INVESTIGATIONS INTO THE IMPACT OF FEED FORM AND AGE ON THE STANDARDISED ILEAL AMINO ACID DIGESTIBILITY OF FEED INGREDIENTS FOR BROILER CHICKENS

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ABSTRACT

The importance of measurement of standardised ileal digestibility coefficients (SIDC) of amino acids (AA) in feed ingredients and their value in improving *inter alia* the precision of broiler feed formulations needs no elaboration. Databases on the SIDC AA of ingredients are accumulating during the past decade and various factors that influence the digestibility have been identified. Two aspects that remain unexplored hitherto in detail are the effects of feed form (FF) and broiler age. Previous AA digestibility assays were accomplished using mash diets because of the simplicity and the fact that research facilities often do not have access to pelleting equipment. As commercial broilers are fed pelleted diets, the data derived from mash diet may not be directly applicable. Another limitation is that, despite the potential age effects on AA digestibility, no study to date has investigated the influence of age on the SIDC AA in ingredients for the whole growth period of broilers. Lack of age-appropriate basal endogenous AA (EAA) flow data for the correction of apparent ileal digestibility coefficients (AIDC) is another constraint. Most of the existing EAA flow data were generated using older birds (21 to 42 d of age). The focus of this thesis research was to address these critical aspects.

The first two experimental studies in Chapters 3 and 4 were conducted to determine the effect of FF (mash vs. pellet) on the SIDC AA in four grains (maize, wheat, sorghum and barley) and three protein sources (PS; soybean meal; SBM, meat and bone meal; MBM, and CM; canola meal) in broilers, respectively. Regardless of the grain type, FF had no significant ($P > 0.05$) influence on the SIDC of any AA, except for Cys and Pro, which were higher ($P < 0.05$) in mash diets. The effect of FF was more pronounced in the SIDC

AA of PS. No influence of FF ($P > 0.05$) was observed for the SIDC of individual indispensable AA (IAA), except for His, the digestibility of which was lower ($P < 0.05$) in the pelleted diets. The SIDC of all individual dispensable AA (DAA), average digestibility of DAA and total AA (TAA) were reduced ($P < 0.05$) by pelleting. Cysteine was the most affected DAA, with a 15.4% reduction in SIDC in pelleted diets in comparison with mash. These results showed that the use of SIDC AA data generated from feeding mash diets may overestimate the AA digestibility in feed ingredients and affect the precision of broiler feed formulations. For this reason, pelleted assay diets were used in succeeding trials.

The third study, reported in Chapter 5, was carried out to investigate basal EAA flow at different ages (7, 14, 21, 28, 35 and 42 d post-hatch) of broilers, following feeding of a nitrogen-free diet (NFD). The basal EAA flow of all individual and total AA were reduced quadratically ($P < 0.05$ to 0.001) as the birds grew older. The EAA flows were higher on d 7, then declined on d 14 and plateaued until d 35. A further decrease in the EAA flow was observed on d 42. These age-specific values were used in the correction of AIDC to SIDC in all ensuing thesis studies.

The study presented in Chapter 6 was conducted to determine the influence of age on the SIDC AA in two grain sources (wheat and sorghum) in broilers. Six broiler ages (d 7, 14, 21, 28, 35, and 42 post-hatch) were investigated. Two assay diets were formulated to contain 938 g/kg of each grain as the only source of AA. Each assay diet, in pelleted form, was offered to birds for 4 d during the six periods namely d 3-7, 10-14, 17-21, 24-28, 31-35 and 38-42 prior to ileal digesta collection. In the case of wheat, no age

influence (P > 0.05) was observed on the SIDC of average of IAA and DAA, though the average of TAA tended (linear, P = 0.09) to increase with advancing broiler age. In sorghum, the average SIDC of IAA, DAA and TAA were higher (linear or quadratic; P < 0.05 to 0.01) at d 7, declined at d 14 and then evened out. Among the IAA, the SIDC of Arg, His, Ile, Leu, Lys, Thr, Val, and the SIDC of all individual DAA (with the exception of Cys) reduced with advancing age (linear or quadratic, P < 0.05 to 0.001).

The study reported in Chapter 7 was conducted to measure the SIDC of AA in maize and barley at six ages (d 7, 14, 21, 28, 35, and 42 post-hatch). Two assay diets were formulated to contain 938 g/kg of either maize or barley as the sole source of dietary AA. In maize, the average SIDC of IAA and TAA was affected in a quadratic manner (P < 0.05) with the values being higher at d 7 that dropped at d 14, increased and evened out between d 21 and 35, and decreased again at d 42. A linear influence (P < 0.05) was observed in the average SIDC of DAA. The SIDC of average IAA, DAA and TAA in barley was influenced (quadratic; P < 0.001) by age. The digestibility values increased from d 7 to 21 and then plateaued until d 42. The SIDC of all AA in barley increased (quadratic; P < 0.05 to 0.001) as the birds grew older.

The final study, presented in Chapter 8, was conducted to determine the influence of broiler age on the SIDC AA in two PS (SBM and CM). Six age groups (d 7, 14, 21, 28, 35, and 42) were utilised. Two assay diets were formulated to contain either SBM (413 g/kg) or CM (553 g/kg) as the only source of dietary AA. In SBM, the average SIDC of IAA and TAA were not affected (linear or quadratic; P > 0.05) by age. The average SIDC of DAA in SBM was affected in a quadratic manner (P < 0.05) and the highest value was

observed at d 7, that followed by a decline from d 14 to 28, and increased beyond d 35. The average SIDC of DAA and TAA in CM were higher (quadratic; $P < 0.05$ to 0.001) from d 7 to 14, reduced at d 21 and then increased beyond d 28. The average SIDC of IAA dropped between d 7 and 28, and followed by an increase thereafter.

The novelties of this thesis research are that (i) no previous study has investigated the SIDC AA of feed ingredients from hatch to the end of broiler growth cycle, (ii) this is the first study reporting age-appropriate basal EAA flows and (iii) the use of pelleted assay diets that resembled the FF commonly used in the broiler industry.

There were several original findings in this thesis. First, pelleting had substantial impact on the SIDC AA of feed ingredients and FF influence was more evident in case of ingredients with higher protein contents. Second, the EAA flow was higher in younger birds and reduced with advancing broiler age. Third, the age effect on the SIDC AA was variable depending on the ingredient and specific AA, and that the age effect on AA digestibility need to be considered in ingredient matrices for precise feed formulation.

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Some studies, completed during the candidature and reported in this thesis have been presented in the following communications:

Refereed scientific papers

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LIST OF ABBREVIATIONS

AA	Amino acids
AID	Apparent ileal digestibility
AIDC	Apparent ileal digestibility coefficients
Ala	Alanine
AME	Apparent metabolisable energy
AMEn	Nitrogen-corrected apparent metabolisable energy
ANF	Antinutritional factor
ANOVA	Analysis of variance
Arg	Arginine
Asp	Aspartic acid
BW	Body weight
Ca	Calcium
CF	Crude fat
CM	Canola meal
CP	Crude protein
cPs	Centipoise
Cys	Cysteine
d	Days
DAA	Dispensable amino acids
DFI	Daily feed intake
DM	Dry matter
DMI	Dry matter intake
DWG	Daily weight gain
EAA	Endogenous amino acids
FF	Feed form
FI	Feed intake
g	Gram
GE	Gross energy
GIT	Gastrointestinal tract
GLM	General linear model
Glu	Glutamic acid
Gly	Glycine
GT	Grain type
h	Hours
HCl	Hydrochloric acid
His	Histidine
HPLC	High performance liquid chromatography
IAA	Indispensable amino acids
Ile	Isoleucine
kg	Kilogram
Leu	Leucine
Lys	Lysine
MBM	Meat and bone meal

Met	Methionine
mg	Milligram
MJ	Mega joule
mL	Millilitre
mm	Millimetre
N	Nitrogen
Na	Sodium
NDF	Neutral detergent fibre
NFD	Nitrogen free diet
NH_3	Ammonia
NRC	National research council
NSP	Non-starch polysaccharide
P	Phosphorus
Pro	Proline
PS	Protein sources
SBM	Soybean meal
S	Seconds
SEM	Standard error of mean
Ser	Serine
SID	Standardised ileal digestibility
SIDC	Standardised ileal digestibility coefficients
TAA	Total amino acids
Ti	Titanium
Thr	Threonine
Trp	Tryptophan
UV	Ultraviolet
Val	Valine

CHAPTER 1 GENERAL INTRODUCTION

The poultry industry has progressed remarkably over the past 60 years. Constant improvements in the production standards of broilers through genetic selection has turned it as the most successful animal industry (Zuidhof *et al.*, 2014). Feed represents 70% of the total cost of modern broiler production (Nolan *et al.*, 2010). Protein is the second most expensive component of poultry diets after energy. Proteins are involved in number of functions (metabolic functions, growth and development, immunity and health), but their main role is to supply the amino acids (AA) for protein synthesis in the body. Dietary supply of correct amounts and ratios of different AA is essential for optimum growth and productivity. Accurate knowledge on the AA digestibility in raw materials is crucial for accuracy and efficiency of feed formulation, and for sustainable use of feed resources.

