INFLUENCE OF FEED FORM AND AGE OF BROILERS ON ENERGY UTILISATION OF FEED INGREDIENTS

A thesis presented in partial fulfilment of the requirements for the degree of

Doctor of Philosophy

in

Animal Science (Poultry Nutrition)

at Massey University, Palmerston North, Manawatu

New Zealand

MAHMOUD MOHAMED KHALIL

2022

ABSTRACT

The accurate determination of the available energy of feed ingredients is crucial to optimise their inclusion in feed formulations and to improve the profitability and sustainability of poultry production. The metabolisable energy (ME) is commonly used for energy evaluation and formulating the diets for poultry. However, there are some limitations and several factors that influence the precision of the evaluation of the ME content of feed ingredients and diets. The current thesis investigated some of the unexplored research gaps on the ME of the commonly used feed ingredients in broiler diets.

The first study presented in Chapter 3 was conducted to investigate the influence of feed form (FF; mash vs. pellet) on the apparent metabolisable energy (AME) and nitrogen-corrected AME (AMEn) of 7 single feed ingredients, four cereal grains (wheat, sorghum, barley, and maize) and three protein sources (soybean meal; SBM, canola meal; CM, and meat and bone meal; MBM). The influence of broiler age AMEn of cereal grains was investigated in Chapter 4 (direct method) and Chapter 5 (substitution method). The fourth experiment reported in Chapter 6 examined the effect of broiler age on the AMEn of protein sources. The experiments discussed in Chapter 7 were unique in that a novel methodology was developed for the quantification of the ileal endogenous energy losses (IEEL) in broiler chickens and for the correction of apparent ileal digestible energy (AIDE) to true (TIDE) ileal digestible energy of cereal grains (wheat, sorghum, barley and maize). The last trial of the thesis reported in Chapter 8 was conducted to refine-tune the IEEL methodology developed in Chapter 7 and to determine the influence of age and dietary cellulose contents on the IEEL estimates in broiler chickens.

Data reported in Chapter 3 demonstrated that FF influenced the AMEn of feed ingredients. Pelleting increased the AMEn of all cereal grains by an average of 0.22 MJ/kg. However, for protein source ingredients, FF influence was ingredient-dependent. Pelleting increased the

AMEn of CM by 0.57 MJ/kg, had no effect on that of SBM and decreased the AMEn of MBM by 0.56 MJ/kg.

The experiment reported in Chapter 4 investigated the influence of broiler age on the AMEn of cereal grains using the direct method. The assay diets were formulated with an inclusion of 962 g/kg of each grain in the diet and pelleted. The data revealed that the age of broiler chickens has a significant impact on the AMEn of cereal grains. The first week of age recorded the highest AMEn for all cereal grains. Thereafter, the AMEn decreased either linearly (sorghum) or quadratically (wheat, barley and maize) with the advancing age of broilers.

In the study reported in Chapter 5, the effect of broiler age on the AMEn of cereal grains, from the same batches used in Chapter 4, was examined using the substitution method. A maize-SBM basal diet was formulated and test diets were developed by replacing (w/w) 300 g/kg of the basal diet with each cereal grain. The results showed that the effect of broiler age on the AMEn varied depending on the grain type. Whilst the AMEn of barley and maize were unaffected by age, the AMEn of wheat and sorghum increased with the advancing age of broiler chickens. The determined AMEn values differed between direct and substitution methods, with the substitution method generating lower AMEn values.

Data reported in Chapter 6 demonstrated that the AMEn content of SBM and CM was influenced by age of broilers. The first week showed the highest AMEn value for both SBM and CM, followed by reductions for both ingredients up to week 3 and increases thereafter.

The studies reported in Chapter 7 present a novel approach to quantify the IEEL in broilers and correct the AIDE of cereal grains (wheat, sorghum, barley and maize) to TIDE enabling comparisons with AMEn. The IEEL was estimated to be 1.45 MJ/kg dry matter intake (DMI) in 21-d old broilers, following the feeding of a glucose-based purified diet and used to

calculate the TIDE. The apparent ileal digestibility of dry matter, nitrogen and starch were positively and highly correlated with the TIDE than the AIDE or AMEn.

The studies reported in Chapter 8 were conducted to refine the proposed methodology for the estimation of IEEL proposed in Chapter 7 and to investigate the influence of age of broilers and the dietary cellulose contents on IEEL estimates. It was found that the age of broilers had no impact on the IEEL estimates. The IEEL was affected by the cellulose content and the IEEL increased from 0.37 MJ/kg DMI for the diet without cellulose to 1.80 MJ/kg DMI for the diet with 75 g/kg inclusion of cellulose.

The findings reported in the current thesis demonstrate that the application of AMEn values determined based on assays using mash diets might result in over- or under-estimation of the available energy content of ingredients in commercial pelleted broiler diets and highlights the need for the use of pelleted diets in energy evaluation assays. The findings also revealed that the effects of age and methodology are relevant in the determination of AMEn of feed ingredients and question the validity of using single AME or AMEn values for feed ingredients in broiler diet formulations across different ages. Another notable contribution was to develop a novel approach to quantify the IEEL in broiler chickens for the first time. The thesis research also provides preliminary data on the TIDE of common cereal grains and highlights the possibility of applying the TIDE as an alternative to the ME system in poultry feed formulation.

ACKNOWLEDGMENTS

This research journey was a challenging and thrilling experience, and I have been so fortunate to have had the mentorship and friendship of so many wonderful people along the way.

First and foremost, I would like to thank God “Allah” for his blessings, mercy and guidance during my PhD journey.

Next, I would like to express my sincere gratitude and appreciations to my main supervisor A/Professor Reza Abdollahi, for his dedicated supervision, support, guidance, and valuable comments. You taught me many things, pushed me to deepen my reasoning and clarify my arguments, and, most importantly, supported me along this journey. I am heartily thankful to my supervisor Professor Ravi Ravindran, for his gentle guidance, inspiring dedication to open science, warm-hearted style of supervision and direction throughout the entire PhD journey. My deepest gratitude to my supervisor Dr Fifi Zaefarian, for her support, caring, and encouragement all the time. Your motivation, confidence and guidance were helpful to complete this project. For me, completion of this project would have not been possible without the help of amazing supervisors and one of the well-known poultry nutritionists in the world. It was really an honour for me working with them.

I am immensely thankful for the support, suggestions, and dedicated assistance I received from Colin Naftel and Ed James from the Massey University Poultry Research Unit. My appreciation extends to Shaun de Malmanche, Kalwyn Pereka, and Elham Ebrazeh for their contribution and assistance in diets preparation, sampling collection and friendship. I also appreciate the support from Felicity Jackson, Karl Dale and other staff of Massey University Nutrition Laboratory.

I wish to express my gratefulness and appreciations to my colleagues and friends; Nipuna, Laura, Obright, Gayatri, Lindon, and especially Mukti for their help, support and fun-times during the course of this journey.

Many friends outside Massey University provided support and camaraderie. First, I would like to say a special thank you to my dear friends Fady Keriakos and Ginger Schuster whose care, laughter, patience, hugs, cards, and encouragement were vital during this whole journey. Special thanks to Rana, Moutasim, Anita, Emma, Jim, David and Raktim for their love, care, support and friendship during my time in New Zealand. I cannot forget the blessing, support and care from my friends back home, who were there whenever needed.

I am deeply thankful to The Ministry of Foreign Affairs and Trade for granting me a New Zealand Development Doctorate Scholarship and AgriFutures Chicken Meat, Australia for the financial support for completing this project. Also, I would like to thank Massey University International office staff, especially Jamie Hopper, for their dedicated support and care throughout the whole journey, even before arriving to New Zealand.

Last but not least, many thanks to my wife Rehab Darwish and children Rehab, Hazem and Mohamed for their love and sacrifice in ensuring I got my PhD completed. No words can express my feeling and appreciation to my mother for her unconditional love, patience, astute ears when the going got tough and her endless prayers. I am deeply thankful for my siblings; Ahmed, Amal, and Doaa for standing by me, offering encouragement, motivation, positivity, care and love.

Finally, I wish to dedicate this PhD thesis to the memory of my father Mohamed Khalil. You are gone but who always trusted in my abilities to be successful in the academic arena. Everything I learnt from you and your belief in me has made this journey possible. You will never be forgotten.

PUBLICATIONS

Studies completed during the candidature, some of which are reported in this thesis have been presented in the following communications:

Refereed scientific papers

Khalil, M.M., Abdollahi, M.R., Zaefarian, F., & Ravindran, V. (2020). Measurement of ileal endogenous energy losses and true ileal digestible energy of cereal grains for broiler chickens. *Poultry Science*, *99*(12), 6809-6817.

Khalil, M.M., Abdollahi, M.R., Zaefarian, F., & Ravindran, V. (2021). Influence of feed form on the apparent metabolisable energy of feed ingredients for broiler chickens. *Animal Feed Science and Technology*, *271*, 114754.

Khalil, M.M., Abdollahi, M.R., Zaefarian, F., Chrystal, P.V., & Ravindran, V. (2021). Apparent metabolizable energy of cereal grains for broiler chickens is influenced by age. *Poultry Science*, *100*(9), 101288.

Khalil, M.M., Abdollahi, M.R., Zaefarian, F., Chrystal, P.V., & Ravindran, V. (2022). Influence of Broiler Age on the Apparent Metabolizable Energy of Cereal Grains Determined Using the Substitution Method. *Animals*, *12*(2), 183.

Khalil, M.M., Abdollahi, M.R., Zaefarian, F., Chrystal, P.V., & Ravindran, V. (2022). Influence of age and dietary cellulose levels on ileal endogenous energy losses in broiler chickens. *Poultry Science*, *101*(7), 101948.

Conference proceedings

Khalil, M.M., Abdollahi, M.R., Zaefarian, F., & Ravindran, V. (2019). Influence of Feed Form on the Metabolisable Energy of Ingredients for Broilers. *Proceedings of the 21st Massey Technical Update Conference*.

Khalil, M.M., Abdollahi, M.R., Zaefarian, F., & Ravindran, V. (2019). Feed form affects the apparent metabolisable energy of individual ingredients to different extents in broiler chickens. In *Symposium of School of Agriculture and Environment.* (Abstract). Massey University, Palmerston North, New Zealand.

Khalil, M.M., Abdollahi, M.R., Zaefarian, F., & Ravindran, V. (2020). Feed form affects the apparent metabolisable energy of individual ingredients to different extents in broiler chickens. In *31st Australian Poultry Science Symposium*.

Khalil, M.M., Abdollahi, M.R., Zaefarian, F., Chrystal, P.V., & Ravindran, V. (2021). Apparent metabolisable energy of common cereal grains for broiler chickens is influenced by bird age. In *32nd Australian Poultry Science Symposium*.

Khalil, M.M., Abdollahi, M.R., Zaefarian, F., Chrystal, P.V., & Ravindran, V. (2021). Estimation of ileal endogenous energy losses at different ages and influence of dietary cellulose levels in broiler chickens. In *33rd Australian Poultry Science Symposium* (Abstract).

Khalil, M.M., Abdollahi, M.R., Zaefarian, F., Chrystal, P.V., & Ravindran, V. (2022). Energy Utilisation Response to Broiler Age Varies Depending on Cereal Grain. In *Proceedings of the 15th New Zealand Poultry Industry Conference.*

TABLE OF CONTENTS

LIST OF FIGURES

Chapter 2

Chapter 4

Chapter 5

Chapter 6

LIST OF TABLES

Chapter 2

Chapter 3

Chapter 4

Chapter 5

Chapter 6

Chapter 7

Chapter 8

Chapter 9

LIST OF ABBREVIATIONS

%	Percent
°C	Degree centigrade
AIDE	Apparent ileal digestible energy
AIA	Acid insoluble ash
AME	Apparent metabolisable energy
AMEn	Nitrogen-corrected apparent metabolisable energy
AOAC	Association of Official Analytical Chemists
BW	Body weight
C	Celsius
Ca	Calcium
CAID	Coefficients of apparent ileal digestibility
CP	Crude protein
CR_2O_3	Chromium oxide
Cu	Copper
CM	Canola meal
d	day/s
DM	Dry matter
DMI	Dry matter intake
EEL	Endogenous energy losses
Fe	Iron
FF	Feed form
FI	Feed intake
g	Gram

GE	Gross energy
h	Hour
HCl	Hydrochloric acid
IEEL	Ileal endogenous energy losses
K	Potassium
kg	Kilogram
KJ	Kilo Joule
MBM	Meat and bone meal
MCP	Monocalcium phosphate
Mg	Magnesium
mg	Milligram
MJ	Mega joule
mm	Millimetre
Mn	Manganese
N	Nitrogen
Na	Sodium
NDF	Neutral detergent fibre
NRC	National Research Council
NSP	Non-starch polysaccharides
P	Phosphorous
P	Probability
PE	Productive energy
PDI	Pellet durability index
SAS	Statistical analysis software
SBM	Soyabean meal

SD	Standard deviation
SEM	Pooled standard error of mean
Ti	Titanium dioxide
TIDE	True ileal digestible energy
UV	Ultraviolet
vs.	versus
w/w	weight/weight
WPSA	World’s Poultry Science Association
Zn	Zinc

CHAPTER 1 GENERAL INTRODUCTION

Feed is the greatest cost item in poultry production, comprising two-thirds of the total production cost. Poultry feeds are formulated by combining an array of ingredients (grains, protein sources, lipid sources, vitamin and mineral premixes, and feed additives) to meet the nutrient and energy requirements for growth and reproduction. The supply of energy represents the costliest component in poultry feed formulation. Energy is not a nutrient, but a property that some nutrients (carbohydrates, lipids and protein) possess. It is recognised that birds tend to eat to satisfy their energy requirements and thus the dietary energy level influences feed intake, which is the major factor driving weight gain in poultry.

Poultry producers rely on grains such as maize, wheat, sorghum, and barley for the provision of energy in poultry feeds. Grains supply 60-70% of the dietary energy and the balance coming from lipids and protein sources (Van der Klis et al., 2010). Protein sources including soybean meal, canola meal and meat and bone meal are used mainly to meet the protein needs, and they also supply about 30% of the energy requirements in broiler diets. The available energy in different ingredients varies widely, depending on variables such as ingredient type, cultivar, location, environment, season and harvest conditions (Hughes and Choct, 1999; Mateos et al., 2019). The available energy content of feed ingredients for poultry could be measured through various methods, with the metabolisable energy (ME) being the most common and accepted procedure. Metabolisable energy can be determined from the differences between the dietary gross energy intake and the gross energy of excreta voided of a specific feed consumed (NRC, 1994), referred to as apparent ME (AME). The AME is frequently corrected for nitrogen (N) retention, referred to as nitrogen-corrected AME (AMEn). The correction is adopted to convert the AME values to a basis of N equilibrium to eliminate the differences in the growth rate of birds across assays or between ingredients differing in protein content (Leeson et al., 1977; Sibbald, 1989; Farrell et al., 1991a, 1997). However, this

correction heavily penalises high-protein ingredients, which correspondingly reduces their energy value.

Feed form (FF) is one of the dietary factors that can affect the AMEn value of diets. Pelleted feed is the most prevalent form of feed used in commercial feed production for broilers (Latshaw, 2008; Dozier III et al., 2010; Abdollahi et al., 2011). All previous studies on energy evaluation of feed ingredients for broilers have been conducted with mash diets and overlook the impact of FF on the estimation of AMEn of feed ingredients. It has been reported that the effects of FF on nutrient digestibility and energy utilisation depend on the ingredient type (Abdollahi et al., 2013a). Moreover, no study to date has investigated the effect of FF on the AMEn of individual ingredients.

When formulating feeds for broilers of different ages, nutritionists use a single AMEn value for each ingredient that is generally sourced from reference tables which have been generated with birds at 5 weeks of age or older (WPSA, 1989; NRC, 1994; Evonik, 2016; FEDNA, 2017). However, age of birds may influence the AMEn of feed ingredients, but the influence of age from hatch until the end of the commercial production cycle has not been investigated thus far. It has been well accepted that chicks go through significant physiological and morphological changes during the first week of life after hatching, with high demand for energy and amino acids (Obst and Diamond, 1992). During this period, birds have limited ability to extract nutrients, as they hatch with an immature digestive system still not fully optimised for nutrient digestion and absorption (Jin et al., 1998). However, the ability of birds to digest and extract nutrients from feed increases with age, as the enzyme production and absorptive capacity increase (Olukosi et al., 2007; Thomas et al., 2008; Stefanello et al., 2016). Published data on age effect on the AMEn of individual ingredients are scant and almost all available data relate to complete diets or are limited to specific ages.

Despite its limitations, the AME has been the system of choice for describing the available energy of feed ingredients for poultry (Hill and Anderson, 1958; Sibbald, 1982; Wu et al., 2020). The apparent ileal digestible energy (AIDE) will eliminate some inherent errors with the classic AMEn methodology and could be a potential energy system for poultry. The AIDE estimates of feed ingredients contain both dietary and non-dietary components. Therefore, correction for the non-dietary energy flow, referred to as ileal endogenous energy loss (IEEL), is necessary for the calculation of true ileal digestible energy (TIDE) of feed ingredients. Currently, no reports are available on the measurement of IEEL for broiler chickens and TIDE estimates of feed ingredients.

The specific objects of the experiments conducted in this thesis were:

1. To evaluate the influence of FF (mash vs. pellet) on the AMEn of main cereal grains (maize, wheat, sorghum, and barley) and protein sources (soybean meal, canola meal, and meat and bone meal) used in broiler diets (Chapter 3).
2. To investigate the effect of age on the AMEn of individual cereal grains (maize, wheat, sorghum, and barley) at six different broiler ages, namely d 7, 14, 21, 28, 35, and 42, measured using the direct method (Chapter 4).
3. To examine the effect of age of broilers from week 1 to 6 post-hatch on the AMEn of individual cereal grains (maize, wheat, sorghum, and barley) using the substitution method (Chapter 5).
4. To determine whether broiler age influences the AMEn estimates of soybean meal and canola meal at six different broiler ages, namely d 7, 14, 21, 28, 35, and 42 using the substitution method (Chapter 6).
5. To develop a methodology for the estimation of IEEL in broiler chickens and measure the AIDE and TIDE of individual cereal grains (maize, wheat, sorghum, and barley) and compare with AMEn (Chapter 7).

6. To measure the IEEL estimates at six different ages, namely d 7, 14, 21, 28, 35, and 42 post-hatch and examine the influence of dietary cellulose content on the IEEL estimates for broiler chickens (Chapter 8).

CHAPTER 2 LITERATURE REVIEW

2.1. Introduction

The energy component represents the major cost item in poultry feed formulations. Poultry diets are formulated on the basis of available energy contents of ingredients, which are obtained from published table values, prediction equations, or *in vivo* assays. Of these, table values are the most commonly used by nutritionists. Table values are based on average from published reports across global institutions and do not account for the wide variations that may exist in locally produced or locally available ingredients.

Predictive equations and *in vivo* bioassays are useful and provide a better alternative than tabulated values. In all methods, energy values reported showed a wide variability due to the various factors that affect energy utilisation, such as bird's age, ingredient type, nutritional composition, heat processing, and the methodology of the bioassays (Van der Klis and Fledderus, 2007; Losada et al., 2010; Noblet, 2015). Therefore, accurate estimation of the factors affecting the energy content of ingredients is crucial to reduce poultry feed costs.

2.2. Available energy systems

2.2.1. Partitioning of energy

Energy partitioning is the sum of physiological and metabolic processes that govern the ultimate use of dietary energy. Available energy can be partitioned into gross energy (GE), digestible energy (DE), metabolisable energy (ME), and net energy (NE; Figure 2.1; Sibbald, 1982).

Gross energy is the chemical energy stored in the ingredient or feed and can be released (measured as calories or joules) when the substance is completely burnt (oxidised) to carbon dioxide and water using 25-30 atmospheres of oxygen in a bomb calorimeter. Estimating the GE is the first step in determining the available energy value. However, GE represents the total

energy in the feed or ingredient and does not consider any of the energy losses that take place during digestion, absorption, and metabolism of ingested feed by the bird.

The energy remaining after the digestion can be defined as the apparent digestible energy (ADE), which is the amount of GE minus the energy excreted in faeces (includes undigested feed residues and unabsorbed endogenous secretions consisting of bile, mucus, and intestinal epithelial cells). Measuring ADE in poultry is not easy as the faeces is mixed with urine and voided together as excreta.

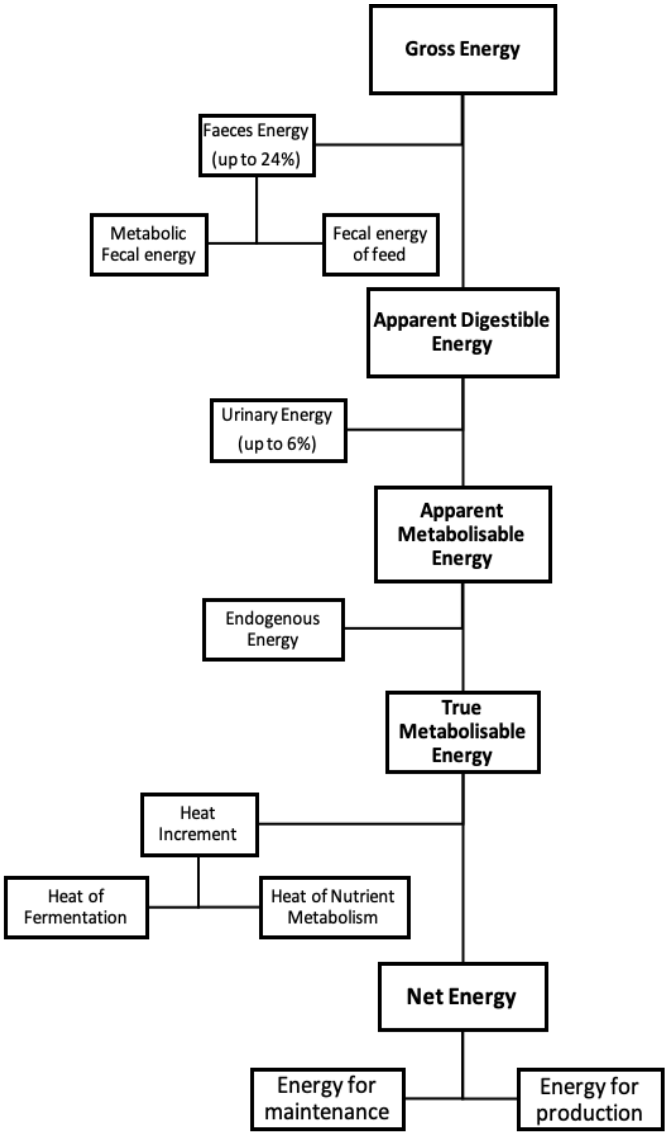


Figure 2.1. Energy partitioning in poultry (Sibbald, 1982)

Another portion of the absorbed energy is lost through urine and gasses produced during intestinal fermentation. The energy voided in urine varies depending on ingredients/diets and energy excreted through gaseous is negligible in poultry and thus not accounted for (Waring and Shannon, 1969, Wu et al., 2020). Deducting these energy losses from ADE is the apparent metabolisable energy (AME).

2.2.2. Apparent metabolisable energy

The AME system was introduced in the mid-1950's, and since then it became the most common system to express the energy requirements for birds and evaluate the available energy of ingredients and diets as it is simple, straightforward and considers most of the energy losses after the digestion and metabolism. The classical method of estimating AME involves the use of growing birds, which have been adapted to the assay diet for at least three consecutive days, followed by a total collection of excreta voided and measurement of feed consumed during the collection period (normally four days). The AME is then calculated using the following formula:

$$\text{AME (MJ/kg diet)} = [(\text{Feed intake} \times GE_{diet}) - (\text{Excreta output} \times GE_{excreta})] / \text{Feed intake}$$

An alternative to the quantitative collection of excreta and feed is the inclusion of a known concentration of a suitable indicator (marker) such as chromic oxide, acid insoluble ash or titanium dioxide in the diet, and the AME is calculated based on the marker ratios in the feed and excreta.

$$\text{GE metabolisability} = [(GE/Ti)_{Diet} - (GE/Ti)_{Excreta}] / (GE/Ti)_{Diet}$$

$$AME_{Diet}\,(MJ/kg) = GE_{Diet} \times \text{GE metabolisability}$$

Estimating the AME is a simple process; however, there are a number of arguments as to whether AME is a perfect system for estimating dietary energy value or if other energy systems need to be adopted (Farrell, 1981; Sibbald, 1985). There are several limitations to the AME system and many factors that can influence the AME of ingredients or diets such as age

of bird, gender, diet composition, feed intake and feed form. For example, dietary AME has been reported to be influenced by the advancing age of birds (Zelenka, 1968; Scott et al., 1998; Batal and Parsons, 2002; Bolarinwa et al., 2012). Another example, fatty acid content and the ratio of unsaturated to saturated fatty acids can influence the AME value of fats (Wiseman et al., 1998; Wiseman, 1999). The AME values of some ingredients will be altered by the presence of other components in the diet (Nitsan et al., 1997).

2.2.3. Nitrogen-corrected apparent metabolisable energy

Nitrogen-corrected AME (AMEn) is defined as AME corrected to zero nitrogen (N) retention. The correction for N retention was proposed to convert AME values to a basis of N equilibrium for comparative purposes, in order to generate consistent AME data for ingredients by eliminating the variation associated with that portion of N that is deposited as protein tissue and not oxidised in the body to provide energy.

The AMEn is widely used as the default value to express the available energy of feed ingredients and for describing the energy requirements of birds (Janssen, 1989; NRC, 1994; Carré et al., 1995; MacLeod, 2002; Rostagno, 2017). Two factors are commonly used for the correction: the GE values of N-containing compounds found in chicken urine, which is 36.54 KJ/g (Titus et al., 1959) or the GE value of uric acid, which is 34.39 KJ/g (Hill and Anderson, 1958; Sibbald, 1989). The difference between the two factors is based on the assumption that the retained N, when catabolised, yields uric acid only as the sole N excretory product in the chicken urine as stated by Hill and Anderson (1958) or yields uric acid along with a mixture of nitrogenous components such as, urea and ammonia according to Titus et al. (1959).

Nitrogen-corrected AME can be calculated based on the formula proposed by Titus et al. (1959):

$$AMEn\ (MJ/kg) = AME_{diet} - (N_{retention\ (g/kg)} \times 36.54 / 1000)$$

The justification for this correction is that feed ingredients should be evaluated for their contribution of supplying energy for birds and not promoting N retention. Another justification is that the N correction is needed to eliminate the variations in N retention associated with experimental factors, such as age of birds, gender, strain, ingredient type, diet composition, and feed additives (McNab, 2000; Lopez and Leeson, 2007). However, criticisms about corrections to N retention have been made by some researchers. McNab (2000) believed the correction unnecessary as the AME is an energy evaluation system *per se*; hence, ingredients or diets should be assessed in terms of their ability to supply energy not promoting N retention. Lopez and Leeson (2008) stated that, as current different broiler strains have similar N accretion, the correction for N retention is not relevant anymore.

Moreover, the correction for zero N retention may result in errors as in many AME assays, the assay diets are amino acids imbalanced and not representative of the balanced commercial diets. The imbalanced diets will decrease the amino acid accretion and the ability of birds to extract energy from those diets. Therefore, the AMEn estimates obtained from such assays may be erroneous, especially if birds are fed diets with higher or lower protein contents (Wu et al., 2020; Abdollahi et al., 2021).

Another issue is that correcting the AME to zero N retention will penalise the energy value of high-protein ingredients such as soybean meal (SBM) over low-protein ingredients such as maize because of the associated higher protein accretion with high-protein ingredients. Therefore, AMEn values might penalise the real contribution of protein sources to the energy of the diet, especially in modern birds fed diets formulated on ileal digestible amino acids, in which a high proportion of the ingested protein is used for muscle accretion and not metabolised or stored as fat (Dale and Fuller, 1984; Mateos et al., 2019). Lopez and Leeson (2008) showed that the AMEn of maize was around 95-99% of its AME, implying an N correction penalty of 1-5%. For SBM, the N correction caused a 7-12% decline in AME.

Abdollahi et al. (2021) also revealed that correcting AME to zero N retention penalised SBM over cereal grains (1.25 vs. 0.30 MJ/kg). The authors explained that the correction for zero N retention will reduce the true energy values of ingredients with high protein contents such as SBM due to their associated high protein accretion. Moreover, the correction for zero N retention could penalise samples with higher protein quality within the same ingredient. Abdollahi et al. (2021) reported that correcting SBM samples for zero N retention highly penalised the AME (1.51 vs. 0.95 MJ/kg) for SBM samples with higher N retention (51.7%) than those of lower N retention (43.6%).

2.2.4. Limitations of applying the apparent metabolisable energy system in feed formulation for broilers

A major criticism of the metabolisable energy system is that the AME values of ingredients are not usually used with equal efficiency by different birds, because there might be considerable fermentation of the dry matter (DM) component, which depends on the chemical composition of the diet and the age of birds (Petersen and Farrell, 1997).

A second criticism is that assigning one AME value for each ingredient is a method commonly used during feed formulation, with the assumption that these energy values are additive, which might not be true. In fact, the effects of substitution levels of ingredient in the diet appear to be additive and not associative (Sibbald, 1977; Wu et al., 2020). Leeson and Summers (2005) and Van der Klis et al. (2010) reported a decrease in the energy level from 115 to 96% when the level of fat was increased from 5 to 40 g/kg.

Moreover, the AME of ingredients or diets must be determined using birds of an age at which the value is to be applied. The AME of the diet or ingredients is dependent on the age of the bird, and hence, the bioassay should be conducted on both growing chickens and mature birds. Farrell et al. (1991b) indicated a gradual increase in the AME of wheat- or oat-based diets

when broiler chickens were fed the same diet from 4 to 44 d of age. Similarly, Lopez and Leeson (2008) found differences in AME and AMEn of maize-SBM diets at different ages of birds.

Finally, the AME system does not account for variations due to the ingredient origin, diet composition, methodologies, and laboratories where the assay is conducted (Choct, 2012, Wu et al., 2020). Olukosi (2021) showed that the AMEn of barley determined using the regression method (10.38 MJ/kg) was greater than that determined by the substitution method (7.71 MJ/kg). Similarly, for SBM, the AMEn measured by the regression method resulted in a greater value than that measured by the substitution method (10.72 vs. 8.71 MJ/kg). Veluri and Olukosi (2020) revealed that the AMEn of protein source ingredients varied depending on the reference diet composition, whereas maize-canola meal basal diet resulted in a greater AMEn value by 0.41 MJ/kg than those determined using the maize-SBM basal diet.

2.3. Methodologies for estimating the apparent metabolisable energy

Accurate determination of the AME of all ingredients is crucial for better feed formulation and for optimising feed efficiency. Several methods have been developed to measure the AME of feed and ingredients for poultry, based on how birds are fed and the excreta is collected, including the *in vitro* method (Valdes and Leeson, 1992). The latter method uses a two-step *in vitro* technique with pepsin, pancreatin, bile acids, and enterokinase. Other methods have been pioneered by various researchers (Hill and Anderson, 1958; Sibbald, 1976; Farrell, 1978; Farrell et al., 1991a). All of these methods vary in their procedures, with the simplest method being feeding only the test ingredient (Lockhart et al., 1967).

2.3.1. Classical total collection method

Total excreta collection is the most preferred method used for the measurement of AME in poultry, and it is based on quantifying feed intake and total excreta for a determined period (Sibbald and Slinger, 1962). In this method, birds housed in metabolic cages are offered a diet

for a period of three to four days to establish a state of digestive equilibrium, followed by a collection period of excreta for four to six days (Vohra, 1972; Farrell, 1999). At the end of the assay, feed intake and excreta weights are recorded and then analysed for GE.

Apparent metabolisable energy can be calculated through the following formula:

$$AME\ (MJ/kg) = (GE_{intake} - GE_{excreta}) / DM_{intake}$$

However, this method has several limitations including adherence of some droppings to the birds' plumage, contamination of excreta with feed, feathers, intestinal mucosa sloughing, changes in excreta chemical composition due to fermentation, and variation in moisture content. Moreover, it greatly relies on the quantitative measurement of feed consumption and excreta output, despite the fact that there are losses in excreta as birds may excrete away from the tray, and excreta losses may occur during removal and transfer from trays to containers. In addition, the assumption is that the excreta voided during the period of collection correlates with the feed ingested during the same period. All of these inherent problems could bias the measurement of AME (McNab, 2000).

2.3.2. Marker method

An alternative method to the classical total collection method is to perform partial excreta collection and determine the AME using the ratio of indigestible substances (markers) present in diets and excreta. This method is based on the assumption that the marker has the same passage rate as the nutrients in the digesta of the birds (Sales and Janssens, 2003). The marker is defined as any material used to estimate nutritional phenomena qualitatively or quantitatively, and it is considered a physical-chemical monitor of digestive processes (Ramos, 2003). Markers should meet specific criteria to be included in any AME assay. They must be non-toxic, and remain unchanged during passage along the gastrointestinal tract, have no

influence on the digestibility of nutrients, be easy to analyse, and be completely recovered in the excreta (Kotb and Luckey, 1972; Sales and Jansen, 2003; Sakomura and Rostagno, 2016).

Chromium oxide (Cr_2O_3) has been the commonly used marker for AME measurements since the 1960s (Vohra, 1966). However, there are some problems associated with using Cr_2O_3, such as the fact that it is difficult to chemically analyse it, and the unequal distribution of Cr_2O_3 in the feed; in addition, the electrostatic characteristic of Cr_2O_3 makes it more difficult to separate feed from the excreta, leading to incomplete recovery and analytical variation when colorimetric methods are used (Vohra, 1972; Sakomura and Rostagno, 2016). Another marker is acid-insoluble ash (AIA), which is an indigestible substance that contains mainly silica treated with hydrochloric acid, and it is considered an internal marker. Cereals contain low levels of AIA, and therefore, external sources of AIA (i.e., Celite^{TM} or sand) may be added to diets to increase the accuracy of the measurements of the AME content of cereals (Sales and Janssens, 2003). In contrast, titanium dioxide (TiO_2), another marker, is very simple to analyse and reproducible (Short et al., 1996; Myers et al., 2004).

According to Sales and Janssens (2003), the marker recovery rate is the most essential criterion to be considered when using markers for AME assays. However, few studies have reported the recovery rates. Marker recovery rate can vary from 96-111%, which affects the energy value of the diet. There are several factors that may affect the marker recovery rate. For example, the amount of marker included in the diet affects diet energy value, as it has been shown that high levels of silica (more than 2%) in the diet affected the digesta passage rate, and hence, the digestibility of nutrients and the resultant AME value of the diet (Cheng and Coon, 1990). Also, markers may remain deposited in different parts of the digestive system, especially when used in high concentrations in the diet (Vohra and Kratzer, 1967; Olukosi et al., 2012). However, if the marker recovery rate is estimated as 100%, this should result in similar AMEn values compared with the total excreta collection method. Roza et al. (2018) reported that the

AMEn of maize-SBM based measured by the total excreta collection (12.21 MJ/kg) is similar to the AMEn value estimated by the marker method with three different indicators; AIA (12.27 MJ/kg), TiO_2 (12.20 MJ/kg), and Cr_2O_3 (12.18 MJ/kg) if the markers recovery rate adjusted to 100%.

This means that the marker method could be a reliable alternative if the analysis achieved high accuracy for the marker recovery rate (McNab, 2000). In general, problems associated with using markers have led to the acceptance of the total excreta collection method as the most applicable method. In the marker method, AME can be calculated using the following formula (Sakomura and Rostagno, 2016):

$$AME\ (MJ/kg) = GE_{diet} - (GE_{excreta} \times IF)$$

where: $IF\ (Indigestibility\ factor) = marker_{feed}/marker_{excreta}$

2.4. Assay diets for estimating the apparent metabolisable energy

2.4.1. Direct method

The direct method is the most widely used method to estimate the AME of cereals, mainly because of the simplicity of the assay diet and calculations. In this method, only the test ingredient is used as the sole source of energy in the test diet (Lockhart et al., 1967).

This method is applicable for specific ingredients (mainly grains); however, it has some limitations. In this method, birds cannot be fed the test diets for prolonged periods due to the unbalanced nature of the diet. The poor palatability of the diet can affect feed consumption, and hence, the estimation of the AME value. Furthermore, most of the ingredients are nutritionally imbalanced; therefore, these diets may result in severe adverse effects on the body functions when fed as a stand-alone diet for several days. McIntosh et al. (1962) reported that the balance of the diet may exert variable influence on the AME of the ingredient, which depends on the

ingredient type and physical form. The authors revealed that increasing the wheat inclusion from 300 to 390 g/kg, decreased the dietary AME from 11.88 MJ/kg to 11.63 MJ/kg.

2.4.2. Difference or substitution method

The difference or substitution method (diet replacement method) is another assay that can be used to evaluate the AME of ingredients (Sibbald et al., 1960). This method is used in case of poor palatability, high protein content, or the presence of high levels of anti-nutritional factors (ANFs) in the test ingredient. This method requires formulating two sets of diets, a basal (reference diet) and the assay diet. The basal diet consists of a mixture of ingredients, typically a maize-SBM basal diet, whereas the assay diet is formulated by replacement of a portion of the basal diet with the test ingredient; in some cases, different substitution levels of the basal diet are considered (Sibbald and Slinger, 1963; Kong and Adeola, 2014). The AME value of the test ingredient is determined by using the following formula:

$$AME_{Basal\ diet}\ (MJ/kg) = [(FI \times GE_{Diet}) - (Excreta\ output \times GE_{Excreta})] / FI$$

The AME of ingredients is then calculated using the following formula:

$$AME_{Ingredient}\ (MJ/kg) = [AME\ of\ test\ ingredient\ diet - (AME\ of\ basal\ diet \times inclusion\ of\ basal\ diet/100)] / inclusion\ of\ test\ ingredient/100$$

This method is a good alternative to the direct method as the reference diet is a standard diet. A comparison between the AME values of wheat, which were estimated following the direct method and the difference method, revealed no differences between the results obtained by either method (McIntosh et al., 1962). Similarly, Lockhart et al. (1967) reported that there were no significant differences between two different methods (direct method, and 33% substitution of a reference diet method) in estimating the AME value of wheat.

However, estimating the AME value following the difference method has some disadvantages as the AME values of the test ingredients can vary according to the composition

of the basal diet. This is because the difference method assumes that there is no interaction between the basal diet and the test ingredients (Sibbald et al., 1960; Wu et al., 2020). Moreover, the inclusion level of the tested ingredient may affect the AME value. Lopez and Leeson (2008) reported that the AME of SBM calculated by the difference method were 10.37, 10.75, or 9.84 MJ/kg, when the SBM substitution level was 100, 200, or 300 g/kg of the basal diet, respectively. Similarly, the AMEn value for a sample of maize distillers' dried grains with solubles decreased from 12.60 MJ/kg and 11.05 MJ/kg for 300 and 600 g/kg substitution levels, respectively, of a maize-SBM diet (Adeola and Ileleji, 2009).

2.4.3. Regression method

An alternative method of estimating the AME values of ingredients is the regression method. In this method, the basal diet is fed to one group of birds, and other diets, with at least two levels of the basal diet replaced by the test ingredient, are fed to several other groups of birds. The energy values of individual diets are compared to the corresponding inclusion level of the ingredient. Extrapolation of energy to the equivalency of 100% inclusion predicts the energy value of the ingredient. Lopez and Leeson (2008) revealed that the AME of maize and SBM can be predicted at different substitution levels of maize and SBM in the test diets following the linear regression method (Figure 2.2).

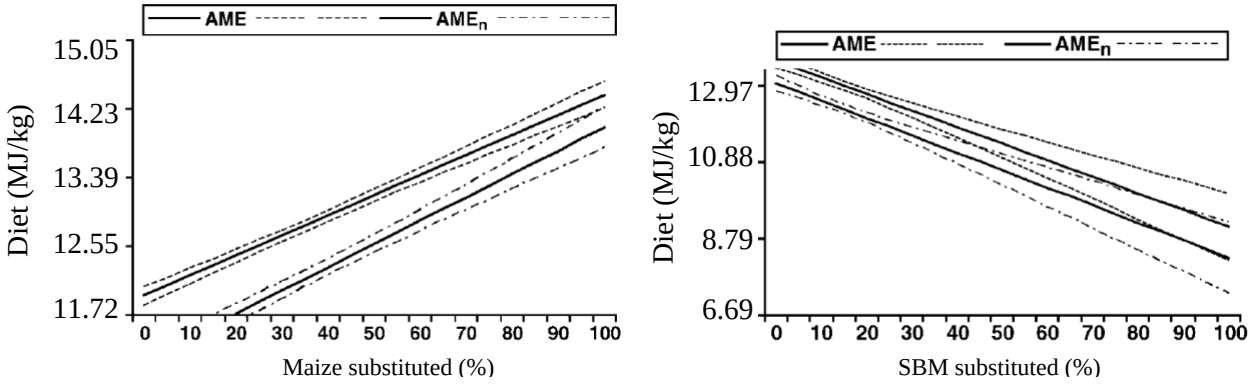


Figure 2.2. The regression of apparent metabolisable energy (AME) and nitrogen-corrected AME (AMEn) of maize and soybean meal (SBM) fed to broilers in different substitution levels from the basal diet

Lee and Kong (2019) found no differences between the direct and regression methods in estimating the AME value of wheat. However, they found a variation between the two methods in the AME value of barley, with AME values of 11.43 MJ/kg and 12.44 MJ/kg for the direct and regression method, respectively. They concluded that the regression method is a good alternative for the direct method, in case the direct method is not applicable due to high ANFs in the feed ingredients.

The AME values obtained from the regression method can vary depending on the diet composition. Adeola and Ileleji (2009) compared the effect of two diet types (maize-SBM and a semi-purified diet) in the determination of the AME and AMEn values of maize distillers' dried grains with solubles at different inclusion levels (0-600 g/kg) for broiler chickens by the regression method. They found that AME and AMEn of maize distillers' dried grains with solubles were affected by the type of diet. The AME value of maize distillers' dried grains with solubles was significantly different between the two types of diet, yielding values of 12.15 MJ/kg versus 12.61 MJ/kg for the maize-SBM diet vs. the semi-purified diet, respectively. Similarly, there was a 0.74 MJ/kg difference in the AMEn value between the two diet types.

2.5. Other energy systems

2.5.1. Rapid metabolisable energy assays

The rapid metabolisable energy assay method was introduced by Sibbald (1976). The rapid true metabolisable assay (TME) was developed to counteract the problems associated with the standard AME method. This assay is relatively rapid, requires a small quantity of test material, and adult roosters used in the bioassay seemed to reach a steady state in terms of body weight and nitrogen balance, and hence, the birds could then be used for many assays. This method is conducted by using several ingredients and by force feeding birds different amounts (30-40 g) through a tube into the crop via the oesophagus, with excreta collection after 24 and

48 hours of feeding, or after sufficient time to allow all feed residue to be voided (Farrell, 1978; Sibbald, 1986). A similar group of birds, with similar body weights, is fasted for the same assay period for the correction of endogenous energy value, determined in excreta. The TME value is then calculated according to the following formula:

$$TME\ (MJ/kg) = (GE_i \times \text{feed intake}) - (GE_f - GE_c) / \text{DM intake (kg)}$$

where:

GE_i is the gross energy of the test ingredient (MJ/kg);

GE_f is the excreta energy of fed birds; and

GE_c is the excreta energy of fasted birds.

However, this assay has been criticised as it ignores the relationship between excreta energy and feed intake, assuming that the level of feed intake is not important in measuring TME, an assumption that cannot be validated within normal ranges of *ad lib* feeding. The TME method of measuring the energy content of ingredients is extremely difficult to validate, and hence, it is also difficult to verify the resultant values, particularly as feed ingredients might be given without any additives. Moreover, only small amounts of excreta are voided, which increases the errors associated with excreta collection (Du Preez et al., 1981; Hätel, 1986; Farrell et al., 1991a). Even minor contamination of foreign materials such as feathers will increase the error in estimating the energy value of ingredients. Furthermore, this method cannot be used in young birds because of the long starvation period and the small quantities of feed provided for birds.

In the late 1970s, Farrell (1978) developed a rapid bioassay for measuring the AME of ingredients. This method relies on training adult roosters to consume their feed allowance in one hour. In this assay, adult birds are offered feed for one hour per day, and then the excreta that is voided during the following 24 h from the feed consumed are collected. Another parallel

assay with continuous feeding is performed by feeding the test diet for three days to allow adaptation, followed by five days for the total collection of excreta. At the end of both bioassays, the ME values of the rapid assays and the continuous feeding assays are compared. Initial results revealed that AME values for SBM and sorghum-based diets obtained from the rapid method were similar to those of the continuous feeding method with young broilers (Farrell, 1978). The advantage of Farrell's (1978) rapid method is that the results of the assays can be achieved in a very short time (three days) and the method does not require adaptation to the test diets before performing the assay. Also, the AME of test ingredients were found to be similar for cockerels and young broilers, without the correction for N retention, and hence, the ME values obtained from the rapid method can be widely applied.

A comparison between the rapid TME assay method of Sibbald (1976), the rapid assay method (AME) of Farrell (1978), and the standard AMEn assay has revealed that TME values are higher than the AME values obtained from Farrell's (1978) rapid assays and the AMEn values of the standard assay. Furthermore, TME values were 17-26% higher than AME values of the standard assay for young broilers (Mollah et al., 1983).

2.5.2. Productive energy

The productive energy (PE) system was the first widely used system to evaluate the energy values of ingredients. This system was first described by Southgate (1930) based on a comparative slaughter method, and it was then refined by Fraps and Carlyle (1939). Following this, PE values of many ingredients were published (Fraps and Carlyle, 1941, 1942; Fraps, 1946). Productive energy of feed is defined as its value to supply energy to birds after deducting all possible sources of energy losses, such as undigested and metabolic components (Fraps and Carlyle, 1939). It is defined as the energy stored as fat or protein in the body, which exceeds the required energy for all maintenance sources (Fraps, 1946).

Measurement of the PE of ingredients involves feeding a complete feed to two groups of birds, either *ad libitum* or 50% *ad libitum*, to measure the maintenance energy and carcass energy gain. After feeding groups for three weeks, carcasses are analysed for energy, protein, fats, and N-free extract contents. The PE can be calculated based on the following simultaneous equations, which means that the PE value of ingredients has to be calculated based on the mean results from a series of trials (Hill and Anderson, 1958):

$$FX = WM + G$$

where:

F = feed consumption;

X = the PE per unit weight of feed;

W = the average body weight during the three weeks' assay;

M = the maintenance requirement per unit weight (assuming that it is constant for different body weights); and

G = carcass energy.

The PE system has several shortcomings; PE values of ingredients are calculated based on the average results from a number of trials, which will increase the variations in PE value. Furthermore, PE values are influenced by the ingredient or feed composition. Variations in the protein content of any diet have an influence on a bird's maintenance requirements; hence, variations in protein content affect the PE value of the diet. For example, birds fed a low-protein diet (170 g/kg crude protein) require 0.0007 MJ/kg body weight/day maintenance energy compared to 0.0006 MJ/kg body weight/day maintenance energy in birds fed a high-protein diet (220 g/kg crude protein). Moreover, the PE of the same ingredient can vary due to bird-to-bird variations in feed digestibility and utilisation (Fraps, 1946). Another criticism is that the procedure requires killing a large number of birds for carcass analyses, which is time consuming and labour intensive (Hill and Anderson, 1958; Davidson et al., 1968).

In 1958, Hill and Anderson (1958) modified the PE procedure proposed by Fraps (1946), and they compared two systems, PE and AME, for broiler chickens. The results showed that there were high variations in PE values between diets and replicates within the same diet, with PE values being about 77% of the AME values. These researchers suggested that the AME system is more accurate for evaluating the energy content of feed compared to the PE system.

2.5.3. Net energy

The net energy (NE) system has received some attention recently as an alternative for the standard AME system in feed formulation for broilers (Swick et al., 2014; Wu et al., 2020). Net energy represents the effective energy for birds used for body maintenance and various forms of production, and it can be defined as the ME minus the energy losses for heat increment (HI), which is the increase in heat production after consumption of food, digestion, metabolism, and excretion of wastes. Heat increment represents almost between 20-25% of the ME of feed for broilers depending on the diet composition (Swennen et al., 2004; Wu et al., 2020).

Dietary NE consists of two components: NE for maintenance (NEm) and NE for production (NEp). Net energy for maintenance can be defined as the energy used to sustain a bird that is neither gaining nor losing weight in a post-absorptive state, in a thermoneutral environment, at rest, and in sexual repose. In a positive energy balance, all NEm is lost as heat and is difficult to separate from other dietary sources of heat. Net energy for production can be defined as the energy required for weight gain, retained in body tissues or products (Zuidhof, 2019; Wu et al., 2020).

