Prediction of the nutritive value of South African maize hybrids for broiler chickens

By

Sibongiseni Siphesihle Mndenuphelele Nkabinde

Submitted in fulfillment of the requirements for the degree

Magister Scientiae Agriculture
MSc (Agric) Animal Science: Animal Nutrition

in the Faculty of Natural and Agricultural Sciences

Department of Animal and Wildlife Sciences

University of Pretoria

2023

Supervisor:

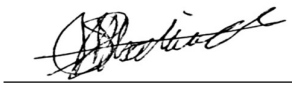
Dr. Christine Jansen van Rensburg

Co-supervisor:

Dr. Peter William Plumstead

Declaration

I, Sibongiseni Siphesihle Mndenuphelele Nkabinde, hereby declare that this thesis, submitted for the MSc (Agric) Animal Science: Animal Nutrition degree at the University of Pretoria, is my own work and has not previously been submitted by me for a degree at any other University.



Pretoria

22 March 2023

Acknowledgements

Thank you, Dr. Christine Jansen van Rensburg, for all your support and guidance and input as my supervisor, and your time and patience were greatly appreciated.

Thank you, Dr. Plumstead, for the guidance and teaching you have given to me throughout the project. Thank you for pushing me to achieve my goals and encouraging me to take them even further.

Thank you to Terry, Peter and the Chemuniqué team for the funding of my MSc. and support throughout the project especially the during the difficult covid lockdown period.

Thank you, Natasha Snyman, for teaching me invaluable life lessons and Monday morning motivation. Thank you for the lessons on workplace etiquette and knowledge on feed formulations as well as presentations.

Thank you, Wiana Louw and the team at SAGL for their knowledge, time, and maize samples.

Thank you, Ernest King, and the team at Nutri Feeds for the maize samples for digestibility, your help was very much appreciated.

Thank you, Sarla, for all the knowledge on sample handling and the NIRS.

Thank you, Ashona for helping with the Promatest and RSD 60 for all my maize samples.

I will always be grateful for the students who assisted me through the trial. Margot Crous, Sarah Harrison, Gareth Wilks, Mbuso Mbukwane, Kubheko Makalima and Gontse Kesaobaka Letlole. I will forever appreciate all the rough times you got me through.

Vernon Ndlovu, thank you for all your effort during my trial the mixing of my treatment diets with the extremely long and sometimes complex sampling days. Your knowledge and muscle power were very much needed and appreciated.

Thank you, Kyle Venter, for all the help and experience when it came to ethics, fetching maize samples, mixing feed, and setting up the trial. Thank you for helping with statistical analysis and interpretation. Your help has been greatly appreciated. You made managing this project a lot easier.

Thank you to Roelf Coertze for your assistance with the statistical analysis, your patience, kindness, and knowledge made the interpretation of the statistics easy and understandable.

Finally, thank you to my mother, brother, and friends for all their love and support during this degree.

Abstract

Maize is a major component of broiler feed and consequently contributes largely to dietary energy values. Previous research showed nitrogen-corrected apparent metabolisable energy (AME_N) of maize varied due to differences in proximate composition, as well as intrinsic kernel factors such as kernel hardness, density, size, and vitreousness. Most studies performed in the past used the total excreta collection method for the determination of AME_N. However, the utilisation of indigestible markers in the feed reduces the errors of the total collection method that occur due to incorrect measurement of feed intake and excreta output. Different markers/indicators have been included in feed such as chromic oxide, titanium dioxide or acid insoluble ash. The present study compared *in vivo* techniques to determine AME_N of European and South African maize samples and prediction of AME_N by using the Danisco Animal Nutrition near-infrared transmittance (NIT). The effects of maize AME_N on the energy uplift of a xylanase, amylase, and protease (XAP) enzyme combination were also investigated. For the first study, white (n = 471) and yellow (n = 639) maize samples from the 2015/2016 (n = 338) and 2016/2017 (n = 772) harvest seasons were collected from different regions in South Africa and analysed for moisture, crude protein, crude fat, milling index, grit yield all, AME_N, and hectolitre mass (L/kg) using near-infrared transmittance (NIT). The Pearson product moment correlation coefficient of all variables was determined using the multivariate analysis test. The relationship between AME_N and all parameters was analysed using the multiple regression model fit test. Factors were included in the final model using stepwise regression to reduce the Bayesian information criterion (BIC). This resulted in the following final model: AME_N = 3589.8 + 37.59 * crude fat + 9.76 * crude protein - 30.32 * moisture + 0.3 * milling index. The R^2 of this model was 0.89 with a root mean square error (RMSE) of 8.95 kcal/kg. For the second study, the treatments comprised of five maize samples using two methods to determine AME_N. For the total collection (TC) method, birds were fasted for 6-hours followed by a 6-hour feeding and an additional 12-hour fasting period. Excreta was collected during feeding and additional fasting periods. For the basal substitution (BS) method, birds were placed onto treatment diets 14 hours before the 3-day collection period. Excreta was collected every 24 hours. Results showed no significant effect of Method between TC and BS, or interaction of Method x NIT AME_N value of maize. This implies that BS can be used as an effective method to determine the AME_N value of maize. Significant differences were observed between NIT AME_N, and measured AME_N, regardless of the method. For the third study, the basal substitution method was used with/without the inclusion of enzyme. On average the XAP enzyme increased the AME_N value of maize by ~60

kcal/kg DM, with absolute effects of XAP on maize varying numerically between maize variants ($P>0.1$).

Table of Contents

List of Abbreviations

AACC	American Association of Cereal Chemists
AME	Apparent Metabolisable Energy
AME_N	Apparent Metabolisable Energy Nitrogen-corrected
AMG	Amyloglucosidase
AOAC	Association of Official Analytical Chemists
ATP	Adenosine Triphosphate
BIC	Bayesian Information Criterion
BS	Basal Substitution
BW	Body Weight
CP	Crude Protein
dCP	Digestible Crude Protein
DE	Digestible Energy
DF	Direct Feeding
dFAT	Digestible Fat
DM	Dry Matter
dNFE	Digestible Nitrogen-free Extract
EEL	Endogenous Energy Loss
EPI	European Production Index
ER	Energy Retention
FHP	Fasting state Heat Production
g	Gram
GE	Gross Energy
GIT	Gastorintestinal Tract
GLM	Generalized Linear Model
GN	Gross Nitrogen
GOPOD	Glucose Oxidase/Peroxidase Reagent
GYA	Grit Yield All
H_2SO_4	Sulfuric Acid
HI	Heat Increment
HP	Heat Production
IMS	Industrial Methylated Spirits
kcal	Kilocalorie

kg	Kilogram
L	Litre
ME	Metabolisable Energy
mg	Miligram
MI	Milling Index
MJ	Megajoule
mTZM	Modified Turbidimetric Zein Method
ND	Not Detectable
NE	Net Energy
NEm	Net Energy for Maintenance
NEp	Net Energy for Production
NER	Net Energy Retention
NFE	Nitrogen-free Extract
NIR	Near-infrared Spectroscopy
NIRS	Near-infrared Reflectance Spectroscopy
NIT	Near-infrared Transmittance
NOx	Nitrogen Oxides
NPU	Net Protein Utilisation
NRF	Net Energy Retention
NSPs	Non-starch Polysaccharides
O2	Opaque2
OMA	Official Methods of Analysis
PSI	Particle Size Index
QPM	Quality Protein Maize
RMSE	Root Square Mean Error
RS	Resistant Starch
RSD	Rate of Starch Digestion
RVA	Rapid Visco Analyser
SAGL	South African Grain Laboratory
SI	International System of Units
SSP	Salt-soluble Protein
TCA	Trichloroacetic Acid
TDF	Total Dietary Fibre
TiO_2	Titanium Dioxide

TME_N	True Metabolisable Energy
XAP	Xylanase Amylase Protease
XCT	X-ray Micro-computed Tomography
XU	Xylanase Units

List of Tables

List of Figures

Chapter 1
General introduction

1.1 Introduction

Maize (*Zea mays* L.) is the primary cereal grain used in South African (SA) broiler diets, contributing approximately 65% of the apparent metabolisable energy (AME_N) and up to 20% of dietary crude protein (CP) consumed by broilers (Cowieson, 2005). In commercial broiler operations, feed accounts for majority of the input costs and dietary energy represents up to three quarters of this cost (Van der Klis & Fledderus, 2007). Due to the large impact of maize on dietary AME_N, the ability to rapidly and accurately estimate the AME_N of maize used in feed formulation is of economic and nutritional importance (Latham *et al.*, 2016). For feed formulation, prediction equations have been developed that have correlated the determined AME_N value of maize fed to broilers with its chemical composition, including dry matter, crude protein, ash, fat, starch, and nitrogen free extract (Rostock, 2010; Brazilian Tables; 2011; CVB, 2012). Previous studies have demonstrated that the AME_N of maize varied due to variations in chemical composition as well as intrinsic kernel factors like kernel hardness, vitreousness, density, and size (Cowieson, 2005). The AME_N of maize is currently calculated by the SA feed industry using prediction equations, but these equations do not take into account variations in digestibility due to variations in kernel hardness or physiochemical structures (Blok et al., 2015), which may reduce the accuracy of these models to account for all observed variance in AME_N.

Compared to other cereals, maize is generally categorised as a highly digestible feed source with low levels of anti-nutritional factors, such as non-starch polysaccharides (NSPs) (Knudsen, 2014). However, contents of phytate phosphorus as a percentage of total phosphorus in cereals are highest for wheat and maize and lowest for oats and rye (Humer *et al*., 2015). Therefore, availability of calcium, trace minerals, energy and protein are possibly compromised by the presences of phytate phosphorous (Selle & Ravindran, 2007). Over the years, the preception that maize has a consistent nutritional value is changing as research has shown that the chemical composition of maize is widely variable from batch to batch (Cowieson, 2005). This implies that generic matrix values for maize are inaccurate (Cowieson, 2005). Studies, such as those conducted by Leeson *et al*. (1993), indicate a variation in energy of up to 500 kcal/kg between different batches of maize. It is essential to understand the factors influencing this variation in pursuit of improved feed formulation accuracy and reduced feed costs. Intrinsic kernel factors contributing to the nutritive value of maize include proximate composition (especially starch, protein, oil, and moisture content), starch-protein interactions, vitreousness, and cultivar. External factors, such as geographical location, harvest season, post-harvest treatment and

storage conditions, also have an impact. The addition of enzymes to maize-based diets to combat the anti-nutritional effects of phytate and NSPs has been shown to increase the AME_N of maize (Namkung & Leeson, 1999).

Metabolisable energy (ME) is the preferred energy system used for poultry in South Africa as it accounts for the energy lost in the form of urine and combustible gases, therefore, giving a better prediction of the energy available to the bird compared to digestible or gross energy (Moehn *et al*., 2005). Metabolisable energy can be expressed as apparent metabolisable energy (AME) and true metabolisable energy (TME), and the nitrogen corrected apparent and true metabolisable energy (AME_N and TME_N, respectively) (Choct, 2004). Despite global efforts made to standardise the most prevalently used AME assay, obtained energy values are widely variable due to the many external environmental and inherent factors affecting grain quality (Choct, 2012). Differences between studies and laboratories also contribute to this variation. The main approaches used by nutritionists in practice to evaluate the energy content of feedstuffs such as maize, are based on a) experimentally determined tabulated values, b) the input of nutrient values obtained from *in vitro* studies, wet chemistry analysis and/or near-infrared technology into evidence-based prediction equations, and c) *in vivo* research experiments (Mateos *et al*., 2015). However, using the AME system has drawbacks, such as poor feed conversion correlation and inaccurate growth rate prediction in broilers (Choct, 2004). There are also factors that contribute to the variation of AME of feedstuff such as feed processing, feed composition, level of feeding, nitrogen-retention, age, strain and species, and environment (Choct, 2004).

Due to the large variations in maize grain quality, the use of rapid, automated systems to determine grain nutrient content and quality is the most economical option to avoid inaccurate feed formulation (Williams *et al.*, 2009). Near-infrared transmittance (NIT) is a quick and accurate method to measure nutrient content with the added benefits that it requires no sample preparation, it is quick, accurate, produces no hazardous chemical waste, requires limited operator input on the final result and is therefore non-destructive, and can be used to analyse whole kernels, improving measurement repeatability and sample preservation (Nduwamungu *et al.*, 2010). It is an ideal piece of equipment to have at the intake offices of silos for quality control purposes where batches of ingredients arrive continuously. However, the predictive power of such technology is highly dependent on the accuracy of the models programmed into the technology, with a larger sample set ensuring better accuracy. Furthermore, it is important to continuously update models to include data from *in vivo* digestibility studies and wet chemistry analysis on a wide range of maize to improve the strength and accuracy of this system (Hruby, 2015).

Most studies performed in the past used the total excreta collection method for the determination of AME_N, however, different markers/indicators have been included in feed such as chromic oxide, titanium dioxide or acid insoluble ash. The use of markers reduces the errors that occur due to incorrect measurement of feed intake and excreta output (Smeets *et al.,* 2015). Titanium dioxide as a marker is generally favoured by researchers as its analysis is accurate, simple, and only a small sample size is required (Short *et al.,* 1996; Sales and Janssens, 2003). Sibbald *et al.* (1960) observed the standard error means of digestibility data were significantly less when a marker method (chromic oxide) was used than those for the data from a total collection method. (Siqueira *et al.,* 2010) observed a significant difference in the AME_N content of maize between total collection method and index method using celite, however, when the AME_N was corrected for recovery rate there was no significant difference observed. Smeet *et al.* (2015) observed a lower AME_N content of wheat when it was calculated using titanium dioxide compared to the total collection method, however, both methods showed the same trend even with different values. Smeets *et al.* (2015) concluded that the use of the marker method with titanium dioxide was the simplest option between the two methods.

1.2. Aim and objectives

The aim of this study was firstly to analyse which intrinsic kernel factors contribute significantly to the observed variance in AME_N and to determine if considering these factors will improve the accuracy of a model to predict the AME_N of maize. The second aim was to determine how accurately NIT technology can measure the AME_N value of various South African maize samples in comparison to *in vivo* AME_N measurements. The third aim was to determine absolute differences in AME_N of maize samples using the total collection method vs. the basal substitution method. The final aim was to determine the effects of maize AME_N on the energy uplift of an enzyme product combining xylanase, amylase, and protease (XAP), using the basal substitution method.

To achieve the four aims set out above, the following objectives were reached:

1. Maize samples were collected, and their AME_N and intrinsic kernel factors analysed. A model was developed using the data collected. The new model was compared to a previous model used to predict the AME_N of maize.
2. FIve maize samples were collected, and their AME_N predicted using NIT technology. The predicted AME_N values were compared to the AME_N values of the maize samples determined *in vivo*.

3. The AME_N values of five maize samples were determined using the total collection method and the basal substitution method. The values from both methods were compared and their differences determined.
4. The AME_N values of five maize samples were determined using the basal substitution method with or without the inclusion of an enzyme combination. The values from both methods were compared and their differences determined.

1.3. Hypotheses

The hypotheses for the current trial were as follows:

H0: Changes in the proximate composition and intrinsic kernel characteristics of maize do not contribute to the variation in AME_N value of maize.
H1: Changes in the proximate composition and intrinsic kernel characteristics of maize contribute to the variation in AME_N value of maize.

H0: NIT technology does not accurately measure the variation in the AME_N value of maize in comparison to *in vivo* obtained results.
H1: NIT technology accurately measures the variation in the AME_N value of maize in comparison to *in vivo* obtained results.

H0: There is not a significant difference in the value of AMEN of maize determined using the total collection method vs. the basal substitution method.
H1: There is a significant difference in the value of AMEN of maize determined using the total collection vs. the basal substitution method.

H0: Maize quality and starting AME_N of maize does not affect enzyme response.
H1: Maize quality and starting AME_N of maize does affect enzyme response.

Chapter 2
Literature Review

2.1. Introduction

Maize makes up the majority of the cereal grains in South African (SA) broiler diets, accounting for up to 65% of the apparent metabolisable energy (AME_N) and 20% of the dietary crude protein (CP) that the birds consume (Cowieson, 2005). The ability to quickly and accurately estimate the AME_N of maize used in feed formulation is of economic and nutritional importance because of the significant impact of maize on dietary AME_N (Latham *et al.,* 2016). Previous studies have demonstrated that the AME_N of maize varied due to variations in chemical composition as well as intrinsic kernel factors like kernel hardness, vitreousness, density, and size (Cowieson, 2005). The SA feed industry currently uses prediction equations that do not take into account variations in digestibility that may arise when calculating the AME_N of maize.

Total excreta collection method is the most frequently used *in vivo* method to determine metabolisable energy (ME) in broiler diets and it is based on measuring feed intake and total faecal excretion for a set period (Siqueira *et al.,* 2010). However, there are factors that interfere with the results from this method, including feed, feathers, intestinal mucosa sloughing (Siqueira *et al*., 2010), as well as loss of excreta during collection due to excreta attached to the wire flooring, and birds excreting away from the collection pans (McNab, 2000).

The marker method involves the inclusion of a marker to the experimental diet. This method determines the ME by the ratio of indigestible substances present in the diet and excreta. This is based on the idea that the total amount of an inert indicator substance that is excreted over time equals the total amount that was consumed during that time (Choct, 2004). Therefore, it is not necessary to measure feed intake and excreta (Vogtmann *et al*., 1975), making this method an attractive alternative.

Titanium dioxide and chromic oxide are commonly used as indigestible markers to determine excreta energy metabolisability and nutrient digestibility in poultry. However, studies comparing different markers as well as comparing the marker technique against the total collection method, are limited (Sales & Janssens, 2003).

2.2. Maize production and use in South Africa

The term maize coming from *Zea mays* is used synonymously with corn, especially in the Western countries (Ranum *et al.*, 2014). Globally, maize production contributes to approximately 35% of total cereal production (Scott & Emery, 2016). North America mostly produces yellow maize, whereas in other countries such Africa, Central America, and South America, white maize is preferred. Yellow maize has a higher concentration of β-carotene and β-cryptoxanthin, therefore, countries with a preference for white maize consume less of these vitamin A precursors (Ranum *et al.*, 2014). The largest producer of maize is the United States of America and controls the world maize trade, with approximately 15% of its production being exported (Ranum *et al.*, 2014).

The maize price has been inflated due to the demand of maize for the animal feed industry as one of the main feedstuffs in poultry and pig feed. At least 55% of the total world maize production is used as livestock feed, 14% for human consumption (Scott & Emery, 2015) and approximately 30% for fuel production (Ranum *et al.*, 2014). This has made maize less affordable for the poor consumers in a number of countries around the world (Shiferaw *et al.*, 2013). The use of higher quantities of fertiliser, water, and pesticides, accompanied by improved crop varieties has led to a two-fold increase in maize production over the past 40 years (Shiferaw *et al.*, 2013).

In 2017, approximately 1.134 billion tonnes of maize was produced throughout the world (FAO, 2019), however, maize production decreased to 1.091 billion tonnes in 2018 (SAGL, 2018). The United States, China and Brazil are the top three producers, with 370, 259, and 97 million tonnes, respectively, in 2017. South Africa ranks 11th with 16.82 million tonnes (FAO, 2019) but production decreased the following year to 12.510 million tonnes in 2018. However, this production value was still close to the previous 10-year average of 12.065 million (SAGL, 2018). The Free State, Mpumalanga and North West were the highest maize-producing provinces, contributing 81% of total production in 2018 (SAGL, 2018). South African - produced maize consists of approximately 48% yellow maize and 52% white maize.

2.3. Chemical composition of maize: Starch, protein, and fat

The pericarp, germ, and endosperm are the three fundamental morphological components of the maize kernel. The morphological component of the maize kernel that contains starch and protein is called the endosperm, which makes up 75–80% of the kernel. Starch comprises around 70% of the weight of the maize kernel on a dry matter basis and contains an estimated 60% of its AME_N (Cowieson, 2005). Zeins and glutelins represent most of the endosperm protein content. At maturity of the kernel, endosperm protein comprises of 50% - 60% zein proteins. Zeins are a varied mixture of

alcohol-soluble proteins, also known as prolamin, that are positively correlated with the vitreousness of the maize kernel. While the pericarp and germ account for 5% - 6% and 10 - 12% of the kernel weight, respectively (FAO, 1992). The pericarp has a high crude fibre content and consists of mainly hemicellulose, cellulose, and lignin. The endosperm contains high amounts of starch and most of the protein contained in the kernel (8% - 11%) and can be proportioned into hard (horny/vitreous) endosperm and soft (floury/opaque) endosperm (FAO, 1992). Most of the crude fat (33.2%) is contained in the germ together with a relatively high level of protein (18.4%) and minerals (FAO, 1992). The starch, crude fat, and crude protein components all contribute to the overall energy value of maize and any variations in these nutritional components will alter the AME_N value.

Table 2.1. Proximate chemical composition of the main components in a maize kernel (%) (Watson, 1987)

Chemical Component	**Pericarp**	**Endosperm**	**Germ**
Protein	3.7	8.0	18.4
Ether extract	1.0	0.8	33.2
Crude fibre	86.7	2.7	8.8
Ash	0.8	0.3	10.5
Starch	7.3	87.6	8.3
Sugar	0.34	0.62	10.8

The primary carbohydrate in maize is starch, the majority of which is in the endosperm. Maize starch contributes around 60% of the AME content in broiler diets. It is therefore important to understand its composition and nutritive value, which ultimately affects digestibility and energy utilisation (FAO, 1992). Simple sugars such as glucose, sucrose, and fructose are carbohydrates present at low levels of between 1% and 3% in the mature kernel. Sucrose is translocated to the endosperm as the primary source of carbon skeletons for starch synthesis and there is an increase in starch content while sugar content decreases as the kernel matures (Leeson and Summers, 1975). Starch is a semi-crystalline polymer of D-glucose bound by α-(1-4) and α-(1-6) glycosidic bonds (Cowieson, 2005). Two molecules found in starch, amylose and amylopectin, are polymers of D-glucose but differ due to the bonds forming between the glucose monomers. If a starch's amylose content is lower or higher than the typical range of 160-350 g/kg, it is categorized as waxy or amylo- (Cowieson, 2005). Starch granules in maize are practically round and the granules range in size between 2 - 30 µm (Tester *et al.*, 2004). Granule size is a significant factor in determining the starch energy value because smaller granules have a greater surface area and are therefore more likely to be

hydrolyzed by endogenous amylase (Cowieson, 2005). The amount of starch gelatinisation that occurs during thermal processing depends on the granule size, moisture content, amylose:amylopectin ratio, heat, and time (Tester *et al.,* 2004). Gelatinisation that occurs during the feed pelleting process is limited to a variable extent by moisture content (Tester *et al.*, 2004). Amylose and amylopectin form double-helical associations during retrogradation to which the extent is dependent on the amylose:amylopectin ratio (Cowieson, 2005).