Describing the available AA in raw materials in terms of digestible AA is superior to total AA in expressing the amounts that actually become available for maintenance and production purposes (Rostagno *et al.*, 1995). The digestibility of AA for poultry can be measured by sampling and analysis of ileal digesta or excreta. Excreta-based digestibility measurements, however, suffer from the modifying and variable effects of caecal microflora on dietary protein utilisation and the contribution of microbial proteins to AA excretion in the excreta. In the ileal digestibility method, these confounding issues can be avoided (Ravindran and Bryden, 1999). Currently, standardised ileal digestibility (SID) is preferred over the apparent ileal digestibility

(AID) method. The calculation of SID involves a correction for the inevitable endogenous AA (EAA) flows from the gastrointestinal tract (Lemme *et al.*, 2004). The additivity of SID over AID AA has been well documented. Formulation of broiler diet based on SID AA is important in terms of accuracy and, reductions of feed cost and nitrogen pollution into the environment (Cowieson *et al.*, 2019).

Age of the bird has a significant influence on the digestion and absorption of dietary AA (Tarvid, 1995). Limited studies, however, have investigated the age effect on the AA digestibility in broilers and the results are inconsistent (Fonolla *et al.*, 1981; Wallis and Balnave, 1984). There is a consensus that the digestibility generally increases with advancing age (Tarvid, 1995; Huang *et al.*, 2005), but some studies have reported a decrease in the AA digestibility in older birds (Fonolla *et al.*, 1981; Zuprizal *et al.*, 1992). Despite the possible age effect, only few reports (Zuprizal *et al.*, 1992; Garcia *et al.*, 2007; Adedokun *et al.*, 2007a, 2008; Toghyani *et al.*, 2015; Szczurek *et al.*, 2020) exist on the standardised AA digestibility estimates and the reports are limited to only two to three broiler ages. No studies to date have investigated the SID coefficients (SIDC) of AA of feed ingredients from hatching to the end of broiler growth cycle. Lack of age-dependant basal EAA flow data for the correction of apparent AA digestibility is a constraint of early studies. Limited studies (Adedokun *et al.*, 2007a, 2008; Toghyani *et al.*, 2015; Szczurek *et al.*, 2020) have used the age-specific basal EAA flow to determine the SIDC AA. Another limitation of previous AA digestibility studies is that the assay

diets used were offered in mash form because of its simplicity. Commercially, broilers are offered pelleted diets and the applicability of data generated with mash diets to pelleted diets could be questioned. There is evidence that feed form (FF; mash vs. pellet) might affect nutrient digestibility (Abdollahi *et al.*, 2013a,b; 2018a; Selle *et al.*, 2012).

The overall aim of this thesis research was to investigate some pivotal factors influencing AA digestibility, refine the protocol of determining SIDC AA using age-specific EAA flows, and establish a comprehensive dataset of the SIDC AA in common feed ingredients for broiler from hatching up to marketing age.

The specific objectives of the studies conducted in this thesis were:

1. To evaluate the influence of FF (mash vs. pellet) on the SIDC of nitrogen (N) and AA in major grains (maize, sorghum, wheat, and barley) used in broiler diets (Chapter 3).
2. To determine the impact of FF (mash vs. pellet) on the SIDC of N and AA in three mostly used protein sources (soybean meal [SBM]; meat and bone meal [MBM]; and canola meal [CM]) in broiler (Chapter 4).
3. To measure the basal ileal endogenous N and AA flows at different broiler ages (d 7, 14, 21, 28, 35, and 42) using an N-free diet (Chapter 5).

4. To examine the age effect on the SIDC of N and AA in wheat and sorghum at six-different broiler ages, namely d 7, 14, 21, 28, 35, and 42 (Chapter 6).
5. To evaluate the SIDC of N and AA in maize and barley at six-different broiler ages, namely d 7, 14, 21, 28, 35, and 42 (Chapter 7).
6. To investigate whether broiler age has an impact on the SIDC of N and AA in two common vegetable protein sources (SBM and CM) (Chapter 8).

CHAPTER 2 LITERATURE REVIEW

2.1. Protein

2.1.1. Introduction

Protein, a polymer of α-amino acids (AA) linked together by peptide bonds, is the building block of different body tissues. Besides the synthesis of body proteins, it acts in the rejuvenation and growth of muscle tissues. Protein is hydrolysed in the digestive system into AA that are re-assembled after absorption and metabolised. In reality, requirements of dietary protein represent the AA needed in the diet. Amino acids are the primary constituents of structural and protective tissues (skin, feathers, bone matrix and ligaments) and the soft tissues (organs and muscles) in the body, and serve a wide range of metabolic roles. Protein is the main component of plasma proteins, enzymes, hormones and antibodies; each AA has a specific role in metabolic functions and play vital roles in the physiology of animals (NRC, 1994; Pond *et al.*, 1995). Since protein is the second most expensive nutrient in poultry diet, knowledge of the requirements for protein and AA is necessary to balance for optimum protein and AA concentrations in broiler diets (Beski *et al.*, 2015). In practical situation and industry practices, accurate measurements of the AA digestibility in broiler feed ingredients are important for increasing efficiency and precision of feed formulation, sustainable use of raw materials, and reducing nitrogenous pollution in the environment (Cowieson *et al.*, 2019).

2.1.2. Protein digestion in poultry

2.1.2.1. Background

To understand the protein digestion in poultry, a description of the anatomy and physiology of the gastrointestinal tract (GIT) of birds is required. The digestive tract of avian species starts at the beak, followed by a toothless mouth, tongue, pharynx, oesophagus, crop, proventriculus, gizzard, small intestine (duodenum, jejunum and ileum), caeca, cloaca and finally vent. There are also accessory organs such as pancreas, intestinal lymphoreticular tissue, bursa and, biliary and salivary systems (Kokoszyński *et al.*, 2017). The GIT plays an important role in breaking down the dietary macromolecules into simple micromolecules ready for absorption. Each segment of the GIT has specific functions that include acquiring, digesting and absorbing nutrients. Animals, including chickens, do not digest or absorb all the ingested dietary protein and AA, and the undigested portion is a substrate of microbial action in the hindgut (Moughan *et al.*, 2014).

Digestion of protein is a highly complex process that combines hydrolysis and absorption of dietary proteins, their processing and concurrent secretion in the body. This process involves the recovery of both the exogenous proteins of dietary origin and endogenous proteins secreted within the bird (Cowieson, 2014). The digestive enzymes are also made of protein and form part of the endogenous losses (Rynsburger, 2009). Various non-protein components in the diet such as the fibre and antinutritional factors have significant influence on protein digestion (Smits and Annison, 1996). Almost all the organs in

the GIT play a role in protein digestion. Though protein digestion in the crop of birds is non-existent, there is presence of acidogenic bacteria in the crop, which could decrease the digesta pH and help protein digestion in the proventriculus (Hinton *et al.*, 1990). In young birds, crop microbiome is not fully developed and the reduced digesta pH is not as notable as in older birds (Hinton *et al.*, 2000).