Net energy can be estimated experimentally by *in vivo* assay, which is an expensive and difficult process, or alternatively, by using predictive equations. The measurement of heat production can be done using calorimetry estimation, in which the heat production of birds can be estimated from the amount of oxygen consumed and carbon dioxide released. Another

method for NE measurement is by determining the energy retention in the carcasses using comparative slaughter and carbon and N balance bioassays (Fraps, 1946). In the 1990s, MacLeod (1994) developed a model to predict the NE content of ingredients. This model predicts the efficiency of utilisation of consumed nutrients for different biological processes such as body maintenance, body growth, and egg production. In this method, the chemical composition of ingredients, and various efficiencies of energy utilisation from ingredients for meat and egg production were considered. Also, this model considered the amino acid composition of ingredients, the protein synthesis needed for egg, body mass, and feathers, and the energy needed for uric acid synthesis.

Theoretically, the NE is the only system that accurately describes the true energy used by birds for maintenance and production; therefore, NE can be considered the most accurate method for estimating the energy content of ingredients, enhancing the precision of feed formulation, efficiency and profitability (Wu et al., 2020; Zaefarian et al., 2021). However, the NE system has been criticised for several reasons. Firstly, measuring the HI of ingredients is labour intensive and complicated compared to the ME method, as it requires comparative slaughter or live animal indirect calorimetry (Moehn et al., 2005; Barzegar et al., 2020). Secondly, the NE method relies on the precise measurements of AME and maintenance requirements for birds, so any errors in the AME procedure will be reflected in the NE measurement. De Lange and Birkett (2005) revealed that the NE system was incapable of determining the NEm and NEp due to the imprecision of the methodology used to estimate the HP. Moreover, the NEp cannot be accurately explained by the NE system.

Parsons (2011) reported that the AME system is the industry-preferred measurement for poultry now and in the near future, as the NE system still needs a lot of research and development. According to Noblet et al. (2021), the NE system is unlikely to be adopted in feed formulation for broilers as it requires intensive research and development. They justified this

as the NE value of a diet or ingredients is highly dependent on the methodology followed, the equation used for the calculation, birds, and environmental conditions for its measurement. However, some research has reported that feeding costs could be reduced by adopting the NE system (Swick et al., 2014).

In summary, there are three reasons that, for poultry, a change from AME to the NE system is not recommended. Firstly, the direct measurement of dietary AME is practical and less expensive. Secondly, the retained energy (in body tissues or products) is identical in the NE and ME systems. Thirdly, it is easier to estimate the total heat production than its various component parts, including NEm, heat increment of feeding, heat of activity, heat of immune response, and heat of thermal regulation (Zuidhof, 2019).

2.5.4. Ileal digestible energy

Digestible energy is the difference between feed GE and the GE of faeces. Approximately 24% of feed GE is lost in the faeces (Larbier and Leclercq, 1994). Digestible energy bioassays are not commonly performed for poultry due to their anatomical differences from other farm animal species. Ileal digestible energy (IDE) of ingredients or diets can be determined by an indicator (marker) method. In the indicator method, the digestibility of energy in the test ingredient can be estimated without measuring the feed intake (Raharjo and Ferrell, 1984). Ileal digestible energy values can be calculated according to the following equation:

$$IDE_{Diet}\ (MJ/kg\ DM) = GE_{Diet} \times [(GE/Ti)_{Diet} - (GE/Ti)_{Digesta]} / (GE/Ti)_{Diet}$$

Hughes et al. (2001) compared AME and IDE values of various samples of barley and sorghum, and they found that the IDE of barley was lower than the AME by 0.40 MJ/kg, whereas for sorghum, the IDE was higher than the AME by 0.30 MJ/kg. In a later study, Hughes (2003) revealed that IDE values of wheat and barley were not affected by the gender of birds, but that AME values were lower in male birds than in females. This implies that the post-

intestinal processes associated with gut microflora are critically influenced by the gender of the birds.

Hancock et al. (2018) estimated the AME, AMEn, and IDE values of dried egg albumen for broiler chickens using a linear regression method and reported that the IDE (20.00 MJ/kg) was lower than AME (20.78 MJ/kg) and higher than AMEn (19.87 MJ/kg). Similarly, same authors revealed that the IDE value of a maize-SBM-based diet (13.78 MJ/kg) was higher than the corresponding AMEn value (13.46 MJ/kg). Furthermore, Gehring et al. (2012) reported an IDE of a maize-based diet was higher than the AMEn by 1.94%.

There are several factors that affect the IDE values of ingredients or diet, such as the inclusion level of the ingredient in the diet. Kong and Adeola (2016) reported that the IDE of canola meal was influenced by the inclusion level in the diet. Ileal digestible energy decreased from 14.58 MJ/kg to 14.05 MJ/kg as the canola meal level increased in the diets from 0 g/kg to 200 g/kg. Moreover, the IDE value of a maize-SBM-based diet was higher than the AME and AMEn values for the same diet by 0.44 and 1.21 MJ/kg, respectively (Kong and Adeola, 2016).

Another factor is the effect of age of birds: Adeola et al. (2018) reported that there were both linear and quadratic decreases ($P < 0.05$) in IDE of meat and bone meal with advancing age from 0 to 21 d of age. Moreover, the IDE value (11.93 MJ/kg) was lower than AME by 0.24 MJ/kg, and higher than AMEn by 0.69 MJ/kg at 0-7 d of age. Similarly with increasing age from 7 to 21 d, the IDE was consistently lower than the AME and higher than AMEn values of meat and bone meal for broiler chickens.

Heat treatment has also been reported to affect the IDE content of diets. Massuquetto et al. (2018) revealed that feeding broiler chickens a pelleted diet increased the IDE by 0.98 MJ/kg compared with the mash diet, which could be related to the mechanical effect of pelleting on ingredient components.

2.6. Factors affecting apparent metabolisable energy

The energy contribution of ingredients to the diet depends on numerous factors. Factors related to birds (i.e., age of the birds, genotype, gender), dietary factors (i.e., feed form, ingredient origin, cultivars, ANF content, and particle size) and the methodology used in the assessment are all influencing the energy contribution of ingredients (Mateos et al., 2019).

2.6.1. Bird-related factors

2.6.1.1. Age

The use of single AME or AMEn values derived from published tables for feed formulation may overestimate the energy utilisation by birds during the early stages of life, and the same tables may underestimate it at older ages. Some of the available tables provide different energy values for birds at different ages (Table 2.1; INRA, 2002; Premier Atlas, 2014; CVB, 2016; Feedipedia, 2017; Rostagno et al., 2017); however, other tables do not distinguish between ages (WPSA, 1989; NRC, 1994; NARO, 2009; RPRI, 2014). Moreover, in practice, not all feed companies use different energy values for broilers at different ages.

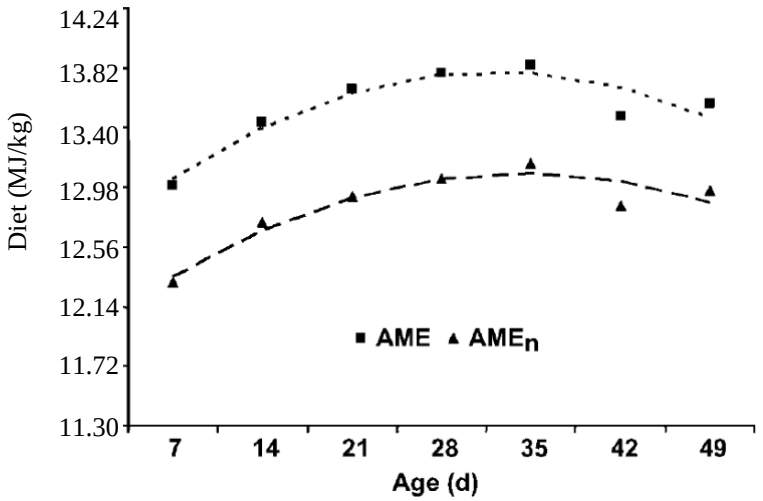
Table 2.1. Energy values (MJ/kg) of feed ingredients according to the growth stage of birds

Ingredient	CVB, 2016		Feedipedia, 2017	
	Young broilers	Adult broilers	Young broilers	Adult broilers
Maize	13.51	13.68	13.01	13.31
Sorghum	12.76	13.26	13.72	13.97
Wheat	12.47	12.80	12.01	12.51
Barley	11.13	11.92	9.83	11.51
Soybean meal	9.04	9.20	9.71	9.87

The differences in energy utilisation by birds at different ages can be explained because mature birds extract more energy than young birds, and with more noticeable differences in nutrients extracted from substances difficult to digest, such as high-fibre ingredients and saturated fats (Black et al., 2005; Mandalawi et al., 2017). Santos et al. (2015) recorded higher AME and AMEn values for sorghum by 7.2% and 7.33%, and for SBM by 3.3% and 4.5%,

respectively, in three-weeks old broilers compared to those aged only two weeks old. However, for maize, bird age had no influence on AME or AMEn. Similarly, Mello et al. (2009) reported that age effect was pronounced for sorghum and SBM, but not for maize or wheat bran. These results suggest that utilisation of energy by birds of different ages changes according to how ingredients are processed by the digestive enzymes at the different stages of the bird's life.

Lopez and Leeson (2007) found that young broilers derived less energy from maize-SBM- based diets than older birds (Figure 2.3). In addition, AME values for broilers were higher than the values for roosters. In contrast, Lopez and Leeson (2008) reported that AME and AMEn values of a maize-SBM-based diet were not affected by age of the broilers.



(AME: $y = 3{,}000 + 18.19 \pm 2.74(x) - 0.281 \pm 0.04(x)^2$; $R^2 = 0.49$.
AMEn: $y = 2{,}850 + 15.95 \pm 2.51(x) - 0.232 \pm 0.04(x)^2$; $R^2 = 0.52$.
y = AME or AMEn (MJ/kg of diet); x = age in days)

Figure 2.3. Apparent metabolisable energy (AME) and nitrogen-corrected AME (AMEn) for broilers fed a standard maize-soybean meal diet

The ability of chicks to digest feed and extract nutrients during the first 2 weeks of age is limited. The development of the microbiota system in the gut increases with age, which influences the adaptation length for dietary AME (Obst and Diamond, 1992; Lu et al., 2003). Furthermore, the ability of birds to utilise non-starch polysaccharides (NSPs) can improve with

age, and hence, increase the AME (Choct et al., 2001). After hatching, chicks go through metabolic adaptations from relying on the embryonic yolk as a source of nutrients to depending on nutrients from exogenous feed; digestive and absorptive capacity increase with age, and thus, the bird's ability to extract nutrients from feeds increases with age as well (Noy and Sklan, 2001). It has been reported that from the second week after hatching, age has significant effects on the total retention of DM and nitrogen, and the AME of various ingredients (Olukosi et al., 2007; Stefanello et al., 2016).

Over the years, several authors have reported contradictory results that energy utilisation either increases or decreases with the advancing age of broilers (Schneider and Lantzsch, 1969; Bartov, 1988; De Groote and Ketels, 1988; Senkoyiu and Janssen, 1988; Bourdillon et al., 1990). The AMEn value of maize SBM-based diet increased by 0.57 MJ/kg from 12.46 MJ/kg to 13.03 MJ/kg from d 1 to d 7, with a further increase to 13.83 MJ/kg at 21 d of age (Batal and Parsons, 2002). Ravindran et al. (2016) reported that the AME values of soybean oil were 18.8 MJ/kg, 34.2 MJ/kg, and 38.4 MJ/kg at weeks one, two, and three, respectively. In contrast, several authors have reported that AME values for growing broilers are not dependent on age (Niegm, 1966; Matterson and Prince, 1969; Siregar and Farrell, 1980; Yaghobfar, 2013).

2.6.1.2. Genotype

Digestibility and utilisation of nutrients and energy vary between bird types and are dependent on the strain of bird (Choct, 2012; Yegani and Korver, 2012; Ball et al., 2013). These variabilities may be due to the differences in the developments of the digestive system organs and/or endogenous nutrient or energy losses. Most of the current energy requirements listed in the published tables are provided in generic terms (INRA, 1989; NRC, 1994; Rostagno et al., 2017; Evonik, 2016), or are provided for specific commercial strains of broiler (Aviagen, 2009;

Hubbard, 2011). However, there is a lack of information on the influence of poultry genotypes on the AME values of many ingredients (cereal by-products, legumes, and lipid mixtures).

Santos et al. (2005) suggested that the higher dietary ME in slow-growing birds could be attributed to the characteristics of the digestive organs, as the relative gizzard weight in slow-growing birds is 26% higher than those in fast-growing birds. Rougiére et al. (2009) and Verdal et al. (2010) explained that birds with larger gastric compartments (proventriculus and gizzard) experience more effective nutrient utilisation compared with birds that show greater development of the small intestine. Rougiére et al. (2009) reported that birds with larger gastric compartments presented a 3.5% higher AMEn compared to birds with smaller proventriculus and gizzard and larger small intestine.

These results have been confirmed by Santos et al. (2015), who revealed that the AME and AMEn values of maize varied between chicken strains (Isa Label vs. Cobb). They found that AME and AMEn values of a maize-based diet fed to the Isa Label were 5.75% and 3.44%, respectively, higher than those values for the Cobb. However, no differences were noted in AME and AMEn between the two strains for sorghum and SBM. The authors explained that considering that the growth rate of Isa Label chickens is slower than that of fast-growing (Cobb) broiler strains, rates of digestive tract growth and enzyme production may be different from those of broilers selected for fast growth. This difference in gastrointestinal development may influence nutrient utilisation, which has implications on the formulation of feeds for slow-growing chickens; feeds formulated for slow growers are commonly based on nutritional data derived from fast-growing strains. Moreover, Lopez and Leeson (2005) showed that broilers yielded 3-4% lower AME values than Plymouth Barred Rocks or Leghorns, suggesting that broilers have higher energy losses than the other two breeds.

However, some researchers reported no differences in energy utilisation between different strains of chickens (Begin, 1967; Washburn et al., 1975). Sibbald and Price (1980)

reported no differences in energy utilisation between Single Comb White Leghorn cockerels and laying hens of different strains. In addition, Yaghobfar (2003) found no differences between two strains (Cornish vs. Rhode Island Red) in dietary AME and AMEn values.

2.6.1.3. Gender

Gender has been reported to have an impact on energy utilisation in poultry. A study by Huang et al. (2007) showed that male Taiwanese chickens utilised about 31% of their diet energy for growth, whereas females utilised around 39% for body growth. Similarly, energy used for feather production was a 4.6% proportion for males and 4% for females from the total energy utilised from the feed.

It has been reported that at the early stages of growth males utilise more of the energy for growth compared to females (Sakomura et al., 2005; Dozier III et al., 2011). However, this difference is narrowed after seven weeks of age. The differences in energy utilisation between males and females can be related to male birds' high growth rates, greater feed consumption, and better feed and energy conversion ratios (Han and Baker, 1994; NRC, 1994; Kidd et al., 2004; Corzo et al., 2005). Moreover, males secrete more testosterone and growth hormone, resulting in increased energy utilisation, which accretes more lean muscle mass (Leenstra et al., 1991; Kühn et al., 1996; Sakomura et al., 2005). In contrast, some reports have shown no differences in energy utilisation between genders during the first three weeks of growth after hatching (Yaghobfar, 2001; Ravindran et al., 2004a).

2.6.2. Dietary factors

There are different ingredients available to be included in poultry diets, and AME values vary between ingredients. The variability between tabulated AME values can be related to differences in the content of energy-yielding components, or the ANF content. Moreover, ingredient cultivars, feed forms (mash vs. pellets), and particle size of the ingredients (fine vs.

coarse) are some of the most relevant factors affecting the efficiency of the energy utilised by birds (Hetland et al., 2005; Amerah et al., 2007; González-Alvarado et al., 2007; Valencia et al., 2009; García-Rebollar et al., 2016).

2.6.2.1. Feed form

Poultry feed manufacturers produce different forms of poultry feed (mash, crumble, and pellets). Mash feed can be produced by mixing ground ingredients with other ingredients (salt, amino acids, minerals, and vitamin premix, etc.) in a mixer to assure homogeneity. Pelleting is the most common thermal processing method in the production of broiler feed. The main aim of pelleting is to agglomerate smaller feed particles by the use of mechanical pressure, moisture, and heat. A major step in the pelleting process is the conditioning of mash prior to pelleting (Skoch et al., 1981), which is generally accomplished by adding steam to the mash feed. A crumble form is the type of feed that can be made by crushing the pellets to a consistency coarser than mash, which is suitable for feeding birds in the first few weeks of age (Cerrate et al., 2009).

A number of factors can be considered as motivations behind the pelleting of broiler feeds. Feed intake (FI) is the major factor driving body weight gain (BWG), and an increased FI is the primary motivation for pelleting broiler diets (Engberg et al., 2002; Svihus et al., 2004). Abdollahi et al. (2011) reported a 14% increase in FI for broilers due to pelleting during the starter phase (1-21 d of age). Lilly et al. (2011) showed that increasing the percentage of intact pellets from 30% to 60% and 90%, in a broiler diet increased FI and consequent BWG. In addition, pelleting reduces feed wastage, which may be attributed to less particles falling from the beak onto the floor or into the water stations (Jensen, 2000). Pelleting also prevents birds from selecting larger particles from mash feed, and the messy sorting poultry engages in when feeding mash diets, which may cause the feed to be pushed out of feeders, thus increasing feed wastage. Pellet-fed birds spend less time and energy on the ingestion of feed and obtain more

nutrients per unit of expended energy than those fed mash diets (Jones et al., 1995; Vtlariño et al., 1996). Pelleting increases the bulk and density of mash feed, allowing more efficient transportation and also enhancing the flow properties of feed (Abdollahi et al., 2013b).

Processing feed affects not only feed intake, but also gastrointestinal tract development, specifically the gizzard, resulting in changes in nutrient utilisation and microbial profile (Frikha et al., 2009; Zang et al., 2009). The effects of pelleting on the energy content of diets are inconsistent, and depend on different factors such as ingredient type used in the diet, heat applied and particle size (Duke, 1986; Mateos et al., 2012; Abdollahi et al., 2013b; Serrano et al., 2013). It has been reported that feeding birds on pelleted diets reduces the AME and AMEn values compared to mash diets, and is attributed to the starch overload in the gut and possible starch retrogradation forming resistant starch (Svihus, 2001; Zimonja et al., 2007; Vicente et al., 2008). Abdollahi et al. (2013a) showed that pelleting reduced the AME value of a wheat-based diet by 0.70 MJ/kg compared to the mash form. However, pelleting showed no effect on the AME value for a maize-based diet. Similarly, Amerah et al. (2007) reported that pelleting reduced the AMEn of a wheat-based diet from 12.5 MJ/kg to 11.8 MJ/kg compared to the mash form.

In contrast, pelleting might increase the energy utilisation of certain ingredients, as heat and mechanical pressure in the pellet die can disrupt the structure of the cell walls, thus releasing some nutrients such as lipids contained in the oil bodies of oil-containing ingredients (González-Alvarado et al., 2007; Jiménez-Moreno et al., 2009).

Svihus et al. (2004) reported that pelleting a wheat-based diet increased the AME from 11.6 MJ/kg to 11.8 MJ/kg, although the increase in AME was not associated with improvements in starch digestibility. Pirgozliev et al. (2016) showed that feeding birds a pelleted wheat-based diet improved dietary AMEn by 4.3%, and the AMEn:GE ratio by 3.6% compared to a mash diet. These findings have been explained as the majority of the available energy in wheat comes

from starch that is stored intracellularly, and this starch is only partly accessible to poultry because they have limited endogenous enzymes, which reduces their ability to degrade plant cell wall material (Ball et al., 2013). Thus, a procedure capable of damaging cell walls, such as steam pelleting, may allow birds' digestive enzymes access to nutrients trapped within the cell. Such a procedure may improve dietary AME. However, the improvement in energy utilisation reported with heat processing of cereals tends to disappear as birds age (García et al., 2008; Gutierrez-Alamo et al., 2008; Frikha et al., 2013).

Although the use of pelleted diets is a common practice in the broiler industry, research on AME assays is usually conducted with mash diets (Lockhart et al., 1967; Adeola and Ileleji, 2009; Lee and Kong, 2019).

It is accepted that feeding pelleted diets has negative consequences on physiological development and functionality of the foregut, especially the gizzard, and nutrient and energy utilisation (Amerah et al., 2007; Abdollahi et al., 2011, 2013a). Therefore, estimates determined in assays using mash diets might over- or under-estimate the AME of individual ingredients when used in pelleted complete diets.

2.6.2.2. Ingredients

Understanding the variation in the efficiency of energy utilisation by birds fed on different ingredients is economically crucial for commercial poultry producers. Variations in the physio-chemical composition of ingredients can significantly influence the digestibility and availability of nutrients, and hence, these variations can affect the AME values of different ingredients (Yegani and Korve, 2012). This variation has been explained as the differences in the proportions and the percentages of the major nutrients: protein, fat, and carbohydrates, which are responsible for significant variation in the birds' abilities to utilise energy from different substances (De Groote 1974; Emmans, 1994).

Cereals are the primary energy sources in poultry diets. Therefore, the accurate measurement of their energy content is of paramount interest. The energy content of the cereals depends on the moisture level and the proportion of the starch and fibre fractions. Higher variability in energy is expected for wheat and barley than for maize. Cereals' AMEn content is recorded in tables according to different institutions' parameters, with consequent wide variations in the AMEn values of cereals and protein source ingredients (Table 2.2). Besides starch content, the variations in AME and AMEn content of cereals could be related to the variation in moisture content in maize; the tannin content and the kafirin proportion of the protein fraction that affect the AMEn of sorghum (Selle et al., 2010); or the ANF content, which affects the AME values for wheat and barley (Choct et al., 1999; Jacob and Pescatore, 2012).

Table 2.2. Nitrogen-corrected AME (MJ/kg) for the common ingredients in poultry diets

Reference*	Barley	Maize	Sorghum	Wheat	Soybean meal	Canola meal	Meat and bone meal
WPSA	11.80	13.60	13.31	12.84	9.46	8.55	10.35
NRC	11.05	14.02	13.77	13.05	9.92	8.37	9.00
Evonik	11.30	13.81	13.60	12.89	9.79	-	-
Feedipedia	9.83	13.01	13.72	12.01	9.70	9.6	11.9
FEDNA	11.63	13.72	13.64	12.97	9.71	7.58	-
Rostagno	11.31	14.08	13.41	12.72	8.88	7.24	7.48

* WPSA (1989); NRC (1994); Evonik Industries (2016); Feedipedia (2017); FEDNA (2017); Rostagno et al. (2017)

Furthermore, variations between different cultivars of the same ingredient can also affect the AME content. Adewole et al. (2017) reported a wide variation in the AMEn values of different canola meal samples, which ranged from 7.08 MJ/kg DM up to 8.55 MJ/kg DM.

These differences could also be related to the differences in the country of origin, light hours supporting plant growth, soil characteristics, and growing, harvesting, and storage conditions (Medic et al., 2014). In the same context, Ravindran et al. (2014) found variations in the AME values of SBM from different countries of origin, where AME values ranged from

8.37 MJ/kg for SBM grown in India to 9.32 and 9.94 MJ/kg for Argentina and US SBM, respectively. Jiang (2007) reported AME values ranging from 12.25 MJ/kg to 14.15 MJ/kg, and AMEn values ranged from 11.91 MJ/kg to 13.77 MJ/kg for maize from different countries of origin: Thailand, Pakistan, China, and Indonesia (Figure 2.4).

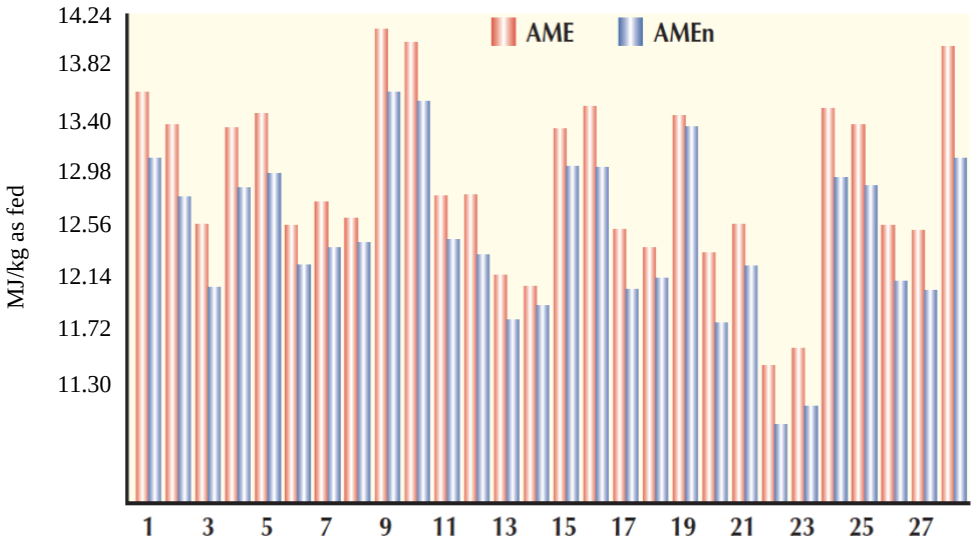


Figure 2.4. Apparent metabolisable energy (AME) and nitrogen-corrected AME (AMEn) of 28 maize samples from different origin

Karunaratne et al. (2018) stated that the AME value of wheat varied between different varieties. The AME content ranged from 13.41 MJ/kg (Canadian wheat, red spring variety) to 14.28 MJ/kg (Canadian wheat, hard white spring variety). Furthermore, the AME values of different batches of the same variety were found to differ between batches, ranging from 12.1 MJ/kg to 16.6 MJ/kg (Sibbald and Slinger, 1962; Schumaier and McGinnis, 1967; Davidson et al., 1978). This variability is expected due to the differences in the starch and protein content of wheat samples, and in agreement with the findings of Azhar et al. (2019), who evaluated 17 wheat samples with different chemical compositions and quality characteristics. They found that the AME values of the samples ranged from 13.68 to14.81 MJ/kg. The AMEn ranged from 13.32 to14.36 MJ/kg, and ash content was negatively correlated with AME and AMEn values.

Another factor influencing the AME values of poultry feeds is the storage period. Choct and Hughes (1997) described that after storage of new-season wheat for three months, the AMEn of wheat increased by nearly 0.30 extra MJ/kg, depending on the variety. Moreover, Yegani and Korver (2012) showed that the AME of wheat increased from 9.19 MJ/kg to 12.03 MJ/kg after storage of wheat samples for 1 year. Consequently, the AME and AMEn values obtained from different varieties of grains may vary depending on the time elapsed from harvest. Therefore, poultry producers should scrutinise the AME and/or AMEn obtained from tables carefully before formulating their birds' diets.

2.6.2.3. Anti-nutritional factors

One of the most common ANFs in feed ingredients is non-starch polysaccharides (NSPs). Non-starch-polysaccharides are a group of polysaccharides with unique physiochemical characteristics of each individual group of NSPs. The level of NSP content varies between different poultry feed ingredients (Table 2.3).The presence of NSPs in any poultry diet is a great concern. Generally, NSPs have different effects on the physiological and intestinal properties of birds. They are non-degradable substances, and remain undigested by the endogenous enzymes in the gastrointestinal tract of birds (Choct, 2015). It has been reported that starch digestibility has a positive correlation with AME values of cereals (Rogel et al., 1987; Wiseman et al., 2000). The detrimental and anti-nutritional effects of NSPs on dietary AME are due to their potential to increase the digesta viscosity, hence negatively affecting the digestibility of nutrients and ultimately, energy utilisation (Choct and Annison, 1992; Bedford, 1995; Van Campenhout, 2007).

The presence of NSPs in wheat samples, specifically soluble NSP, is one of the reasons for variation in the energy values of wheat (Annison 1993; Choct et al., 1999; McCracken and Quintin, 2000). In fact, Smeets et al. (2015) found that the presence of high NSP content (102

g/kg DM) in wheat reduced AMEn values for broiler chicken to 12.62 MJ/kg, compared to 13.36-13.09 MJ/kg for wheat with low-NSP content (83.4 g/kg DM-73.9 g/kg DM).

Table 2.3. Non-starch polysaccharide contents in some feed ingredients (g/kg, DM); (Choct, 1997)

Ingredient	Insoluble NSP	Soluble NSP	Total NSP
Maize	8.0	0.1	8.1
Wheat	9.0	2.4	11.4
Barley	12.2	4.5	16.7
Sorghum	4.6	0.2	4.8
Soybean meal	11.9	3.0	14.0
Canola meal	15.5	5.0	20.5

There are various types of ANFs in poultry feeds that affect energy utilisation: trypsin in SBM, tannins in sorghum, xylans and β-glucans in barley, and phytate. All of these ANFs not only affect AME values, but also affect birds' gastrointestinal tract function and energy utilisation of other components in their diet. Liu et al. (2015) suggested that the reasons for the poor energy utilisation of sorghum-based diets are related to the presence of kafirin, the primary protein portion in sorghum; phenolic compounds, which occur as condensed tannin and phytate.

The adverse effects of NSPs can be exaggerated by processing, as pelleting at a high temperature can solubilise the insoluble NSP fractions in wheat and barley, leading to higher viscosity of the digesta compared to the unprocessed form of feed, hence reducing digestibility and AME values (Mateos et al., 2019).

2.7. Research gaps and conclusions

Enhancing feed formulation and optimising feed costs necessitate the accurate estimation of the energy content of feed ingredients. Pelleted feed is the prominent feed form used by the poultry industry. However, the AME value of feed ingredients applied in diet formulation has been obtained from assays with unprocessed mash form diets, which neglects the prominent influence of feed form on energy utilisation of diets and feed ingredients for broiler chickens. Moreover, the current single AME estimates of feed ingredients used in feed

formulation are estimated from birds at a specific growth stage, mostly 3 weeks old. This neglects the fact that birds' ability to digest and utilise feed, including energy-yielding nutrients, varies with advancing age. Considering the current shortcomings of the AME system, the most preferred method until now by the poultry industry, an alternative system that could eliminate those shortcomings could be obtained by the true ileal digestible energy (TIDE) system. The TIDE system requires estimation of the ileal endogenous energy losses in broilers, which have not been reported yet. Therefore, the current literature review discussed the various assay methods which could be applied for evaluating dietary energy content. Various factors have an effect on the energy values of diets (i.e., age of birds, gender, genotype, feed form and the ingredient type). Therefore, the main focus of this research is to precisely understand the factors affecting the energy content of ingredients and diets, and additionally, to evaluate the assay method for the evaluation of the dietary energy content.

CHAPTER 3 Influence of feed form on the apparent metabolisable energy of feed ingredients for broiler chickens

3.1. Abstract

The influence of feed form (FF) on the apparent metabolisable energy (AME) of seven feed ingredients for broiler chickens was examined in two experiments. The first experiment was conducted to investigate the interaction between four cereal grains (maize, sorghum, wheat and barley) and two FF (mash vs. pellet) on the nitrogen-corrected AME (AMEn) contents using the direct method. The test diets contained 962 g/kg of each grain. Each diet was randomly allocated to six replicate cages (six birds per cage) and fed for 7 d from 21 to 28 d post-hatch. The feed intake and total excreta output for each replicate cage were measured over the last 4 d of the assay. No interaction ($P > 0.05$) between grain type and FF was found for the AMEn. Maize and sorghum showed the highest AMEn, barley the lowest and wheat being intermediate. Regardless of the grain type, pelleting increased ($P < 0.05$) the AMEn of the grains by 0.22 MJ/kg, compared with mash diets. The second experiment was conducted to examine the effect of FF on the AMEn of three protein sources (meat and bone meal [MBM], soybean meal [SBM], and canola meal [CM]) using the substitution method. A maize-SBM basal diet was formulated and the test diets were developed by replacing (w/w) 30% of the basal diet with MBM, SBM or CM. Each diet was randomly allocated to six replicate cages (six birds per cage) and fed for 7 d (d 21 to 28) and, feed intake and total excreta output were measured over the last 4 d. There was a significant ($P < 0.05$) interaction between the protein source and FF. Pelleting reduced the AMEn of MBM, had no effect on that of SBM, but increased ($P < 0.05$) the AMEn of CM. Overall, pelleting process increased the AMEn of all cereal grains and influenced those of protein sources with the effect varied depending on the ingredient. The current findings suggest that the application of AMEn values determined based on assays using mash diets might result in over- or under-estimation of the available energy content of ingredients in commercial

pelleted broiler diets and highlights the need for the use of pelleted diets in energy evaluation assays.

3.2. Introduction

Different feed ingredients are combined in poultry feed formulations to meet the nutrient and energy requirements of birds for production. Dietary energy is the most important factor to be considered when formulating poultry diets. The fact that feed cost represents about 70% of the total production cost and the energy represents two-thirds of the feed cost necessitates the need for proper energy evaluation of ingredients to optimise the formulations.

Energy is not a nutrient, but a property that some nutrients (carbohydrates, lipids and protein) possess. Various feed ingredients, with differing available energy contents, are available to use as energy sources in poultry diets. It is crucial from the standpoint of formulating a well-balanced diet, as energy regulates, inter alia, the feed consumption of birds. Therefore, accurate estimation of the energy content of different dietary ingredients is essential to enhance bird performance and efficiency.

The available energy content of ingredients for poultry could be measured through different methods. Measurement of apparent metabolisable energy (AME) is the accepted standard and most common procedure as it is simple and straightforward and considers most of the energy losses after digestion and metabolism (Hill and Anderson, 1958; Zelenka, 1970). Another system is the net energy (NE) system, which is however, a very complicated method due to the requirement for special equipment and complex measurements (Moehn et al., 2005; Noblet et al., 2010; Choct, 2012). Moreover, the NE system relies on the precise measurement of AME and the maintenance requirements for birds; any errors in the AME procedure will, therefore, affect the NE measurement. There is consensus that the AME system will remain the preferred measurement for poultry for the foreseeable future, as the NE system still requires a

lot of research and development (Carré et al., 2014).

Three different methods can be used for the determination of AME of feed ingredients, namely the direct, substitution (difference) and regression methods. The direct method is the most commonly used, mainly because of the simplicity of the assay diet and calculations. In this method, the test ingredient is used as the sole source of energy in the assay diet. The substitution method is used to evaluate the AME of ingredients with poor palatability, high protein content, or high level of anti-nutritional factors in the test ingredient (Sibbald et al., 1960). The regression method is based on feeding a basal diet and assay diets, with at least two levels of the basal diet replaced by the test ingredient. The energy values of individual diets are then compared to the corresponding inclusion level of the ingredient. Extrapolation of energy to the equivalency of 100% inclusion of the test ingredient predicts the energy value of the ingredient (Fan and Sauer, 1995).

The measurement of AME content of feed ingredients for broilers, regardless of the assay method, is normally conducted using mash diets (Lockhart et al., 1967; Batal and Parsons, 2004; Adeola and Ileleji, 2009; Lee and Kong, 2019) and the impact of feed form (FF; mash vs. pellet) on AME estimates of individual feed ingredients is largely overlooked. Despite its indisputable advantages on broiler performance, feeding pelleted diets has negative consequences on physiological development and functionality of the foregut, especially the gizzard, and nutrient and energy utilisation (Amerah et al., 2007; Abdollahi et al., 2011, 2013a). Naderinejad et al. (2016) clearly showed that the improvement in AME of a maize-based diet was associated with higher gizzard weights and lower gizzard pH, when birds were fed pelleted diets. To the author's knowledge, no previous study has investigated the effect of FF on the AME of individual ingredients. Published data on the effects of FF on AME of complete diets in broilers, however, have been equivocal (Svihus et al., 2004; Amerah et al., 2007; Abdollahi et al., 2013a, 2014; Roza et al., 2018). Hussar and Robblee (1962) reported that pelleting had no effect on

the dietary AME of a wheat-based diet. In contrast, Svihus et al. (2004) reported an increase of 0.2 MJ/kg (from 11.6 to 11.8 MJ/kg) in the AME of a wheat-based diet in pellet form compared to mash form. Negative effects of pelleting on the AME have also been reported in wheat- and sorghum-based diets (Amerah et al., 2007; Abdollahi et al., 2011, 2013a, 2014). These findings suggest that the estimates determined in assays using mash diets might over- or under-estimate the AME of individual ingredients when used in pelleted complete diets. The data above call into question the application of AME values obtained with mash diets to commercial situations where the majority of broiler feeds is pelleted and highlights the need to investigate the impact of FF on energy evaluation of individual feed ingredients. It was hypothesised that FF will influence the energy utilisation of individual feed ingredients in broilers. Therefore, the objective of this study was to investigate the impact of FF on the nitrogen-corrected AME (AMEn) of four cereal grains (maize, sorghum, wheat and barley) and three protein sources (meat and bone meal [MBM], soybean meal [SBM], and canola meal [CM]) for broiler chickens.

3.3. Materials and methods

The experiment was conducted according to the New Zealand Revised Code of Ethical Conduct for the use of live animals for research, testing and teaching and approved by the Massey University Animal Ethics Committee.

3.3.1. Ingredients and laboratory evaluation

Four cereal grains (maize, sorghum, wheat and barley) and three protein sources (MBM, SBM and solvent-extracted CM) were obtained from a local commercial supplier. The maize and barley were sourced from New Zealand, and wheat and sorghum samples were of Australian origin. The MBM was sourced from New Zealand, SBM was of Argentinian origin, and CM was of Australian origin. The protein sources were in ground form and the cereal grains were ground in a hammer mill to pass through a screen size of 3.0 mm. Representative samples

of each ingredient were analysed, in duplicate, for dry matter (DM), nitrogen (N), crude fat, neutral detergent fibre (NDF), ash, calcium (Ca), phosphorus (P) and gross energy (GE). The cereal samples were also analysed for starch.

3.3.2. Birds and housing

Day-old male broiler chicks (Ross 308) were obtained from a commercial hatchery, raised in a floor pen until 21 d of age and fed a commercial broiler starter diet (230 g/kg crude protein and 12.56 MJ/kg AMEn). The temperature was maintained at 31℃ on d 1 and was gradually reduced to 22 ℃ by the end of the third week. Central ceiling extraction fans and wall inlet ducts controlled the ventilation. Feed and water were offered *ad libitum*.

3.3.3. Experiment 1- Cereal grains

The AMEn of the grains (maize, sorghum, wheat and barley) were determined using the direct method. In this method, four basal diets were formulated to contain 962 g/kg of each grain (Table 3.1). Each basal diet was then divided into two equal batches and, one was offered in mash form and the second batch was pelleted, to develop 4 × 2 factorial arrangements of eight dietary treatments. On d 18, birds were individually weighed and 288 birds of uniform body weight (946 g) were randomly allocated to 48 cages with six replicates per treatment (six birds/cage). Birds were fed the experimental diets from 21 until 28 d of age. The cages were located in an environmentally controlled room with 20 h of fluorescent illumination per day.

3.3.4. Experiment 2- Protein sources

The AMEn were determined for MBM, SBM and CM by the substitution method. In this method, a maize-SBM basal diet was formulated (Table 3.1) and the test diets, containing different protein sources, were developed by replacing (w/w) 300 g/kg of the basal diet with one of the protein sources. The basal and test diets were divided into two equal batches and, one was offered in mash form and the second batch was pelleted, to develop 3 × 2 factorial

arrangements of six dietary treatments. On d 18, the birds were individually weighed and 288 birds of uniform body weight (968 g) were randomly allocated to 48 cages with six replicates per treatment (six birds/cage). The birds were fed a commercial broiler starter diet as in experiment 1 until 21 d of age and, the experimental diets were offered from 21 until 28 d of age.

Table 3.1. Composition (g/kg, as fed basis) of the cereal-based test diets used in experiment 1 and the basal diet used in experiment 2 (protein sources)

Ingredient	Composition (g/kg)				
	Experiment 1				Experiment 2
	Maize	Sorghum	Wheat	Barley	Basal diet
Test ingredient	962	962	962	962	-
Maize	-	-	-	-	590
Soybean meal	-	-	-	-	356
Soybean oil	-	-	-	-	18.0
Dicalcium phosphate	19.0	19.0	19.0	19.0	22.0
Limestone	13.0	13.0	13.0	13.0	8.0
Sodium Chloride	2.0	2.0	2.0	2.0	2.0
Sodium bicarbonate	2.0	2.0	2.0	2.0	2.0
Trace mineral-vitamin premix[1]	2.0	2.0	2.0	2.0	2.0

[1]Vitamin and trace mineral premix supplied the following per kilogram of diet: antioxidant, 100 mg; biotin, 0.2 mg; calcium pantothenate, 12.8 mg; vitamin D_3 (cholecalciferol), 2400 IU; cyanocobalamin, 0.017 mg; folic acid, 5.2 mg; menadione, 4 mg; niacin, 35 mg; pyridoxine, 10 mg; vitamin A (trans-retinol), 11100 IU; riboflavin, 12 mg; thiamine, 3.0 mg; vitamin E (dl-α-tocopheryl acetate), 60 IU; choline chloride, 638 mg; Co, 0.3 mg; Cu, 3.0 mg; Fe, 25 mg; I, 1 mg; Mn, 125 mg; Mo, 0.5 mg; Se, 200 µg; Zn, 60 mg.

For both experiments, diets were mixed in a single-screw paddle mixer (Bonser Engineering Co. Pty. Ltd., Merrylands, Australia). Following mixing, the diets were split into two batches. The first batch was retained in mash form. The second batch was pelleted using a pellet mill (Model Orbit 15; Richard Sizer., Kingston-upon-Hull, UK) capable of manufacturing 180 kg of feed/h and equipped with a die ring with 3-mm holes and 35 mm thickness. The pellet diameter and length were 3.0 mm and 5.0 mm, respectively. Representative samples of all diets were collected after pelleting for physical pellet quality measurements.

3.3.5. Pellet durability index and pellet hardness

Pellet durability index (PDI) was determined in a Holmen Pellet Tester (New Holmen NHP100 Portable Pellet Durability Tester, TekPro Ltd., Willow Park, North Walsham, Norfolk, UK) using the method described by Abdollahi et al. (2011). Briefly, clean pellet samples (100 g; five replicates per diet), with no fines, were rapidly circulated in an air stream around a perforated test chamber for 30 seconds. Fines were removed continuously through the perforations using the test cycle. After the test cycle, the subject pellets were ejected and weighed manually. The PDI was calculated as the percentage of weight of pellets not passing through the perforations at the end of the test to weight of whole pellets at the start.

Pellet hardness was tested using a motor-driven pellet hardness tester (KAHL Pellet-Härtetester, Amandus Kahl GmbH & Co. KG, Hamburg, Germany). Individual pellet samples (12 per diet) were inserted between a pressure piston and a bar and by increasing pressure applied by means of the pressure piston, the force (kg) needed to break the pellets was determined.

3.3.6. Determination of the apparent metabolisable energy

The AME was determined using the total excreta collection procedure from d 25-28. Diets were fed for 7 d (21 to 28 d post-hatch), with the first 3 d serving as an adaptation period. The feed intake (FI) and total excreta output for each replicate were recorded over the last 4 d of the assay. Daily excreta collections were pooled within replicates, mixed in a blender and sub-sampled. Sub-samples were lyophilised (Model 0610, Cuddon Engineering, Blenheim, New Zealand), and dried excreta samples were ground to pass through a 0.5-mm sieve and stored in airtight plastic containers at 4 ºC pending analysis. The diet and excreta samples were analysed for DM, GE, and N.

3.3.7. Gizzard pH and relative weight of proventriculus and gizzard

On d 28, two birds from each cage, with body weights closest to the mean weight of the cage, were selected, weighed and euthanised by cervical dislocation. Proventriculus and gizzard were excised for further analysis. Gizzard pH was measured as described by Singh et al. (2014). The pH of gizzard content was measured with a calibrated digital pH meter (Model IQ120, ISFET pH Meter, Shindengen, Japan) by inserting the pH meter directly into three different parts (proximal, middle, and distal) of the gizzard from each bird. Readings were recorded after stabilisation of value and the average of the three readings was considered as the gizzard pH value. The proventriculus and gizzard were dissected, emptied and the adhering fat was removed. The proventriculus and gizzard weights were dried and organ-to-body weight ratios were calculated.

3.3.8. Chemical analysis

Dry matter was determined using standard procedures (Method 930.15; AOAC, 2016). Ash was determined by a standard procedure (Method 942.05; AOAC, 2016) using a muffle furnace at 550ºC for 16 h. Nitrogen was determined by combustion (Method 968.06; AOAC, 2016) using a carbon nanosphere-200 carbon, N and sulphur auto analyser (rapid MAX N exceed, Elementar, Donaustraze, Hanau, Germany). The crude protein (CP) content was calculated as N × 6.25. The crude fat was determined by Soxtec extraction procedure (Method 2003.06; AOAC, 2016) using (Soxtec System HT 1043 Extraction Unit, Höganäs, Sweden). Starch was measured using a Megazyme kit (Method 996.11; AOAC, 2016) based on thermostable α-amylase and amyloglucosidase (McCleary et al., 1997). The NDF (Method 2002.04; AOAC, 2016) was determined using Tecator Fibertec™ (FOSS Analytical AB, Höganäs, Sweden). For minerals, samples were ashed and then calcium (Ca) and phosphorus (P) were determined colorimetrically following digestion with HCl (Method 968.08D; AOAC,

2005). Gross energy was determined by an adiabatic bomb calorimeter (Gallenkamp Autobomb, Weiss Gallenkamp Ltd, Loughborough, UK) standardised with benzoic acid.

3.3.9. Calculations

All data were expressed on a DM basis and the AME was determined using the following formula:

$$AME_{Diet}\ (MJ/kg) = [(FI \times GE_{Diet}) - (\text{Excreta output} \times GE_{Excreta})] / FI$$

The AME of the cereal grains and protein sources were determined as follows:

$$\text{Experiment 1: } AME_{Grain}\ (MJ/kg) = \text{AME of test grain diet} \times (100/96.2)$$

$$\text{Experiment 2: } AME_{\text{Protein source}}\ (MJ/kg) =$$

$$[\text{AME of test protein source diet} - (\text{AME of basal diet} \times 0.70)] / 0.30$$

In experiment 2, the AME of protein sources was calculated using the AME of the mash basal diet for the diets in mash form and the AME of the pelleted basal diet for diets in pelleted form.

Nitrogen retention, as a percentage of intake, was determined as follows:

$$N\ \text{retention}\ (\%) = 100 \times [((FI \times N_{diet}) - (\text{Excreta output} \times N_{excreta})) / (FI \times N_{diet})]$$

The AMEn was then calculated by correction for zero N retention by assuming 36.54 KJ per g N retained in the body as described by Titus et al. (1959).

3.3.10. Statistical analysis

The data were analysed by two-way ANOVA to determine the main effects (grain type and FF in experiment 1, and protein source and FF in experiment 2) and their interaction using the General Linear Models procedure of the SAS Institute Inc. (version 9.4; 2015. SAS Institute Inc., Cary, NC). Cages served as the experimental unit. Significant differences between means were separated by the Least Significant Difference test. Significance of effects was declared at $P \leq 0.05$.

3.4. Results

3.4.1. Experiment 1- Cereal grains

3.4.1.1. Proximate and nutrient compositions

The proximate and nutrient compositions of the cereal grains are summarised in Table 3.2. The results are presented in 'as received' basis. There was a wide variation in protein content among the ingredients with barley had the highest CP (125.0 g/kg), followed by wheat (123.1 g/kg) and sorghum (106.3 g/kg), and the lowest CP was recorded for maize (80.6 g/kg). The starch content in maize, sorghum, wheat and barley was 590, 606, 532 and 499 g/kg, respectively. The highest NDF content was recorded for wheat (103.0 g/kg) followed by barley (90.1 g/kg), maize (83.1 g/kg) and sorghum (62.2 g/kg).

Table 3.2. Proximate, carbohydrate and mineral compositions of the tested ingredients (g/kg; as received basis)

Item	Experiment 1				Experiment 2		
	Maize	Sorghum	Wheat	Barley	MBM[b]	SBM[b]	CM[b]
DM[a]	909	909	899	925	968	921	911
Ash	20.5	15.5	18.4	16.1	149	65.4	72.5
Nitrogen	12.9	17.0	19.7	20.0	103	77.4	56.9
Protein	80.6	106.3	123.1	125.0	644	489	356
Fat	32.4	32.6	18.5	22.0	142	12.3	48.5
Starch	590	606	532	499	-	-	-
NDF[a]	83.1	62.2	103	90.1	154	84.6	250
Ca[a]	0.17	0.10	0.21	0.19	41.3	3.26	5.78
P[a]	2.47	2.89	3.51	2.65	23.3	6.61	10.5
GE[a] (MJ/kg)	16.25	16.68	16.18	16.69	21.19	17.67	17.83

[a] Ca, calcium; DM, dry matter; GE, gross energy; NDF, neutral detergent fibre; P, phosphorus.
[b] CM, canola meal; MBM, meat and bone meal; SBM, soybean meal.