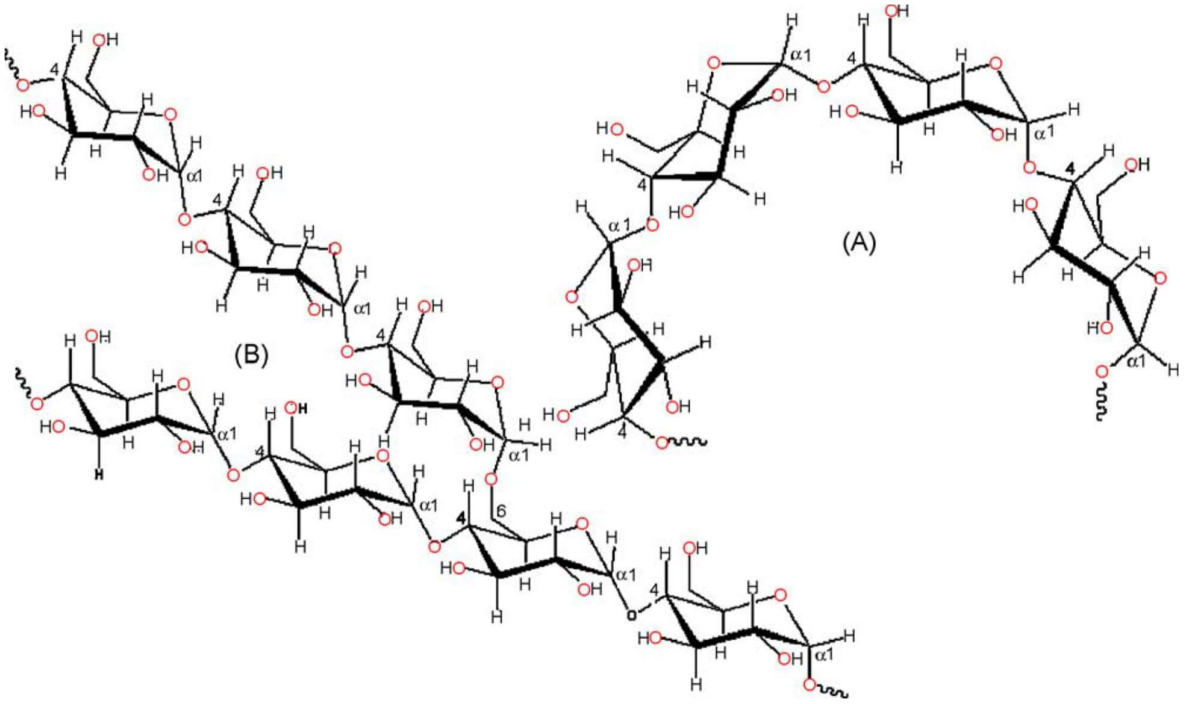


Figure. 2.1. Representation of the basic structure of amylose (A) and amylopectin (B) (Cowieson, 2005)

Resistant starch (RS) cannot be readily accessed by digestive enzymes, such as α-amylase and amyloglucosidase, and consequently hydrolysis is delayed (Jiang *et al.*, 2010). There are four types of resistant starch that may be broadly defined as follows:

- RS 1 represents the starch granules embedded in plant tissue. It resists digestion due to them being in a physically inaccessible form such as partially milled grains and seeds.
- RS 2 represents certain native starch granules and are particularly resistant to digestion compared with other structures.

- RS 3 represents the starch fraction that is most resistant and formed during the cooling process of gelatinised starch, therefore, producing retrograde amylose.
- RS 4 when novel chemical bonds are formed (other than α - (1 - 4) or α - (1 - 6)).

The structure and size of starch granules must be accounted for when determining feeding value of maize as smaller granules will have a relatively larger surface area, allowing greater potential for starch hydrolysis by endogenous amylase (Carre, 2004). Generally, softer endosperm has smaller starch granules resulting in a higher percentage of fine particles when milled (Fox and Manley, 2009) thereby allowing more hydrolysis and better gelatinisation. However, this can also lead to increased intestinal viscosity in broilers which can suppress nutrient digestibility and thereby reduce energy availability to the bird. In comparison, hard maize contains predominantly coarser particles when milled, resulting in slower hydrolysis and limited swelling of the starch granules (Almeida-Dominguez *et al*., 1997). This translates to lower intestinal viscosity but also incomplete nutrient digestion and absorption due to limited hydrolysis by amylase. Englyst *et al*. (1992) categorised starch according to differences in their rate of digestion as either rapidly digestible starch (RDS), slowly digestible starch (SDS) or resistant starch (RS).

Protein is the second largest chemical component in the maize kernel after starch, varying from 8% - 11% of kernel weight, and is found in the endosperm and germ (FAO, 1992). The relative concentration of protein is highest in the kernel germ. However, because the endosperm occupies more space, it contains the highest amount of protein in the kernel (FAO, 1992). There are four types of proteins present which can be differentiated by solubility: prolamins (zeins), glutelins, albumins and globulins (FAO, 1992). The zein proteins can be subdivided into four types: alpha (α), beta (β), gamma (γ), and delta (δ). Prolamins and glutelins are endosperm-specific proteins and make up about 52% and 30% of kernel nitrogen, respectively, while albumins, globulins and nonproteins contribute 7%, 5% and 6% to total kernel nitrogen content respectively (FAO, 1992).

Maize fat content has been genetically controlled for many years and is found mainly in the maize kernel germ. fat content varies from 3% - 18%, depending on the cultivar. Maize fat contains low levels of saturated fatty acids and higher levels of unsaturated fatty acids (FAO, 1992). According to estimates from Summers (2001), maize's available energy content increases by about 4.5 kcal/kg for every 0.1% increase in fat content. For poultry, lipids are a key source of energy, essential fatty acids, and fat-soluble vitamins. The fatty acid composition and the proportion of saturated to unsaturated fatty acids in the dietary lipid fraction have an impact on the digestibility and AME_N values of fats in broiler diets (Ketels & De Groote, 1989). Generally, maize which is more vitreous has a higher

proportion of poly-unsaturated fatty acids (PUFA), specifically linoleic and linolenic acid, while floury endosperm contains less PUFA but higher proportions of saturated and mono - unsaturated fatty acids (Gayral *et al*., 2015). Lysophospholipids (LPL) and free fatty acids (FFA) are contained in the starch of cereal grains as integral lipids and are positively correlated with the amylose fraction of starch (Tester *et al*., 2004). In maize with high amylose content, LPL can account for about 2% of starch weight.

2.4. Factors influencing the variability of maize

2.4.1. Variation and distribution of zein protein

Zein proteins can be further subdivided into α -, β -, γ -, and σ - zein. Zeins and glutelins represent most of the endosperm protein content. At maturity of the maize kernel, endosperm protein comprises of 50 - 60% zein proteins (Monjardino *et al.*, 2005; Gayral *et al.*, 2016). Zeins are a varied mixture of alcohol-soluble proteins, also known as prolamin (Esen, 2008), that are positively correlated with the vitreousness of the maize kernel (Wu *et al.*, 2010). Zein protein contains a high concentration of the amino acid proline. Proline has a highly hydrophobic nature making zeins predominately water insoluble. Zein proteins are distributed to different parts of the kernel; α- and σ-zeins are predominantly stored in the centre of protein bodies of the endosperm, and γ - and β - zeins are deposited in peripheral region (Wu *et al.*, 2010). Zein is found in the endosperm with starch, encapsulating it to form a starch matrix (Larson & Hoffman, 2008). High moisture, floury, and opaque maize contains low levels of zein proteins compared to those of flint and dent maize (Larson & Hoffman, 2008). However, high levels of zeins results in poor protein quality in maize as they are deficient in lysine and tryptophan (Wu *et al.*, 2010).

2.4.2. Vitreousness, kernel hardness and kernel density

There are two types of endosperm, one is of higher density, harder, more translucent, called the vitreous endosperm, located on the outside of the kernel. The other is of lower density, softer, mealy textured, called the floury endosperm, located in the centre of the kernel (Guelpa *et al.,* 2015; Gayral *et al.,* 2016). Maize can be categorised into five classes by kernel hardness that range by decreasing vitreousness: flint, pop maize, flour, dent, and sweet (Kaczmarek *et al.,* 2013). Vitreousness and kernel hardness play an important role in the processing of maize as different industries or milling processes require different hardness levels. Dry milling requires hard maize with large kernels and with pericarps and germs that are easy to remove during processing. Softer maize is required for wet

milling as this usually requires less steeping and leads to a higher starch-protein division. Different physical characteristics of maize that have been linked to hardness as well as influencing processing are density, weight, kernel size and shape, resistance to grinding or to abrasion and percentage of coarse vs fine material after grinding and sieving (Blandino *et al.*, 2010). Vitreousness may be influenced by γ - zein and α - zeins; with less α - zeins there are more water - soluble proteins being found in the floury endosperm of normal opaque - 2 and floury - 2 cultivars (Mestres & Matencio, 1996). Correa *et al.* (2002) discovered increased vitreousness in m aize caused a linear decrease in the availability of starch. Endosperm protein composition and distribution determines vitreousness. Maize that contains more horny endosperm is more vitreous caused by older protein bodies and increased protein synthesis (Gehring *et al.*, 2013). Baidoo *et al.* (1991) observed a decrease in crude fibre, crude protein, and ash contents including an increase in starch content with an increase in kernel density.

Kernel hardness is mainly due to genetic expression, but transportation, drying, storage and environment may also play a role in hardness properties. Kernel density, bulk density, breakage sensitivity caused by drying, storage, handling and processing are all related to kernel hardness (Williams *et al.*, 2009). Hard kernel have a higher percentage of glossy endosperm; soft kernels have a higher percentage of floury endosperm while intermediate kernels have an even distribution of glossy and floury endosperm (Williams *et al.*, 2009). Kernel density is a sign of hardness and maturity (Tilley, 1998). This is positively correlated to starch content and negatively correlated to crude fibre, crude protein, and ash content (Baidoo *et al.*, 1991).

2.4.3. Phytate

Phosphorous is an important mineral for bone mineralisation and metabolic processes (Menezes-blackburn *et al.*, 2015). Phytate (myo - inositol - 1,2,3,4,5,6 - hexakisphosphate) phosphorus is the mixed salt of phytic acid found in plant-sourced ingredients and constitutes approximately 60% of the total phosphorus. In complete diets that include Ca, phytate is predominantly unavailable to monogastric animals (Selle & Ravindran, 2007; Gehring *et al.*, 2013). Disturbance of absorption of nutrients in the gastrointestinal tract (GIT) is aided by the interference of endogenous enzymatic activity, chelation of minerals, and sodium imbalance (Gehring *et al.*, 2013). Mono- and divalent mineral cations are readily chelated by phytate due its polyanionic nature at gastric pH, thus, protein and starch digestibility is decreased (Gehring *et al.*, 2013). It has been suggested that phytate can bind directly or indirectly to starch and even has the ability to inhibit α-amylase activity. Starch can be bound directly through hydrogen bond formation or indirectly through proteins associated with starch (Humer *et al.,* 2015). Thompson *et al*. (1987) found a negative effect of phytate on starch digestion in

humans *in vitro* and a reduced blood glucose response *in vivo*, with the addition of Ca having a disadvantageous effect. The authors hypothesised that phytic acid may interact with the enzyme amylase or with a protein closely associated with starch. Phytic acid should also be structurally capable of binding starch through phosphate bonds and should directly reduce starch digestion. In addition, since Ca is required for amylase activity, its complexation by phytate can result in decreased enzyme activity. Conversely, Ca addition can react with phytic acid, making it less available for amylase binding (Humer *et al.,* 2015). Absorption of calcium, trace minerals, energy and protein is possibly compromised by the presences of phytate phosphorous (Selle & Ravindran, 2007). According to Humer *et al.* (2015), phytate reduces protein/amino acid (AA) utilization by forming complexes that alter protein structure and, as a result, decrease protein solubility, enzymatic activity, and proteolytic digestibility. Because phytic acid can react directly with charged protein groups or indirectly with negatively charged protein groups of proteins via a mineral cation, it can form complexes with proteins at both low and high pH. Complex formation occurs at low pH (around 2.8) via binary phytate-protein complexes. Ternary complexes (bonds via a cationic bridge, mediated for example by Ca, Mg, or Zn) form at relatively alkaline pH (around 8.4) (Humer *et al*., 2015). Under acidic conditions, where the pH is below the isoelectric point of the protein, the polyanionic phytate molecule is negatively charged and has the ability to form strong electrostatic bonds with the cationic group of the basic lysine, arginine, and histidine residues (Humer *et al.,* 2015).

Contents of phytate phosphorus as a percentage of total phosphorus in cereals are highest for wheat and maize and lowest for oats and rye (Humer *et al.*, 2015). From approximately 3 weeks post pollination, the synthesis of phytate begins and continues with maturity (Gehring *et al.*, 2013). The availability of phytate phosphorus as well as phytases are predominately associated with the outer layers of most cereal grains: however, the distribution differs between cereals (Humer *et al.*, 2015). Phytate is predominately found in the germ of maize with more than 85% present in the germ (Gehring *et al.*, 2013). The content of phytate phosphorus varies between varieties of maize, the variation can be from 1.9 - 3.5 g/kg phytate phosphorous (Kasim & Jr, 2000) and the phytate content in poultry diets is 2.5 - 4.0 g/kg phytate phosphorous (Selle & Ravindran, 2007). Classen *et al.* (2010) analysed 200 maize samples and found that phytate concentrations ranged from 0.2 - 0.9% with a mean of 0.6%. Protein and starch digestibility are decreased by phytate concentration in maize and the severity is increased by other factors affecting nutrient utilization in maize.

2.4.4. Harvest season, drying temperature and storage

Harvest season and geography

Optimum harvest time is dependent on the weather conditions and geographical location of a certain harvest season. Additionally, harvest time will also determine the yield and quality of maize (Gaile, 2008). A study by Gaile (2008) on maize production in Latvia, showed that there can be large differences in maize yield between harvest years (e.g., 14.17 t/ha in 2005 vs. 12.11 t/ha in 2006). This variation in yield was caused by dry weather conditions in Lativa in 2006. Protein solubility and successive processes may be affected by the time of harvest, as early harvested or immature maize has a higher moisture content but a lower fraction of zein and glutelin proteins compared to mature maize (Gehring *et al.*, 2013). Early harvested maize is heat sensitive and contains a higher proportion of reducing sugar content compared with mature maize (Gehring *et al.*, 2013). Other than growing region, fertilisation and soil conditions are also important agronomic characteristics determining crop quality. High temperature within a region may cause heat stress during the lag phase. The lag phase is characterised by rapid growth but very little dry matter (DM) accumulation (Outtar *et al.,* 1987), therefore, heat stress during this phase leads to the reduction of cell division and the amount of endosperm cells and starch granules. The accumulation of protein is less affected by heat and water stress compared to starch metabolism (Monjardino *et al.*, 2005).

Soil nutrient profile, available moisture and environmental conditions before planting and during the growing stages of maize can influence starch and protein content (Fox & Manley, 2009), which in turn will affect kernel hardness and vitreousness. Increased use of nitrogenous fertilisers leads to an increase in final protein content and increased hardness (Fox & Manley, 2009).

Drying temperature

Wet grain is harvested in most countries with a moisture content of 25 - 36% or higher. The maize is sun-dried or artificially dried using dryers that use air heated to 50˚C to 130˚C (Odjo *et al.*, 2015). Solubility and molecular structure of protein is changed due to thermal denaturation by improper drying methods, resulting in decreased availability of amino acids and the impairment of water and α-amylase to the starch granules (Gehring *et al.*, 2013). Kaczmarek *et al.* (2013) observed decreased protein solubility and increased resistant starch concentration in maize when the drying temperature was increased from 60˚C to 140˚C. In addition, ileal digestibility of protein decreased but no interaction was found between hardness and drying temperature for resistant starch or protein solubility. AME_N is reduced by increased drying temperature (Kaczmarek *et al.*, 2013). It is widely accepted that maize dried aggressively at high temperatures, especially maize harvested with a high moisture content will have a lower nutritional value (Kaczmarek *et al.*, 2007) due to reduced bioavailability of nutrients such as starch, protein and oil. This results in poor broiler performance and economic losses. In a study conducted by Kaczmarek *et al.* (2007), maize was dried at 80°C, 120°C

and 140°C to investigate the influence of drying temperature on broiler performance. Results of the study suggested that higher drying temperatures negatively affected body weight gain, feed conversion ratio (FCR) and AME values. Heating may modify the structure of certain components in the maize kernel, which collectively reduces its nutritive value. This refers to retrogradation of starch, denaturation of heat labile vitamins, damage of protein due to interactions with reducing sugars during the Maillard reaction (Kaczmarek *et al.,* 2007) and inaccessibility of oil due to its interactions with non-salt soluble proteins (Gehring *et al.,* 2012). Therefore, reduced protein and starch solubility negatively affect energy utilisation. Starch digestion is dependent on the degree of enzymatic hydrolysis of starch, which is enhanced by gelatinisation and inhibited by retrogradation. Gelatinisation theoretically occurs at the start of the drying process when there is high temperature and kernel moisture content is high, while retrogradation could occur during cooling at the end of drying (Odjo *et al.,* 2015).

Storage

The impact of storage time and conditions on maize chemical composition and nutritive value has been extensively studied. Bartov (1996) reported that maize stored under good conditions can be stored for at least up to 110 months and the value of AME_N was not affected by storage duration. A decrease in fat concentration can be observed when maize is stored with 15% moisture content (Bartov, 1996).

To prevent microbial growth and decay of maize, storage temperature of 12 - 20˚C and moisture of 14% is recommended. Unfavourable conditions create the perfect breeding ground for fungi on the maize kernels, with a resultant contamination with mycotoxins (Nuss & Tanumihardjo, 2010).

According to Yin *et al.* (2017), maize should not be utilised in broiler feed if it has been stored for four years or more. This was concluded after conducting a study to determine the effect of different storage times, ranging from two to five years, on chemical composition, broiler body weight gain (BWG), FCR and European production index (EPI). Starch, crude protein, amino acids, fatty acids, and test weight were discovered to generally decrease with longer storage times. When maize was stored for more than four years, there were decreased catalase and peroxidase activities as well as an increase in fat acidity. It was found that BWG and EPI decreased from 0 to 6 weeks of broiler age, while FCR increased with storage time. Broiler performance, FCR and EPI seemed to be positively correlated with catalase activity and negatively correlated with fat acidity (Yin *et al.,* 2017). However, while the study noted a general deterioration in maize quality, it was concluded that storage time had no significant effect on the AME_N value of maize.

2.4.5. Processing: grinding and pelleting

Amerah *et al.* (2007) reported pelleting of maize resulted in better feed intake, feed efficiency and weight gain by broilers. Better starch digestibility brought by the chemical changes during pelleting, increased intake of nutrients, changes in physical form, decreased feed wastage and energy used for ingestion. Pelleting of feed reduces the effect of particle size on broiler performance. Both fine and coarse grinding of maize improves broiler performance, however, no differences were seen in feed intake. Grinding reduces particle size that leads to a changes in structure and surface area of particles, in addition to, chemical changes (Shi *et al.*, 2016). An increase in surface area when maize is ground, makes for better enzymatic or bacterial activity (Kilburn & Edwards, 2001). However, grinding of maize may cause damage to starch by the negative change in granular structure (Shi *et al.*, 2016). Better performance is seen in broiler chickens fed pelleted diets compared to mash due to better bone ash and higher metabolisable energy value (Kilburn & Edwards, 2001). However, pelleting can result in poor broiler performance when the incorrect temperature is used during the steam-conditioning process (Abdollahi, 2011). High conditioning temperatures could induce negative effects such as reduced energy availability through the following ways: retrogradation of starch to form enzyme-resistant starch, increased intestinal viscosity due to solubilisation of NSPs and losses in lysine and arginine due to Maillard reactions in which they form complexes with sugars (Cutlip *et al.*, 2008; Amerah *et al.*, 2013). Kilburn & Edwards (2001) observed an increased incidence of rickets and decreased tibia ash of a significant level when poultry diets were pelleted. Douglas *et al.* (1990) reported better performance but no significant difference in feed intake of broiler chickens on a pelleted diet. Douglas *et al.* (1990) suggested that the difference in performance but not feed intake was possibly because of better nutrient availability, feed conversion and less feed wastage.

2.4.6. Cultivar

Genetically modified maize cultivars have been developed over the years for different purposes. These cultivars have inbred qualities such as drought-resistance, increased yield and resistance to pests and diseases that results in their wide use in South Africa. Protein, starch and fibre levels as well as nutrient digestibility and metabolisable energy may differ between cultivars (Augustyn *et al.*, 2016).

Eyherabide *et al*. (2004) assessed genotype by environment interactions on maize hardness and yield using hybrid cultivars. It was found that hybrids with lower yields across different locations had harder endosperms than those with higher yields, and when considering each hybrid, endosperm

hardness increased as environmental conditions for grain yield improved. Numerous maize mutants with high lysine content that are floury or opaque mutants have been developed but showed poor agronomical attributes. However, opaque/floury mutants were developed based on the biosynthesis of storage proteins and their control; secondly, quality protein maize (QPM) was developed to restore vitreousness of the endosperm while maintaining a good level of lysine. The Opaque2 (O2) lines were used to develop the QPM lines (Krivanek *et al.*, 2007; Gayral *et al.*, 2016). Yellow maize was produced through biofortification of maize with provitamin A carotenoids that changed the colour of maize from white to yellow-orange as well as the aroma and flavour of the maize (Pillay *et al.*, 2011).

2.4.7. Moisture content

Moisture content is an important characteristic to consider in the grain grading system as it influences mould growth, infestation by insects and the monetary value of the grain (Singh *et al.*, 2006). Moisture content is inversely correlated to bulk density and positively correlated to both true density and porosity (Seifi & Alimardani, 2010). Starch gelatinisation during thermal processing is dependent on moisture content among other factors and may be limited by higher moisture content (Cowieson, 2004). Barnwal *et al.* (2012) reported a decrease in kernel density with an increase in moisture content ranging from 12.8 - 29.0%. Moisture content is inversely correlated to bulk density and positively correlated to both true density and porosity (Seifi & Alimardani, 2010). Starch gelatinisation during thermal processing is dependent on moisture content among other factors and may be limited by higher moisture content (Cowieson, 2004). Post-harvest drying of maize is important to conserve nutritional value as many countries harvest maize at high moisture contents varying between 25% and 36%, sometimes even higher (Odjo *et al.,* 2015).

The formation of resistant starch is linked to retrogradation (Odjo *et al.,* 2015) and is amplified when initial moisture content of the maize is higher. Furthermore, storing maize that was harvested with high moisture content prior to drying can lead to the partial degradation of carbohydrates (e.g., starches), increasing the concentration of reducing sugars in the maize, which will drive the production of Maillard products during the drying process (Kaczmarek *et al.,* 2007).