Proventriculus is the first site of protein digestion where the ingested dietary proteins are exposed to hydrochloric acid (HCl), denaturing protein and exposing peptide bonds to enzyme hydrolysis. The distended proventriculus causes the release of acetylcholine that binds to G cells in the proventriculus stimulating the release of gastrin (Rynsburger, 2009). Gastrin stimulates the release of histamine from enterochromaffin- like cells. Acetylcholine, gastrin and histamine bind to parietal cells that stimulate the release of HCl, reducing the pH of both proventriculus and gizzard contents (Bohak, 1973; Khan and James, 1998). Gastrin also stimulates the release of pepsinogen that is converted to pepsin at pH of ≥ 2. Adequate secretion of HCl is important for this conversion. The first enzyme that initiates protein digestion is pepsin that cleaves the N-terminal of aromatic AA (Phe, Trp and Tyr). The net result of acid denaturation and pepsin hydrolysis is the formation of peptides of smaller molecular weight (Auer and Glick, 1984). These peptides are further degraded in the small intestine by enzymes secreted by the pancreas and the small intestine. Besides pepsin, several other enzymes play roles in protein digestion and each of these enzymes target specific peptide bonds.

Pancreatic proteinases include trypsin, chymotrypsin, elastase and, carboxypeptidase A and B. These enzymes are stored as inactive zymogens in the pancreas and activated by enterokinases located in the brush border of enterocytes (Puigserver and Desnuell, 1975; Wang *et al.*, 1995). Secretion of these enzymes in the intestine causes the activation of trypsinogen into trypsin by enteropeptidase (Light and Fonseca, 1984). Presence of trypsin then activates the conversion of chymotrypsinogen to chymotrypsin (Desnuelle, 1960), proelastase to elastase (Brown and Wold, 1973), and procarboxypeptidase to carboxypeptidase (Puigserver and Desnuell, 1975). These enzymes catalyse specific bonds and AA. For example, trypsin only binds to the positively charged side chains of Lys and Arg residues, facilitating the hydrolysis of these peptide bonds. Elastase binds small side chain AA such as Gly and Ala (Brown and Wold, 1973). The side chains of aromatic or large hydrophobic AA residue are bound by chymotrypsin that catalyses leucyl, methionyl, asparaginyl, and glutamyl residues (Desnuelle, 1960).

Carboxypeptidase A hydrolyses only the peptide bond adjacent to the C-terminal end of a polypeptide chain, whereas carboxypeptidase B catalyses the hydrolysis of basic AA (Lys and Arg) that are also from the C-terminal end of polypeptide chains. Carboxypeptidase A and B catalyse the release of free AA from oligopeptides. Membrane hydrolysis is the final step in protein digestion and, facilitated by the pancreatic exopeptidases (carboxypeptidase A and B) and two types of brush border enzymes namely the aminopeptidases and dipeptidases

(Desnuelle and Figarella, 1979). These enzymes, working together, catalyse the reactions that break small oligopeptides and dipeptides to AA for absorption through the small intestinal wall.

After absorption, extensive catabolism of AA occurs in small intestine that modulates the entry of dietary AA into the portal circulation and plasma (Figure 2.1; Riedijk *et al.*, 2007; Chen, 2017). Every AA has its unique catabolic pathway. A series of reactions initiate AA catabolism including transamination, deamination, oxidative deamination, decarboxylation, dehydroxylation, hydroxylation, oxidation, reduction and dehydrogenation (Wu, 2009). The notable metabolites of AA catabolism are ammonia (NH_3), carbon dioxide, ketone bodies, glucose, long and short-chain fatty acids, urea and uric acid, polyamines and other nitrogenous substances (Morris, 2007; Montanez *et al.*, 2008). Physiological concentration of AA and their metabolites are important for different metabolic pathways of nutrition and physiology of animals. Increased level of some metabolites (NH_3, homocysteine) may also cause some pathological disorders (Wu, 2009). Ammonia, the potentially toxic metabolites produced by proteolytic fermentation in chicken, might disturb the intestinal mucosal development and decrease the villus height (Nousiainen, 1991; Chen, 2017). In poultry houses, gaseous NH_3 is emitted from the breakdown of undigested protein and uric acid in the litter (David *et al.*, 2015) which is a significant air quality concern. Uric acid converts to urea which hydrolyses to NH_3 in poultry litter (Liang *et al.*, 2014). Exposure to high NH_3 environment generates toxic effects in the

respiratory tract (Liu *et al.*, 2020), spleen (Zhao *et al.*, 2020), liver (Sa *et al.*, 2018) and intestine (Wang *et al.*, 2019). Ammonia can also alter the composition of intestinal microflora (Han *et al.*, 2021), negatively affecting bird performance (Caveny *et al.*, 1981; Miles *et al.*, 2004).

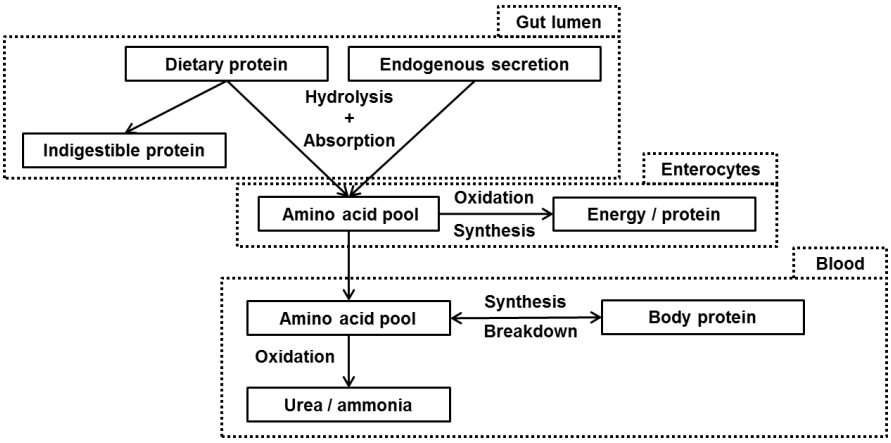

Figure 2.1. Schematic overview of post-absorption metabolism of dietary protein (Chen, 2017).

2.1.2.2. Endogenous nitrogen and amino acid secretion

Significant amounts of endogenous nitrogen (N) are secreted from the different segments of GIT during digestion and absorption of feed. The sources of these inevitable endogenous N secretion are digestive secretions (i.e. mucin, bile, gastric, pancreatic and intestinal secretions and microbial protein); metabolites of protein catabolism (peptides and free AA); mucoproteins and desquamated epithelial cells lining the gut (Nyachoti *et al.*, 1997; Moughan *et al.*, 1998). It has been reported that approximately 85% of the endogenous protein is recovered and retained in the body and the remainder is either excreted or modified by the hindgut microbiota. Hydrophobic or refractory endogenous proteins are poorly recovered, though the recovery rate of other

endogenous proteins is high (Fuller and Reeds, 1998). Forstner and Forstner (1994) stated that glycosylated domains of mucin that are rich in Ser, Thr and Pro are poorly recovered. However, a large proportion of endogenous protein (70%) is secreted distally from the gizzard and, therefore, does not undergo gastric digestion (Fuller and Reeds, 1998).

Amino acids from endogenously secreted proteins that escape the intestinal digestion and re-absorption, and enter the terminal ileum are lost to the animal (Moughan *et al.*, 1998). Digesta at the terminal ileum also contain microbial proteins that originate from the microbial activity in the small intestine. Since this bacterial protein is of non-dietary origin, it is included in the estimation of endogenous protein though it is not strictly endogenous (Moughan *et al.*, 2005; Miner-Williams *et al.*, 2009). Two distinct types of endogenous AA (EAA) losses, namely basal and specific occur at the terminal ileum. Basal endogenous losses are independent of diet type, composition of feedstuff and proportional to dry matter (DM) intake by the animals (Jansman *et al.*, 2002; Lemme *et al.*, 2004). These are inevitable losses and associated with the metabolic functions of animals (Cowieson *et al.*, 2009). On the other hand, specific endogenous losses include the losses induced by specific components in feedstuffs such as fibre and antinutritional factors (ANF; Blok *et al.*, 2017). There are different fractions that make up the recovered AA at the terminal ileum (Figure 2.2; Lemme *et al.*, 2004).

Quantification of these losses is important, especially for the estimation of true AA digestibility of feed ingredients (Ravindran and Bryden,

1999; Ravindran *et al.*, 2009). Quantification is also important to measure the metabolic costs related to protein turnover in the gut. Furthermore, better understanding of the factors affecting these losses will help to develop effective strategies to improve the utilisation of protein and AA (Ravindran *et al.*, 2009).