3.4.1.2. Nutrient retention and energy utilisation

The influence of grain type and FF on the FI, retention of DM and N, and AME and AMEn is summarised in Table 3.3. The AME values followed the same trend as the AMEn and, for this reason, only the AMEn data will be discussed below.

A significant ($P < 0.01$) grain type × FF interaction was observed for FI. Feeding pelleted diets reduced the FI in the sorghum-based diet, had no effect in maize- and wheat-based diets, but increased FI in the barley-based diet. Neither the DM and N retention, nor the AMEn were subjected to a grain type × FF interaction. The DM retention was influenced by ($P < 0.001$) grain type, with similar DM retention for birds fed sorghum- and maize-based diets, which were higher than wheat- and barley-based diets. Grain type also influenced ($P < 0.001$) the N retention, with maize-based diets having higher N retention compared to those fed the other grains. Grain type ($P < 0.001$) and FF ($P < 0.01$) had significant effects on the AMEn. The AMEn of maize and sorghum were similar and higher ($P < 0.05$) than those of wheat and barley. Barley showed the lowest AMEn values. Pelleting increased ($P < 0.05$) the AMEn values, regardless of the grain type.

3.4.1.3. Gizzard pH and, relative weights of proventriculus and gizzard

The main effects of grain type and FF were significant ($P < 0.05$ to 0.001) for the pH and relative weight of gizzard (Table 3.3). Wheat-based diets resulted in the lowest ($P < 0.05$) gizzard pH compared to other grains. Broilers fed maize- and sorghum-based diets had similar but higher ($P < 0.05$) gizzard weights than those fed wheat and barley diets. Pelleting increased ($P < 0.05$) the gizzard pH but reduced the relative gizzard weight, regardless of the grain type. Grain type had significant ($P < 0.01$) effects on the relative weight of proventriculus. Birds fed maize-based diets had proventriculus weights similar ($P > 0.05$) to sorghum-based diets, but higher ($P < 0.05$) than those fed wheat- and barley-based diets. There was a tendency ($P = 0.061$) for lower proventriculus weights in pellet-fed birds compared to those fed mash diets.

3.4.1.4. Pellet durability index and pellet hardness

There was a significant ($P < 0.01$) effect of grain type on the PDI and pellet hardness as shown in Table 3.3. Wheat produced the most durable pellets followed by sorghum, maize and

barley. Sorghum and wheat-based diets showed higher (P < 0.05) pellet hardness than the maize- and barley-based diets.

Table 3.3. Influence of grain type on pellet durability index (PDI, %) and pellet hardness (kg)

Grain type	PDI[1]	Pellet hardness[2]
Maize	82.8[c]	1.49[b]
Sorghum	84.7[b]	2.05[a]
Wheat	91.8[a]	1.94[a]
Barley	81.5[c]	1.51[b]
SEM[3]	0.50	0.097
Probability, P ≤	0.001	0.001

Means in a column not sharing a common letter (a-c) are significantly different (P < 0.05).
[1] Each value represents the mean of five replicates.
[2] Each value represents the mean of 12 replicates.
[3] Pooled standard error of mean.

3.4.2. Experiment 2- Protein sources

3.4.2.1. Proximate and nutrient compositions

The proximate and nutrient compositions of the protein sources are summarised in Table 3.2. The protein content in MBM, SBM and CM was 644, 489 and 356 g/kg, respectively. There were marked differences in the fat content of MBM (142 g/kg), SBM (12.3 g/kg) and CM (48.5 g/kg). The highest NDF content was observed in CM (250 g/kg) followed by MBM (154 g/kg) and SBM (84.6 g/kg).

Table 3.4. Influence of grain type and feed form on feed intake (g/bird), retention (% of intake) of dry matter (DM) and nitrogen (N), apparent metabolisable energy (AME; MJ/kg DM basis), nitrogen-corrected apparent metabolisable energy (AMEn; MJ/kg DM basis)[1] in broilers measured from 24 to 28 d post-hatch and, gizzard pH[2] and relative weight (g/kg body weight) of gizzard and proventriculus[3]

Grain type[4]	Feed form	Feed intake	DM retention	N retention	AME	AMEn	Gizzard pH	Proventriculus weight	Gizzard weight
Maize	Mash	419^{b}	82.0	52.7	15.26	15.00	2.57	3.5	13.3
	Pellet	418^{b}	83.0	53.2	15.48	15.22	2.86	3.5	11.9
Sorghum	Mash	348^{d}	81.1	36.9	15.50	15.24	2.48	3.3	14.5
	Pellet	297^{e}	81.9	42.1	15.69	15.40	3.05	3.3	11.6
Wheat	Mash	400^{bc}	75.7	36.3	14.01	13.72	2.27	3.3	12.0
	Pellet	350^{cd}	76.7	41.8	14.30	13.96	2.46	3.0	10.4
Barley	Mash	421^{b}	71.9	41.5	13.29	12.96	2.40	3.1	11.9
	Pellet	491^{a}	72.7	41.0	13.57	13.24	3.19	2.8	9.50
SEM[5]		17.7	0.77	3.15	0.126	0.108	0.161	0.11	0.46
Main effects									
Grain type									
Maize		419	82.5^{a}	53.0^{a}	15.37^{a}	15.11^{a}	2.71^{a}	3.5^{a}	12.6^{a}
Sorghum		322	81.5^{a}	39.5^{b}	15.60^{a}	15.32^{a}	2.76^{a}	3.3^{ab}	13.0^{a}
Wheat		375	76.2^{b}	39.1^{b}	14.15^{b}	13.84^{b}	2.37^{b}	3.1^{bc}	11.2^{b}
Barley		456	72.3^{c}	41.2^{b}	13.43^{c}	13.10^{c}	2.80^{a}	3.0^{c}	10.7^{b}
Feed form									
Mash		397	77.7	41.9	14.51^{b}	14.23^{b}	2.43^{b}	3.3	12.9^{a}
Pellet		389	78.6	44.5	14.76^{a}	14.45^{a}	2.89^{a}	3.1	10.9^{b}
Probabilities, P ≤									
Grain type		0.001	0.001	0.001	0.001	0.001	0.034	0.002	0.001
Feed form		0.506	0.108	0.244	0.009	0.006	0.001	0.061	0.001
Grain type × Feed form		0.004	1.000	0.698	0.981	0.949	0.237	0.452	0.356

Means in a column not sharing a common letter (a-e) are significantly different (P < 0.05).

[1] Each value represents the mean of six replicates (six birds per replicate).

[2] Each value represents the mean of six replicates (two birds per replicate).

[3] Each value represents the mean of six replicates (two gizzards per replicate, three pH readings per gizzard).

[4] DM (g/kg) of ingredients: maize, 909; sorghum, 909; wheat, 899; barley, 925.

[5] Pooled standard error of mean.

3.4.2.2. Nutrient retention and energy utilisation

The influence of protein source and FF on the FI, retention of DM and N, AME and AMEn is summarised in Table 3.4. Birds fed pelleted diets consumed more ($P < 0.001$) feed in all protein sources compared with those fed mash diets. However, the magnitude of FI responses to pelleting was greater for birds fed CM diet than those fed SBM and MBM diets, resulting in a significant ($P < 0.05$) interaction between protein source and FF. Both the protein source ($P < 0.001$) and FF ($P < 0.05$) had significant effects on the DM retention. Soybean meal recorded the highest ($P < 0.05$) DM retention, followed by CM and MBM. Feeding pelleted diets reduced ($P < 0.05$) the DM retention. Significant ($P < 0.05$) protein source × FF interactions were observed for the N retention and AMEn. Feeding pelleted diets increased ($P < 0.05$) the N retention for MBM, but had no effect on those for SBM and CM. Pelleting did not have any effect ($P > 0.05$) on the AMEn of SBM, reduced ($P < 0.05$) that of MBM, and increased ($P < 0.05$) the AMEn of CM.

3.4.2.3. Gizzard pH and, relative weights of proventriculus and gizzard

Significant ($P < 0.05$) protein source × FF interaction was observed for the gizzard pH and the relative gizzard weight as shown in Table 3.5. Pelleting increased ($P < 0.05$) gizzard pH in birds fed the SBM diet, but had no effect in those fed MBM or CM diets. In all protein sources, the gizzard weight in pellet-fed birds was lower than those fed diets in mash form. However, the magnitude of the differences between feed forms were higher in the MBM and CM diets than the SBM diets. Neither the main effects nor the interaction was significant ($P > 0.05$) for relative proventriculus weight.

Table 3.5. Influence of protein source and feed form on feed intake (g/bird), retention (% of intake) of dry matter (DM) and nitrogen (N), apparent metabolisable energy (AME; MJ/kg DM basis), nitrogen-corrected apparent metabolisable energy (AMEn; MJ/kg DM basis)[1] in broilers measured from 24 to 28 d post-hatch and, gizzard pH[2] and relative weight (g/kg body weight) of gizzard and proventriculus[3]

Protein source[4]	Feed form	Feed intake	DM retention	N retention	AME	AMEn	Gizzard pH	Proventriculus weight	Gizzard weight
Meat and bone meal	Mash	473^{d}	63.6	47.2^{d}	17.03^{a}	14.79^{a}	3.67^{ab}	3.4	12.6^{a}
	Pellet	583^{b}	62.6	51.4^{bc}	16.42^{b}	14.23^{b}	4.01^{a}	3.1	9.10^{c}
Soybean meal	Mash	529^{c}	72.5	55.9^{a}	11.47^{c}	10.06^{c}	2.90^{c}	3.2	10.7^{b}
	Pellet	652^{a}	72.5	54.2^{ab}	11.05^{c}	9.88^{c}	3.92^{a}	3.3	9.50^{c}
Canola meal	Mash	503^{cd}	69.3	51.4^{bc}	9.04^{d}	7.87^{e}	3.40^{b}	3.2	12.2^{a}
	Pellet	688^{a}	66.7	49.9^{cd}	9.48^{d}	8.44^{d}	3.36^{bc}	3.4	9.20^{c}
SEM[5]		15.1	0.56	1.16	0.202	0.182	0.174	0.13	0.41
Main effects									
Protein source									
Meat and bone meal		528	63.1^{c}	49.3	16.72	14.51	3.83	3.2	10.8
Soybean meal		591	72.5^{a}	55.0	11.26	9.97	3.41	3.2	10.1
Canola meal		595	68.0^{b}	50.7	9.26	8.16	3.38	3.3	10.7
Feed form									
Mash		502	68.5^{a}	51.5	12.51	10.91	3.32	3.2	11.8
Pellet		641	67.3^{b}	51.8	12.32	10.85	3.76	3.3	9.20
Probabilities, P ≤									
Protein source		0.001	0.001	0.001	0.001	0.001	0.018	0.883	0.138
Feed form		0.001	0.013	0.738	0.245	0.719	0.003	0.878	0.001
Protein source × Feed form		0.040	0.084	0.024	0.033	0.014	0.012	0.168	0.014

Means in a column not sharing a common letter (a-e) are significantly different (P < 0.05).
[1] Each value represents the mean of six replicates (six birds per replicate).
[2] Each value represents the mean of six replicates (two birds per replicate).
[3] Each value represents the mean of six replicates (two gizzards per replicate, three pH readings per gizzard).
[4] DM (g/kg) of ingredients: meat and bone meal, 968; soybean meal, 921; canola meal, 911.
[5] Pooled standard error of mean.

3.4.2.4. Pellet durability index and pellet hardness

Protein sources had significant ($P < 0.001$) effects on the PDI and pellet hardness (Table 3.6). Soybean meal and CM showed similar ($P > 0.01$) PDI and higher than MBM diets. Diets made of SBM showed the highest ($P < 0.05$) pellet hardness, followed by CM and MBM.

Table 3.6. Influence of protein source on pellet durability index (PDI, %) and pellet hardness (kg)

Protein source	PDI[1]	Pellet hardness[2]
Meat and bone meal	67.4[b]	0.91[c]
Soybean meal	83.4[a]	2.93[a]
Canola meal	83.3[a]	1.77[b]
SEM[3]	0.625	0.107
Probability, P ≤	0.001	0.001

Means in a column not sharing a common letter (a-c) are significantly different ($P < 0.05$).
[1] Each value represents the mean of five replicates.
[2] Each value represents the mean of 12 replicates.
[3] Pooled standard error of mean.

3.5. Discussion

3.5.1. Experiment 1- Cereal grains

The proximate and nutrient composition of the four cereal grains were, in general, within the range reported in the literature. Hoai et al. (2011) reported a higher starch content (658 g/kg) in maize compared to the sample used in this study. Barzegar et al. (2019) presented higher starch and CP values of 489 g/kg and 86.0 g/kg, respectively, in a sample of maize compared to those in the current study. Losada et al. (2009) revealed that the starch and CP content in sorghum were 648 g/kg and 89.0 g/kg, respectively. Moss et al. (2017) reported a lower CP content (97.7 g/kg) in sorghum. Amerah (2015) reported a range of 402-712 g/kg of starch in wheat samples. Barzegar et al. (2019) had higher values for starch (639 g/kg) and lower CP content (112 g/kg) in wheat compared to those of wheat used in the current study. Losada et al. (2009) reported a range of 88-122 g/kg for CP in barley. Perera et al. (2019) presented higher starch content (610 g/kg) and lower CP content (101 g/kg) for barley compared to those reported in the present study. However, values outside the range have also been reported by some

researchers (Cowieson, 2005; Losada et al., 2009; Lasek et al., 2012; Moss et al., 2017; Truong et al., 2017; Perera et al., 2019).

The benefits of feeding pelleted diets to broilers in terms of increasing feed consumption, growth rate and feed efficiency are well documented in diets based on maize (Naderinejad et al., 2016; Abdollahi et al., 2018b; Roza et al., 2018; Massuquetto et al., 2019), sorghum (Selle et al., 2013; Abdollahi et al., 2014; Truong et al., 2017), wheat (Svihus et al., 2004; Amerah et al., 2007; Abdollahi et al., 2011) and barley (McIntosh et al., 1962; Bennett et al., 2002; Brickett et al., 2007). In fact, the enhanced performance of pellet-fed broilers can be, to a major extent, explained by the increases in FI, as positive growth responses to pelleting always parallel the increases in feed consumption (Abdollahi et al., 2018a). However, some studies have reported no effect of pelleting on FI of birds fed maize-based diets (Fujita, 1973; Parsons et al., 2006; Stark et al., 2009). Hamilton and Kennie (1997) reported lower FI in turkeys fed a wheat-based pelleted diet compared to the mash diet. In the current study, the effect of pelleting on FI varied between the cereal grains. Pelleting had no effect on the FI of birds fed maize- or wheat-based diets, but reduced the FI in sorghum-based diet by 14.7%. The FI reduction in sorghum-based diet might have been due, partly, to the higher hardness of pellets made of sorghum (Abdollahi et al., 2018a). Pelleting, however, increased the FI of broilers fed barley-based diet by 16.6%. Barley has been reported to be less palatable compared to other cereal grains tested in the present study (Engberg et al., 2002; Svihus et al., 2004; Scott, 2005), and the effect of pelleting in improving feed palatability (Behnke, 1998; Behnke and Beyer, 2002; Abdollahi et al., 2013c) could, to some extent, explain the FI increase. Although the contradictory effect of pellet feeding on FI in different cereal grains is not readily explainable, it could be due to the differences in the nutrient composition or the presence of anti-nutritional factors such as non-starch polysaccharides and tannins in the grains, which could potentially affect the palatability

of assay diets and the FI responses to pelleting (Engberg et al., 2002; Svihus et al., 2004; Scott, 2005).

The lack of FF effect on DM and N retention is in agreement with the finding of Woyengo et al. (2010) and Favero et al. (2012), who observed no effect on N retention in a maize-SBM based diet fed to broilers and turkeys, respectively. Similarly, FF had no effect on the N retention of a sorghum-based diet fed to broilers (Selle et al., 2012). In contrast, Zatari and Sell (1990) showed that pelleting a maize-based diet increased the retention of DM and N by 3.0% and 6.9%, respectively. An increase in DM (3.8%) and N by (5.2%) retentions of wheat-based diet was also reported by Pirgozliev et al. (2016). However, Serrano et al. (2013) reported that DM retention was higher for a mash diet than for crumbled and pelleted SBM diets. The contradictory results for the FF effect on DM and N retention could be related, inter alia, to differences in diet composition, type of cereal grain and pelleting conditions.

Regardless of the methodology (direct, difference or regression method) used to evaluate the AME content of cereal grains, all previous assays with individual ingredients have been conducted using mash diets (Lopez and Leeson, 2008; Dozier III et al., 2011; Kong and Adeola, 2016; Olukosi et al., 2017) and neglected the influence of feed processing and FF on energy utilisation (Abdollahi et al., 2013c; Naderinejad et al., 2016) despite the fact that pelleted diets are the most prevalent form of feed used in the broiler industry. Previous studies on the effect of FF (mash vs. pellet) on AME and AMEn have revealed contradictory results. Similar to the current findings, Farrell et al. (1983) detected an increase in AME by 0.67, 0.64 and 0.28 MJ/kg due to pelleting of maize-, wheat- and barley-based diets, respectively, compared to the mash diets for broiler chickens. Feeding the same diets to cockerels showed no effect of pelleting on AME in barley-based diet, a reduction of 0.34 MJ/kg in maize-based diet and an increase of 0.57 MJ/kg in wheat-based diet. Increases in metabolisable energy of wheat-based diets in broilers due to pelleting were also reported by Svihus et al. (2004; AME by 0.20 MJ/kg) and

Pirgozliev et al. (2016; AMEn by 0.57 MJ/kg). Roza et al. (2018), in a study with maize-based diet, reported that pelleting increased the AME and AMEn values by 0.27 and 0.26 MJ/kg, respectively. In contrast, Amerah et al. (2007) reported a significant negative effect of pelleting on the AMEn (11.81 MJ/kg) of a wheat-based diet compared to the mash diet (12.54 MJ/kg). Pelleting has been reported to reduce the AME of a wheat-based diet by 0.46 MJ/kg (Abdollahi et al., 2011) and AMEn of a maize-based diet by 0.17 MJ/kg (Abdollahi et al., 2018b). However, all these studies have been conducted with complete diets, and, to our knowledge, there are no reports available on the effect of FF on AME evaluation of individual ingredients.

The current results demonstrated that pelleting increased the AME and AMEn of the cereal grains by an average of 0.25 and 0.22 MJ/kg, respectively, compared to their mash counterparts, regardless of the grain type. The present findings suggest that the application of AME or AMEn values determined based on assays using mash diets might result in inaccurate estimation of metabolisable energy in feed ingredients when used in pelleted diets. Therefore, to formulate precise diets, the effect of feed processing and FF should be considered in metabolisable energy measurements of individual cereal grains.

A negative correlation ($r = -0.214$, $P < 0.05$) was observed between the gizzard pH and the relative gizzard weight. This is in agreement with the findings of Liu et al. (2015), which revealed a similar negative correlation ($r = -0.451$). The gizzard pH increased in pellet-fed birds and this was associated with a reduction in the gizzard weight. In agreement, Naderinejad et al. (2016) reported that pelleting a maize-based diet increased the gizzard pH by 0.27 and reduced the gizzard weight by 4.5 g/kg BW. García-Rebollar et al. (2016) revealed that feeding broiler chickens a wheat-based diet in mash form decreased the gizzard pH (3.04 vs. 3.52) and increased the gizzard weight (14.2 vs. 10.6 g/kg BW) when compared to pelleted diets. Frikha et al. (2009) reported that the gizzard pH increased from 3.46 in mash diets to 3.99 in pelleted diets and the gizzard weight reduced from 23.1 to 19.4 g/kg BW in pullets. Similar findings in

pullets were reported by Saldaña et al. (2015). The lower gizzard pH in mash-fed birds could be a general response to the longer retention time of mash feed in the foregut, allowing increased secretion of hydrochloric acid by the proventriculus (Engberg et al., 2002; Mateos et al., 2012).

In the current study, the grain type influenced the pellet quality (PDI and pellet hardness). This is in agreement with the findings of Stevens (1987), which showed that wheat-based pellets were more durable (90.3-91.0%) than those based on maize (57.5-57.6%). The variations in pellet quality could be related to the differences in grain CP content, as wheat had higher CP content (123.1 g/kg) compared to maize (80.6 g/kg). These results are consistent with those of Briggs et al. (1999) who found that increasing the CP content from 163 to 210 g/kg increased the average pellet durability from 75.8 to 88.8% in maize-based diet. This is due likely to the formation of hydrogen bonds between the hydrophilic portion of the protein and water molecules provided from the injected steam (Maier and Briggs, 2000). Although not measured in the present study, the variation in PDI between cereal grains could also be related to the variation in starch gelatinisation degree. According to Lund and Lorenz (1984), starches from different cereals have different gelatinisation characteristics. Wheat starch has a low gelatinisation temperature (52-65 ◦C) compared to that of maize (65-70.6 ◦C). Furthermore, gelatinisation heat depends on the starch source. Gelatinisation heat of wheat starch (10.05 J/g) is lower than that of maize (13.82 J/g).

3.5.2. Experiment 2- Protein sources

The nutrient compositions of protein sources were, in general, within the range reported in the literature for MBM (Anwar et al., 2016; Mutucumarana and Ravindran, 2016), SBM (Ravindran et al., 2014; Barzegar et al., 2019) and CM (Barekatain et al., 2015; Kong and Adeola, 2016).

In the current study, the CP content of MBM was 644 g/kg, which is higher than the CP content of 492.5 g/kg reported by Adeola et al. (2018). The fat content of MBM in the current study (142.0 g/kg) was also higher than those reported (88-128 g/kg) by Anwar et al. (2016). The variations in MBM nutrient composition could be related to the effects of processing techniques (Wang and Parsons, 1998), the source of raw materials (Kirstein, 1999) and the proportion of muscles to bones.

The current work confirms the fact that feeding pelleted diets facilitates easy prehension and increases FI of broilers. Benefits of pellet feeding on FI are the major driving factor behind improvements in growth performance (Lemme et al., 2006; Amerah et al., 2007; Abdollahi et al., 2011, 2013c; Serrano et al., 2013). However, the magnitude of FI advantages in favour of pelleted diets was inconsistent among the different protein sources, as evidenced by the significant protein source and FF interaction. Feeding pelleted diets supported higher FI but the advantage was greater (36.8%) in CM diets and eroded to 23.2% in MBM and SBM diets.

Pelleting reduced DM retention for protein sources by 1.75% compared to mash form. Feeding pelleted diets increased the N retention only for MBM and had no effect on the N retention for SBM and CM compared to mash diets.

A 3.94% disadvantage in the AMEn of MBM diets was observed as a result of pelleting. However, pelleting increased the AMEn of CM by 7.25% compared to the mash form, which might be attributed to the effect of heat and mechanical pressure during the pelleting process in disrupting the structure of the cell walls, thus releasing the nutrients, especially lipids, entrapped in the oil bodies (Jiménez-Moreno et al., 2009). Adewole et al. (2017), however, reported that the effect of pelleting on AMEn of CM differed depending on the source of CM. It was suggested that the variable effects of pelleting on the AMEn of CM samples are related to the variations in the chemical composition, particularly in fat and NDF contents. No other studies are available

comparing the effect of FF on the AME or AMEn of individual protein source ingredients for broilers.

Unlike cereal grains, there was no correlation between gizzard pH and gizzard weight ($P > 0.05$) in birds fed protein source diets. Gizzard pH increased for birds fed pelleted SBM diet only, with no effect for birds fed MBM and CM pelleted diets. The increase in gizzard pH associated with feeding pelleted diet could be due to the fact that pelleting increases the consumption of feed, the pH of which is usually close to neutral, and can elevate gizzard pH, unless gastric juice secretion is able to increase in accordance with intake. This is likely to be the main reason for the higher gizzard pH reported for pelleted diets when compared with mash diets (Engberg et al., 2002; Huang et al., 2006; Jiménez-Moreno et al., 2009).

Pelleting lowered the relative gizzard weights in birds fed the protein sources. The magnitude of reduction varied among protein sources, with gizzard weight being reduced by 3.5, 3.0, and 1.2 g/kg BW for birds fed pelleted MBM, CM, and SBM, respectively, compared to the corresponding mash diets. The reduction in gizzard weight of birds fed pelleted diets could be due to the reduction in feed particle size because of the pelleting process and the resultant reduction in the mechanical stimulation of the gizzard musculature (Svihus, 2011b; Mateos et al., 2012).

The protein source influenced PDI and pellet hardness. Meat and bone meal diet resulted in the lowest PDI and pellet hardness. Whilst SBM and CM diets showed similar PDI, the SBM diet created the hardest pellets. This high variation in pellet quality could be related to the differences in the chemical composition of the protein sources, especially the fat content. The MBM sample had a higher fat content (142 g/kg) than SBM (12.3 g/kg) and CM (48.5 g/kg). This finding is consistent with those of Briggs et al. (1999) who demonstrated a negative impact of increasing oil content from 29 to 75 g/kg on pellet quality. High dietary fat inclusions can partially coat feed particles, making a barrier to steam penetration of feed particles and thus

preventing the development of binding adhesions (Lowe, 2005). Moreover, due to lubricating effects, fat can reduce the heat generated by friction in the pellet mill resulting in lesser pellet quality.

3.6. Conclusions

In summary, the present data showed that the effect of feed form on AME and AMEn of feed ingredients varies depending on the ingredient type. Pelleting increased the AMEn of all four cereal grains tested but reduced the AMEn of meat and bone meal and increased that of canola meal. The inference from the present study is that the metabolisable energy values for feed ingredients estimated in assays using mash diets are not accurate to be used in a complete diet fed in pellet form. It is proposed that the energy evaluation of feed ingredients should consider the impact of feed processing and that the AME data should be generated using pelleted diets to resemble the feeding practice in the broiler industry.

CHAPTER 4 Apparent metabolisable energy of cereal grains for broiler chickens is influenced by age

4.1. Abstract

The current study was conducted to investigate the influence of broiler age on the apparent metabolisable energy (AME) and nitrogen-corrected AME (AMEn) of four common cereal grains (wheat, sorghum, barley and maize), measured using the direct method. In this method, four experimental diets with the same inclusion (962 g/kg) of each grain were developed. Six groups of broiler chickens aged 1-7, 8-14, 15-21, 22-28, 29-35 or 36-42 d post-hatch, were utilised. Each diet, in pellet form, was randomly allocated to six replicate cages in each age group. Birds were fed a starter (d 1-21) and/or a finisher (d 22-35) diet before the experimental diets were introduced. The number of birds per cage was 10 (d 1-7) and 8 (d 8-42). Excreta were collected over the last 4 d of each age period. The AME and AMEn of the grains were determined by total excreta collection. Bird age had significant ($P < 0.001$) effects on AME and AMEn of all cereal grains. The AMEn of wheat declined quadratically ($P < 0.01$) with advancing age, from 14.48 MJ/kg in week 1 to 13.47 MJ/kg in week 2 and then plateaued. The AMEn of sorghum grain declined linearly ($P < 0.001$) with advancing age, from 15.74 MJ/kg in week 1 to 15.12 MJ/kg in week 2, plateaued to week 5 and then declined to 14.88 MJ/kg in week 6. A quadratic ($P < 0.001$) reduction in the AMEn of barley was observed as birds grew older, with the AMEn decreasing between week 1 (13.75 MJ/kg) and week 2 (12.50 MJ/kg), increasing in week 3 (13.04 MJ/kg) and then plateauing. The AMEn of maize declined quadratically ($P < 0.05$) with advancing broiler age; the highest AMEn was observed in weeks 1 and 5, the lowest AMEn in week 2, with the other weeks being intermediate. In conclusion, the present results showed that broiler age has a substantial impact on the AME and AMEn of cereal grains and the effect varied depending on the cereal grain. These data suggest that age-

dependent AME and AMEn values may need to be considered when formulating broiler diets to enhance the precision of feed formulation and production efficiency.

4.2. Introduction

Cereal grains such as wheat, sorghum, barley, and maize are commonly used in poultry diets as the major source of energy. Knowledge of the metabolisable energy content of cereal grains is critical for their efficient and sustainable use and precise poultry feed formulation. Despite several limitations (Mateos et al., 2019; Wu et al., 2020), the apparent metabolisable energy (AME) is the globally accepted system for describing the available energy for poultry (Hill and Anderson, 1958; Sibbald, 1982). The simplicity, relatively easy measurements, and the fact that it accounts for most of the energy losses after digestion and metabolism are the major reasons for its worldwide acceptance.

When formulating commercial poultry diets, the current practice in the feed industry is to use the AME or nitrogen-corrected AME (AMEn) values of ingredients from equations or reference tables which have been determined using older birds (WPSA, 1989; NRC, 1994; Rostagno et al., 2017). Most published data on the AMEn in feed ingredients have been generated with older broilers (typically 5-weeks old) and used in feed formulations for all the phases of broiler growth. This practice however, overlooks the potential effect of bird age on the AMEn content of feed ingredients. Bird age has been shown to have a substantial effect on the digestion and absorption of energy-yielding nutrients (Bennett et al., 1995; Batal and Parsons, 2002; Wiseman, 2006). Birds of different ages have variable abilities to digest and metabolise feed ingredients, especially those containing anti-nutritive substances such as soluble non-starch polysaccharides (Ravindran et al., 2004a; Adeola et al., 2018). Moreover, the capacity of the digestive tract to digest and absorb nutrients is limited during the early life of broilers and, there is consensus that nutrient digestibility generally increases with advancing age (Brumano et al., 2006; Olukosi et al., 2007). Several studies have reported higher dietary

AME values in older broilers than younger birds (Zelenka, 1968; Scott et al., 1998; Batal and Parsons, 2002; Bolarinwa et al., 2012). A positive linear correlation between age and AME of a wheat-based diet has also been reported in broilers from 8 to 16 d (Scott et al., 1998). In contrast, some studies showed a negative or no age effect on AME of diets. Fonolla et al. (1981) revealed that the AME value of a maize-based diet was not influenced by the age of birds up to d 52. Bartov (1995) showed that AMEn values of maize- and sorghum-based diets were reduced with advancing age of the birds from 11 to 22 d. Despite the importance of the age effect, a scan of the literature reveals that there is no data on age-related values for the AMEn of cereal grains used in poultry diets. The relevance of using a single value of AMEn obtained with older birds to all growth phases, especially the early life, of broilers is questionable and highlights the need for age-dependent estimates for use in feed formulations.

All previous studies on age-related responses have been conducted with grain-based complete diets (Olukosi and Bedford 2019; Yang et al., 2020). To our knowledge, no published data are available on age effects for the AMEn of single cereal grains. Therefore, the aim of this study was to investigate whether the age of broiler chickens has any effect on the AMEn of commonly used cereal grains (wheat, sorghum, barley and maize) using the direct method by the total excreta collection (Hill and Anderson, 1958).

4.3. Materials and methods

The experiment was conducted according to the New Zealand Revised Code of Ethical Conduct for the use of live animals for research, testing and teaching and approved by the Massey University Animal Ethics Committee.

4.3.1. Ingredients

Four cereal grains (wheat, sorghum, barley, and maize) were obtained from a local commercial supplier. The proximate and nutrient compositions of the cereal grains are presented in Chapter 3. The wheat and sorghum samples were of Australian origin, and maize and barley were sourced from New Zealand. All grains were ground in a hammer mill to pass through a screen size of 3.0 mm.

4.3.2. Diets, birds and housing

Day-old male broilers (Ross 308) were obtained from a local hatchery and raised on floor pens in an environmentally controlled room until assigned weekly to the experimental treatments. Except for the 1-7 d age group, birds were fed broiler starter mini pellets (230 g/kg crude protein and 12.56 MJ/kg AMEn) until d 21 and finisher pellets (207 g/kg crude protein and 13.0 MJ/kg AMEn) from d 22 to 35 (Table 4.1). At the beginning of each week (d 1, 8, 15, 22, 29 and 36), new batches of birds were selected randomly from the floor pens, weighed individually and allocated to cages so that the average bird weight per cage was similar. For each cereal grain, the assay diet was fed to six replicate cages of broilers during six periods, namely week 1 (d 1-7), week 2 (d 8-14), week 3 (d 15-21), week 4 (d 22-28), week 5 (d 29-35) or week 6 (d 36-42). Each replicate cage housed 10 birds during week 1, and 8 birds during weeks 2 to 6 post-hatch.

The AME was determined using the direct method. In this method, four basal diets were formulated to contain the same inclusion level (962 g/kg) of each grain, and fortified with macro minerals and, vitamin and trace mineral premixes. Diets were mixed in a single-screw paddle mixer (Bonser Engineering Co. Pty. Ltd., Merrylands, Australia), then pelleted using a pellet mill (Model Orbit 15; Richard Sizer, Kingston-upon-Hull, UK) capable of manufacturing 180 kg of feed/h and equipped with a die ring with 3-mm holes and 35 mm thickness.

4.3.3. Determination of the apparent metabolisable energy

The AME was determined using the total excreta collection procedure (Hill and Anderson, 1958). During each week, diets were fed for 7 d, with the first 3 d in each week serving as an adaptation period. The feed intake (FI) and total excreta output for each replicate cage were recorded over the last 4 consecutive d of the assay. Daily excreta collections were pooled within a replicate cage, mixed in a blender and sub-sampled. Sub-samples were lyophilised (Model 0610, Cuddon Engineering, Blenheim, New Zealand), and dried excreta samples were ground to pass through a 0.5-mm sieve and stored in airtight plastic containers at 4 ºC pending analysis. The diet and excreta samples were analysed for dry matter (DM), gross energy (GE), and nitrogen (N).

Table 4.1. Composition (g/kg, as fed basis) of the cereal-based test diets, and the broiler starter (d 1 to 21) and finisher (d 22 to 35) diets

Ingredient	Wheat	Sorghum	Barley	Maize	Starter diet	Finisher diet
Test ingredient	962	962	962	962	-	
Maize	-	-	-	-	574.2	660.0
Soybean meal, 460 g/kg	-	-	-	-	381.4	295.6
Soybean oil	-	-	-	-	8.8	13.6
Dicalcium phosphate	19.0	19.0	19.0	19.0	10.7	8.2
Limestone	13.0	13.0	13.0	13.0	11.3	9.9
L Lysine HCl	-	-	-	-	2.0	1.9
DL Methionine	-	-	-	-	3.3	3.0
L Threonine	-	-	-	-	1.0	0.7
Sodium chloride	2.0	2.0	2.0	2.0	2.5	2.5
Sodium bicarbonate	2.0	2.0	2.0	2.0	2.7	2.5
Trace mineral premix	1.0	1.0	1.0	1.0	1.0	1.0
Vitamin premix[1]	1.0	1.0	1.0	1.0	1.0	1.0
Ronozyme HiPhos (Phytase)	-	-	-	-	0.1	0.1

[1] Vitamin and trace mineral premix supplied the following per kilogram of diet: antioxidant, 100 mg; biotin, 0.2 mg; calcium pantothenate, 12.8 mg; vitamin D_3 (cholecalciferol), 2400 IU; cyanocobalamin, 0.017 mg; folic acid, 5.2 mg; menadione, 4 mg; niacin, 35 mg; pyridoxine, 10 mg; vitamin A (trans-retinol), 11100 IU; riboflavin, 12 mg; thiamine, 3.0 mg; vitamin E (dl-α-tocopheryl acetate), 60 IU; choline chloride, 638 mg; Co, 0.3 mg; Cu, 3.0 mg; Fe, 25 mg; I, 1 mg; Mn, 125 mg; Mo, 0.5 mg; Se, 200 µg; Zn, 60 mg.

4.3.4. Chemical analysis

Dry matter was determined using standard procedures (Methods 930.15; AOAC, 2016). Nitrogen was determined by combustion (Method 968.06; AOAC, 2016) using a carbon nanosphere-200 carbon, N and sulphur auto analyser (rapid MAX N exceed, Elementar, Donaustraze, Hanau, Germany). Gross energy was determined by an adiabatic bomb calorimeter (Gallenkamp Autobomb, Weiss Gallenkamp Ltd, Loughborough, UK) standardised with benzoic acid.

4.3.5. Calculations

All data were expressed on a DM basis, and the AME was determined using the following formula:

$$AME_{Diet}\ (MJ/kg) = [(FI \times GE_{Diet}) - (\text{Excreta output} \times GE_{Excreta})] / FI$$

The AME of the cereal grains was then calculated using the following formula:

$$AME_{Grain}\ (MJ/kg) = \text{AME of test grain diet} \times (100/96.2)$$

Nitrogen retention, as a percentage of intake, was determined as follows:

$$N\ \text{retention}\ (\%) = 100 \times [((FI \times N_{Diet}) - (\text{Excreta output} \times N_{Excreta})) / (FI \times N_{Diet})]$$

The AMEn was then calculated by correction for zero N retention by assuming 36.54 KJ per g N retained in the body as described by Titus et al. (1959).

4.3.6. Statistical analysis

The data for each grain were analysed by one-way ANOVA using the General Linear Models procedure of the SAS (version 9.4; 2015. SAS Institute Inc., Cary, NC). Cages served as the experimental unit. Significant differences between means were separated by the Least Significant Difference test. In addition, the data were subjected to orthogonal polynomial

contrasts using the General Linear Models procedure of SAS (2015) to study whether responses to increasing bird age were of linear or quadratic nature. Significance of effects was declared at $P \leq 0.05$.

4.4. Results

The influence of broiler age on the retention of DM and N, AME and AMEn of wheat is summarised in Table 4.2. The retention of both DM and N declined quadratically ($P < 0.001$) with advancing age. The highest DM and N retentions were recorded in week 1.

Bird age had a significant ($P < 0.001$) effect on the AME and AMEn of wheat. The AMEn of wheat declined quadratically ($P < 0.01$) with advancing age, from 14.48 MJ/kg in week 1 to 13.47 MJ/kg in week 2 and then plateauing to week 6 (Figure 4.1A).

Table 4.2. Influence of broiler age on the retention (% of intake) of dry matter (DM) and nitrogen (N), apparent metabolisable energy (AME; MJ/kg DM basis) and nitrogen-corrected AME (AMEn; MJ/kg DM basis) of wheat grain[1]

Age (week)	DM retention	N retention	AME	AMEn
1	82.1	57.7	14.92	14.48
2	74.2	28.9	13.69	13.47
3	73.9	41.4	13.62	13.31
4	73.9	37.5	13.66	13.37
5	75.2	39.0	13.81	13.51
6	74.1	32.2	13.69	13.44
SEM[2]	1.08	1.61	0.193	0.190
Orthogonal polynomial contrast, P ≤				
Linear	0.001	0.001	0.001	0.004
Quadratic	0.001	0.001	0.001	0.002

[1] Each value represents the mean of six replicates. The number of birds per replicate cage was 10 (week 1) and 8 (weeks 2-6).
[2] Pooled standard error of mean.

The influence of broiler age on the retention of DM and N, AME and AMEn of sorghum is presented in Table 4.3. Dry matter ($P < 0.01$) and N ($P < 0.001$) retentions decreased quadratically with the advancing age of birds, where week 1 showed the highest DM and N retentions, and then both DM and N retentions declined at week 6. The AMEn of sorghum

declined linearly (P < 0.001) with advancing age, from 15.74 MJ/kg in week 1 to 15.12 MJ/kg in week 2, plateaued to week 5 and then declined further to 14.88 MJ/kg in week 6 (Figure 4.1B).

Table 4.3. Influence of broiler age on the retention (% of intake) of dry matter (DM) and nitrogen (N), apparent metabolisable energy (AME; MJ/kg DM basis) and nitrogen-corrected AME (AMEn; MJ/kg DM basis) of sorghum grain[1]

Age (week)	DM retention	N retention	AME	AMEn
1	86.5	56.6	16.16	15.74
2	80.9	32.8	15.36	15.12
3	80.8	34.4	15.40	15.15
4	80.7	35.1	15.46	15.20
5	79.3	36.5	15.18	14.92
6	79.1	33.8	15.13	14.88
SEM[2]	0.66	1.80	0.110	0.100
Orthogonal polynomial contrast, P ≤				
Linear	0.001	0.001	0.001	0.001
Quadratic	0.002	0.001	0.019	0.072

[1] Each value represents the mean of six replicates. The number of birds per replicate cage was 10 (week 1) and 8 (weeks 2-6).
[2] Pooled standard error of mean.

The influence of broiler age on the retention of DM and N, AME and AMEn of barley is presented in Table 4.4. Dry matter and N retentions showed a significant (P < 0.001) quadratic response to bird age, where birds retained the highest DM and N during week 1. Bird age had a significant (P < 0.001) effect on the AME and AMEn of barley. A quadratic (P < 0.001) reduction in the AMEn of barley was observed as birds grew older, with the AMEn decreasing between week 1 (13.75 MJ/kg) and week 2 (12.50 MJ/kg), increasing in week 3 (13.04 MJ/kg) and then plateauing (Figure 4.1C).

The retention of DM and N, AME and AMEn of maize measured in broilers at different ages are presented in Table 4.5. The highest DM and N retentions were observed in week 1 and declined linearly (P < 0.05) for DM retention and quadratically (P < 0.05) for N retention with advancing age. The AMEn of maize declined quadratically (P < 0.05) with advancing broiler

age; the highest AMEn was observed in weeks 1 and 5, the lowest AMEn in week 2, with other weeks being intermediate (Figure 4.1D).

Table 4.4. Influence of broiler age on the retention (% of intake) of dry matter (DM) and nitrogen (N), apparent metabolisable energy (AME; MJ/kg DM basis) and nitrogen-corrected AME (AMEn; MJ/kg DM basis) of barley grain[1].

Age (week)	DM retention	N retention	AME	AMEn
1	77.9	61.5	14.19	13.75
2	69.3	40.9	12.79	12.50
3	72.9	46.2	13.37	13.04
4	71.6	43.8	13.24	12.93
5	71.0	42.5	12.99	12.70
6	71.6	41.3	13.18	12.89
SEM[2]	0.46	1.31	0.079	0.073
Orthogonal polynomial contrast, P ≤				
Linear	0.001	0.001	0.001	0.001
Quadratic	0.001	0.001	0.001	0.001

[1] Each value represents the mean of six replicates. The number of birds per replicate cage was 10 (week 1) and 8 (weeks 2-6).
[2] Pooled standard error of mean.

Table 4.5. Influence of broiler age on the retention (% of intake) of dry matter (DM) and nitrogen (N), apparent metabolisable energy (AME; MJ/kg DM basis) and nitrogen-corrected AME (AMEn; MJ/kg DM basis) of maize grain[1].

Age (week)	DM retention	N retention	AME	AMEn
1	85.3	62.9	15.78	15.48
2	80.2	40.5	15.01	14.82
3	82.7	53.2	15.38	15.12
4	83.2	52.2	15.56	15.31
5	83.5	53.5	15.66	15.41
6	81.4	48.7	15.42	15.18
SEM[2]	0.42	1.77	0.077	0.070
Orthogonal polynomial contrast, P ≤				
Linear	0.017	0.034	0.651	0.455
Quadratic	0.119	0.013	0.036	0.043

[1] Each value represents the mean of six replicates. The number of birds per replicate cage was 10 (week 1) and 8 (weeks 2-6).
[2] Pooled standard error of mean.

4.5. Discussion

To the author's knowledge, no published data are available on age-related energy utilisation responses of individual cereal grains from weeks 1 to 6 of broiler chickens. A perusal of the literature showed that previous studies comparing age effects have been conducted with grain-based complete diets at specific ages and most do not include week 1. Scott et al. (1998) showed that birds at d 8 of age had a lower AME of wheat compared to d 16 (14.31 vs. 14.77 MJ/kg); the same trend was observed for barley, where the AME increased by 0.42 MJ/kg at d 16. Batal and Parsons (2004) found that the AMEn of a maize-soy diet increased numerically by 0.41 MJ/kg from d 14 to 21.

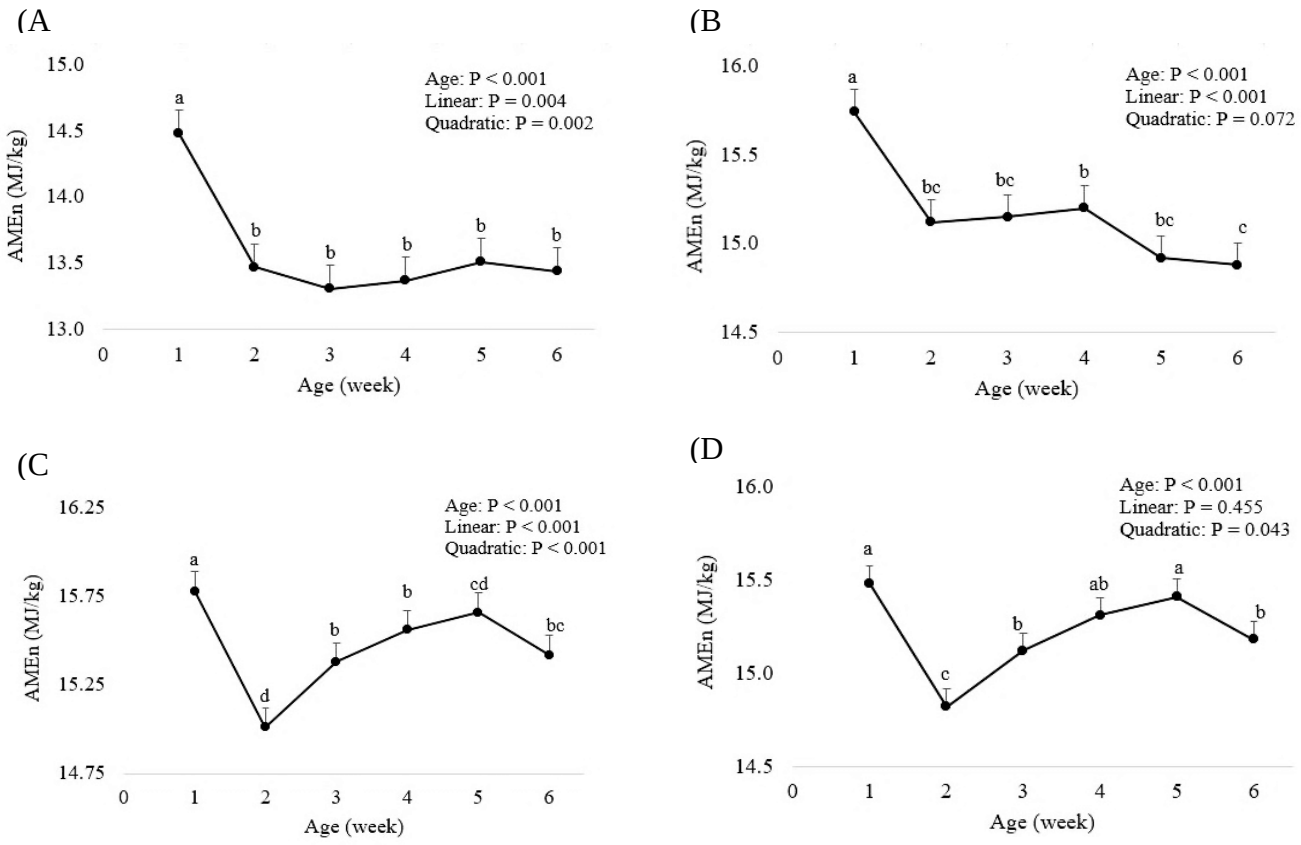


Figure 4.1. Effect of broiler age on the nitrogen-corrected apparent metabolisable energy (AMEn) of wheat (A), sorghum (B), barley (C) and maize (D) grains, mean ± standard error .[a-d] Values with different superscripts differ significantly (P < 0.05)

Thomas et al. (2008) reported that the AMEn of a maize-based diet was 12.28 MJ/kg at d 7 and then increased to 13.01 MJ/kg at d 14 of age. These researchers also observed similar trends for wheat- and sorghum-based diets. Moreover, N retention decreased with the advancing age of birds from 75.5% at d 3 to 63.3% at d 7. In contrast, Bartov (1995) reported a significant reduction in the AMEn of a sorghum-maize-based diet from 12.23 MJ/kg at 13 d of age to 12.10 MJ/kg at 22 d of age.

The principal motive for the present study was therefore to investigate whether (i) the age of broilers influences the AME content of common cereal grains and (ii) the age effect, if present, is similar for all grains. The results showed that the AMEn contents of cereal grains were influenced by bird age, regardless of cereal type. The highest AMEn values were observed at the first week for all cereal grains and then the AMEn declined, either linearly (sorghum) or quadratically (wheat, barley, and maize). Similarly, the highest DM and N retention values were observed at the first week and then declined quadratically for all cereal grains. The only exception was the DM retention in maize, which declined linearly. These reductions were associated with reductions in the AMEn of all grains.