2.4.8. Kernel maturity

Kernel maturity at harvest has a major impact on the proximate composition of maize, therefore, it is important to take both climatic conditions and crop maturity into consideration when evaluating the nutritive value of maize. Maize harvested earlier contains higher proportions of reducing sugars

and a lower proportion of starch in comparison to 'mature' maize (Leeson & Summers, 1975). Immature maize contains higher levels of crude protein and a lower proportion of zein proteins in comparison to mature maize (Leeson & Summers, 1975). Theoretically, immature maize therefore has higher protein quality, however, studies suggest feeding quality of maize protein is unaffected by stage of maturity at harvest (Leeson & Summers, 1975). Furthermore, with increasing maturity in maize, kernel vitreousness and density increases resulting in reduced starch digestibility (Correa *et al.*, 2002).

2.4.9. Exogenous enzymes

The GIT of broilers lacks the necessary enzymes to completely digest the nutrients of some ingredients and the inclusion of exogenous enzymes is practiced to improve access to nutrients otherwise unavailable. The use of exogenous enzymes such as xylanase, amylase and protease to manage the variability of maize and improve nutrient digestibility are becoming increasingly common in maize-soy based broiler diets (Cowieson, 2010).

Xylanase (X) degrades non-starch polysaccharides (NSPs) in feed, including soluble and insoluble arabinoxylans, lowering digesta viscosity in viscous grains. Amylase (A) improves starch digestibility and increases hydrolysis, complementing the secretion of endogenous amylases. Protease (P) increases protein digestibility by hydrolyzing storage and structural proteins, as well as disrupting protein interactions with starch and fiber in the diet. XAP is the commercial name for the combination of these enzymes (Coweison, 2005).

Another exogenous enzyme used frequently in the poultry industry to breakdown the substrate phytate or phytic acid is called phytase. The availability of phosphorus in the feed increases with the addition of phytase to poultry diets, and nutrients like calcium, sodium, and amino acids may also increase (Cowieson, 2005).

A study was conducted by Tang *et al.* (2014) to determine the effect of two exogenous enzyme combinations and four different maize sources on the growth performance and nutrient digestibility of broilers. Twelve diets were formulated based on four maize sources (sources based on rainfall: sufficient, medium, medium, and drought) and with or without the addition of combination enzyme A or B. Both enzyme A and B were XAP. Supplementation of enzyme A and B improved BWG by 8.6% and 4.9%, respectively, and decreased FCR by 5.0% and 1.9%, respectively. Diets containing either of the enzyme combinations had significantly improved ileal protein and energy digestibility

compared to the group without an enzyme. The addition of enzyme A or B improved AME and AME_N; furthermore, nitrogen retention, AME and AME_N were influenced by the interaction of maize source x enzyme. Amerah *et al.* (2017) observed significantly improved starch, nitrogen, and gross energy digestibility. A significant improvement was also observed for AME_N of ~58 (kcal/kg DM) between the negative control (NC) and XAP treatment diet.

2.5. Variability of AME_N of maize fed to broilers

It has been shown by several studies that ME values vary significantly not only among different feed ingredients, but also among different batches of the same feed ingredient within the same region. When formulating feed using tabulated values, it can result in the variation of ME of more than 50 kcal/kg between batches of complete feeds (Jiang, 2010). The excessive or insufficient feeding of dietary energy due to inaccurate diet formulation has huge economic implications and reduces profitability due to poor broiler performance, carcass quality and increased feed costs. Although regression equations can be used to predict ME based on proximate analyses, the accuracy of these prediction equations is questionable. In most instances, a limited number of maize samples, usually from a certain region in the world, were analysed and used to derive these equations. Thus, it is clear that performing biological assays to validate regression equations is necessary (Jiang, 2010).

A study conducted by Carpenter & Clegg (1956) on metabolisable energy values of common poultry feedstuffs stated that the energy value of feedstuffs is not dictated by a single chemical component. Therefore, estimated energy values obtained from the application of formulas based on proximate analysis of the ingredient must be correlated to experimentally (*in vivo*) determined energy values. It investigated how protein, fat, starch, polysaccharides, and sugar contents could be used to predict experimentally determined energy values of certain ingredients. A total of seventeen samples, including cereals, cereal by-products, and mixed feeds, were analysed for proximate composition, as well as starch and sugar content. The metabolisable energy values of the samples were determined via total excreta collection of laying hens. ME values of the different samples ranged from 1.320 to 3.500 kcal/kg and there was a standard deviation of about 60 kcal/kg between individual ME values for the same ration. It was concluded that starch and sugars in the nitrogen-free extract (NFE) fraction are major contributors to the energy value of mixed poultry diets.

Leeson & Summers (1975) investigated the reduced yield of the 1974 Ontario maize crop due to poor growing and harvesting conditions. It was suspected that the crop had lower than normal energy levels as there were reports of increased feed to weight gain ratios in broilers. A total of seventeen

maize samples from this area were dried to a moisture content of approximately 15% prior to determining the ME and net protein utilisation (NPU) and proximate analysis. The total collection method was used to determine ME values which were corrected to zero nitrogen retention. The average ME value of the samples was recorded as 3.219 kcal/kg, which was 3% below the norm. However, the ME values ranged from 3.051 kcal/kg to 3.410 kcal/kg among the samples, indicating significant variations. It was concluded that differences were due to the effect of adverse growing conditions on maturity at harvest and may not be applicable for varietal effects of mature samples grown under more ideal conditions. As a result, variations in maize maturity at harvest are correlated with sugar, starch, and maize composition. The "immature" samples had lower ME values, lower starch proportions, and higher sugar proportions when compared to the mature samples. It was discovered that crude protein, starch, sugar, pigment score, and bushel weight were all related to ME levels. The expected changes in maize ME with unit changes in the various composition parameters are shown by the study's multiple linear regression equations.

Leeson *et al.* (1977) conducted a follow up study on the 1974 maize crop to determine the energy content of three different maize hybrids of known moisture levels representing a range of kernel maturity. The ME content of twelve samples (four for each type of hybrid) was determined by the total collection method and correlation coefficients were calculated, relating ME values to bushel weight, 100 kernel weight and moisture content at harvest. ME values of the three hybrids ranged between 2.972 kcal/kg and 3.344 kcal/kg. The conclusions confirmed previous assumptions that immature maize provides less ME for poultry in comparison to mature maize. Regression analysis revealed that for every 1% increase in moisture at harvest, the ME value of immature maize drops by about 12 kcal/kg. It was proposed that measurements of kernel moisture at harvest, bushel weight, and 100 kernel weight, rather than conventional proximate analyses, provide a better indication of changes in maize energy value with maturity. When immature maize must be used in broiler feed due to poor harvesting conditions, formulations should be adjusted (Leeson *et al.,* 1977).

In a later study, Leeson *et al.* (1993) investigated the apparent reduction in yield and feeding value of the 1992 maize crop of Eastern Canada and the northern states of the USA. These areas experienced adverse harvesting conditions due to unusually cool and wet weather. Harsh drying conditions were required due to the high moisture content of the crop. The study involved the proximate analysis and ME determination of twenty-six maize samples varying in bushel weight. Majority of the samples came from the same region. The total collection method was used for ME determination and, although the mean analysed ME value was about 3% less than the common standard value, the ME values were surprisingly high considering the harsh growing, harvesting, and

drying conditions. The ME values of the maize samples ranged from 2,926 kcal to 3.473 kcal/kg with an average of 3.218 kcal/kg. The calculated standard deviation was 162 kcal/kg, indicating large variations in ME values of the same maize crop. According to the study's findings (Leeson *et al.,* 1993), there is no correlation between ME and bushel weights or between ME and any other chemical estimates of maize's nutritional value. However, it was determined that low bushel weight is not a reliable indicator of the nutritional value of maize because it is correlated with low crude protein but not with the availability of any specific amino acid.

Gehring *et al.* (2012) conducted an experiment to evaluate maize quality both *in vitro* and *in* vivo and determine relationships between salt - soluble protein content (SSP) and vitreousness with nutrient as well as energy digestibility. This was in response to findings by authors such as Leeson *et al.* (1993) indicating that grading systems used to evaluate maize, such as bushel weight, are poor estimators of feeding value as they do not account for inherent variation in nutrient content and digestibility. The study analysed twelve maize samples for proximate composition followed by quality evaluation based on SSP and vitreousness. Thereafter, six of the twelve samples that varied in predicted quality but had similar proximate compositions were fed to broilers and the apparent ileal digestibility of nitrogen, starch, crude fat were determined, as well as AME_N via excreta analysis. AME_N ranged from 3.262 to 3.342 kcal/kg and was correlated with SSP and apparent ileal digestibility. The authors concluded that maize sources with a similar proximate composition may vary in their digestible energy content but, because protein solubility is related to energy utilisation, SSP can be used as a tool to differentiate between maize sources of wide - ranging AME_N values.

Other literature providing evidence of the variable energy values of maize include a study by Sibbald and Slinger (1962), which reported that the ME values from 11 yellow maize samples ranged from 1.580 – 1.800 Cal/lb. Cowieson (2005) reported on the variations in the chemical composition of maize and placed emphasis on the effect of starch, protein and anti-nutritional factors on digestibility and the AME content of poultry diets. The study concluded that the variations in the chemical composition of maize from batch-to-batch result in significant variations in energy availability to poultry and that the use of exogenous enzymes, such as xylanase, amylase, protease and phytase could assist in alleviating the problem. Latham *et al.* (2016) investigated the effect of maize AME value on broiler performance when associated with geographical location, xylanase inclusion and formulation. Broiler performance was compared when broilers were fed diets formulated using predicted AME values of maize from different regions based on NIR calibrations, and when fed diets formulated using the NRC (1994) AME value for maize. These diets were also tested with and without the addition of xylanase. The maize used in the experiment had an AME variation of 105 kcal/kg from the highest to

the lowest quality maize. Differences in growth performance were observed during the study to support the theory that using accurate AME values in formulations will assist in equalising broiler performance when feed ingredients vary in nutrient content.

2.6. *In vivo* determination of AME

AME is determined *in vivo* using the total excreta collection method. Total excreta collection method is the most frequently used method to determine ME in broiler diets and it is based on measuring feed intake and total faecal excretion for a determined period of time (Dourado *et al.*, 2010). However, there are factors that interfere with the results from this method that are feed, feathers, and intestinal mucosa sloughing, thus, limiting the accuracy of the method (Dourado *et al.*, 2010).

The following equations can be used to calculate AME and AME_N when the total excreta collection method is used:

$$AME\ /\ g\ feed = \frac{(GE\ feed\ x\ Feed\ consumed(g)) - (GE\ excreta\ x\ Excreta(g))}{(Feed\ consumed(g))} \quad (1)$$

$$AME_N/g\ feed = \frac{((GE\ feed\ x\ Feed\ consumed(g)) - (GE\ excreta\ x\ Excreta(g))) - (NR\ x\ K)}{Feed\ consumed(g)} \quad (2)$$

Where: 'GE feed' = gross energy content per gram of feed; 'GE excreta' = gross energy content per gram of excreta output; 'NR' = (feed intake(g) x N/g of feed(g)) – (excreta(g) x N/g of excreta(g)) and 'K' is a constant (either 8.22 kcal/g or 34.4 kJ/g as proposed by Hill and Anderson (1958)).

A marker is added to the experimental diet as part of the marker method. According to the theory that the total amount of inert indicator substance excreted equals the total amount ingested over a specific time period, this method calculates the ME by comparing the ratio of indigestible substances present in the diet and excreta (Choct, 2004). Therefore, there is no requirement for the measurement of feed intake and excreta (Vogtmann *et al*., 1974). For the marker to meet all necessary requirements it has to be a known substance, non-toxic, remain unchanged during the movement along the gastro-intestinal tract, have no physiological effect on the animal, have no interaction with other substances, be easy to analyse and be recovered completely in the excreta (Dourado *et al.*, 2010).

Most studies performed in the past used the total excreta collection method for the determination of AME_N, however, the use of markers reduces the errors that occur due to incorrect measurement of feed intake and excreta output (Smeets *et al*., 2015). The advantages of using a marker is that it is accurate, simple, and only a small sample size is required (Short *et al*., 1996; Sales & Janssens, 2003). Sibbald *et al*. (1960) observed the standard error means of the marker (chromic oxide) were significantly less than those for the data from total collection. Dourado *et al*. (2010) observed a significant difference in the AME_N content of maize between total collection method and index method using celite, however, when the AME_N was corrected for recovery rate there was no significant difference observed. Smeet *et al*. (2015) observed a lower AME_N content of wheat when it was calculated using titanium dioxide compared to the total collection method, however, both methods showed the same trend even with the different values.

The following formulas were adapted from Sibbald *et al*. (1960) to determine ME/g of feed and test ingredient:

$$ME/(g).\,feed = GE/(g).\,feed - \left(\frac{marker/(g)feed}{(marker/(g)excreta} x\ GE/(g)excreta\right)\ (1)$$

$$ME_N/(g).feed =$$

$$ME/(g).feed - (GN/(g).feed - (\frac{marker/(g).feed}{marker/(g).excreta}\ x\ GN/(g).excreta))\ x\ 8.22$$

$$ME/(g).\,test\ ingredient = \frac{(M.E./(g)test\ diet - (M.E/(g)basal\ diet\ x \frac{\%\ basal\ in\ test\ diet}{100}))}{\%\ test\ material\ in\ test\ diet}$$

Where: ME = metabolisable energy, ME_N = nitrogen-corrected metabolisable energy, GE = gross energy and GN = gross nitrogen.

The rapid AME bioassay has been found to be easier, faster, and cheaper than conventional methods. In this bioassay, individually caged broilers are trained to consume their daily pelleted feed allowance in one hour. At least 70g of feed is required to ensure that endogenous excreta does not significantly reduce ME (Choct, 2004). The total amount of excreta voided by each bird over the next 24 hours represents all of the feed consumed, providing an accurate measurement of the ME of the diet. To eliminate possible differences in ME values due to age and the need to adjust ME values based on nitrogen retention, the rapid bioassay requires that both chicks and cockerels be fed the same diet for comparison (Choct, 2004). The advantages of the rapid bioassay are that birds are easily trained, and the assay can quickly be completed. In addition, the cockerels do not require an adaptation diet before the test diet (Choct, 2004). However, there are disadvantages, such as, some birds may not be easily trained and once trained must be maintained in that state, which is time-

consuming and labour intensive. The amount of feed consumed also affects the AME values for the diets, a lower amount of feed consumed results in a lower AME value (Choct, 2004).

2.7. Current prediction equations are used for the calculation of maize energy value

Energy is used as a common currency in nutrition and is not a nutrient itself, but rather the collective amount of energy derived from various nutrients in the diet. Laws of thermodynamics: "Energy is defined as the potential capacity to perform work. There are several forms of energy: chemical, mechanical, kinetic, positional, electrical and heat. The first law of thermodynamics states that energy can be neither created nor destroyed but can be transformed from one form to another. The second law states that all forms of energy are convertible to heat, so the driving force in all systems is to release energy as heat". Nutritionists typically focus on the conversion of chemical energy stored in food molecules to forms that are utilised by the animal body for important processes such as maintenance, growth, and production. The Second Law of Thermodynamics emphasizes that all forms of energy are convertible to heat and that as energy is transferred and transformed, more and more is wasted or released as heat. Heat released as a by-product from the animal body is unavoidable and is due to biochemical reactions occurring during digestion, absorption, basal metabolism, physical activity, thermal regulation and the formation and excretion of waste products (NRC, 1981). Energy is derived in the form of adenosine triphosphate (ATP) from the Krebs/Citric acid cycle occurring in the mitochondrion organelle of cells in the body. Energy in animal feeds can be quantified in calories – where 1 calorie is defined as the energy required to raise the temperature of 1-gram water by 1˚C. However, the International System of Units (SI) makes use of a joule as the standard unit of energy. Therefore, the energy content of most feeds is expressed as either kilocalories per kilogram (kcal/kg) or megajoules per kilogram (MJ/kg). The following conversion factor can be used to convert calories to joules: 1 calorie = 4.185 joules.

The common systems that describe dietary energy are gross energy (GE), digestible energy (DE), metabolisable energy and net energy.

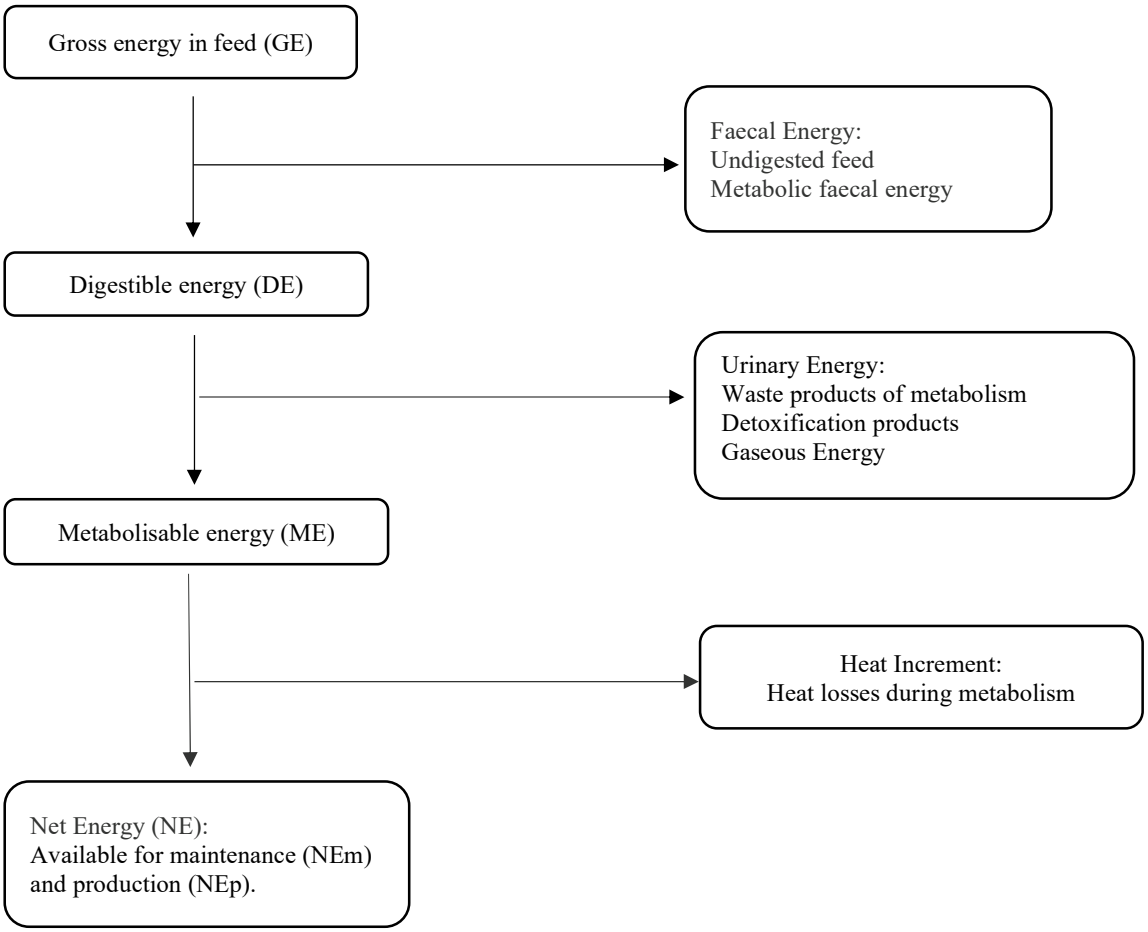


Figure 2.2. Basic sub-dividing of gross energy adapted from McDonald *et al*. (1995)

Gross energy (GE) is the energy expelled during combustion of feed in the presence of excess oxygen. Losses of energy due to the digestive processes of feed are not accounted for and for this reason GE is not used for poultry. However, GE is the first step in the evaluation of feed energy (Moehn *et al.*, 2005).

Digestible energy (DE) is the gross energy of feed excluding the energy lost in the form of feaces. This considers the digestibility of feed and is easy to determine. However, the loss of energy through urine, metabolism and combustible gases is not considered. Protein and fibre energy contribution is also overestimated by DE (Moehn *et al.*, 2005).

ME is the digestible energy of feed excluding the energy lost form of urine and combustible gases such as methane. ME gives a better prediction of energy by taking these factors into account (Moehn *et al.*, 2005). ME is the energy system used for poultry in South Africa and can be expressed as apparent metabolisable energy (AME) and true metabolisable energy (TME), and the nitrogen corrected apparent and true metabolisable energy (AME_N and TME_N, respectively) (Choct, 2004). The reason energy metabolised is called apparent is because the excreta collected for analysis of energy is only partly derived from food, thus, a portion of the excreta consists of undigested and unmetabolised dietary residues. A portion of the excreta that is derived from the bird is of endogenous origin and the energy derived from this is known as the endogenous energy loss (EEL) (Choct, 2004). Energy from faecal matter, sloughed-off gut lining, bile excretions and unabsorbed enzymes and the excretory products of nitrogen metabolism contributes towards the EEL (Choct, 2004). However, the use of the AME system has its disadvantages that include poor correlation with feed conversion and poor prediction of growth rate in broilers (Choct, 2004). There are also factors that contribute to the variation of AME of feedstuff, such as, feed processing, feed composition, level of feeding, nitrogen-retention, age, strain and species, and environment (Choct, 2004).

Net Energy (NE) is the metabolisable energy minus the energy lost in the form of heat or heat increment (HI) produced during feed digestion, nutrient metabolism, and waste excretion. The remaining energy after these losses is used for maintenance and production (growth, gestation, and lactation). As a result, net energy is the most accurate and unbiased system for estimating feed energy content. However, determining NE is more difficult and complex than determining DE or ME (Moehn *et al.,* 2005).

2.7.1. Energy systems

There are several energy systems used around the world to estimate the energy values of poultry diets. However, there are three main methods to determine the energy value of ingredients in poultry diets including values attained from tables, prediction equations and *in vivo* experiments. These methods are widely available for use by the animal feed industry (Mateos *et al.*, 2015).

The Dutch CVB is an energy system modified for AME systems that account for the efficiency of utilisation of various nutrients. A correction factor was introduced in this system to account for the differences in energy utilisation between fats and carbohydrates. Digestibility coefficients for crude protein, crude fat and nitrogen free extract derived from trials conducted with adult cockerels and young broilers are given in the CVB feed tables. In the experimental trials, it was determined which

part of the ingested feed ingredient did not appear in the faeces and was therefore apparently digested. Since crude fiber cannot be digested by poultry, its energy contribution is thought to be insignificant (CVB, 2016). According to the Dutch CVB (2016) the AME_N content for broilers and adult poultry can be calculated as follows:

Broilers: AME_N (kJ/kg) = 15.56 dCP + 38.83 dC.FAT + 17.32 dNFE

Adult poultry: AME_N (kJ/kg) = 18.03 dCP + 38.83 dC.FAT + 17.32 dNFE

(dCP = digestible Crude Protein, dC FAT = digestible Crude Fat, NFE = digestible Nitrogen-free Extract)

In broilers, digestible crude protein has a lower energy value. This value was determined by performing regression analyses on the measured AMEN value and the grams of digested protein, fat, and NFE in 15 important poultry feed ingredients (Van der Klis & Fledderus, 2007). According to this regression equation, the energy coefficient for digestible protein in broilers was lower than in adult cockerels, while the energy coefficients for the other nutrients were comparable (Klis & Fledderus, 2007).