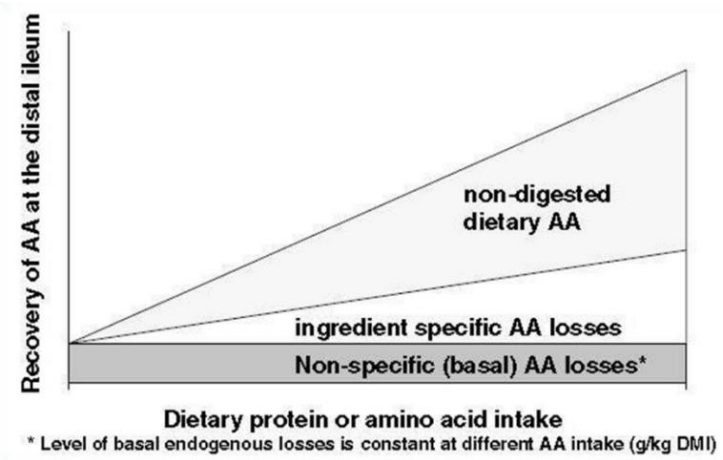

Figure 2.2. Amino acid losses at the distal ileum affected by the dietary protein and amino acid intake (Lemme *et al.*, 2004).

2.1.2.3. Limitations to protein digestion

As dietary protein sources are heterogeneous mixtures of proteins, protein is digested at different rates causing variation in the rates at which different AA are absorbed (Bryden *et al.*, 2009a). Proteins vary in their chemical composition and have different linkages with carbohydrates, lipids and other proteins. Therefore, the digestion of dietary protein is affected by these interactions and diet composition (Hughes and Choct, 1999).

The digestibility of protein or AA depends on the type of feed ingredient (Bryden *et al.*, 2009a). Most feedstuffs commonly used in poultry diets contain ANFs that reduce the digestion and absorption of

protein. The ANFs include protease inhibitors, lectins, polyphenolic compounds, saponins and non-starch polysaccharide (NSP; Bryden, 1996; Hughes and Choct, 1999). The three major NSPs found in feeds are cellulose, hemicellulose and pectin. The indigestible NSP in the cell wall matrix shields the digestion of protein and other nutrients (Ward, 2014). The ANFs also increase endogenous losses, resulting in lower apparent AA digestibility estimates.

High and non-optimal temperatures during feed processing can reduce the digestibility of or destroy the protein and AA (Abdollahi *et al.*, 2011; Frikha *et al.*, 2012). The most heat sensitive AA is Cys that is negatively affected at high processing temperatures, followed by Lys (Papadopoulos, 1989; Shirley and Parsons, 2000). The two major factors influencing Lys digestibility are the Maillard reaction and formation of Lys complexes during thermal processing (Huang *et al.*, 2005; Abdollahi *et al.*, 2010a). Raw soybeans contain several ANFs, but these are heat-labile and most are destroyed by heat treatment (Liener, 1994; Newkirk, 2010).

Age is one of the major factors influencing protein digestion in poultry (Tarvid, 1995; Rynsburger, 2009). The digestive tract and nutrient absorptive functions are not sufficiently developed in newly hatched chicks (Noy and Uni, 2010). Young birds are sensitive to inadequacies in protein digestion as there is a high AA demand for organ and muscle development (Ward, 2014).

Class, gender, and health status of birds may also affect the protein digestibility. Published data on the influence of bird type on AA digestibility are equivocal, but it appears that AA digestibility in broilers is generally higher compared to layers and roosters (Almirall *et al.*, 1995; Huang *et al.*, 2006). Male broilers showed lower AA digestibility than the females in a study by ten Doeschate *et al.* (1993).

2.2. Amino acids

2.2.1. Introduction

Amino acids are organic moieties containing both amino and acid groups. All AA, except Pro, have a primary amino group and a carboxyl group linked to the α-carbon atom (α-AA) (Wu, 2009). In β-AA, the amino group links to the β-carbon atom. Due to variations in their side chains, AA differ in their biochemical properties and functions. They are generally stable in aqueous solution at physiological pH (Wu *et al.*, 2007; Suenaga *et al.*, 2008).

Amino acids are recognised as the substrates for the synthesis of various nitrogenous compounds in animal body and key regulators of metabolism by controlling major metabolic pathways in various tissues and organs (Tesseraud *et al.*, 2011; Wu, 2013). These pathways are important for regulation of hormone secretion, gene expression, cell signalling, maintenance, growth, production, reproduction and immunity (Wu, 2009). Amino acids play various significant roles in the body homeostasis of animals (Figure 2.3; Wu, 2010).

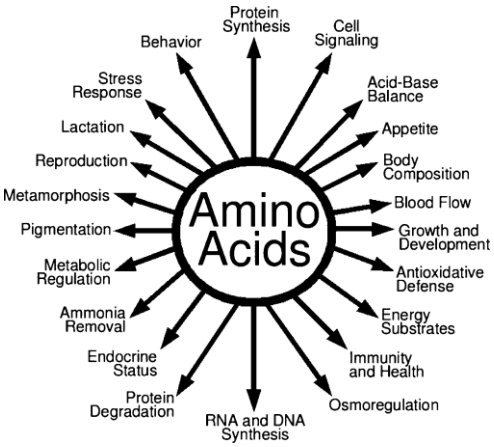

Figure 2.3. Roles of amino acids in nutrition and body homeostasis (Source: Wu, 2010).

Amino acids are the anabolic factors that induce protein synthesis rather than inhibiting proteolysis (Belloir *et al.*, 2015). Amino acids exhibit their roles in tissues and organs including visceral tissues and regulate the distribution of nutrients between tissues. Visceral tissues and organs are the first to be exposed to nutrients because of their anatomical position (Nieto and Lobley, 1999).

2.2.2. Essential (indispensable) and non-essential (dispensable) amino acids

All 22 AA present in body proteins are physiologically essential (Ravindran and Bryden, 1999). However, AA have been traditionally classified as nutritionally “essential” and “non-essential” based on growth and N balance of animals (Wu, 2013). Essential or indispensable AA (IAA) are those that poultry cannot synthesise at all or rapidly enough to meet metabolic requirements and must be supplied in diet. The 10 essential AA are Arg, His, Ile, Leu, Lys, Met, Thr, Trp,

Phe, and Val. Methionine (or Met + Cys) is considered as the first and Lys is the second AA limiting the growth and production in poultry. For young broilers, Gly (or Gly + Ser) is also considered indispensable, since the synthesis rate of this AA is inadequate to support optimum growth. Non-essential or dispensable AA (DAA) are those that can be synthesised from other AA. Tyrosine and Cys are semi-indispensable as they can be synthesised from Phe and Met, respectively. Adequate dietary supply of DAA reduces the necessity of synthesising those from essential AA. Therefore, combining dietary requirements of both protein and essential AA is an appropriate way for ensuring that all AA needed physiologically are supplied (Ravindran and Bryden, 1999).

2.2.3. An overview of different AA and their function in poultry

Amino acids unquestionably influence the protein metabolism, regulation of different metabolic pathways and are precursors of important molecules and, therefore, their deficiency reduce protein synthesis. Provision of all IAA in the diet in proper amounts and ratios is a prerequisite to maintain optimal rates of protein synthesis. The carcass composition, breast meat yield and abdominal fat content are also influenced by dietary AA contents (Leclercq, 1995; Conde-Aguilera *et al.*, 2013).

Methionine and Cysteine

Methionine, an IAA for poultry and the precursor of Cys, is the first limiting AA in practical broiler diets. The functional groups in the side chain of Met and Cys are thiol and sulphide, respectively, and both

contain sulphur in their side chains (Figure 2.4). Deficiencies of Met and Cys have a huge negative impact on protein and AA content of tissues in chickens (Conde-Aguilera *et al.*, 2013). Supply of these sulphur AA modifies the susceptibility of meat to oxidation (Belloir *et al.*, 2015). Methionine is needed for almost all biological methylation reactions that include methylation of DNA and histones, and is also involved in epigenetic regulation (Waterland and Michels, 2007; Tesseraud *et al.*, 2009). Oxidative stress and lipid peroxidation are reduced with additional Met in the diet (Martı´n-Venegas, *et al.*, 2006; Willemsen *et al.*, 2011). Methionine requirement is relatively higher for optimum feed efficiency than the requirement for maximal weight gain (Mack *et al.*, 1999). The requirement for Met + Cys is higher in birds to supply the sulphur for feather synthesis (Belloir *et al.*, 2015). Cysteine is essential for the synthesis of glutathione and taurine, which are essential compounds for host defence against oxidative stress (Métayer-Coustard *et al.*, 2010).

