1.13	0.95
HO-Proline	0.04	0.08	0.06
Methionine	0.22	0.32	0.32
Valine	0.80	1.18	1.12
Phenylalanine	0.94	1.37	1.3
Isoleucine	0.73	1.03	1.03
Leucine	1.25	1.82	1.76
Histidine	0.62	1.11	1.32
Lysine	1.38	1.68	1.93

[1] Sprouting; 12-hour pre-soaking, four-day open-air sprouting at ambient conditions; [2]Roasting: cylindrical (L =1.5 m; Diameter= 0.50 m) manually rotating, cast-iron, gas heated drum, 20 kg cowpeas, initial maximal constant interior drum temperature 150°C, 20 minutes roasting to internal drum temperatures at sampling 105°C.

*Trypsin inhibitor activity (TIA) was determined according to the American Oil Chemists' Society official (AOCS) (1998; method Ba 12-75).

Sprouting; 12-hour pre-soaking in water in water, four-day open-air sprouting at ambient conditions;

Roasting: cylindrical (L =1.5 m; Diameter= 0.50 m) manually rotating, cast-iron, gas heated drum, 20 kg cowpeas, initial maximal constant interior drum temperature 150°C, 20 minutes roasting to 105°C terminal grain temperature.

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Table 4.2 Ingredient, nutrient and energy composition of raw, sprouted and roasted and cowpea diets

	Cowpea Diets		
Composition	**Raw**	**[1]Sprouted**	**[2]Roasted**
Ingredients (% as fed)			
Maize meal	56.8	63.6	57.1
Cowpea meal	40.4	33.6	40.1
*Pig grower micronutrient pack	2.8	2.8	2.8
Total	100.0	100.0	100.0
Analised chemical and calculated energy composition (DM Basis)			
Dry matter (g kg $^{-1}$)	932	933	934
Organic matter (g kg $^{-1}$)	851	886	847
Ash (g kg $^{-1}$)	8.1	4.7	4.7
Acid detergent fibre (g kg $^{-1}$)	59	65	63
Neutral detergent fibre (g kg $^{-1}$)	165	197	252
Fat (ether extract) (g kg $^{-1}$)	30	29	30
[3]Metabolizable Energy (MJ kg ^{1})	14.5	14.1	14.3
Crude protein (g kg $^{-1}$)	150	150	150
Lysine (g/100g DM)	1.0	0.8	1.0
Methionine (g/100g DM)	0.3	0.3	0.2
Calcium (g kg $^{-1}$)	5.4	5.4	5.2
Phosphorus (g kg $^{-1}$)	3.3	3.4	3.4

[1] Sprouting; 12-hour pre-soaking in water in water, four-day open-air sprouting at ambient conditions

[2] Roasting: cylindrical (L =1.5 m; Diameter= 0.50 m) manually rotating, cast-iron, gas heated drum, 20 kg cowpeas, initial maximal constant interior drum temperature 150°C, 20 minutes roasting to 105°C terminal grain temperature

[3] Metabolizable Energy (MJ kg ^{1}) = 1000 x DE – 0.68 x CP. Noblet and Perez. 1993, equation. 43), where digestible Energy (DE) (MJ kg $^{-1}$) = 4.168 – 9.1 x Ash + 1.9 x CP + 3.9 x EE – 3.6 x NDF (Noblet and Perez, 1993, equation 23)

* Trouw Nutrition, South Africa, containing nutrients per kg of supplement: Vitamin A-6500IU, Vitamin D3-1200IU, Vitamin E Equivalent- 400U, Vitamin K3 (43%)-0.0002g, Vitamin B1 (Thiamine Mononitra)-0.00015g, Vitamin B2 80% (Riboflavin)- 0.00045g, Niacin (99.5%) -0.0025g, Calcium Pantothenate (98%)-0.0012g, Vitamin B12 (1g/kg)-0.0000003g, Vitamin B6 (98% pyridoxine HCL)-0.00025g, Choline (Chloride 60%)-0.019048g, Folic Acid (96%)-0.00006g, Biotin (2%)-0.000005g, L-Lysine 98%-0.00019g, DL-Methionine (98%)-0.02g, Phytase (10 000 FTU/g)-0.005g, Manganese (Manganese Sulphate 31%)-0.004g, Zinc (Zinc So4-Mono 35.5%)- 0.001g, Copper (Copper So4-25.2% Penta)-0.0125g, Iodide (Potassium Iodide 76.45)- 0.0001g, Ferrous (ferrous $S0_4$-30% Mono)- 0.01g, Selenium (Sodium Selenite 4.5%)-0.00003g, Limestone powder-0.1782g, Mono Dicalcium Phos. (21%)-0.8g, Salt-0.6g

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4.2.2 Determination of dietary nutrient digestibility and N balance

The dietary components’ digestibility was determined using *in vitro (*dry matter*) and in vivo (*fibre, nutrients) techniques. *In Vitro* Dry Matter Digestibility (*IVDMD*), evaluation was included to separately predict the effects of cowpea processing on compartmental, upper versus lower gut digestion, while the *in vivo* evaluation enabled the evaluation of dietary effects on intake, on the apparent total tract digestibility of the complete diet and of its specific components, and effects on measured and calculated parameters of N utilisation.

4.2.2.1 Determination of *in vitro* dry matter digestibility of cowpea diets

The procedures applied in chapter three were applied to estimate the dietary *IVDMD*.

4.2.2.2 Determination of *in vivo* digestibility and nitrogen balance

Nine male pigs, three each of Windsnyer (W), Large White (LW) X Landrace (LR), and 3-way crossbred (W X LW X LR) pig genotypes were used. The pigs were bred at the Agricultural Research Council-Irene Pig Breeding Unit, from where they were selected from different litters born after natural sow mating, which had been weaned onto a commercial weaner diet at 4 weeks. Mature weights for the parent commercial (LW and LR), and the indigenous, W type pure breed populations were previously estimated between 300 – 350 kg, and 100 to 150 kg, respectively (Kanengoni *et al.,* 2014). At the start of the experiment, the W, LW x LR and W x L W x LR groups weighed in at 11 ± 1.15 kg, 14 ± 1.15 kg, and 12 ± 1.15 kg live weight, respectively. The terminal weights were 27.3 ± 1.15 kg, 28.3 ± 1.15 kg and 27.01 ± 2.0 kg respectively. Prior to the trial, all pigs received a 1 ml subcutaneous injection of Ivomec antiparasitic drug (Reg. No. G2858). The trial was setup in a naturally ventilated house in which each pig was placed within a 57 cm x 118 cm metabolism cage fitted with individual feeders and nipple drinkers. Pigs of the three genotypes were randomly assigned to test diets a 3 (diet) x 3 (genotype) factorial experiment within three Latin squares, each square with one pig of each genotype rotated through the diets in three feeding periods, in a balanced crossover experiment as depicted in Table 4.3. The first seven days of each feeding period in a Latin square were used to adapt the pigs to the dietary treatments, followed by five days of measuring the voluntary feed intake, and total faeces and urine collection. Faeces and urine collection and sampling were performed between 08:00 – 09:00 hours. The daily urine was collected in 50ml of 20% HCl, to prevent N volatilization AOAC. 2005.

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Faecal and urine samples were stored at −04°C until analyzed. Frozen faecal samples were dried in a forced-air oven to constant weight at 60°C, and ground to pass through a 3-mm screen AOAC. 2000.

Feed intake (FI), dry matter (DM), crude protein (CP), ash, fat, Neutral detergent fibre (NDF) and acid detergent fibre (ADF) nutrient digestibility and nitrogen balance parameters [Nitrogen intake (NI), Faecal nitrogen output (FNO), Urinary nitrogen output (UNO), Total nitrogen excretion (TNE), Nitrogen retention (NR), Absorbed nitrogen (AN), Nitrogen utilization (NU) and The biological value of feed protein (BVFP)] were estimated from the difference between dietary intake and excretion in faeces and urine. The intake and N parameters were expressed on a metabolic body weight ($BW^{0.75}$) basis.

Table 4.3 Treatment assignment within a three balanced Latin Square, changeover feeding experiment.

Pig	Genotype	Period 1	Period 2	Period 3
A1	W	Control (Raw)	Roasted	Sprouted
A2	LW x LR	Roasted	Sprouted	Control (Raw)
A3	W x LW x LR	Sprouted	Control (Raw)	Roasted
A4	W x LW x LR	Control (Raw)	Roasted	Sprouted
A5	W	Roasted	Sprouted	Control (Raw)
A6	LW x LR	Sprouted	Control (Raw)	Roasted
A7	LW x LR	Control (Raw)	Roasted	Sprouted
A8	W x LW x LR	Roasted	Sprouted	Control (Raw)
A9	W	Sprouted	Control (Raw)	Roasted

4.2.2.3 Chemical analyses

A 1 mm sieve hammer-milled the feed samples. Dry matter was determined using the AOAC (2000; method 976.050. Ash was determined using the AOAC (2000; method 923.03). Nitrogen was determined using the micro-Kjeldahl method (AOAC, 2000; method 976.05). Ether extract (EE) was determined by soxhlet extraction (AOAC, 2000; method 920.39). Neutral detergent fibre (NDF) and acid detergent fibre (ADF) were analysed according to (Goering & Van Soest, 1970).

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4.2.3 Mathematical analysis

The nitrogen balance parameters were calculated as outlined in Table 5.4. The nitrogen content level of faeces and urine were determined according to the method described by Kjeldahl (AOAC, 1990). Nitrogen balance was therefore calculated as follows:

Table 4.4 Equations for estimating nitrogen balance parameters

Component	[1]Formulae
Nitrogen intake (NI)	$N\frac{feed}{100} \times dailyfeed\ intake$
Faecal nitrogen output (FNO)	$N\frac{feces}{100} \times daily\ faecal\ output$
Urinary nitrogen output (UNO)	$N\frac{urine}{100} \times dailyurine\ output$
Total nitrogen excretion (TNE)	$FNO + UNO$
Nitrogen retention (NR)	$NI - TNE$
Absorbed nitrogen (AN)	$NI - FNO$
Apparent nitrogen digestibility (ND)	AN/NI
Nitrogen utilization (NU)	$NR/NI \times 100$
The biological value of feed protein (BVFP)	$NR/ND \times 100$

[1]Source: Patráš *et al.* (2009)

4.2.3.2 Statistical analysis

The *IVDMD* digestibility coefficients of diets were subjected to One-Way ANOVA using the GLM of MINITAB software (Version 17.0) using the model:

$$\boldsymbol{Y_{ij} = \mu + T_i + \mathcal{E}_{ij}}$$

Where Y_{ij} is the the jth observation on the ith diet, μ *the* overall mean, T_i the effect of the ith cowpea processing method and $\mathcal{E}_{ij}$ the random error.