Other AME Formulas, such as the Rostock equation, consider the differences of the energy value of the different carbohydrate fractions. The Rostock Feed Evaluation System is based on the ATP generating capacity (Beyer *et al.*, 2003). It includes net energy retention (NER) values for poultry and tables containing NE values for poultry feedstuffs. In the equation for AME_N, the NFE fraction is split into starch, sugars and NRF, where NRF = 1000 – (moisture + crude ash + CP + C. Fat + Starch + Sugars). This relates the variation in the starch content of an ingredient to its AME_N value.

AME_N (kJ/kg) = 18.8 x dig.CP + 39.8 x dig.C. Fat + 17.3 x dig. Starch + 16.0 x dig.Sugars + 17.2 x dig.NRF

The Brazilian Tables of Feedstuff Composition made use of the total excreta collection method to determine the ME values of poultry feedstuffs (Rostagno *et al.,* 2011). Chromium oxide and acid insoluble ash were used as faecal markers when determining the ME of several feedstuffs. These values, presented as $ME_{poultry}$, were determined in broilers of different ages, and were corrected for nitrogen retention. The system uses two equations, one for poultry in general and another for hens or mature poultry, as prior studies indicated that ME values for vegetable feedstuffs obtained from mature poultry were generally higher in comparison to growing broilers. The following equation can be used to estimate the metabolisable energy of vegetable feedstuffs fed to broilers and young poultry:

$ME_{poultry}$ = 4.31 CPd + 9.29 Fd + 4.14 NFEd

(CPd = digestible protein (g/kg), Fd = digestible fat (g/kg), NFEd = digestible nitrogen-free extract (g/kg))

2.7.2. Net energy system

DE and ME are frequently used to assess the energy content of feed for monogastric animals. The net energy (NE) value of feed energy content, on the other hand, is the closest estimate to the 'true' energy value of feed, as it accounts for differences in metabolic utilisation of ME of nutrients for maintenance and production. Furthermore, NE is the only system in which energy requirements and diet energy values are expressed on a theoretically independent of feed characteristics basis. The NE system has been widely implemented in the pig industry, but it is rarely used in the poultry industry (Noblet *et al.,* 2010).

The NE/ME ratio (or k) corresponds to the efficiency of ME utilisation for NE. The NE/ME ratio also corresponds to 1-(HI/ME). However, due to animal species and several physiological factors the HI/ME ratio is not constant for any given feed over a large range of ME intake. The HI is lower for ME supplied below the maintenance energy requirement than for ME supplied above maintenance. When ME is used for fat deposition there is a lower HI compared to protein deposition (Noblet *et al.*, 2010).

De Groote (1974) found the net availability of the ME of glucose, maize oil, and isolated soybean protein to be 75%, 90% and 60% respectively for maintenance and growth of both young and mature chickens. Therefore, relative efficiencies of energy utilisation for carbohydrates, fat and protein are 100%, 113% and 78%. This leads to the conclusion that, in comparison to carbohydrates, the ME system in fact underestimates the productive energy value of feedstuffs high in fat and overestimates this value for feedstuffs high in protein (Swick *et al.*, 2014). Another concern is that the ME system assumes the energy values obtained for individual feed ingredients to be additive, when they are not. Ingredients differ in their metabolisability, chemical compositions, anti - nutritional factors, and interactions with one another, which all affect energy availability to the bird.

In practice, HI is said to be the difference between heat production (HP) of the animal in a ‘normal’ fed state and HP in a fasting state (FHP), which corresponds with the rate of increase in HP per unit of ME intake increase (Noblet, 2013). To determine the extent to which the ME of the feed is utilised by the bird, either its HP or ER must be measured. HP can be measured using calorimetry and ER in the carcass can be measured through the comparative slaughter technique or the carbon and

nitrogen balance technique (Priyankarage *et al*, 2011). Closed - circuit indirect calorimetry is the most common technique used to calculate heat production from the amount of oxygen consumed and the amount of carbon dioxide expired by the animal (Choct, 2004). Heat production is estimated by measuring gas exchange and the excretion of urinary nitrogen in a respiratory chamber, according to the Brouwer equation (McLean, 2005):

Heat production (kcal) = (3.866 × O_2 consumption, l) + (1.200 × CO_2 production, l) – (0.518 × CH_4 production, l) – (1.431 N urine, production, g)

Where O_2, CO_2 and CH_4 represent volumes of oxygen consumed and of carbon dioxide and methane produced (l), and N is the quantity of urinary nitrogen excreted (g).

According to the principle of conservation of energy, the ME the bird consumes in the diet is either retained in the body (ER) or is lost as heat (Sibbald, 1982; Choct, 2004). Therefore, ME = HI + ER (Choct, 2004). One can then use experimentally determined values for ME and HP via bomb and indirect calorimetry to calculate the unknown variable: ER = ME – HP. Essentially, subtracting the HI component from ME provides the NE value of the feed (Choct, 2004).

2.8. Technology to predict maize nutritive value

Near-infrared reflectance spectroscopy (NIRS) has been used as a real time grain analyser that is fast, reliable, accurate, non-destructive and is of economic benefit (Singh *et al.*, 2006). NIRS measures grain quality through the determination of kernel hardness, vitreousness, moisture content, colour classification, identification of damaged grain, detection of insects and mycotoxins and proximate composition analysis (Singh *et al.*, 2006). NIRS measures the wavelength and absorption of near-infrared light by a material. The visible light and the infrared light region is in the wavelength region of 700 to 2500 nm, that is also known as the near-infrared region (Singh *et al.*, 2006). The principle around NIRS is that molecules with different proximate compositions absorb light in the NIR region differently causing them to vibrate at different frequencies. Energy absorbed by the material is measured by the amount of reflected or transmitted light collected by a spectrometer. The concentration of a particular parameter of a material is measured through the relationship of the near-infrared spectra obtained from multiple wavelengths (Singh *et al.*, 2006). NIRS is first calibrated before use for quantitative measurement through the analyses of different parameters of material that involves the creation of reference data by the use of other methods such as wet chemistry analysis (Osborne, 2006; Singh *et al.*, 2006). The three basic functions of analytical chemistry that are separation, identification and quantification are provided by the calibration. Therefore, it is important

that the procedure to obtain the calibration equation for each application is accurate and reliable (Osborne, 2006).

NIRS is currently routinely used for prediction of chemical composition (moisture, crude protein, crude fibre, fat, and ash), as well as amino acid content of feed ingredients. It can also be used to measure other factors contributing to the quality of maize, which include bulk density, kernel size, kernel hardness, vitreousness, damaged kernels, mycotoxins and foreign material (Singh *et al.*, 2006). Calibrations to determine AME_N values of cereal grains, such as maize, have also been developed by scanning samples with known *in vivo* AME_N using a NIRS (Van Kempen & Simmins, 1997). Large databases containing information about ingredients that vary in their composition profiles and are from different geographical regions, are used to derive NIRS calibrations. These calibrations are therefore only as good as the methods used to collect the reference data. Accuracy is highly dependent on large sample databases collected from chemical analysis and *in vivo* validation trials. NIRS uses a regression technique and predictions are based on correlations between spectral information from absorbed light and reference data (Van Kempen & Simmins, 1997). In order to improve the accuracy of calibrations it is necessary that the reference data be continually updated with new samples of varying profiles.

Near-infrared reflectance (NIR) and near-infrared transmittance (NIT) are the two types of NIRS. When radiation passes through a sample, NIR measures the ratio of the intensity of light reflected from the sample, whereas NIT records the decrease in radiation intensity as a function of wavelength (Hruby, 2015). NIRS analysis requires samples to have been ground to smaller particles size which requires sample preparation that is time - consuming and can affect the accuracy of the results. Therefore, it is recommended that each operation calibrate their NIR technology over time according to the processing and particle size generally used to ensure more accurate results (Singh *et al.*, 2006). NIT is advantageous as it requires no sample preparation, it is quick, accurate, produces no hazardous chemical waste, limited operator input on the final result and is therefore non-destructive, and can be used to analyse whole kernels, improving measurement repeatability and sample preservation (Nduwamungu *et al.*, 2009). It is an ideal piece of equipment to have at the intake offices of silos for quality control purposes where batches of ingredients arrive continuously.

NIRS has the potential for commercial use in the grain industry through its ability to perform real-time analysis of both physical and chemical properties of grains. Calibrations for the NIRS are only as accurate as the reference data and analysis methods used (Singh *et al.*, 2006). Using these rapid methods to evaluate the nutrient content of maize would benefit the broiler industry in terms of

amino acid addition and would assist in determining the optimal quantity of amino acids to add and to prevent the over feeding of amino acids (Van Kempen & Simmins, 1997).

2.9. Methods used to measure the quality of maize

The analysis of the nutritional components of maize is not necessarily a simple procedure due its heterogenous nature and the interactions between different nutrients that affect the accessibility of certain nutrients. Research has been conducted over the years to develop reliable methods that best reflect *in vivo* conditions and result in accurate physiological measurements. This has led to the publication of standardised methods that are available to analytical scientists worldwide to use. Examples of publications that are widely used include the Official Methods of Analysis (OMA) of the Association of Official Analytical Chemists (AOAC) International and the American Association of Cereal Chemists (AACC) International. The analytical methods used to determine the nutrient components in maize samples differ between studies and laboratories, making direct comparisons of results difficult.

Crude protein is usually analysed as a mandatory nutrient and can be estimated by multiplying the N content of a sample by a nitrogen to protein conversion factor (Simonne *et al*., 1997). This conversion factor is generally accepted as 6.25, as the amino-N accounts for approximately 16% of the protein weight in most feed sources (Simonne *et al*., 1997, 2002). This is considered an indirect method of protein determination, whereas direct protein determination is based on the analysis of amino acid residues (Mæhre *et al*., 2018). Official methods for total N determination in routine feed analysis include the Kjeldahl technique and the Dumas technique. The Kjeldahl technique involves hydrolysing a 1 g ground feed sample in concentrated sulphuric acid (H_2SO_4) combined with catalysts and salts (Mæhre *et al*., 2018). The mixture is digested at a high temperature, causing organic and inorganic forms of N to convert to ammonium (Simonne *et al*., 1997). Ammonium is then analytically quantified by titrimetry, colorimetry or by using an ion-specific electrode. The method assumes that N recovered during digestion is mainly amino - N from proteins (total organic N) and ignores the contribution of inorganic N (Simonne *et al*., 1997). However, there are many variations of the steps involved in this method and it tends to be a time - consuming procedure requiring the use of harmful chemicals. The Dumas technique, also referred to as the combustion technique, is a quicker technique of N determination which does not involve wet chemistry (Simonne *et al*., 1997). This method uses automated lab equipment to convert all the forms of N present in a feed sample into gaseous nitrogen oxides (NOx) by complete combustion. The NOx gases are then reduced to N_2, which is quantified via a thermal conductivity detector (Sweeney & Rexroad, 1987). Results are given as % or mg N and can be converted to a crude protein value by multiplying total nitrogen by 6.25 (AOAC, 1990).

In a study combining advances in cereal chemistry with rapid turbidity methods in a procedure termed the modified turbidimetric zein method (mTZM), Larson & Hoffman (2008) were able to quantify the zein content in whole maize. The mTZM method involves solubilising a dried, defatted, acetone-insoluble maize sample in 55% aqueous isopropyl alcohol containing 0.6% 2-mercapto-ethanol (Larson & Hoffman, 2008). Trichloroacetic acid (TCA) at 0.15 M is then added to the aqueous alcohol-solubilised zein to achieve turbidity and the degree of turbidity of the sample is measured by log absorbance at 440 nm in a spectrophotometer (Larson & Hoffman, 2008). The study included the development of a standard absorbance curve from purified zein at different concentrations prior to the testing of maize samples. This method can therefore be used to 'quantify starch granule encapsulation by hydrophobic prolamin proteins' in whole maize kernels (Larson & Hoffman, 2008).

Total starch content can be determined by using the AOAC Method 996.11 or AACC Method 76-13.01. This is an enzymatic procedure and is referred to as the Amyloglucosidase/α-Amylase assay (Megazyme, 2017). The principle of the method involves the complete solubilisation of starch in the presence of thermostable α-amylase at 100ºC. The α-amylase hydrolyses the starch into soluble branched and unbranched maltodextrins, which are then subsequently hydrolysed by amyloglucosidase (AMG) to D-glucose (Megazyme, 2017). One mole of hydrogen peroxide is used to oxidise D-glucose to D-gluconate, which is quantified with the use of quinoneimine dye. Samples that typically contain high levels of resistant starch (i.e., high amylose maize) should be pre-treated with KOH followed by neutralisation with sodium acetate buffer and hydrolysis with α-amylase (Megazyme, 2017). The residual starch after 120 minutes of incubation with pancreatin and amyloglucosidase is defined as the resistant starch (Englyst *et al*., 1992).

The principle of the AOAC Method 2002.02/AACC Method 32 - 40 to determine resistant starch content is as follows: After determining the moisture content of a 50 g ground grain sample, it is incubated with pancreatic α-amylase and AMG for 16 hours at 37°C. These enzymes cause the solubilisation and hydrolysation of non-resistant starch to D-glucose. Ethanol or industrial methylated spirits (IMS) is used to terminate the reaction and the RS is recovered as a pellet once centrifuged. This is followed by further centrifugation after the pellet has been washed twice by suspension in aqueous IMS or ethanol (50% v/v). Free liquid is then removed and the RS in the pellet is dissolved in 2 M KOH and stirred vigorously in an ice-water bath. The resultant solution is neutralised with acetate buffer and the starch is hydrolysed to glucose using AMG. Glucose oxidase/peroxidase reagent (GOPOD) is used to measure the amount of D-Glucose which is equivalent to the RS content of the sample. Non-resistant starch (solubilised starch) can be measured by adding the original supernatant

and the washings, adjusting the volume to 100 mL, and measuring D-glucose content with GOPOD. The total starch content is the sum of resistant starch and non-resistant (solubilised) starch.

There are several analytical techniques to quantify the total fat content of a feed sample; one of which is the gravimetric determination of total fat, which is based on the mass of lipid extracted from a sample (Srigley & Mossoba, 2017). One kind of variation of this technique is the solvent extraction procedure. The Soxhlet procedure is a conventional solvent extraction method in which a dried and homogenized sample is washed semi-continuously with an organic solvent (e.g. ethers such as ethyl and petroleum) (Srigley & Mossoba, 2017). This technique can be conducted with the use of specialised glassware or by using automated instrumentation in which the solvent undergoes heating and condensation. Samples are mixed with anhydrous sodium sulphate in preparation for extraction and then placed into an extraction chamber. The solvent is heated in reflux to travel up the distillation arm as vapour and then condenses in the extraction chamber with the solid sample. As the chamber fills up with solvent, the lipids contained in the sample dissolve into the solvent and this portion is then extracted and undergoes filtration to remove excess water, leaving the concentrated extract behind. Total crude fat can be indirectly determined by calculating the difference in sample weight before and after extraction according to AOAC 985.15.

The Megazyme total dietary fibre (TDF) assay is based on a combination of AOAC and AACC procedures (AOAC Method 985.29, AOAC Method 991.42, AOAC Method 991.43, AOAC Method 993.19, AACC Method 32-05.01, AACC Method 32-06.01, AACC Method 32-07.01, AACC Method 32-21.01). The assay is done in duplicate and involves cooking dried samples with heat stable α-amylase at 100°C, resulting in the gelatinisation, hydrolysis and depolymerisation of starch molecules. This is followed by incubation at 60°C with protease (to solubilise and depolymerise proteins) and amyloglucosidase (to hydrolyse starch fragments to glucose) (Megazyme, 2017). The samples are then treated with ethanol at four varied volumes to allow the precipitation of soluble fibre and the displacement of depolymerised protein and glucose. The leftover residue is washed with 78% ethanol, 95% ethanol, and acetone, and is dried and weighed. Analysis of protein is done on one duplicate sample while the other is incubated at 525°C to determine the ash content. Subtracting the weight of the protein and ash from the weight of the residue that was filtered and dried results in the TDF weight.

Various methods can be used to determine maize hardness/vitreousness and include the determination of grit yield, particle size index (PSI), starch gelatinization properties and resistance to

grinding and abrasion (Fox & Manley, 2009). Hand dissection, although tedious, is another method that can be used to determine the ratio of vitreous to floury endosperm (Guelpa, 2015). Other indirect methods include near-infrared spectroscopy (NIR), the rapid visco analyzer (RVA) and X-ray micro-computed tomography (XCT). The Rapid Visco Analyser (RVA) technique relates starch properties such as paste viscosity, gelatinisation temperature and time to maize hardness (Fox and Manley, 2009). The hardness of whole kernels can be measured at a wavelength of 860 nm as it will depict differences in particle size (Lee *et al*., 2006). On the other hand, milled samples should be scanned at 2230 nm, as reflectance at this wavelength is independent of chemical composition and only considers differences in particle size (Downey *et al*., 1986; Guelpa, 2015).

In vitro rate of starch digestion within 60 minutes (RSD60) is based on the method by Ebsim (2013) by preparing two enzyme solutions, benzoic acid solution, and sodium acetate buffer. The glucose oxidase peroxidase determination (GOPOD) reagent from Megazyme (D-glucose assay procedure - GOPOD format, K-GLUC 07/11, Megazyme International Ireland, Bray, Co. Wicklow, Ireland) is used for the glucose oxidase method. Samples are ground finely using a screen-hole size of 0.5 mm; fine grinding is used to stimulate the impact of the chicken's gizzard. Triplicates of approximately 700 mg of each sample are weighed, and added into 50 mL polypropylene centrifuge tubes, and 50 mg of guar gum powder is also added to each tube to standardize the viscosity. A blank tube containing 50 mg of guar gum powder is used to regulate glucose content in the amyloglucosidase solution and is used as the blank sample. A starch standard is prepared by adding regular maize starch and guar gum powder into a tube. *In vitro* starch digestion is completed on a set of nine samples at a time. Initially, 1.5 mL of enzyme solution I (2000 U mL - 1 of pepsin - HCl solution) is added to each centrifuge tube. Then, tubes are capped, homogenised on a vortex mixer, and placed horizontally in a water bath (41 °C) for 30 min. The enzyme solution II is prepared during this time. Tubes are removed from the water bath after 30 min, and three glass balls (1.5 cm diameter) are added to each tube. Then, 20 mL of sodium acetate buffer (41 °C) is added to each sample, standard and blank tube, capped and rotated. 5 mL of enzyme solution II is added to each tube, and then the tubes are capped, rotated, and immediately securely positioned in a shaking water bath (41 °C). Aliquots (0.5 mL) are taken from each tube at 60 min and added to 50 mL polypropylene centrifuge tubes containing 20 mL of absolute ethanol (stop the enzyme reaction). The amount of released glucose is measured colourimetrically according to a glucose oxidase method of a Megazyme kit (D-glucose assay procedure - GOPOD format, K-GLUC 07/11, Megazyme International Ireland, Bray, Co. Wicklow, Ireland).

Salt-soluble protein content is determined according to the official method (method NF-V03–741; AFNOR, 2008) as described by Janas *et al.* (2010) (Promatest). The samples are milled. Flour (5.4 g ± 0.01 g) is then introduced in an ernelmeyer with 100 mL of extraction solution and shaken for 3 min. The samples are filtered on filters Whatman 595 1/2 (Germany), the first 10 mL is pulled off and the following 20 mL kept for analysis. The PMMA at a quantity of 4 mL from VWR (Belgium) are added, 40 µL of standard, 40 µL of sample and 3 mL of Coomassie blue into a macro cuvet. The cuvets are shaken slowly and then the optical density at 595 nm is measured. The optical density at 595 nm is measured after the cuvets have been slowly shaken.

The NIT calibration created by the Southern African Grain Laboratory (SAGL) is used to calculate milling index (MI) and grit yield all (GYA). A greater milling index indicates a greater extraction of the high grade and most economical products like samp, maize rice, and maize grits (degermed products) that are produced from the vitreous part of the endosperm. Grit yield all is defined as the sum of the Roff B2 grits, B3 fine grits, and B3 coarse grits fractions calculated as a weight percentage of the total weight of the maize before milling. Grit yield all is linearly correlated with the MI and expresses the real amount of total hard endosperm that can be removed from the maize during Roff milling (SAGL., 2018).

2.10. Conclusion

Maize is a major component of broiler feed and consequently contributes largely to dietary energy values. It is therefore imperative to understand and quantify the nutritive value of maize to better estimate AME_N values. This will assist in improving feed formulation accuracy and thereby improve broiler performance, profitability, and sustainability. Research has shown that the nutritional value of maize is widely variable, which leads to either an over- or undersupply of energy in broiler diets due to formulation inaccuracies. Therefore, in addition to proximate composition, intrinsic factors of the kernel that contribute to observed variation in the AME_N of maize should be considered when predicting AME_N for broilers.

The intrinsic factors vitreousness and kernel hardness are not considered when calculating AME_N, play an important role in the processing of maize. Different industries and/or milling processes require different hardness levels. Physical characteristics of maize that have been linked to hardness are density, weight, kernel size and shape, resistance to grinding or abrasion, and the amount of coarse vs

fine material after grinding and sieving. Kernel hardness is mainly due to genetic expression, but transportation, drying, storage, and environment may also play a role in hardness properties.

The use of near-infrared technology may help combat the effect of the large variations in maize grain quality, as the use of a rapid, automated system to determine grain nutrient content and quality may help to avoid inaccurate feed formulation. However, this technology is only as good as the sample reference data included in its calibrations. The method mostly used to collect *in vivo* data on the AME_N of maize has been the total collection method. However, the total collection method is subjected to a high rate of contamination and birds have to be fasted for hours at a time. Another method investigated is the basal substitution method that currently involves the use of indigestible markers in order to avoid contamination and in accuracies.

Chapter 3
Methods and Materials

This experiment was conducted at the IA@UP Research Park (University of Pretoria, Hillcrest, Pretoria) using the broiler and poultry metabolic facilities. The study was approved by the Animal Ethics Committee of the University of Pretoria (Project number: NAS250/2019).

3.1. Collection of maize samples for model development

The samples were collected from different regions in South Africa by the Southern African Grain Laboratory (The Willows, Pretoria, Gauteng). A total of 837 white and 1 068 yellow maize samples were collected from the 2015/2016 (n=338), 2016/2017 (n=772), and 2017/2018 (n=787) harvest years.