The higher AMEn and retention of DM and N determined for week 1 are counter-intuitive, since the digestive tract of the newly hatched chick is immature and must undergo dramatic changes before it can efficiently digest and absorb nutrients. The obvious limiting factors are the secretion and activities of digestive enzymes, and the surface area for absorption. These limitations are overcome with advancing age, resulting in concurrent improvements in nutrient utilisation (Noy and Sklan, 2001). Uni et al. (1995) reported that the N retention increased from 70% at d 4 to 90% at d 14 post-hatching. Similarly, they reported that the starch digestibility increased by 14% between d 4 and d 14 post-hatching. Olukosi et al. (2007) observed that DM and N retention increased by 20.9% and 19.9%, respectively, between weeks 1 and 2. A study by Murakami et al. (1992) showed a lower AME during the first few days of life followed by

an increase after the first week. In contrast, Moss et al. (2020) reported higher ME:GE ratios in broiler chicks (7-9 d) than older broilers (33-34 d) in diets based on wheat (0.799 vs. 0.765), sorghum (0.782 vs. 0.713) or maize (0.796 vs. 0.785).

Three possible explanations may be provided to justify the high AMEn values determined during week 1. First, the presence of residual yolk sac may be imparting beneficial effects on the digestion and utilisation of lipids and protein, contributing to the high AMEn (Zelenka, 1968). The newly hatched chick relies on residual egg yolk lipids as the primary source of energy up to d 5 post-hatch (Sell et al., 1991; Murakami et al., 1992). Noy and Sklan (2001) estimated that the yolk represents about 20% of the body weight of the hatchling and contains about 50% lipids, providing immediate energy post-hatch. Break-down of yolk lipids, by the lipolytic enzymes in the yolk sac, provides more than 90% of the total energy required for the hatchling (Speake et al., 1998; Dzoma and Dorrestein, 2001; Sato et al., 2006). Murakami et al. (1992) estimated that the residual yolk contributed for approximately 30% towards the total dietary energy intake from d 0-3 of age. However, the exact mechanism or contribution of the yolk sac towards nutrient intake and utilisation remains unclear (van der Wagt et al., 2020).

Second, FI during the first week post-hatch is low as the hatchling adapts to the move from yolk nutrition to oral nutrition (Bartov, 1995). It has been accepted that dietary energy has a regulatory role on FI and birds alter their feed consumption to primarily meet their energy requirements (Leeson et al., 1993; Lamot et al., 2017). Zelenka (1997) stated that the decrease in AMEn with advancing age could be due to increased feed consumption. Scott (2005) reported a negative correlation ($r = -0.64$) between feed consumption and AME for wheat-based diets.

Lower FI reduces the feed passage rate in the digestive tract, which improves the digestibility of nutrients by increasing the time of contact with digestive enzymes and absorptive cells (Washburn, 1991; Choct et al., 1996). In a study, Ten Doeschate et al. (1993) found that the DM and N digestibility of maize-soy diet was higher in birds with low FI. With

advancing age, feed consumption of birds fed diets with high inclusions of cereals increases, overloading the digestive tract with starch, resulting in incomplete digestion of starch. Under these conditions, a negative correlation was observed between FI and starch digestibility in wheat, causing reductions in the AME (Svihus and Hetland, 2001; Svihus 2006, 2011a). Vergara et al. (1989) noted that the increase in digesta passage rate with age is related to the increased FI. Hughes (2008) reported a linear correlation between the AME and total tract digesta transit time (r = 0.31). Rougière and Carré (2010) reported a decrease in digesta retention time in the digestive tract of broilers from 339 min at d 9 to 254 min at d 29. In contrast, Uni et al. (1995) showed that the passage rate time of digesta in the digestive tract increased from 74 mins at d 7 to 122 mins at d 22 of age.

Finally, the role of the microbiome, or the lack of it, in the newly hatched chick cannot be discounted. The digestive tract of the hatchlings is sterile (Gabriel et al., 2006; Thomas et al., 2008) but rapidly colonised by the microbiome via the feed and environment. The intestinal microbiome contains various bacterial species that are heavy consumers of amino acids and energy (McBurney et al., 2003; Harrow et al., 2007) for their rapid growth and colonisation. Thus, the absence or low bacterial population during week 1 may provide an advantage in terms of AME. The subsequent decline in the AMEn after week 1 may be partly attributed to the increasing load of the microbiome.

The current findings disagree with the widely assumed perception that energy utilisation increases with age (Tancharoenrat et al., 2013; Yang et al., 2020). The lack of positive effect of age on the AMEn of all grains in the current study was also confirmed by the lack of positive effects on DM and N retention. Energy is not a nutrient per se, but a property of energy-yielding nutrients (carbohydrates, lipids, and protein). The energy derived differs between carbohydrates, lipids, and protein. Studies on the influence of age of birds on nutrient digestibility have shown contradictory results. Uni et al. (1995) revealed that the digestibility of starch, N and fatty acids

increased with advancing age. Szczurek et al. (2020), on the other hand, reported that the apparent and standardised ileal digestibility coefficients of amino acids of wheat were higher in birds at 14 d of age vs. 27 d of age. Similarly, Olukosi and Bedford (2019) showed a reduction in fat digestibility by 45% between d 7 to d 14 of age of broiler chickens, which was attributed to the faster passage rate in older birds. In contrast, Tancharoenrat et al. (2013) found that fat digestibility increased by 50% at week 2 compared to week 1 post-hatch (0.807 vs. 0.532), which was related to the limitation of the physiological ability of hatchlings to utilise fats.

One possible reason for the discrepancies reported between studies is the use of different methodologies. Several approaches are available to determine the AME, with the main consideration being the inclusion level of the test ingredient in the test diet (Wu et al., 2020). Calculations of the AME value of ingredients also differ in different methods. Even within the same method, the AME of ingredients can vary depending on how the basal diet is formulated and, the inclusion rate of ingredient rate of the test ingredient in the test diet (Wu et al., 2020). According to Sibbald (1982), the AME estimates of feed ingredients are lowered with increasing inclusion levels in assay diets. McIntosh et al. (1962), however, concluded that the diet composition (balanced or unbalanced) has no influence on the AME of ingredients.

The simplest method to measure AME of ingredients is feeding birds a diet that is composed solely of the test ingredient, referred to as the direct method. This method eliminates the need for a reference diet. However, some ingredients are unpalatable and cannot be tested as a single ingredient. An alternative method is the substitution method, where the test ingredient is substituted for a portion of a reference diet composed of practical ingredients.

Differences between methodologies have contributed to significant variations among AME values of ingredients. Lockhart et al. (1967) revealed that the AME of wheat measured by the substitution method was 0.34 MJ/kg higher than that measured by the direct method. Olukosi (2021) reported that the AMEn value of barley measured by the regression method was

2.96 MJ/kg greater than that measured by the substitution method (10.97 vs. 8.01 MJ/kg), however, the AMEn of maize was not influenced by the methodology; suggesting that the influence of the methodology on AME estimations is ingredient dependent. Similarly, Lee and Kong (2019), comparing direct vs. regression methods, and Veluri and Olukosi (2020), studying difference vs. regression methods, showed that the assay method can influence the AME and AMEn value of feed ingredients, and that should be considered in cross-studies comparisons. In the current work, the direct method was employed to determine the AME of four cereals. Given that the methodology can influence AME, future studies will be of interest to measure the AMEn of these cereals using the substitution method at different broiler ages.

4.6. Conclusions

The results of the present study confirmed that, regardless of grain type, age of broiler has a substantial impact on the AMEn values of cereal grains. These results question the validity of applying a single AME or AMEn value for broilers of different ages, which can under- or over-estimate the energy utilisation. Therefore, to improve the precision of feed formulations and production efficiency, age-dependent AME and AMEn values may need to be considered when formulating broiler diets. Moreover, as the methodology (direct vs. substitution) can impact the measurement of AME content of feed ingredients, future studies are required to evaluate the AME and AMEn contents of cereal grains using the substitution method.

CHAPTER 5 Influence of broiler age on the apparent metabolisable energy of cereal grains determined using the substitution method

5.1. Abstract

The present study investigated the influence of broiler age on the apparent metabolisable energy (AME) and nitrogen-corrected AME (AMEn) of four common cereal grains (wheat, sorghum, barley and maize) using the substitution method. A maize-soybean meal basal diet was formulated, and the test diets were developed by replacing (w/w) 300 g/kg of the basal diet with wheat, sorghum, barley or maize. Six groups of broiler chickens, aged 1-7, 8-14, 15-21, 22-28, 29-35 or 36-42 d post-hatch, were utilised. Each diet, in pellet form, was randomly allocated to six replicate cages in each age group. Except for the 1-7 d age group, birds were fed a starter (d 1-21) and/or a finisher (d 22-35) diet prior to the introduction of experimental diets. The number of birds per cage was 10 (d 1-7), 8 (d 8-14) and 6 (d 15-42). The AME and AMEn of the grains were determined by total excreta collection. Data for each grain were subjected to orthogonal polynomial contrasts using the General Linear Models procedure. The retention of dry matter (DM) and nitrogen linearly decreased ($P < 0.001$) with advancing broiler age in all cereals. Bird age influenced ($P < 0.001$) the AMEn of wheat and sorghum but had no effect ($P > 0.05$) on those of barley and maize. The AMEn of wheat increased with age ($P < 0.001$) from 12.53 MJ/kg DM in week 1 to 14.55 MJ/kg DM in week 2, then declined for following weeks, but no linear or quadratic responses were observed. The AMEn of sorghum demonstrated a quadratic response ($P < 0.05$), increasing from 12.84 MJ/kg DM in week 1 to 13.95 MJ/kg DM in week 2, and then plateauing to week 6. Overall, the present results suggest that the effect of broiler age on the AMEn of cereal grains varies depending on the grain type. The current data demonstrate that the application of age-dependent AME or AMEn values of grains in diet formulations is conditional on the type of grain.

5.2. Introduction

Available energy in feed or feed ingredients for poultry can be measured by different systems, with the apparent metabolisable energy (AME; Hill and Anderson, 1958), despite its limitations (Mateos et al., 2019; Wu et al., 2020), being the commonly accepted and extensively used system.

Three methods namely, direct, substitution (or difference) and regression, have been used to determine the AME of ingredients for poultry. In each method, the excreta can be collected by total collection, which is the preferred method, or partial collection (marker method) using the ratio of an indigestible marker present in diet and excreta. Each method has its own merits and drawbacks. The main difference between these methods being how the test ingredients are included in the assay diets (Wu et al., 2020).

The direct method, which is used mainly to estimate the AME of cereal grains (Chapter 4), is based on feeding the test ingredient as the sole source of energy in the assay diet. A limitation of this method is that it cannot be used for longer feeding periods due to the nutritionally unbalanced assay diet. Moreover, it cannot be used for ingredients with poor palatability (Fraps et al., 1940; Sibbald, 1976).

The substitution method is used to determine the AME of poorly palatable ingredients, or those containing high protein content or anti-nutrients. This method requires formulating two sets of diets, a basal (or reference diet) and a test diet, which is developed by replacing a portion of the basal diet with the test ingredient (Sibbald et al., 1960; Farrell, 1978). Because the reference diet is a nutritionally balanced diet, this method overcomes the main limitation of the direct method. However, the substitution method suffers from some disadvantages in that the AME of the test ingredient can be influenced by the composition of the basal diet, the assumption of additivity and the inclusion level of the test ingredient (Wu et al., 2020; Olukosi, 2021).

Another alternative for estimating the AME of ingredients is the regression method. In this method, a basal diet and several test diets in which the basal diet is replaced by at least two levels of the test ingredient are fed. The energy value of individual diets is compared to the corresponding inclusion level of the test ingredient and extrapolation of energy to the equivalency of 100% inclusion of test ingredient predicts the AME (Kong and Adeola, 2016; Noblet et al., 2021). However, this method is rarely used due to the errors associated with the calculations and the high cost involved in running the *in vivo* trials.

Estimation of AME of a feed ingredient, therefore, can vary depending on the methodology (Lockhart et al., 1967; Veluri and Olukosi, 2020; Olukosi, 2021). In the previous study (Chapter 4), the direct method was employed to determine the AME of wheat, sorghum, barley and maize at different ages (1-6 weeks) of broiler chickens. It was found that broiler age has a substantial influence on the AME and nitrogen-corrected AME (AMEn) of all grains, but the effect varied depending on the grain type. The aim of the present study was to determine the impact of age on AMEn of the same batch of cereal grains (wheat, sorghum, barley and maize) as those used in Chapter 4 for broiler chickens using the substitution method.

5.3. Materials and methods

The experiment was conducted according to the New Zealand Revised Code of Ethical Conduct for the use of live animals for research, testing and teaching and approved by the Massey University Animal Ethics Committee.

5.3.1. Ingredients

Four cereal grains (wheat, sorghum, barley, and maize) were obtained from a local commercial supplier. The proximate and nutrient compositions of the cereal grains are presented in Chapter 3. The wheat and sorghum samples were of Australian origin, and maize

and barley were sourced from New Zealand. All grains were ground in a hammer mill to pass through a screen size of 3.0 mm.

5.3.2. Diets, birds and housing

A total number of 1,260 day-old male broilers (Ross 308) were obtained from a local hatchery and raised on floor pens in an environmentally controlled room until assigned weekly to the experimental treatments. Except for the 1-7 d age group, birds were fed broiler starter mini pellets (230 g/kg crude protein and 12.56 MJ/kg AMEn) until d 21 and finisher pellets (207 g/kg crude protein and 13.0 MJ/kg AMEn) from d 22 to 35 (Table 5.1). At the beginning of each week (d 1, 8, 15, 22, 29 and 36), new batches of birds were selected randomly from the floor pens, weighed individually, and allocated to cages so that the average bird weight per cage was similar. For each cereal grain, the assay diet was fed to six replicate cages of broilers during the six periods, namely week 1 (d 1-7), week 2 (d 8-14), week 3 (d 15-21), week 4 (d 22-28), week 5 (d 29-35) or week 6 (d 36-42). Each replicate cage housed 10 birds during week 1, 8 birds during week 2, and 6 birds during weeks 3 to 6 post-hatch.

The AME was determined using the substitution method. In this method, a maize-soybean meal basal diet was formulated (Table 5.1), and then four test diets were developed by replacing (w/w) 300 g/kg of the basal diet with one of the cereal grains (Wu et al., 2020). Diets were mixed in a single-screw paddle mixer (Bonser Engineering Co. Pty. Ltd., Merrylands, Australia), then pelleted using a pellet mill (Model Orbit 15; Richard Sizer, Kingston-upon-Hull, UK) capable of manufacturing 180 kg of feed/h and equipped with a die ring with 3-mm holes and 35 mm thickness.

5.3.3. Determination of the apparent metabolisable energy

The AME was determined using the total excreta collection procedure (Hill and Anderson, 1958). During each week, diets were fed for 7 d, with the first 3 d serving as an

adaptation period. The feed intake (FI) and total excreta output for each replicate cage were recorded over the last 4 consecutive d of the assay. Daily excreta collections were pooled within a replicate cage, mixed in a blender and sub-sampled. Sub-samples were lyophilised (Model 0610, Cuddon Engineering, Blenheim, New Zealand), and dried excreta samples were ground to pass through a 0.5-mm sieve and stored in airtight plastic containers at 4 ºC pending analysis. The diet and excreta samples were analysed for dry matter (DM), gross energy (GE), and nitrogen (N).

Table 5.1. Composition (g/kg, as fed basis) of the basal diet used in the apparent metabolisable energy assay and, of pre-assay diets fed to broiler starters (d 1 to 21) and finishers (d 22 to 35)

Ingredient	Basal diet	Starter diet	Finisher diet
Maize	604.4	574.2	660.0
Soybean meal, 460 g/kg	338.1	381.4	295.7
Soybean oil	14.2	8.8	13.6
Dicalcium phosphate	15.8	10.7	8.2
Limestone	10.4	11.3	9.9
L Lysine HCl	3.7	2.0	1.9
DL Methionine	3.1	3.3	3.0
L Threonine	2.0	1.0	0.7
L Valine	0.7	-	-
Sodium chloride	1.0	2.5	2.5
Sodium bicarbonate	3.9	2.7	2.5
Trace mineral premix	1.0	1.0	1.0
Vitamin premix[1]	1.0	1.0	1.0
Choline Chloride 60%	0.7	-	-
Ronozyme HiPhos (Phytase)	-	0.1	0.1

[1] Vitamin and trace mineral premix supplied the following per kilogram of diet: antioxidant, 100 mg; biotin, 0.2 mg; calcium pantothenate, 12.8 mg; vitamin D_3 (cholecalciferol), 2400 IU; cyanocobalamin, 0.017 mg; folic acid, 5.2 mg; menadione, 4 mg; niacin, 35 mg; pyridoxine, 10 mg; vitamin A (trans-retinol), 11100 IU; riboflavin, 12 mg; thiamine, 3.0 mg; vitamin E (dl-α-tocopheryl acetate), 60 IU; choline chloride, 638 mg; Co, 0.3 mg; Cu, 3.0 mg; Fe, 25 mg; I, 1 mg; Mn, 125 mg; Mo, 0.5 mg; Se, 200 µg; Zn, 60 mg.

5.3.4. Chemical analysis

Dry matter was determined using standard procedures (Method 930.15; AOAC, 2016). Nitrogen was determined by combustion (Method 968.06; AOAC, 2016) using a carbon

nanosphere-200 carbon, N and sulphur auto analyser (rapid MAX N exceed, Elementar, Donaustraze, Hanau, Germany). Gross energy was determined by an adiabatic bomb calorimeter (Gallenkamp Autobomb, Weiss Gallenkamp Ltd, Loughborough, UK) standardised with benzoic acid.

5.3.5. Calculations

All data were expressed on a DM basis and the AME was determined using the following formula:

$$AME_{Diet}\ (MJ/kg) = [(FI \times GE_{Diet}) - (Excreta\ output \times GE_{Excreta})] / FI$$

The AME of the cereal grains was then calculated using the following formula:

$$AME_{Grain}\ (MJ/kg) = [AME\ of\ test\ grain\ diet - (AME\ of\ basal\ diet \times 0.70)] / 0.30$$

Nitrogen retention, as a percentage of intake, was determined as follows:

$$N\ retention\ (\%) = 100 \times [((FI \times N_{Diet}) - (Excreta\ output \times N_{Excreta})) / (FI \times N_{Diet})]$$

The AMEn was then calculated by correction for zero N retention by assuming 36.54 KJ per g N retained in the body as described by Titus et al. (1959).

5.3.6. Statistical analysis

The data for each grain were analysed separately by one-way ANOVA using the General Linear Models procedure of the SAS (version 9.4; 2015. SAS Institute Inc., Cary, NC). Cages served as the experimental unit. Significant differences between means were separated by the Least Significant Difference test. The data were subjected to orthogonal polynomial contrasts using the General Linear Models procedure of SAS (2015) to examine whether the responses to increasing bird age were of linear or quadratic nature. Significance of effects was declared at $P \leq 0.05$.

5.4. Results

The influence of broiler age on the retention of DM and N, AME and AMEn of wheat is summarised in Table 5.2. The highest DM and N retentions were recorded in weeks 1 and 2. The retention of both DM and N showed a linear response ($P < 0.001$) with advancing age, with the retentions decreasing as the birds grew older. Although the bird age did not exhibit any linear or quadratic response ($P > 0.05$; Figure 5.1A), the AMEn of wheat was observed to increase ($P < 0.001$) from 12.53 MJ/kg DM in week 1 to 14.55 MJ/kg DM in week 2, then declined in following weeks compared to week 2.

Table 5.2. Influence of broiler age on the retention (% of intake) of dry matter (DM) and nitrogen (N), apparent metabolisable energy (AME; MJ/kg DM basis) and nitrogen-corrected AME (AMEn; MJ/kg DM basis) of wheat[1].

Age (week)	DM retention	N retention	AME	AMEn
1	77.6	76.2	13.30	12.53
2	81.6	76.4	15.28	14.55
3	73.6	64.5	13.35	12.75
4	75.3	65.7	13.84	13.31
5	75.4	65.7	14.03	13.48
6	74.3	59.6	13.76	13.20
SEM[2]	0.66	1.23	0.273	0.240
Orthogonal polynomial contrast, P ≤				
Linear	0.001	0.001	0.667	0.731
Quadratic	0.271	0.157	0.274	0.111

[1] Each value represents the mean of six replicates. The number of birds per replicate cage was 10 (week 1), 8 (week 2) and 6 (weeks 3-6).
[2] Pooled standard error of mean.

The influence of broiler age on the retention of DM and N, AME and AMEn of sorghum is presented in Table 5.3. Dry matter and N retentions decreased ($P < 0.001$) linearly with the advancing age of birds. The DM retention declined from 77.8% in week 1 to 74.7% in week 6 and the highest N retention of 70.9% was recorded in week 1, declining to 58.1% in week 6. The AMEn of sorghum increased quadratically ($P < 0.05$) with advancing age, from 12.84 MJ/kg DM in week 1 to 13.95 MJ/kg DM in week 2, then plateaued up to week 6 (Figure 5.1B).

Table 5.3. Influence of broiler age on the retention (% of intake) of dry matter (DM) and nitrogen (N), apparent metabolisable energy (AME; MJ/kg DM basis) and nitrogen-corrected AME (AMEn; MJ/kg DM basis) of sorghum[1]

Age (week)	DM retention	N retention	AME	AMEn
1	77.8	70.9	13.32	12.84
2	78.0	68.2	14.38	13.95
3	75.9	65.7	14.39	13.90
4	77.2	65.2	14.86	14.45
5	75.6	63.6	14.29	13.87
6	74.7	58.1	14.48	14.13
SEM[2]	0.61	1.25	0.344	0.321
Orthogonal polynomial contrast, P ≤				
Linear	0.001	0.001	0.047	0.017
Quadratic	0.468	0.376	0.043	0.039

[1] Each value represents the mean of six replicates. The number of birds per replicate cage was 10 (week 1), 8 (week 2) and 6 (weeks 3-6).
[2] Pooled standard error of mean.

The influence of broiler age on the retention of DM and N, AME and AMEn of barley is presented in Table 5.4. The retention of DM and N showed linear decreases ($P < 0.001$) with advancing age. The birds retained the highest DM and N in week 1 and the lowest in week 6. Broiler age had no influence ($P > 0.05$) on the AME or AMEn of barley (Figure 5.1C).

Table 5.4. Influence of broiler age on the retention (% of intake) of dry matter (DM) and nitrogen (N), apparent metabolisable energy (AME; MJ/kg DM basis) and nitrogen-corrected AME (AMEn; MJ/kg DM basis) of barley[1]

Age (week)	DM retention	N retention	AME	AMEn
1	75.8	74.2	11.98	11.26
2	75.6	72.2	12.46	11.78
3	72.8	65.7	12.09	11.46
4	72.9	64.3	12.06	11.56
5	72.5	65.3	12.15	11.59
6	71.3	59.3	12.24	11.67
SEM[2]	0.63	1.03	0.360	0.325
Orthogonal polynomial contrast, P ≤				
Linear	0.001	0.001	0.906	0.561
Quadratic	0.429	0.278	0.972	0.796

[1] Each value represents the mean of six replicates. The number of birds per replicate cage was 10 (week 1), 8 (week 2) and 6 (weeks 3-6).
[2] Pooled standard error of mean.

The retention of DM and N, AME and AMEn of maize measured in broilers at different ages are presented in Table 5.5. The DM retention declined linearly ($P < 0.001$) from 80.3% in week 1 to 76.9% in week 6. A similar trend was observed for N retention, wherein N retention decreased linearly ($P < 0.001$) as the birds grew older from 76.8% in week 1 to 63.1% in week 6. The AME and AMEn of maize were unaffected ($P > 0.05$) by the age of broilers (Figure 5.1D).

Table 5.5. Influence of broiler age on the retention (% of intake) of dry matter (DM) and nitrogen (N), apparent metabolisable energy (AME; MJ/kg DM basis) and nitrogen-corrected AME (AMEn; MJ/kg DM basis) of maize[1]

Age (week)	DM retention	N retention	AME	AMEn
1	80.3	76.8	14.68	14.12
2	79.8	73.1	15.09	14.62
3	77.2	69.6	14.79	14.28
4	78.4	69.8	15.33	14.85
5	77.8	68.3	15.24	14.80
6	76.9	63.1	15.38	14.92
SEM[2]	0.53	1.12	0.366	0.359
Orthogonal polynomial contrast, P ≤				
Linear	0.001	0.001	0.152	0.099
Quadratic	0.233	0.930	0.879	0.814

[1] Each value represents the mean of six replicates. The number of birds per replicate cage was 10 (week 1), 8 (week 2) and 6 (weeks 3-6).
[2] Pooled standard error of mean.

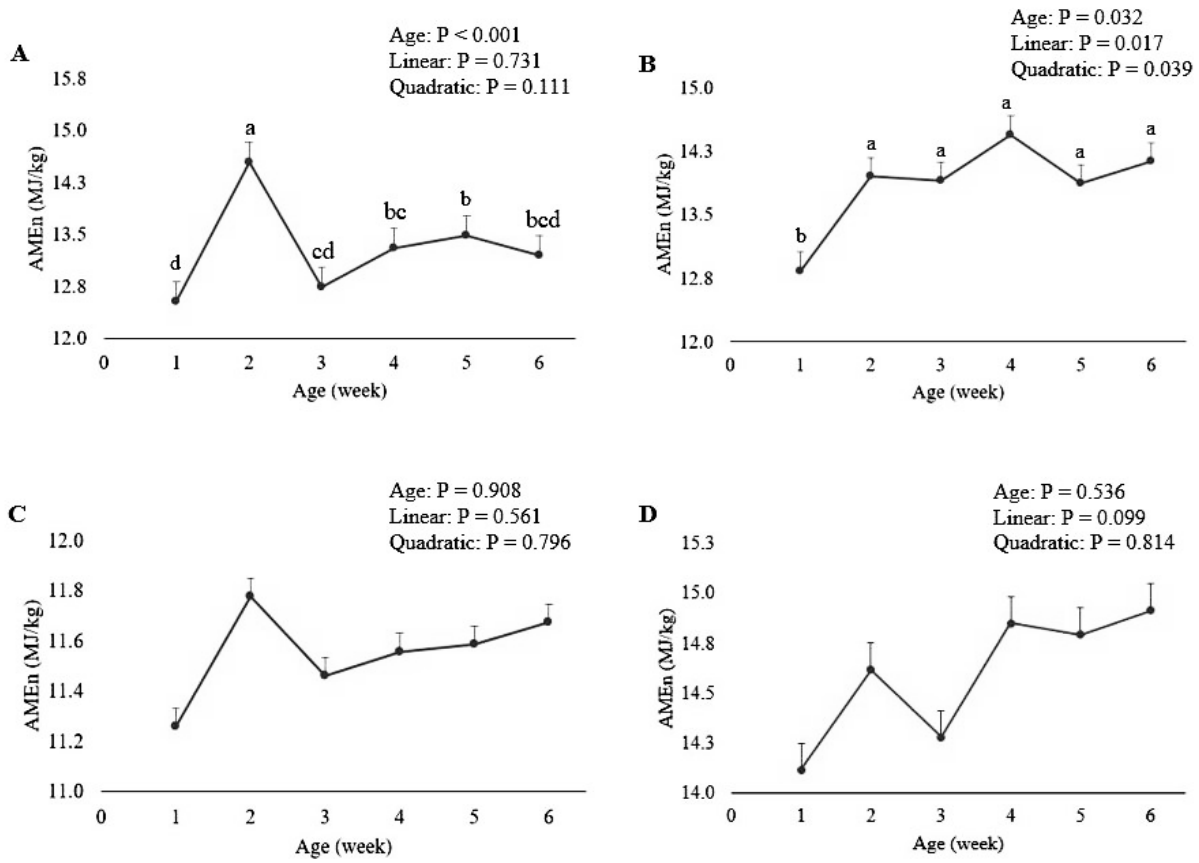


Figure 5.1. Effect of broiler age on the nitrogen-corrected apparent metabolisable energy (AMEn) of wheat (A), sorghum (B), barley (C) and maize (D) grains; mean ± standard error.
[a-d] Values with different superscripts differ significantly (P < 0.05)

5.5. Discussion

The objectives of the present study were to investigate whether (i) the AMEn of commonly used cereal grains measured by the substitution method is influenced by the age of broilers and (ii) the AMEn estimates of cereals are comparable to those determined using the direct method in a previous study reported in Chapter 4.

When the direct method was employed in the AME assay (Chapter 4), the highest DM and N retention were observed in week 1 and then declined with age for all cereal grains. Somewhat similar trends were observed for retention values in the current AME assay using the substitution method. The highest retention of DM and N were recorded in weeks 1 and 2 and declined thereafter as the birds grew older. These findings are similar to those of Lopez and

Leeson (2007) who showed that the retention of N in a maize-soybean meal diet declined as broilers grew older, especially after 28 d of age. Similarly, Aderibigbe et al. (2020) reported significant reductions in the retention of DM and N in a maize-soybean meal diet from 1 to 42 d of age of broiler chickens. Yang et al. (2020) reported that the advancing age of broilers significantly decreased the N retention of cereal-based diets from 68.8% at 7 d of age to 60.9% at 35 d of age. The observed age-related reductions in the N retention in the current study, and the previous ones, are to be expected, reflecting surplus N from increasing feed consumption and decreasing needs of N for growth (Bartov, 1995).

In the previous chapter (Chapter 4), following the direct method, the highest AMEn values were observed in week 1 for all cereal grains and then declined with age. In the present study, with the substitution method, the trends were exactly the opposite. In general, the lowest AMEn values were recorded in week 1 for all four cereal grains (statistically significant for wheat and sorghum, and numerically for barley and maize) and increased thereafter. Published data on the influence of broiler age on the AME of cereals are limited and all available data relate to complete diets. Current findings agree with previous studies in broilers fed complete practical diets, where the utilisation of energy-yielding nutrients improved with age (Zelenka, 1968; Batal and Parsons, 2002). Batal and Parsons (2002) showed that the AMEn of a maize-soybean meal diet increased with age (from 13.33 MJ/kg at 7 d to 14.35 MJ/kg at 14 d) and, then plateaued after 14 d of age. However, Batal and Parsons (2004) observed no differences were in the AMEn of a maize-soybean meal diet between 7 and 14 d of age. Thomas et al. (2008) showed that the AMEn of wheat- and maize-based diets increased between d 7 (11.06 and 12.28 MJ/kg, respectively) and d 14 (13.24 and 13.01 MJ/kg, respectively), with no further change between 14 and 21 d of age. Aderibigbe et al. (2020) observed that the AMEn of a maize-soybean meal diet increased from 13.60 to 13.80 MJ/kg between 11 to 21 d of age, then plateaued until 42 d of age.

In diets containing adequate levels of protein, AME is a function of the utilisation of lipids and starch. Available data on fat and starch digestion patterns lend support to the increase in AME with age. Tancharoenrat et al. (2013), investigating several fat sources, found that the total tract fat digestibility was low in week 1 and increased with advancing age. A similar observation was reported by Lessire et al. (1982) who examined the influence of age on the fat digestibility and AME of beef tallow. It was found that the apparent fat digestibility and AME of beef tallow increased by 8.5% and 4.3%, respectively, between weeks 2 and 6. Scheele et al. (1997) also revealed that the apparent digestibility of animal fat increased after the second week post-hatch and the AME increased by 1.0 MJ/kg between weeks 2 and 4. Batal and Parsons (2002) showed that the apparent digestibility of fat in a maize-soybean meal diet increased with advancing age from 59% at week 1 to 74% at week 2 post-hatch. These researchers attributed the increase in AMEn to the increase in fat digestibility with the advancing age of broilers. Svihus (2011a) indicated that there is a strong correlation (r = 0.984) between the AME and the digestibility of starch, the main source of energy in cereal-based diets. Hatchlings can digest starch rapidly due to high activity levels and accumulation of starch-degrading endo-enzymes such as α-amylase and disaccharidase in the pancreas during embryonic development (Mahagna and Nir, 1996; Sklan and Noy, 2000). Akiba and Murakami (1995) stated that the activity of amylase increased by 10% between 1 and 21 d post-hatch. Noy and Sklan (1995) also reported that the secretion of amylase was low at 4 d post-hatch and increased by 100 folds at 21 d post-hatch; however, there was no difference in starch digestibility between 4-21 d of age. Uni et al. (1995) found that starch digestibility of a maize-soybean meal diet increased from 90% at d 4 to 95% at d 14 of age. Similar increases in starch digestibility with advancing broiler age have been reported by Batal and Parsons (2002) and Zelenka and Ceresnakova (2005).

It is acknowledged that the data from the current study (substitution method) cannot be statistically compared with those from the study (direct method) reported in Chapter 4. However,

since the same samples of the four cereals were evaluated in both studies, a general inference of the effect of methodology could be made. Consistent with previously published research, the AME estimates were influenced by the methodology (Lockhart et al., 1967; Lee and Kong, 2019; Veluri and Olukosi, 2020). There were two key differences between the findings of the two studies. First, the AMEn of cereal grains determined by the substitution method were lower, with average differences ranging from 2.0 MJ/kg at week 1 to 0.45 MJ/kg at later ages. The average difference between the AMEn value determined by the direct and substitution method was the highest for barley (1.42 MJ/kg) followed by sorghum (1.21 MJ/kg), and the lowest difference was recorded for wheat (0.29 MJ/kg). The greatest difference in AMEn estimates between the methods was recorded for the first week post-hatch, which could be related to the differences in FI in the two methods. Higher FI is recognised to have a negative influence on dietary AMEn (Zelenka 1997; Scott, 2005). Second, trends of AMEn with advancing broiler age differed between the two methods. In the direct method, the AMEn was greater in week 1 and then declined, whereas the AMEn was lower in week 1 and then increased in the substitution method. Such a divergence was unexpected and has not been reported previously. Overall, these findings add further complications to the existing inconsistencies in AMEn determination assays (Wu et al., 2020).

Possible explanations for the variation in AME estimates among methodologies lie primarily in the differences in assay diet composition and calculation methods. Only one study has compared the substitution and direct methods. Lockhart et al. (1967) reported that the AME of wheat was lower when measured by the direct method (12.91 vs. 13.09 MJ/kg). Veluri and Olukosi (2020), comparing the substitution and regression methods, found that the assay method can influence the AME and methodology differences should be considered in comparisons across studies. Lee and Kong (2019) found that the AME of barley measured by the direct method was lower than that measured by the regression method (11.42 vs. 12.43

MJ/kg). The observed difference was attributed to the high inclusion level of barley in the direct method, leading to greater β-glucan content and digesta viscosity, hence decreasing the digestion and absorption of nutrients (Pettersson and Aman, 1989; Annison, 1991). Olukosi (2021) reported that the AMEn of barley measured by the regression method was 2.96 MJ/kg greater than that of the substitution method (10.97 vs. 8.01 MJ/kg), but the AMEn of maize was not influenced by the methodology, suggesting that the influence of the methodology is ingredient-dependent. However, Lee and Kong (2019) observed no significant differences for the AME of wheat when measured by the direct vs. regression method.

5.6. Conclusions

The current findings, along with those previous studies, demonstrate that the effects of age and methodology are relevant in the determination of AMEn of cereal grains. The influence of age of birds on the AMEn of cereal grains was grain-dependent. Whilst AMEn of wheat and sorghum were influenced by age, the AMEn of barley and maize were unaffected. The direct method yielded higher AMEn estimates than the substitution method, but this does not necessarily make the method that gave greater values more robust. Importantly, current findings question the validity of using single AME or AMEn values for feed ingredients in broiler diet formulations across different ages.

CHAPTER 6 Influence of age on the apparent metabolisable energy of soybean meal and canola meal for broilers

6.1. Abstract

The present study investigated the influence of broiler age on the apparent metabolisable energy (AME) and nitrogen-corrected AME (AMEn) of soybean meal (SBM) and canola meal (CM). A maize-SBM basal diet was formulated and the test diets were developed by replacing (w/w) 300 g/kg of the basal diet with SBM or CM. Six groups of broiler chickens, aged 1-7, 8-14, 15-21, 22-28, 29-35 or 36-42 d post-hatch, were utilised. Each diet, in pellet form, was randomly allocated to six replicate cages in each age group. Except for the 1-7 d age group, birds were fed a starter (d 1-21) and/or a finisher (d 22-35) diet prior to the introduction of experimental diets. The number of birds per cage was 10 (d 1-7), 8 (d 8-14) and 6 (d 15-42). The AME and AMEn of the protein source ingredients were determined by total excreta collection. Data for each protein source were subjected to orthogonal polynomial contrasts using the General Linear Models procedure. Bird age decreased the retention of dry matter quadratically ($P < 0.001$) for both SBM and CM. The retention of nitrogen decreased linearly ($P < 0.001$) with the advancing age of broilers for SBM and CM. The AMEn of SBM and CM decreased quadratically ($P < 0.001$) as birds grew older. The highest AMEn was observed during week 1 for both SBM and CM, then declined until week 3, followed by increases thereafter. The current results showed that the age of broiler chickens influenced the AMEn of SBM and CM. These findings support the need to consider the age-dependent AMEn of feed ingredients in diet formulations.

6.2. Introduction

Determining the available energy of feed ingredients for broilers is crucial to optimise their dietary inclusion rate and improve feed efficiency. The most prevalent system in

evaluating the energy availability of feed ingredients is the apparent metabolisable energy (AME) or the nitrogen-corrected-AME (AMEn; Hill and Anderson, 1958; Sibbald, 1982).

Dietary energy is provided mainly by the starch in cereal grains, accounting for over 60% of the energy requirements for broilers. Plant-based protein sources also cover up to 30% of the energy supply in broiler diets and their interaction with main energy sources impacts the overall energy utilisation (Hossain et al., 2012; Veluri and Olukosi, 2020).

The two plant protein sources commonly used in poultry diets are soybean meal (SBM) and canola meal (CM). Soybean meal remains the sovereign protein source used globally due to its high protein content, excellent amino acid profile that complements cereal grains, and high amino acid digestibility (Mateos et al., 2008). Canola meal contains lower AME than SBM (Khajali and Slominski, 2012), but is increasingly included in broiler diets as an alternative to SBM, due to the ever-increasing cost of SBM.

Commercial nutritionists have been using a single AME or AMEn value of feed ingredients obtained from tabulated values, predictive regression equations or bioassays (Mateos et al., 2019). This approach ignores the fact that energy utilisation is influenced by a number of factors, including age of birds. Bird age has been reported to influence the digestion and absorption of energy-yielding nutrients in feed ingredients (Batal and Parsons, 2003; Leslie et al., 2007; Lopez and Leeson, 2008). During the early growth stages of life, the intestinal tract is immature and less developed (Sklan and Noy, 2000). Moreover, low concentrations and low activities of digestive enzymes during the first two weeks of age reduce the ability of birds to digest and utilise nutrients. Studies have shown that the age of birds impacts the AME of complete diets and individual feed ingredients (Bartov, 1995; Batal and Parsons, 2003). Lopez and Leeson (2008) observed that the AMEn of SBM increased by 0.50 MJ/kg between d 12 and d 33 of age of broilers.

The applicability of a single AME value, obtained with older birds, to all growth phases, especially the early life, of broilers is debatable and underlines the need for age-dependent estimates of AMEn of ingredients in feed formulations. Despite this importance, studies examining the age effects on AMEn of SBM and CM in broilers are scant and none have investigated the changes from the hatch to marketing stage. The aim of the present study was to determine the AMEn in SBM and CM from week 1 to 6 post-hatch.

6.3. Materials and methods

The experiment was conducted according to the New Zealand Revised Code of Ethical Conduct for the use of live animals for research, testing and teaching and approved by the Massey University Animal Ethics Committee.

6.3.1. Ingredients

Two protein sources (SBM and solvent-extracted CM) were obtained from a local commercial supplier and ground in a hammer mill to pass through a screen size of 3.0 mm. The SBM was of Argentinean origin and the CM of Australian origin. The proximate and nutrient composition of SBM and CM are presented in Chapter 3.

6.3.2. Diets, birds and housing

Day-old male broilers (Ross 308) were obtained from a local hatchery and raised on floor pens in an environmentally controlled room until assigned weekly to the experimental treatments. Except for the 1-7 d age group, birds were fed broiler starter mini pellets (230 g/kg crude protein and 12.56 MJ/kg AME) until d 21 and finisher pellets (207 g/kg crude protein and 13.0 MJ/kg AME) from d 22 to 35 (Table 6.1). At the beginning of each week (d 1, 8, 15, 22, 29 and 36), new batches of birds were selected randomly from the floor pens, weighed individually, and allocated to cages so that the average bird weight per cage was similar. For

each protein source, the assay diet was fed to six replicate cages of broilers during the six periods, namely week 1 (d 1-7), week 2 (d 8-14), week 3 (d 15-21), week 4 (d 22-28), week 5 (d 29-35) or week 6 (d 36-42). Each replicate cage housed 10 birds during week 1, 8 birds during week 2, and 6 birds during weeks 3 to 6 post-hatch.

The AME was determined using the substitution method. In this method, a maize-SBM basal diet was formulated (Table 6.1), and then two test diets were developed by replacing (w/w) 300 g/kg of the basal diet with one of the protein sources. Diets were mixed in a single-screw paddle mixer (Bonser Engineering Co. Pty. Ltd., Merrylands, NSW, Australia) and then pelleted using a pellet mill (Model Orbit 15; Richard Sizer, Kingston-upon-Hull, UK) capable of manufacturing 180 kg of feed/h and equipped with a die ring with 3-mm holes and 35 mm thickness.

Table 6.1. Composition (g/kg, as fed basis) of the basal diet used in the apparent metabolisable energy assay and, of pre-assay diets fed to broiler starters (d 1 to 21) and finishers (d 22 to 35)

Ingredient	Basal diet	Starter diet	Finisher diet
Maize	604.4	574.2	660.0
Soybean meal, 460 g/kg	338.1	381.4	295.7
Soybean oil	14.2	8.8	13.6
Dicalcium phosphate	15.8	10.7	8.2
Limestone	10.4	11.3	9.9
L Lysine HCl	3.7	2.0	1.9
DL Methionine	3.1	3.3	3.0
L Threonine	2.0	1.0	0.7
L Valine	0.7	-	-
Sodium chloride	1.0	2.5	2.5
Sodium bicarbonate	3.9	2.7	2.5
Trace mineral premix	1.0	1.0	1.0
Vitamin premix[1]	1.0	1.0	1.0
Choline Chloride 60%	0.7	-	-
Ronozyme HiPhos (Phytase)	-	0.1	0.1

[1]Vitamin and trace mineral premix supplied the following per kilogram of diet: antioxidant, 100 mg; biotin, 0.2 mg; calcium pantothenate, 12.8 mg; vitamin D_3 (cholecalciferol), 2400 IU; cyanocobalamin, 0.017 mg; folic acid, 5.2 mg; menadione, 4 mg; niacin, 35 mg; pyridoxine, 10 mg; vitamin A (trans-retinol), 11100 IU; riboflavin, 12 mg; thiamine, 3.0 mg; vitamin E (dl-α-tocopheryl acetate), 60 IU; choline chloride, 638 mg; Co, 0.3 mg; Cu, 3.0 mg; Fe, 25 mg; I, 1 mg; Mn, 125 mg; Mo, 0.5 mg; Se, 200 µg; Zn, 60 mg.

6.3.3. Determination of the apparent metabolisable energy

The AME was determined using the total excreta collection procedure. During each week, diets were fed for 7 d, with the first 3 d serving as an adaptation period. The feed intake (FI) and excreta output for each replicate cage were recorded over the last 4 consecutive d of the assay. Daily excreta collections were pooled within a replicate cage, mixed in a blender and sub-sampled. Sub-samples were lyophilised (Model 0610, Cuddon Engineering, Blenheim, New Zealand), and dried excreta samples were ground to pass through a 0.5-mm sieve and stored in airtight plastic containers at 4 ºC pending analysis. The diet and excreta samples were analysed for dry matter (DM), gross energy (GE), and nitrogen (N).

6.3.4. Chemical analysis

Dry matter was determined using standard procedures (Method 930.15; AOAC, 2016). Nitrogen was determined by combustion (Method 968.06; AOAC, 2016) using a carbon nanosphere-200 carbon, N and sulphur auto analyser (rapid MAX N exceed, Elementar, Donaustraze, Hanau, Germany). Gross energy was determined by an adiabatic bomb calorimeter (Gallenkamp Autobomb, Weiss Gallenkamp Ltd, Loughborough, UK) standardised with benzoic acid.

6.3.5. Calculations

All data were expressed on a DM basis and the AME was determined using the following formula:

$$AME_{Diet}\ (MJ/kg) = [(FI \times GE_{Diet}) - (\text{Excreta output} \times GE_{Excreta})] / FI$$

The AME of the protein sources was then calculated using the following formula:

$$AME_{protein\ source}\ (MJ/kg) =$$

$$[\text{AME of test protein source diet} - (\text{AME of basal diet} \times 0.70)] / 0.30$$

Nitrogen retention, as a percentage of intake, was determined as follows:

$$N\ retention\ (\%) = 100 \times [((FI \times N_{Diet}) - (Excreta\ output \times N_{Excreta})) / (FI \times N_{Diet})]$$

The AMEn was then calculated by correction for zero N retention by assuming 36.54 KJ per g N retained in the body as described by Titus et al. (1959).

6.3.6. Statistical analysis

The data for each protein source were analysed separately by one-way ANOVA using the General Linear Models procedure of the SAS (version 9.4; 2015. SAS Institute Inc., Cary, NC). Cages served as the experimental unit. Significant differences between means were separated by the Least Significant Difference test. The data were subjected to orthogonal polynomial contrasts using the General Linear Models procedure of SAS (2015) to examine whether the responses to increasing bird age were of linear or quadratic nature. Significance of effects was declared at $P \leq 0.05$.

6.4. Results

The influence of broiler age on the retention of DM and N, AME, AMEn and the ratio between AMEn and gross energy of SBM is summarised in Table 6.2. The retention of DM was the highest in week 1, declined in week 2, plateaued between weeks 3 and 5, and declined further in week 6, resulting in quadratic ($P < 0.001$) age effect. A linear decrease ($P < 0.01$) in the N retention was observed as birds grew older from 66.3% in week 1 down to 47.1% in week 6. The AME, AMEn and AMEn:GE of SBM showed a quadratic response with age (Figure 6.1A). The AMEn of SBM decreased from 11.38 MJ/kg in week 1 to 9.46 MJ/kg in week 3, followed by an increase to week 5 to 10.33 MJ/kg. Similar trend was observed for the AMEn:GE ratio.

Table 6.2. Influence of broiler age on the retention (% of intake) of dry matter (DM) and nitrogen (N), apparent metabolisable energy (AME; MJ/kg DM basis), nitrogen-corrected AME (AMEn; MJ/kg DM basis) and the ratio between AMEn and gross energy (AMEn:GE; MJ/MJ) of soybean meal[1]

Age (week)	DM retention	N retention	AME	AMEn	AMEn:GE
1	76.2	66.3	12.99	11.38	0.624
2	70.9	58.3	11.70	10.40	0.570
3	66.8	55.9	10.17	9.46	0.519
4	67.3	56.6	10.98	9.86	0.541
5	68.0	56.3	11.64	10.33	0.567
6	63.9	47.1	10.60	9.75	0.535
SEM[2]	0.55	1.10	0.280	0.191	0.0105
Orthogonal polynomial contrast, P ≤					
Linear	0.001	0.001	0.001	0.001	0.001
Quadratic	0.001	0.811	0.001	0.001	0.001

[1] Each value represents the mean of six replicates. The number of birds per replicate cage was 10 (week 1), 8 (week 2) and 6 (weeks 3-6).
[2] Pooled standard error of mean.

The retention of DM and N, AME and AMEn and AMEn:GE of CM measured at different ages post-hatch for broiler chickens are presented in Table 6.3. The DM retention of CM showed a quadratic decrease ($P < 0.001$) as birds grew older. Birds retained the highest DM in week 1, decreased to week 2, plateaued between weeks 3 and 5, and declined further in week 6. For N retention, birds retained 66.3% N in week 1, which decreased linearly ($P < 0.001$) to 48.1% in week 6. The AME, AMEn and AMEn:GE of CM decreased quadratically ($P < 0.001$) with advancing age (Figure 6.1B). The AMEn of CM decreased from 9.10 MJ/kg in week 1 to 6.44 MJ/kg in week 3, increased to 7.30 MJ/kg in week 4, and then plateaued up to week 6.

The influence of broiler age on excreta GE and the ratio between excreta output to FI in birds fed SBM and CM diets are presented in Table 6.4. There was a quadratic ($P < 0.001$) response to broiler age for excreta GE content and the ratio between excreta output to FI. The GE of excreta increased between week 1 and week 4, followed by a decrease up to week 6 of age. The excreta to FI ratio increased from 0.24 and 0.27 kg:kg at week 1 to 0.36 and 0.39 kg:kg at week 6, respectively, for SBM and CM.