Lysine

Lysine is the most limiting AA in cereals and second limiting in practical broilers diets (Ravindran and Bryden, 1999). The side chain of Lys is basic and the functional group in the side chain is amino group (Figure 2.4). The primary function of Lys is building of muscle protein. As a result, Lys is the major determinant of breast meat yield (Leclercq, 1995) with a direct effect on carcass characteristics in broilers and its deficiency reduces the proportion of breast muscle (Tesseraud *et al.*,

1996a,b). Lysine is important for improved feed conversion and breast meat yield rather than body weight (BW) gain in broilers (Dozier *et al*., 2009, 2010). Amino acid balance has a significant effect on the proportion of nutrients used for glycogen storage and hence the ultimate pH (Belloir *et al.*, 2015). Hypertrophy of breast muscles in broilers has been suggested to be associated with a decrease in glycogen storage (Berri *et al.*, 2007). Berri *et al.* (2008) reported that an increased level of digestible Lys (from 8.3 to 10.3 g/kg) in broiler diets increased the pH and reduced the drip loss of breast muscle during storage.

Lysine is the reference AA in ideal protein concept in broiler nutrition. For optimum performance, birds require AA in specific amounts. If the absorbed AA increases in relation to the first limiting AA, the excess AA will be oxidised and N will be excreted in environment. Hence, the dietary AA should be adjusted following the "Ideal Protein Concept". Though Met and Cys are the first limiting AA in broiler diets, there are several aspects make Lys as the reference AA in this concept. Firstly, Lys is almost exclusively utilised for body protein synthesis and its requirement is unaffected by other metabolic functions (i.e. maintenance, feather synthesis in the case of Met and Cys). Secondly, Lys has no metabolic interactions with other AA. For instance, Met can be converted to Cys. In addition, the analysis of Lys is easier than Met and Cys (Lemme, 2003). The ratio of AA requirements to Lys in broilers, however, may vary depending on strain, gender, age and criterion of response (Baker, 2009). As could be expected, males have

higher requirements of Lys than the females for optimum growth and feed efficiency (Garcia *et al.*, 2006; Dozier *et al.*, 2008).

Arginine, Histidine, Glycine, Serine, Alanine, Proline, Glutamic acid, Aspartic acid

The side chains of Arg and His are basic and contain guanidine group and imidazole groups, respectively. On the other hand, Gly and Ala have nonpolar side chains and contain hydrogen and alkyl groups. In the case of Ser, the side chain is a hydroxyl group and in Pro, the polar side chain contains a functional rigid cyclic structure (Figure 2.4).

Unlike in mammals, Arg cannot be synthesised via the urea cycle in birds. However, Arg, Glu, Gly and Pro are involved in several critical functions in the body, including nutrient transport and metabolism, gene expression, cell signalling, blood flow, intestinal microbial growth, innate and cell mediated immune response, and antioxidative responses (Wu, 2013). Glycine is not synthesised rapidly enough to fulfil the metabolic needs, especially in younger birds. Glycine is a precursor for synthesis of uric acid that is important for the excretion of N in bird and should be supplied sufficiently in diet either alone or in combination with Ser (as these two AA are reversible; Belloir *et al.*, 2015).

The side chains of Asp and Glu contain a carboxylic acid (Figure 2.4). High concentrations of Asp, Ser and Glu in ileal endogenous secretions have been reported in poultry (Bielorai and Iosif, 1987; Siriwan *et al.*, 1994). Ravindran *et al.* (2009) stated the highest content of Glu and the

lowest content of Met among AA in the ileal endogenous flow of broilers. The same study reported relatively higher concentrations of Gly, Thr, Asp, Ser, Val and Leu, and lower concentrations of Lys and Cys in endogenous secretions.

Threonine, Tryptophan, Valine, Leucine, Isoleucine, Phenylalanine, Tyrosine

Threonine is considered as the third limiting AA for poultry diets, especially in maize-soybean meal (SBM)-based diets. Arginine and Val are deficient in low protein diets and considered as fourth limiting AA (Han *et al.*, 1992; Baker, 2009). Threonine is a major AA found in endogenous secretions of poultry (Kadim *et al.*, 2002; Ravindran *et al.*, 2004; Huang *et al.*, 2005). The body composition of broilers is also influenced by Thr and Val, but the effect is lesser than that of Lys (Leclercq, 1998).

Tryptophan is an important AA influencing hormonal secretions (Pan *et al.*, 2013; Bello *et al.*, 2018), immune organ development (Khanipour *et al.*, 2019), meat quality (Wang *et al.*, 2014) and egg production (Dong *et al.*, 2007), and in the mitigation of oxidative stress (Bello *et al.*, 2018; Hao *et al*., 2020). There is a large volume of data on the digestibility of AA in different ingredients for poultry, but these often lack values for Trp. The reason behind this is the analytical difficulty in Trp determination because of labile nature of this AA at acidic pH (Wu *et al.*, 2009). However, the side chains of Trp, Thr and Val contain functional indole, hydroxyl and alkyl groups, respectively.

The side chains of Leu and Ile contain functional alkyl groups. Phenylalanine has aromatic group in its side chain. Tyrosine contains a phenolic hydroxyl group in its side chain (Figure 2.4).

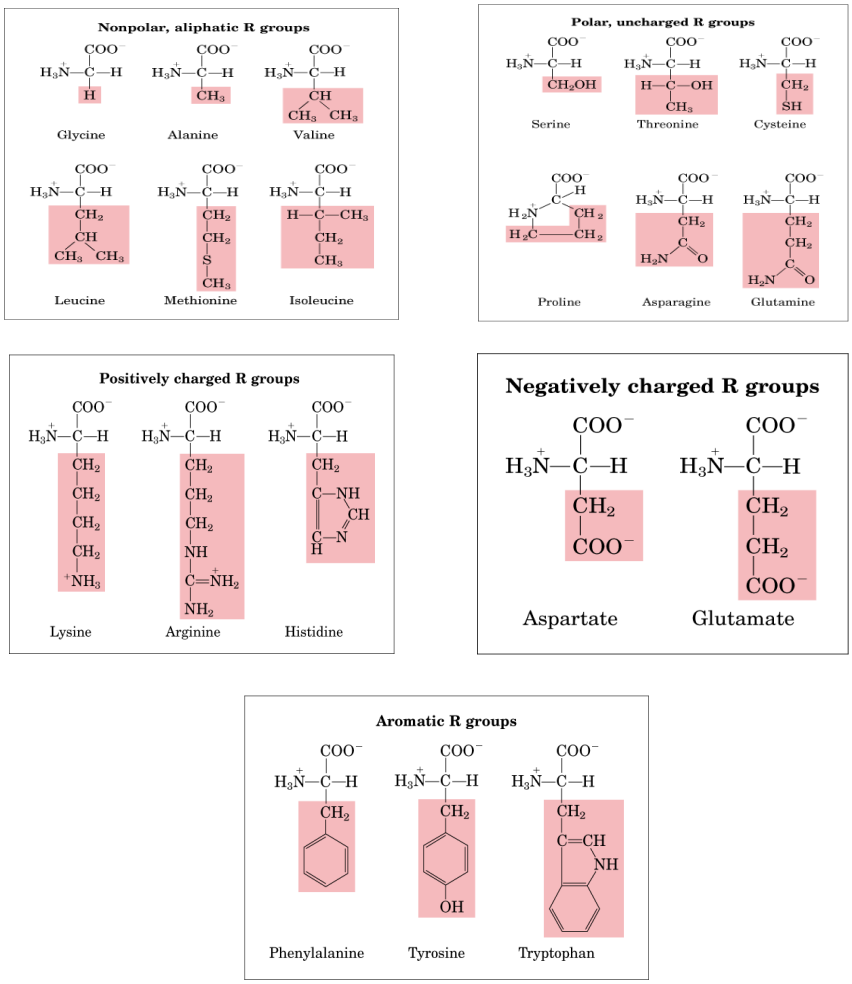

Figure 2.4. Molecular structure of the different amino acids (Source: Nelson and Cox, 2000).