3.2. Laboratory work and model development

Maize samples from the 2016, 2017 and 2018 harvest years that have been collected by the South African Grain Laboratory were analysed using a near-infrared transmittance (NIT) grain analyser (Infratec^{TM} 1241 Grain Analyser, Foss, Denmark). Pioneer Seeds (Danisco Animal Nutrition, IFF, Marlborough, UK) developed a method to predict the nitrogen-corrected apparent metabolisable energy (AME_N) of maize directly using NIT spectroscopy. This was done by selecting over 300 maize samples from different countries that differed in their chemical and physical characteristics. The AME_N of these samples was then determined *in vivo*, while the same sample was scanned using an NIT instrument. Equations were then developed that showed that NIT technology could accurately predict the AME_N of maize, with a standard error of prediction of less than 10 kcal/kg maize.

For the first part of the current study, AME_N values were obtained using NIT for 60 South African maize samples from the 2015/2016 harvest season. These same samples were also chemically analysed for moisture, gross energy, crude protein, crude fat, starch, and ash content according to methods described by the Association of Official Agricultural Chemists (AOAC). The ME values of the samples were calculated using the CVB (2012) prediction equation for poultry: ME po (MJ/kg) = 18.03dCP + 38.83dCFat + 17.32dNFE. The following digestibility coefficients applied to the equation: DCCP = 83%, DCCFat = 84% and DCNFE = 91%.

For model development, 1 110 maize samples from the 2015/2016 and 2016/2017 harvest seasons were collected from different regions in South Africa. Moisture, crude protein, crude fat,

milling index (MI), grit yield all (GYA), and AME_N values for the samples were obtained using NIT spectroscopy. A prediction model was subsequently developed to predict AME_N from proximate analysis as well as intrinsic kernel characteristics, including MI for kernel vitreousness and hardness. The Pearson product-moment correlation coefficients of all variables were determined using the multivariate analysis test (JMP Pro 13.1). The Multiple Regression Model Fit test (JMP Pro 13.1) was used to analyse the relationship between AME_N and all parameters, and for model development using stepwise regression to minimise the Bayesian Information Criterion (BIC).

Thereafter, 50 samples from three harvest seasons (2015/2016, 2016/2017 and 2017/2018) were collected for *in vitro* analysis and a further ten samples were collected for the animal trial, during the 2018/2019 harvest season. These samples were analysed for starch digestibility and salt-soluble proteins using RSD60 and Promatest, respectively, at the Chemunique (Pty) Ltd Laboratory (Lanseria Business Park, Lanseria, Gauteng).

3.3. Collection and scanning of maize samples for *in vivo* validation

Maize sub-samples collected from four different feed mills from the 2018/2019 harvest season were scanned using an NIT grain analyser at Chemunique (Pty) Ltd Laboratory to determine NIT AME_N of the maize. Thereafter, five samples were selected that varied in NIT AME_N from 3892.94 to 3979.18 kcal/kg (DM). The samples were collected in a quantity of 35 kg.

The NIT-predicted and chemically analysed nutrient profiles of the maize collected for the study are shown in Table 3.1 and Table 3.2, respectively. Table 3.3 shows the results for the NIT-predicted milling index and grit yield all, in addition, the chemically analysed rate of starch digestion (RSD60) and salt-soluble protein content (Promatest).

Table 3.1. Near infrared transmittance predicted nutrient profile of maize variants fed to broiler chicks (All values except moisture are on a dry matter basis).

Maize Variant	Moisture (%)	Crude protein (%)	Crude fat (%)	Starch (%)	GE (kcal/g)	DE (kcal/g)	AME_N (kcal/g)
Test maize 1	11.80	8.87	3.62	69.82	4473.45	3996.19	3892.94
Test maize 2	12.65	7.60	3.74	72.09	4450.90	4007.84	3905.60
Test maize 3	10.88	8.23	4.01	70.26	4489.23	4028.78	3928.36
Test maize 4	11.32	9.56	4.18	68.56	4510.53	4048.59	3949.90
Test maize 5	10.70	9.45	4.12	69.08	4526.61	4075.53	3979.18

GE: Gross energy
DE: Digestible energy
AME_N: Nitrogen-corrected apparent metabolisable energy

Table 3.2. Chemically analysed nutrient profile of five maize variants used in the total collection and basal substitution methods[1]

Maize Variant	Moisture (%)	Crude protein (%)	Crude fat (%)	Crude fibre (%)	Ash (%)
Test maize 1	10.57	7.53	3.08	2.36	1.16
Test maize 2	11.35	6.78	2.76	1.83	1.22
Test maize 3	10.69	7.29	3.49	2.04	1.12
Test maize 4	11.08	7.59	3.07	2.21	1.16
Test maize 5	9.31	8.22	4.14	2.00	1.25

[1] all values except moisture are on a dry matter basis.

Table 3.3. Near-infrared transmittance predicted milling index and grit yield all and wet chemistry analyses of rate starch digestibility over 60 minutes (RSD60) and salt-soluble proteins denaturation index (Promatest) of maize sources fed to broiler chicks

Maize Variant	Milling index	Grit yield all	RSD60 (%)	Promatest (mg protein/100 mL)
Test maize 1	77.96	64.25	37.44	69.82
Test maize 2	70.87	62.57	30.58	72.09
Test maize 3	71.75	62.76	29.62	70.26
Test maize 4	81.37	65.10	33.38	68.56
Test maize 5	62.31	60.45	42.75	69.08

3.4. Milling of maize

The five maize sources were milled with the use of a Bliss Experimental hammer mill (Bliss Manufacturing, Ponca City, OK, USA) with a 3.18 mm screen. One kg sample was collected from each ground maize variant to determine the final particle size in duplicate. Prior to milling the maize sources, the mill was cleaned to remove any feedstuff. At the end of milling, the entire system was cleaned again.

3.5. Animals

900-day-old male Ross 308 chicks were sourced from a commercial hatchery (National Chicks, Pretoria) where they were sexed and checked for any health defects before arrival on the farm. The chick placement procedure was as follows: 50 males were randomly selected, weighed together in a portable crate (weight was recorded) and placed in a pen. Birds were weighed per pen in portable

crates at 7 and 14 days-of-age. At 14 days-of-age, all birds were individually weighed and assigned to one of six body weight (BW) groups. Only 768 birds were moved on 14 days-of-age. One bird from each BW group was transferred to a metabolic cage at random, that resulted in each metabolic cage having had six birds and each pen a similar average body weight. The group BW of birds in each cage were recorded at 14, 19, 21 and 22 days-of-age.

3.6. Test facilities and husbandry

The experimental trial was conducted on the IA@UP Research Park (University of Pretoria, Hillcrest, Pretoria). From 0 to 14 days-of-age, broilers were housed in a small environmentally controlled broiler house, which contained 34 pens with a floor area of 3 m x 1.3 m per pen. Only 18 of the pens were used for this trial. The house was cleaned and pre-heated to approximately 36°C to ensure a litter temperature of 34°C prior to chick arrival and placement. The pens contained clean pine shavings as bedding material. Each pen contained two bell drinkers and two tube feeders. Additionally, two pan feeders, two scratch papers and two fountain drinkers were provided to ensure easy access to feed and water during the brooding period. House temperature was gradually reduced to reach 28°C by 14 days-of-age. The artificial lighting program was 23 hours of light and 1 hour of darkness for the first seven days, after which it was adjusted to six hours of darkness and 18 hours of light.

The broilers were moved at 14 days-of-age to the environmentally controlled metabolic house consisting of 128 cages, all 84 x 69 x 52 cm in size. Cages were separated by a wire partition, and each contained one feeder spanning the front of the cage and two nipple drinkers. Temperature of the house was gradually adjusted from 28°C to 22°C from 14 to 22 days-of-age. The lighting regiment was kept at six hours of darkness and 18 hours of light. Each cage served as an experimental unit/replicate when treatment diets were fed. In both houses, temperature and ventilation was monitored twice daily and adjusted accordingly if needed.

3.7. Exogenous enzymes

The enzyme combination of xylanase, amylase, and protease (XAP) included in treatment 11-15 were endo-1,4-β-xylanase (EC 3.2.1.8), α-amylase (EC 3.2.1.1), and a serine protease (EC 3.4.21.62). The xylanase originated from *Tricoderma reesei*, the amylase from *Bacillus licheniformis* and the protease from *Bacillus subtilis*. The product used was Axtra® XAP 101 TPT (Danisco Animal Nutrition, IFF, Marlborough, UK) at an inclusion rate of 100 mg/kg. The enzyme activity of xylanase in feed samples (500g) was measured at the Chemuniqué (Pty) Ltd laboratories (Lanseria, South

Africa; Table 3.7) in duplicate. One xylanase unit was defined as the amount of enzyme that releases 0.48 µmol of reducing sugar as xylose from wheat arabinoxylan per minute at 4.2 pH and 50˚C.

Table 3.4. Expected and measured xylanase activity in feed samples.

Dietary Treatment*	Expected (XU/kg of feed)	Measured (XU/kg of feed)
6	0	ND
7	0	ND
8	0	ND
9	0	ND
10	0	ND
11	2000	1879
12	2000	2840
13	2000	2639
14	2000	2497
15	2000	1539
16	0	ND

Xylanase from *Tricoderma ressei* (2,000 U/kg).
ND = not detectable.
XU = xylanase units defined as the quantity of enzyme that dispenses 0.48 µmol of reducing sugar as xylose from wheat arabinoxylan per minute pH 4.2 and 50˚C.
*Treatments 1 to 5 were not analysed for xylanses as these treatments were made of the maize variants only.

3.8. Mixing of treatment diets

The basal diet was formulated based on commercial specifications using Format NC software, Format International UK, by Feed First (Pty) Ltd (Centurion, South Africa) and was mixed at Simple Grow (Centurion, South Africa) using a 1.5-ton fountain blender. The reference diet (Table 3.5) was first mixed with a maize/TiO_2 premix for 3 minutes using a Hobart floor mixer (701 South Ridge Ave. Troy, OH, USA). Thereafter, the maize sample and XAP enzyme combination were added and mixed for 3 minutes. This was repeated for each treatment and the equipment that was used was cleaned between each treatment diet. The treatment diets were pelleted using a 4 mm die.

The pre-starter was fed from 0 to 7 days-of-age, starter from 7 to 20 days-of-age and the reference diet was used to produce treatments 11-15, the formulations of the diets are as shown in Table 3.5.

Table 3.5. Raw material inclusion of the pre-starter, starter, and reference diet

Ingredients (%)	Pre-starter (0-7 days)	Starter (7-20 days)	Reference diet
Yellow maize	49.93	53.67	53.67
Soya oilcake (46%)	24.0	20.3	20.3
Full-fat soya	12.0	12.0	12.0
Gluten 60	3.20	3.00	3.00
Monodicalcium phosphate (MDCP)	2.25	2.00	2.00
Oil crude soya (degummed)	2.09	2.58	2.58
Sunflower oilcake (36%)	2.00	2.00	2.00
Limestone	1.56	1.56	1.56
Pellibond[2]	1.00	1.00	0
Sodium Bicarbonate ($NaHCO_3$)	0.522	0.515	0.515
Premix[1]	0.345	0.345	0.345
Lysine HCL (99%)	0.326	0.305	0.305
L-Methionine (78.8%)	0.268	0.227	0.227
Threonine (98.5%)	0.141	0.121	0.121
Salt (Fine)	0.13	0.135	0.135
Choline chloride (60%)	0.12	0.12	0.12
Zinc Bacitracin (15%)[3]	0.067	0.067	0
Cycostat (Robenidine 6.6%)[4]	0.05	0.05	0.05
Titanium dioxide	0	0	0.9

[1]Provided per kg of complete diet: Vitamin A, 12000 IU; Vitamin D3, 5000 IU; Vitamin E, 60.00 mg; Vitamin K3, 2.00 mg; Vitamin B1, 2.00 mg; Vitamin B2, 5.00 mg; Niacin (B3), 50.00 mg; B5, 12.00 mg; Pyridoxine, 3.00 mg; Folic acid, 2.00 mg; Vitamin B12, 0.01 mg; Biotin, 0.10 mg; Manganese, 110mg; Iron, 41.2 mg; Zinc, 100mg; Copper, 10 mg; Cobalt, 0.5 mg; Iodine, 2. mg; Selenium 0.3 mg.
[2]Pellibond: pellet binder produced from refined starch used to improve pellet and crumble quality (Simple Grow Agricultural Services, Centurion, South Africa).
[3]Zinc Bacitracin: each kilogram contains feed grade zinc bacitracin equivalent to 150 g bacitracin (Albac, Asia Pacific, Shanghai, China).
[4]Cycostat: contains 66 g robenidine hydrochloride per kg premix (Zoetis South Africa (Pty) Ltd, Gaunteng, South Afirca).

The treatments included in the study are shown in Table 3.6. Treatments 1 - 5 were made from the five maize variants only and were fed using the total collection method; the nutrient profiles of these samples are shown in Table 3.1, 3.2 and 3.3. Treatments 6 - 10 were made from a 40% substitution of the reference diet with the five test maize and treatments 11 - 15 were made by substituting 40% of the reference diet with the five maize variants and includes XAP at a rate of 100 mg/kg. These were fed using the basal substitution method. Treatment 16 was made from the reference diet only and also fed using the basal substitution method. The nutrient profiles of these diets are shown in Table 3.7.

Table 3.6. Treatments used in the metabolic study with one of five maize sources and exclusion or inclusion of an enzyme combination (xylanase, amylase, and protease)

Treatment	Composition	Method to determine AME_N
1	Test maize 1	Total collection
2	Test maize 2	Total collection
3	Test maize 3	Total collection
4	Test maize 4	Total collection
5	Test maize 5	Total collection
6	60% Reference diet + 40% Test maize 1	Basal substitution
7	60% Reference diet + 40% Test maize 2	Basal substitution
8	60% Reference diet + 40% Test maize 3	Basal substitution
9	60% Reference diet + 40% Test maize 4	Basal substitution
10	60% Reference diet + 40% Test maize 5	Basal substitution
11	60% Reference diet + 40% Test maize 1 + 100 mg/kg XAP	Basal substitution
12	60% Reference diet + 40% Test maize 2 + 100 mg/kg XAP	Basal substitution
13	60% Reference diet + 40% Test maize 3 + 100 mg/kg XAP	Basal substitution
14	60% Reference diet + 40% Test maize 4 + 100 mg/kg XAP	Basal substitution
15	60% Reference diet + 40% Test maize 5 + 100 mg/kg XAP	Basal substitution
16	Reference diet	Basal substitution

Basal substitution treatment diets consisted of 60% reference diet + 40% Test maize.
Titanium dioxide (TiO_2) was included at the rate of 0.45%, the reference diet had an inclusion of 0.9%.
The reference diet included was used in the basal substitution method treatment diets.

The chemically analysed nutrient profile of treatments 6 -16 are shown in Table 3.7.

Table 3.7. Chemically analysed nutrient profile of dietary treatments fed to broiler chicks[1]

Dietary treatments	Moisture (%)	Crude protein (%)	Crude fat (%)	Crude fibre (%)	Ash (%)	Calcium (%)	Phosphorus (%)
6	9.27	15.01	5.48	2.64	3.90	0.54	0.46
7	11.29	14.90	5.40	2.37	4.08	0.59	0.49
8	11.14	14.85	5.60	2.60	3.85	0.55	0.46
9	10.58	15.74	5.81	2.45	4.07	0.58	0.49
10	10.69	15.53	5.59	2.42	3.98	0.59	0.48
11	10.75	14.93	5.63	2.56	3.88	0.55	0.46
12	10.83	15.01	5.49	2.54	3.94	0.55	0.46
13	10.88	15.34	5.97	2.74	3.87	0.56	0.47
14	10.80	16.12	5.93	2.67	4.13	0.61	0.51
15	10.30	15.81	5.92	2.66	3.91	0.54	0.47
16	11.09	21.18	7.36	3.25	5.94	0.94	0.68

[1]all values except moisture are on a dry matter basis.

3.9. *In vivo* methods to determine AME_N

The study was composed of five maize samples that were analysed for proximate composition and scanned using a Foss Grain analyser (InfratecTM 1241 Grain Analyser, Foss, Denmark) to determine NIT AME_N. Maize samples were fed to broilers using two methods (total collection and basal substitution) to determine *in vivo* AME_N with an additional five treatments using the basal substitution (BS) method to determine AME_N uplift from an added enzyme combination of xylanase, amylase, and protease (XAP). An experimental unit was a cage of six male Ross 308 broilers with eight replicate cages/treatment. For the total collection (TC) method, birds (n = 240) were fasted for six hours followed by a six hour feeding and an additional 12 hour fasting period, after which total excreta was collected. For the basal substitution (BS) method, birds (n = 528) received treatment diets 24 hours before the three-day collection period during which excreta samples were collected every 24 hours.

3.9.1. Total collection method

For the total collection (TC) method, all birds were fed a single standard broiler pre-starter and starter diet formulated to achieve optimum performance for all birds according to Industry breed standards for 0 to 7 and 8 to 20 days-of-age, respectively. At 14 days-of-age, all birds were weighed individually and divided into six groups of birds based on body weight (BW). A single chick from each group was allocated to a cage resulting in six birds per cage with each cage having had a similar mean BW and BW distribution. The trial was conducted with five treatments and eight replicates (metabolic cages) per treatment. Six birds of 14 days-of-age were placed in each metabolic cage. Birds was allocated to cages such that the average BW and range in BW between cages was similar. The metabolic cages (n = 40) are in a closed building (Experimental Farm, University of Pretoria) fitted with lights, ventilation, and air conditioning. Temperature was closely controlled for optimum bird comfort. Birds were placed in the metabolism cages at the start of day 14.

At 21 days-of-age, feed was removed, and birds were fasted for six hours, after which excreta collection pans were cleaned out. A fixed quantity of 300 g/test source maize was allocated to each battery cage feeder (50 g/bird or 300 g/feeder) immediately following the fasting period. Birds were fed the test diets (ground maize) for a six-hour feeding period. Test diets were removed and weighed back after six hours. After which birds were left in cages with only access to water for a further twelve hours. Excreta was collected during the six hours feeding as well as during the twelve hours after test source removal (post-feeding portion). The birds were removed after the twelve hours fast and the excreta was allowed to air dry for 24 hours before it was collected.

3.9.2. Basal substitution method

For the basal substitution method, all birds were fed a single standard broiler pre-starter and starter diet formulated to achieve optimum performance for all birds according to Industry breed standards for 0 to 7 and 8 to 19 days-of-age, respectively. At 14 days-of-age, all birds were weighed individually and divided into six groups of birds based on body weight (BW). A single chick from each group was allocated to a cage resulting in six birds per cage with each cage having had a similar mean BW and BW distribution.

The trial was conducted as a 5 x 2 factorial with five test maize sources replacing 40% of a reference diet with/without XAP enzyme. The reference diet was also fed alone as an additional treatment. There were eight replicates (metabolic cages, n = 88) per treatment.

From 19 days-of-age, at 08:00 am birds in the first block (Tier) were put onto the test diets and clean excreta pans were placed. Each tier of six cages was staggered in time by 5 minutes to ensure feed was provided to birds at an exact time. On 20, 21, and 22 days-of-age after 24h, 48h and 72h periods, excreta collection began at 08:00 am and followed the same time-frame of feed allocation by removing excreta trays from each tier in 5 min. intervals.

3.10. Chemical analysis

All excreta were removed, weighed, and dried in a force draught oven at 80˚C for 24 hours. Dried excreta was be weighed and ground (mortar and Pestle), then double-bagged and labelled with sample ID. 30 g of sample in duplicate were weighed into sealed plastic jars and were grouped together by treatment, and then sent to the University of Arkansas Central Analytical Laboratory (1260 W. Maple, POSC L-209. University of Arkansas Fayetteville, AR) for analysis. The University of Arkansas Central Analytical Laboratory analysed excreta samples for moisture, protein (as nitrogen), and gross energy. Chem Nutri Analytical Laboratory (4 Porcelain Road, Clayville, Johannesburg, Gauteng) analysed excreta samples for titanium marker.

Three samples of approximately 500 g each were taken at the end of the diet preparation run of both the common pre-starter and common starter diets. One sample of each diet was retained and the other two were sent to Chem Nutri Analytical Laboratory for proximate analysis, as well as calcium, and phosphorus. One sample (of approximately 100 g) of each ground test source and treatment diet was sent to University of Arkansas Central Analytical Laboratory and analysed for moisture, protein

(as nitrogen) and gross energy along with the prepared excreta samples. Gross energy for the feed and maize was determined using a method adapted from ANSI/ASTM Method D2015-77 (1978), ANSI/ASTM Method D240 – 76 (1980), and ANSI/ASTM E144-64 (1976). Dry matter and ash content of the feed and maize samples were determined according to method 942.05 of the AOAC's official method of analysis (AOAC, 2000). Moisture was determined according to method 943.01 of the AOAC's official method of analysis (AOAC, 2000). Crude fibre was analysed according to method 962.09 of the AOAC's official method of analysis (AOAC, 2000). The crude fat content was determined in agreement to method 920.39 of the AOAC's official method of analysis (AOAC, 2000). Crude protein was analysed according to method 988.05 of the AOAC's official method of analysis (AOAC, 2000). Chem Nutri Analytical adapted the titanium analysis method from AOAC 985.01 and AOAC 2006.03 (AOAC, 2000); the technique was based on Microwave Digestion and Inductively Coupled Plasma – Optical Emission Spectrometry (ICP-OES). Salt-soluble protein content was determined in agreement to the official method (method NF-V03–741; AFNOR, 2008) as defined by Janas *et al.* (2010) (Promatest). *In vitro* rate of starch digestion within 60 minutes (RSD60) was determined according to the method by Ebsim (2013) by preparing two enzyme solutions, benzoic acid solution, and sodium acetate buffer and the use of a Megazyme kit (D-glucose assay procedure — GOPOD format, K-GLUC 07/11, Megazyme International Ireland, Bray, Co. Wicklow, Ireland).

3.11. Calculations

AME and AME_N were calculated using the following equations:

$$AME\,/\,g\,feed = \frac{(GE\,feed\;x\;Feed\;consumed(g)) - (GE\;excreta\;x\;Excreta(g))}{(Feed\;consumed(g))} \quad (1)$$

$$AME_N\,/\,g\,feed = \frac{\left((GE\,feed\;x\;Feed\;consumed(g)) - (GE\;excreta\;x\;Excreta(g))\right) - (NR\;x\;K)}{Feed\;consumed(g)} \quad (2)$$

where: 'GE feed' = gross energy content per gram of feed; 'GE excreta' = gross energy content per gram of excreta output; 'NR' = (feed intake(g) x N/g of feed(g)) – (excreta(g) x N/g of excreta(g)) and 'K' is a constant (either 8.22 kcal/g or 34.4 kJ/g as proposed by Hill & Anderson (1958)).