The *in vivo* apparent dry matter, fibre, nutrient digestibility coefficients, and N balance parameters were analysed using repeated measures within PROC MIXED procedures in Minitab (Version 17, 2016), the genotype, diet, period, and Latin square as fixed effects, while the pig was treated as a random effect using the model:

$$\boldsymbol{Y_{ijklm} = \beta Cov_{j(ilm)} + \mu + G_i + D_k + P_l + LS_m + (G \times D)_{ik} + Animal_{j(ilm)} + \mathcal{E}_{iklm}}$$

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Where Y_{ijjklm} is the observed response of the j^th^ pig, of the i^th^ genotype, assigned to the k^th^ diet in the l^th^ period of the m^th^ Latin square, $\beta Cov_{j(ilm)}$ the covariate, the initial weight of the j^th^ pig of the i^th^ genotype in the l^th^ period of m^th^ Latin square, μ the overall mean of the response variable, G_i the effect of the i^th^ genotype, D_k the effect of the k^th^ diet, P_l the effect of the l^th^ period, LS_m the effect of the m^th^ Latin square, $(G\times D)_{ik}$ the interaction between the genotype and the diet, $Animal_{j(lmi)}$ the effect of the j^th^ pig within the i^th^ genotype in the m^th^ Latin square and l^th^ period and ϵ_{iklm} the residual error.

Tukey's test was used to compare means where significant main effects were detected.

4.3 Results

4.3.1 In vitro DM digestibility of raw, roasted and sprouted cowpea-growing pig diets

Table 4.5 shows effects of cowpea processing on *in vitro* dietary DM digestibility (*IVDMD*). Roasting cowpeas and sprouting cowpeas showed no (*P>0.05*) processing effect on step-1-2 and step-3. The total *IVDMD* was significantly (*P<0.05*) high for raw cowpea and roasted.

Table 4.5 ***In vitro* digestibility of raw, roasted and sprouted cowpea-growing pig diets**

		Cowpea Diets				
	N	**Raw**	**^{2}Sprouted**	**^{3}Roasted**	**SEM**	**Significance**
^{1}*In vitro* dry matter digestibility						
Step 1-2	14	0.670	0.669	0.642	0.00486	ns
Step 3	14	0.215	0.196	0.238	0.00605	ns
Total	14	0.885^{a}	0.865^{b}	0.880^{ab}	0.00336	***

^{ab} Treatment means within a row with different letter superscripts are significantly different at (*P<0.05*). SEM- Standard error of the mean. *Significant at *P<0.05*; *P>0.05*- not significant (ns)

^{1}*Step 1 (gastric digestion)*: 87.5 ml phosphate buffer (pH 7.2, 0.1 M, pH 6.0), 35 ml 0.2 M HCl, pH adjusted to 2.0 using 1 M HCl/M NaOH solutions, 3.5 ml pepsin solution (10 mg/ml pepsin (Pepsin from porcine gastric mucosa powder, ≥250 units/mg solid (Sigma-Aldrich® product P7000), 1.7 ml of a chloramphenicol solution (0.5 g Chloramphenicol ≥98% (HPLC) (Sigma-Aldrich® product C0378, per 100 ml ethanol), 2 hours digestion. *Step 2 (Small intestine digestion);* After the pepsin digestion, pH adjusted to 6.8 by adding 35 ml of sodium phosphate buffer solution (0.2 M, pH 6.8), 17.5 ml NaOH (0.6 M, pH 13.8), 3.5 ml aliquot freshly prepared pancreatin solution containing 50 mg pancreatin (Pancreatin from porcine pancreas (Sigma-Aldrich® product P3292), 5 h digestion. *Step 3 (Colon or large intestine digestion):* step 2 media discarded, 218.75 ml freshly prepared phosphate buffer (0.1 M, pH 4.8), 1.75 ml Viscozyme (Viscozyme® L, mixture of beta-glucanase, pectinase, hemicellulose and xylanase enzymes (Sigma-Aldrich® product V2010), 18 hours digestion.

^{2}12-hour soaking, 1, 2, 3 and 4-day open air sprouting, sun-dried

^{3}Roasting: cylindrical (L =1.5 m; Diameter= 0.50 m) manually rotating, cast-iron, gas heated drum, 20 kg cowpeas, initial maximal constant interior drum temperature 150°C.

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4.3.2 *In vivo* evaluation of cowpea diets

Table 4.6 shows treatment effects on *in vivo* diet utilization. The Latin square had an effect on feed intake g/day and covariate had an effect on feed intake g/kg LW. On scaled bases, feed consumption was higher on the sprouted cowpeas compared to the raw diet ($P<0.05$). Sprouting and roasting both reduced the ash digestibility. Ash digestibility was lower ($P<0.05$) for LW x LR compared to W x LW x LR pigs.

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Table 4.6. **Dietary intake and nutrient digestibility of raw, roasted^{1} and sprouted^{2} cowpea-maize diets by different pig genotypes**

Treatments		Intake			Digestibility					
		g/day	g/kg LW	g/kg LW$^{0.75}$	DM	CP	Ash	Fat	ADF	NDF
^{3}Genotype	**Diet**									
LW x LR	Raw	234.8	12.7	26.1	0.90	0.91	0.16^{ab}	0.071	0.27	0.46
	Sprouted	243.9	12.0	25.5	0.83	0.88	0.17^{ab}	0.079	0.29	0.53
	Roasted	265.6	13.4	28.4	0.85	0.89	0.20^{ab}	0.103	0.36	0.64
W	Raw	233.0	12.3	25.4	0.88	0.88	0.25^{a}	0.093	0.41	0.69
	Sprouted	227.9	12.4	25.4	0.84	0.90	0.13^{b}	0.071	0.27	0.49
	Roasted	226.7	11.1	23.6	0.81	0.89	0.17^{ab}	0.067	0.34	0.76
W x LW x LR	Raw	246.1	12.1	25.8	0.89	0.91	0.22^{ab}	0.089	0.40	0.68
	Sprouted	227.9	114.0	29.3	0.82	0.90	0.23^{a}	0.093	0.42	0.69
	Roasted	226.7	112.7	26.4	0.86	0.89	0.16^{ab}	0.078	0.28	0.48
SEM		12.7	0.818	1.51	0.006	0.00633	1.12	0.600	2.47	6.20
Diet										
Raw		238.2	12.8	25.8	0.89	0.90	0.21^{a}	0.084	0.36	0.61
Sprouted		246.7	12.4	26.7	0.83	0.89	0.18^{b}	0.080	0.33	0.57
Roasted		244.7	12.4	26.1	0.84	0.89	0.18^{b}	0.082	0.33	0.63
SEM		7.24	0.326	0.794	0.342	0.00414	0.705	0.413	1.53	3.96
Genotype										
W		229.7	11.9	24.8	0.86	0.89	0.19^{ab}	0.077	0.34^{ab}	0.65
LW X LR		247.6	12.7	26.6	0.85	0.89	0.18^{b}	0.084	0.30^{b}	0.55
W x LW x LR		252.5	13.0	27.2	0.86	0.90	0.20^{a}	0.086	0.37^{a}	0.62
SEM		7.15	0.394	0.784	0.312	0.00411	0.652	0.383	1.42	3.68
Period										
1		259.1	13.4	28.1	0.85	0.89	0.23^{a}	0.097	0.40^{a}	0.69
2		238.9	11.7	25.0	0.85	0.89	0.19^{b}	0.077	0.34^{ab}	0.64
3		231.6	12.5	25.6	0.85	0.90	0.15^{c}	0.073	0.27^{b}	0.48
SEM		7.14	0.392	0.783	0.243	0.00409	0.651	0.381	1.41	3.66
P Values										
Covariate		0.386	0.015	0.085	0.417	0.423	0.749	0.965	0.878	0.997
Diet		0.691	0.709	0.706	0.533	0.327	0.004	0.784	0.189	0.609
Genotype		0.088	0.174	0.123	0.606	0.174	0.041	0.212	0.017	0.156
Latin Square		0.045	0.170	0.096	0.533	0.870	0.129	0.810	0.572	0.685
Period		0.608	0.070	0.165	0.402	0.100	0.000	0.116	0.000	0.372
Animal		0.171	0.259	0.233	0.174	0.184	0.148	0.771	0.728	0.763
Diet X Genotype		0.851	0.257	0.432	0.413	0.332	0.000	0.016	0.001	0.009

[1] Sprouting; 12-hour pre-soaking in water in water, four-day open-air sprouting at ambient conditions

[2] Roasting: cylindrical (L =1.5 m; Diameter= 0.50 m) manually rotating, cast-iron, gas heated drum, 20 kg cowpeas, initial maximal constant interior drum temperature 150°C, 20 minutes roasting to 105°C terminal grain temperature.

[abcd] Within factors or their interaction, treatment means with different letter superscripts are significantly different at (*$P<0.05$*)

[3] W: Windsnyer, LW = Large White; LR = Landrace

SEM- Standard error of the mean

[*] Significant at $P<0.05$; P>0.05- not significant

Treatment effects on nitrogen balance parameters scaled to the pig metabolic weight are shown in Table 4.6. Dietary effect was not observed for all the parameters. Genotype x Diet interaction was significant (*$P<0.05$*) for NI, ND, UNO, FNO, TNE, AN and NR.

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Table 4.7. Nitrogen balance from raw, roasted and sprouted cowpea-maize diets by different pig genotypes

Treatment		g/kg $W^{0.75}$							NU (%)	BVFP (%)
		NI	[4]ND	UNO	FNO	TNE	AN	NR		
[3]Genotype	**Diet**									
LW x LR	Raw	2.1^{b}	0.91	0.11^{de}	0.18^{c}	0.29^{c}	1.8^{ab}	8.4^{c}	34.4	40.0
	Sprouted	2.5^{ab}	0.88	0.07^{cde}	0.29^{ab}	0.36^{abc}	2.1^{ab}	9.6^{ab}	38.6	45.3
	Roasted	3.1^{a}	0.90	0.20^{abc}	0.31^{a}	0.53^{ab}	2.6^{ab}	9.4^{ab}	29.5	35.5
W	Raw	3.0^{ab}	0.89	0.24^{ab}	0.32^{a}	0.56^{ab}	2.5^{ab}	10.0^{ab}	32.4	40.2
	Sprouted	2.1^{b}	0.90	0.17^{abcd}	0.20^{bc}	0.37^{c}	1.7^{b}	7.2^{c}	34.0	45.3
	Roasted	2.3^{ab}	0.88	0.13^{abcde}	0.26^{abc}	0.40^{abc}	1.9^{ab}	10.0^{a}	41.5	35.5
W x LW x LR	Raw	2.5^{ab}	0.90	0.14^{abcde}	0.24^{abc}	0.38^{abc}	1.8^{ab}	10.1^{a}	38.9	45.7
	Sprouted	3.3^{a}	0.90	0.24^{a}	0.31^{a}	0.55^{a}	2.8^{a}	9.8^{ab}	28.7	34.5
	Roasted	2.2^{ab}	0.89	0.13^{bcd}	0.23^{abc}	0.36^{bc}	1.9^{ab}	7.7^{bc}	33.7	40.1
SEM		0.151	0.00633	0.139	0.0139	0.0224	0.0392	0.0349	15.6	21.1
Diet (D)										
Raw		2.6	0.90	0.16	0.24	0.41	2.1	9.1	33.2	41.9
Sprouted		2.6	0.89	0.16	0.27	0.43	2.2	8.8	33.7	40.4
Roasted		2.6	0.89	0.15	0.27	0.43	2.1	9.0	34.9	41.9
SEM		0.0874	0.00414	0.00815	0.00950	0.0132	0.0793	0.176	8.25	11.4
Genotype										
W		2.5	0.89	0.18^{a}	0.26	0.44^{a}	2.0	9.0	35.9	43.8
LW X LR		2.6	0.89	0.13^{b}	0.26	0.39^{b}	2.2	8.8	34.1	40.3
W x LW x LR		2.7	0.90	0.17^{a}	0.26	0.43^{ab}	2.3	9.2	33.8	40.1
SEM		0.0849	0.00411	0.00805	0.00938	0.0129	0.0783	0.175	8.15	11.3
Period										
1		3.2^{a}	0.90	0.24^{a}	0.30^{a}	0.54^{a}	2.6^{a}	9.6^{a}	29.8	36.2
2		2.4^{ab}	0.89	0.10^{b}	0.28^{a}	0.38^{ab}	2.1^{ab}	10.0^{a}	40.0	47.5
3		2.1^{b}	0.89	0.14^{b}	0.20^{b}	0.34^{b}	1.8^{b}	7.4^{b}	34.0	40.5
SEM		0.0848	0.00409	0.00804	0.00937	0.0128	0.0782	0.174	8.14	11.2
P Values										
Covariate		0.874	0.265	0.234	0.288	0.997	0.860	0.013	0.046	0.083
Diet		0.865	0.206	0.775	0.173	0.580	0.875	0.501	0.409	0.551
Genotype		0.220	0.406	0.001	0.998	0.029	0.149	0.295	0.169	0.056
Latin Square		0.916	0.337	0.882	0.477	0.614	0.958	0.893	0.427	0.655
Period		0.000	0.120	0.001	0.000	0.000	0.000	0.000	0.065	0.130
Animal		0.232	0.231	0.023	0.439	0.561	0.197	0.156	0.270	0.237
Diet x Genotype		0.000	0.051	0.002	0.000	0.000	0.001	0.000	0.223	0.373