Table 6.3. Influence of broiler age on the retention (% of intake) of dry matter (DM) and nitrogen (N), apparent metabolisable energy (AME; MJ/kg DM basis), nitrogen-corrected AME (AMEn; MJ/kg DM basis) and the ratio between AMEn and gross energy (AME:GE; MJ/MJ) of canola meal[1]

Age (week)	DM retention	N retention	AME	AMEn	AME:GE
1	72.9	66.3	10.56	9.10	0.501
2	68.7	63.2	9.53	7.99	0.440
3	63.0	56.9	7.27	6.44	0.355
4	63.9	58.5	8.30	7.30	0.402
5	64.8	58.7	9.22	7.78	0.428
6	61.0	48.1	8.18	7.46	0.411
SEM[2]	0.58	0.81	0.348	0.229	0.0127
Orthogonal polynomial contrast, P ≤					
Linear	0.001	0.001	0.001	0.001	0.001
Quadratic	0.001	0.140	0.001	0.001	0.001

[1] Each value represents the mean of six replicates. The number of birds per replicate cage was 10 (week 1), 8 (week 2) and 6 (weeks 3-6).
[2] Pooled standard error of mean.

Table 6.4. Influence of broiler age on excreta gross energy (GE; MJ/kg DM) and excreta output:feed intake (kg:kg); in birds fed soybean meal and canola meal diets[1]

	Soybean meal		Canola meal	
Age (week)	Excreta GE (MJ/kg DM)	Excreta output: Feed intake	Excreta GE (MJ/kg DM)	Excreta output: Feed intake
1	15.79	0.24	16.29	0.27
2	16.12	0.29	16.80	0.31
3	16.12	0.33	16.62	0.37
4	15.80	0.33	16.33	0.36
5	15.56	0.32	15.99	0.35
6	15.44	0.36	15.94	0.39
SEM[2]	0.048	0.006	0.079	0.006
Orthogonal polynomial contrast, P ≤				
Linear	0.001	0.001	0.001	0.001
Quadratic	0.001	0.001	0.001	0.001

[1] Each value represents the mean of six replicates. The number of birds per replicate cage was 10 (week 1), 8 (week 2) and 6 (weeks 3-6).
[2] Pooled standard error of mean.

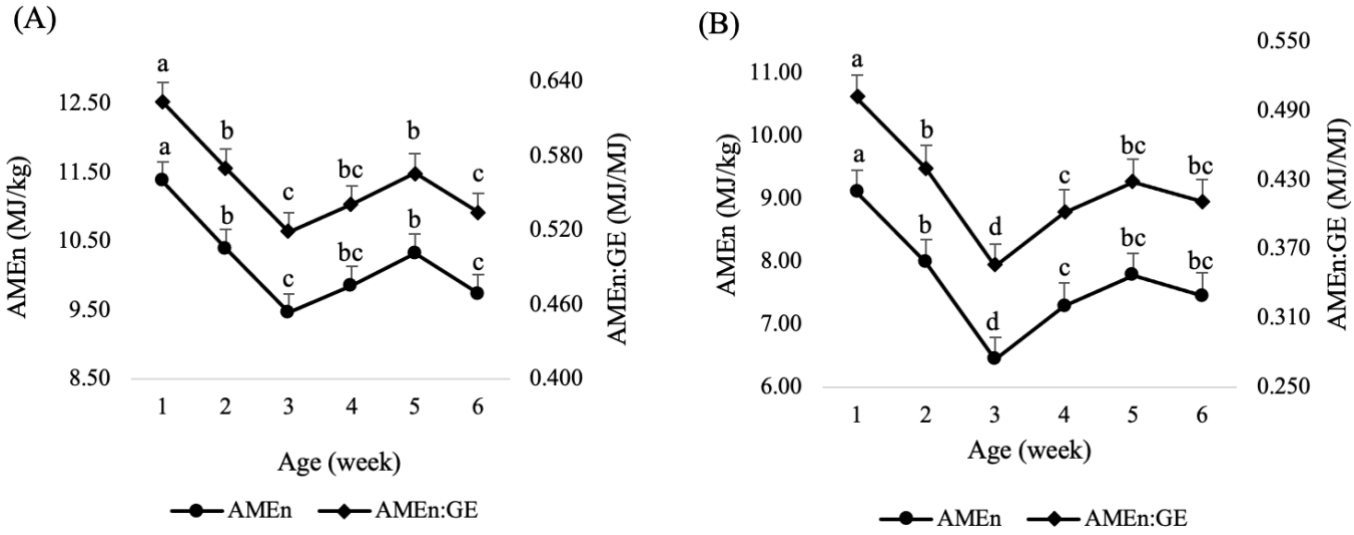


Figure 6.1. Effect of broiler age on the nitrogen-corrected apparent metabolisable energy (AMEn) and the relation between AMEn and gross energy (GE) of soybean meal (A) and canola meal (B); mean ± standard error. [a-d] Values with different superscripts differ significantly ($P < 0.05$)

6.5. Discussion

Accurate determination of the metabolisable energy content of feed ingredients is crucial to achieve an optimum energy level based on the energy content of feed ingredients, and, therefore, a central factor in least-cost feed formulations. Focus is usually placed on the inclusion level of dietary energy ingredients in feed formulation as changes in the dietary energy play a pivotal role in determining not only the feed consumption but also the cost of the diet (Dozier III et al., 2007; Abdollahi et al., 2021). Protein source ingredients, such as SBM and CM, besides supplying the majority of dietary protein, are secondary sources of supplying energy in broiler diets after cereals.

To the author's knowledge, no published data are available on age-related energy utilisation responses, from week 1 to 6 post-hatch, of individual protein source ingredients. The main objective of the current study was to examine whether the AMEn of SBM and CM is influenced by broiler age and if the age effect varies between these two ingredients. The results showed that the AMEn of SBM and CM decreased with advancing age, with the first week post-hatch recording the highest AMEn value for both protein sources, then declining to the

lowest value at d 21 and increasing thereafter to d 42. The retention of DM (quadratically) and N (linearly) also declined with the advancing age. Furthermore, the ratio of AMEn:GE declined between d 7 and d 21 and increased with the advancing age of broilers.

The observed reductions in AMEn and, the retention of DM and N with the advancing age of broilers were unexpected as it is well accepted that the digestive tract of newly hatched chicks is immature and lacks the appropriate enzymatic capabilities for efficient nutrient digestion and absorption. Rapid development and maturation of the digestive system, increased secretion of digestive enzymes and morphological surface area for nutrient absorption, along with improvements in nutrient transporter activities, are observed as the broilers grew older (Sklan and Noy, 2000; Ravindran and Abdollahi, 2021).

The influence of broiler age on the AMEn of SBM and CM has not been previously evaluated. Published data on age influence on the AME of complete diets, although mostly limited to 2 age groups, present contradictory patterns. Some studies showed an increasing trend (Bourdillon et al., 1990; Batal and Parsons, 2003; Lopez and Leeson, 2008; Stefanello et al., 2016; Adeola et al., 2018), while others revealed a decrease (Schneider and Lantzsch, 1969; Bartov, 1988). In several, no influence of age was observed (Niegm, 1966; Matterson and Prince, 1969; Siregar and Farrell, 1980, Fonolla et al., 1981). Batal and Parsons (2002) showed that the AMEn of a maize-SBM diet increased from 13.33 MJ/kg at d 7 to 14.35 MJ/kg at d 14, with no further increase up to d 21 post-hatch. Yang et al. (2020) found that there was a linear AMEn response to age with AMEn increasing from d 7 to 28 and then slightly decreasing at 35 d of age. Krás et al. (2013) found that the AMEn of diets with low and high fibre contents decreased by 0.24-0.30 MJ/kg, respectively, between d 10 and 20 post-hatch. The higher AMEn values at d 10 compared to d 20 were attributed to the longer digesta retention time and therefore, better digestion of nutrients. Bartov (1995) reported a decline in dietary AMEn of 0.32 MJ/kg

with broiler age between d 13 and 22 post-hatch. The reduction was ascribed mainly to the diet composition, specifically to the dietary ratio between crude protein and energy.

In agreement with the present findings, Thomas et al. (2008) reported that AMEn of a wheat-based diet decreased from 13.90 MJ/kg at d 3 to 12.17 MJ/kg at d 5 post-hatch, with a further decrease to 11.06 MJ/kg at d 7. Similarly, it was observed that the AMEn of a sorghum-based diet declined between d 3 and d 5 (14.49 vs. 12.76 MJ/kg) followed by a 0.62 MJ/kg reduction at d 7 post-hatch. Moreover, the AMEn of maize-based diet reduced with advancing age from 13.87 MJ/kg at d 3 to 12.28 at d 7 of age. These researchers also reported an increase in dietary AMEn from d 7 to 14 post-hatch. Regardless of the cereal-base, the highest N retention and total tract fat digestibility were recorded at d 3, which decreased gradually to the lowest values at d 7. The exact cause for this reduction in the utilisation of energy-yielding components towards the end of the first week is unclear. However, as discussed below, a number of factors may have contributed to this finding including the yolk sac contribution to dietary energy, changes in microbiota, availability of digestive enzymes, digesta passage rate and endogenous energy losses (EEL).

Zelenka (1968) revealed that the AME of a maize-SBM diet was the highest at d 3 post-hatch, declined to the lowest value by d 7, followed by an increase at d 14 post-hatch. In a follow-up study, this researcher reported a similar reduction of dietary AME from hatch to 7 d of age followed by an increase at 14 d post-hatch. Murakami et al. (1992) reported a decline in energy utilisation during the first few d post-hatch, followed by an increase after d 7. Gracia et al. (2003) reported that the dietary AME declined between d 4 and 8, which was associated with a reduction in the retention of N and fats on d 8, followed by an increase in dietary AMEn on d 15 post-hatch.

In the current study, the ratio between AME and GE for both SBM and CM declined with the advancing age of broilers. A similar trend was reported by Moss et al. (2020) who showed

that the AMEn:GE ratio for a maize-based diet was higher at d 7 than at d 34 (0.807 vs. 0.778), with similar trend observed for sorghum-based (0.778 vs. 0.718) and wheat-based (0.798 vs. 0.761) diets.

The higher AMEn of SBM and CM at d 7 post-hatch may be attributed, as discussed previously in Chapter 4, to a combination of three factors: First, FI is very low during the first week compared to subsequent weeks. Lower FI restricts the uniform flow of digesta in the digestive tract and increases the digesta retention time, allowing better digestion and absorption of nutrients (Washburn, 1991; Choct et al., 1996). With the advancing age of broilers, the rapid increase in FI could lead to increase digesta passage rate in the digestive tract, which decreases the duration of contact with digestive enzymes, hence reducing nutrient digestibility and utilisation (Thomas et al., 2008). Vergara et al. (1989) related the increase in digesta passage rate to the increase in FI as birds grew older. Uni et al. (1995) observed that the digesta passage rate increased from 74 mins to 122 mins between d 7 and 22 of age. In agreement, Rougière and Carré (2010) demonstrated that the digesta passage rate increased by 25% between d 9 and 29 of age for broiler chickens. Moreover, at low levels of FI, the EEL (measured as g/kg FI), will be proportionally higher than at higher FI levels, which decreases the AMEn of feed ingredients (Lima et al., 1988). Similarly, Murakami et al. (1995) stated that the EEL estimates increased with decreasing FI levels.

The gastrointestinal tract (GIT) matures as birds grow older and the secretion and activities of enzymes increase improving the digestibility of nutrients (Sell et al., 1991; Jin et al., 1998). However, the development of GIT and increases in the digestive and enzymatic activities might not be able to keep up with the marked increase in FI with the advancing age of broiler, reducing energy utilisation compared to the first week. Moreover, the relative contribution of FI to the excreted energy content at low FI is higher than at higher FI (Hätel, 1986). As observed in the current study, the FI to excreta output ratio for SBM and CM

increased quadratically with the advancing age of broilers. The lowest ratio of excreta to FI for both SBM and CM diets was recorded in d 7, suggesting that less nutrients are excreted per unit of the feed consumed during the first week, which might have contributed to the higher AMEn on d 7.

Second, the presence of the residual yolk sac could have contributed to the higher AMEn during week 1. The lipids of the yolk provide the chick embryo with over 90% of its energy requirement for development (Noble and Cocchi, 1990). Yolk lipids are utilised extensively during the last week of incubation, but 25% still remain unutilised at the time of hatch (Noble and Cocchi, 1990). The residual yolk sac plays an important role in the overall nutrition, growth and development during the first few days post-hatch. Chamblee et al. (1992) showed that body weight significantly increased only after the absorption of 20% of the residual yolk sac. Ravindran and Abdollahi (2021) discussed that the presence of the residual yolk sac may be beneficial for the utilization of protein and energy. It has been speculated that the residual yolk sac contributes to the breakdown of lipids through the lipolytic enzymes, providing 90% of the total energy required for the hatching and 30% of the energy required during the first 3 d post-hatch (Speake et al., 1998; Dzoma and Dorrestein, 2001; Sato et al., 2006). This enzymatic influence of the yolk sac towards lipid digestion could have extended for a period after hatch. The absorption through the membrane of the yolk sac been via direct release into the blood circulation, and/or the expulsion through the yolk stalk into the GIT are some possible explanations for the beneficial effects of the yolk sac on lipid utilisation. However, the exact mechanism or contribution of the yolk sac towards nutrient utilisation remains unclear (van der Wagt et al., 2020; Ravindran and Abdollahi, 2021).

The third possible explanation for the reductions in AMEn of SBM and CM with advancing age of broilers could be related to the development of the microbiota. During the first week post-hatch, the sterile intestinal environment and the absence of microbial population

in the neonatal chick may, in part, provide apparent advantages in terms of nutrient utilisation and AME. As the birds grow older, the intestinal microbiome population increases, hence competing for energy and nutrients from diets resulting in a reduction in the AMEn of diets (Thomas et al., 2008).

Substitution is the commonly used method for the measurement of AME of protein sources. The direct method is unsuitable because of issues of palatability and anti-nutritional factors. The regression method could be used but is costly. Published data on the AME of SBM and CM have generally originated with assay diets wherein the ingredient was substituted at 200-300 g/kg and with older broilers (21-35 d of age). In the current study, the AME of SBM during 21-35 d ranged from 10.17- 11.64 MJ/kg, and those of CM ranged from 7.27- 9.22 MJ/kg. These findings compare closely with the AME values of both ingredients reported in the literature. Ravindran et al. (2014) showed that the AME of SBM from different origins ranged from 8.39 - 9.94 MJ/kg. Olukosi (2021) revealed that the AME of SBM was 10.07 MJ/kg at 21 d of age. Ahiwe et al. (2018) discussed that the AME of CM ranged from 8.39-9.15 MJ/kg. Khajali and Slominski (2012) showed that the AME of CM varied among different canola varieties ranging from 7.27 to 9.16 MJ/kg. MJ/kg). The lower AME content of CM, compared to SBM, could be due to differences in the oligosaccharides (2.0 vs. 5.6%, respectively) and fibre content (11.2 vs. 5.4%, respectively).

Current findings showed that age influences the AMEn, partly, through the effect on the digestion and utilisation of energy-yielding nutrients, wherein the retention of DM and N were reduced with advancing age of broilers. Bartov (1995) showed that the retention of DM decreased by 2.4% between d 13 and 22 of age of broilers. It was also shown that the N retention decreased from 60.1% at d 13 to 57.9% at d 22. Yang et al. (2020) reported that DM retention increased with age from 75.3% at d 7 to 78.4% at d 28, then declined to 76.2% at d 35 of age. These researchers also reported that the N retention decreased only between d 28 and d 35 from

82.5% to 81.8%, respectively. Fonolla et al. (1981) demonstrated that the N retention was higher for younger birds (21-26 d) than in older birds (52-57 d) in a maize SBM diet (60.7% vs. 54.0%). Moss et al. (2020) observed a reduction in the retention of N between young (7-9 d) and older broilers (33-34 d) in maize-based (70.7 vs. 61.7%), sorghum-based (70.4 vs. 57.3%) and wheat-based (69.4 vs. 60.2%) diets.

6.6. Conclusions

The present findings demonstrate that the AME and AMEn of SBM and CM were influenced by the age of broilers. The AME was determined to be highest during week 1 compared to subsequent weeks. This finding was contrary to expectations and could be ascribed to low feed intake, longer digesta retention time, yolk sac contribution to dietary energy and changes in microbiota population. These findings confirm that the use of a single AME value of feed ingredients in diet formulations is questionable and age-dependent AMEn values need to be considered in formulations to optimise economic returns.

CHAPTER 7 Measurement of ileal endogenous energy losses and true ileal digestible energy of cereal grains for broiler chickens

7.1. Abstract

Two experiments were conducted to determine the ileal endogenous energy losses (IEEL) in broiler chickens and, apparent metabolisable energy (AME) and true ileal digestible energy (TIDE) of four cereal grains (maize, sorghum, wheat and barley) for three-week-old broilers. In experiment 1, a glucose-based purified diet was used to determine the IEEL for correcting the apparent ileal digestible energy (AIDE) values to TIDE. Titanium dioxide (5.0 g/kg) was added to the diet as an indigestible marker. The diet was randomly allocated to six replicate cages (six birds per cage) of male broilers and fed from 18 to 21 d post-hatch and, jejunal and ileal digesta were collected on d 21. Jejunal and ileal EEL (mean ± SE; n = 6) were determined to be 2.71 ± 0.173 and 1.45 ± 0.173 MJ/kg dry matter (DM) intake, respectively. Experiment 2 was conducted to determine the nitrogen-corrected AME (AMEn), AIDE and TIDE of the four cereal grains. Four experimental diets with similar inclusion (957 g/kg) of grains were developed. Titanium dioxide (5.0 g/kg) was added to all diets as an indigestible marker. Each diet was randomly allotted to six replicate cages (eight birds per cage) and fed for 7 d from 14 to 21 d post-hatch. The AMEn was measured by both total excreta collection and marker methods. The ileal digesta were collected on d 21 for the measurement of AIDE and TIDE. With the marker method, the TIDE was higher ($P < 0.05$) than both AMEn and AIDE for all cereals, with no differences between the AMEn and AIDE. However, with the total excreta collection method, TIDE was higher ($P < 0.05$) than AME and AMEn of maize, lower ($P < 0.05$) than those of barley and showed no difference ($P > 0.05$) with AME and AMEn of sorghum and wheat. The highest and lowest coefficients of apparent ileal digestibility (CAID) for nitrogen and starch were obtained with maize and barley, respectively, with sorghum and wheat being intermediate. The highest ($P < 0.05$) AIDE and TIDE values were observed for

maize, followed by sorghum, wheat and barley. The CAID of DM, nitrogen and starch were positively correlated ($P < 0.001$) with TIDE ($r = 0.990$, 0.703 and 0.705, respectively) than the AMEn measured by marker method ($r = 0.873$, 0.483 and 0.656, respectively) or the AMEn measured by total excreta collection ($r = 0.778$, 0.332 and 0.546, respectively). Strong positive correlations were observed between the TIDE and AMEn measured by both the marker ($r = 0.912$) and the total excreta collection ($r = 0.831$). In conclusion, IEEL can be quantified in the ileal digesta of birds by feeding a glucose-based purified diet. Overall, TIDE values were higher than AMEn, and AIDE, and showed strong correlations with ileal digestibility of nutrients. Further studies are warranted to determine the TIDE of a range of ingredients and to investigate the application of TIDE as a potential available energy system in poultry feed formulation.

7.2. Introduction

Efficient poultry production relies on supplying the birds with an adequate amount of nutrients and energy. Special attention should be given to dietary energy, because of its importance in controlling feed intake, which drives bird growth and diet cost. An accurate evaluation of the available energy content of ingredients is, therefore, critical. Since the 1950's, the apparent metabolisable energy (AME) has been the system of choice for describing available energy for poultry (Hill and Anderson, 1958; Sibbald, 1982). It is not a perfect system, with a number of limitations (Mateos et al., 2019; Wu et al., 2020). In particular, it is an excreta-based measurement containing urine that is voided along with faeces and also includes the energy loss or gain due to the presence of microbial mass from the caecal fermentation. But it is simple, easy to measure, accounts for most of the energy losses after digestion and metabolism, and these features have positioned the AME well ahead of other energy measurements. Currently, the general approach for the measurement of AME is by total excreta collection; however, partial collection of excreta with the use of an inert marker is an alternative for the total excreta collection method (Scott and Boldaji, 1997; Sales and Janssens, 2003).

An alternative energy system that has received some attention is apparent ileal digestible energy (AIDE). The AIDE is measured at the ileal level and reflects digestibility rather than metabolisability as in the case of the AME (Gehring et al., 2012). A switch of available energy measurement to AIDE will overcome the limitations of AME and also align energy availability with the current trend of using digestible content of nutrients in feed formulations (Lemme et al., 2004; Mutucumarana et al., 2015). The ileal approach would also eliminate some inherent errors with the classic AME methodology, including the effect of feed intake, contamination from feathers and scales and potential loss of some excreta during collection (Wu et al., 2020). The lack of relationship between the AME and growth responses sometimes seen in feed enzyme research (Hong et al., 2002; Wu et al., 2004; González-Ortiz et al., 2016) lends further credence to investigate AIDE as an alternative option.

The digesta collected from the terminal ileum contains both dietary undigested nutrients and endogenous materials that are not derived from the feed, e.g., digestive juices, bile, mucin, sloughed intestinal epithelial cells and bacterial mass (Simon et al., 1986; Nyachoti et al., 1997; Ravindran et al., 2004b). Thus, the available energy measured at the terminal ileum is apparent digestible energy, and correction for the non-dietary energy flow, referred to as ileal endogenous energy loss (IEEL), is necessary for the calculation of true ileal digestible energy (TIDE). Currently, there is no methodology available for the measurement of IEEL in poultry. In the case of nutrients, basal ileal endogenous flows have been usually determined following the feeding of respective nutrient-free purified diets; for example, protein-free diets (Muztar and Slinger, 1980; Ravindran et al., 2004b; Adedokun et al., 2007) and calcium and phosphorus-free diets (Mutucumarana and Ravindran, 2016; Anwar et al., 2017). The development of an energy-free diet, however, is not practical and other approaches therefore need to be explored. Feeding of almost 100% digestible protein sources, such as casein or enzymatically-hydrolysed casein (Lemme et al., 2004; Ravindran et al., 2008; Ravindran, 2016),

has been used to measure endogenous protein losses in poultry and a similar approach using a completely digestible simple sugar, such as glucose (Herman, 1974; Riesenfeld et al., 1980), may be employed to quantify the IEEL in poultry.

The aims of the studies reported herein were three-fold: (i) to investigate whether IEEL in broiler chickens can be quantified following feeding a glucose-based purified diet, (ii) if this methodology proves successful, then to estimate the AIDE and TIDE contents of commonly used cereal grains (maize, sorghum, wheat and barley); only limited and scattered published data are available for the AIDE of individual ingredients (Leslie et al., 2007; Gehring et al., 2012; Woyengo and Wilson, 2019) and (iii) to compare the AIDE and TIDE with the nitrogen-corrected AME (AMEn) contents of these grains.

7.3. Materials and methods

The experiment was conducted according to the New Zealand Revised Code of Ethical Conduct for the use of live animals for research, testing and teaching and approved by the Massey University Animal Ethics Committee.

7.3.1. Ingredients

The four cereal grains (maize, sorghum, wheat and barley) were obtained from a local commercial supplier and were ground in a hammer mill to pass through a screen size of 3.0 mm. The maize and barley were sourced from New Zealand, and wheat and sorghum samples were of Australian origin. The proximate and nutrient compositions of the cereal grains are presented in Chapter 3.

7.3.2. Diets, birds and housing

7.3.2.1. Experiment 1- Determination of ileal endogenous energy loss

To determine the IEEL, a glucose-based purified diet, containing 900 g/kg glucose, was developed (Table 7.1). Titanium dioxide (Ti, Merck KGaA, Darmstadt, Germany) was included in the diet as an indigestible marker at an inclusion rate of 5.0 g/kg.

Day-old male broilers (Ross 308) were obtained from a local hatchery and raised on floor pens in an environmentally controlled room. The temperature was maintained at 31 °C on d 1 and gradually reduced to 22 °C by 21 d post-hatch. Birds were fed commercial broiler starter pellets (230 g/kg crude protein and 12.56 MJ/kg AME). On d 14, birds were moved to grower cages for acclimatisation. Between d 14 and 18 of age, pellets were gradually changed to mash as the purified diet was in mash form. On d 18, birds were individually weighed and allocated to six cages (six birds per cage). The glucose-based purified diet was offered *ad libitum* for 3 d from 18 to 21 d post-hatch.

Table 7.1. Composition of the glucose-based purified diet (g/kg, as received basis) used in experiment 1

Ingredient	Inclusion, g/kg
Glucose[1]	900
Solkafloc (Cellulose)[2]	50
Dicalcium phosphate	20
Limestone	13
Titanium dioxide	5.0
Vitamin-trace mineral-premix[3]	5.0
Sodium bicarbonate	3.0
Sodium chloride	3.0
Dipotassium phosphate	1.0

[1]Glucose, Dexmonc, Davis food ingredients, Victoria, Australia.
[2]Solkafloc, Ceolus PH-102, Asahi Kasei Corporation, Tokyo, Japan. Added to maintain uniform passage and consistency of digesta in the digestive tract.
[3]Vitamin and trace mineral premix supplied the following per kilogram of diet: antioxidant, 100 mg; biotin, 0.2 mg; calcium pantothenate, 12.8 mg; vitamin D_3 (cholecalciferol), 2400 IU; cyanocobalamin, 0.017 mg; folic acid, 5.2 mg; menadione, 4 mg; niacin, 35 mg; pyridoxine, 10 mg; vitamin A (trans-retinol), 11100 IU; riboflavin, 12 mg; thiamine, 3.0 mg; vitamin E (dl-α-tocopheryl acetate), 60 IU; choline chloride, 638 mg; Co, 0.3 mg; Cu, 3.0 mg; Fe, 25 mg; I, 1 mg; Mn, 125 mg; Mo, 0.5 mg; Se, 200 µg; Zn, 60 mg.

7.3.2.2. Experiment 2- Determination of the apparent metabolisable energy and ileal digestible energy of cereal grains

The AME was determined using the direct method. In this method, four basal diets were formulated to contain the same inclusion level (957 g/kg) of each grain (Table 7.2). Titanium dioxide was included in all diets as an indigestible marker at an inclusion rate of 5.0 g/kg. Diets were mixed in a single-screw paddle mixer (Bonser Engineering Co. Pty. Ltd., Merrylands, Australia), then pelleted at 60 °C using a steam pellet mill (Model Orbit 15; Richard Sizer., Kingston-upon-Hull, UK) capable of manufacturing 180 kg of feed/h and equipped with a die ring with 3-mm holes and 35 mm thickness.

Table 7.2. Composition (g/kg, as received basis) of the cereal-based test diets used in experiment 2 and the broiler starter diet (experiments 1 and 2)

Ingredient	Maize	Sorghum	Wheat	Barley	Starter diet
Test grain	957	957	957	957	-
Maize	-	-	-	-	574.2
Soybean meal, 460 (g/kg)	-	-	-	-	381.4
Soybean oil	-	-	-	-	8.8
Titanium dioxide	5.0	5.0	5.0	5.0	-
Dicalcium phosphate	19.0	19.0	19.0	19.0	10.7
Limestone	13.0	13.0	13.0	13.0	11.3
L Lysine HCl	-	-	-	-	2.0
DL Methionine	-	-	-	-	3.3
L Threonine	-	-	-	-	1.0
Sodium chloride	2.0	2.0	2.0	2.0	2.5
Sodium bicarbonate	2.0	2.0	2.0	2.0	2.7
Trace mineral premix	1.0	1.0	1.0	1.0	1.0
Vitamin premix[1]	1.0	1.0	1.0	1.0	1.0
Ronozyme HiPhos (Phytase)	-	-	-	-	0.1

[1] Vitamin and trace mineral premix supplied the following per kilogram of diet: antioxidant, 100 mg; biotin, 0.2 mg; calcium pantothenate, 12.8 mg; vitamin D_3 (cholecalciferol), 2400 IU; cyanocobalamin, 0.017 mg; folic acid, 5.2 mg; menadione, 4 mg; niacin, 35 mg; pyridoxine, 10 mg; vitamin A (trans-retinol), 11100 IU; riboflavin, 12 mg; thiamine, 3.0 mg; vitamin E (dl-α-tocopheryl acetate), 60 IU; choline chloride, 638 mg; Co, 0.3 mg; Cu, 3.0 mg; Fe, 25 mg; I, 1 mg; Mn, 125 mg; Mo, 0.5 mg; Se, 200 µg; Zn, 60 mg.

Day-old male broiler chicks (Ross 308) were obtained from a commercial hatchery, raised on a floor pen and fed the same commercial broiler starter diet as experiment 1 until 14 d of age. On d 14, a total number of 192 birds were individually weighed and randomly allocated to 24 cages with six replicates per treatment (eight birds per cage). Birds were fed the experimental diets from d 14 to 21 d of age.

In both experiments, the cages were housed in environmentally controlled rooms with 20 h of fluorescent illumination per d and feed and water were offered *ad libitum*. The temperature was maintained at 31℃ on d 1 and was gradually reduced to 22 ℃ by the end of the third week. Central ceiling extraction fans and wall inlet ducts controlled ventilation.

7.3.2.2.1. Determination of the apparent metabolisable energy

The four experimental diets were fed for 7 d (14 to 21 d), with the first 3 d serving as an adaptation period. Feed intake (FI) and total excreta output were recorded during the last four days of the assay. Daily excreta collections were pooled within a cage, mixed in a blender and sub-sampled. Sub-samples were frozen and then lyophilised (Model 0610, Cuddon Engineering, Blenheim, New Zealand). Dried excreta samples were ground to pass through a 0.5-mm sieve and stored in airtight plastic containers at 4 ºC pending analysis. The diet and excreta samples were analysed for dry matter (DM), gross energy (GE), nitrogen (N) and Ti.

7.3.2.2.2. Jejunal and ileal digesta collection

At the end of each experiment (d 21), all birds were euthanised by intravenous injection (1 ml per 2 kg live weight) of sodium pentobarbitone (Provet NZ Pty Ltd., Auckland, New Zealand). The small intestine was isolated and the digesta from the terminal jejunum and terminal ileum (in experiment 1) and terminal ileum (in experiment 2) were collected. The jejunum was defined as the portion from the distal-most point of insertion of the duodenal

mesentery to that descending down to Meckel's diverticulum. The ileum was defined as the portion of the small intestine extending from Meckel's diverticulum to ~40 mm proximal to the ileo-caecal junction and the ileal digesta were collected from the lower half towards the ileo-caecal junction. The digesta were removed by gentle flushing with distilled water, as described by Ravindran et al. (2005). Digesta were pooled within a cage, lyophilised (Model 0610, Cuddon Engineering, Blenheim, New Zealand), ground to pass through a 0.5-mm sieve and stored at 4ºC until laboratory analysis. The digesta samples were analysed for DM, GE, glucose and Ti in experiment 1, and for DM, N, starch, GE and Ti in experiment 2.

7.3.3. Chemical analysis

Dry matter was determined using standard procedures (Methods 930.15; AOAC, 2016). Nitrogen was determined by combustion (Method 968.06; AOAC, 2016) using a carbon nanosphere-200 carbon, N and sulphur auto analyser (rapid MAX N exceed, Elementar, Donaustraze, Hanau, Germany). Samples were assayed for Ti on a UV spectrophotometer following the method of Short et al. (1996). Glucose was determined using an assay kit (Rx Daytona Plus, Randox Laboratories Ltd, Crumlin, UK) following enzymatic oxidation in the presence of glucose oxidase. Gross energy was determined by an adiabatic bomb calorimeter (Gallenkamp Autobomb, Weiss Gallenkamp Ltd, Loughborough, UK) standardised with benzoic acid.

7.3.4. Calculations

All data were expressed on a DM basis, and the AME content of the test diets was calculated using the following formulas:

- Total excreta collection method:

$$AME_{Diet}\ (MJ/kg) = [(Feed\ intake \times GE_{Diet}) - (Excreta\ output \times GE_{Excreta})] / Feed\ intake$$

- Marker method:

$$\text{GE metabolisability} = [(GE/Ti)_{Diet} - (GE/Ti)_{Excreta}] / (GE/Ti)_{Diet}$$

$$AME_{Diet}\,(MJ/kg) = GE_{Diet} \times \text{GE metabolisability}$$

The AME of the cereal grains, in both methods, was then calculated as follows:

$$AME_{Grain}\,(MJ/kg) = \text{AME of test diet} \times (100/95.7)$$

Nitrogen-corrected AME was determined by correction for zero N retention by assuming 36.54 KJ per g N retained in the body as described by Titus et al. (1959).

Apparent jejunal and ileal absorption of glucose were calculated using the Ti ratios in the diet and digesta as shown below. All concentrations were expressed as g per kg DM.

$$\text{Apparent absorption} = 1 - [(Ti_{Diet}/Ti_{Digesta}) \times (Glucose_{Digesta}/Glucose_{Diet})]$$

The coefficient of apparent ileal digestibility (CAID) of the nutrients was calculated from the dietary ratio of nutrient to Ti relative to the corresponding ratio in the ileal digesta:

$$\text{CAID of nutrient} = [(\text{Nutrient}/Ti)_{Diet} - (\text{Nutrient}/Ti)_{Digesta}] / (\text{Nutrient}/Ti)_{Diet}$$

where, $(\text{Nutrient}/Ti)_{Diet}$ = ratio of nutrient to Ti in diet and $(\text{Nutrient}/Ti)_{Digesta}$ = ratio of nutrient to Ti in ileal digesta.

The CAID of GE and AIDE were calculated using the following formulas:

$$\text{CAID of GE} = [(GE/Ti)_{Diet} - (GE/Ti)_{Digesta}] / (GE/Ti)_{Diet}$$

$$AIDE\,(MJ/kg) = GE_{Diet} \times \text{CAID of GE}$$

The flow of jejunal and ileal endogenous energy, as MJ lost per kilogram of feed DM intake (DMI), was calculated by using the following formula:

$$\text{Endogenous energy losses (MJ/kg DMI)} = GE_{Digesta}\,(MJ/kg) \times [Ti_{Diet}\,(g/kg)/Ti_{Digesta}\,(g/kg)]$$

Apparent ileal digestibility data for GE were then converted to true digestibility values, using IEEL determined from birds fed the glucose-based purified diet.

Coefficient of true ileal energy digestibility =

$$\text{CAID of GE} + [\text{Basal IEEL (MJ/kg DMI)}/GE_{Diet}\,(MJ/kg)]$$

$TIDE_{Diet}$ = Coefficient of true ileal energy digestibility × GE_{Diet}

$TIDE_{Grain} = TIDE_{Diet} \times (100/95.7)$

7.3.5. Statistical analysis

The data were analysed as a one-way ANOVA using the General Linear Model procedure of SAS (version 9.4; SAS Institute Inc., Cary, NC). The data from experiment 2 were subjected to two sets of one-way ANOVA to compare the differences in different energy measurements among grains and within a grain. In both experiments, cage served as the experimental unit. Significant differences between means were separated by the Least Significant Difference test. Significance of effects was declared at $P \leq 0.05$. Linear relationships between measured parameters were evaluated using Pearson's correlation analysis.

7.4. Results

Estimates for the EEL and coefficient of apparent glucose absorption in the jejunum and ileum are shown in Table 7.3. The results showed that jejunal EEL was higher ($P < 0.05$) than those measured at the terminal ileum and coincided with lower ($P < 0.05$) glucose absorption in the jejunum. Glucose completely disappeared by the time digesta reached the terminal ileum.

Table 7.3. Endogenous energy flow (MJ/kg DM intake) and coefficient of apparent glucose absorption in the jejunum and ileum of broilers of 21 d old broilers fed glucose-based purified diet

Segment	Endogenous energy loss	Apparent glucose absorption coefficient[1]
Jejunum	2.71[a]	0.945[b]
Ileum	1.45[b]	1.00[a]
Probabilities, P ≤	0.001	0.001
SEM[2]	0.173	0.010

Means in a column not sharing a common letter (a-b) are significantly different ($P < 0.05$).
[1] Analysed glucose values: diet, 892 g/kg; jejunal digesta, 255 ± 125 g/kg (mean ± SD; 6 replicates); ileal digesta, 5.2 ± 5.2 g/kg (mean ± SD; 6 replicates).
[2] Pooled standard error of mean.

For all cereal grains, the TIDE value was higher ($P < 0.05$) than that of AMEn determined using the marker method, while no differences ($P > 0.05$) were observed between the AMEn and AIDE values (Table 7.4). The highest ($P < 0.05$) AIDE and TIDE values were recorded for maize, followed by sorghum and wheat and, the lowest ($P < 0.05$) values for barley.

Table 7.4. Apparent metabolisable energy (AME; MJ/kg DM)[1], nitrogen-corrected AME (AMEn; MJ/kg DM)[1] determined using the marker method and apparent ileal digestible energy (AIDE; MJ/kg DM)[1] and true ileal digestible energy (TIDE; MJ/kg DM)[1,2] in different cereal grains in broilers at 21 d of age

Method	Maize	Sorghum	Wheat	Barley	Probabilities, P ≤	SEM[2]
AME	14.64^{Ab}	14.00^{Ab}	11.10^{Bb}	10.24^{Cb}	0.001	0.258
AMEn	14.39^{Ab}	13.74^{Ab}	10.78^{Bb}	9.92^{Cb}	0.001	0.252
AIDE	14.83^{Ab}	13.79^{Bb}	11.54^{Cb}	10.54^{Db}	0.001	0.284
TIDE	16.40^{Aa}	15.27^{Ba}	13.13^{Ca}	12.07^{Da}	0.001	0.286
Probabilities, P ≤	0.001	0.001	0.001	0.001		
SEM[2]	0.226	0.218	0.318	0.305		

Means in a column not sharing a common letter (a-b) are significantly different ($P < 0.05$).
Means in a row not sharing a common letter (A-D) are significantly different ($P < 0.05$).
[1] Each value represents the mean of six replicates (eight birds per replicate).
[2] Apparent ileal digestible energy values were corrected to true ileal digestible energy using the ileal endogenous energy flow value of 1.45 MJ/kg DM intake, determined by feeding glucose-based diet.
[3] Pooled standard error of mean.

The values for AME and AMEn determined using the total excreta collection method, AIDE and TIDE in the tested cereal grains are summarised in Table 7.5. In maize grain, the highest and lowest values were observed for TIDE and AIDE, respectively, with AME and AMEn being intermediate. In sorghum and wheat, no differences ($P > 0.05$) were observed between AME, AMEn and TIDE values, and these values were higher ($P < 0.05$) than the AIDE value. However, in barley, AME and AMEn values were the highest ($P < 0.05$) followed by TIDE and AIDE.

The influence of cereal grain type on the ratios between AMEn (measured by marker and total excreta collection methods) and AIDE and TIDE is shown in Table 7.6. With the marker method, no influence ($P > 0.05$) of cereal grain type on the AMEn:AIDE or AMEn:TIDE ratios. However with the total excreta collection method, the cereal grain type affected ($P < 0.05$) the

AMEn:AIDE and the AMEn:TIDE ratios, whereas a tendency (P = 0.059) was observed for cereal effect for the relationship between AMEn and TIDE. The AMEn:TIDE tended to be lower for the viscous grains (wheat and barley) than that for nonviscous grains (maize and sorghum).

Table 7.5. Apparent metabolisable energy (AME; MJ/kg DM)[1], nitrogen-corrected AME (AMEn; MJ/kg DM)[1] determined using the total excreta collection method and apparent ileal digestible energy (AIDE; MJ/kg DM)[1] and true ileal digestible energy (TIDE; MJ/kg DM)[1,2] in different cereal grains in broilers at 21 d of age

Method	Maize	Sorghum	Wheat	Barley	Probabilities, P ≤	SEM[2]
AME	15.38^{Ab}	15.40^{Aa}	13.62^{Ba}	13.37^{Ba}	0.001	0.128
AMEn	15.12^{Abc}	15.15^{Aa}	13.31^{Ba}	13.04^{Ba}	0.001	0.123
AIDE	14.83^{Ac}	13.79^{Bb}	11.54^{Cb}	10.54^{Dc}	0.001	0.284
TIDE	16.40^{Aa}	15.27^{Ba}	13.13^{Ca}	12.07^{Db}	0.001	0.286
Probabilities, P ≤	0.001	0.001	0.001	0.001		
SEM[3]	0.186	0.205	0.269	0.212		

Means in a column not sharing a common letter (a-c) are significantly different (P < 0.05).
Means in a row not sharing a common letter (A-D) are significantly different (P < 0.05).
[1] Each value represents the mean of six replicates (eight birds per replicate).
[2] Apparent ileal digestible energy values were corrected to true ileal digestible energy using the ileal endogenous energy flow value of 1.45 MJ/kg DM intake, determined by feeding purified glucose-based diet.
[3] Pooled standard error of mean.

Table 7.6. The effect of cereal grain type on the relationship between nitrogen-corrected apparent metabolisable energy (AMEn) and apparent ileal digestible energy (AIDE) and true ileal digestible energy (TIDE) in broilers at 21 d of age[1]

Grain type	$AMEn_{Marker}$: AIDE	$AMEn_{Marker}$: TIDE	$AMEn_{Total\ collection}$: AIDE	$AMEn_{Total\ collection}$: TIDE
Maize	0.942	0.878	1.021^{c}	0.923^{c}
Sorghum	0.999	0.902	1.101^{bc}	0.994^{bc}
Wheat	0.939	0.825	1.159^{ab}	1.018^{ab}
Barley	0.942	0.822	1.242^{a}	1.084^{a}
Probabilities, P ≤	0.401	0.059	0.001	0.001
SEM[2]	0.028	0.023	0.033	0.026

Means in a column not sharing a common letter (a-c) are significantly different (P < 0.05).
[1] Each value represents the mean of six replicates (eight birds per replicate).
[2] Pooled standard error of mean.

The influence of cereal grain type on the CAID of DM, N and starch for broiler chickens at 21 d of age is summarised in Table 7.7. Among the cereal grains, maize showed the highest ($P < 0.05$) CAID of DM, followed by sorghum, wheat and barley, with similar CAID between for wheat and barley. Maize and barley had the highest and lowest N and starch digestibility, respectively, with wheat and sorghum being intermediate. The GE digestibility was affected ($P < 0.05$) by the cereal type, with the highest GE digestibility for maize, followed by sorghum, wheat, and the lowest for barley.

Table 7.7. Influence of cereal grain type on the coefficients of apparent ileal digestibility of dry matter, nitrogen, starch and gross energy in broilers at 21 d of age[1]

Grain type	Dry matter	Nitrogen	Starch	Gross energy
Maize	0.787[a]	0.766[a]	0.991[a]	0.814[a]
Sorghum	0.704[b]	0.703[b]	0.967[b]	0.749[b]
Wheat	0.609[c]	0.743[ab]	0.973[b]	0.642[c]
Barley	0.564[c]	0.644[c]	0.943[c]	0.585[d]
Probabilities, P ≤	0.001	0.001	0.001	0.001
SEM2	0.0170	0.0151	0.0051	0.0156

Means in a column not sharing a common letter (a-d) are significantly different ($P < 0.05$).
[1] Each value represents the mean of six replicates (eight birds per replicate).
[2] Pooled standard error of mean.

Linear correlations between CAID of DM, N, starch, and AIDE, TIDE, AMEn measured following the marker and total excreta collection methods are presented in Table 7.8. There were significant positive correlations ($P < 0.05$ to 0.001) among all measured parameters, except between the CAID of N and AMEn determined by the total excreta collection method ($r = 0.332$; $P > 0.05$). The TIDE was highly correlated with the CAID of DM, N and starch ($r = 0.990$, 0703 and 0.705, respectively; $P < 0.001$) than the AIDE, AMEn determined by marker and AMEn determined by the total excreta collection method.

Table 7.8. Pearson correlation coefficients (r-values) between measured biological parameters[1]

	DM	N	Starch	AIDE	TIDE	$AMEn_M$[2]	$AMEn_{TC}$[3]
DM	1.00						
N	0.725	1.00					
	(0.001)						
Starch	0.698	0.669	1.00				
	(0.001)	(0.001)					
AIDE	0.988	0.690	0.695	1.00			
	(0.001)	(0.001)	(0.001)				
TIDE	0.990	0.703	0.705	1.00	1.00		
	(0.001)	(0.001)	(0.001)	(0.001)			
$AMEn_M$[2]	0.873	0.483	0.656	0.915	0.912	1.00	
	(0.001)	(0.017)	(0.001)	(0.001)	(0.001)		
$AMEn_{TC}$[3]	0.778	0.332	0.546	0.838	0.831	0.960	1.00
	(0.001)	(0.112)	(0.006)	(0.001)	(0.001)	(0.001)	

[1] P-values are in parentheses.
[2] M, Marker.
[3] TC, Total collection.

7.5. Discussion

7.5.1. Ileal endogenous energy losses

It is well accepted that true digestibility values provide a better measure of true utilisation potential than apparent values (Lemme et al., 2004). The difference between these two measures is the contribution of non-dietary materials of endogenous origin to the undigested matter in the ileal digesta. One objective of the current work was to develop and test a methodology for the measurement of IEEL, which could be used to correct the AIDE to true values. Currently, no methodology exists for the determination of IEEL. The simplest approach, employed for the measurement of ileal endogenous losses of other major nutrients (protein, fat, calcium and phosphorus), had been to feed respective nutrient-free purified diets or diets based on purified nutrient sources with ~ 100% digestibility. It is evident that the only possible option is to test a diet based on a simple monosaccharide, such as glucose, which is the major end product and

absorbed form of carbohydrate digestion. As the basic absorbable form, glucose requires no enzymatic digestion and, is quickly and completely absorbed with most of the absorption taking place in the duodenum and jejunum (Herman, 1974).

In the current work, glucose absorption was measured at the jejunum and terminal ileum to investigate its absorption dynamics. The data clearly showed that the absorption of glucose continues beyond the jejunum and is completed only in the ileum. At the terminal ileal level, 100% of the glucose provided in the assay diet was absorbed and, therefore, the energy determined could be considered to have come only from endogenous sources. These results are in agreement with those of Riesenfeld et al. (1980) who used a glucose-based diet and reported that glucose absorption in the lower jejunum was 90% and was almost completely absorbed in the lower ileum. The complete disappearance of glucose, the sole energy source in the assay diet, demonstrates the possibility of using a glucose-based purified diet for the measurement of IEEL in broilers. In the present study, the IEEL was estimated to be 1.45 MJ/kg DMI. As this is the first study reporting the IEEL in poultry, no comparable data are available in the literature.

There have been previous studies estimating the endogenous energy losses in poultry, but all were determined following fasting with precision feeding in the excreta of adult roosters for the calculation of true metabolisable energy (Sibbald, 1982). Average excreta endogenous energy output of fasted adult roosters of 2 kg body weight has been reported to range between 0.04 to 0.06 MJ/bird/d (Sibbald, 1982). These estimates, however, are not comparable to the IEEL determined in the current study. First, the correction at the excreta level includes metabolic as well as endogenous energy contained in both the faeces and urine. Second, the unit of measurement is a function of time (MJ/bird/d) rather than intake and, therefore, cannot be used for the calculation of TIDE in any of the currently used methods of digestibility measurements. Future research investment is warranted to validate the methodology developed

in the current work and to further explore the subject of IEEL. It is recognised, as with endogenous amino acid losses (Adeola et al., 2016; Ravindran, 2016), IEEL will be influenced by a number of factors including genotype, age of birds, diet composition, environmental conditions or the ileal digesta collection method.

An unavoidable limitation in the composition of the assay diet used needs to be acknowledged at this point. Cellulose (50 g/kg) was included in the diet to ensure diet structure and uniform passage and consistency of digesta in the digestive tract. Cellulose, being indigestible, would have contributed to the undigested fraction remaining in the terminal ileum, causing some overestimation of IEEL.

7.5.2. Determination of true ileal digestible energy

A number of scattered published data are available on the AIDE, but most are for the AIDE of complete diets (Camden et al., 2001; de Coca-Sinova et al., 2008; Romero et al., 2014; Yang et al., 2020). Limited studies have also reported the AIDE of individual cereal grains, including maize (Gehring et al., 2012), wheat and barley (Scott et al., 1998). In the present study, the AIDE of maize, sorghum, wheat and barley were determined to be 14.83, 13.79, 11.54 and 10.54 MJ/kg, respectively. The differences in AIDE among the four cereals closely paralleled those in respective values for the ileal digestibility of DM, N (except in wheat) and starch. The AIDE value of maize was close to the range of 13.59 to 14.25 MJ/kg reported for 12 samples by Gehring et al. (2012). The current AIDE values for wheat and barley were lower than those reported by Scott et al. (1998). In their study, the AIDE value of different wheat samples ranged from 13.89 to 14.69 MJ/kg and those for hull-less and hulled barley were 11.80 and 12.72 MJ/kg, respectively.