2.2.4. Specific amino acid relationships

2.2.4.1. Synergism

Synergistic relationship among different AA is beneficial, though each AA may be metabolised independently. This relationship is often helpful to convert one AA to another when there is a metabolic need. During biological processes, Met can donate its methyl group that results in homocysteine, a sulphur-containing compound. Together with Ser, it can be used for the synthesis of Cys via cystathionine. Two Met molecules are needed to supply the two sulphur atoms of Cys. This conversion is irreversible meaning that the Met requirement cannot be met with Cys (Baker, 1976). Biological degradation of Phe often produces Tyr. Therefore, Phe is able to fulfil the need for Tyr on a mole-for-mole basis. This reaction may be reversed to a small extent which is not significant practically (NRC, 1994). Though poultry can synthesise Gly, the rate of synthesis is not adequate (Featherston, 1976) in young birds. There is synergism between Ser and Gly where Ser may be converted to Gly and this conversion is reversible (Sugahara and Kandatsu, 1976).

2.2.4.2. Antagonism

Antagonism exists between AA having side chains with similar structural or chemical characteristics. An increase in dietary concentration of an AA can adversely affect the absorption and metabolism of another AA. These antagonisms are not practically relevant unless high inclusions of supplemental AA are used in low-

protein diets (NRC, 1994). Several AA such as Leu-Ile-Val, Arg-Lys and Thr-Trp are known to exhibit antagonisms. The antagonism between Leu and Ile may be significant in some feedstuff combinations (i.e. maize plus maize gluten meal) leading to a high level of Leu and marginal level of Ile (D'Mello and Lewis, 1970).

2.2.4.3. Toxicity

Toxicity occurs when there is high level of one AA. Such an occurrence is rare, as excess levels of AA are never intentionally added in practical diets. For example, supplemental forms of Met and Lys are routinely used, but normal additions are too low to cause toxicity. Amino acid toxicity, if observed, is an indication of improper or inadequate diet mixing. Addition of excess Met (40 g/kg) in a maize-SBM-based diet led to 92% reduction of weight gain (118 vs. 11g) in broilers. Similar levels of Trp, Lys, and Thr were less toxic (Edmonds and Baker, 1987).

2.3. Amino acid availability

2.3.1. Digestibility vs. availability

Though the terms ‘digestibility’ and ‘availability’ are used interchangeably, these two terms are distinctly different. Amino acid bioavailability refers to the form of AA that is suitable for digestion, absorption and utilisation by the bird. Availability can only be measured by the animal growth assays (i.e. slope-ratio method; Ravindran and Bryden, 1999). On the other hand, digestible AA is the fraction of ingested AA absorbed by the bird and not excreted in the

excreta. The AA digestibility assay, however, represents the practical way of determining AA availability (Lemme *et al.*, 2004).

Accurate assessment of AA digestibility in different raw materials for poultry is crucial for the formulation of balanced diets that ensures the supply of available AA for the maintenance and production (Lemme *et al.*, 2004; Sczurek, 2009). However, under some conditions (i.e. heat-damaged protein sources), AA might be absorbed in forms not suitable for utilisation. These AA make no contribution to the maintenance, growth and production (Ravindran *et al.*, 2005).

2.3.2. Total versus digestible AA

There is a wide variation among the individual feed ingredients in respect to AA digestibility. When a diet is formulated based on total AA, the prediction of bird performance becomes less reliable, especially in formulations with poorly digested feed ingredients (Fernandez *et al.*, 1995). Digestible AA contents more closely describe the availability of AA to the birds than total AA contents and help in precise diet formulations. Formulations based on digestible AA will also lower the N output from poultry operations (Rostagno *et al.*, 1995; Lemme *et al.*, 2004). Maize and SBM, are the commonly used ingredients globally, have high AA digestibility and only a small benefit might be observed by switching to the digestible AA system. However, the use of digestible AA contents will deliver huge advantages when alternative raw materials, with lower AA digestibility, are used (Lemme *et al.*, 2004).

2.3.3. Measuring AA availability in poultry

2.3.3.1. Direct *in vitro* methods

Protein quality or AA availability in feed ingredients can be evaluated by *in vitro* methods that include enzymatic, chemical, microbiological, near infrared reflectance (NIR) spectroscopy and immunological assays (Ravindran and Bryden, 1999). Several *in vitro* assays (i.e. pepsin N digestibility and protein solubility in KOH) have been used as predictors of protein quality in specific feedstuffs (Parsons *et al.*, 1991, 1997).

In vitro techniques are simple, rapid, reproducible, avoid the use of animals and, provide useful information on heat damage to protein and rank of protein sources. However, the general criticism is that most *in vitro* techniques suffer from some degree of uncertainty and the data are not acceptable for practical diet formulations. Simulation of protein digestion in the laboratory using enzymatic assays do not reflect what actually happens in the digestive tract (Swaisgood and Catigni, 1991). The NIR spectroscopy is however more rapid than other techniques but requires the calibration of the equipment (Ravindran and Bryden, 1999).

2.3.3.2. Indirect *in vivo* methods

After digestion and absorption of dietary proteins, free AA and peptides are transported by blood circulation to the target tissues. The principle underlying indirect *in vivo* methods is that the AA in blood reflects the dietary protein availability. Constant plasma free AA concentration

during starvation is used as reference in this method that is compared with AA concentration after meal (Ng and Pascaud, 1990). This method is simple, quick and convenient. However, there are also some drawbacks. Free AA levels in the plasma depend on different factors such as age, species, physiological status of the bird, site and time of blood sampling, ambient temperature, frequency of feeding, supply of energy yielding nutrients during starvation and AA imbalances etc. (Sibbald, 1987; Low, 1990). During protein synthesis, free AA can be removed from circulation when there is positive N balance. In contrast, excess AA are released into the plasma during high catabolism (Ravindran and Bryden, 1999). Therefore, the interpretation of data from changes in plasma AA level and the quantitative evaluation of AA availability is tenuous (Papadopoulos, 1985).

2.3.3.3. Direct *in vivo* methods

Direct *in vivo* methods for determining protein availability include growth or digestibility assays (Ravindran and Bryden, 1999). Generally, growth bioassays are considered as the only direct means of evaluating nutritional value obtained by other procedures. Growth bioassay determines the level of dietary AA that is digested, absorbed and finally incorporated into body proteins or metabolites for body usage (Carpenter, 1973).

Availability in poultry can be determined using growth assays by the slope-ratio method that are normally costly and time-consuming. In

addition, growth assays determine the availability of only one AA per assay (Ravindran and Bryden, 1999).

2.4. Determination of AA digestibility in poultry

2.4.1. Background

The digestibility assays are conducted with the assumption that the difference between input and output of nutrients in animals is a valid indicator of bioavailability. The digestibility assay is the most favoured method nowadays as all the AA can be determined in one assay and the digestibility values are directly applied to the bird. The digestibility assays are divided into excreta and ileal assays (Bryden *et al.*, 2009a).

2.4.2. Excreta digestibility assay

Excreta AA digestibility method was a preferred method at past because of its simplicity. In this method, a large number of birds can be used without any surgical modification or sacrificing them. Moreover, the birds may be used for several assays (Lemme *et al.*, 2004; Bryden *et al.*, 2009a). However, excreta digestibility measurements have some major limitations. This assay measures 'metabolisability' rather than 'digestibility' as the excreta contains both faeces and urine (Lemme *et al.*, 2004). As there may be some AA in avian urine, estimation of AA digestion from excreta may be a source of error (Sibbald, 1987). On the other hand, several reports indicate that the renal AA excretion is negligible (Terpstra, 1978; Parsons, 2002). Another drawback of excreta collection is that the samples may be contaminated by feathers, scales, and other foreign materials (Ravindran and Bryden, 1999).