The following equations were used to calculate AME and AME_N for the basal substitution method (adapted from Sibbald *et al.* (1960)):

$$AME/(g).feed = GE./(g).feed - \left(\frac{marker/(g)feed}{(marker/(g)excreta} x\ GE/(g)excreta\right) \quad (1)$$

$$AME_N/(g).feed =$$

$$AME/(g).feed - (GN/(g).feed - (\frac{marker/(g).feed}{marker/(g).excreta}\ x\ GN/(g).excreta))\ x\ 8.22$$

$$AME/(g).test\ ingredient = \frac{(ME/(g)test\ diet \quad (M.E/(g)basal\ diet\ x \frac{\%\ basal\ in\ test\ diet}{100}))}{\%\ test\ material\ in\ test\ diet}$$

Where: AME = apparent metabolisable energy, AME_N = nitrogen-corrected apparent metabolizable energy, GE = gross energy and GN = gross nitrogen and 'K' is a constant (either 8.22 kcal/g or 34.4 kJ/g as proposed by Hill & Anderson (1958)).

Total tract digestibility was calculated using the following equation (Kong & Adeola, 2014):

$$\text{Digestibility (\%)} = [1 - (Mi/Mo) \times (Eo/Ei)] \times 100,$$

where Mi represents the concentration of titanium dioxide in the diet in grams per kilogram of DM; Mo represents the concentration of titanium dioxide in the excreta and ileal digesta in grams per kilogram of DM output; Ei represents the concentration of DM, CP, energy, or EE in the diet in milligrams per kilogram of DM; and Eo represents the concentration of DM, CP, energy, or EE in the excreta and ileal digesta in milligrams per kilogram of DM.

The AME, or AME_N, of the test ingredients was calculated using the 'difference' method, using AME as an example (Olukosi, 2020):

$$AME_{tf} = \{AME_{td} - (AME_{rd}\ \mathbf{x}\ PC_{bd})\}/\ PC_{tf}$$

where AME_{tf}, AME_{td} and AME_{rd} were the metabolisable energy content (kcal/kg) of the test ngredient, test diet and reference diet, respectively. PC_{tf} was the proportion (adjusted for additional energy) of the test ingredient in the test diet. PC_{bd} (proportion of reference diet, corrected for additional energy, in the test diet) was, by definition, $1 - PC_{tf}$.

3.12. Statistical analysis

For the first study, the Pearson product-moment correlation coefficients of all variables were determined using the multivariate analysis test (JMP Pro 13.1). The Multiple Regression Model Fit test (JMP Pro 13.1) was used to analyse the relationship between AME_N and all parameters, and for model development using stepwise regression to reduce the Bayesian Information Criterion (BIC).

For the second study, the data was analysed as two experiments, 1) evaluating the method and maize variant effect 2) evaluating the XAP response on the maize variants. Data was analysed as a full factorial with 5 maize, and 2 methods and 5 maize and 2 enzyme inclusions. All data was analysed in SAS (version 12.1) using full factorial to evaluate main and interaction effects. Block was included as a random effect. Where the model was significant, means were separated using protected LS means at $P<0.05$. To evaluate the response in measured AME_N vs. predicted NIT AME_N, data was analysed using Proc Mixed of SAS, with Predicted NIT AME_N as a dependent continuous variable and measured AME_N as a class variable. Block was included in the model as a random effect.

Chapter 4: Results

4.1. Pearson correlation coefficients

Significant positive correlations were observed (Table 4.1) between AME_N and crude fat (r = 0.63, *P<0.0001*), crude protein (r = 0.58, *P<0.0001*), and milling index (MI) (r = 0.55, *P<0.0001*). A significant negative correlation between AME_N and maize starch (r = -0.60, *P<0.0001*) and hectolitre mass (L/kg) (r = -0.47, *P<0.0001*).

Table 4.1. Pearson correlation between variables using 1110 maize samples (all values for nutrients are on a dry matter basis)

Variable	by Variable	Correlation	Signif Prob
Crude fat (%)	AME_N (kcal/kg)	0.6305	<0.001
Crude protein (%)	AME_N (kcal/kg)	0.5843	<0.001
Crude protein (%)	Crude fat (%)	0.1301	<0.001
Starch (%)	AME_N (kcal/kg)	-0.603	<0.001
Starch (%)	Crude fat (%)	-0.3487	<0.001
Starch (%)	Crude protein (%)	-0.8567	<0.001
Hectolitre mass (L/kg)	AME_N (kcal/kg)	-0.4696	<0.001
Hectolitre mass (L/kg)	Crude fat (%)	-0.1617	<0.001
Hectolitre mass (L/kg)	Crude protein (%)	-0.6345	<0.001
Hectolitre mass (L/kg)	Starch (%)	0.6895	<0.001
Milling, index	AME_N (kcal/kg)	0.5501	<0.001
Milling, index	Crude fat (%)	0.1307	<0.001
Milling, index	Crude protein (%)	0.5707	<0.001
Milling, index	Starch (%)	-0.3934	<0.001
Milling, index	Hectolitre mass L/kg	-0.4556	<0.001

Signif prob = significant probability *P<0.05*.

4.2. NIT AME_N vs CVB (2012)

The comparison of near-infrared transmittance (NIT) AME_N values of 50 samples from three harvest seasons (2015/2016, 2016/2017 and 2017/2018) that were collected for *in vitro* analysis and a further 10 more samples that were collected for the animal trial, during the 2018/2019 harvest season. The AME_N values of the samples were plotted on a scatter against the CVB ME values. The CVB (2012) prediction equation was not able to adequately explain the measured variation in maize AME_N ($R^2 = 0.29$) (Figure 4.1.)

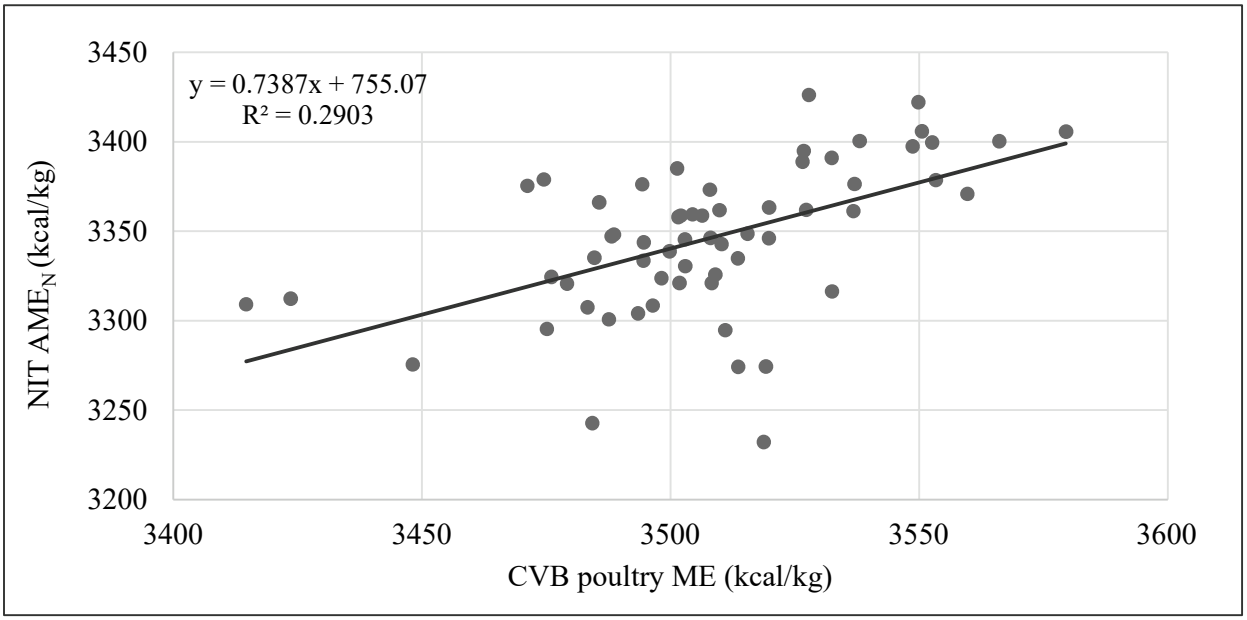


Figure 4.1. Near-infrared transmittance predicted nitrogen-corrected apparent metabolisable energy (AME_N) plotted against CVB poultry metabolisable energy of the 60 maize samples from four harvest seasons (2015/2016, 2016/2017, 2017/2018, and 2018/2019)

4.3. Final prediction model

The Multiple Regression Model Fit test (JMP Pro 13.1) was used to analyse the relationship between AME_N and all parameters, and for model development using stepwise regression to reduce the Bayesian Information Criterion (BIC). The final model, AME_N = 3589.8 + 37.59 * crude fat + 9.76 * crude protein – 30.32 * moisture + 0.30 * MI, had an R^2 of 0.875 and root mean square error (RMSE) of 8.94 kcal/kg. The final model was validated on an independent dataset of 787 samples and predicted AME_N with an R^2 of 0.949 (Figure 4.2). The R^2 of this model was 0.89 with a root mean square error (RMSE) of 8.95 kcal/kg. The starch component of maize did not significantly affect AME_N (*P = 0.07*) after other independent variables were included. The final model was validated on an independent dataset of 787 samples from the 2017/2018 harvest year and predicted AME_N with an R^2 of 0.949 (Figure 4.2).

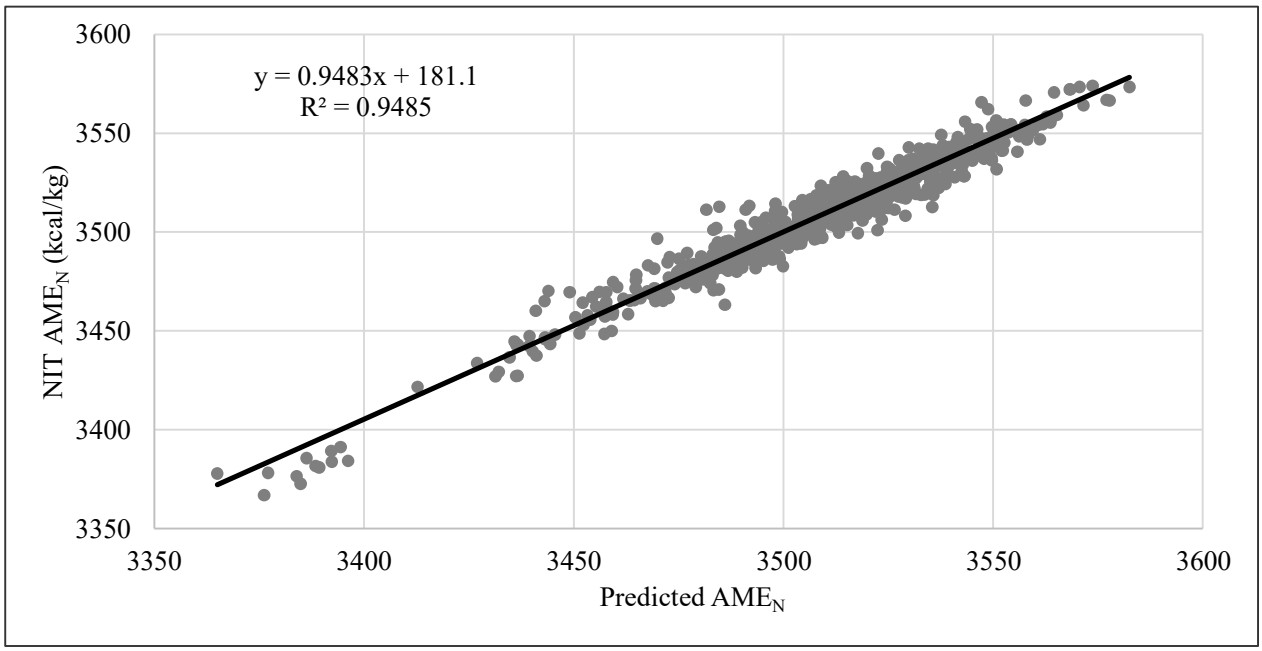


Figure 4.2. Near-infrared transmittance predicted nitrogen-corrected apparent metabolisable energy (AME_N) plotted against the predicted nitrogen corrected apparent metabolisable energy of 787 maize samples from the 2017/2018 harvest year

4.4. Results from digestibility study using two different methods

A significant (*P<0.05*) difference was observed (Table 4.2) between total collection (TC) and basal substitution with XAP (BS-XAP) for AME_N. However, no significant (P<0.05) difference was observed between TC and BS for AME_N. Significant (*P<0.05*) differences were observed between maize variants for AME_N. A significant (*P<0.05*) difference was observed (Table 4.3) between methods for TC and BS and BS-XAP for DM digestibility. Similar was observed (Table 4.5) between methods for GE digestibility. A significant (*P<0.05*) difference was observed between methods (Table 4.4) between TC and BS as well as TC and BS-XAP for N digestibility between methods.

Table 4.2. Effect of method on different maize variants on AME_N

Maize Variant	TC (kcal/kg)	BS (kcal/kg)	BS-XAP (kcal/kg)	Mean (kcal/kg)
1	3655.74	3754.98	3850.04	3753.59^{C}
2	3752.32	3839.98	3868.77	3820.36^{BC}
3	3803.84	3837.93	3836.28	3826.02^{BC}
4	3812.52	3848.66	3907.25	3856.15^{AB}
5	3834.85	3918.48	4044.66	3932.66^{A}
Mean	3771.86^{b}	3840.01^{ab}	3901.40^{a}	
SEM	*57.52*	*58.78*	*57.36*	*33.74*

[a,b] Row means without a common superscript differ (*P < 0.05*).
[A,B] Column means without a common subscript differ (*P < 0.05*).
TC = Total Collection, BS = Basal substitution without XAP, BS-XAP = Basal substitution with XAP.

Table 4.3. Effect of method on different maize variants on DM digestibility

Maize Variant	TC (%)	BS (%)	BS-XAP (%)	Mean (%)
1	86.38	76.81	76.25	79.82
2	87.45	75.11	74.91	79.30
3	88.01	75.39	75.60	79.82
4	87.45	73.85	75.95	79.08
5	86.09	74.66	77.73	79.49
Mean	87.16^{a}	75.16^{b}	76.09^{b}	
SEM	*0.543*	*0.543*	*0.543*	*0.702*

[a, b] Row means without a common superscript differ (*P < 0.05*).
TC = Total Collection, BS = Basal substitution without XAP, BS-XAP = Basal substitution with XAP.

Table 4.4. Effect of method on different maize variants on N digestibility

Maize Variant	TC (%)	BS (%)	BS-XAP (%)	Mean (%)
1	47.96	63.48	61.81	57.75
2	53.31	59.95	60.84	58.03
3	59.09	61.76	60.02	60.29
4	60.24	59.08	59.13	59.48
5	51.99	62.85	63.43	59.42
Mean	54.52[b]	61.59[a]	61.05[a]	
SEM	*4.049*	*4.161*	*4.049*	*2.360*

[a, b] Row means without a common superscript differ ($P < 0.05$).
TC = Total Collection, BS = Basal substitution without XAP, BS-XAP = Basal substitution with XAP.

Table 4.5. Effect of method on different maize variants on GE digestibility

Maize Variant	TC (%)	BS (%)	BS-XAP (%)	Mean (%)
1	86.44	79.94	80.25	82.20
2	87.96	79.41	78.94	82.10
3	88.17	79.22	79.50	82.30
4	87.59	77.91	79.91	81.91
5	86.32	79.07	81.60	82.33
Mean	87.29[a]	79.11[b]	80.10[b]	
SEM	*1.140*	*1.140*	*1.155*	*0.658*

[a, b] Row means without a commonsuperscript differ ($P < 0.05$).
TC = Total Collection, BS = Basal substitution without XAP, BS-XAP = Basal substitution with XAP.

Several correlations (Table 4.6) were observed amongst the measured proximates, AME_N, RSD 60, promatest and predicted MI and GYA. A significant positive correlation was observed between protein and crude fat (r = 0.91, *P<0.0001*). A significant positive correlation was observed between crude fibre and crude fat (r = 0.86, *P<0.0001*), crude protein (r = 0.72), and moisture (r = 0.37, *P<0.0001*). A significant positive correlation was observed between MI and moisture (r = 0.35, *P<0.0001*) and promatest (r = 0.77, *P<0.0153*). A significant positive correlation was observed between GYA and moisture (r = 0.35, *P<0.0001*) and promatest (r = 0.77, *P<0.0001*). A significant negative correlation was observed MI and RSD 60 (r = -0.43, P<0.0001). A significant positive correlation was observed between measured AME_N and crude fat (r = 0.34, *P<0.0010*) and crude protein (r = 0.38, *P<0.0003*). A significant negative correlation was observed between measured AME_N and MI (r = -0.25, *P<0.0164*) and GYA (r = -0.25, *P<0.0162*).

Table 4.6. Pearson correlation between variables using 5 maize samples from the digestibility study (all values are on a dry matter basis except for moisture)

Variable	by Variable	Correlation	Count	Lower 95%	Upper 95%	Signif Prob
Crude protein (%)	Crude fat (%)	0.9139	120	0.8786	0.9393	<0.0001
Moisture (%)	Crude fat (%)	0.1708	120	-0.0087	0.3396	0.0622
Moisture (%)	Crude protein (%)	-0.1175	120	-0.2906	0.0631	0.2014
Crude fibre (%)	Crude fat (%)	0.8363	120	0.7730	0.8832	<0.0001
Crude fibre (%)	Crude protein (%)	0.7205	120	0.6215	0.7968	<0.0001
Crude fibre (%)	Moisture (%)	0.3747	120	0.2095	0.5190	<0.0001
RSD 60 (%)	Crude fat (%)	0.0971	120	-0.0836	0.2716	0.2913
RSD 60 (%)	Crude protein (%)	0.0066	120	-0.1729	0.1856	0.9431
RSD 60 (%)	Moisture (%)	-0.1470	120	-0.3178	0.0331	0.1092
RSD 60 (%)	Crude fibre (%)	0.0317	120	-0.1484	0.2097	0.7315
Promatest (mg protein/100 mL)	Crude fat (%)	-0.0501	120	-0.2273	0.1303	0.5868
Promatest (mg protein/ 100 mL)	Crude protein (%)	0.0271	120	-0.1528	0.2054	0.7686
Promatest (mg protein/ 100 mL)	Moisture (%)	0.0721	120	-0.1086	0.2481	0.4340
Promatest (mg protein/ 100 mL)	Crude fibre (%)	0.0567	120	-0.1238	0.2335	0.5388
Promatest (mg protein/ 100 mL)	RSD 60 (%)	-0.7413	120	-0.8126	-0.6482	<0.0001
NIT MI	Crude fat (%)	-0.0676	120	-0.2439	0.1130	0.4634
NIT MI	Crude protein (%)	-0.0983	120	-0.2727	0.0824	0.2856
NIT MI	Moisture (%)	0.3515	120	0.1838	0.4993	<0.0001
NIT MI	Crude fibre (%)	0.2209	120	0.0434	0.3849	0.0153
NIT MI	RSD 60 (%)	-0.4312	120	-0.5667	-0.2731	<0.0001
NIT MI	Promatest (mg protein/ 100 mL)	0.7671	120	0.6816	0.8320	<0.0001
NIT GYA	Crude fat (%)	-0.0685	120	-0.2448	0.1121	0.4570
NIT GYA	Crude protein (%)	-0.0975	120	-0.2720	0.0832	0.2894
NIT GYA	Moisture (%)	0.3508	120	0.1831	0.4987	<0.0001
NIT GYA	Crude fibre (%)	0.2184	120	0.0408	0.3827	0.0165
NIT GYA	RSD 60 (%)	-0.4383	120	-0.5726	-0.2811	<0.0001
NIT GYA	Promatest (mg protein/ 100 mL)	0.7714	120	0.6871	0.8351	<0.0001
NIT GYA	NIT MI	1.0000	120	0.9999	1.0000	<0.0001
In vivo AME_N of Maize (kcal/kg)	Crude fat (%)	0.3401	90	0.1431	0.5112	0.0010
In vivo AME_N of Maize (kcal/kg)	Crude protein (%)	0.3761	90	0.1833	0.5411	0.0003

In vivo AME_N of Maize (kcal/kg)	Moisture (%)	-0.1771	90	-0.3706	0.0311	0.0949
In vivo AME_N of Maize (kcal/kg)	Crude fibre (%)	0.2021	90	-0.0052	0.3928	0.0561
In vivo AME_N of Maize (kcal/kg)	RSD 60 (%)	0.1850	90	-0.0230	0.3776	0.0809
In vivo AME_N of Maize (kcal/kg)	Promatest (mg protein/ 100 mL)	-0.1073	90	-0.3076	0.1020	0.3140
In vivo AME_N of Maize (kcal/kg)	NIT MI	-0.2524	90	-0.4367	-0.0478	0.0164
In vivo AME_N of Maize (kcal/kg)	NIT GYA	-0.2528	90	-0.470	-0.0483	0.0162

Signif prob: significant probability (*P<0.05*).
NIT: near-infrared transmittance.
MI: milling index.
GYA: grit yield all.
AMEN: apparent metabolisable energy nitrogen corrected.
RSD 60: rate of starch digestion over 60 minutes.

Maize AME_N determined by the total collection (TC) and basal substitution (BS) methods were similar (Figure 4.3) but significantly lower than the NIT AME_N ($P<0.05$) (Figure 4.4) for all five maize variants. On average, the XAP enzyme significantly increased the AME_N value of maize by approximately 60 kcal/kg dry matter (DM), with no interaction of XAP enzyme effect and maize variant ($P>0.1$) (Figure 4.5).