[abcde] Within factors or their interaction, treatment means with different letter superscripts are significantly different at (*P<0.05*).

[3] W-Windsnyer, LW - Large White; LR- Landrace

[4] Nitrogen intake (NI) -(N feed/100) × daily feed intake; Faecal nitrogen output (FNO)- (N faeces / 100) × daily faecal output; Urinary nitrogen output (UNO)- (N urine / 100) × daily urine output; Total nitrogen excretion (TNE) FNO+UNO - Nitrogen retention (NR) - NI – TNE; Absorbed nitrogen (AN)- NI – FNO; Apparent nitrogen digestibility (ND) - (AN / NI);
Nitrogen utilization (NU) - NR / NI × 100; The biological value of feed protein (BVFP) – NR / ND × 100
SEM- Standard error of the mean
[*] Significant at *P<0.05*; P>0.05- not significant

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4.4 Discussion

In this study, a combination of compartmental pig gut *in vitro* dry matter digestibility and *in vivo* fibre, nutrient digestibility and N balance evaluation were employed to evaluate the effects of sprouting versus roasting of cowpeas on diet utilisation by weaned pigs of diverse genotypes. Low partial step-three *IVDMD* of sprouted, compared to roasted cowpeas, likely reflected the cumulative effect of greater quantitative steps 1-2 digestion, and escape residues consisting of poorly or slowly digestible, largely fibrous cell-wall structural matter in the sprouts. Unfortunately, the micro-gravimetric digestion setup excluded further molecular insight into the nature of the biochemical effects, which could have important implications for pig nutrition. In contrast, both roasting and sprouting cowpeas similarly reduced the *in vivo* apparent DM digestibility, which implied outflow rate-dependent escape of slowly digested plant embryonic secondary tissues in cowpea sprouts, and of cross-linked compounds in roasted cowpeas from all, including spontaneous disintegration and the endogenous enzyme hydrolytic (steps 1-2) or microbial (step 3) fermentative digestion. In pigs, the fate of feed passage and nutrient digestibility in the gut are subject to the dietary chemical and physical properties, such as the type and levels of dietary fibre and the feeding level (Le Goff *et al.,* 2002). The DM for the raw, sprouted or roasted cowpea diets that escaped the foregut could be of variable bulkiness, which is known to influence gut emptying in growing pigs (Kyriazakis & Emmans, 1995). However, *in vivo*, less escape, particularly of fibrous matter, should be expected since, commercial product such as Viscozyme out perform the counterpart, the greater diversity of pig gut microbial fibrolytic activity (Fushai *et al.,* 2019) in the colon fibrous compounds are highly digestible. A fibrolytic advantage of the indigenous pig gut was previously reported from the genomic evaluation of faecal microbiota (Kanengoni *et al.,* 2015). The researcher hypothesized the superior fibrolytic capacity that could theoretically present a mechanism for broader tolerance of toxic feed ANFs.

Genotype x Diet interaction was observed for ash, fat, ADF and NDF, however the main mean showed non-significant ($P<0.05$) for fat, ADF and NDF. The antinutrients like trypsin inhibitors, phytic acid, saponins, heamagglutinins and tannins are some of the undesirable components in legumes that could hinder utilization of important minerals including calcium, magnesium, iron and zinc (Vasagam & Rajkumar, 2011). It interferes with their absorption and utilization and thereby contributes to mineral deficiency (Vasagam & Rajkumar, 2011). In the current study most of the mineral in coweas might have reacted during processing due to generally increase reaction rates and resulted in low digestibility as compared to raw cowpeas. Fat digestibility of the

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processed and raw cowpea diet could be due to greater endogenous wastage on the cowpea diet and or greater flow of fat substrates for bacterial fat synthesis in the hindgut (Boeck *et al.,* 2021). While starch and fats are easily digested in the small intestine (Svihus & Hervik, 2016), cell-wall-encased and membrane-covered fat cells are protected from emulsification for efficient enzymatic digestion than free fats (Zhang *et al.,* 2018). Dietary fat is largely digested and exclusively absorbed before the end of the ileum (Guéraud *et al.,* 2015). Wang Lin *et al.* (2020) observed that the apparent and true digestibility of fat at the end of the ileum and over the entire intestinal tract is similar, confirming that there is no net absorption of fat in the hindgut of pigs.

In livestock production, efficient utilisation of protein requirement for growth depends more on understanding the optimum levels that meet protein needs, reproduction, and maintenance (Paul *et al.,* 2007). An incorrect supply of protein is not ideal for optimal protein deposition and can be detrimental to pig performance (Whittemore *et al.,* 2001; Noblet *et al.,* 2004; Norgaard *et al.,* 2014). Furthermore, excess dietary protein increases nitrogen excretion through the urine (Carpenter *et al.,* 2004). Therefore, conserving dietary N is critical to meet environmental and economic objectives (Rotz, 2004). To achieve these objectives, diet formulation should strike the correct balance between the quantitative and qualitative protein intake and the animal requirement, minimize N excretion, and optimize animal productivity. Therefore, the critical, typically limiting factors are the feed protein quality in relation to often poorly defined specific animal requirements. Slow-growing pigs have lower protein requirements compared to lean, fast-growing genotypes (Aquilani *et al.*, 2019).

On standard, multiphase-feeding diets, like poultry, commercial growing pigs are highly N efficient, with as high as 40% nitrogen use efficiency (Rotz, 2004). In the current study, NU ranged between 29.5% and 41.5%, with a BVFP (%) of 40.0% – 45.7%. The values at the low end of these parameters suggested inferior protein quality in digestion and or imbalance in the profile absorbed amino acids, resulting in low tissue utilization (Smiricky *et al.,* 2002).

A significant amount of dietary N is lost due to inadequate digestion and subsequent tissue assimilation (Rotz, 2004). Then, variable protein extraction takes place in the stomach combined with variable loss via the urine or faeces channels depending on the food (Ball *et al*., 2013). Depending on meal quality and quantity, complex gut-systemic exchange of endogenous and dietary protein, amino acids, and urea occurs (Ball *et al*., 2013). ND was generally high in the current study for the roasted (89%) and raw (90%), sprouted (89%) cowpea diets. El-Jasser

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(2011) observed that sprouted cowpeas had a protein digestibility of 75-79% *in vitro*. Cowpeas' protein digestibility was reported to be enhanced *in vivo* by sprouting (Urbano *et al*., 2005) and thermal processing (Doblado *et al*., 2007), but its digestibility was also shown to be decreased. Excessive heating reduces its digestibility due to non-enzymatic (Maillard) reactions between proteins and reducing sugars and thermally induced amino acid cross-linking (Tunio *et al*., 2017). Mosenthin *et al*., (2000) and Święch (2017) concluded based on the fact that neither the *in vitro* nor the *in vivo* evaluations can accurately predict the effects of processing on protein quality because neither can measure ileal protein or amino acid digestibility.

Legume seeds contain ANFs that affect feed intake and nutrient digestibility and also compromise functions of the liver, kidneys and intestines (Ndou *et al.,* 2015). Variable dietary DM, protein and amino acid digestibility should be expected of diets in which the legume feed contains significant antinutritional factors (Kumar *et al.,* 2006; Kayembe, 2013). In the present study, pigs were fed diets equally balanced in N and energy raw, sprouted or roasted cowpea diets. Chemical analyses suggested significant residual trypsin inhibitors in the sprouted, but less in the roasted cowpeas.

The pattern of NI across the genotype x diets treatments reflected that of the feed intake. However, different diet x genotype interactions were observed for ND, FNO, TNE, NU and NR, which suggested genotype differentiated N metabolism depending on cowpea processing. Nitrogen extraction from the gut seemed more efficient in all the pig genotypes on all the cowpea diets. Pigs were similarly efficient in extracting N from the cowpea diets. The pattern of ND directly reflected the dietary N availability or inversely reflected the endogenous wastage, a function of dietary ANFs. Moreira *et al.* (2004) reported that the total nitrogen excretion is determined by certain factors, primarily the crude protein content of the diet. Cahn *et al.* (1998) reported a 45% reduction in urinary nitrogen excretion when the dietary crude protein content decreased from 16.5 to 12.5%. In this study, faecal N wastage was equally for all pigs on the cowpea diets. Similar to the ND, the pattern of faecal N excretion likely reflected the undigested fraction, along with the endogenous N wastage. Urinary wastage was low for LW x LR pigs on all the cowpea diets. The high urine N excretion for W x LW x LR and W could reflect excess dietary N or an imbalanced amino acid profile relative to the pig requirement, or a dietary energy deficit requiring partial protein utilization for energy. Excess N is converted to urea, which increases its excretion in urine, as a result of an imbalance in the amino acids supplied for protein synthesis for growth and other functions (Ball *et al.,* 2013).

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On the other hand, protein indigestion in the upper tract sends N to the fermentation process in the colon (Bindelle *et al.,* 2009). Colon bacteria assimilate both endogenous and dietary N to lock it and shift excretion from urea in urine to microbial protein in faeces with an adequate fermentable energy supply (Bindelle *et al.,* 2009). Hlongwana *et al.* (2021) confirmed high N excretion through faeces because of either dietary or endogenous N diverted to microbial protein synthesis, thereby increasing microbial biomass in the hindgut. If energy is deficient, increased colon protein fermentation may also produce toxic nitrogenous metabolites (Tuśnio *et al.,* 2017). The pattern of TNE consisted of the summative FNO and UNO, the net effect of which was similarly low total N excretion for LW x LR pigs on the cowpea diets. The AN mirrored the ND, with the equally observed values for cowpea diet. Measured by the NU, protein efficiency was not differentiated by the pig genotype or the diet and was not subject to the genotype x diets interaction. BVFP, protein efficiency was fair enough, which suggested a balance dietary amino acids or the protein-energy ratio in relation to the genotype requirement. The “period” in the experimental setup represented the age of the pigs, which decreased the NI, UNO, FNO and NR, with a fairly inverse trend for the TNE and AN. Given different maturing patterns, the pig degree of maturity effects were evident in the period x diet interaction for UNO, whereby LW x LR excreted less N with time, consistent with their maturation.