Excreta digestibility method ignores the influence of hindgut microorganisms on protein digestion and utilisation, and, therefore, the contribution of microbial proteins to the AA profile and concentrations in the faeces is underestimated (Bryden *et al*., 1990; Lemme *et al.*, 2004). Caecectomised birds were used later to overcome the problem of microbial modification of protein but the effects of caecectomy on digestibility values vary (Ravindran and Bryden, 1999; Bryden *et al.*, 2009a). The caecal environment is anaerobic and has a pH range of 6.5-7.5. This pH range together with evacuation of caecal contents every 6-8 h creates a favourable medium for bacteria proliferation (Ravindran and Bryden, 1999). Caecal microorganisms modify the AA composition of the excreta with significant effects on digestibility estimates. The caecal microflora may also show proteolytic activity and hydrolyse proteins (Isshiki *et al.*, 1974).

A large volume of published data on digestible AA in feed ingredients, primarily based on the adult rooster excreta assays, was produced during the 1980s (Green *et al.*, 1987; NRC, 1994). It was assumed that the nutrient digestibility is similar among different classes of birds and AA digestibility values generated with roosters were used, during this period, in diet formulations for broilers and layers. Recent studies, however, indicate that differences exist in the AIDC and SIDC of AA among roosters, layers and broilers (Huang *et al.* 2006; Adedokun *et al.*, 2009a, 2014).

2.4.3. Ileal digestibility assay

2.4.3.1. Background

The pioneers of the idea using ileal contents rather than excreta were Payne *et al*. (1968). These researchers suggested that ileal protein and AA digestibility in poultry may be more reliable than excreta digestibility. Currently, the ileal assay is considered as the preferred method because of its distinct advantages over the digestibility values based on excreta analysis (Ravindran and Bryden, 1999; Adedokun *et al.*, 2009a; Huang *et al.*, 2006). This method involves collecting digesta from the distal part of the ileum. Therefore, the modifying effects of hindgut microbial fermentation on protein composition and urine AA as sources of error are eliminated. In addition, the difficulty arising from combined voiding faeces and urine is removed (Ravindran and Bryden, 1999; Kadim *et al.*, 2002; Bryden *et al.*, 2009a).

2.4.3.2. Determination of ileal amino acid digestibility

There are two methods of collecting ileal digesta of birds: intestinal cannulation and after killing birds by cervical dislocation or euthanasia (Ravindran and Bryden, 1999). The former method includes insertion of a cannula into the distal ileum (Thomas and Crissey, 1983; Raharjo and Farrell, 1984). The limitation of this method is that it is laborious and often sufficient digesta cannot be obtained. Furthermore, this method can be used only in mature birds and considerable surgical skills are required (Lemme *et al.*, 2004). Tanksley *et al.* (1981) stated

that the physical alteration to the intestine from ileal cannulation will interfere with the normal physiological processes of animals.

Cervical dislocation of birds is not also preferred because the loss of endogenous protein is increased during the killing as there is increased shedding of mucosal cells into the gut lumen causing errors in digestibility calculation (Ravindran and Bryden, 1999). Summer and Robblee (1985) reported that the apparent ileal AA digestibility was underestimated in birds when killed by cervical dislocation in comparison with euthanised birds.

Euthanasia is the preferred method that involves killing birds humanely using anaesthetics such as pentobarbitone sodium. It reduces peristalsis that prevents movement of digesta and mucosal shedding (Badaway, 1964). After euthanasia, the small intestine is exposed surgically and the ileum is separated (Ravindran and Bryden, 1999). The ileum is defined as that portion of the small intestine extending from the Meckel's diverticulum to a point 40 mm or a few centimetres proximal to the ileo-caecal junction (McLelland, 1979; Ravindran *et al.*, 2017). The content of the lower half of the ileum is collected by gently flushing with distilled water. The digesta samples are immediately frozen at -20 ^{o}C after collection and freeze-dried subsequently.

Indigestible markers, having a high recovery rate of about 100% with no effect on nutrient digestibility, are used in assay diets for digestibility calculations. Using a marker facilitates the use of *ad libitum* feeding and eliminates the need to record the feed intake (FI;

Lemme *et al.*, 2004). An ideal marker should be inert and must not be digested or absorbed by the bird. Choice of the marker is important as available evidence shows that the digestibility estimates vary depending on the marker (Scott and Boldaji, 1997). Chromium oxide, titanium dioxide and acid insoluble ash are the commonly used markers.

2.4.3.3. Apparent ileal digestibility assay

The apparent ileal digestibility (AID) estimates the digestibility of AA of both dietary and endogenous (non-dietary) origins and does not make any correction for the contribution of endogenous component (Ravindran and Bryden, 1999). The AID coefficients (AIDC) are calculated from the dietary ratio of AA to indigestible marker relative to the corresponding ratio in the ileal digesta (Bryden *et al.*, 2009a). It can also be expressed as a percentage (%).

$$\text{AIDC of AA} = [(\text{AA/Marker})_d - (\text{AA/Marker})_i]/(\text{AA/Marker})_d$$

Where $(\text{AA/Marker})_d$ = ratio of AA to marker in the diet, and

$(\text{AA/Marker})_i$ = ratio of AA to marker in ileal digesta.

The major concern of AIDC method is that the level of FI and dietary AA concentrations both can influence the digestibility (Bielorai and Iosif, 1987; Fan *et al.*, 1994; Angkanaporn *et al.*, 1997). When the assay diet contains low AA concentration, AIDC would underestimate the digestibility because the proportion of endogenous AA relative to dietary AA will be high in the ileal digesta (McNab, 1989). However, some argue that the use of AIDC is suitable when poultry diets are

formulated to least-cost using linear programming because the cost of endogenous AA loss should be included (Bryden *et al.*, 2009a). Several compilations of data on the apparent ileal AA digestibility of feed ingredients for broilers are available (Ravindran *et al.*, 2002, 2005, 2014a,b; Huang *et al.*, 2005; Bryden *et al.*, 2009a; Adedokun *et al.*, 2009a; Kim *et al.*, 2012a,b).

2.4.3.4. Standardised ileal digestibility

The ‘‘standardised digestibility’’ overcomes the limitations of apparent digestibility and has become ‘the golden standard’ in current feed formulations. Though the terms ‘‘standardised’’ or ‘‘true’’ digestibility are used interchangeably in the literature; they are slightly different. Standardised ileal digestibility (SID) values are the AID values that are corrected for basal (diet independent) EAA flow. On the other hand, the true digestibility estimates correct for both basal and diet specific EAA flows (Lemme *et al*., 2004).

The basal EAA flow at the terminal ileum, expressed as grams of AA flow per kilogram of DM intake (DMI), is calculated using the following the formula described by Moughan *et al*. (1992).

Basal EAA flow (g/kg DMI) = AA concentration in ileal digesta (g/kg) × [Diet marker (g/kg)/Ileal digesta marker (g/kg)]

The AIDC data are then converted to standardised ileal digestibility coefficients (SIDC), as below (Ravindran *et al.*, 2014a).

SIDC = AIDC + [Basal EAA (g/kg DMI)/ Ing. AA (g/kg DM)]

Where, SIDC = standardised ileal digestibility coefficient of the AA;

AIDC = apparent ileal digestibility coefficient of the AA;

Basal EAA = basal endogenous flow of the AA, and

Ing. AA = concentration of the AA in the ingredient.

Several published reports are now available on the SIDC AA in cereals and protein ingredients for broilers (Lemme *et al.*, 2004; Adedokun *et al.*, 2007a, 2009a; Bandegan *et al.*, 2009, 2011; Ravindran *et al.*, 2014b, 2017; Blok and Dekker, 2017).

2.4.3.5. Estimation of endogenous amino acid (EAA) flow

Some details of endogenous N and AA flows have been reviewed previously in section 2.1.3.2. There are several methods for quantifying EAA losses in poultry and exhaustive reviews are available on these methods (Ravindran and Bryden, 1999; Blok *et al.*, 2017). The methods are broadly classified as feeding N-free diet (NFD), diets with highly digestible protein sources or low molecular weight protein sources, radioisotope-labelling and regression methods (Ravindran, 2021). The analytical steps of most of these methods are cumbersome or involve using large number of animals or both (Adedokun *et al.*, 2007b; Cowieson *et al.*, 2009). Each method has its own advantages and limitations (Ravindran and Bryden, 1999). Of these methods, basal EAA estimates determined following the feeding of NFD is now accepted as being valid values for the correction of apparent digestibility values (Stein *et al.*, 2007; Ravindran *et al.*, 2017). For this

reason, only the NFD method is discussed in detail. Compared to other methods, this technique is simple and less laborious.