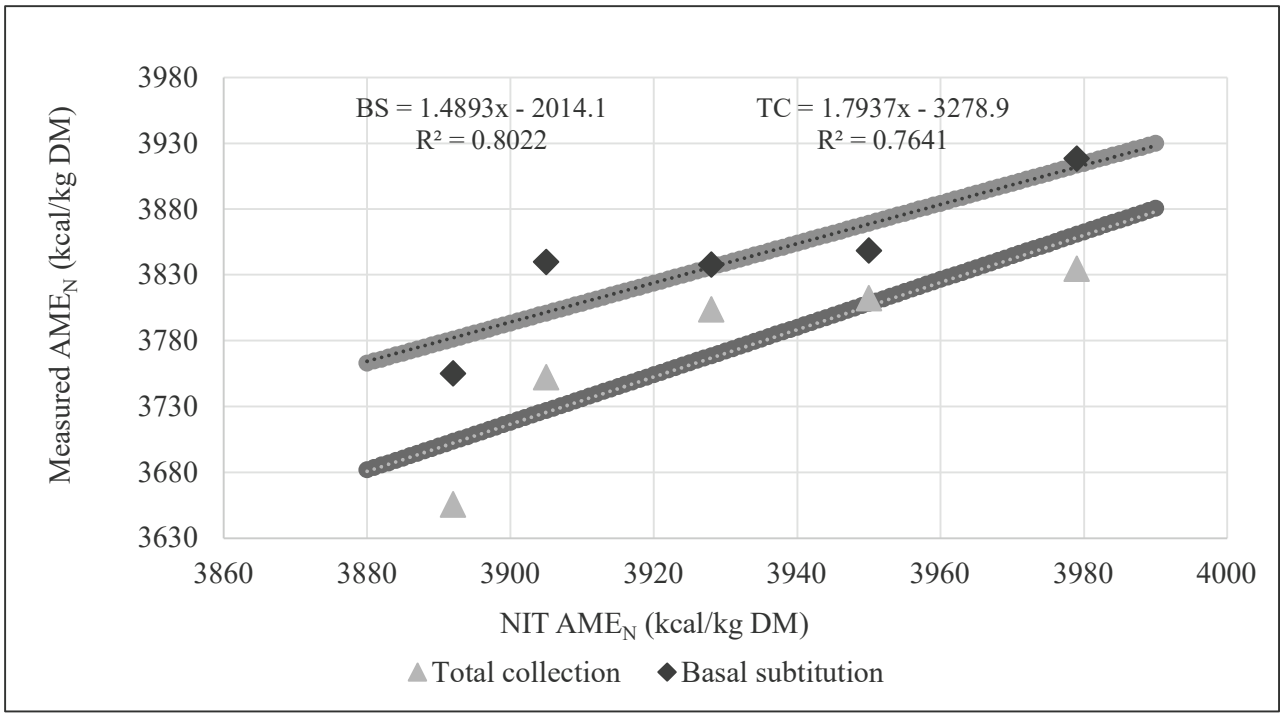


Figure 4.3. Comparison of measured nitrogen-corrected metaboliasable energy (AME_N) of the total collection (TC) method against the basal substitution method of five maize samples used in the digestibility study

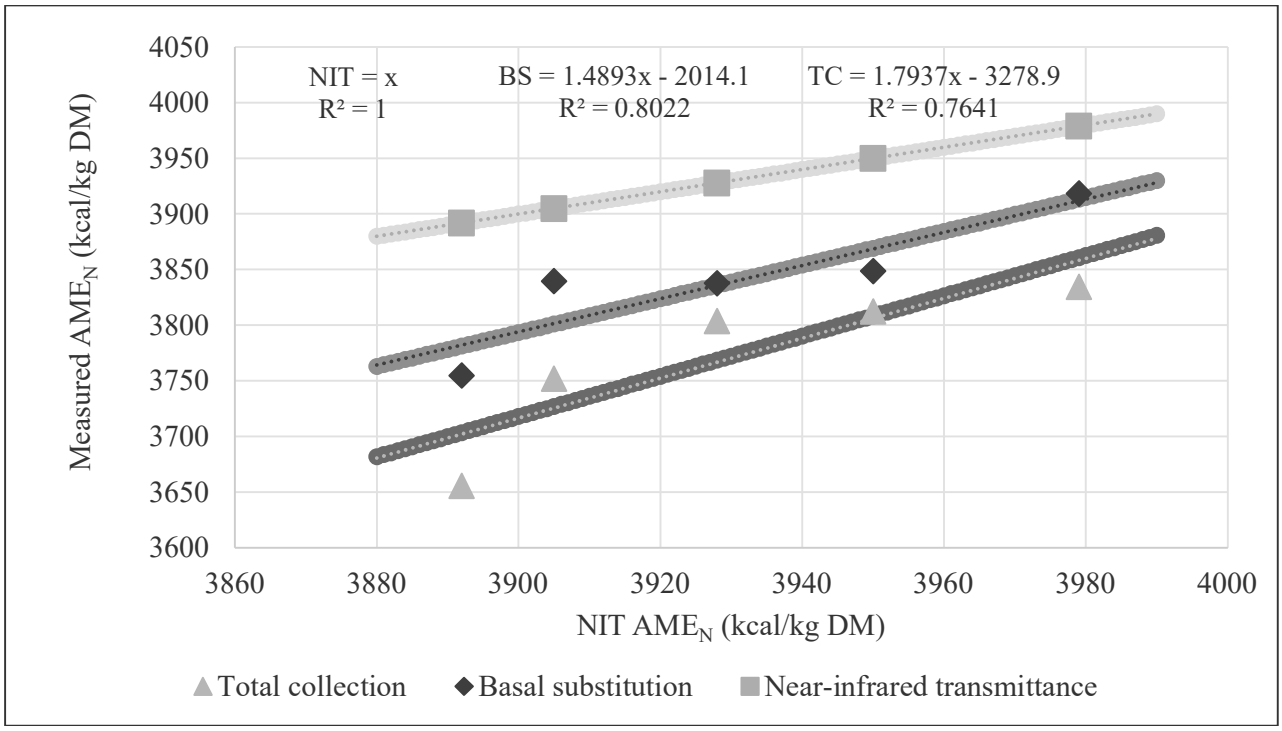


Figure 4.4. Comparison of near-infrared transmittance (NIT) predicted nitrogen-corrected apparent metabolisable energy against measured nitrogen-corrected metaboliasable energy (AME_N) of the total collection (TC) method and the basal substitution method of five maize samples used in the digestibility study

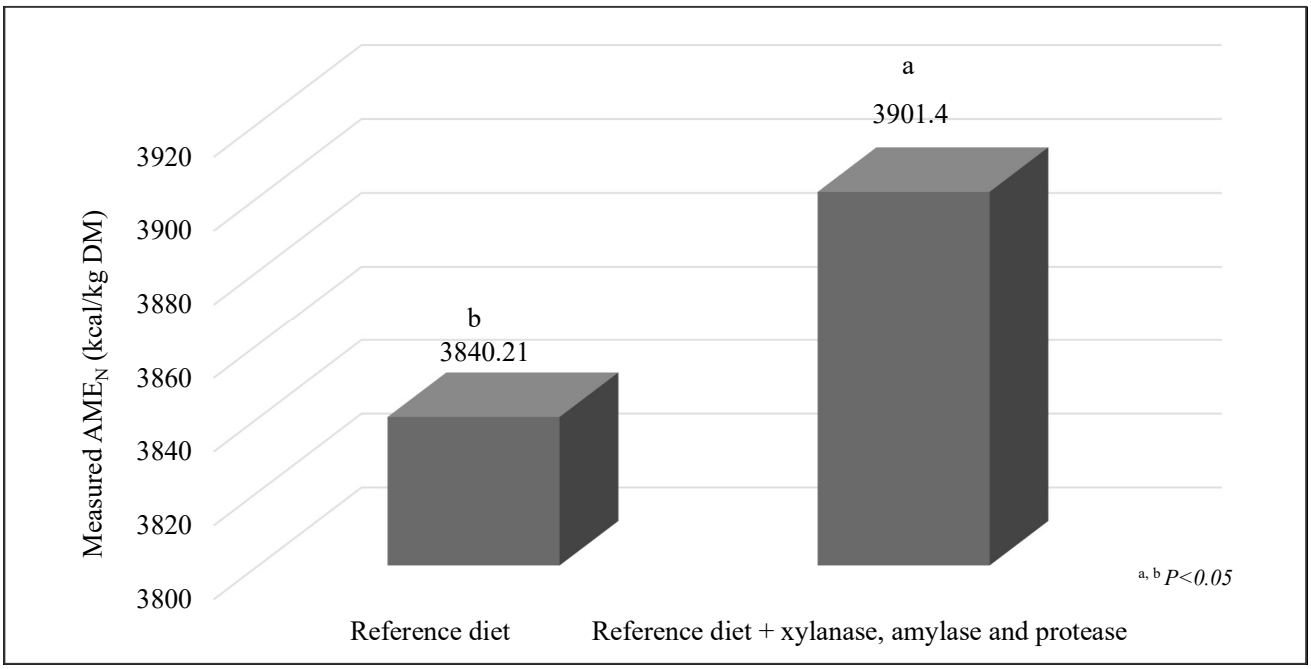


Figure 4.5. A bar graph of the effect of xylanase, amylase, and protease (XAP) enzyme combination on the nitrogen-corrected apparent metabolisable energy of five maize samples used in the digestibility study

Chapter 5: Discussion

5.1. Intrinsic factors contributing to the AME_N of maize

The objective of this study was to determine the AME_N of maize samples and investigate the intrinsic factors that contribute to the AME_N of maize for broiler chickens.

The study demonstrates a positive correlation (Table 4.1) between AME_N and crude fat (r = 0.63, *P<0.0001*), and crude protein (r = 0.58, *P<0.0001*). A significant negative correlation between AME_N and maize starch (r = -0.60, *P<0.0001*) can be explained by starch in-turn being negatively correlated with crude fat (r = -0.34, *P<0.0001*) and crude protein (r = -0.85, *P<0.0001*) of maize. Physical kernel properties of maize that were influential in explaining variance in AME_N included hectolitre mass (L/kg) (r = -0.47, *P<0.0001*); milling index (MI) (r = 0.55, *P<0.0001*); and grit yield all (GYA) (r = 0.52, *P<0.0001*). Correa *et al.* (2002) discovered increased vitreousness in maize caused a linear decrease in the availability of starch. This in an agreement with the findings from this study as a significant negative correlation was observed between MI and starch (r = 0.39, *P<0.001*). Kernel density is a sign of hardness and maturity (Tilley, 1998). This is positively correlated to starch content and negatively correlated to crude fibre, crude protein, and ash content (Baidoo *et al.,* 1991). This was also observed in this study as positive correlation was observed between hectolitre mass and starch (r = 0.69, *P<0.0001*) and negative correlation with crude protein (r = -0.63, *P<0.0001*).

The final model (Figure 4.2), AME_N = 3589,8 + 37,59 * crude fat + 9,76 * crude protein – 30,32 * moisture + 0,30 * MI, had an R^2 of 0,875 and root mean square error (RMSE) of 8,94 kcal/kg. The final model was validated on an independent dataset of 787 samples and predicted AME_N with an R^2 of 0,949 (Figure 4.2). The R^2 of this model was 0.89 with a root mean square error (RMSE) of 8.95 kcal/kg. The starch component of maize did not significantly affect AME_N (*P = 0.07*) after other independent variables were included.

Several correlations (Table 4.6) were observed amongst the measured proximates, AME_N, RSD 60, promatest and predicted MI and GYA. A significant positive correlation was observed between crude protein and crude fat (r = 0.91, *P<0.0001*) can be explained by protein and oil being found in the germ. The relative concentration of crude protein and crude fat is highest in the kernel germ (FAO, 1992). A significant positive correlation was observed between crude fibre and crude fat (r = 0.86, *P<0.0001*), crude protein (r = 0.72), and moisture (r = 0.37, *P<0.0001*). A significant positive

correlation was observed between MI and moisture (r = 0.35, *P<0.0001*) and promatest (r = 0.77, *P<0.0153*). A significant positive correlation was observed between GYA and moisture (r = 0.35, *P<0.0001*) and promatest (r = 0.77, *P<0.0001*) (Williams *et al*., 2009). Hard kernels have a higher percentage of glossy endosperm; soft kernels have a higher percentage of floury endosperm, while intermediate kernels have an even distribution of glossy and floury endosperm (Williams *et al*., 2009). The majority of the protein in maize is found in the glossy/hard endosperm of the kernel. Solubility and molecular structure of protein is changed due to thermal denaturation by improper drying methods, resulting in decreased availability of amino acids and the impairment of water and α-amylase to the starch granules (Gehring *et al.,* 2013), thus, impairing the separation of starch from protein explaining the correlation observed between MI, moisture, and protein. GYA is linearly correlated with the MI and indicates the real quantity of total hard endosperm that can be removed from the maize during Roff milling (SAGL., 2021), therefore, they have similar correlations. A significant negative correlation was observed between MI and RSD 60 (r = -0.43, *P<0.0001*) as well as between GYA and RSD 60 (r = -0.44, *P<0.0001*). In the previous study we observed that starch is negatively correlated to protein and protein is found in the horny endosperm that is majority of the MI and GYA milled fractions. Therefore, the negative correlation can be explained by the fact that a higher horny endosperm content can result in a lower rate of starch digestion due to a higher percentage of starch granules that are encapsulated in the endosperm (Gehring *et al.*, 2013). A significant positive correlation was observed between measured AME_N and crude fat (r = 0.34, *P<0.0010*) and crude protein (r = 0.38, *P<0.0003*). The crude fat is found almost entirely in the germ portion of the kernel and for every 0.1% increase in crude fat content, the available energy content of the maize is estimated to increase by approximately 4.5 kcal/kg (Summers, 2001). A significant negative correlation was observed between measured AME_N and MI (r = -0.25, *P<0.0164*) and GYA (r = -0.25, *P<0.0162*). For these reasons the null hypothesis stating, Changes in the proximate composition and intrinsic kernel characteristics of maize do not contribute to the variation in AME_N value of maize, was rejected. This result contradicts the results from the previous study as a significant positive correlation was observed. This can only be explained by the negative correlation between MI and crude fat (r = -0.0676, *P = 0.4634*) and crude protein (r = -0.0983, *P = 0.2856*), however, not significant. Based on this results and correlations the null hypothesis stating, Changes in the proximate composition and intrinsic kernel characteristics of maize do not contribute to the variation in AMEN value of maize, was rejected.

5.2. Effect of method on the AME_N and digestibility of maize

Estimating the AME of a feed ingredient accurately is important for poultry nutritionists because it allows them to formulate diets that accurately meet the bird's energy requirements. As a result, it is critical to evaluate the AME values of feed ingredients. Because different energy sources in poultry diets have different physicochemical compositions, their interaction in the gastrointestinal tract with other cereal grains or alternative energy sources may affect digestibility and energy utilization in different feed ingredients. Furthermore, current feed ingredients and birds are different than in previous decades, necessitating a re-evaluation of AME diets and feed ingredients, as well as research into the various in vivo methods for determining AME. The most commonly used method to determine ME in broiler diets is total excreta collection/direct feeding, which is based on measuring feed intake and total faecal excretion over a set period (Dourado *et al.,* 2010). However, there are factors that interfere with the results from this method such as contamination with feed, feathers, and intestinal mucosa sloughing, thus limiting the accuracy of the method (Dourado *et al*., 2010). The marker/basal substitution method involves the inclusion of a marker to the experimental diet. This method determines the ME by the ratio of indigestible substances present in the diet and excreta which depends on the principle that the total amount of inert indicator substance excreted equals the amount ingested during a given period (Choct, 2004). Therefore, there is no requirement for the measurement of feed intake and excreta (Vogtmann *et al*.,1974). The utilisation of markers reduces the errors that occur due to incorrect measurement of feed intake and excreta output (Smeets *et al*., 2015). The convenience of using a marker is that it is accurate, simple, and only a small sample size is required (Short *et al*., 1996; Sales & Janssens, 2003). A basal diet is formulated to meet all nutrient requirements and energy, and a proportion of the basal diet is substituted by the test ingredient to produce a test diet (Sibbald *et al*., 1960).

The objective of this study was to determine absolute differences in AME_N of maize samples using the total collection method vs. the basal substitution method. The difference that was determined can be used as an adjustment factor to migrate predicted AME_N values of the NIT to the new system of determination. No significant ($P<0.05$) difference was observed between TC and BS for AME_N (Table 4.2). However, their slopes are similar, indicating that basal substitution can be used as an effective method to determine the AME_N value of maize (Figure 4.3). This was in agreement with Dourado *et al.* (2010) that found a significant difference in AME between the two methods, however, when the AME values of the basal substitution method were corrected by the marker recovery rate there was no longer a significant difference between the two methods. Liu *et al.,* (2019) conducted a study that compared the direct and substitution methods to determine the nutrient digestibility of maize and soyabean meal in growing pigs and observed no significant differences in gross energy between the two methods. Based on the results from the study the null hypothesis stating that, there is

not a significant difference in the value of AME_N of maize determined using the total collection method vs. the basal substitution method, was rejected. Significant (*P<0.05*) differences were observed between TC and BS for all maize variants for DM digestibility (Table 4.3). Similar was observed for GE digestibility as well as a significant (*P<0.05*) difference was observed between TC and BS for N digestibility between methods (Table 4.5). This may be due to the BS method having an adaptation period and sampling being conducted over several days compared to the TC method only having a single sampling and several fasting periods. This would change the dynamics of the digestive system. Significant (*P<0.05*) differences were observed between maize variants for AME_N, N and GE digestibility. When comparing the NIT predicted AME_N to the measured AME_N, we observed that the slope of the lines was similar meaning the NIT AME_N was able to describe the increment in measured AME_N with a similar slope (Figure 4.4). Significant (*P<0.05*) differences were observed between NIT predicted AME_N, and measured AME_N, regardless of the method, therefore, a bias adjustment was needed for the NIT prediction of AME_N to correct for this. Therefore, based on the results the null hypothesis stating that, NIT technology does not accurately measure the variation in the AME_N value of maize in comparison to *in vivo* methods, was rejected.

5.3. Effect of xylanase, amylase and protease enzyme combination on the AME_N and digestibility of maize

The gastro-intestinal tract (GIT) of broilers lacks the necessary enzymes to completely digest the nutrients of some ingredients, so exogenous enzymes are used to improve access to nutrients that would otherwise be unavailable. Exogenous enzymes such as xylanase, amylase, and protease are increasingly being used in maize-soy based broiler diets to manage maize variability and improve nutrient digestibility (Cowieson, 2010). The effects of modified maize diets on starch digestibility have been linked to the development of new enzymes that can improve the digestion and access to the cell contents of resistant starches. These new enzymes can also mitigate the negative effects of antinutritional factors derived from soy and/or maize (Tang *et al.,* 2014). The objective of this study was to determine the effects of maize AME_N on XAP enzyme energy uplift and matrix values using the Basal substitution method. In current diets formulated with maize and soyabean meal, an estimated 450 kcal/kg of energy is released for use by exogenous enzymes, that can contain up to 37% from undigested starch (Stefanello *et al.,* 2019). Carbohydrases have been progressively used in maize soyabean meal-based diets, with exogenous amylases being able to supplement endogenous amylase output and allow for greater starch breakdown (Stefanello *et al.,* 2019).

In this study, the Estimates of Main effect of Method (BS or BS-XAP) had a difference of 61.39 kcal/kg DM (3840.01 vs. 3901.40 kcal/kg DM) that shows that on average the XAP enzyme increased the AME_N value of maize by ~60 kcal/kg DM, with absolute effects of XAP on maize varying numerically between maize variants (*P>0.1*). This may have been due to the lack of a reference diet that included XAP as could have been used as a reference point for the energy uplift from the reference diet alone. This is in accordance with Amerah *et al*. (2017) with an improved AME_N of ~58 (kcal/kg DM) between the negative control (NC) and XAP treatment diet. Tang et al. (2014) reported on average an improvement of ~110 and ~ 79 kcal/kg DM for enzyme A and B, respectively. A significant difference was also observed for DM digestibility between the negative control and enzyme treatments. Liu *et al.* (2014) observed a significant increase in maize AME_N with the addition of a xylanase, amylase, and protease enzyme combination. A significant difference may not have been observed in this study, however, there was an effect on the AME_N of the maize variants. Based on this the null hypothesis stating, the maize quality and starting AME_N of maize does not affect enzyme response, was rejected. No significant differences were observed between BS and BS-XAP for DM, N and GE digestibility, however, numerical differences were observed within maize variants. Amerah *et al*. (2017) and Tang *et al.* (2014) reported a significant difference in N and GE digestibility between the NC and XAP treatment.

Chapter 6: Conclusion

Results showed that variance in the AME_N of maize arose from differences in intuitive parameters, as well as physical kernel properties. In addition to proximate composition, intrinsic factors of the kernel that contribute to observed variation in the AME_N of maize should be considered when predicting AME_N for broilers. Results showed no significant effect of Method between TC and BS, or interaction of Method x NIT AME_N value of maize. The NIT AME_N was able to describe the increment in measured AME_N with a similar slope. Significant differences were observed in the intercept between NIT predicted AME_N, and measured AME_N, regardless of the method. Based on this a biased adjustment on the NIT prediction is needed to correct for this. There was a significant effect of XAP on the AME_N of maize, with no interaction of XAP addition and maize variant.

Chapter 7

Critical review and recommendation

The birds were only given a day to adapt to the treatment diets and collection began the day afterwards for 3 days due to the low quantity of treatment diets. There was no reference diet for XAP that possibly influenced the results. Future studies should have a 4-day adaptation with a 3-day collection and possibly also collect ileal content for apparent ileal digestible energy. Marker recovery rate should be considered for as correction factor between total collection and basal substitution method.

References

Abdollahi, M.R., 2011. Influence of feed processing on the performance, nutrient utilisation, and gut development of poultry and feed quality. A thesis presented in partial fulfillment of the requirements for the degree of Doctor of Philosophy in Poultry Nutrition.

Almeida-Dominguez, H. D., Suhendro, E. L., & Rooney, L. W. 1997. Factors affecting rapid visco analyser curves for the determination of maize kernel hardness. J. Cereal Sci. 25, 93–102.

Amerah, A. M., Ravindran, V., Lentle, R. G., & Thomas, D. G. 2007. Feed particle size: Implications on the digestion and performance of poultry. Worlds. Poult. Sci. J. 63, 439–455.

Amerah, A. M., Romero, L. F., Awati, A., & Ravindran, V. 2017. Effect of exogenous xylanase, amylase, and protease as single or combined activities on nutrient digestibility and growth performance of broilers fed maize / soy diets. Poult. Sci. 96, 807–816.

Anderson, T. J., & Lamsa, B. P. 2011. Zein extraction from maize, maize products, and coproducts and modifications for various applications: A review. Cereal Chem. 88, 159–173.

AOAC. 2000. Official Methods of Analysis. Association of Official Analytical Chemists, Washington, DC.

Augustyn, R., Lasek, O., Barteczko, J., Borowiec, F., & Smulikowska, S. 2016. The nutritive value of maize cultivars for broiler chickens. J. Anim. Feed Sci. 21, 345–360.

Baidoo, S. K., Shires, A., & Robblee, A. R. 1991. Effect of kernel density on the apparent and true metabolizable energy value of maize for chickens. Poult. Sci. 70, 2102–2107.

Barnwal, P., Kadam, D.M. & Singh, K.K., 2012. Influence of moisture content on physical properties of maize. Int. Agrophys. 26(3), 331-334.

Bartov, I. 1996. Effect of storage duration on the nutritional value of maize kernels for broiler chicks. Poult. Sci. 75, 1524–1527.

Beyer, M., Chudy, A., Hoffmann, L., Jentsch, W., Laube, W., Nehring, K. and Schiemann, R., 2003. Rostock feed evaluation system. Research Institute for the Biology of Farm Animals (FBN), Dummerstorf, Germany.

Blandino, M., Mancini, M. C., Peila, A., Rolle, L., Vanara, F., & Reyneri, A. 2010. Determination ofmaize kernel hardness: Comparison of different laboratory tests to predict dry-milling performance. J. Sci. Food Agric. 90, 1870–1878.