4.5 Conclusion

In this study, the pattern of compartmental and the total *IVDMD* suggested that, compared to roasting, sprouting increased and or shifted fibrous substrates and their digestion to the lower gut, without effect on total *IVDMD*. In contrast, *in vivo*, roasting, and sprouting of cowpeas similarly reduced dietary DM digestibility compared to raw cowpeas, which implied outflow rate-dependent escape from total digestion of slowly digested plant embryonic secondary tissues in cowpea sprouts, and of cross-linked compounds in roasted cowpeas. The W x LW x LR expressed higher capacity to digest ADF than the LW x LR pigs, though W x LW x LR had comparable ADF digestibility to the W pigs. Though partly, the effect of intake induced differences in outflow rate, the genotype differentiation in capacity to digest different fibre fractions could reflect different microbiota ecosystems in the hindgut. The nitrogen balance responses were characterized by interaction of diet with the pig genotype. Nitrogen extraction from the gut seemed more efficient in W pigs and W x LW x LR pigs from the cowpeas diet. In the present study, we attributed the pattern of urine N excretion to excess dietary amino acids relative to the pig requirement, or a dietary energy deficit, which triggered protein utilization for energy. The pattern of TNE was

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consisted with the summated FNO and UNO, the net effect of which was similarly low total N excretion for LW x LR pigs on the cowpea diets. Overall, measured by the NU, protein efficiency was not differentiated by the pig genotype, or the diet, and not subject to genotype x diet interaction. However, measured as the BVFP, protein efficiency was comparable among the pigs, which suggested balance in either the dietary amino acids, or the protein-energy ratio, in relation to the genotype requirement. We concluded that both sprouting and roasting caused chemical changes with no decrease on the dietary intake and depress mineral digestibility with better DM digestibility. The LW x LR expressed lower digestibility of the more recalcitrant ADF. Diet x genotype interaction with the biological versus thermal cowpea processing resulted in significant variation in pig responses in terms N balance parameters which relate to different amino acid metabolism, effects which suggested unique digestive and metabolic adaptive traits among the experimental pig genotypes to the diets. The implications need verification in large-scale performance trials with more extensive dietary chemical characterization.

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CHAPTER 5

GROWTH, SLAUGHTER PERFORMANCE, VISCERAL ORGAN SIZES AND PLASMA METABOLIC MARKER PROFILES OF DIFFERENT PIG GENOTYPES FED ROASTED OR SPROUTED COWPEA (*VIGNA UNGUICULATA*) BASED DIETS

Abstract

Inefficiently processed cowpeas may contain residual enzyme inhibitors and toxins that can affect growing pigs. The choice of methods to process the cowpeas in low-resource settings should consider factors such as technology, cost, and modification of nutrients in relation to the pig's response. This study evaluated the efficacy of roasted, versus sprouted cowpea diets in Windsnyer (W), Large White (LW) X Landrace (LW)] and the three-way crossbred (W X LW X LR) growing pig genotypes. The study used 36 animals, twelve male, 28-day weaned pigs of each genotype, of respective initial live weights 15.0±2.3, 39.0±1.4 and 37.0±1.2 kg (10-11% degree of maturity). To balance initial weights across dietary treatments, pigs were first blocked by weight within genotype, and were allocated randomly within the weight blocks to experimental diets for a 3 (genotype) X 3 (diets) factorial experiment replicated four times. The experimental diets were iso-nutrient (17.5 ± 1 MJ ME kg, 160 ± 2g CP kg) standard, commercial maize-soybean pig grower (control), and either roasted or sprouted whole cowpea-maize grower pig diets. Pigs were individually fed in crates housed within an open, naturally ventilated trial house. Pig growth was differentiated by genotype in the order LW x LR ≥ LW x LR x W > W and by the dietary treatments in the order control > sprouted cowpeas > roasted cowpeas diet ($P<0.05$). Feed intake was differentiated in the genotype order W x LW x LR ≥ LW x LR > W and by the dietary order control > sprouted cowpeas ≥ roasted cowpeas diet ($P<0.05$). The FCR was similar ($P>0.05$) across the genotypes but was differentiated by diet in the order control (3.41 ± 0.551) < sprouted cowpeas (4.94 ± 0.551) ≤ roasted cowpeas (5.93 ± 0.551) diet ($P<0.05$). While back fat was thickest on the control diet (9.91 ± 0.321) compared to the other diets ($P<0.05$). The W pigs dressed inferior ($P<0.05$) compared to the other genotypes. Scaled on metabolic weight basis (weight$^{0.75}$), the liver and kidneys were relatively large ($P<0.05$) in W pigs, and the kidneys enlarged 0.567 ± 0.0105 % ($P<0.05$) in pigs on the roasted cowpea diet. W x L x LR had low ALP (99.50 ± 6.090) U/L, W had low creatinine (44.94 ± 2.32 μmol/L) and Cholesterol (1.23 ± 0.0833 mmol/L) and LW x LR had low plasma total protein (69.20 ± 0.915 g/L). Sprouted cowpea diet had high urea (5.30 ± 0.255 mmol/L) and cholesterol (1.82 ± 0.0833 mmol/L). Albumin was low for sprouted diet (35.58 ± 0.902 g/L) and roasted diet (35.60 ± 0.902 g/L), while ALP was high for sprouted diet (110.92 ±

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6.090 U/L) and roasted diet (130.13 ± 6.090 U/L). The roasted and sprouted cowpea diets supported similar, but less pig growth compared to the commercial (soybean-based) diet. Plasma metabolic biomarkers aligned with the pig performance. The findings suggested both roasted and sprouted cowpea can be included in the diet of growing pigs without adverse effects on developing pig health and productivity.

Keywords: *Antinutritional factors, Legume, Pig genotype, Plasma metabolites, Roasting, Sprouting*

5.1 Introduction

Globally, soybean (*Glycine max*) oil cake is considered the conventional, primary plant protein source for stock feeding. However, simulation models predict negative climate-change impacts in most rain-fed, low-technology, arid tropical soybean production systems (Ma *et al.,* 2021; Gong *et al.,* 2022). Despite the dire implications of climate change on future stockfeed supplies, native legume alternatives such as the cowpea (*Vigna unguiculata*) remain marginalised from mainstream animal nutrition research. Unlike the soybean, the cowpea oil content is too low to justify detoxifying expeller extraction produces the high protein oilcake as a byproduct. Therefore, there are no options but to feed whole grain cowpea to pigs, which requires efficient, cost effective and technologically appropriate processing methods for the most poorly resourced tropical settings.

Tropical small-holder pig farming systems are typically endowed with a diversity of indigenous, exotic, and crossed pig genotypes, which likely express unique naturally (indigenous pigs) or artificially (improved pigs) acquired nutritional traits of potential production importance (Bovo *et al.,* 2016). Due to feed shortage and the high cost, pigs in poorly resourced, extensive systems are often exposed to inferior diets, constituted from unconventional (Carter *et al.,* 2016). The extent to which pigs of different genotypes may adapt to the anti-nutrients or metabolically compensate for the nutrient deficiencies typical of such diets is uncertain.

In species such as the pig, a range of plasma metabolites and enzymes are considered biomarkers of animal nutritional or disease status (Yang *et al.,* 2011). Compared to large-scale, long term on-farm performance trials, metabolic exploration of nutrient metabolism using blood biomarkers is considered suitable for on-farm or on-station nutrigenomic studies, given the

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convenience and the more controlled, rapid and low-cost application (Montoro *et al.,* 2022). A spectrum of metabolite biomarker for protein and energy utilisation, and key enzyme signals of digestive, metabolic and metabolite excretory tissue and organ functions have been characterised. Protein metabolic markers include total protein, albumin, urea nitrogen and creatinine, while the indicators for energy metabolism include glucose, triglycerides, and cholesterol (Montoro *et al.,* 2022). In pigs, total plasma protein is considered strongly predictive of protein metabolism (Hassan *et al.,* 2020). Plasma albumin and urea correlate to quantitative and/or qualitative protein adequacy (Akinfala & Tewe, 2001). Creatinine, a product of muscle metabolism, positively correlates to muscle, particularly striated muscle metabolism (Montero *et al.,* 2022). In mammals, urea is the primary nitrogenous end-product of amino acid catabolism, and the excretory plasma urea nitrogen is predictive of dietary protein quality or animal protein status, with high levels indicating amino acid excess or imbalance (Liu *et al.,* 2015). Blood ALP activity is primarily from the liver and bone tissue. It is also linked to lipid transport in the intestines (Chinmaya *et al.,* 2017) and a signal for its metabolism (Liu *et al.,* 2015). Plasma ALP activity is positively correlated to pig weight gain (Pond *et al.,* 1997; Yang *et al.,* 2011; Liu *et al.,* 2015). Among others, liver function is routinely evaluated through blood alanine transaminase (ALT), aspartate transaminase (AST), alkaline phosphatase (ALP) activities, in combination with bilirubin, total protein and albumin (Ozer *et al.,* 2008). Elevated ALT and AST activities which are disproportionate to ALP activity and plasma bilirubin, suggest hepatocellular disease, while the opposite characterizes cholestasis (Meyer & Harvey, 1998; Milinković-Tur *et al.,* 2005). Elevated ALT activity also signifies heart and skeletal muscle degeneration (Nathwani *et al.,* 2005). In a healthy subject, albumin degradation should be in equilibrium with its synthesis (Peters, 1996). Albumin catabolism occurs primarily in the muscle and the skin, which respectively account for 40% to 60% of albumin degradation (Juliene *et al.,* 2004), with as much as 10% of the albumin secreted from the gastrointestinal (GI) epithelia and skin secretory epithelia (Juliene *et al.,* 2004). The AST is widely distributed in tissues and organs, with high activity in the liver (Zimmerman *et al.,* 1968) cited by (Milinković-Tur *et al.,* 2005).

Apart from lectin, tannin, phytase and protease digestive enzyme inhibitors, feeding raw cowpeas to growing pigs causes impaired pancreatic exocrine secretion, with wastage of endogenous amino acids, coupled to pathophysiological changes in gut epithelial morphology, affects which affect nutrient absorption sufficiently to severely affect pig health and growth (Makinde *et al.,*1996; Umapathy & Erlwanger, 2008). In addition to impaired digestion, poor growth seems principally due to a reduction in feed intake, primarily due to tannins (Umapathy & Erlwanger, 2008).