The notable feature of the current work is that the TIDE was determined for four common cereals with the hope of initiating an interest in the development of matrix values for individual

ingredients, which could then be used in feed formulations if the ileal digestible energy system proves to be more predictive of bird performance than the AME. To the author's knowledge, no previous study has determined TIDE for ingredients or diets due to the lack of IEEL quantification. The TIDE values were higher than the corresponding AIDE values by 1.57 MJ/kg (average of all cereal grains). This finding was expected, based on the definition of AIDE and TIDE.

Interestingly, correction for IEEL resulted in TIDE values that were higher than AME and AMEn in maize, similar in sorghum and wheat, and lower in barley. These findings could be, in part, explained by the differences between non-starch polysaccharides (NSP), especially the soluble NSP, contents in these cereal grains (Pettersson and Aman, 1989; Choct and Annison, 1990). It is well documented that the NSP content varies between cereal grains, with an average of 0.1, 0.2, 2.4 and 4.5 g/kg DM for maize, sorghum, wheat and barley, respectively (Choct, 1997). Increased digesta viscosity generated by the high soluble NSP content of viscous cereal grains, such as barley, will obstruct the digestion process and reduce the digestibility of nutrients and consequently digestible energy content (Choct and Annison, 1992; Steenfeldt, 2001).

The AME and AMEn values measured by the marker method for all cereals were similar to their counterpart AIDE values. Taking into consideration that the widely used method for the determination of AMEn is the total excreta collection method, a comparison was also established between AIDE, TIDE and AME and AMEn values measured by the total excreta collection. Like the marker method, the total excreta collection method resulted in similar AME and AMEn values for all cereals but lower AIDE values than AME and AMEn (except in maize). Similar values for viscous cereals were not anticipated as the hindgut fermentation of undigested components would have added more microbial mass and energy, and decreased the

AME estimate (Shires et al., 1980). Masood et al. (2011) reported that the AMEn of sunflower meal determined through the total excreta collection and marker methods were higher than the IDE value for broiler chickens of 34 d of age (10.17 and 10.00 vs. 9.46 MJ/kg). In contrast, Kong and Adeola (2016) showed that the IDE of a maize-soybean basal diet containing 100 and 200 g/kg canola meal, was higher than AME and AMEn by 0.45 and 1.21 MJ/kg, respectively, measured by the regression method.

The fermentation of undigested dietary nutrients and the differences in NSP contents in barley and wheat provide a plausible explanation for the observed trends in AME and AMEn values among the cereal grains. The microbial populations in the caeca of chickens multiply in the presence of NSP and undigested nutrients and ferment these substances with the resultant increase in microbial mass in the excreta (Sugahara et al., 2004).

The AMEn:AIDE was higher than 1.0 for all cereal grains, with the highest and similar ratio for barley and wheat and the lowest for maize. It is difficult to provide reasons for the differences among cereal grains, but may be related to the variation in ingredients composition and digestibility of nutrients, which contributed to higher AMEn than AIDE. Despite that, the AMEn:AIDE ratio was lower than 1.0 when AMEn was measured by the marker method for all cereal grains. A range of AMEn:AIDE ratio (0.980-1.004) was observed for 12 maize samples (Gehring et al., 2012).

Cereal grain type influenced the CAID of DM, N, starch, AIDE and TIDE. In general, viscous cereal grains (wheat and barley) had lower CAID of DM and starch compared to maize, with the lowest CAID of N, starch and AIDE and TIDE for barley compared to other cereal grains. These results are in agreement with those of Romero et al. (2014) who reported that a maize-based diet had higher AIDE compared to a wheat-based diet (13.78 vs. 13.42 MJ/kg) in 21 d old broilers. Abdollahi et al. (2013a) similarly found that a maize-based diet had a higher

CAID of N (0.766 vs. 0.676) and starch (0.984 vs. 0.920) compared to a wheat-based diet. Perera et al. (2019) reported a higher CAID of DM and starch for wheat (0.738 and 0.987) than those of waxy starch hull-less barley (0.624 and 0.870). The differences between CAID of nutrients for different cereal grains could be related to the anti-nutritive characteristics of NSP (Annison and Choct, 1991), which are found in higher concentrations in wheat and barley than in maize and sorghum, resulting in lower nutrient digestibility in birds fed wheat- or barley-based diets.

Strong correlations were found between the CAID of N and TIDE and AIDE (r = 0.703 and 0.690) than AMEn (r = 0.483). Similarly, CAID of starch was highly correlated with TIDE (r = 0.705) and AIDE (r = 0.695) than AMEn measured by the marker and total collection (r = 0.656 and 0.546, respectively. These results are in contrast with those of Gehring et al. (2012) who reported no correlation between CAID of starch and AIDE or AMEn of maize, which could be related to the high starch digestibility and limited availability of undigested starch for caecal fermentation by the microflora in the present work. Positive correlations among AIDE, TIDE and AMEn were evident in the current study. Scott et al. (1998) similarly observed a positive correlation between the AME and AIDE of wheat and barley (r = 0.80 and 0.55, respectively).

Finally, a limitation in employing just one IEEL estimate for the correction of AIDE of all ingredients, regardless of their available energy contents, must be acknowledged. Such an approach may penalise low-energy ingredients, but this is inevitable. A parallel situation exists in the use of one set of ileal endogenous amino acid flow values, measured after the feeding of a protein-free diet, for standardisation of amino acid digestibility values, regardless of varying digestible contents.

7.6. Conclusions

The present data proposes a novel approach to quantify IEEL in broiler chickens using a glucose-based purified diet and provides preliminary data on the TIDE of common cereal grains. To the author's knowledge, this is the first report on the quantification of endogenous energy flow at the ileal level. Furthermore, it was demonstrated that the energy evaluation of feed ingredients is influenced by the assay method. True IDE was highly correlated with the CAID of nutrients and, higher than the AME and AMEn of cereal grains. Future research is warranted to establish the TIDE of a range of ingredients and evaluation of TIDE as a potential available energy system, and its suitability to be applied in poultry diet formulations merits further research investment.

CHAPTER 8 Influence of age and dietary cellulose levels on ileal endogenous energy losses in broiler chickens

8.1. Abstract

Two experiments were conducted to investigate the influence of age and dietary cellulose levels on the ileal endogenous energy losses (IEEL) in broiler chickens. In experiment 1, a glucose-based purified diet was used to determine the IEEL. Titanium dioxide (5.0 g/kg) was added to the diet as an indigestible marker. Six groups of broiler chickens aged 1-7, 8-14, 15-21, 22-28, 29-35 or 36-42 d post-hatch, were utilised. Except during 1-7 d, the birds were fed a starter (d 1-21) and/or a finisher (d 22-35) diet before the experimental diet was introduced. The diet was randomly allocated to six replicate cages, and the number of birds per cage was 12 (d 1-7), 10 (d 8-14) and 8 (d 15-42). The ileal digesta were collected on the last day of each week (d 7, 14, 21, 28, 35 and 42). Bird age had no effect ($P > 0.05$) on the IEEL estimates. The IEEL estimates ranged from 1.10 to1.32 MJ/kg dry matter intake (DMI) during weeks 1 to 6. In Experiment 2, four glucose-based purified diets were developed using 0, 25, 50 and 75 g/kg cellulose. Titanium dioxide (5.0 g/kg) was added to the diets as an indigestible marker. The diets were randomly allocated to six replicate cages (eight birds per cage) and fed from 18 to 21 d post-hatch and, ileal digesta were collected on d 21. The IEEL estimates of broiler chickens at 21 d of age showed a quadratic response ($P < 0.05$) to increasing cellulose contents. The lowest IEEL (0.37 MJ/kg DMI) was recorded for the diet without cellulose and the highest IEEL (1.80 MJ/kg DMI) was observed for the diet with 75 g/kg cellulose. Overall, the present findings confirmed the observation that IEEL in broiler chickens can be quantified using a glucose-based purified diet. Bird age has no influence on IEEL estimates in broiler chickens. The IEEL increased with increasing dietary cellulose contents and the IEEL determined using a purified diet without cellulose represents a better estimate of IEEL.

8.2. Introduction

Dietary energy is the most important aspect to be considered in diet formulations, as it represents the costliest component in poultry feeds and regulates feed intake, highlighting the need for an accurate evaluation of the available energy for poultry. Energy availability from feed ingredients or complete diets can be evaluated by several systems, with the apparent metabolisable energy (AME) being the commonly accepted system by the poultry industry. However, the AME suffers from several limitations (Mateos et al., 2019; Wu et al., 2020) and apparent ileal digestible energy (AIDE) has recently been investigated as a potential alternative (Chapter 7). Moreover, the AIDE system aligns with the current evaluation of ileal digestible content of other nutrients such as amino acids (Lemme et al., 2004), phosphorus (Mutucumarana et al., 2015), and calcium (David et al., 2021) in feed ingredients.

Development of the AIDE system for describing the energy availability of feed ingredients for poultry has been proposed in Chapter 7. In Chapter 7, the AIDE of cereal grains, compared to nitrogen-corrected AME (AMEn), showed strong positive correlations with the digestibility of nutrients including dry matter, nitrogen and starch. The true ileal digestible energy (TIDE), calculated by correcting the AIDE for ileal endogenous energy losses (IEEL), the non-dietary energy flow, showed a stronger correlation with nutrient digestibility than with the AIDE.

A novel approach for the quantification of IEEL in broiler chickens was developed and proposed in the previous study (Chapter 7). Feeding a glucose-based purified diet to broiler chickens was proved to be an acceptable method for the estimation of IEEL, as glucose was shown to be completely absorbed before the lower ileum, indicating that the energy determined from the digesta collected at the lower ileum could have been originated only from non-dietary components.

The IEEL may be affected by factors similar to those affecting endogenous amino acid losses and dietary energy availability for birds (Adedokun et al., 2011; Adeola et al., 2016; Ravindran, 2016). Age of birds has been reported to influence the endogenous energy losses determined in the excreta (Murakami et al., 1995). Silva et al. (2006; 2011) observed that the endogenous and metabolic energy losses increased linearly with the advancing age of broiler chickens. However, all previous studies have determined the endogenous energy losses in the excreta (Dale and Fuller, 1982; Sibbald, 1982; Pirgozliev et al., 2009), and, to the author's knowledge, there is no published report investigating the effect of broiler age on the IEEL.

Moreover, in the previous study (Chapter 7), to ensure the diet texture and uniform digesta passage in the digestive tract of the birds, 50 g/kg cellulose was included in the glucose-based purified diet. Cellulose is not digested by birds and would have contributed to the undigested components in the terminal ileum, resulting in an overestimation of the IEEL. Therefore, the objectives of the current study were two-fold. First to investigate whether the age of broilers influences IEEL estimates and second to examine whether inclusion levels of cellulose influence the IEEL.

8.3. Materials and Methods

The experiments were conducted according to the New Zealand Revised Code of Ethical Conduct for the use of live animals for research, testing and teaching and approved by the Massey University Animal Ethics Committee.

8.3.1. Diets, birds and housing

8.3.1.1. Experiment 1- Determination of ileal endogenous energy loss in broiler chickens from 1-6 weeks of age

A glucose-based purified diet, containing 900 g/kg glucose, was developed (Table 8.1). Titanium dioxide (Ti, Merck KGaA, Darmstadt, Germany) was included in the diet as an indigestible marker at an inclusion rate of 5.0 g/kg.

Table 8.1. Composition of the glucose-based purified diet (g/kg, as fed basis), Experiment 1

Ingredients	Inclusion, g/kg
Glucose[1]	900
Solkafloc (Cellulose)[2]	50
Dicalcium phosphate	20
Limestone	13
Titanium dioxide	5.0
Sodium bicarbonate	3.0
Sodium chloride	3.0
Dipotassium phosphate	1.0
Vitamin -trace mineral-premix[3]	5.0

[1]Glucose, Dexmonc, Davis food ingredients, Victoria, Australia.
[2]Solkafloc, Ceolus PH-102, Asahi Kasei Corporation, Tokyo, Japan. Added to maintain uniform passage and consistency of digesta in the digestive tract.
[3]Vitamin and trace mineral premix supplied the following per kilogram of diet: antioxidant, 100 mg; biotin, 0.2 mg; calcium pantothenate, 12.8 mg; vitamin D_3 (cholecalciferol), 2400 IU; cyanocobalamin, 0.017 mg; folic acid, 5.2 mg; menadione, 4 mg; niacin, 35 mg; pyridoxine, 10 mg; vitamin A (trans-retinol), 11100 IU; riboflavin, 12 mg; thiamine, 3.0 mg; vitamin E (dl-α-tocopheryl acetate), 60 IU; choline chloride, 638 mg; Co, 0.3 mg; Cu, 3.0 mg; Fe, 25 mg; I, 1 mg; Mn, 125 mg; Mo, 0.5 mg; Se, 200 µg; Zn, 60 mg.

A total number of 324, day-old male broilers (Ross 308) were obtained from a local hatchery and raised on floor pens. Except for the 1-7 d age group, birds were fed broiler starter pellets (Table 8.2; 225 g/kg crude protein and 12.14 MJ/kg AME) until d 21 and finisher pellets (Table 8.2; 190 g/kg crude protein and 12.68 MJ/kg AME) from d 22 to 35 before they switched to the assay diet (Table 8.1). At the beginning of each week (d 1, 8, 15, 22, 29 and 36), birds

were selected randomly from floor pens, individually weighed, and allocated to six replicate cages during six periods, namely week 1 (d 1-7), week 2 (d 8-14), week 3 (d 15-21), week 4 (d 22-28), week 5 (d 29-35) or week 6 (d 36-42). For each age period, birds were offered the starter or finisher diet in mash form for the first 4 d as the purified diet was in mash form. The glucose-based purified diet was offered *ad libitum* for the last 3 d of each week. Each replicate cage housed 12 birds during week 1, 10 birds during week 2, and 8 birds during weeks 3 to 6 post-hatch.

8.3.1.2. Experiment 2- Influence of dietary cellulose content on ileal endogenous energy loss estimates in broiler chickens

This experiment was initiated to determine the effect of dietary cellulose inclusion levels in a glucose-based purified diet on the IEEL estimates in broiler chickens. Four assay diets were developed using 0, 25, 50 or 75 g/kg cellulose at the expense of glucose (Table 8.3). Diets were mixed individually in a single-screw paddle mixer (Bonser Engineering Co. Pty. Ltd., Merrylands, Australia). Titanium dioxide (Merck KGaA, Darmstadt, Germany) was included in the diet as an indigestible marker at an inclusion rate of 5.0 g/kg.

A total number of 144, day-old male broilers (Ross 308) were obtained from a local hatchery and raised on floor pens. Birds were fed broiler starter pellets (225 g/kg crude protein and 12.14 MJ/kg AME) until d 21 when they switched to assay diets. On d 14, birds were weighed individually, allocated to cages and offered the starter diet in mash form from d 14 to 17, as the purified diets were in mash form. The assay diets were then offered from d 18 to 21. Each assay diet was fed to six replicate cages (six birds per cage).

In both experiments, the floor pens and cages were housed in environmentally controlled rooms with 20 h of fluorescent illumination per day, and feed and water were offered *ad libitum*.

The temperature was maintained at 31 °C on d 1 and was gradually reduced to 22°C by the end of the third week. Central ceiling extraction fans and wall inlet ducts-controlled ventilation.

Table 8.2. Composition (g/kg, as fed basis) of the broiler starter (d 1 to 21) and finisher (d 22 to 35) diets, Experiment 1

Ingredient	Starter diet	Finisher diet
Maize	574.2	660.0
Soybean meal, 460 g/kg	381.4	295.6
Soybean oil	8.8	13.6
Dicalcium phosphate	10.7	8.2
Limestone	11.3	9.9
L Lysine HCl	2.0	1.9
DL Methionine	3.3	3.0
L Threonine	1.0	0.7
Sodium chloride	2.5	2.5
Sodium bicarbonate	2.7	2.5
Trace mineral premix[1]	1.0	1.0
Vitamin premix[1]	1.0	1.0
Ronozyme HiPhos (Phytase)	0.1	0.1
Calculated analysis		
AME (MJ/kg)	12.14	12.68
CP	225	190
Digestible lysine	11.0	9.2
Digestible methionine	6.2	5.6
Digestible methionine + cysteine	9.2	8.3
Digestible threonine	7.2	6.0
Crude fat	32	39
Crude fibre	29.3	27.5
Calcium	9.8	8.5
Available phosphorus	4.9	4.2
Sodium	2.2	2.1
Chloride	2.3	2.3
Potassium	11.5	9.7

[1]Vitamin and trace mineral premix supplied the following per kilogram of diet: antioxidant, 100 mg; biotin, 0.2 mg; calcium pantothenate, 12.8 mg; vitamin D_3 (cholecalciferol), 2400 IU; cyanocobalamin, 0.017 mg; folic acid, 5.2 mg; menadione, 4 mg; niacin, 35 mg; pyridoxine, 10 mg; vitamin A (trans-retinol), 11100 IU; riboflavin, 12 mg; thiamine, 3.0 mg; vitamin E (dl-α-tocopheryl acetate), 60 IU; choline chloride, 638 mg; Co, 0.3 mg; Cu, 3.0 mg; Fe, 25 mg; I, 1 mg; Mn, 125 mg; Mo, 0.5 mg; Se, 200 µg; Zn, 60 mg.

8.3.2. Ileal digesta collection

On the final day of each week (d 7, 14, 21, 28, 35 and 42) in experiment 1 and d 21 in experiment 2, all birds in a cage were euthanised by intravenous injection (1 mL per 2 kg live weight) of sodium pentobarbitone (Provet NZ Pty Ltd., Auckland, New Zealand).

The small intestine was isolated and the digesta from the terminal ileum were collected. The ileum was defined as the portion of the small intestine extending from Meckel's diverticulum to ~40 mm proximal to ileo-caecal junction and the ileal digesta were collected from the lower half towards the ileo-caecal junction. The digesta were removed by gentle flushing with distilled water, as described by Ravindran et al. (2005). Digesta were pooled within a cage, lyophilised (Model 0610, Cuddon Engineering, Blenheim, New Zealand), ground to pass through a 0.5-mm sieve and stored at 4ºC until laboratory analysis. The feed and digesta samples were analysed for dry matter (DM), gross energy (GE), glucose and Ti.

Table 8.3. Composition of the glucose-based purified diets (g/kg, as fed basis), Experiment 2

Ingredients	Cellulose content (g/kg)			
	No Cellulose	25	50	75
Glucose[1]	950	925	900	875
Solkafloc (Cellulose)[2]	0.0	25	50	75
Dicalcium phosphate	20	20	20	20
Limestone	13	13	13	13
Titanium dioxide	5.0	5.0	5.0	5.0
Sodium bicarbonate	3.0	3.0	3.0	3.0
Sodium chloride	3.0	3.0	3.0	3.0
Dipotassium phosphate	1.0	1.0	1.0	1.0
Vitamin-trace mineral-premix[3]	5.0	5.0	5.0	5.0

[1]Glucose, Dexmonc, Davis food ingredients, Victoria, Australia.
[2]Solkafloc, Ceolus PH-102, Asahi Kasei Corporation, Tokyo, Japan.
[3]Supplied per kg diet: antioxidant, 100 mg; biotin, 0.2 mg; calcium pantothenate, 12.8 mg; cholecalciferol, 60 µg; cyanocobalamin, 0.017 mg; folic acid, 5.2 mg; menadione, 4 mg; niacin, 35 mg; pyridoxine, 10 mg; trans-retinol, 3.33 mg; riboflavin, 12 mg; thiamine, 3.0 mg; dl-α-tocopheryl acetate, 60 mg; choline chloride, 638 mg; Co, 0.3 mg; Cu, 3.0 mg; Fe, 25 mg; I, 1 mg; Mn, 125 mg; Mo, 0.5 mg; Se, 200 µg; Zn, 60 mg.

8.3.3. Chemical analysis

Dry matter was determined using standard procedures (Methods 930.15; AOAC, 2016). Samples were assayed for Ti on a UV spectrophotometer following the method of Short et al. (1996). Glucose was determined using an assay kit (Rx Daytona Plus, Randox Laboratories Ltd, Crumlin, UK) following enzymatic oxidation in the presence of glucose oxidase. Gross energy was determined by an adiabatic bomb calorimeter (Gallenkamp Autobomb, Weiss Gallenkamp Ltd, Loughborough, UK) standardised with benzoic acid.

8.3.4. Calculations

Apparent ileal absorption of glucose was calculated using the Ti ratios in the diet and digesta as shown below. All concentrations were expressed as g per kg DM.

$$\text{Apparent absorption} = 1 - [(Ti_{Diet}/Ti_{Digesta}) \times (Glucose_{Digesta}/Glucose_{Diet})]$$

The flow of ileal endogenous energy, as MJ lost per kilogram of DM intake (DMI), was calculated by using the following formula:

$$\text{Endogenous energy losses (MJ/kg DMI)} = GE_{Digesta}\,(MJ/kg) \times [Ti_{Diet}\,(g/kg)/Ti_{Digesta}\,(g/kg)]$$

8.3.5. Statistical analysis

The data were analysed as a one-way ANOVA using the General Linear Model procedure of SAS (version 9.4; SAS Institute Inc., Cary, NC). Cage served as the experimental unit. Significant differences between means were separated by the Least Significant Difference test. In addition, the data were subjected to orthogonal polynomial contrasts using the General Linear Models procedure of SAS (2015) to study whether responses to increasing bird age (experiment 1) or cellulose level (experiment 2) were of linear or quadratic nature. Significance of effects was declared at $P \leq 0.05$.

8.4. Results

The estimates for the IEEL and coefficient of apparent glucose absorption in the ileum are presented in Table 8.4. The IEEL was unaffected ($P > 0.05$) by bird age. During weeks 2 to 6, the coefficient of apparent glucose absorption was determined to be 1.00, confirming complete glucose absorption by the time digesta reaches the terminal ileum. There were insufficient digesta samples at week 1 for glucose analysis.

Table 8.4. Influence of age on the ileal endogenous energy loss (MJ/kg dry matter intake) and coefficient of apparent glucose absorption in broilers fed a glucose-based purified diet, Experiment 1[1]

Age (week)	Endogenous energy loss	Apparent glucose absorption coefficient[2]
1	1.32	-
2	1.17	1.00
3	1.16	1.00
4	1.22	1.00
5	1.10	1.00
6	1.19	1.00
SEM[3]	0.057	0.001
P-value	0.193	0.426
Orthogonal polynomial contrast, P ≤		
Linear	0.104	-
Quadratic	0.162	-

[1] Each value represents the mean of six replicates. The number of birds per replicate cage was 12 (week 1), 10 (week 2) and 8 (week 3-6).
[2] Analysed glucose values: diet, 892 g/kg; ileal digesta, week 1, not enough sample; week 2, 2.01 ± 1.69; week 3, 0.43 ± 0.11; week 4, 0.56 ± 0.26; week 5, 0.28 ± 0.08; week 6, 2.35 ± 0.94 g/kg (mean ± SD; 6 replicates).
[3] Pooled standard error of mean.

The estimates for the IEEL and coefficient of apparent glucose absorption in the ileum of birds fed glucose-based purified diets with different inclusion levels of cellulose are presented in Table 8.5. The IEEL increased ($P < 0.01$) with increasing dietary cellulose inclusions and the magnitude of responses differed between inclusion levels, resulting in a quadratic effect ($P < 0.05$). The lowest IEEL (0.37 MJ/kg DMI) was recorded for the diet without cellulose and the highest (1.80 MJ/kg DMI) for the diet with 75 g/kg cellulose. Complete glucose absorption was determined at the ileal level, regardless of dietary cellulose content.

Table 8.5. Influence of dietary cellulose content on the ileal endogenous energy loss (MJ/kg dry matter intake) and coefficient of apparent glucose absorption in broilers fed glucose-based purified diets, Experiment 2[1]

Cellulose content (g/kg)	Endogenous energy loss	Apparent glucose absorption coefficient[2]
No cellulose	0.37d	1.00
25	0.76c	1.00
50	1.21b	1.00
75	1.80a	1.00
SEM[4]	0.039	0.001
P-value	0.001	0.254
Orthogonal polynomial contrast, P ≤		
Linear	0.001	-
Quadratic	0.015	-

Means in a column not sharing a common letter (a-d) are significantly different (P < 0.05).
[1] Each value represents the mean of six replicates (eight birds per replicate).
[2] Analysed glucose values: No cellulose diet, 942 g/kg; 25 g/kg cellulose diet, 917 g/kg; 50 g/kg cellulose diet, 892 g/kg; 75 g/kg cellulose diet, 867 g/kg; ileal digesta for No cellulose diet, 3.04 ± 3.28; 25 g/kg cellulose diet, 4.58 ± 5.52; 50 g/kg cellulose diet, 0.85 ± 0.17; 75 g/kg cellulose diet, 0.79 ± 0.22 g/kg (mean ± SD; 6 replicates).
[3] Pooled standard error of mean.

8.5. Discussion

The methodology for the determination of IEEL by feeding broiler chickens with a glucose-based purified diet (Chapter 7) was validated in the current study. The previous estimate of IEEL (1.45 MJ/kg DMI) in Chapter 7 was determined using 21 d old broiler chickens and used to correct the AIDE values to TIDE in birds of the same age. However, the application of a single IEEL estimate as a correction factor for different broiler ages may be challenged. A recent study in our laboratory (Barua et al., 2021) revealed that the basal ileal endogenous amino acid flow was influenced by broiler age and that the flows decreased quadratically with age; the values being higher on d 7, decreasing on d 14, plateauing until d 35 and decreasing further on d 42. It was plausible to assume that the IEEL in broilers may also vary with age and, therefore, an objective of the present work was to investigate the effect of bird age on IEEL estimates. The current findings, however, showed that bird age had no effect on IEEL estimates. Similar to the findings in Chapter 7 and regardless of bird age, glucose

completely disappeared at the terminal ileal level, suggesting that the dietary glucose was completely absorbed by the time the digesta reached the terminal ileum. Riesenfeld et al. (1980), in a study with a glucose-based diet, reported complete absorption of glucose in the lower ileum. These findings could be explained by the fact that glucose, a simple monosaccharide, is the end-product of carbohydrate digestion and is completely absorbed from the intestine without any enzymatic digestion (Herman, 1974). Therefore, glucose absorption is not influenced by age-related developments and maturation of the intestinal tract, and it is well absorbed from the early days post-hatch (Moran et al., 2010; Ravindran and Abdollahi, 2021). In agreement, Bogner and Haines (1964) stated that the maximum absorption of glucose occurred during the first week post-hatch, with minor changes in the following weeks. Obst and Diamond (1992) reported that the absorption of glucose in the intestine was constant from 1 to 84 d age in broiler chickens.

In Experiment 1, the IEEL estimates in broilers of 1-6 weeks age ranged from 1.10 to 1.32 MJ/kg DMI. The IEEL value of 1.16 MJ/kg DMI at 3 weeks of age was substantially lower than the IEEL of 1.45 MJ/kg DMI determined in broilers of similar age in Chapter 7. No comparable data on IEEL in poultry are available in the literature. All previous studies estimating the endogenous energy losses in poultry were determined in the excreta of broilers or adult roosters (Sibbald, 1982; Murakami et al., 1995; Silva et al., 2006, 2011). Sibbald (1981) estimated the endogenous energy losses in fasted adult birds and found that the metabolic plus endogenous energy losses increased by 14.3% between weeks 19 and 22 of age. Similarly, Murakami et al. (1995) showed that the endogenous energy losses increased with the advancing age of broiler chickens that was attributed to increasing dietary energy consumption. Silva et al. (2006) found that the endogenous and metabolic energy losses in broilers increased from 60.79 KJ/bird at d 7 to 150.46 KJ/bird at d 37. In a subsequent study, Silva et al. (2011) reported that the endogenous and metabolic energy losses increased linearly with the advancing age of

broilers from 87.32 KJ/bird at d 5 to 320.24 KJ/bird at d 35 post-hatch. These endogenous energy losses estimated at the excreta level include both metabolic and endogenous losses in the urine and faeces, and therefore cannot be compared with the IEEL estimates from the current study. Moreover, the metabolic and endogenous losses were expressed as a function of time (KJ/bird/d) rather than as per DMI.

In studies with purified diets, a source of fibre, usually cellulose, is included as a structural component and to texturise the feed. Therefore, cellulose was included at 50 g/kg in the glucose-based purified diet in the previous study (Chapter 7) and Experiment 1 in this chapter. Cellulose is an insoluble fibre material composed of a linear chain of β-1,4-linked D-glucopyranosyl residues (O'Sullivan, 1997; Gilbert, 2010). Cellulose has no nutritional value for poultry, but is typically included in purified diets for its physiological role as a bulking agent to enable a steady and uniform digesta passage rate (Siri et al., 1992). Cellulose being indigestible in poultry may have contributed to the gross energy generated from undigested materials in the ileum, resulting in overestimation of IEEL values reported in Chapter 7 and Experiment 1. Therefore, to fine-tune the methodology for the quantification of IEEL, Experiment 2 was designed to investigate whether the IEEL estimates are impacted by different inclusion levels of cellulose in the glucose-based purified diet. The current data clearly demonstrated the impact of cellulose inclusion level on IEEL estimates, with increasing the inclusion of cellulose from 0 to 25, 50 and 75 g/kg increased the IEEL estimates by 0.39, 0.84 and 1.43 MJ/kg DMI, respectively. The GE content of cellulose used in the current study was 16.10 MJ/kg, which was similar to the GE value of 17.2 MJ/kg for cellulose powder reported by Kienzle et al. (2001). Tasaki and Kibe (1959) found that cellulose was excreted almost completely undigested when birds were fed a basal diet supplemented with 200 g/kg cellulose. Siri et al. (1992) reported that increasing the dietary cellulose content from 50 to 100 g/kg increased the excreta energy output

by 50%. As cellulose is not digested by the bird, it can contribute to the undigested matter in the ileal digesta, resulting in an overestimation of the IEEL.

The endogenous materials are derived mainly from proteins, including desquamated epithelial cells, gastrointestinal secretions (bile, gastric, pancreatic, and intestinal secretions), and mucoproteins. The increase in the IEEL associated with increasing the cellulose inclusions could be related to two possible reasons; first, the increase in the mechanical damage of the absorptive surface of the intestinal epithelial cell wall caused by greater inclusions of cellulose (Hegde et al., 1982). Okumura et al. (1982) found that the excretion of nitrogen increased with increasing dietary cellulose levels from 0 to 50 g/kg. A similar influence of cellulose on ileal endogenous amino acid losses was reported in a study by Kluth and Rodehutscord (2009). These researchers reported that the ileal endogenous losses of amino acids increased by 29% when the dietary cellulose level increased from 30 to 80 g/kg. Second, cellulose is reported to increase gastrointestinal secretions, mainly mucin. Mucin is the main glycoprotein of the mucus layer secreted by the goblet cells and, plays a major role in protecting the gut from physical, chemical and enzymatic damages, along with the removal of pathogenic bacteria (Sharma and Schumacher, 1995; Satchithanandam et al., 1996; Montagne et al., 2003). The secretion of mucin is altered by several factors, including dietary fibre and physical properties. Several studies have demonstrated that dietary fibre increased mucin secretion (Fuller and Cadenhead, 1991; Mariscal-Landin et al., 1995; Lien et al., 2001). Montagne et al. (2003) stated that insoluble dietary fibre is more aggressive in scraping the mucin from the gut wall as it passes through the digestive tract. In addition, Jha and Mishra (2021) speculated that dietary fibre may increase the stimulation and secretion of endogenous digestive enzymes in broilers.

Aderibigbe et al. (2021) calculated the IEEL originating from endogenous amino acids in broilers following the feeding of a nitrogen-free diet to be around 0.24-0.25 MJ/kg DMI, which

was lower than the IEEL estimate (0.37 MJ/kg DMI) in the current study. The higher IEEL estimate in the current study could be related to the contribution of ileal non-protein components to the IEEL.

The TIDE was proposed (Chapter 7) as a potential energy system in poultry feed formulation as it not only overcomes the limitations of the AME but also aligns energy availability with the current trend of using digestible nutrient contents in feed formulations (Lemme et al., 2004; Mutucumarana et al., 2015; David et al., 2021). Moreover, the findings from Chapter 7 showed that, compared to the AME and AMEn of cereal grains, TIDE was highly correlated with the coefficient of apparent ileal digestibility of DM, starch and N.

The prime application of the quantified IEEL is for the correction of AIDE to TIDE, and therefore, an accurate estimation of the IEEL is needed for the calculation of the TIDE value of feed ingredients. Two specific concerns regarding IEEL were raised from the previous study (Chapter 7) namely, the use of a single IEEL value for broilers of different ages and the overestimation of IEEL due to the inclusion of cellulose in the purified test diet. The current data showed that age had no influence on IEEL estimates. The lack of age effect on IEEL estimates suggests that a single basal IEEL value could be used for the correction of TIDE across broiler ages. However, dietary cellulose inclusion had a marked impact on IEEL estimates. In Chapter 7, the TIDE values of cereal grains were calculated using the IEEL value of 1.45 MJ/kg DMI, determined following the feeding of a glucose-based purified diet with 50 g/kg cellulose. The resultant TIDE values were higher than their counterpart AIDE, AME and AMEn values for all cereal grains. Using the AIDE values of the cereal grains from Chapter 7, the TIDE of cereal grains was re-calculated using the IEEL value of 0.37 MJ/kg DMI, determined by feeding the glucose-based purified diet without cellulose (Table 8.6). Although no significant differences were observed between AME, AMEn, AIDE and TIDE for all cereal

grains, the TIDE values tended to be greater in wheat (P = 0.053), barley (P = 0.111) and maize (P = 0.077).

Table 8.6. Apparent metabolisable energy (AME; MJ/kg DM)[1], nitrogen-corrected AME (AMEn; MJ/kg DM)[1] determined using the marker method and apparent ileal digestible energy (AIDE; MJ/kg DM)[1] and true ileal digestible energy (TIDE; MJ/kg DM)[2] in different cereal grains in broilers at 21 d of age

Method	Wheat	Sorghum	Barley	Maize
AME	11.10	14.00	10.24	14.64
AMEn	10.78	13.74	9.92	14.39
AIDE	11.54	13.79	10.54	14.83
TIDE	12.06	14.20	11.00	15.26
Probabilities, P ≤	0.053	0.442	0.111	0.077
SEM[3]	0.318	0.218	0.305	0.227

[1] Each value represents the mean of six replicates (eight birds per replicate). Values were obtained from the previous study in Chapter 7.
[2] Apparent ileal digestible energy values were corrected to true ileal digestible energy using the ileal endogenous energy flow of 0.37 MJ/kg DM intake, determined by feeding a glucose-based diet without cellulose.
[3] Pooled standard error of mean.

8.6. Conclusions

The present study provides, for the first time, data on the IEEL in broiler chickens from 1 to 6 weeks of age. The findings confirm that the IEEL in broiler chickens can be quantified using a glucose-based purified diet and that the age of birds has no impact on the IEEL. The dietary cellulose content had a substantial impact on IEEL estimates and it is suggested that the IEEL determined using a purified diet with no added cellulose represents a better estimate. Some aspects relevant to the determination of TIDE were explored in the studies reported in the current work and Chapter 7, but further research is warranted before TIDE could be adopted as a better energy system in poultry feed formulations. In particular, comprehensive feeding trials comparing formulations based on metabolisable energy (AME and AMEn) versus ileal digestible energy (AIDE and TIDE) and, their impact on broiler growth performance and the production economics will be instructive.

CHAPTER 9 GENERAL DISCUSSION

9.1. Introduction

In commercial poultry production, feed represents about 70% of the total production cost, with energy contributing to two-thirds of the feed cost. Dietary energy is the first item to consider while formulating poultry feeds, as it is required for maintenance, physiological functions, metabolism and growth. Several systems have been developed for the estimation of the available energy content of feed ingredients for birds, with the apparent metabolisable energy (AME) being the system commonly used in poultry nutrition to describe the energy requirements and dietary energy content for poultry (NRC, 1994). However, the AME system has several shortcomings, with several aspects of practical relevance remain unexplored. This project aimed to address some of the current research gaps and possible solutions.

The AME of feed ingredients can be corrected for zero nitrogen retention to estimate the nitrogen-corrected AME (AMEn). This correction has been introduced to eliminate the variations in nitrogen retention, within the same AME assay, due to the experimental factors, such as ingredient types, dietary treatments, and age of birds. Whilst the need for such correction has been debated for over 60 years, no definitive conclusion has been reached. Because the majority of poultry nutritionists formulate diets based on AMEn of feed ingredients rather than AME, and the trends for the influence of factors investigated in this thesis on AME and AMEn were similar, AMEn has been the main focus of discussion in all studies in this thesis.

First, to the author's knowledge, all previous experiments on AMEn in broilers have been conducted using mash diets because of the simplicity and the fact that most research stations do not have pelleting facilities. However, in commercial practice, pelleted diets are the most prevalent feed form (FF) used for broiler feeding worldwide. Therefore, the applicability of the AMEn values of feed ingredients generated with mash diets to pelleted diets could be

challenged. The first study (Chapter 3) aimed to determine the effect of FF (mash vs. pellet) on the AME and AMEn of four common cereal grains (wheat, sorghum, barley and maize) and three main protein sources (PS; soybean meal, SBM; canola meal, CM; and meat and bone meal, MBM) for broilers. The notable findings of this experiment were that pelleting has a considerable effect on the AMEn of feed ingredients, highlighting the need for the use of pelleted diets in energy evaluation assays.

Second, several previous studies indicated that broiler age has a major impact on the AME content of complete broiler diets (Zelenka, 1968; Batal and Parsons, 2002; Bolarinwa et al., 2012). To the best of the author's knowledge, only a few age-related studies exist on the AME or AMEn of individual feed ingredients (Lopez and Leeson, 2008; Lee and Kong, 2019; Woyengo and Wilson, 2019; Veluri and Olukosi, 2020), but these were limited to only two or three ages and none have examined AME of feed ingredients from hatching to the end of broiler growth cycle.

Currently, AMEn estimates are generated from older birds (mostly 22 to 35 d) and used in feed formulations regardless of broiler age. Using a single AMEn estimate of feed ingredients for all ages will lead to over- or under-estimation of the available energy content of ingredients and energy requirement of broilers, and ultimately influence the precision of feed formulations. Experiments reported in Chapters 4, 5 and 6 determined the AMEn of feed ingredients at different ages of broilers (d 7, 14, 21, 28, 35 and 42) in four cereal grains (wheat, sorghum, barley and maize; Chapters 4 and 5) and two PS (SBM and CM; Chapter 6). These studies demonstrated that age has a significant influence on the AMEn of feed ingredients and that the age effect is variable depending on the ingredient type.

Third, it has been reported previously that the methodology has a significant impact on the estimated AMEn content of diets or feed ingredients (Lockhart et al., 1967; Veluri and Olukosi, 2020; Olukosi, 2021). Three main methodologies, namely direct, substitution and

regression methods (Wu et al., 2020) can be employed for the determination of AMEn. Limited research has been conducted on the individual feed ingredients comparing the direct and substitution methods. In this project, both the direct (Chapter 4) and substitution (Chapter 5) methods were employed for the determination of AMEn of cereal grains. Although the data generated in Chapters 4 and 5 could not be statistically compared, differences were observed in the estimated AMEn values of each individual cereal grain between the two methodologies.

Finally, in recent years, novel approaches have gained attention to refine energy evaluation methods for poultry. The apparent ileal digestible energy (AIDE) is one such system. It is measured at the ileal level and reflects digestibility rather than metabolisability. A switch of metabolisable energy measurement to digestible energy system will eliminate some of the errors associated with the determination of AME/AMEn and aligns the energy evaluation system with the current trend of using ileal digestible content of nutrients in feed formulation. However, most of the research for the AIDE were conducted on complete diets (Romero et al., 2014; Yang et al., 2020) and individual cereal grains (Scott et al., 1998; Gehring et al., 2012). Moreover, a correction of the AIDE values for the non-dietary energy contribution, referred to as ileal endogenous energy loss (IEEL), is crucial for the calculation of the true ileal digestible energy (TIDE) content of feed ingredients. However, IEEL estimates have never been previously determined for broiler chickens. In this project, a novel methodology was developed for the first time for the quantification of the IEEL estimates in broiler chickens (Chapter 7) and was further refined with the estimation of the IEEL at different broiler ages and various cellulose inclusions (Chapter 8). Following the estimation of IEEL value, the AIDE of wheat, sorghum, barley and maize were corrected to the TIDE values in Chapter 7. This study confirmed that the TIDE is correlated more with the ileal digestibility of nutrients than AIDE, AME and AMEn. Findings from Chapter 8 demonstrated that the age of birds has no impact on

the IEEL estimates and that the IEEL determined using a purified diet with no added cellulose represents a better estimate.

9.2. Influence of feed form on the metabolisable energy of feed ingredients

The main findings of the first experiment reported in Chapter 3 revealed that, regardless of the cereal grain type, FF influenced the AMEn of cereal grains, wherein pelleting increased the AMEn of the grains by 1.6 % compared with mash form. The results of the second experiment in Chapter 3 revealed that FF exhibited a significant impact on the AMEn of individual PS and the response varied depending on the PS. Compared to mash form, pelleting reduced the AMEn of meat and bone meal by 0.56 MJ/kg, had no effect on that of soybean meal, but increased the AMEn of canola meal by 0.57 MJ/kg.

It is evident from these two experiments that the influence of FF on the AMEn of feed ingredients varies depending on the ingredient type. Jiménez-Moreno et al. (2009) demonstrated that the influence of pelleting on AMEn of ingredients could be related to the impact of pelleting process in breaking down the structure of cell walls, thus releasing the nutrients, especially lipids, entrapped in oil bodies. Adewole et al. (2017) stated that the influence of pelleting on AMEn depends on the source of ingredient, chemical composition, particularly the fat and neutral detergent fibre contents.

9.3. Do we need to pellet the assay diets in energy evaluation assays?

It is known that the FF has a significant impact on the AME of complete broiler diets (Abdollahi et al., 2011, 2014). However, no studies to date have investigated the effect of FF on the AMEn content of single feed ingredients. From the first study (Chapter 3), it is evident that FF has a substantial impact on the AMEn of feed ingredients for broilers. The effect was pronounced for all four cereal grains (wheat, sorghum, barley and maize); however, the effects were observed only for two PS (MBM and CM). These findings indicate that the current trend

of using the AMEn data derived from mash assay diets may lead to over- or under-estimation of the AMEn, affecting precise feed formulations. It is, therefore, recommended that AMEn assay diets should be pelleted to resemble the prevalent FF in the broiler industry. Based on these findings, assay diets in subsequent age-related studies (Chapters 4, 5, 6 and 7) were offered in pellet form.

9.4. Influence of broiler age on the AMEn of cereal grains and protein sources

The AMEn of wheat, sorghum, barley and maize at six different broiler ages (d 7, 14, 21, 28, 35 and 42) are reported in Chapters 4 and 5. In Chapter 4, the AMEn of wheat, sorghum, barley and maize were measured following the direct method. The data showed that the age of broilers significantly impacted the AMEn of all four cereal grains. The AMEn of all cereal grains declined (quadratically or linearly) with advancing age. For wheat, the AMEn was the highest at d 7 then dropped at d 14 and plateaued up to d 42 of age. A linear decline in the AMEn of sorghum was observed, in which the AMEn decreased between d 7 and 14, plateaued thereafter until d 35, and then declined again at d 42. For barley, the AMEn decreased from d 7 to d 14, increased at d 21, followed by a plateau to d 42. A different trend was observed for maize, where the highest AMEn was observed at d 7, then dropped to the lowest value at d 14, followed by an increase up to d 35 and declining at d 42.

A different trend was observed in Chapter 5, where the AMEn of cereal grains, the same batches as those tested in Chapter 4, were determined using the substitution method. The data showed that the influence of age on AMEn was grain-dependent. The AMEn of wheat increased between d 7 and d 14 and then dropped afterwards. For sorghum, AMEn increased from d 7 to d 14, and plateaued thereafter up to d 42. In the case of barley and maize, age did not exhibit any effect on the AMEn.

In Chapter 6, the AMEn of two protein sources, namely SBM and CM, were estimated at six broiler ages (d 7, 14, 21, 28, 35 and 42) following the substitution method. The results

revealed that d 7 represented the highest AMEn for both SBM and CM, followed by a drop at d 14, and increased up to d 35, and evened out between d 35 and 42.

The current findings of AMEn estimates with advancing age from hatching to broiler market age generated in this thesis are novel and the only available data to date. The reasons for the variations of AMEn response to the age of broilers in different feed ingredients are difficult to justify, but could be related to factors including the methodology of estimating the AMEn (direct vs. substitution; Lockhart et al., 1967), diet composition (Wu et al., 2020), feed intake (Scott, 2005) and digesta passage rate (Noy and Sklan, 1995). The data from this project has verified, to a large extent, that the estimates of AME or AMEn rely on the methodology. Wu et al. (2020) attributed the AME variations from different studies to the methodology used, inclusion level of the test ingredient and, the formulation of basal and test diets.

9.5. How age-dependent AMEn estimates determined using pelleted diets can benefit the broiler industry?

This thesis research explored an important area in energy utilisation investigating the age-dependency of AMEn estimates of six common feed ingredients (four cereal grains and two protein sources) used in broiler feeds. The results of this work are both of scientific and practical interest.

It is well accepted that the digestibility and utilisation of nutrients change with age. This critical information, however, has not been capitalised in feed formulations due mainly to the fact that only limited and scattered published data are available. Being the first study reporting the AMEn estimates of single feed ingredients over the whole broiler growth cycle, this is a timely addition to the currently existed AMEn estimates database. It creates an opportunity to apply the AMEn values for specific ages of broilers that will increase the precision of feed formulations.

As discussed previously, several factors associated with pelleting significantly influenced the AMEn of feed ingredients. The positive effects of pelleting are more illustrated in the AMEn of cereal grains than ingredients with higher protein contents. In commercial practice, pellets are the commonly used FF in broiler production. Thus, the application of existing AME or AMEn data derived from mash diets will result in over- or under-estimation of the available energy content of feed ingredients. Considering the FF (mash vs. pellet) effect on the AMEn of individual feed ingredients (Chapters 3), the diets were pelleted in all assays reported in this thesis. Due to the resemblance of the physical form of diets used in industry practice, the AMEn data generated in this thesis are clearly more applicable in practical feed formulations for broilers.

The age-specific AMEn data generated in this thesis provide an opportunity to modify existing AMEn databases for different feed ingredients. This will eliminate the risk of under- or over- estimation of the AMEn in different growth stages and will improve the precision of feed formulations and the sustainability of broiler production.

Commercially, AME or AMEn values incorporated in diet formulations, are obtained mostly from birds at 21 d of age. One possible application of the current findings would be to incorporate the weekly values obtained from the current thesis into a coefficient of the AMEn values determined at 21 d of age. Table 9.1. summarises the coefficients that could be used to calculate the weekly AMEn values for the six feed ingredients from the AMEn values estimated at 21 d of age (coefficient of 1.0). Table 9.1 clearly suggests that protein sources are influenced by the age of birds more than cereal grains. For example, following the direct method, only at d 7 the AMEn content of wheat varies compared to other age stages and the AMEn of wheat at d 7 could be calculated based on the current reported AMEn value at d 21 multiplied by 1.09. However, for high protein ingredients, the influence of age is more pronounced, which may

require to consider three or four AMEn values to more precisely formulate the diets from hatch to market age.

Table 9.1. The nitrogen-corrected apparent metabolisable energy (AMEn) of tested feed ingredients as coefficients of AMEn determined at d 21 of age

Methodology	Ingredient	Age (days)					
		7	14	21	28	35	42
Direct method (Chapter 4)	Wheat	1.09	1.01	1.00	1.00	1.02	1.01
	Sorghum	1.04	1.00	1.00	1.00	0.98	0.98
	Barley	1.05	0.96	1.00	0.99	0.97	0.99
	Maize	1.02	0.98	1.00	1.01	1.02	1.00
Substitution method (Chapter 5)	Wheat	0.98	1.14	1.00	1.04	1.06	1.04
	Sorghum	0.92	1.00	1.00	1.04	1.00	1.02
	Barley	0.98	1.03	1.00	1.01	1.01	1.02
	Maize	0.99	1.02	1.00	1.04	1.04	1.04
Substitution method (Chapter 6)	Soybean meal	1.20	1.10	1.00	1.04	1.09	1.03
	Canola meal	1.41	1.24	1.00	1.13	1.21	1.16

From Table 9.1, it is evident that applying a single AMEn value in feed formulation is not a good practice. On the other hand, the development of six separate broiler diets is neither practical nor economical, and the application of weekly data over the 6-week growth period in feed formulations will be a challenge for poultry nutritionists.