Blok, M.C., Brandsma, G., Bosch, G., Gerrits, W.J., Jansman, A.J. and Everts, H., 2015. A new Dutch net energy formula for feed and feedstuffs for growing and fattening pigs (56). Wageningen UR Livestock Research.

Carpenter, K. J., & Clegg, K. M. 1956. The metabolizable energy of poultry feeding stuffs in relation to their chemical composition. J. Sci. Food Agric. 7, 45–51.

Carré, B. 2010. Causes for variation in digestibility of starch among feedstuffs. Worlds. Poult. Sci. J. 60, 76–89.

Choct, M., 2004. The net energy value for commonly used plant ingredients for poultry in Australia. Australian Government. Rural Industries Research and Develoment Corporation. RIRDC, (04), 58.

Choct, M., 2012. Feed energy-what system to use and prospects for evaluation. In XXIV World's Poult. Congress. Salvador de Bahia, Brazil (pp. 1-8).

Classen, H.L., Maenz, D.D. and Caruthers, C., 2010. Ingredient considerations, total phytate concentrations and susceptibility of phytate to hydrolysis. Proceedings of the 1st International Phytase Summit, 173-177.

Correa, C. E. S., Shaver, R. D., Kohn, K., Pereira, M. N., & Lauer, J. G. 2002. Relationship Between Maize Vitreousness and Ruminal in Situ Starch Degradability. J. Dairy Sci. 85, 3008–3012.

Cowieson, A. J. 2005. Factors that affect the nutritional value of maize for broilers. Anim. Feed Sci. Technol. 119, 293–305.

Cowieson, A. J. 2010. Strategic selection of exogenous enzymes for maize/soy-based poultry diets. J. Poult. Sci. 47, 1–7.

CVB, T. P. 2012. Chemical composition and nutritional value of feedstuffs and feeding standards. Hague, Netherlands CVB Ser.

Douglas, J. H., Sullivan, T. W., Bond, P. L., Struwe, F. J., Baier, J. G., & Robeson, L. G. 1990. Influence of grinding, rolling, and pelleting on the nutritional-value of grain sorghums and yellow maize for broilers. Poult. Sci. 69, 2150–2156.

Dourado, L. R. B., Siqueira, J. C., Sakomura, N. K., Pinheiro, S. R. F., Marcato, S. M., Fernandes, J. B. K., & Silva, J. H. V. 2010. Poultry feed metabolizable energy determination using total or partial excreta collection methods. Br. J. Poult. Sci. 12, 129–132.

Downey, G., Byrne, S., & Dwyer, E. 1986. Wheat trading in the republic of Ireland: The utility of a hardness index derived by near infrared reflectance spectroscopy. J. Sci. Food Agric. 37, 762–766.

Erasmus, C., 2003. Maize kernel translucency measurement by image analysis and its relationship to vitreousness and dry performance (Doctoral dissertation, University of Pretoria).

Esen, A. 2008. Separation of Alcohol-Soluble Proteins (Zeins) from Maize into Three Fractions by Differential Solubility. Plant Physiol. 80, 623–627.

Eyhérabide, G.H., Robutti, J.L., Percibaldi, N.M., Presello, D.A. and DEL P ALVAREZ, M., 2004. Association between grain yield and endosperm hardness in maize cultivars. Maydica, 49(4), 319-326.

Food and Agriculture Organisation (FAO)., 1992. Maize in human nutrition.
Available at: https://www.fao.org/3/t0395e/t0395e00.htm [Accessed 09 October 2019]

Food and Agriculture Organisation (FAO) Maize Production Quantity - list for 2019. Available at: https://www.fao.org/faostat/en/#data/QCL [Accessed 09 October 2019]

Fox, G., & Manley, M. 2009. Hardness methods for testing maize kernels. J. Agric. Food Chem. 57, 5647–5657.

Gaile, Z. 2008. Harvest time effect on yeild and quality of maize (Zea mays L.) grown for silage. Latv. J. Agron. 20, 104–111.

Gayral, M., Gaillard, C., Bakan, B., Dalgalarrondo, M., Elmorjani, K., Delluc, C., Brunet, S., Linossier, L., Morel, M. H., & Marion, D. 2016. Transition from vitreous to floury endosperm in maize (Zea mays L.) kernels is related to protein and starch gradients. J. Cereal Sci. 68, 148–154.

Gehring, C. K., Bedford, M. R., Cowieson, A. J., & Dozier, W. A. 2012. Effects of maize source on the relationship between in vitro assays and ileal nutrient digestibility. Poult. Sci. 91, 1908–1914.

Gehring, C. K., Cowieson, A. J., Bedford, M. R., & Dozier, W. A. 2013. Identifying variation in the nutritional value of maize based on chemical kernel characteristics. Worlds. Poult. Sci. J. 69, 299–312.

Gonzalez Mateos, G., Cámara, L., Saldaña, B., Guzmán, P., & Lazaro Garcia, R. P. 2015. Evaluating the energy content of ingredients in poultry diets. An update.

Groote, G. D. E. 1974. A comparison of a new net energy system with the metabolisable energy system in broiler diet formulation, performance, and profitability. Br. Poult. Sci. 15, 75–95.

Guelpa, A., du Plessis, A., Kidd, M., & Manley, M. 2015. Non-destructive Estimation of Maize (Zea mays L.) Kernel Hardness by Means of an X-ray Micro-computed Tomography (µCT) Density Calibration. Food Bioprocess Technol. 8, 1419–1429.

Hill, F. W., & Anderson, D. L. 1958. Comparison of metabolizable energy and productive energy determinations with growing chicks. J. Nutr. 64, 587–603.

Hruby, M. 2015. Maize nutritive value key. Feedstuffs 86, 2014–2016.

Humer, E., Schwarz, C., & Schedle, K. 2015. Phytate in pig and poultry nutrition. J. Anim. Physiol. Anim. Nutr. (Berl). 99, 605–625.

Janas, S., Boutry, S., Malumba, P., Vander Elst, L., & Béra, F. 2010. Modelling dehydration and quality degradation of maize during fluidized bed drying. J. Food Eng. 100, 527–534.

Jiang, H., Lio, J., Blanco, M., Campbell, M., & Jane, J. L. 2010. Resistant-starch formation in high-amylose maize starch during kernel development. J. Agric. Food Chem. 58, 8043–8047.

Kaczmarek, S., Cowieson, A.J., Józefiak, D., Bochenek, M. and Rutkowski, A., 2007, August. The effect of drying temperature and exogenous enzymes supplementation on the nutritional value of maize for broiler chickens. In Proc. 16th Euro. Symp. Poult. Nutr., Strasbourg, France.

Kaczmarek, S., Cowieson, A. J., Józefiak, D., & Rutkowski, A. 2013. Effect of maize endosperm hardness, drying temperature and microbial enzyme supplementation on the performance of broiler chickens. Anim. Prod. Sci. 54, 956–965.

Van Kempen, T. A. T. G., & Simmins, P. H. 1997. Near-infrared reflectance spectroscopy in precision feed formulation. J. Appl. Poult. Res. 6, 471–477.

Kilburn, J., & Edwards, H. M. 2001. The response of broilers to the feeding of mash or pelleted diets containing maize of varying particle sizes. Br. Poult. Sci. 42, 484–492.

Kingman, C. E. 1992. Classification and measurement of nutritionally important starch fractions.pdf.

Klis, J. D. van der, & Fledderus, J. 2007. Evaluation of raw materials for poultry: What's up? Eur. Symp. Poult. Nutr., 123–130.

Knudsen, B. 2014. Fiber and nonstarch polysaccharide content and variation in common crops used in broiler diets. Poult. Sci. 93, 2380–93.

Kong, C. and Adeola, O., 2014. Evaluation of amino acid and energy utilization in feedstuff for swine and poultry diets. Asian-australas. J. Anim. Sci., 27(7), 917.

Krivanek, A. F., Groote, H. De, Gunaratna, N. S., & Diallo, A. O. 2007. Breeding and disseminating quality protein maize (QPM) for Africa. African J. Biotechnol. 6, 312–324.

Larson, J., & Hoffman, P. C. 2008. Technical Note: A Method to Quantify Prolamin Proteins in Maize That Are Negatively Related to Starch Digestibility in Ruminants. J. Dairy Sci. 91, 4834–4839.

Latham, R. E., Williams, M. P., Flores, C., Masey O'Neill, H. V., York, T. W., & Lee, J. T. 2016. Impact of variable maize nutrient content, AME prediction, and xylanase inclusion on growth performance. J. Appl. Poult. Res. 25, 338–351.

Leeson, S., & Summers, J. D. 1975b. Effect of Adverse Growing Conditions on Maize Maturity and Feeding Value for Poultry. Poult. Sci. 55, 588–593.

Leeson, S., Summers, J. D., & Daynard, T. B. 1977. The Effect of Kernel Maturity at Harvest as Measured by Moisture Content, on the Metabolizable Energy Value of Maize. Poult. Sci. 56, 154–156.

Leeson, S., Yersin, A., & Volker, L. 1993. Nutritive value of the 1992 maize crop. J. Appl. Poult. Res. 2, 208–213.

Liu, S.Y., Cadogan, D.J., Péron, A., Truong, H.H. and Selle, P.H., 2014. A combination of xylanase, amylase and protease influences growth performance, nutrient utilisation, starch and protein digestive dynamics in broiler chickens offered maize-, sorghum-and wheat-based diets. Animal Production Science, 55(10), 1255-1263.

Liu, D., Liu, H., Li, D. and Wang, F., 2019. Determination of nutrient digestibility in maize and soybean meal using the direct and substitution methods as well as different basal diets fed to growing pigs. Journal of Applied Animal Research, 47(1),184-188.

Mæhre, H.K., Dalheim, L., Edvinsen, G.K., Elvevoll, E.O. and Jensen, I.J., 2018. Protein determination—method matters. Foods, 7(1), 5.

Marion, D., Elmorjani, K., Linossier, L., Brunet, S., Dalgalarrondo, M., Morel, M.-H., Bakan, B., Gayral, M., & Delluc, C. 2015. Lipid Partitioning in Maize (Zea mays L.) Endosperm Highlights Relationships among Starch Lipids, Amylose, and Vitreousness. J. Agric. Food Chem. 63, 3551–3558.

McLean, J. A. 2005. On the calculation of heat production from open-circuit calorimetric measurements. Br. J. Nutr. 27, 597.

Megazyme Total Dietary Fiber Procedure. Megazyme International, Ireland. 2017.

Megazyme Resistant Starch Assay Procedure. Megazyme International, Ireland. 2017.

Megazyme Total Starch Assay Procedure (Amyloglucosidase/α-Amylase Method).

Megazyme International, Ireland. 2017.

Menezes-Blackburn, D., Gabler, S. and Greiner, R., 2015. Performance of seven commercial phytases in an in vitro simulation of poultry digestive tract. J. Agric. Food Chem. 63, 6142-6149.

Mestres, C., & Matencio, F. 1996. Biochemical basis of kernel milling characteristics and endosperm vitreousness of maize. J. Cereal Sci. 24, 283–290.

Moehn, S., Atakora, J., & Ball, R. O. 2005. Using net energy for diet formulation: Potential for the Canadian pig industry. Adv. Pork Prod. 116, 119–129.

Monjardino, P., Smith, A. G., & Jones, R. J. 2005. Heat stress effects on protein accumulation of maize endosperm. Crop Sci. 45, 1203–1210.

Namkung, H., & Leeson, S. 1999. Effect of phytase enzyme on dietary nitrogen-corrected apparent metabolizable energy and the ileal digestibility of nitrogen and amino acids in broiler chicks. Poult. Sci. 78, 1317–1319.

Nduwamungu, C., Ziadi, N., Parent, L.-É., Tremblay, G. F., & Thuriès, L. 2010. Opportunities for, and limitations of, near infrared reflectance spectroscopy applications in soil analysis: A review. Can. J. Soil Sci. 89, 531–541.

Noblet, J., 2013, June. Use of net energy vs metabolizable energy in swine and poultry. In Paper presented at the Southeast Asian Feed Technology and Nutrition Workshop (Vol. 3, p. 7).

Noblet, J., Dubois, S., Labussière, E., Carré, B., & Van Milgen, J. 2010. Metabolic utilization of energy in monogastric animals and its implementation in net energy systems. EAAP Sci. Ser. 127, 573–582.

Nuss, E. T., & Tanumihardjo, S. A. 2010. Maize: A paramount staple crop in the context of global nutrition. Compr. Rev. Food Sci. Food Saf. 9, 417–436.

Odjo, S. D. P., Malumba, P. K., Beckers, Y., & Béra, F. 2015. Impact of drying and heat treatment on the feeding value of maize. A review. Biotechnol. Agron. Soc. Environ. 19, 301–312.

Olukosi, O. A. 2020. Investigation of the effects of substitution levels, assay methods and length of adaptation to experimental diets on determined metabolisable energy value of maize, barley, and soya bean meal. Br. Poult. Sci. 62, 278–284.

Osborne, B. G. 2006. Near-Infrared Spectroscopy in Food Analysis. Encycl. Anal. Chem., 1–14.

Ouattar, S., Jones, R.J., and Crookston, R.K., 1987. Effect of water deficit during grain filling on the pattern of maize kernel growth and development 1. Crop science, 27, 726-730.

Pillay, K., Derera, J., Siwela, M., & Veldman, F. J. 2011. Consumer acceptance of yellow, provitamin a-biofortified maize in KwaZulu-Natal. South African J. Clin. Nutr. 24, 186–191.

Priyankarage, N., Rose, S. P., & Pirgozliev, V. R. 2011. Energy, energy requirement and different energy systems in poultry. SL Vet. Journal 58, 1–22.

Ranum, P., Peña-Rosas, J. P., & Garcia-Casal, M. N. 2014. Global maize production, utilization, and consumption. Ann. N. Y. Acad. Sci. 1312, 105–12.

Rostagno, H.S., Gomes, P.C., de Oliveira, R.F., Lopes, D.C., Ferreira, A.S., de Toledo Barreto, S.L. and rico Euclides, R.F., 2011. Composition of feedstuffs and nutritional requirements. Universidade Federal de Viçosa.

The Southern African Grain Laboratory (SAGL), 2018. South African Maize Crop Quality Report, Pretoria: The Southern African Grain Laboratory.

Sales, J. and Janssens, G.P., 2003. Methods to determine metabolizable energy and digestibility of feed ingredients in the domestic pigeon (Columba livia domestica). Poult. Sci, 82(9), 1457-1461.

Scott, M.P. and Emery, M., 2016. Maize: overview. Encyclopiedia of food grains, 99-104.

Seifi, M.R. and Alimardani, R., 2010. The moisture content effect on some physical and mechanical properties of maize (Sc 704). J. Agric. Sci., 2(4), 125.

Selle, P.H. and Ravindran, V., 2007. Microbial phytase in poultry nutrition. Anim. Feed Sci. Technol., 135(1-2), 1-41.

Shi, L., Li, W., Sun, J., Qiu, Y., Wei, X., Luan, G., Hu, Y., & Tatsumi, E. 2016. Grinding of maize: The effects of fine grinding on compositional, functional, and physicochemical properties of maize flour. J. Cereal Sci. 68, 25–30.

Shiferaw, B., Smale, M., Braun, H. J., Duveiller, E., Reynolds, M., & Muricho, G. 2013. Crops that feed the world 10. Past successes and future challenges to the role played by wheat in global food security. Food Secur. 5, 291–317.

Short, F. J., Gorton, P., Wiseman, J., & Boorman, K. N. 1996. Determination of titanium dioxide added as an inert marker in chicken digestibility studies. Anim. Feed Sci. Technol. 59, 215–221.

Sibbald, I. R., Summers, J. D., & Slinger, S. J. 1960. Factors Affecting the Metabolizable Energy Content of Poultry Feeds. Poult. Sci. 39, 544-556.

Sibbald, I. R., Slinger, S. J., Czarnocki, J., Wilson, A., Pudelkiewiez, W. J., & Singsen, E. P. 1962. Research Notes: The metabolizable energy of materials fed to growing chicks, 1612–1613.

Sibbald, I.R. and Slinger, S.J., 1962. The metabolizable energy of materials fed to growing chicks. Poult. Sci. 41(5), 1612-1613.

Sibbald, I. R. 1982. Measurement of bioavailable energy in poultry feeding stuffs: A review. Can. J. Anim. Sci. 62, 983–1048.

Simonne, A. H., Eitenmiller, R. R., Mills, H. A., Simonne, E. H., & Cresman, C. P. 2002. Could the Dumas Method Replace the Kjeldahl Digestion for Nitrogen and Crude Protein Determinations in Foods? J. Sci. Food Agric. 73, 39–45.

Singh, C.B., Paliwal, J., Jayas, D.S. and White, N.D.G., 2006. Near-infrared spectroscopy: Applications in the grain industry. In 2006 ASAE Annual Meeting (p. 1). American Society of Agricultural and Biological Engineers.

Siqueira, J., Sakomura, N., Dourado, L., Marcato, S., Silva, J., Fernandes, J., & Pinheiro, S. 2010. Poultry feed metabolizable energy determination using total or partial excreta collection methods. Rev. Bras. Ciência Avícola 12, 129–132.

Smeets, N., Nuyens, F., Van Campenhout, L., Delezie, E., Pannecoucque, J., & Niewold, T. 2015. Relationship between wheat characteristics and nutrient digestibility in broilers: Comparison between total collection and marker (titanium dioxide) technique. Poult. Sci. 94, 1584–1591.

Srigley, C. T., & Mossoba, M. M. 2016. Current analytical techniques for food lipids. Food Saf. Innov. Anal. Tools Saf. Assess., 33–64.

Stefanello, C., Vieira, S.L., Soster, P., Dos Santos, B.M., Dalmoro, Y.K., Favero, A. and Cowieson, A.J., 2019. Utilization of maize-based diets supplemented with an exogenous α-amylase for broilers. Poultry Science, 98(11), 5862-5869.

Summers, J.D., 2001. Maize: factors affecting its digestibility and variability in its feeding value. Enzymes in farm animal nutrition, 109-124.

Swick, R. A., Wu, S., Rodgers, N., & Choct, M. 2014. Energy systems for broilers - recent development and relevance for feed formulation. Int. Broiler Nutr. Conf.

Tang, D., Hao, S., Liu, G., Nian, F., & Ru, Y. 2014. Effects of maize source and complex enzymes on performance and nutrient utilization of broilers. Asian-Australasian J. Anim. Sci. 27, 1755–1762.

Tester, R. F., Karkalas, J., & QI, X. 2004. Starch structure and digestibility Enzyme-Substrate relationship. Worlds. Poult. Sci. J. 60, 186–195.

Tilley, K.A., 1998. Cereal Grain Quality. Crop Science, 38(1), 275-277.

Vogtmann, H., Pfirter, H. P., & Prabucki, A. L. 1975. A new method of determining metabolisability of energy and digestibility of fatty acids in broiler diets. Br. Poult. Sci. 16, 531–534.

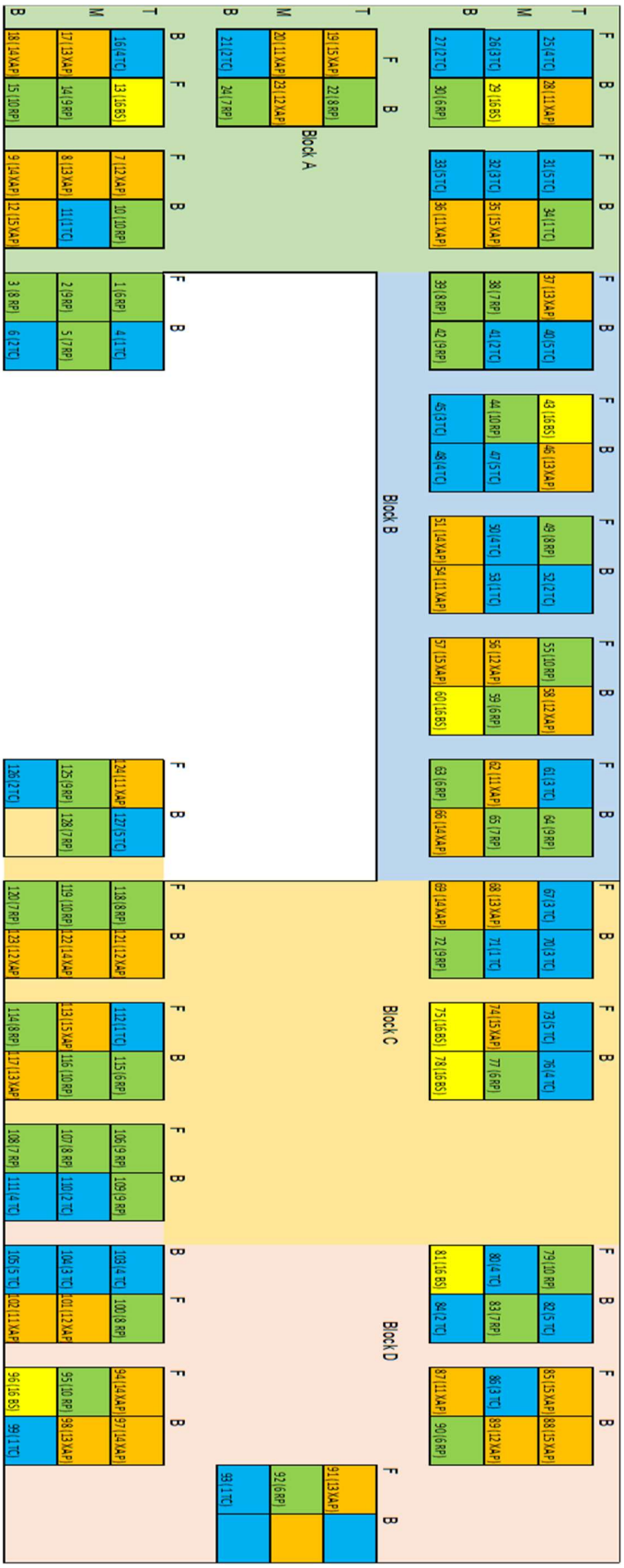
Watson, S. A. 1987. Structure and composition. Maize Chem. Technol., 53–82.

Williams, P., Geladi, P., Fox, G., & Manley, M. 2009. Maize kernel hardness classification by near infrared (NIR) hyperspectral imaging and multivariate data analysis. Anal. Chim. Acta 653, 121–130.

Wu, Y., Holding, D. R., & Messing, J. 2010. -Zeins are essential for endosperm modification in quality protein maize. Proc. Natl. Acad. Sci. 107, 12810–12815.

Appendix

Metabolic house blocking design.



Addendum

A

B

C

D

A) Metabolic cages
B) Maize samples with sub-samples for analysis.
C) NIT with AMEN calibration used to analyse maize samples.
D) NIR with cereal grains calibration, used to anaylse proximates of
E) Basal diet used to mix treatment diets.
F) Chicks in small broiler house.

E

F