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Depending on resources, there are several on-farm bio and thermal processing options for legume grains (Amaefule *et al.,* 2004; Amaefule *et al.,* 2006; Udensi *et al.,* 2007; Drulyte & Orlien, 2019). In principle, chemical effects unique to the method or efficacy of processing cowpeas for pig feeding may influence nutrient extraction and the pathways of intermediary metabolic pathways sufficiently to alter the profile of intermediate and or final plasma metabolites to provide insight into genotype-discriminated nutritional phenotypes (Bovo *et al.,* 2016). Results from the metabolic trial confirmed that the fibre, nutrient digestibility, and N balance parameters subject to genotype X diet interactions, suggested unique adaptive traits among the experimental genotypes.

Therefore, a performance trial evaluated the potential interaction of the pig genotype with the method of cowpea processing in terms of pig growth and slaughter performance parameters, effects on visceral organs and on blood metabolite and enzyme biomarkers for protein and energy metabolism.

5.2 Materials and methods

The Ethics Committees of the University of Venda (SARDF/17/ANS/07/0412) approved the experimental protocols for the management and care of animals.

5.2.1 Site of study

The study was conducted at the Pig Research Unit of the Agricultural Research Council Animal Production Institute, Irene, South Africa. The Agricultural Research Council-Irene Research Station is located at 25° 55' South; 28° 12' East. The location is in the Highveld region of South Africa, at an altitude of 1525 m above sea level.

5.2.3 Diet

Maize based test diets (Table 5.1) were formulated using roasted or sprouted cowpeas as the primary plant protein sources. The method applied in chapter 4 was applied in cowpea processing. A commercial maize-soybean diet was the control against which iso-nutrient cowpea diets were formulated to meet the minimum nutrients recommended for commercial growing pigs (National Research Council, 2012). To prepare the different diet mixes, cowpeas and maize were hammer-milled (Jacobson model P160 Teordrop 10HP) through a 3 mm screen. Ingredients were

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then blended into the balanced dietary composites by 20-minute mixing in 1000 kg lots in a MORHLANG VERTA MIX 1200VM vertical mixer.

Table 5.1 Analysed ingredients and chemical composition of experimental diets

Components	**[1]Sprouted cowpeas**	**[2]Roasted cowpeas**
Ingredients (% as fed)		
Maize	61.2	55.4
Sprouted Cowpea	36.0	-
Roasted Cowpea	-	42
Soybean meal	-	-
NaCl	0.57	0.51
Mineral & Vitamin Mix	2.23	2.09
Total	100.0	100.0
Analysed chemical (g kg $^{-1}$) and calculated energy (MJ kg^{-1}) composition		
Crude protein	155.0	155.0
Crude fibre	21.0	20.8
Nitrogen detergent fibre	195.3	164.4
Acid detergent fibre	84.9	72.1
Ash	27.3	27.8
Crude fat (ether extract)	24.7	24.8
[3]Metabolisable energy	14.1	14.3
Calcium	5.4	5.4
Phosphorus	3.4	3.4

[1]Sprouting; 12-hour pre-soaking in water in water, 4-day open-air sprouting at ambient conditions;

[2]Roasting: cylindrical (L =1.5 m; Diameter= 0.50 m) manually rotating, cast-iron, gas heated drum, 20 kg cowpeas, initial maximal constant interior drum temperature 150°C, 20 minutes roasting to 105°C terminal grain temperature.

[5]Metabolizable Energy (ME) MJ kg^{1} : ME = 1.000 x DE – 0.68 x CP ….eq. 43. Noblet and Perez. 1993, where [4]Digestible Energy (DE) MJ kg^{-1} : DE = 4.168 - 9.1 x Ash + 1.9 x CP + 3.9 x EE - 3.6 x NDF….eq. 23. Noblet and Perez. 1993

5.2.4 Experimental design and animal management

The study was performed in a customized open trial house in which pigs were in individual 1.2 m × 1.4 m clear view steel crates, from which feed and water were freely dispensed from individual self-feeders and nipple drinkers as ad lib. The pigs were bred at the Agricultural Research Council-Irene Pig Breeding Unit. They were selected from litters born by naturally serviced sows and weaned at 28 days onto a commercial weaner diet. Previously, for the same pig populations, Kanengoni *et al.* (2014) estimated 300 – 350 kg mature weight for the LW x LR genotype, and 100 to 150 kg for the Windsnyer type pigs. These estimates guided the weight selection of 36 males, 12 each of Windsnyer (W) (15 ± 2.3 kg), Large White (LW) X Landrace (LR) (37 ± 1.4 kg), and W X L X LR crossed (39 ± 1.2 kg). The selected pigs were all within an estimated 10-11% degree of maturity. The pigs were further blocked by weight within breed into 3-pig groups, from which they were randomly allocated to dietary treatments for a live-weight balanced, 3 × 3 factorial experiment replicated four times. Early in the trial, some pigs showed signs of exposure to elevated ammonia levels, which was promptly addressed by optimizing ventilation. As a precaution, all pigs received treatment for potential mild pneumonia. The pigs were first acclimatized to the experimental setup for a period of 7-days. The feeding trial lasted for 8 weeks, after which pigs were humanely slaughtered by standard procedures.

5.2.5 Measurement of growth, slaughter performance and vital visceral organ sizes

Pigs were weighed at the beginning of the experiment and fortnightly thereafter to monitor growth to the end of the experimental period. Average daily weight gain (ADG), the unscaled average daily feed intake (ADFI), live and metabolic scaled feed to unitary (kg) live weight intake, and the feed: gain ratio (FCR) were calculated. The pigs were slaughtered following humane, standard protocols. Upon slaughter, the heart, liver, kidneys, spleen, stomach and lungs were removed and weighed. The hot carcass weight was recorded after dressing, and the cold carcass weight were measured after 24-hour 3 – 4°C cold storage.

5.2.6 Plasma metabolite and enzyme biomarkers

Blood samples were collected at slaughter from the severed anterior *vena cava* into heparin tube tubes were placed in ice for approximately four to six h prior to centrifuging at 3,000 x g for 15 min at 4°C for plasma separation. The plasma was frozen to -4°C prior to metabolite analysis. An IDEXX analyser with Catalyst Dx Chemistry Analyser was used to determine Glucose, Urea, Creatinine, Total protein (TP), albumin, globulin, alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), total bilirubin-total, cholesterol (Chol) and triglycerides (TG).

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5.2.7 Statistical analysis

Growth and slaughter were subjected to ANOVA for a 3 x 3 factorial experiment using the PROC MIXED procedures in Minitab version 17.0 (2014). The initial (growth parameters) and slaughter (visceral organs) pig weight were used as covariates in the following mixed model:

$$\boldsymbol{Y_{ijkl}} = \boldsymbol{\beta Cov_{k(ij)}} + \boldsymbol{\mu} + \boldsymbol{G_i} + \boldsymbol{D_J} + (\boldsymbol{G} \times \boldsymbol{D})_{\boldsymbol{ij}} + \mathcal{E}_{\boldsymbol{ijkl}}$$

Where Y_{ijk} is the l^{th} observation for the k^{th} pig on the j^{th} diet, $\beta Co_{ik(ij)}$ is the covariate term representing the initial /slaughter weight of the k^{th} pig within the i^{th} genotype on the j^{th} diet, μ is the overall mean, G_i and D_j are the fixed effects of the i^{th} genotype and j^{th} diet, respectively, $(GxD)_{ij}$ is the interaction between the i^{th} genotype and j^{th} diet, and $\mathcal{E}_{ijk}$ is the random error associated with each observation.

Plasma metabolites were analised using the following model:

$$\boldsymbol{Y_{ijk}} = \boldsymbol{\mu} + \boldsymbol{G_i} + \boldsymbol{D_J} + (\boldsymbol{G} \times \boldsymbol{D})_{\boldsymbol{ij}} + \mathcal{E}_{\boldsymbol{ijk}}$$

Where Y_{ijk} is the k^{th} observation for the j^{th} genotype and i^{th} pig/animal, μ is the overall mean, G_i and D_j are the fixed effects of the i^{th} genotype and j^{th} diet, respectively, $(GxD)_{ij}$ is the interaction between the i^{th} genotype and j^{th} diet, and $\mathcal{E}_{ijk}$ is the random error associated with each observation.

Tukey's post hoc test was used to compare different means ($\alpha \leq 0.05$).

5.3 Results

Dietary and pig genotype effects on the pig growth performance indices are presented in Table 5.2. The growth responses of the different pig genotypes on different cowpea diets is depcted in Figure 5.1. Feed intake was in the genotype order W x LW x LR ≥ LW x LR > W and by the dietary order control > sprouted cowpeas ≥ roasted cowpeas diet ($P<0.05$). The FCR was similar ($P>0.05$) across the genotypes but was in the dietary order control (3.220 ± 0.551) < sprouted cowpeas (4.529 ± 0.551) ≤ roasted cowpeas (6.077 ± 0.551) diet ($P<0.05$). While back fat was thickest on the control diet (9.91 ± 0.321) compared to the other diets ($P<0.05$). The W pigs dressed inferior ($P<0.05$) compared to the other genotypes.

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Dietary and pig genotype effects on vital visceral organ sizes are presented in Table 5.3. The LW x LR and W x LW x LR pigs had enlarged ($P < 0.05$) kidneys and spleen. Pigs on the roasted cowpeas had enlarged kidneys diet ($P<0.05$). Scaled on metabolic weight basis (weight$^{0.75}$), the kidneys and spleen were relatively large ($P<0.05$) in W x LW x LR pigs, and the kidneys enlarged 0.410 ± 0.0105 % ($P<0.05$) in pigs on the roasted cowpea diet.

Dietary and pig genotype effects on the measured plasma parameters are presented in Table 5.4. W x L x LR had low ALP 99.50 ± 6.090 U/L, W had low creatinine 44.94 ± 2.32 µmol/L and Chol. 1.23 ± 0.0833 mmol/L; and LWxLR had low plasma total protein 69.20 ± 0.915 g/L. Sprouted cowpea diet had high urea 5.30 ± 0.255 mmol/L and cholesterol 1.82 ± 0.0833 mmol/L. Albumin was low for sprouted diet 35.58 ± 0.902 g/L and roasted diet 35.60 ± 0.902 g/L, while ALP was high for sprouted diet 110.92 ± 6.090 U/L and roasted diet 130.13 ± 6.090 U/L. Plasma glucose, globulin, triacyclglycerols, alanine aminotransferase (AST), and aspartate aminotransferase (ALT) activities were not ($P>0.05$) affected by the treatments.