Table 9.2 proposes age-dependent AMEn values for different phases for each feed ingredient, based on the variations between the coefficients (Table 9.1) of the AMEn for each individual ingredient. It is clear from Table 9.2 that the AMEn value of cereal grains varied between different methodologies. Moreover, Table 9.2 illustrates that diets formulated for the first week post-hatch should incorporate a different AMEn value for each ingredient than later ages. For ingredients with high protein contents, more than 3 dietary phases with different AMEn values may be needed for a more precise formulation. It is proposed that the age of broiler should be a variable within the ingredient matrix. Table 9.2. presents an example of the

AMEn values, obtained from the current project, that might be incorporated in formulating the commercial broiler diets. Modern feed formulation softwares could easily include and implement the age-dependent values of AMEn for different ingredients. Formulation to age-dependent AMEn values of feed ingredients could potentially reduce the feed cost, however, the benefits of this approach must be tested and confirmed in well-planned feeding trials considering broiler performance, market weight, and economic return.

Table 9.2. Proposed age-dependent nitrogen-corrected apparent metabolisable energy values (MJ/kg) of feed ingredients for broiler feed formulations

Methodology	Ingredient	1-7 d	8-14 d	15-21 d	22-42 d
Direct method (Chapter 4)	Wheat	14.48	13.47	13.31	13.44
	Sorghum	15.74	15.12	15.15	15.00
	Barley	13.75	12.50	13.04	12.84
	Maize	15.48	14.82	15.12	15.30
Substitution method (Chapter 5)	Wheat	12.53	14.55	12.75	13.33
	Sorghum	12.84	13.95	13.90	14.15
	Barley	11.26	11.78	11.46	11.61
	Maize	14.12	14.62	14.28	14.86
Substitution method (Chapter 6)	Soybean meal	11.38	10.40	9.46	9.98
	Canola meal	9.10	7.99	6.44	7.51

9.6. True ileal digestible energy system for poultry: An alternative to metabolisable energy system?

Since the 1950s, AME has been the common system for evaluating the available energy of ingredients and diets as it is simple, straightforward and considers most of the energy losses after digestion and metabolism. As previously discussed, there are many factors that affect the determination of AME of feed ingredients or diets such as feed form, age of bird, and methodology. Moreover, there are several inherent errors associated with the estimation of AME such as the effect of feed intake, contamination from feathers and scales and potential loss of some excreta during collection (Wu et al., 2020). The AME system is an excreta-based

measurement containing urine voided along with faeces and includes the energy loss or gain due to the presence of microbial mass from caecal fermentation.

Because of the above-mentioned reasons, an innovative approach was proposed in this project suggesting the true ileal digestible energy system (TIDE) as a potential alternative for the AME that eliminates major errors in the AME methodology (Chapters 7 and 8). A number of previous studies have determined the AIDE of broiler diets, but not the TIDE due to the lack of established methodology to measure ileal endogenous energy losses (IEEL). Estimation of IEEL, which represents energy from endogenous materials that are not derived from the feed, e.g., digestive enzymes, bile, mucin, sloughed intestinal epithelial cells and bacterial mass, is required for the calculation of TIDE. In Chapter 7, an attempt was made to estimate the IEEL in broilers following the feeding of a glucose-based purified diet. The data showed that the glucose was completely absorbed by the time the digesta reaches the terminal ileum and the energy measured from digesta could be used as an estimate of IEEL. The IEEL was estimated to be 1.45 MJ/kg DMI (Chapter 7), and used for correcting the AIDE of cereal grains to TIDE. It was found that the TIDE of individual cereal grains is significantly higher than the AME and AMEn. Moreover, strong positive correlations were observed between the TIDE and coefficient of apparent ileal digestibility of nutrients than those with the AIDE, AME or AMEn. These findings on the IEEL and TIDE of feed ingredients are novel and represent the first published data to date.

In Chapter 8, the influence of age of broilers and dietary cellulose contents on the IEEL estimates was examined. The age of birds had no influence on the IEEL estimates. However, cellulose inclusion levels influenced the IEEL, wherein increasing dietary cellulose inclusions increased the IEEL estimates. It is suggested that the IEEL estimate with no cellulose inclusion represents a better estimate than those of purified diets supplemented with cellulose.

9.7. Recommendations for future energy (AMEn or TIDE) assays

This project was initiated to estimate the AMEn of commonly used individual feed ingredients in broiler diets. Moreover, this project investigated some of the factors that influence the AMEn of individual feed ingredients. The current project confirmed that the methodology has a marked impact on the AMEn estimation of feed ingredients, and further studies are needed to compare the growth performance and economical returns of feeding broiler chickens with diets formulated based on the AMEn values of feed ingredients generated from different methodologies.

In the direct method, viscous grains such as wheat and barley that contain high levels of non-starch polysaccharides (NSPs) were used as the sole cereal and at a high inclusion rate (962 g/kg) in the direct method (Chapter 4). The NSPs have major implications on the digestion of nutrients and intestinal health. No exogenous NSP-degrading enzymes were used in the assay diets in this project, as the objective was to investigate the true age effects on the AMEn of feed ingredients. Future studies are warranted using exogenous NSP-degrading enzymes to investigate the extent of AMEn responses at different ages and to develop age-dependent enzyme matrix values.

In this project, the IEEL estimates for broiler chickens were determined. Further research on the factors influencing the IEEL in broilers and layers is required. Another issue of interest is the TIDE system, which has been proposed in this project still needs further validation. In future TIDE assays, the evaluation must be extended to estimate the TIDE of other feed ingredients, especially the common protein sources. Factors that can influence the TIDE, such as age of birds, gender, and enzyme supplementation are aspects worth exploring.

9.8. Summary and main conclusions

In the main, the work reported in this thesis research is new and, for the first time, provides comprehensive information on two important factors contributing to variation in AMEn of

single feed ingredients: feed form and age of broilers. Feed form substantially impacted the AMEn of feed ingredients. The influence of pelleting on the AMEn was more pronounced in cereal grains. The AMEn estimates from hatch to market age in 6 most commonly used feed ingredients in broiler feeds were reported. A key finding was that the age effect on the AMEn was variable depending on the ingredient type and methodology. The direct method generated AMEn values lower than those of the substitution method. It is proposed that the age of broilers should be accommodated as a variable within the ingredient matrix in feed formulation packages. The application of age-appropriate AMEn data might enable the poultry industry to improve the precision of feed formulations, broiler performance, profitability and sustainability of poultry production. It was also shown that the IEEL can be quantified in broilers by feeding a glucose-based purified diet and that age has no effect on the IEEL estimates. The IEEL estimate from the purified diet containing no cellulose provides the best estimate for IEEL for broiler chickens. Evaluation of TIDE, proposed as a potential available energy system in this project, and its suitability for application in broiler feed formulations merits further research investment.

REFERENCES

Abdollahi, M. R., Ravindran, V., & Svihus, B. (2013a). Influence of grain type and feed form on performance, apparent metabolisable energy and ileal digestibility of nitrogen, starch, fat, calcium and phosphorus in broiler starters. *Animal Feed Science and Technology*, *186*(3-4), 193-203.

Abdollahi, M. R., Ravindran, V., & Svihus, B. (2013c). Pelleting of broiler diets: An overview with emphasis on pellet quality and nutritional value. *Animal Feed Science and Technology*, *179*(1-4), 1-23.

Abdollahi, M. R., Ravindran, V., & Svihus, B. (2014). Influence of feed form on growth performance, ileal nutrient digestibility, and energy utilisation in broiler starters fed a sorghum-based diet. *Livestock Science*, *165*, 80-86.

Abdollahi, M. R., Ravindran, V., Wester, T. J., Ravindran, G., & Thomas, D. V. (2011). Influence of feed form and conditioning temperature on performance, apparent metabolisable energy and ileal digestibility of starch and nitrogen in broiler starters fed wheat-based diet. *Animal Feed Science and Technology*, *168*(1-2), 88-99.

Abdollahi, M. R., Ravindran, V., Wester, T. J., Ravindran, G., & Thomas, D. V. (2013b). Influence of pellet diameter and length on the quality of pellets and performance, nutrient utilisation and digestive tract development of broilers fed on wheat-based diets. *British Poultry Science, 54*(3), 337-345.

Abdollahi, M. R., Wiltafsky-Martin, M., & Ravindran, V. (2021). Application of apparent metabolizable energy versus nitrogen-corrected apparent metabolizable energy in poultry feed formulations: a continuing conundrum. *Animals*, *11*(8), 2174.

Abdollahi, M. R., Zaefarian, F., & Ravindran, V. (2018a). Feed intake response of broilers: Impact of feed processing. *Animal Feed Science and Technology*, *237*, 154-165.

Abdollahi, M. R., Zaefarian, F., Ravindran, V., & Selle, P. H. (2018b). The interactive influence of dietary nutrient density and feed form on the performance of broiler chickens. *Animal Feed Science and Technology*, *239*, 33-43.

Adedokun, S. A., Adeola, O., Parsons, C. M., Lilburn, M. S., & Applegate, T. J. (2011). Factors affecting endogenous amino acid flow in chickens and the need for consistency in methodology. *Poultry Science*, *90*(8), 1737-1748.

Adedokun, S. A., Parsons, C. M., Lilburn, M. S., Adeola, O., & Applegate, T. J. (2007). Standardized ileal amino acid digestibility of meat and bone meal from different sources in broiler chicks and turkey poults with a nitrogen-free or casein diet. *Poultry Science*, *86*(12), 2598-2607.

Adeola, O., & Ileleji, K. E. (2009). Comparison of two diet types in the determination of metabolizable energy content of corn distillers dried grains with solubles for broiler chickens by the regression method. *Poultry Science*, *88*(3), 579-585.

Adeola, O., Anwar, M. N., Abdollahi, M. R., & Ravindran, V. (2018). Age-related energy values of meat and bone meal for broiler chickens. *Poultry Science*, *97*(7), 2516-2524.

Adeola, O., Xue, P. C., Cowieson, A. J., & Ajuwon, K. M. (2016). Basal endogenous losses of amino acids in protein nutrition research for swine and poultry. *Animal Feed Science and Technology*, *221*, 274-283.

Aderibigbe, A. S., Cowieson, A. J., Ajuwon, K. M., & Adeola, O. (2021). Contribution of purified soybean trypsin inhibitor and exogenous protease to endogenous amino acid losses and mineral digestibility. *Poultry Science*, *100*(12), 101486.

Aderibigbe, A., Cowieson, A. J., Sorbara, J. O., & Adeola, O. (2020). Growth phase and dietary α-amylase supplementation effects on nutrient digestibility and feedback enzyme secretion in broiler chickens. *Poultry Science*, *99*(12), 6867-6876.

Adewole, D. I., Rogiewicz, A., Dyck, B., & Slominski, B. A. (2017). Effects of canola meal source on the standardized ileal digestible amino acids and apparent metabolizable energy contents for broiler chickens. *Poultry Science*, *96*(12), 4298-4306.

Ahiwe, E. U., Omede, A. A., Abdallh, M. B., & Iji, P. A. (2018). Managing dietary energy intake by broiler chickens to reduce production costs and improve product quality. *Animal Husbandry and Nutrition*, *115*, 145.

Akiba, Y. & Murakami, H. (1995). Partitioning of energy and protein during early growth of broiler chicks and contribution of vitteline residue. In *Proceedings of 10th Symposium on Poultry Nutrition* (pp. 44-52).

Amerah, A. M. (2015). Interactions between wheat characteristics and feed enzyme supplementation in broiler diets. *Animal Feed Science and Technology*, *199*, 1-9.

Amerah, A. M., Ravindran, V., Lentle, R. G., & Thomas, D. G. (2007). Influence of feed particle size and feed form on the performance, energy utilization, digestive tract development, and digesta parameters of broiler starters. *Poultry Science*, *86*(12), 2615-2623.

Annison, G. (1991). Relationship between the levels of soluble nonstarch polysaccharides and the apparent metabolizable energy of wheats assayed in broiler chickens. *Journal of Agricultural and Food Chemistry*, *39*(7), 1252-1256.

Annison, G. (1993). The role of wheat non-starch polysaccharides in broiler nutrition. *Australian Journal of Agricultural Research*, *44*(3), 405-422.

Annison, G., & Choct, M. (1991). Anti-nutritive activities of cereal non-starch polysaccharides in broiler diets and strategies minimizing their effects. *World's Poultry Science Journal*, *47*(3), 232-242.

Anwar, M. N., Ravindran, V., Morel, P. C. H., Ravindran, G., & Cowieson, A. J. (2016). Measurement of true ileal calcium digestibility in meat and bone meal for broiler chickens using the direct method. *Poultry Science*, *95*(1), 70-76.

Anwar, M. N., Ravindran, V., Morel, P. C. H., Ravindran, G., & Cowieson, A. J. (2017). Effect of calcium source and particle size on the true ileal digestibility and total tract retention of calcium in broiler chickens. *Animal Feed Science and Technology*, *224*, 39-45.

AOAC. (2005). Official Methods of Analysis 18th Edition.

AOAC. (2016). Official Methods of analysis 20th ed.

Aviagen. (2009). Ross Nutrient Supplement. Aviagen, Scotland, UK.

Azhar, M. R., Rose, S. P., Mackenzie, A. M., Mansbridge, S. C., Bedford, M. R., Lovegrove, A., & Pirgozliev, V. R. (2019). Wheat sample affects growth performance and the apparent metabolisable energy value for broiler chickens. *British Poultry Science*, *60*(4), 457-466.

Ball, M. E. E., Owens, B., & McCracken, K. J. (2013). Chemical and physical predictors of the nutritive value of wheat in broiler diets. *Asian-Australasian Journal of Animal Sciences*, *26*(1), 97.

Barekatain, M. R., Wu, S. B., Toghyani, M., & Swick, R. A. (2015). Effects of grinding and pelleting condition on efficiency of full-fat canola seed for replacing supplemental oil in broiler chicken diets. *Animal Feed Science and Technology*, *207*, 140-149.

Bartov, I. (1988). Effect of age of broiler chicks and method of determination on the metabolizable energy of corn. In *Proceedings of the 18 th World Poultry Congress* (pp. 787-789).

Bartov, I. (1995). Differential effect of age on metabolisable energy content of high protein-low energy and low protein-high energy diets in young broiler chicks1. *British Poultry Science*, *36*(4), 631-643.

Barua, M., Abdollahi, M. R., Zaefarian, F., Wester, T. J., Girish, C. K., Chrystal, P. V., & Ravindran, V. (2021). Basal ileal endogenous amino acid flow in broiler chickens as influenced by age. *Poultry Science*, *100*(8), 101269.

Barzegar, S., Wu, S. B., Choct, M., & Swick, R. A. (2020). Factors affecting energy metabolism and evaluating net energy of poultry feed. *Poultry Science*, *99*(1), 487-498.

Barzegar, S., Wu, S. B., Noblet, J., & Swick, R. A. (2019). Metabolizable energy of corn, soybean meal and wheat for laying hens. *Poultry Science*, *98*(11), 5876-5882.

Batal, A. B., & Parsons, C. M. (2002). Effects of age on nutrient digestibility in chicks fed different diets. *Poultry Science*, *81*(3), 400-407.

Batal, A. B., & Parsons, C. M. (2003). Utilization of different soy products as affected by age in chicks. *Poultry Science*, *82*(3), 454-462.

Batal, A. B., & Parsons, C. M. (2004). Utilization of various carbohydrate sources as affected by age in the chick. *Poultry Science*, *83*(7), 1140-1147.

Bedford, M. R. (1995). Mechanism of action and potential environmental benefits from the use of feed enzymes. *Animal Feed Science and Technology*, *53*(2), 145-155.

Begin, J. J. (1967). The relation of breed and sex of chickens to the utilization of energy. *Poultry Science*, *46*(2), 379-383.

Behnke, K. C. (1998). Why pellet. In *Proceedings of Kansas State University/American Feed Industry Association Pellet Conference*.

Behnke, K. C., & Beyer, R. S. (2002). Effect of feed processing on broiler performance. In *VIII. International Seminar on Poultry Production and Pathology, Santiago, Chile*.

Bennett, C. D., Classen, H. L., & Riddell, C. (1995). Live performance and health of broiler chickens fed diets diluted with whole or crumbled wheat. *Canadian Journal of Animal Science*, *75*(4), 611-614.

Bennett, C. D., Classen, H. L., & Riddell, C. (2002). Feeding broiler chickens wheat and barley diets containing whole, ground and pelleted grain. *Poultry Science*, *81*(7), 995-1003.

Black, J. L., Hughes, R. J., Nielsen, S. G., Tredrea, A. M., MacAlpine, R., & Van Barneveld, R. J. (2005). The energy value of cereal grains, particularly wheat and sorghum, for poultry. *In Proceedings of the Australian Poultry Science Sympsoium*, 17, 21-29.

Bogner, P. H., & Haines, I. A. (1964). Functional development of active sugar transport in the chick intestine. *American Journal of Physiology-Legacy Content*, *207*(1), 37-41.

Bolarinwa, O. A., Olukosi, O. A., & Adeola, O. (2012). Metabolizable energy value of porcine meat and bone meal for broiler chickens. *Canadian Journal of Animal Science*, *92*(1), 73-78.

Bourdillon, A., Carré, B., Conan, L., Francesch, M., Fuentes, M., Huyghebaert, G., ... & Wiseman, J. (1990). European reference method of in vivo determination of metabolisable energy in poultry: reproducibility, effect of age, comparison with predicted values. *British Poultry Science*, *31*(3), 567-576.

Brickett, K. E., Dahiya, J. P., Classen, H. L., & Gomis, S. (2007). Influence of dietary nutrient density, feed form, and lighting on growth and meat yield of broiler chickens. *Poultry Science, 86*(10), 2172-2181.

Briggs, J. L., Maier, D. E., Watkins, B. A., & Behnke, K. C. (1999). Effect of ingredients and processing parameters on pellet quality. *Poultry Science, 78*(10), 1464-1471.

Brumano, G., Gomes, P. C., Albino, L. F. T., Rostagno, H. S., Generoso, R. A. R., & Schmidt, M. (2006). Chemical composition and metabolizable energy values of protein feedstuffs to broilers at different ages. *Revista Brasileira de Zootecnia, 35*, 2297-2302.

Camden, B. J., Morel, P. C. H., Thomas, D. V., Ravindran, V., & Bedford, M. R. (2001). Effectiveness of exogenous microbial phytase in improving the bioavailabilities of phosphorus and other nutrients in maize-soya-bean meal diets for broilers. *Animal Science*, *73*(2), 289-297.

Carré, B., Gomez, J., & Chagneau, A. M. (1995). Contribution of oligosaccharide and polysaccharide digestion, and excreta losses of lactic acid and short chain fatty acids, to dietary metabolisable energy values in broiler chickens and adult cockerels. *British Poultry Science*, *36*(4), 611-629.

Carré, B., Lessire, M., & Juin, H. (2014). Prediction of the net energy value of broiler diets. *Animal, 8*(9), 1395-1401.

Cerrate, S., Wang, Z., Coto, C., Yan, F., & Waldroup, P. W. (2009). Effect of pellet diameter in broiler starter diets on subsequent performance. *Journal of Applied Poultry Research, 18*(3), 590-597.

Chamblee, T. N., Brake, J. D., Schultz, C. D., & Thaxton, J. P. (1992). Yolk sac absorption and initiation of growth in broilers. *Poultry Science, 71*(11), 1811-1816.

Cheng, T. K., & Coon, C. N. (1990). Research note: calcium digestibility studies utilizing acid-insoluble ash measurements. *Poultry Science*, *69*(12), 2228-2230.

Choct, M. (1997). Feed non-starch polysaccharides: chemical structures and nutritional significance. *Feed Milling International*, *191*(1), 13-26.

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).

Choct, M. (2015). Feed non-starch polysaccharides for monogastric animals: classification and function. *Animal Production Science*, *55*(12), 1360-1366.

Choct, M., & Annison, G. (1990). Anti-nutritive activity of wheat pentosans in broiler diets. *British Poultry Science*, *31*(4), 811-821.

Choct, M., & Annison, G. (1992). Anti-nutritive effect of wheat pentosans in broiler chickens: Roles of viscosity and gut microflora. *British Poultry Science*, *33*(4), 821-834.

Choct, M., & Hughes, R. J. (1997). The nutritive value of new season grains for poultry. *Recent Advances in Animal Nutrition in Australia*, *11*, 146-150.

Choct, M., Hughes, R. J., & Annison, G. (1999). Apparent metabolisable energy and chemical composition of Australian wheat in relation to environmental factors. *Australian Journal of Agricultural Research*, *50*(4), 447-452.

Choct, M., Hughes, R. J., Perez-Maldonado, R., & Van Barneveld, R. J. (2001). The metabolisable energy value of sorghum and barley for broilers and layers. *In Proceedings of the Australian Poultry Science Sympsoium*, 10, 39-42.

Choct, M., Hughes, R. J., Wang, J., Bedford, M. R., Morgan, A. J., & Annison, G. (1996). Increased small intestinal fermentation is partly responsible for the anti-nutritive activity of non-starch polysaccharides in chickens. *British Poultry Science*, *37*(3), 609-621.

Corzo, A., Kidd, M. T., Burnham, D. J., Miller, E. R., Branton, S. L., & Gonzalez-Esquerra, R. (2005). Dietary amino acid density effects on growth and carcass of broilers differing in strain cross and sex. *Journal of Applied Poultry Research*, *14*(1), 1-9.

Cowieson, A. J. (2005). Factors that affect the nutritional value of maize for broilers. *Animal Feed Science and Technology*, *119*(3-4), 293-305.

CVB (Centraal Veevoeder Bureau)., 2016. Veevoedertabel (Livestock Feed Table). Wageningen, the Netherlands.

Dale, N. M, & Fuller, H. L. (1984). Correlation of protein content of feedstuffs with the magnitude of nitrogen correction in true metabolizable energy determinations. *Poultry Science*, *63*(5), 1008-1012.

Dale, N. M., & Fuller, H. L. (1982). Endogenous energy losses of fed versus fasted roosters. *Poultry Science*, *61*(5), 898-901.

David, L. S., Abdollahi, M. R., Bedford, M. R., & Ravindran, V. (2021). True ileal calcium digestibility in soybean meal and canola meal, and true ileal phosphorous digestibility in maize-soybean meal and maize-canola meal diets, without and with microbial phytase, for broiler growers and finishers. *British Poultry Science*, *62*(2), 293-303.

Davidson, J., Banfield, C. G., Duguid, J. G., & Leitch, E. G. (1978). The measurement of metabolisable energy in samples of oats, barley and wheat using chicks. *Journal of the Science of Food and Agriculture*, *29*(4), 339-344.

Davidson, J., Hepburn, W. R., Mathieson, J., & Pullar, J. D. (1968). Comparisons of heat loss from young cockerels by direct measurement and by indirect assessment involving body analysis. *British Poultry Science*, *9*(1), 93-109.

de Coca-Sinova, A., Valencia, D. G., Jiménez-Moreno, E., Lázaro, R., & Mateos, G. G. (2008). Apparent ileal digestibility of energy, nitrogen, and amino acids of soybean meals of different origin in broilers. *Poultry Science*, *87*(12), 2613-2623.

De Groote, G. (1974). A comparison of a new net energy system with the metabolisable energy system in broiler diet formulation, performance and profitability. *British Poultry Science*, *15*(1), 75-95.

De Groote, G., & Ketels, E. (1988). The metabolisable energy content of different meat-and meat and bone meals as influenced by dietary inclusion level and age of the chick. In *Proc. XVIII World's Poultry Congress, Nagoya, Japan* (pp. 781-783).

De Lange, C. F. M., & Birkett, H. (2005). Characterization of useful energy content in swine and poultry feed ingredients. *Canadian Journal of Animal Science*, *85*(3), 269-280.

Dozier III, W. A., Behnke, K. C., Gehring, C. K., & Branton, S. L. (2010). Effects of feed form on growth performance and processing yields of broiler chickens during a 42-day production period. *Journal of Applied Poultry Research*, *19*(3), 219-226.

Dozier III, W. A., Gehring, C. K., Corzo, A., & Olanrewaju, H. A. (2011). Apparent metabolizable energy needs of male and female broilers from 36 to 47 days of age. *Poultry Science*, *90*(4), 804-814.

Dozier III, W. A., Purswell, J. L., Kidd, M. T., Corzo, A. L. E. X., & Branton, S. L. (2007). Apparent metabolizable energy needs of broilers from two to four kilograms as influenced by ambient temperature. *Journal of Applied Poultry Research*, *16*(2), 206-218.

Du Preez, J. J., Hayes, J. P., & Duckitt, J. S. (1981). Endogenous energy, a cause of biased true metabolisable energy values. *South African Journal of Animal Science*, *11*(3), 269-272.

Duke, G. E. (1986). Alimentary canal: secretion and digestion, special digestive functions, and absorption. In *Avian Physiology* (pp. 289-302). Springer, New York, NY.

Dzoma, B. M., & Dorrestein, G. M. (2001). Yolk sac retention in the ostrich (Struthio camelus): histopathologic, anatomic, and physiologic considerations. *Journal of Avian Medicine and Surgery*, *15*(2), 81-89.

Emmans, G. C. (1994). Effective energy: a concept of energy utilization applied across species. *British Journal of Nutrition*, *71*(6), 801-821.

Engberg, R. M., Hedemann, M. S., & Jensen, B. B. (2002). The influence of grinding and pelleting of feed on the microbial composition and activity in the digestive tract of broiler chickens. *British Poultry Science*, *43*(4), 569-579.

Evonik Industries. (2016). AMINODat 5.0 platinum version.

Fan, M. Z., & Sauer, W. C. (1995). Determination of apparent ileal amino acid digestibility in barley and canola meal for pigs with the direct, difference, and regression methods. *Journal of Animal Science*, *73*(8), 2364-2374.

Farrell, D. J. (1978). Rapid determination of metabolisable energy of foods using cockerels. *British Poultry Science*, *19*(3), 303-308.

Farrell, D. J. (1981). An assessment of quick bioassays for determining the true metabolizable energy and apparent metabolizable energy of poultry feedstuffs. *World's Poultry Science Journal*, *37*(2), 72-83.

Farrell, D. J. (1999). In vivo and in vitro techniques for the assessment of the energy content of feed grains for poultry: a review. *Australian Journal of Agricultural Research*, *50*(5), 881-888.

Farrell, D. J., Smulders, A., Mannion, P. F., Smith, M., & Priest, J. (1997). The effective energy of six poultry diets measured in young and adult birds. In *Proceedings Symposium of Energy Metabolism* (Vol. 14, pp. 371-374).

Farrell, D. J., Takhar, B. S., Barr, A. R., & Pell, A. S. (1991b). Naked oats: their potential as a complete feed for poultry. In Recent Advances in Animal Nutrition in Australia.. (Ed. D. J. Farrell.) pp. 312.25. (University of New England: Armidale, NSW.)

Farrell, D. J., Thomson, E., Choice, A., Ashes, J. R., Peck, N. J., & Hogan, J. P. (1983). Effects of milling and pelleting of maize, barley and wheat on their metabolizable energy value for cockerels and chicks. *Animal Feed Science and Technology*, *9*(2), 99-105.

Farrell, D. J., Thomson, E., Du Preez, J. J., & Hayes, J. P. (1991a). The estimation of endogenous excreta and the measurement of metabolisable energy in poultry feedstuffs using four feeding systems, four assay methods and four diets. *British Poultry Science*, *32*(3), 483-499.

Favero, A., Maiorka, A., Silva, A. V. F. D., Valle, F. L. D. P., Santos, S. A. D., & Muramatsu, K. (2012). Influence of feed form and corn particle size on nutrient digestibility and energy utilization by young turkeys. *Revista Brasileira de Zootecnia*, *41*, 86-90.

FEDNA (Fundación Española para el Desarrollo de la Nutrición Animal). (2017). Revisión de las Normas FEDNA de Composición y Valor Nutritivo de Alimentos para la Fabricación de Piensos Compuestos.

Feedipedia. (2017). Animal feed resources information system.

Fonolla, J., Prieto, C., & Sanz, R. (1981). Influence of age on the nutrient utilization of diets for broilers. *Animal Feed Science and Technology*, *6*(4), 405-411.

Fraps, G. S. (1946). Composition and productive energy of poultry feeds and rations. *Texas FARMER Collection*.

Fraps, G. S., & Carlyle, E. C. (1939). The utilization of the energy of feed by growing chickens. Texas Agricultural Experiment Station Bulletin No 571.

Fraps, G. S., & Carlyle, E. C. (1941). Productive energy of corn meal, alfalfa leaf meal, dried buttermilk, and casein as measured by production of fat and flesh in growing chickens. Texas Agricultural Experiment Station Bulletin No *600*.

Fraps, G. S., & Carlyle, E. C. (1942). Productive Energy of Some Feeds and Foods as Measured by Gains of Energy by Growing Chickens. Texas Agricultural Experiment Station Bulletin No 625.

Fraps, G. S., Carlyle, E. C., & Fudge, J. F. (1940). Metabolizable energy of some chicken feeds. Texas Agricultural Experiment Station Bulletin No 589.

Frikha, M., Safaa, H. M., Serrano, M. P., Arbe, X., & Mateos, G. G. (2009). Influence of the main cereal and feed form of the diet on performance and digestive tract traits of brown-egg laying pullets. *Poultry Science*, *88*(5), 994-1002.

Frikha, M., Valencia, D. G., de Coca-Sinova, A., Lázaro, R., & Mateos, G. G. (2013). Ileal digestibility of amino acids of unheated and autoclaved pea protein concentrate in broilers. *Poultry Science*, *92*(7), 1848-1857.

Fujita, H. (1973). Quantitative studies on the variations in feeding activity of chickens II. Effect of the physical form of the feed on the feeding activity of laying hens. *Japanese Poultry Science*, *10*(2), 47-55.

Fuller, M. F., & Cadenhead, A. (1991). Effect of the amount and composition of the diet on galactosamine flow from the small intestine. In *Proceedings of the Vth International Symposium on Digestive Physiology in Pigs* (pp. 330-333). EAAP Publication no. 54 Pudoc, Wageningen.

Gabriel, I., Lessire, M., Mallet, S., & Guillot, J. F. (2006). Microflora of the digestive tract: critical factors and consequences for poultry. *World's Poultry Science Journal*, *62*(3), 499-511.

García-Rebollar, P., Cámara, L., Lázaro, R. P., Dapoza, C., Pérez-Maldonado, R., & Mateos, G. G. (2016). Influence of the origin of the beans on the chemical composition and nutritive value of commercial soybean meals. *Animal Feed Science and Technology*, *221*, 245-261.

García, M., Lázaro, R., Latorre, M. A., Gracia, M. I., & Mateos, G. G. (2008). Influence of enzyme supplementation and heat processing of barley on digestive traits and productive performance of broilers. *Poultry Science*, *87*(5), 940-948.

Gehring, C. K., Bedford, M. R., Cowieson, A. J., & Dozier Iii, W. A. (2012). Effects of corn source on the relationship between in vitro assays and ileal nutrient digestibility. *Poultry Science*, *91*(8), 1908-1914.

Gilbert, H. J. (2010). The biochemistry and structural biology of plant cell wall deconstruction. *Plant Physiology*, *153*(2), 444-455.

González-Alvarado, J. M., Jiménez-Moreno, E., Lázaro, R., & Mateos, G. G. (2007). Effect of type of cereal, heat processing of the cereal, and inclusion of fiber in the diet on productive performance and digestive traits of broilers. *Poultry Science*, *86*(8), 1705-1715.

González-Ortiz, G., Olukosi, O., & Bedford, M. R. (2016). Evaluation of the effect of different wheats and xylanase supplementation on performance, nutrient and energy utilisation in broiler chicks. *Animal Nutrition*, *2*(3), 173-179.

Gracia, M. I., Latorre, M. A., Garcia, M., Lazaro, R., & Mateos, G. G. (2003). Heat processing of barley and enzyme supplementation of diets for broilers. *Poultry Science*, *82*(8), 1281-1291.

Gutierrez-Alamo, A., De Ayala, P. P., Verstegen, M. W. A., Den Hartog, L. A., & Villamide, M. J. (2008). Variability in wheat: factors affecting its nutritional value. *World's Poultry Science Journal*, *64*(1), 20-39.

Hamilton, R. M. G., & Kennie, J. (1997). The effects of lighting program, ingredient particle size and feed form on the performance of broiler turkeys. *Canadian Journal of Animal Science*, *77*(3), 503-508.

Han, Y., & Baker, D. H. (1994). Digestible lysine requirement of male and female broiler chicks during the period three to six weeks posthatching. *Poultry Science*, *73*(11), 1739-1745.

Hancock, S., Zhang, F., & Adeola, O. (2018). Regression method-derived energy value of dried egg albumen for broiler chickens. *Poultry Science*, *97*(5), 1677-1680.

Harrow, S. A., Ravindran, V., Butler, R. C., Marshall, J., & Tannock, G. W. (2007). The influence of farming practices on ileal Lactobacillus salivarius populations of broiler chickens measured by real-time quantitative PCR. *Applied and Environmental Microbiology*, *73*, 7123-7127.

Hätel, H. (1986). Influence of food input and procedure of determination on metabolisable energy and digestibility of a diet measured with young and adult birds. *British Poultry Science*, *27*(1), 11-39.

Hegde, S. N., Rolls, B. A., & Coates, M. E. (1982). The effects of the gut microflora and dietary fibre on energy utilization by the chick. *British Journal of Nutrition*, *48*(1), 73-80.

Herman, R. H. (1974). Hydrolysis and absorption of carbohydrates, and adaptive responses of the jejunum. *Sugars in Nutrition. HL Sipple & KW McNutt, eds*.

Hetland, H., Svihus, B., & Choct, M. (2005). Role of insoluble fiber on gizzard activity in layers. *Journal of Applied Poultry Research*, *14*(1), 38-46.

Hill, F. W., & Anderson, D. L. (1958). Comparison of metabolizable energy and productive energy determinations with growing chicks. *Journal of Nutrition*, *64*, 587-603.

Hoai, H. T., Kinh, L. V., Viet, T. Q., Sy, P. V., Hop, N. V., Oanh, D. K., & Yen, N. T. (2011). Determination of the metabolizable energy content of common feedstuffs in meat-type growing ducks. *Animal Feed Science and Technology*, *170*(1-2), 126-129.

Hong, D., Burrows, H., & Adeola, O. (2002). Addition of enzyme to starter and grower diets for ducks. *Poultry Science*, *81*(12), 1842-1849.

Hossain, M. A., Islam, A. F., & Iji, P. A. (2012). Energy utilization and performance of broiler chickens raised on diets with vegetable proteins or conventional feeds. *Asian Journal of Poultry Science*, *6*, 117-128.

Huang, C. C., Hsieh, C. C., & Chiang, S. H. (2007). Estimating the energy partitioning of Taiwanese native chickens by mathematical model. *Animal Feed Science and Technology*, *134*(3-4), 189-197.

Huang, D. S., Li, D. F., Xing, J. J., Ma, Y. X., Li, Z. J., & Lv, S. Q. (2006). Effects of feed particle size and feed form on survival of Salmonella typhimurium in the alimentary tract and cecal S. typhimurium reduction in growing broilers. *Poultry Science*, *85*(5), 831-836.

Hubbard, L. L. C. (2011). Management guide for broiler. *Hubbard LLC, Duluth GA, 30096*.

Hughes, R. J. (2003). Sex and the single chicken. *Proceedings of the Australian Poultry Science Sympsoium*, *15*, 172-176.

Hughes, R. J. (2008). Relationship between digesta transit time and apparent metabolisable energy value of wheat in chickens. *British Poultry Science*, *49*(6), 716-720.

Hughes, R. J., & Choct, M. (1999). Determinants of nutritional value-Chemical and physical characteristics of grains related to variability in energy and amino acid availability in poultry. *Australian Journal of Agricultural Research*, *50*(5), 689-702.

Hughes, R. J., Choct, M., & Van Barneveld, R. J. (2001). Factors influencing the energy values of Australian cereal grains fed to broilers. *Proceedings of the Australian Poultry Science Sympsoium*, *13*, 30-38.

Hussar, N., & Robblee, A. R. (1962). Effects of pelleting on the utilization of feed by the growing chicken. *Poultry Science*, *41*(5), 1489-1493.

INRA (Institut national de la recherche agronomique, France). (1989). *L'alimentation des animaux monogastriques: porc, lapin, volailles*. Editions Quae.

INRA (Institut national de la recherche agronomique, France). (2002). *Tables de Composition et de Valeur Nutritive des Matieres Premi'eres Destin'ees aux Animaux d''elevage*

Jacob, J. P., & Pescatore, A. J. (2012). Using barley in poultry diets—A review. *Journal of Applied Poultry Research*, *21*(4), 915-940.

Janssen, W. M. M. A. (1989). European table of energy values for poultry feedstuffs. *Spelderholt Center for Poultry Research and Information Services, Beekbergen, Netherlands*.

Jensen, L. S. (2000). Influence of pelleting on the nutritional needs of poultry. *Asian-Australasian Journal of Animal Sciences*, *13*(Special iss.), 35-46.

Jha, R., & Mishra, P. (2021). Dietary fiber in poultry nutrition and their effects on nutrient utilization, performance, gut health, and on the environment: a review. *Journal of Animal Science and Biotechnology*, *12*(1), 1-16.

Jiang, Z. (2007). Putting Metabolizable Energy into Context. *International Poultry Production*, *12*(6), 13-19.

Jiménez-Moreno, E., González-Alvarado, J. M., Lázaro, R., & Mateos, G. G. (2009). Effects of type of cereal, heat processing of the cereal, and fiber inclusion in the diet on gizzard pH and nutrient utilization in broilers at different ages. *Poultry Science*, *88*(9), 1925-1933.

Jin, S. H., Corless, A., & Sell, J. L. (1998). Digestive system development in post-hatch poultry. *World's Poultry Science Journal*, *54*(4), 335-345.

Jones, F. T., Anderson, K. E., & Ferket, P. R. (1995). Effect of extrusion on feed characteristics and broiler chicken performance. *Journal of Applied Poultry Research*, *4*(3), 300-309.

Karunaratne, N. D., Abbott, D. A., Chibbar, R. N., Hucl, P. J., Pozniak, C. J., & Classen, H. L. (2018). In vitro assessment of the starch digestibility of western Canadian wheat market classes and cultivars. *Canadian Journal of Animal Science*, *98*(3), 463-476.

Khajali, F., & Slominski, B. A. (2012). Factors that affect the nutritive value of canola meal for poultry. *Poultry Science*, *91*(10), 2564-2575.

Kidd, M. T., McDaniel, C. D., Branton, S. L., Miller, E. R., Boren, B. B., & Fancher, B. I. (2004). Increasing amino acid density improves live performance and carcass yields of commercial broilers. *Journal of Applied Poultry Research*, *13*(4), 593-604.

Kienzle, E., Schrag, I., Butterwick, R., & Opitz, B. (2001). Calculation of gross energy in pet foods: new data on heat combustion and fibre analysis in a selection of foods for dogs and cats. *Journal of Animal Physiology and Animal Nutrition*, *85*(5-6), 148-157.

Kirstein, D. D. (1999). Composition and quality of porcine meat and bone meal. *Nutrition and Animal Health*, 222-236.

Kluth, H., & Rodehutscord, M. (2009). Effect of inclusion of cellulose in the diet on the inevitable endogenous amino acid losses in the ileum of broiler chicken. *Poultry Science*, *88*(6), 1199-1205.

Kong, C., & Adeola, O. (2014). Evaluation of amino acid and energy utilization in feedstuff for swine and poultry diets. *Asian-Australasian Journal of Animal Sciences*, *27*(7), 917.

Kong, C., & Adeola, O. (2016). Determination of ileal digestible and apparent metabolizable energy contents of expeller-extracted and solvent-extracted canola meals for broiler chickens by the regression method. *Springerplus*, *5*(1), 1-7.

Kotb, A. R. & TD Luckey. 1972. Markers in nutrition. In *Nutrition Abstracts and Reviews, (42),* 813.

Krás, R. V., Kessler, A. D. M., Ribeiro, A. M. L., Henn, J. D., Bockor, L., & Sbrissia, A. F. (2013). Effect of dietary fiber, genetic strain and age on the digestive metabolism of broiler chickens. *Brazilian Journal of Poultry Science*, *15*, 83-90.

Kühn, E. R., Darras, V. M., Gysemans, C., Decuypere, E., Berghman, L. R., & Buyse, J. (1996). The use of intermittent lighting in broiler raising.: 2. Effects on the somatotrophic and thyroid axes and on plasma testosterone levels. *Poultry Science*, *75*(5), 595-600.

Lamot, D. M., Sapkota, D., Wijtten, P. J. A., van den Anker, I., Heetkamp, M. J. W., Kemp, B., & van den Brand, H. (2017). Diet density during the first week of life: Effects on energy and nitrogen balance characteristics of broiler chickens. *Poultry Science*, *96*(7), 2294-2300.

Larbier, M., & Leclercq, B. (1994). *Nutrition and feeding of poultry*. Nottingham (UK), Nottingham University Press.

Lasek, O., Barteczko, J., Borowiec, F., Smulikowska, S., & Augustyn, R. (2012). The nutritive value of maize cultivars for broiler chickens. *Journal of Animal and Feed Sciences*, *21*(2), 345-360.

Latshaw, J. D. (2008). Daily energy intake of broiler chickens is altered by proximate nutrient content and form of the diet. *Poultry Science*, *87*(1), 89-95.

Lee, J., & Kong, C. (2019). Comparison of energy values estimated by direct and indirect methods for broiler chickens. *International Journal of Poultry Science*, *18*, 244-248.

Leenstra, F. R., Decuypere, E., Beuving, G., Buyse, J., Berghman, L., & Herremans, M. (1991). Concentrations of hormones, glucose, triglycerides and free fatty acids in the plasma of broiler chickens selected for weight gain or food conversion. *British Poultry Science*, *32*(3), 619-632.

Leeson, S., & Summers, J. D. (2005). *Commercial poultry nutrition*. Nottingham University Press.

Leeson, S., Boorman, K. N., Lewis, D., & Shrimpton, D. H. (1977). Metabolisable energy studies with Turkeys: Nitrogen correction factor in metabolisable energy determinations. *British Poultry Science*, *18*(4), 373-379.

Leeson, S., Summers, J. D., & Caston, L. (1993). Growth response of immature brown-egg strain pullets to varying nutrient density and lysine. *Poultry Science*, *72*(7), 1349-1358.

Lemme, A., Ravindran, V., & Bryden, W. L. (2004). Ileal digestibility of amino acids in feed ingredients for broilers. *World's Poultry Science Journal*, *60*(4), 423-438.

Lemme, A., Wijtten, P. J. A., Van Wichen, J., Petri, A., & Langhout, D. J. (2006). Responses of male growing broilers to increasing levels of balanced protein offered as coarse mash or pellets of varying quality. *Poultry Science*, *85*(4), 721-730.

Leslie, M. A., Moran Jr, E. T., & Bedford, M. R. (2007). The effect of phytase and glucanase on the ileal digestible energy of corn and soybean meal fed to broilers. *Poultry Science*, *86*(11), 2350-2357.

Lessire, M., Leclercq, B., & Conan, L. (1982). Metabolisable energy value of fats in chicks and adult cockerels. *Animal Feed Science and Technology*, *7*(4), 365-374.

Lien, K. A., Sauer, W. C., & He, J. M. (2001). Dietary influences on the secretion into and degradation of mucin in the digestive tract of monogastric animals and humans. *Journal of Animal and Feed Sciences*, *10*(2), 223-246.

Lilly, K. G. S., Gehring, C. K., Beaman, K. R., Turk, P. J., Sperow, M., & Moritz, J. S. (2011). Examining the relationships between pellet quality, broiler performance, and bird sex. *Journal of Applied Poultry Research*, *20*(2), 231-239.

Lima, I. L., Silva, D., Rostagno, H. S., & Tafuri, M. L. (1988). Composicao quiimica e valores energeticos de alguns alimentos determinados com pintos e galos, utilizando duas metodologias. *18*, 546-556.

Liu, S. Y., Fox, G., Khoddami, A., Neilson, K. A., Truong, H. H., Moss, A. F., & Selle, P. H. (2015). Grain sorghum: a conundrum for chicken-meat production. *Agriculture*, *5*(4), 1224-1251.

Lockhart, W. C., Bryant, R. L., & Bolin, D. W. (1967). A comparison of several methods in determining the metabolizable energy content of durum wheat and wheat cereal by chicks. *Poultry Science*, *46*(4), 805-810.

Lopez, G., & Leeson, S. (2005). Utilization of metabolizable energy by young broilers and birds of intermediate growth rate. *Poultry Science*, *84*(7), 1069-1076.

Lopez, G., & Leeson, S. (2007). Relevance of nitrogen correction for assessment of metabolizable energy with broilers to forty-nine days of age. *Poultry Science*, *86*(8), 1696-1704.

Lopez, G., & Leeson, S. (2008). Assessment of the nitrogen correction factor in evaluating metabolizable energy of corn and soybean meal in diets for broilers. *Poultry Science*, *87*(2), 298-306.

Losada, B., García-Rebollar, P., Álvarez, C., Cachaldora, P., Ibáñez, M. A., Méndez, J., & De Blas, J. C. (2010). The prediction of apparent metabolisable energy content of oil seeds and oil seed by-products for poultry from its chemical components, in vitro analysis or near-infrared reflectance spectroscopy. *Animal Feed Science and Technology*, *160*(1-2), 62-72.

Losada, B., Rebollar, P. G., Cachaldora, P., Álvarez, C., & De Blas, J. C. (2009). A comparison of the prediction of apparent metabolisable energy content of starchy grains and cereal by-products for poultry from its chemical components, in vitro analysis or near-infrared reflectance spectroscopy. *Spanish Journal of Agricultural Research*, (4), 813-823.

Lowe, R. (2005). Judging pellet stability as part of pellet quality. *Feed Technology*, *9*, 15-19.

Lu, J., Idris, U., Harmon, B., Hofacre, C., Maurer, J. J., & Lee, M. D. (2003). Diversity and succession of the intestinal bacterial community of the maturing broiler chicken. *Applied and Environmental Microbiology*, *69*(11), 6816-6824.

Lund, D., & Lorenz, K. J. (1984). Influence of time, temperature, moisture, ingredients, and processing conditions on starch gelatinization. *Critical Reviews in Food Science & Nutrition*, *20*(4), 249-273.

MacLeod, M. G. (1994). Dietary energy utilisation: prediction by computer simulation of energy metabolism. *Publication-European Association For Animal Production*, *76*, 237-237.

Macleod, M. G. (2002). Energy utilization: measurement and prediction. *Poultry Feedstuffs: Supply, Composition and Nutritive Value*, 191-217.

Mahagna, M., & Nir, I. (1996). Comparative development of digestive organs, intestinal disaccharidases and some blood metabolites in broiler and layer-type chicks after hatching. *British Poultry Science*, *37*(2), 359-371.

Maier, D. E., & Briggs, J. L. (2000). Making better. *Feed and Grain*, *1*, 12–15.

Mandalawi, H. A., Mallo, J. J., Menoyo, D., Lázaro, R., & Mateos, G. G. (2017). Metabolizable energy content of traditional and re-esterified lipid sources: Effects of inclusion in the diet on nutrient retention and growth performance of broilers from 7 to 21 days of age. *Animal Feed Science and Technology*, *224*, 124-135.

Mariscal-Landín, G., Sève, B., Colléaux, Y., & Lebreton, Y. (1995). Endogenous amino nitrogen collected from pigs with end-to-end ileorectal anastomosis is affected by the method of estimation and altered by dietary fiber. *The Journal of Nutrition*, *125*(1), 136-146.

Masood, W., Khan, S. H., Bhatti, S. A., & Parveen, A. (2011). Comparison of sample source (excreta or ileal digesta) and age of broiler chick on measurement of apparent metabolisable energy of local feed ingredients. *Journal of Applied Animal Research*, *39*(4), 359-366.

Massuquetto, A., Durau, J. F., Schramm, V. G., Netto, M. T., Krabbe, E. L., & Maiorka, A. (2018). Influence of feed form and conditioning time on pellet quality, performance and ileal nutrient digestibility in broilers. *Journal of Applied Poultry Research*, *27*(1), 51-58.

Massuquetto, A., Panisson, J. C., Marx, F. O., Surek, D., Krabbe, E. L., & Maiorka, A. (2019). Effect of pelleting and different feeding programs on growth performance, carcass yield, and nutrient digestibility in broiler chickens. *Poultry Science*, *98*(11), 5497-5503.

Mateos, G. G, Sueiro, S., González, M. R., Safaa, H. M., & Frikha, M. (2008). Recognizing the nutritional quality of soybean meal in diets for poultry. *Ukrania*, *62*, 79-101.

Mateos, G. G., Cámara, L., Fondevila, G., & Lázaro, R. P. (2019). Critical review of the procedures used for estimation of the energy content of diets and ingredients in poultry. *Journal of Applied Poultry Research*, *28*(3), 506-525.