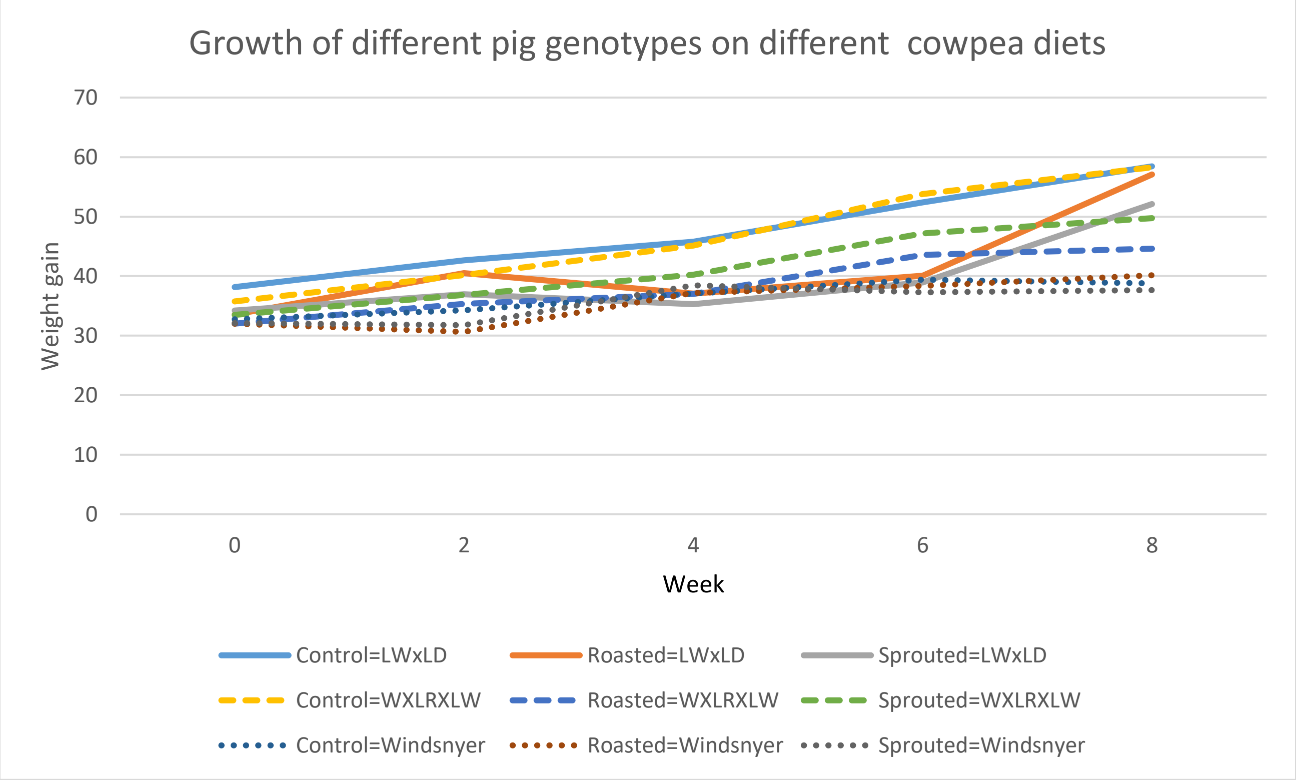

Figure 5. 1 Growth of different pig genotypes on different cowpea diets

Table 5.2 **Growth and slaughter performance of indigenous, exotic, and crossbred growing pigs fed a standard (control), and roasted or sprouted cowpea diets**

Treatment		Slaughter Weight (kg)	Dressing %		Feed intake		Weight gain (kg/day)	FCR	Back Fat (mm)
			Warm carcass	Cold carcass	(kg/day)	kg/ LW$^{0.75}$			
Genotype	**Diets**								
LW x LR	Control	65.6	66.161	63.553	2.211	0.67	0.837^{a}	2.978	8.25^{cde}
	Sprouted Cowpeas	59.7	83.311	80.859	1.871	0.61	0.408^{ab}	7.646	7.50^{de}
	Roasted Cowpeas	55.1	75.561	73.732	1.522	0.62	0.222^{b}	9.357	6.50^{e}
W	Control	34.2	78.662	74.0392	1.182	0.72	0.0431^{b}	7.496	11.00^{ab}
	Sprouted Cowpeas	29.4	46.403	41.7503	1.103	0.66	0.439^{ab}	0.297	9.25^{cde}
	Roasted Cowpeas	26.6	73.256	65.153	1.510	0.78	0.447^{ab}	3.387	9.75^{bcd}
W x LW x LR	Control	66.4^{a}	71.183	64.253	2.012	0.60	0.923^{a}	0.513	10.50^{abc}
	Sprouted Cowpeas	61.0^{a}	88.121	83.285	2.031	0.64	0.383^{ab}	6.239	11.75^{a}
	Roasted Cowpeas	57.5b^{c}	77.572	74.039	1.510	0.62	0.302^{ab}	5.487	9.75^{abcd}
SEM		0.549	0.511	0.551	0.0339	0.0144	0.0234	0.361	0.164
Genotype	LW x LR	60.2^{a}	75.012	72.71	1.868	0.63^{b}	0.489	6.661	7.41^{b}
	Windsnyer	30.1^{b}	66.111	60.308	1.241	0.72^{a}	0.310	3.529	9.75^{a}
	W x LW x LR	61.7^{a}	78.958	73.858	1.849	0.62^{b}	0.536	3.737	10.66^{a}
SEM		0.503	12.5	14.0	0.494	0.468	0.321	4.63	0.146
Diet	Control	55.4^{a}	72.463	67.275	1.801^{a}	0.66	0.601^{a}	3.320^{b}	9.91^{a}
	Sprouted Cowpeas	50.0^{b}	72.611	68.632	1.603^{ab}	0.64	0.410^{b}	4.529^{ab}	8.50^{b}
	Roasted Cowpeas	46.4^{c}	75.463	70.974	1.554^{b}	0.68	0.324^{b}	6.077^{a}	9.41^{ab}
SEM		0.841	0.723	0.780	0.0479	0.0204	0.0331	0.551	0.321
P-values	*Covariate*	-	0.822	0.900	0.482	0.935	0.999	0.105	0.922
	Genotype	0.000	0.355	0.279	0.976	0.012	0.828	0.231	0.000
	Diet	0.000	0.620	0.788	0.154	0.539	0.012	0.025	0.005
	Genotype x Diet	0.949	0.633	0.718	0.153	0.450	0.099	0.151	0.037

^{abcde} Within each factor or combination of factors means within a column with different letter superscripts are significantly different at (*P<0.05*), SEM- Standard error of the mean, W- Windsnyer, Large White (LW), Landrace (LR), FCR = Feed conversion ratio. ; [1]Control –Standard commercial maize-soybean grower diet, [2]Sprouted- 12-hour soaking, 4-day open air sprouting, sun-dried, , [3]Roasted- 150° C initial, empty cast-iron drum temperature, 20-minute roasting to 105° C terminal grain temperature

Table 5.3 Visceral organ sizes of indigenous, exotic and crossbred growing pigs fed a standard and roasted or sprouted cowpea diets

Treatments		Heart	Liver	Kidneys	Spleen	Stomach	Lungs
		% Live Weight					
Genotype	**Diet**						
LW x LR	Control	0.520	1.34	0.115	0.215	3.45	1.52
	Sprouted cowpeas	0.424	2.34	0.533	0.224	0.234	1.84
	Roasted cowpeas	0.431	1.44	0.410	0.200	1.54	2.10
W	Control	0.133	2.94	0.210	0.321	1.24	2.11
	Sprouted cowpeas	0.0244	2.83	0.323	0.520	8.31	1.71
	Roasted cowpeas	0.132	3.44	0.144	0.570	1.14	1.21
W x LW x LR	Control	0.324	1.34	0.220	0.342	2.13	2.54
	Sprouted cowpeas	0.310	1.61	0.553	0.024	2.33	1.43
	Roasted cowpeas	0.420	1.80	0.450	0.213	0.910	1.21
SEM		0.0224	0.0768	0.0105	0.0109	0.147	0.0638
Genotype (G)							
LW x LR		0.440	1.54	0.311^{a}	0.223^{a}	1.73	1.83
W		0.313	2.93	0.040^{b}	0.132^{b}	1.64	1.80
W x LW x LR		0.944	1.61	0.411^{a}	0.410^{ab}	0.220	1.00
SEM		0.0317	0.109	0.0148	0.0155	0.208	0.0902
Diet (D)							
Control		0.340	1.84	0.214	0.152	2.30	1.284
Sprouted cowpeas		0.234	2.04	0.242	0.104	2.054	1.51
Roasted cowpeas		0.302	2.13	0.303	0.104	1.21	1.62
SEM		0.0317	0.109	0.0148	0.0155	0.208	0.0902
P-values	*Covariate*	0.760	0.819	0.297	0.121	0.238	0.881
	Genotype	0.483	0.959	0.013	0.031	0.448	0.627
	Diet	0.948	0.905	0.126	0.939	0.075	0.592
	Genotype x Diet	0.976	0.922	0.236	0.885	0.076	0.860

[abc] Within each factor or combination of factors, means within a column with different letter superscripts are significantly different at ($P<0.05$), SEM- Standard error of the mean, W- Windsnyer, Large White (LW), Landrace (LR), FCR = Feed conversion ratio; [1]Control –Standard commercial maize-soybean grower diet, [2]Sprouted- 12-hour soaking, 4-day open air sprouting, sun-dried, [3]Roasted- 150º C initial, empty cast-iron drum temperature, 20-minute roasting to 105° C terminal grain temperature

Table 5.4 Plasma metabolite and enzyme biomarkers of indigenous, exotic and crossbred growing pigs fed a standard and roasted or sprouted cowpea diets

Treatment		Glucose mmol/L	Creatinine µmol/L	Urea mmol/L	Total Protein g/L	Albumin g/L	Globulin U/L	ALT U/L	AST U/L	ALP U/L	Bilirubin l/L	Choesterol mmol/L	Triglycerides mol/L
		Normal range											
		4.72 - 8.88 mmol/L	44.2 - 185.64 µmol/L	2.14 - 10.71 mmol/L	60 - 80 g/L	18 - 33 g/L	9 - 43 U/L	16 - 65 U/L	92 - 294 U/L	92 - 294 U/L	1.71 - 5.13 µmol/L	0.47 - 2.04 mmol/L	0.46 - 0.94 mmol/L
Genotype	**Diet**												
LWxLR	[1]Control	6.61	75.14	3.21	71.75	37.00	34.75	51.00	62.00	100.50	8.12	1.77	0.63
	[3]Sprouted	6.02	72.93	4.46	67.25	32.75	34.50	42.50	74.00	136.00	8.55	2.15	0.43
	[2]Roasted	5.89	66.30	4.46	68.50	33.00	35.50	42.00	50.25	140.50	5.99	2.13	0.71
W	Control	7.27	46.41	3.84	73.50	39.75	33.75	56.75	62.75	118.75	5.13	0.85	0.39
	Sprouted	6.16	41.99	5.71	68.25	35.25	33.00	56.25	62.00	102.75	5.13	1.28	0.40
	Roasted	6.62	46.41	5.54	67.75	35.25	32.50	66.25	69.25	136.50	5.56	1.42	0.37
WxLWxLR	Control	6.56	64.09	3.66	75.00	41.50	33.50	53.50	62.50	91.25	8.12	1.53	0.40
	Sprouted	6.30	59.67	5.62	73.50	39.00	34.50	49.75	56.25	94.00	5.56	2.02	0.50
	Roasted	6.36	63.30	3.57	72.25	38.50	33.75	53.25	70.75	113.25	10.64	1.48	0.47
SEM		0.197	1.64	0.180	0.647	0.637	0.446	2.420	3.901	4.310	0.618	0.0589	0.0387
Genotype (G)	LWxLR	6.17	71.46^{a}	4.05	69.20^{b}	34.25^{b}	34.92	45.20	62.13	125.70^{a}	7.55	2.02^{a}	0.62
	W	6.68	44.94^{b}	5.03	69.83^{ab}	36.75^{ab}	33.13	59.75	64.70	119.33^{ab}	5.27	1.23^{b}	0.40
	WxLWxLR	6.23	63.35^{a}	4.28	73.63^{a}	39.67^{a}	33.92	59.20	63.20	99.50^{b}	8.11	1.77^{a}	0.43
SEM		0.395	3.28	0.360	1.29	1.27	0.892	4.83	7.80	8.62	1.24	0.118	0.774
Diet (D)	Control	6.81	61.88	3.57^{b}	73.42	39.42^{a}	34.00	53.75	62.42	103.50^{b}	7.13	1.41^{b}	0.53
	Sprouted	6.22	58.20	5.30^{a}	69.67	35.58^{b}	34.00	49.50	64.13	110.92^{a}	6.41	1.82^{a}	0.44
	Roasted	6.29	59.67	4.52^{ab}	69.50	35.60^{b}	33.92	53.83	63.42	130.13^{a}	7.43	1.72^{ab}	0.52
SEM		0.279	2.32	0.255	0.915	0.902	0.631	3.420	5.510	6.090	0.874	0.0833	0.0547
P-values	Genotype	0.579	0.000	0.085	0.020	0.007	0.261	0.064	0.964	0.050	0.160	0.000	0.112
	Diet	0.373	0.658	0.003	0.320	0.032	0.996	0.707	0.985	0.049	0.801	0.016	0.737
	Genotype x Diet	0.953	0.670	0.288	0.986	0.986	0.901	0.798	0.561	0.410	0.373	0.326	0.589

^{abc} for each factor, and interactions, ^{means} within a column with different letter superscripts are significantly different at ($P<0.05$). SEM- Standard error of the mean, ALT - Alanine aminotransferase, AST - Aspartate aminotransferase, ALP - Alkaline phosphatase, LW- Large White LR- Landrace, W- Windsnyer, ; [1]Control –Standard maize-soybean grower diet, [2]Sprouted- 12-hour soaking, 4-day open air sprouting, sun-dried, , [3]Roasted- 150° C initial, empty cast-iron drum temperature, , 20-minute roasting to 105° C terminal grain temperature.

5.4 Discussion

In the present study, the pig genotype affected the pig size, growth rate, dressing percentage, liver and kidney sizes, creatinine, cholesterol, plasma total protein and albumin, while the diet affected the growth rate, weight gain, feed intake, FCR, dressing percentage, kidney size, cholesterol urea, albumin and ALP profiles, with genotype X diet interactions for back-fat. The literature is scant of studies which similarly combined the measurement of growth, slaughter performance and effects on scaled organ sizes with the chemical profiling of plasma biomarkers to specifically evaluate effects of sprouting versus sprouting whole cowpeas for total dietary replacement of expeller soybean cake in the spectrum of pig genotypes used in this study. Comparability of findings from plasma biomarker based nutrigenetic studies is however complicated by experimental factors which may influence metabolism, such as the actual or physiological animal age, growth stage, and the feeding level or period (Montoro *et al.,* 2022). In the present study, growth rate was in the genotype order LW x LR ≥ W x LW x LR > W, feed intake in the order W x LW x LR ≥ LW x LR > W, with higher backfat recorded by W, compared to LW x LR pigs. These effects were consistent with the superior, artificially improved genetics of the exotic pigs (Len *et al.,* 2008; Jiang *et al*., 2011), and the heterosis expected in the 3-way crossbreed. Measured by pig growth rate and feed intake, both cowpea diets were considered inferior to the commercial control diet. Slower growth on the cowpea diets was likely the result of intrinsically inferior protein quality compared to soybean cake (Khattab, 2009; Frota *et al.,* 2017). If the processing was suboptimal, the poor utilisation of the cowpea diets could result from residual anti-nutrients which impaired digestion, caused endogenous protein wastage, and importantly, could be the effect of tannins related low palatability, and consequently low intake (Umapathy and Erlwanger, 2008). The tannin effect is supported by fact that pigs consumed more of the control diet compared to the cowpea diets. The higher intake of the control diet explained the thicker back fat. The higher back fat in LW x LR and LW x LR on the roasted cowpea diet compared more back fat on the sprouted cowpea diet in W pigs suggested unique energy or lipid metabolism on the different diets. The enlarged liver and kidney in W pigs, and the enlarged kidney in pigs on the roasted cowpea diet could be unique adaptive traits to suboptimal or toxic diets. The W x LW x LR pigs had higher plasma total protein compared to the commercial type, LW X LR pigs, with an intermediate level for the W pigs, which suggested the same order in protein demand or the reverse order in the efficiency of protein utilization. These effects need further investigation.

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In the present study, across the treatments, the plasma creatinine, glucose, urea, Chol, total protein 67.25 - 75.00 g/L and the ALP and AST activities were considered within the reference normal pig ranges (Milinković-Tur *et al.,* 2005 Ozer *et al.,* 2008). However, plasma albumin, bilirubin and the ALT activity exceeded the normal ranges (Nathwani *et al.,* 2005), which could be effects of infection or non-infectious hepatocellular disease. However, in the present study, the elevated plasma metabolites were not diet related, and were attributed to an inflammatory response triggered by the transient high ammonia levels to which the pigs had been exposed. The observed genotype influences on plasma parameters such as the total protein, ALP activity and the variable lipid components were overall, generally consistent with indications from more robust metabolomic exploration, which similarly differentiated both protein and lipid metabolism in fast growing, lean versus unimproved indigenous pigs (Yang *et al.,* 2011; Bovo *et al*., 2016). The intensity of metabolic changes, mainly of protein, is also reflected in the concentration of other biochemical indicators of blood, such as total protein, urea, aspartate aminotransferase or alanine aminotransferase (Kapelański *et al.,* 2000, Więcek and Skomiał 2000). In this study, plasma total protein was in the range 68.5-75 g/L across the treatments. As can be seen, most of the biochemical parameters of blood related to protein metabolism were higher (73.63 g/L) in W x LW x LR pigs characterized by a high rate of growth compared to the commercial type LW X LR pigs (69.20 g/L), with an intermediate level (69.83) for the slow growing W pigs. The relative imbalance in dietary amino acids in relation to the animal requirement, likely sufficiently to compromise the liver or whole-body protein synthesis (Hassan *et al.,* 2020). Friendship *et al*., (1984) previously reported such variations in range of plasma total protein of 52-83 g/L in Ontario pigs on maize diets. Harapin *et al*., (2003) reported higher values of 76-88 g/L in wild boar pigs3 – 5 years old scavenging in protected camps. Albumin constitutes up to 60% of the total plasma proteins (Shen *et al.,* 2004) and is involved in the transport of plasma lipids (Matejtschuk, Dash, & Gascoigne, 2000). Protein nutrition influences albumin synthesis, which decreases with protein malnutrition (Juliene, *et al.,* (2004). In the present study, a range of 32.5-39.5 g/L plasma albumen was observed across the treatments. The sprouted, and roasted cowpea diets similarly (35.58-35.60 g/L) decreased pig plasma albumin compared to the control (39.42 g/L). The LW x LR pigs had lower (34.25 g/L) plasma albumen than the W x LW x LR pigs (39.67 g/L), with an intermediate level (36.75 g/L) for the W pigs. Low plasma albumen suggests less plasma transport of lipid compounds (Juliene, *et al.,* 2004). In the present study, pigs on the sprouted diet had higher plasma urea compared to those on the control diet, which suggested either excess or imbalance in dietary amino acids. Surprisingly, despite dietary influences on urea N, plasma urea N was not differentiated by genotype, to reflect effects of higher demand for amino acids expected

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of the lean-type LW x LR genotype (Liu *et al.,* 2015). However, though the diet did not influence total plasma total protein, an opposite trend to plasma total protein was observed for plasma ALP activity among the pig genotypes. The W is a smaller, fatter breed, with slower growth potential compared to the genetically improved commercial pig breeds (Qin *et al.,* 2002). Both cowpea diets increased plasma ALP, regardless of the cowpea processing method. Plasma ALP activity is positively correlated to pig weight gain (Pond *et al.,* 1997; Yang *et al.,* 2011; Liu *et al.,* 2015), and is a signal for lipid metabolism (Liu *et al.,* 2015), likely the effects of the pig growth rate, given that, compared to the LW X LR, creatinine was lower in W pigs, consistent with a lower muscle mass, or less turnover for the slow growing breed. (Fisher, 1954; Broda, 2014) indicated that, most incoming creatinine is condensed to form tissue proteins and that catabolic processes are secondary to anabolic one. Creatinine may pass immediately out of the body, therefore a little may be used directly for endogenous processes Broda, 2014.

The primary mammalian energy repository are the lipids. Plasma TG, total, low and high density lipoprotein Chol levels reflect patterns of both lipid absorption and utilization (Ma *et al.,* 2020) The principal storage lipid classes are the TG, phospholipids, and steroids, with the TG quantitatively the most important (Godsland, 2004). Mammals revert to breaking down stored lipids to meet energy requirements only when in dietary energy deficit, or to preserve glucogenic fuel substrates for the dependent vital organs (Lomb *et al.,* 2010). The TG are considered indices of total body fat (Griffin *et al.,* 1982; Whitehead and Griffin 1984). (Yang *et al.,* 2011) stated that, in animal species, the blood levels of molecules related to lipid, glucose and protein metabolism, such as non-esterified fatty acids, triglyceride, glucose and alanine aminotransferase (ALT), reflect nutritional and disease status. Though not statistically significant, the genetically improved LW X LR genotype had higher plasma TG and consistent with a higher feed intake. Apart from dietary supply, plasma TG include those mobilized from adipocytes to increase the plasma levels during energy deficit (Jensen *et al.,* 2000). In the fed state, unsaturated fatty acids, saturated fatty acids, and glycerol are used to synthesize triglycerides (Miyazaki *et al.,* 2001). During fasting, fatty acids are released from triacylglycerol stored in adipocytes of growing pigs, resulting in increased levels in plasma (Jansen *et al.,* 2001). Therefore, the higher plasma TG in the LW x LR pigs could also partially indicate marginal energy deficit. Thyroid hormones and their receptors they are reported to stimulate reverse cholesterol transport in animal models (Pedrelli *et al.,* 2010) and such increased was observed when cowpea diets increased plasma Chol, particularly the sprouted cowpea diet, likely an effect of low dietary intake. Adequate cholesterol level in blood is therefore crucial for achieving maximum weight gain in growing animals. The blood levels of lipids and

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cholesterol depend on the breed of pigs, their genotype in relation to lipoproteins, sex and the type of feed given (Migdał *et al.,* 1999, Barowicz *et al.,* 2000, Migdał *et al.,* 2003). In the present study differences were shown in the concentration of blood serum cholesterol between the pig genotype studied, whereby plasma Chol was low in W pigs. Similarly, in chicken, slow-growing genotypes had low plasma Chol (Tudorache *et al.,* 2022). Therefore, it is believable that, for the W pigs, low plasma Chol levels suggested either low intake, or greater or more efficient transport and or clearance of dietary Chol through catabolism in the liver, or via deposition in adipose tissues. In the present study, the treatments did not affect plasma glucose, which suggests the insulin-glucagon axis control of glucose or energy metabolism was not active.