Mateos, G. G., Jiménez-Moreno, E., Serrano, M. P., & Lázaro, R. P. (2012). Poultry response to high levels of dietary fiber sources varying in physical and chemical characteristics. *Journal of Applied Poultry Research*, *21*(1), 156-174.

Matterson, L. D., & Prince, R. (1969). The effect of age and environment on the metabolizable energy value of feedstuffs. *Proceedings Maryland Nutrition Conference for Feed Manufacturers*, *97*.

McBurney, W., Tannock, G., & Ravindran, V. (2003). A culture-independent approach to the analysis of the gut microflora of broilers. In *Proceedings of Austuralian Poultury Science Symposium,15,*131-134.

Mccleary, B. V., Gibson, T. S., & Mugford, D. C. (1997). Measurement of total starch in cereal products by amyloglucosidase-α-amylase method: Collaborative study. *Journal of AOAC International, 80*(3), 571-579.

McCracken, K. J., & Quintin, G. (2000). Metabolisable energy content of diets and broiler performance as affected by wheat specific weight and enzyme supplementation. *British Poultry Science, 41*(3), 332-342.

McIntosh, J. I., Slinger, S. J., Sibbald, I. R., & Ashton, G. C. (1962). Factors affecting the metabolizable energy content of poultry feeds: 7. The effects of grinding, pelleting and grit feeding on the availability of the energy of wheat, corn, oats and barley; 8. A study on the effects of dietary balance. *Poultry Science, 41*(2), 445-456.

McNab, J. M. (2000). Rapid metabolizable energy assays. *Farm Animal Metabolism and Nutrition,* 307.

Medic, J., Atkinson, C., & Hurburgh, C. R. (2014). Current knowledge in soybean composition. *Journal of the American oil chemists' society, 91*(3), 363-384.

Mello, H. H., Gomes, P. C., Rostagno, H.S ., Albino, L. F. T., Souza, R. M., & Calderano, A.A. (2009). Metabolizable energy values of some foods obtained from birds of different ages. *Revista Brasileira de Zootecnia, 38,* 863-868.

Moehn, S., Atakora, J., & Ball, R. O. (2005). Using net energy for diet formulation: Potential for the Canadian pig industry. *Advances in Pork Production, 16,* 119-129.

Mollah, Y., Bryden, W. L., Wallis, I. R., Balnave, D., & Annison, E. F. (1983). Sudies on low metabolisable energy wheats for poultry using conventional and rapid assay procedures and the effects of processing. *British Poultry Science*, *24*(1), 81-89.

Montagne, L., Pluske, J. R., & Hampson, D. J. (2003). A review of interactions between dietary fibre and the intestinal mucosa, and their consequences on digestive health in young non-ruminant animals. *Animal Feed Science and Technology*, *108*(1-4), 95-117.

Moran, A. W., Al-Rammahi, M. A., Arora, D. K., Batchelor, D. J., Coulter, E. A., Ionescu, C., ... & Shirazi-Beechey, S. P. (2010). Expression of Na+/glucose co-transporter 1 (SGLT1) in the intestine of piglets weaned to different concentrations of dietary carbohydrate. *British Journal of Nutrition*, *104*(5), 647-655.

Moss, A. F., Khoddami, A., Chrystal, P. V., Sorbara, J. O. B., Cowieson, A. J., Selle, P. H., & Liu, S. Y. (2020). Starch digestibility and energy utilisation of maize-and wheat-based diets is superior to sorghum-based diets in broiler chickens offered diets supplemented with phytase and xylanase. *Animal Feed Science and Technology*, *264*, 114475.

Moss, A. F., Sydenham, C. J., Truong, H. H., Liu, S. Y., & Selle, P. H. (2017). The interactions of exogenous phytase with whole grain feeding and effects of barley as the whole grain component in broiler diets based on wheat, sorghum and wheat-sorghum blends. *Animal Feed Science and Technology*, *227*, 1-12.

Murakami, H., Akiba, Y., & Horiguchi, M. (1992). Growth and utilization of nutrients in newly-hatched chick with or without removal of residual yolk. *Growth, development, and aging: Growth Development and Aging*, *56*(2), 75-84.

Murakami, H., Akiba, Y., & Horiguchi, M. (1995). A Modified Bioassay for Energy Utilization in Newly Hatched Chicks.: 2. Determination of Feed Input and Procedures to Estimate Endogenous Energy Loss. *Poultry Science, 74*(2), 343-351.

Mutucumarana, R. K., & Ravindran, V. (2016). Measurement of true ileal phosphorus digestibility in meat and bone meal for broiler chickens using the direct method. *Animal Feed Science and Technology, 219,* 249-256.

Mutucumarana, R. K., Ravindran, V., Ravindran, G., & Cowieson, A. J. (2015). Measurement of true ileal phosphorus digestibility in meat and bone meal for broiler chickens. *Poultry Science, 94*(7), 1611-1618.

Muztar, A. J., & Slinger, S. J. (1980). Bioavailable amino acids in corn and alfalfa as measured by applying the true metabolizable energy assay. *Poultry Science, 59*(8), 1873-1877.

Myers, W. D., Ludden, P. A., Nayigihugu, V., & Hess, B. W. (2004). A procedure for the preparation and quantitative analysis of samples for titanium dioxide. *Journal of Animal Science, 82*(1), 179-183.

Naderinejad, S., Zaefarian, F., Abdollahi, M. R., Hassanabadi, A., Kermanshahi, H., & Ravindran, V. (2016). Influence of feed form and particle size on performance, nutrient utilisation, and gastrointestinal tract development and morphometry in broiler starters fed maize-based diets. *Animal Feed Science and Technology, 215,* 92-104.

NARO (National Agriculture and Food Research Organization). (2009). Standard Tables of Feed Composition in Japan, Japan Livestock Industry Association.

Niegm, H. M. (1966). Verdaulichkeit des Huhnerfutters und ihre Beeinflussung durch Tiermaterial und Futterform. PhD Thesis, Stuttgart-Hohenheim

Nitsan, Z., Dvorin, A., Zoref, Z., & Mokady, S. (1997). Effect of added soyabean oil and dietary energy on metabolisable and net energy of broiler diets. *British Poultry Science*, *38*(1), 101-106.

Noble, R. C., & Cocchi, M. (1990). Lipid metabolism and the neonatal chicken. *Progress in Lipid Research*, *29*(2), 107-140.

Noblet, J. (2015). Comparative interests and limits of metabolizable energy and net energy for evaluating poultry and pig feeds. In *20. European Symposium on Poultry Nutrition (ESPN), 20*, 48-56

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*, *127*, 573-582.

Noblet, J., Wu, S. B., & Choct, M. (2021). Methodologies for energy evaluation of pig and poultry feeds: A review. *Animal Nutrition*, *8*, 185-203.

Noy, Y., & Sklan, D. (1995). Digestion and absorption in the young chick. *Poultry Science*, *74*(2), 366-373.

Noy, Y., & Sklan, D. (2001). Yolk and exogenous feed utilization in the posthatch chick. *Poultry Science*, *80*(10), 1490-1495.

NRC (National Research Council). (1994). *Nutrient requirements of poultry: 1994*. National Academies Press.

Nyachoti, C. M., Lange, C. D., McBride, B. W., & Schulze, H. (1997). Significance of endogenous gut nitrogen losses in the nutrition of growing pigs: a review. *Canadian Journal of Animal Science*, *77*(1), 149-163.

O'sullivan, A. C. (1997). Cellulose: the structure slowly unravels. *Cellulose*, *4*(3), 173-207.

Obst, B. S., & Diamond, J. (1992). Ontogenesis of intestinal nutrient transport in domestic chickens (Gallus gallus) and its relation to growth. *The Auk, 109*(3), 451-464.

Okumura, J. I., Isshiki, Y., & Nakahiro, Y. (1982). Influence of dietary cellulose and indigestible dry matter on metabolic and endogenous nitrogen excretion in chickens. *Japanese Poultry Science, 19*(5), 300-304.

Olukosi, O. A. (2021). 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. *British Poultry Science, 62*(2), 278-284.

Olukosi, O. A., & Bedford, M. R. (2019). Comparative effects of wheat varieties and xylanase supplementation on growth performance, nutrient utilization, net energy, and whole-body energy and nutrient partitioning in broilers at different ages. *Poultry Science, 98*(5), 2179-2188.

Olukosi, O. A., Adedokun, S. A., & Agboola, J. O. (2017). Species-dependent response to the influence of adaptation length during assay for metabolisable energy of cereal grains employing the difference method. *Animal Feed Science and Technology, 231*, 111-118.

Olukosi, O. A., Bolarinwa, O. A., Cowieson, A. J., & Adeola, O. (2012). Marker type but not concentration influenced apparent ileal amino acid digestibility in phytase-supplemented diets for broiler chickens and pigs. *Journal of Animal Science, 90*(12), 4414-4420.

Olukosi, O. A., Cowieson, A. J., & Adeola, O. (2007). Age-related influence of a cocktail of xylanase, amylase, and protease or phytase individually or in combination in broilers. *Poultry Science, 86*(1), 77-86.

Parsons, A. S., Buchanan, N. P., Blemings, K. P., Wilson, M. E., & Moritz, J. S. (2006). Effect of corn particle size and pellet texture on broiler performance in the growing phase. *Journal of Applied Poultry Research*, *15*(2), 245-255.

Parsons, C. M. (2011). Energy and Amino Acid Digestibility Assays: Where Have We Been, Where Are We Now, and What Does the Future Hold. *In Proceedings of Arkansas Nutrition Conference.*

Perera, W. N. U., Abdollahi, M. R., Ravindran, V., Zaefarian, F., Wester, T. J., & Ravindran, G. (2019). Nutritional evaluation of two barley cultivars, without and with carbohydrase supplementation, for broilers: metabolisable energy and standardised amino acid digestibility. *British Poultry Science*, *60*(4), 404-413.

Petersen, S. T., & Farrell, D. J. (1997). A comparison of cereal and grain legumes on several digestive parameters in broilers. *Proceedings of the Australian Poultry Science Sympsoium*, *9*(149), 52.

Pettersson, D., & Åman, P. (1989). Enzyme supplementation of a poultry diet containing rye and wheat. *British journal of Nutrition*, *62*(1), 139-149.

Pirgozliev, V., Acamovic, T., & Bedford, M. R. (2009). Previous exposure to dietary phytase reduces the endogenous energy losses from precision-fed chickens. *British Poultry Science*, *50*(5), 598-605.

Pirgozliev, V., Mirza, M. W., & Rose, S. P. (2016). Does the effect of pelleting depend on the wheat sample when fed to chickens?. *Animal*, *10*(4), 571-577.

PremierAtlas, P. (2014). Premier Nutrition Ingredients Matrix. Products Ltd, AB Agri Ltd, Rugeley Staffs, UK.

Raharjo, Y., & Farrell, D. J. (1984). A new biological method for determining amino acid digestibility in poultry feedstuffs using a simple cannula, and the influence of dietary fibre on endogenous amino acid output. *Animal Feed Science and Technology*, *12*(1), 29-45.

Ramos, S. (2003). *Comparação de diferentes indicadores com método de coleta total para determinação da digestibilidade de diferentes dietas para eqüinos. 2003. 69p*(Doctoral dissertation, Dissertação (Mestrado em Medicina Veterinária)-Universidade de São Paulo, Pirassununga).

Ravindran, V. (2016). Feed-induced specific ileal endogenous amino acid losses: Measurement and significance in the protein nutrition of monogastric animals. *Animal Feed Science and Technology*, *221*, 304-313.

Ravindran, V., & Abdollahi, M. R. (2021). Nutrition and digestive physiology of the broiler chick: State of the art and outlook. *Animals*, *11*(10), 2795.

Ravindran, V., Abdollahi, M. R., & Bootwalla, S. M. (2014). Nutrient analysis, metabolizable energy, and digestible amino acids of soybean meals of different origins for broilers. *Poultry Science*, *93*(10), 2567-2577.

Ravindran, V., Hew, L. I., & Ravindran, G. (2004b). Endogenous amino acid flow in the avian ileum: quantification using three techniques. *British Journal of Nutrition*, *92*(2), 217-223.

Ravindran, V., Hew, L. I., Ravindran, G., & Bryden, W. L. (2005). Apparent ileal digestibility of amino acids in dietary ingredients for broiler chickens. *Animal Science*, *81*(1), 85-97.

Ravindran, V., Morel, P. C., Rutherfurd, S. M., & Thomas, D. V. (2008). Endogenous flow of amino acids in the avian ileum as influenced by increasing dietary peptide concentrations. *British Journal of Nutrition*, *101*(6), 822-828.

Ravindran, V., Tancharoenrat, P., Zaefarian, F., & Ravindran, G. (2016). Fats in poultry nutrition: Digestive physiology and factors influencing their utilisation. *Animal Feed Science and Technology*, *213*, 1-21.

Ravindran, V., Wu, Y. B., & Hendriks, W. H. (2004a). Effects of sex and dietary phosphorus level on the apparent metabolizable energy and nutrient digestibility in broiler chickens. *Archives of Animal Nutrition*, *58*(5), 405-411.

Riesenfeld, G., Sklan, D., Bar, A., Eisner, U., & Hurwitz, S. (1980). Glucose absorption and starch digestion in the intestine of the chicken. *The Journal of Nutrition*, *110*(1), 117-121.

Rogel, A. M., Annison, E. F., Bryden, W. L., & Balnave, D. (1987). The digestion of wheat starch in broiler chickens. *Australian Journal of Agricultural Research*, *38*(3), 639-649.

Romero, L. F., Sands, J. S., Indrakumar, S. E., Plumstead, P. W., Dalsgaard, S., & Ravindran, V. (2014). Contribution of protein, starch, and fat to the apparent ileal digestible energy of corn-and wheat-based broiler diets in response to exogenous xylanase and amylase without or with protease. *Poultry Science*, *93*(10), 2501-2513.

Rostagno, H. S., Albino, L. F. T., Donzele, J. L., Gomes, P. C., Oliveira, R. F., Lopes, D. C., ... & Euclides, R. F. (2017). Brazilian tables for poultry and swine: composition of feedstuffs and nutritional requirements. *Animal Science Department UFV, Viçosa, MG, Brazil*.

Rougière, N., & Carré, B. (2010). Comparison of gastrointestinal transit times between chickens from D+ and D− genetic lines selected for divergent digestion efficiency. *Animal*, *4*(11), 1861-1872.

Rougière, N., Gomez, J., Mignon-Grasteau, S., & Carré, B. (2009). Effects of diet particle size on digestive parameters in D+ and D− genetic chicken lines selected for divergent digestion efficiency. *Poultry Science*, *88*(6), 1206-1215.

Roza, L. F., Tavernari, F. D. C., Surek, D., Sordi, C., Albino, L. F. T., Paiano, D., D., Boiago, M., Petrolli, T., & Júnior, A. C. (2018). Metabolizable energy and amino acid digestibility of mash and pelleted diets for broilers determined under different methodologies. *Animal Feed Science and Technology*, *235*, 1-7.

RPRI (Russian Poultry Research Institute). (2014). Manual for Optimization of Formulation of Compound Feeds for Poultry. Russian Poultry Research Institute, Sergiev Posade, Russian Federation.

Sakomura, N. K., & Rostagno, H. S. (2016). Research methods in monogastric nutrition. *Jaboticabal, SP, Brazil: Funep*.

Sakomura, N. K., Longo, F. A., Oviedo-Rondon, E. O., Boa-Viagem, C., & Ferraudo, A. (2005). Modeling energy utilization and growth parameter description for broiler chickens. *Poultry Science*, *84*(9), 1363-1369.

Saldaña, B., Guzmán, P., Safaa, H. M., Harzalli, R., & Mateos, G. G. (2015). Influence of the main cereal and feed form of the rearing phase diets on performance, digestive tract, and body traits of brown-egg laying pullets from hatch to 17 weeks of age. *Poultry Science*, *94*(11), 2650-2661.

Sales, J., & Janssens, G. P. J. (2003). The use of markers to determine energy metabolizability and nutrient digestibility in avian species. *World's Poultry Science Journal*, *59*(3), 314-327.

Santos, A. L. D., Sakomura, N. K., Freitas, E. R., Fortes, C. M. L. S., Carrilho, E. N. V. M., & Fernandes, J. B. K. (2005). Growth, performance, carcass yield and meat quality of three broiler chickens strains. *Revista Brasileira de Zootecnia*, *34*, 1589-1598.

Santos, F. R., Stringhini, J. H., Oliveira, P. R., Duarte, E. F., Minafra, C. S., & Café, M. B. (2015). Values of metabolizable energy and metabolization of nutrients for slow- and fast-growing birds at different ages. *Brazilian Journal of Poultry Science*, *17*, 517-522.

SAS Institute. (2015). SAS® 9.4 Qualification Tools User's Guide. SAS Institute Inc., Cary, NC, USA.

Satchithanandam, S., Klurfeld, D. M., Calvert, R. J., & Cassidy, M. M. (1996). Effects of dietary fibers on gastrointestinal mucin in rats. *Nutrition Research*, *16*(7), 1163-1177.

Sato, M., Tachibana, T., & Furuse, M. (2006). Heat production and lipid metabolism in broiler and layer chickens during embryonic development. *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology*, *143*(3), 382-388.

Scheele, C. W., Kwakernaak, C., van der Klis, J. D., & Bakker, G. C. M. (1997). Effects of different factors including enzymes on the nutritional value of fats for poultry. In P. C. Gansworthy, & J. Wiseman (Eds.), *Recent Advances in Animal Nutrition* (pp. 59-75).

Schneider, W., & Lantzsch, H. J. (1969). Zum Einfluss des Alters auf das Verdauungsvermogen von Masthybriden. *Landwirt Forsch*, *22*, 182-186

Schumaier, G., & McGinnis, J. (1967). Metabolizable energy values of wheat and some by-product feedstuffs for growing chicks. *Poultry Science*, *46*(1), 79-82.

Scott, T. A. (2005). Variation in feed intake of broiler chickens. *Recent Advances in Animal Nutrition in Australia*, *15*, 237-244.

Scott, T. A., & Boldaji, F. (1997). Comparison of inert markers [chromic oxide or insoluble ash (Celite)] for determining apparent metabolizable energy of wheat-or barley-based broiler diets with or without enzymes. *Poultry Science*, *76*(4), 594-598.

Scott, T. A., Silversides, F. G., Classen, H. L., Swift, M. L., & Bedford, M. R. (1998). Comparison of sample source (excreta or ileal digesta) and age of broiler chick on measurement of apparent digestible energy of wheat and barley. *Poultry Science*, *77*(3), 456-463.

Sell, J. L., Angel, C. R., Piquer, F. J., Mallarino, E. G., & Al-Batshan, H. A. (1991). Developmental patterns of selected characteristics of the gastrointestinal tract of young turkeys. *Poultry Science*, *70*(5), 1200-1205.

Selle, P. H., Cadogan, D. J., Li, X., & Bryden, W. L. (2010). Implications of sorghum in broiler chicken nutrition. *Animal Feed Science and Technology*, *156*(3-4), 57-74.

Selle, P. H., Gill, R. J., & Cowieson, A. J. (2013). Steam-pelleting temperatures, sorghum variety and feed form influence growth performance, nutrient utilisation and nitrogen digestibility in broiler chickens. *In Proceedings of the Australian Poultry Science Symposium*, *12*, 142.

Selle, P. H., Liu, S. Y., Cai, J., & Cowieson, A. J. (2012). Steam-pelleting and feed form of broiler diets based on three coarsely ground sorghums influences growth performance, nutrient utilisation, starch and nitrogen digestibility. *Animal Production Science*, *52*(9), 842-852.

Senkoyiu, N., & Janssen, W. M. (1988). The effect of age, basic feed, fat type, and inclusion levels upon apparent metabolizable energy values in chicken feeds. In *Proceedings of the 18 th World's Poultry Congress* (pp. 784-786).

Serrano, M. P., Frikha, M., Corchero, J., & Mateos, G. G. (2013). Influence of feed form and source of soybean meal on growth performance, nutrient retention, and digestive organ size of broilers. 2. Battery study. *Poultry Science*, *92*(3), 693-708.

Sharma, R., & Schumacher, U. (1995). Morphometric analysis of intestinal mucins under different dietary conditions and gut flora in rats. *Digestive Diseases and Sciences*, *40*(12), 2532-2539.

Shires, A., Robblee, A. R., Hardin, R. T., & Clandinin, D. R. (1980). Effect of the age of chickens on the true metabolizable energy values of feed ingredients. *Poultry Science*, *59*(2), 396-403.

Short, F. J., Gorton, P., Wiseman, J., & Boorman, K. N. (1996). Determination of titanium dioxide added as an inert marker in chicken digestibility studies. *Animal Feed Science and Technology*, *59*(4), 215-221.

Sibbald, I. R. (1976). A bioassay for true metabolizable energy in feedingstuffs. *Poultry Science*, *55*(1), 303-308.

Sibbald, I. R. (1977). The true metabolizable energy values of some feedingstuffs. *Poultry Science*, *56*(1), 380-382.

Sibbald, I. R. (1981). Metabolic plus endogenous energy and nitrogen losses of adult cockerels: the correction used in the bioassay for true metabolizable energy. *Poultry Science*, *60*(4), 805-811.

Sibbald, I. R. (1982). Measurement of bioavailable energy in poultry feedingstuffs: a review. *Canadian Journal of Animal Science*, *62*(4), 983-1048.

Sibbald, I. R. (1985). The true metabolizable energy bioassay as a method for estimating bioavailable energy in poultry feedingstuffs. *World's Poultry Science Journal*, *41*(3), 179-187.

Sibbald, I. R. (1986). *The TME system of feed evaluation: methodology, feed composition data and bibliography*. Agriculture Canada, Research Branch.

Sibbald, I. R., & Price, K. (1980). Variability in metabolic plus endogenous energy losses of adult cockerels and in the true metabolizable energy values and rates of passage of dehydrated alfalfa. *Poultry Science*, *59*(6), 1275-1279.

Sibbald, I. R., & Slinger, S. J. (1962). The metabolizable energy of materials fed to growing chicks. *Poultry Science*, *41*(5), 1612-1613.

Sibbald, I. R., & Slinger, S. J. (1963). A biological assay for metabolizable energy in poultry feed ingredients together with findings which demonstrate some of the problems associated with the evaluation of fats. *Poultry Science, 42*(2), 313-325.

Sibbald, I. R., Summers, J. D., & Slinger, S. J. (1960). Factors affecting the metabolizable energy content of poultry feeds. *Poultry Science*, *39*(3), 544-556.

Sibbald, I.R. (1989). Metabolizable energy evaluation of poultry diets. In: Recent Development in Poultry Nutrition, Cole, D.J.A. and W. Haresing (Eds.). Anchor Press Ltd., Tiptree, Essex, pp. 12-26

Silva, E. P., Rabello, C. B. V., de Lima, M. B., de Lima, S. B. P., Lima, R. B., & de Lima, T. S. (2011). Age effect on the endogenous and metabolic losses in broiler chickens and free range. *Ciência Animal Brasileira, 12*(1), 37-47.

Silva, E. P., Rabello, C. B., Lira, R. C., Farias Filho, R. V., Albuquerque, C. S., & Silva, D. A. (2006). Estimativas das perdas endógenas e metabólicas em frangos de corte. *Revista Brasileira de Ciências Agrárias*, *1*(1), 115-121.

Simon, O., Zebrowska, T., Münchmeyer, R., Bergner, H., & Rohrmann, H. (1986). Studies on the secretion of amino acids and of urea into the gastro intestinal tract of pigs. *Archives of Animal Nutrition*, *36*(1), 5-16.

Singh, Y., Rao, S. R., & Ravindran, V. (2014). Effect of feeding diets based on coarse maize on productive performance, gizzard development and energy utilisation of laying hens. *British Poultry Science*, *55*(2), 221-227.

Siregar, A. P., & Farrell, D. J. (1980). A comparison of the energy and nitrogen metabolism of fed ducklings and chickens. *British Poultry Science*, *21*(3), 213-227.

Siri, S., Tobioka, H., & Tasaki, I. (1992). Effects of dietary cellulose level on nutrient utilization in chickens. *Asian-Australasian Journal of Animal Sciences*, *5*(4), 741-746.

Sklan, D., & Noy, Y. (2000). Hydrolysis and absorption in the small intestines of posthatch chicks. *Poultry Science*, *79*(9), 1306-1310.

Skoch, E. R., Behnke, K. C., Deyoe, C. W., & Binder, S. F. (1981). The effect of steam-conditioning rate on the pelleting process. *Animal Feed Science and Technology*, *6*(1), 83-90.

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. *Poultry Science*, *94*(7), 1584-1591.

Southgate, B. A. (1930). The maintenance requirement of the fattening cockerel with a note on a proposed new method for the determination of the surface area of birds. *The Journal of Agricultural Science*, *20*(2), 206-212.

Speake, B. K., Murray, A. M., & Noble, R. C. (1998). Transport and transformations of yolk lipids during development of the avian embryo. *Progress of Lipid Research, 37(1),* 1-32.

Stark, C. R., Spencer, B. E., Shih, J. C. H., Chewning, C. G., & Wang, J. J. (2009). Evaluation of keratinase stability in pelleted broiler diets. *Journal of Applied Poultry Research, 18*(1), 30-33.

Steenfeldt, S. (2001). The dietary effect of different wheat cultivars for broiler chickens. *British Poultry Science, 42*(5), 595-609.

Stefanello, C., Vieira, S. L., Xue, P., Ajuwon, K. M., & Adeola, O. (2016). Age-related energy values of bakery meal for broiler chickens determined using the regression method. *Poultry Science, 95*(7), 1582-1590.

Stevens, C. A. (1987). *Starch gelatinization and the influence of particle size, steam pressure and die speed on the pelleting process*. Kansas State University.

Sugahara, K., Matsumoto, M., Yamada, M., Yaoshizawa, F., & Karasawa, Y. (2004). Effect of bilateral cecal ligation on use of dietary energy in growing chickens. *Animal Science Journal, 75*(5), 431-434.

Svihus, B. (2001). Research note: a consistent low starch digestibility observed in pelleted broiler chicken diets containing high levels of different wheat varieties. *Animal Feed Science and Technology, 92*(1-2), 45-49.

Svihus, B. (2006). The role of feed processing on gastrointestinal function and health in poultry. *Avian Gut Function in Health and Disease, 28,* 183-194.

Svihus, B. (2011a). Limitations to wheat starch digestion in growing broiler chickens: a brief review. *Animal Production Science, 51*(7), 583-589.

Svihus, B. (2011b). The gizzard: function, influence of diet structure and effects on nutrient availability. *World's Poultry Science Journal*, *67*(2), 207-224.

Svihus, B., & Hetland, H. (2001). Ileal starch digestibility in growing broiler chickens fed on a wheat-based diet is improved by mash feeding, dilution with cellulose or whole wheat inclusion. *British Poultry Science*, *42*(5), 633-637.

Svihus, B., Kløvstad, K. H., Perez, V., Zimonja, O., Sahlström, S., Schüller, R. B., Jeksrud, W., & Prestløkken, E. (2004). Physical and nutritional effects of pelleting of broiler chicken diets made from wheat ground to different coarsenesses by the use of roller mill and hammer mill. *Animal Feed Science and Technology*, *117*(3-4), 281-293.

Swennen, Q., Janssens, G. P. J., Decuypere, E., & Buyse, J. (2004). Effects of substitution between fat and protein on feed intake and its regulatory mechanisms in broiler chickens: energy and protein metabolism and diet-induced thermogenesis. *Poultry Science*, *83*(12), 1997-2004.

Swick, R., Wu, S. B., Rodgers, N., Choct, M., & Poultry, C. R. C. (2014). Energy systems for broilers-recent development and relevance for feed formulation. In *Conference paper. Queenstown, New Zealand*.

Szczurek, W., Szymczyk, B., Arczewska-Włosek, A., & Świątkiewicz, S. (2020). Apparent and standardised ileal digestibility of amino acids in wheat, triticale and barley for broiler chickens at two different ages. *British Poultry Science*, *61*(1), 63-69.

Tancharoenrat, P., Ravindran, V., Zaefarian, F., & Ravindran, G. (2013). Influence of age on the apparent metabolisable energy and total tract apparent fat digestibility of different fat sources for broiler chickens. *Animal Feed Science and Technology*, *186*(3-4), 186-192.

Tasaki, I., & Kibe, K. (1959). A study on the digestion of cellulose in poultry. *Poultry Science*, *38*(2), 376-379.

Ten Doeschate, R., Scheele, C. W., Schreurs, V. V. A. M., & Van Der Klis, J. D. (1993). Digestibility studies in broiler chickens: influence of genotype, age, sex and method of determination. *British Poultry Science*, *34*(1), 131-146.

Thomas, D. V., Ravindran, V., & Ravindran, G. (2008). Nutrient digestibility and energy utilisation of diets based on wheat, sorghum or maize by the newly hatched broiler chick. *British Poultry Science*, *49*(4), 429-435.

Titus, H. W., Mehring Jr, A. L., Johnson Jr, D., Nesbitt, L. L., & Tomas, T. (1959). An evaluation of MCF (Micro-Cel-Fat), a new type of fat product. *Poultry Science*, *38*(5), 1114-1119.

Truong, H. H., Moss, A. F., Liu, S. Y., & Selle, P. H. (2017). Pre-and post-pellet whole grain inclusions enhance feed conversion efficiency, energy utilisation and gut integrity in broiler chickens offered wheat-based diets. *Animal Feed Science and Technology*, *224*, 115-123.

Uni, Z., Noy, Y., & Sklan, D. (1995). Posthatch changes in morphology and function of the small intestines in heavy-and light-strain chicks. *Poultry Science*, *74*(10), 1622-1629.

Valdes, E. V., & Leeson, S. (1992). Measurement of metabolizable energy in poultry feeds by an in vitro system. *PoultrySscience*, *71*(9), 1493-1503.

Valencia, D. G., Serrano, M. P., Lázaro, R., Jiménez-Moreno, E., & Mateos, G. G. (2009). Influence of micronization (fine grinding) of soya bean meal and full-fat soya bean on the ileal digestibility of amino acids for broilers. *Animal Feed Science and Technology*, *150*(3-4), 238-248.

Van Campenhout, L. (2007). The role of carbohydrases in diets for non-ruminants. *Polysaccharides and polysaccharidases in food and agriculture*, 169-201.

Van der Klis, J. D., & Fledderus, J. (2007). Evaluation of raw materials for poultry: what's up. In *Proceedings of the European Symposium on Poultry Nutrition*. *16*, 123-130.

Van der Klis, J. D., Kwakernaak, C., Jansman, A., & Blok, M. (2010). Energy in poultry diets: adjusted AME or net energy. . *In Proceedings of the Australian Poultry Science Sympsoium*, *21*, 44-49.

van der Wagt, I., de Jong, I. C., Mitchell, M. A., Molenaar, R., & van den Brand, H. (2020). A review on yolk sac utilization in poultry. *Poultry Science*, *99*(4), 2162-2175.

Veluri, S., & Olukosi, O. A. (2020). Metabolizable energy of soybean meal and canola meal as influenced by the reference diet used and assay method. *Animals*, *10*(11), 2132.

Verdal, H., Mignon-Grasteau, S., Jeulin, C., Le Bihan-Duval, E., Leconte, M., Mallet, S., Martin, C., & Narcy, A. (2010). Digestive tract measurements and histological adaptation in broiler lines divergently selected for digestive efficiency. *Poultry Science*, *89*(9), 1955-1961.

Vergara, P., Jimenez, M., Ferrando, C., Fernandez, E., & Gonalons, E. (1989). Age influence on digestive transit time of particulate and soluble markers in broiler chickens. *Poultry Science*, *68*(1), 185-189.

Vicente, B., Valencia, D. G., Pérez-Serrano, M., Lázaro, R., & Mateos, G. G. (2008). The effects of feeding rice in substitution of corn and the degree of starch gelatinization of rice on the digestibility of dietary components and productive performance of young pigs. *Journal of Animal Science*, *86*(1), 119-126.

Vohra, P. (1966). Energy concepts for poultry nutrition. *World's Poultry Science Journal*, *22*(1), 6-24.

Vohra, P. (1972). Evaluation of metabolizable energy for poultry. *World's Poultry Science Journal*, *28*(2), 204-214.

Vohra, P., & Kratzer, F. H. (1967). Absorption of barium sulfate and chromic oxide from the chicken gastrointestinal tract. *Poultry Science*, *46*(6), 1603-1604.

Vtlariño, M., Picard, M. L., Melcion, J. P., & Faure, J. M. (1996). Behavioural adaptation of laying hens to dilution of diets under mash and pellet form. *British Poultry Science*, *37*(5), 895-907.

Wang, X., & Parsons, C. M. (1998). Effect of raw material source, processing systems, and processing temperatures on amino acid digestibility of meat and bone meals. *Poultry Science*, *77*(6), 834-841.

Waring, J. J., & Shannon, D. W. F. (1969). Studies on the energy and protein values of soya bean meal and two varieties of field beans using colostomised laying hens. *British Poultry Science*, *10*(4), 331-336.

Washburn, K. W. (1991). Efficiency of feed utilization and rate of feed passage through the digestive system. *Poultry Science*, *70*(3), 447-452.

Washburn, K. W., Guill, R. A., & Edwards Jr, H. M. (1975). Influence of genetic differences in feed efficiency of young chickens on derivation of metabolizable energy from the diet and nitrogen retention. *The Journal of Nutrition*, *105*(6), 726-732.

Wiseman, J. (1999). Optimizing the role of fats in diet formulation. In *Proceedings of the Australian Poultry Science Symposium*,*11*, 8-15.

Wiseman, J. (2006). Variations in starch digestibility in non-ruminants. *Animal Feed Science and Technology*, *130*(1-2), 66-77.

Wiseman, J., Nicol, N. T., & Norton, G. (2000). Relationship between apparent matabolisable (AME) values and in vivo/in vitro strach digestibility of wheat for broilers. *World's Poultry Science Journal*, *56*(4), 305-318.

Wiseman, J., Powles, J., & Salvador, F. (1998). Comparison between pigs and poultry in the prediction of the dietary energy value of fats. *Animal Feed Science and Technology*, *71*(1-2), 1-9.

Woyengo, T. A., & Wilson, J. (2019). Enhancing nutrient utilization in maize for broiler chickens by superdosing phytase. *Animal Feed Science and Technology*, *252*, 34-40.

Woyengo, T. A., Emiola, A., Owusu-Asiedu, A., Guenter, W., Simmins, P. H., & Nyachoti, C. M. (2010). Performance and nutrient utilization responses in broilers fed phytase supplemented mash or pelleted corn-soybean meal-based diets. *The Journal of Poultry Science*, *47*(4), 310-315.

WPSA (WORLD'S POULTRY SCIENCE ASSOCIATION).(1989). *European table of energy values for poultry feedstuffs*. Nutrition of the European Federation of Branches. Subcommittee Energy of the Working Group (Beekbergen).

Wu, S. B., Choct, M., & Pesti, G. (2020). Historical flaws in bioassays used to generate metabolizable energy values for poultry feed formulation: a critical review. *Poultry Science*, *99*(1), 385-406.

Wu, Y. B., Ravindran, V., Thomas, D. G., Birtles, M. J., & Hendriks, W. H. (2004). Influence of method of whole wheat inclusion and xylanase supplementation on the performance, apparent metabolisable energy, digestive tract measurements and gut morphology of broilers. *British Poultry Science*, *45*(3), 385-394.

Yaghobfar, A. (2001). Effect of genetic line, sex of birds and the type of bioassay on the metabolisable energy value of maize. *British Poultry Science*, *42*(3), 350-353.

Yaghobfar, A. (2003). The effects of strain, age and sex of birds on the determined metabolisable energy of maize and fishmeal. *British Poultry Science*, *44*(S1), 38-39.

Yaghobfar, A. (2013). Effects of Bioassay and Age on Amino Acid Digestibility and Metabolizable Energy of Soybean, Sunflower and Canola Meals. *Iranian Journal of Applied Animal Science*, *3*(2), 249-261.

Yang, Z., Pirgozliev, V. R., Rose, S. P., Woods, S., Yang, H. M., Wang, Z. Y., & Bedford, M. R. (2020). Effect of age on the relationship between metabolizable energy and digestible energy for broiler chickens. *Poultry Science*, *99*(1), 320-330.

Yegani, M., & Korver, D. R. (2012). Prediction of variation in energetic value of wheat for poultry. *Canadian Journal of Animal Science*, *92*(3), 261-273.

Zaefarian, F., Cowieson, A. J., Pontoppidan, K., Abdollahi, M. R., & Ravindran, V. (2021). Trends in feed evaluation for poultry with emphasis on in vitro techniques. *Animal Nutrition*, *7*(2), 268-281.

Zang, J. J., Piao, X. S., Huang, D. S., Wang, J. J., Ma, X., & Ma, Y. X. (2009). Effects of feed particle size and feed form on growth performance, nutrient metabolizability and intestinal morphology in broiler chickens. *Asian-Australasian Journal of Animal Sciences*, *22*(1), 107-112.

Zatari, I. M., & Sell, J. L. (1990). Effects of pelleting diets containing sunflower meal on the performance of broiler chickens. *Animal Feed Science and Technology*, *30*(1-2), 121-129.

Zelenka, J. (1968). Influence of the age of chicken on the metabolisable energy values of poultry diets. *British Poultry Science*, *9*(2), 135-142.

Zelenka, J. (1970). Comparison of the classical and N-corrected metabolizable energy values of a diet for chickens during their growth. *Acta Universitatis Agriculturae, Facultas Agronomica*, *18*, 113-120.

Zelenka, J. (1997). Effects of sex, age and food intake upon metabolisable energy values in broiler chickens. *British Poultry Science*, *38*(3), 281-284.

Zelenka, J., & Ceresnakova, Z. (2005). Effect of age on digestibility of starch in chickens with different growth rate. *Czech Journal of Animal Science*, *50*, 411-415.

Zimonja, O., Stevnebø, A., & Svihus, B. (2007). Nutritional value of diets for broiler chickens as affected by fat source, amylose level and diet processing. *Canadian Journal of Animal Science*, *87*(4), 553-562.

Zuidhof, M. (2019). A review of dietary metabolizable and net energy: Uncoupling heat production and retained energy. *Journal of Applied Poultry Research*, *28*(2), 231-241.

APPENDIX

Statement of contribution to doctoral thesis containing publications

Statement of contribution to doctoral thesis containing publications from Chapters 3, 4, 5, 6, 7 and 8 are attached.

MASSEY UNIVERSITY
TE KUNENGA KI PŪREHUROA
UNIVERSITY OF NEW ZEALAND

GRADUATE RESEARCH SCHOOL

STATEMENT OF CONTRIBUTION
DOCTORATE WITH PUBLICATIONS/MANUSCRIPTS

We, the candidate and the candidate's Primary Supervisor, certify that all co-authors have consented to their work being included in the thesis and they have accepted the candidate's contribution as indicated below in the *Statement of Originality*.

Name of candidate:	Mahmoud Khalil
Name/title of Primary Supervisor:	Dr. M. Reza Abdollahi
In which chapter is the manuscript /published work:	Chapter 3

Please select one of the following three options:

(●) The manuscript/published work is published or in press

- Please provide the full reference of the Research Output:

Khalil, M. M., Abdollahi, M. R., Zaefarian, F., & Ravindran, V. (2021). Influence of feed form on the apparent metabolisable energy of feed ingredients for broiler chickens. Animal Feed Science and Technology, 271, 114754.

( ) The manuscript is currently under review for publication – please indicate:

- The name of the journal:
- The percentage of the manuscript/published work that was contributed by the candidate:
- Describe the contribution that the candidate has made to the manuscript/published work:

( ) It is intended that the manuscript will be published, but it has not yet been submitted to a journal

Candidate's Signature:	Mahmoud Khalil Digitally signed by Mahmoud Khalil Date: 2022.06.30 14:55:57 +12'00'
Date:	30-Jun-2022
Primary Supervisor's Signature:	Reza Abdollahi Digitally signed by Reza Abdollahi Date: 2022.07.01 08:47:36 +12'00'
Date:	1-Jul-2022

This form should appear at the end of each thesis chapter/section/appendix submitted as a manuscript/ publication or collected as an appendix at the end of the thesis.

MASSEY UNIVERSITY
TE KUNENGA KI PŪREHUROA
UNIVERSITY OF NEW ZEALAND

GRADUATE RESEARCH SCHOOL

STATEMENT OF CONTRIBUTION
DOCTORATE WITH PUBLICATIONS/MANUSCRIPTS

We, the candidate and the candidate's Primary Supervisor, certify that all co-authors have consented to their work being included in the thesis and they have accepted the candidate's contribution as indicated below in the *Statement of Originality*.

Name of candidate:	Mahmoud Khalil
Name/title of Primary Supervisor:	Dr. M. Reza Abdollahi
In which chapter is the manuscript /published work:	Chapter 4

Please select one of the following three options:

(●) The manuscript/published work is published or in press

- Please provide the full reference of the Research Output:

Khalil, M. M., Abdollahi, M. R., Zaefarian, F., Chrystal, P. V., & Ravindran, V. (2021). Apparent metabolizable energy of cereal grains for broiler chickens is influenced by age. Poultry Science, 100, 101288.

( ) The manuscript is currently under review for publication – please indicate:

- The name of the journal:
- The percentage of the manuscript/published work that was contributed by the candidate:
- Describe the contribution that the candidate has made to the manuscript/published work:

( ) It is intended that the manuscript will be published, but it has not yet been submitted to a journal

Candidate's Signature:	Mahmoud Khalil Digitally signed by Mahmoud Khalil Date: 2022.06.30 14:55:57 +12'00'
Date:	30-Jun-2022
Primary Supervisor's Signature:	Reza Abdollahi Digitally signed by Reza Abdollahi Date: 2022.07.01 08:48:53 +12'00'
Date:	1-Jul-2022

This form should appear at the end of each thesis chapter/section/appendix submitted as a manuscript/ publication or collected as an appendix at the end of the thesis.

MASSEY UNIVERSITY
TE KUNENGA KI PŪREHUROA
UNIVERSITY OF NEW ZEALAND

GRADUATE RESEARCH SCHOOL

STATEMENT OF CONTRIBUTION
DOCTORATE WITH PUBLICATIONS/MANUSCRIPTS

We, the candidate and the candidate's Primary Supervisor, certify that all co-authors have consented to their work being included in the thesis and they have accepted the candidate's contribution as indicated below in the *Statement of Originality*.

Name of candidate:	Mahmoud Khalil
Name/title of Primary Supervisor:	Dr. M. Reza Abdollahi
In which chapter is the manuscript /published work:	Chapter 5

Please select one of the following three options:

(●) The manuscript/published work is published or in press

- Please provide the full reference of the Research Output:

Khalil, M. M., Abdollahi, M. R., Zaefarian, F., Chrystal, P. V., & Ravindran, V. (2022). Influence of Broiler Age on the Apparent Metabolizable Energy of Cereal Grains Determined Using the Substitution Method. Animals, 12, 183.

( ) The manuscript is currently under review for publication – please indicate:

- The name of the journal:
- The percentage of the manuscript/published work that was contributed by the candidate:
- Describe the contribution that the candidate has made to the manuscript/published work:

( ) It is intended that the manuscript will be published, but it has not yet been submitted to a journal

Candidate's Signature:	Mahmoud Khalil Digitally signed by Mahmoud Khalil Date: 2022.06.30 14:55:57 +12'00'
Date:	30-Jun-2022
Primary Supervisor's Signature:	Reza Abdollahi Digitally signed by Reza Abdollahi Date: 2022.07.01 08:49:32 +12'00'
Date:	1-Jul-2022

This form should appear at the end of each thesis chapter/section/appendix submitted as a manuscript/ publication or collected as an appendix at the end of the thesis.

GRS Version 5 – 13 December 2019
DRC 19/09/10

MASSEY UNIVERSITY
TE KUNENGA KI PŪREHUROA
UNIVERSITY OF NEW ZEALAND

GRADUATE RESEARCH SCHOOL

STATEMENT OF CONTRIBUTION
DOCTORATE WITH PUBLICATIONS/MANUSCRIPTS

We, the candidate and the candidate's Primary Supervisor, certify that all co-authors have consented to their work being included in the thesis and they have accepted the candidate's contribution as indicated below in the *Statement of Originality*.

Name of candidate:	Mahmoud Khalil
Name/title of Primary Supervisor:	Dr. M. Reza Abdollahi
In which chapter is the manuscript /published work:	Chapter 6

Please select one of the following three options:

○ The manuscript/published work is published or in press

- Please provide the full reference of the Research Output:

○ The manuscript is currently under review for publication – please indicate:

- The name of the journal:
- The percentage of the manuscript/published work that was contributed by the candidate:
- Describe the contribution that the candidate has made to the manuscript/published work:

◉ It is intended that the manuscript will be published, but it has not yet been submitted to a journal

Candidate's Signature:	Mahmoud Khalil Digitally signed by Mahmoud Khalil Date: 2022.06.30 14:55:57 +12'00'
Date:	30-Jun-2022
Primary Supervisor's Signature:	Reza Abdollahi Digitally signed by Reza Abdollahi Date: 2022.07.01 08:50:04 +12'00'
Date:	1-Jul-2022

This form should appear at the end of each thesis chapter/section/appendix submitted as a manuscript/ publication or collected as an appendix at the end of the thesis.

MASSEY UNIVERSITY
TE KUNENGA KI PŪREHUROA
UNIVERSITY OF NEW ZEALAND

GRADUATE RESEARCH SCHOOL

STATEMENT OF CONTRIBUTION
DOCTORATE WITH PUBLICATIONS/MANUSCRIPTS

We, the candidate and the candidate's Primary Supervisor, certify that all co-authors have consented to their work being included in the thesis and they have accepted the candidate's contribution as indicated below in the *Statement of Originality*.

Name of candidate:	Mahmoud Khalil
Name/title of Primary Supervisor:	Dr. M. Reza Abdollahi
In which chapter is the manuscript /published work:	Chapter 7

Please select one of the following three options:

(●) The manuscript/published work is published or in press

- Please provide the full reference of the Research Output:

Khalil, M. M., Abdollahi, M. R., Zaefarian, F., & Ravindran, V. (2020). Measurement of ileal endogenous energy losses and true ileal digestible energy of cereal grains for broiler chickens. Poultry Science, 99(12), 6809-6817.

( ) The manuscript is currently under review for publication – please indicate:

- The name of the journal:
- The percentage of the manuscript/published work that was contributed by the candidate:
- Describe the contribution that the candidate has made to the manuscript/published work:

( ) It is intended that the manuscript will be published, but it has not yet been submitted to a journal

Candidate's Signature:	Mahmoud Khalil Digitally signed by Mahmoud Khalil Date: 2022.06.30 14:55:57 +12'00'
Date:	30-Jun-2022
Primary Supervisor's Signature:	Reza Abdollahi Digitally signed by Reza Abdollahi Date: 2022.07.01 08:50:43 +12'00'
Date:	1-Jul-2022

This form should appear at the end of each thesis chapter/section/appendix submitted as a manuscript/ publication or collected as an appendix at the end of the thesis.

MASSEY UNIVERSITY
TE KUNENGA KI PŪREHUROA
UNIVERSITY OF NEW ZEALAND

GRADUATE RESEARCH SCHOOL

STATEMENT OF CONTRIBUTION
DOCTORATE WITH PUBLICATIONS/MANUSCRIPTS

We, the candidate and the candidate's Primary Supervisor, certify that all co-authors have consented to their work being included in the thesis and they have accepted the candidate's contribution as indicated below in the *Statement of Originality*.

Name of candidate:	Mahmoud Khalil
Name/title of Primary Supervisor:	Dr. M. Reza Abdollahi
In which chapter is the manuscript /published work:	Chapter 8

Please select one of the following three options:

(●) The manuscript/published work is published or in press

- Please provide the full reference of the Research Output:

Khalil, M. M., Abdollahi, M. R., Zaefarian, F., Chrystal, P. V., & Ravindran, V. (2022). Influence of age and dietary cellulose levels on ileal endogenous energy losses in broiler chickens. Poultry Science, 101 (7), 101948.

( ) The manuscript is currently under review for publication – please indicate:

- The name of the journal:
- The percentage of the manuscript/published work that was contributed by the candidate:
- Describe the contribution that the candidate has made to the manuscript/published work:

( ) It is intended that the manuscript will be published, but it has not yet been submitted to a journal

Candidate's Signature:	Mahmoud Khalil Digitally signed by Mahmoud Khalil Date: 2022.06.30 14:55:57 +12'00'
Date:	30-Jun-2022
Primary Supervisor's Signature:	Reza Abdollahi Digitally signed by Reza Abdollahi Date: 2022.07.01 08:51:22 +12'00'
Date:	1-Jul-2022

This form should appear at the end of each thesis chapter/section/appendix submitted as a manuscript/ publication or collected as an appendix at the end of the thesis.