In addition to the chemical modification of feed nutrients, their absorption in the gut, and the subsequent pig intermediary metabolism, the efficacy of processing legume grains is also a function of potential residual toxic compounds. The liver is a primary nutrient processing organ, including the digestion, metabolism for assimilation or detoxification, and excretion, which exposes it to dietary toxins, effects which are indirectly indicated by serum biomarkers or by biopsy (Chen *et al.,* 2017). However, in the present study, despite genotype differences in liver and kidney size, and dietary effects on kidney size, the profile of the clinical biomarkers did not suggest dietary or genotype discrimination.

5.5 Conclusion

Pig growth rate was not affected either by diet nor genotype differences. However, pigs fed control diet performed better than those fed sprouted or roasted cowpea diet. The pig growth rate was in the genotype order LW x LR ≥ W x LW x LR > W, and the dietary order control > sprouted cowpeas > roasted cowpeas diet. Feed intake was in the genotype order W x LW x LR ≥ LW x LR > W, and dietary order control > sprouted cowpeas ≥ roasted cowpeas diet. Despite the enlarged liver and kidney in W pigs, and the enlarged kidney in pigs on the roasted cowpea diet, the relevant plasma biomarkers for the organs' functional or disease status did not suggest dietary or genotype discrimination, respectively. Overall, pig performance was consistent with the productive efficiency expected of the genetically improved, compared to the native pig genotypes, and also reflected beneficial non-additive gene anticipated effects of their crossed genotype. The findings suggested cowpeas, either roasted or sprouted can substitute soybean oil cake in growing pig diets with minimal compromise to pig productivity. However, genotype x diet interactions implied pigs either naturally (indigenous pigs) or artificially (improved pigs) acquired or lacked some

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metabolic or physiological functional traits to differently process dietary antinutrients or compensate for nutrient deficiencies in the cowpea diets. Genotype-differentiated pig utilisation of differently processed cowpeas justify further investigation to guide producer choices in pig selection criteria, of alternative legumes to soybean, and of methods for processing different legumes for pig feeding.

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CHAPTER 6

GENERAL CONCLUSION AND RECOMMENDATIONS

6.1. General conclusion

The study determined the capacity of different pig genotypes to use sprouted or roasted cowpea as a dietary protein source for increasingly expensive soybeans to provide options for both small-scale and commercial pig producers to maintain pig productivity and economic viability. To determine the effects of different extents of sprouting or roasting of cowpeas (*Vigna unguiculata Walp (L.)),* changes in gut compartmental and the total *in vitro* enzymatic dry matter digestibility were evaluated, supported by analyses for the organic and mineral composition, and trypsin inhibitor activity. The *in vivo* nutrient digestion, nitrogen balance, feeding intake and efficiency, salughter and blood biochemical parameters of optimally roasted and sprouted cowpeas were compared.

Overall, the trend of *IVDMD* suggested that under the experimental conditions, sprouting beyond four -days was necessary to determine the threshold *IVDMD* for the test cowpeas. The roasting experiment suggested 20 minutes of roasting from 150^{o}C initial empty drum temperature to 105^{o} C terminal grain temperature was most effective. In contrast, *in vivo*, roasting and sprouting cowpeas similarly reduced the digestibility of DM, which implied outflow rate-dependent escape from total digestion of slowly digested plant embryonic secondary tissues in cowpea sprouts, and of cross-linked compounds in roasted cowpeas.

The nitrogen balance responses were characterized by interaction of diet with the pig genotype. Nitrogen extraction from the gut seemed more efficient in W pigs and W x LW x LR pigs from the cowpeas diet. In the present study, we attributed the pattern of urine N excretion to excess dietary amino acids relative to the pig requirement, or a dietary energy deficit, which triggered protein utilization for energy. The pattern of TNE was consisted with the summated FNO and UNO, the net effect of which was similarly low total N excretion for LW x LR pigs on the cowpea diets. Overall, measured by the NU, protein efficiency was not differentiated by the pig genotype, or the diet, and not subject to genotype x diet interaction. However, measured as the BVFP, protein efficiency was comparable among the pigs, which suggested balance in either the dietary amino acids, or the protein-energy ratio, in relation to the genotype requirement.

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Pig growth rate was not affect either by diet nor genotype differences. However pigs fed control diet performed better than those fed sprouted or roasted cowpea diet. Despite the enlarged liver and kidney in W pigs, and the enlarged kidney in pigs on the roasted cowpea diet, the relevant plasma metabolites biomarkers for the organs' functional or disease status did not suggest dietary or genotype discrimination in viscera tissue development, respectively. Overall, pig performance was consistent with the productive efficiency expected of the genetically improved, compared to the native pig genotypes. It also reflected beneficial non-additive gene anticipated effects of their crossed genotype.

In summary, sprouting deferred the digestion of fibrous substrates to the lower gut compared to roasting. Neither processing method affected the total *IVDDM* compared to the raw cowpea diet. Therefore, it is evident that the fibre, nutrient digestibility, and N balance parameters, subject to genotype X diet interactions, suggested unique adaptive traits among the experimental genotypes. The effects require validation in a performance trial. The findings suggested cowpeas, either roasted or sprouted can substitute soybean oil cake in growing pig diets with minimal compromise to pig productivity. However, the observed genotype x diet interactions implied pigs either naturally or artificially acquired or lacked some metabolic or physiological functional traits to differently process dietary antinutrients or compensate for nutrient deficiencies in the cowpea diets. The pig genotype differentiated its utilisation of differently processed cowpeas observed in the present study, which justifies further investigation to guide producer choices in pig selection criteria, in relation to alternative legumes to soybean cake, and regarding methods for processing the legumes.

6.2. Recommendations and scope for future research

The following recommendations are based on the findings of the research:

- The *IVDMD* and quantitative TIA and chemical changes suggested opportunity for a longer sprouting period to improve the quality of the sprouts. Therefore, investigation of the effects of sprouting beyond four days is recommended, which could either confirm a minimum or indicate the optimum cowpea sprouting period.
- Genotype X diet interactions were observed for some parameters, which suggested unique adaptive traits among the experimental genotypes. These effects were supported by findings from the growth trial, but require further validation in larger, on-station, followed by on-farm performance trials.

- Cost-benefit analyses of replacing the conventional soybean with cowpea diets, and of roasting or sprouting the cowpeas is recommended across different farming systems in order to validate the viability of these interventions for long-term adoption.
- Subject to confirmation of both biological and economic efficiencies, farmers may be encouraged to grow more cowpeas, to provide local surpluses which can be used for feeding pigs.

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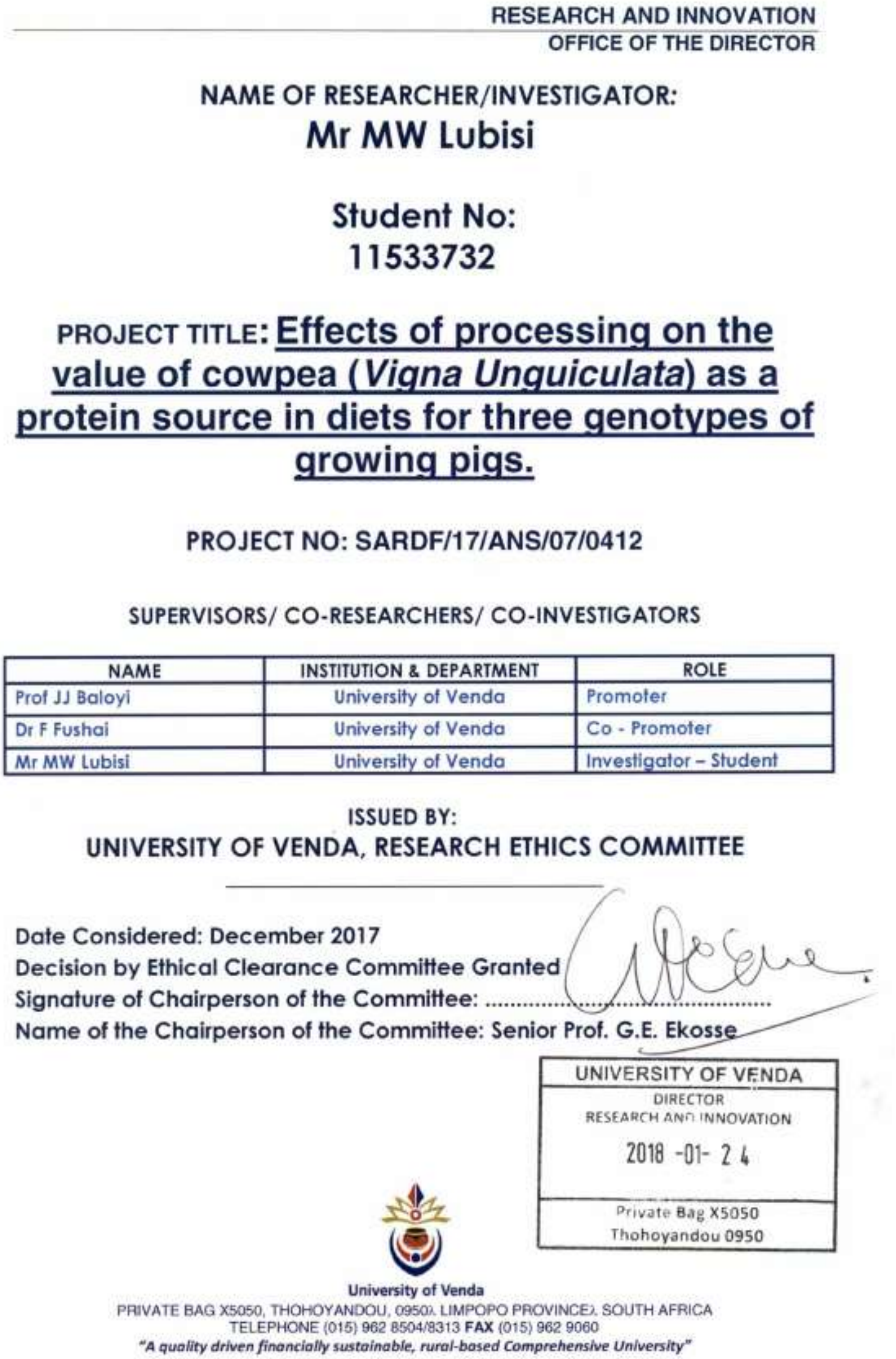
CHAPTER 7

APPENDIX



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NAME OF RESEARCHER/INVESTIGATOR:
Mr MW Lubisi

Student No:
11533732

PROJECT TITLE: Effects of processing on the value of cowpea (*Vigna Unguiculata*) as a protein source in diets for three genotypes of growing pigs.

PROJECT NO: SARDF/17/ANS/07/0412

SUPERVISORS/ CO-RESEARCHERS/ CO-INVESTIGATORS

NAME	INSTITUTION & DEPARTMENT	ROLE
Prof JJ Baloyi	University of Venda	Promoter
Dr F Fushai	University of Venda	Co - Promoter
Mr MW Lubisi	University of Venda	Investigator – Student

ISSUED BY:
UNIVERSITY OF VENDA, RESEARCH ETHICS COMMITTEE

Date Considered: December 2017
Decision by Ethical Clearance Committee Granted
Signature of Chairperson of the Committee: ....
Name of the Chairperson of the Committee: Senior Prof. G.E. Ekosse

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