In the NFD method, a diet composed of starch or sugar (> 80%), small amounts of fibre (i.e. cellulose), vegetable oil and trace vitamin-mineral premix is fed to birds for a short period and the ileal digesta is collected. The assumption is that, since no protein was fed, the protein and AA in the ileal digesta are of endogenous origin and represent the basal losses. Feeding of NFD is, however, physiologically abnormal affecting protein catabolism and digestive enzyme secretions in the GIT (Jansman *et al.*, 2002; Lemme *et al.*, 2004; Stein *et al.*, 2007). It is well documented that the NFD severely underestimates EAA flows in birds (Lemme *et al.*, 2004; Cowieson *et al.*, 2009).

The EAA flows at the terminal ileum of broilers has been reported to be higher in 5 d compared to 15-d-old broiler chicks (Adedokun *et al.*, 2007b). Since the EAA flow is a balance between the secretion and reabsorption of AA (Ravindran, 2021), factors like increased mucin production and lower AA digestibility at early ages were attributed for the higher EAA flows. Therefore, it can be hypothesised that the correction of AIDC for age-specific EAA flows might impact the SIDC AA at different ages of broiler. The published data on basal EAA flows in broilers of different ages are compiled in Table 2.1. With the exception of some reports (Adedokun *et al.*, 2007b,c; Szczurek, 2010; Toghyani *et al.*, 2015), all previous studies measured EAA flows in older birds (21 to 42 d of age).

Table 2.1. Published data on basal ileal endogenous amino acid flow values (g/kg dry matter intake) in broilers

					Indispensable AA										Dispensable AA							
References	Age (d)	CP	Total AA	N	Arg	His	Ile	Leu	Lys	Met	Phe	Thr	Trp	Val	Ala	Asp	Cys	Glu	Gly	Pro	Ser	Tyr
					Nitrogen free diet Method (NFD)																	
Siriwan *et al.* (1993)	-	-	-	-	0.23	0.10	0.39	0.49	0.22	0.07	0.25	0.45	-	0.37	0.25	0.49	-	0.63	0.28	-	0.39	0.66
Ravindran *et al.* (2004)	35	7.33	5.82	1.17	0.28	0.16	0.29	0.44	0.21	0.10	0.29	0.51	0.09	0.42	0.29	0.61	0.23	0.72	0.51	-	0.42	0.25
Adedokun *et al.* (2007b)	5	-	8.69	-	0.44	0.18	0.34	0.64	0.49	0.15	0.39	0.54	0.06	0.49	0.44	0.79	0.28	1.00	0.41	0.43	0.51	0.28
	15	-	3.73	-	0.16	0.07	0.15	0.24	0.18	0.05	0.15	0.27	-	0.21	0.17	0.34	0.12	0.38	0.19	0.22	0.23	0.12
	21	-	3.95	-	0.17	0.07	0.16	0.25	0.18	0.05	0.15	0.27	-	0.22	0.18	0.34	0.14	0.42	0.21	0.24	0.26	0.13
Adedokun *et al.* (2007c)	5	-	9.05	-	0.46	0.19	0.39	0.67	0.52	0.15	0.41	0.57	-	0.52	0.45	0.84	0.23	1.04	0.43	0.45	0.54	0.29
	15	-	4.24	-	0.18	0.08	0.17	0.28	0.21	0.06	0.18	0.29	-	0.23	0.19	0.38	0.13	0.43	0.22	0.25	0.26	0.14
	21	-	3.94	-	0.16	0.07	0.16	0.24	0.18	0.04	0.15	0.26	-	0.21	0.17	0.33	0.14	0.43	0.19	0.25	1.03	0.64
Adedokun *et al.* (2008)	-		3.08	-	0.12	0.06	0.12	0.18	0.14	0.05	0.12	0.24	-	0.18	0.14	0.25	0.09	0.33	0.16	0.16	0.17	0.09
Golian *et al.* (2008)	21	-	4.37	-	0.20	0.90	0.20	0.29	0.17	0.07	0.42	0.43	0.07	0.27	0.22	0.43	0.14	0.49	0.24	0.29	0.34	-
Szczurek (2009)	30	-	5.85	-	0.28	0.21	0.29	0.49	0.27	0.09	0.28	0.29	0.10	0.38	0.23	0.58	0.15	0.75	0.28	0.47	0.38	0.36
Szczurek (2010)	14	-	-		0.17	0.16	0.16	0.29	0.19	0.07	0.16	0.29	0.07	0.21	0.17	0.41	0.11	0.45	0.19	0.20	0.23	0.21
Ravindran *et al.* (2009)	35	-	6.20	1.23	0.24	0.13	0.25	0.36	0.23	0.09	0.19	0.53	-	0.28	0.29	0.55	0.20	0.69	0.48	1.07	0.41	0.21
Soleimani *et al.* (2010)	42	-	6.81	-	0.47	0.20	0.33	0.54	0.38	0.08	0.39	0.54	0.05	0.34	0.35	0.58	0.10	0.85	0.40	0.34	0.55	0.27
Kong and Adeola (2013a)	-	-	-	2.08	0.58	0.25	0.54	0.88	0.63	0.17	0.50	0.80	0.07	0.66	0.57	1.02	1.09	1.34	0.65	0.68	0.71	0.39
Kong and Adeola (2013b)	-	-	8.76	1.96	0.37	0.16	0.38	0.56	0.38	0.10	0.35	0.58	0.09	0.53	0.37	0.59	0.22	0.92	0.43	0.52	0.51	0.29
Adedokun *et al.* (2014)	21	10.5	-	-	0.39	0.15	0.34	0.57	0.35	0.11	0.61	0.56	-	0.46	0.39	0.72	0.24	0.93	0.45	0.49	0.56	-
Toghyani *et al.* (2015)	10	8.18	-	-	0.29	0.16	0.29	0.45	0.33	0.10	0.52	0.51	-	0.39	0.29	0.63	0.19	0.63	0.33	0.39	0.47	-
	24	6.51	-	-	0.22	0.09	0.21	0.30	0.16	0.05	0.44	0.39	-	0.29	0.21	0.49	0.19	0.49	0.26	0.33	0.44	-
Abdollahi *et al.* (2015)		8.16	-	-	0.37	0.17	0.34	0.51	0.29	0.10	0.26	0.60	-	0.45	0.37	0.59	0.22	0.87	0.62	0.33	0.52	0.28
Adeola *et al.* (2016)	-	11.3	8.40	10	0.39	0.18	0.37	0.56	0.39	0.11	0.37	0.60	-	0.51	0.39	0.73	0.41	0.98	0.47	0.50	0.56	0.30
Ullah *et al.* (2016)	21	-	-	-	0.18	0.19	0.35	0.34	0.23	0.05	0.20	0.41	-	0.39	0.11	0.17	0.14	0.24	0.12	-	0.14	-
Ravindran *et al.* (2017)	35	12.3	-	-	0.49	0.20	0.42	0.69	0.52	0.16	0.49	0.62	0.16	0.55	0.48	0.84	0.25	0.11	0.50	0.55	0.63	-
Cowieson *et al.* (2019)	28	-	-	-	0.37	0.19	0.33	0.55	0.35	0.12	0.34	0.70	-	0.47	0.41	0.96	0.29	0.91	0.44	0.53	0.59	0.34
Average		9.18	5.92	3.29	0.30	0.18	0.29	0.45	0.30	0.09	0.32	0.47	0.08	0.38	0.30	0.57	0.23	0.67	0.35	0.41	0.45	0.89

Highly Digestible Protein Method (HDP)																					
Adedokun *et al.* (2009b)	-	-	8.81	-	0.26	0.17	0.55	0.45	0.48	0.17	0.23	0.49	0.07	0.54	0.36	0.68	0.15	1.80	0.31	0.47	0.76	0.19
Guanidinated Casein Method (GuC)																					
Ravindran *et al.* (2004)	35	19.6	17.1	3.14	0.78	0.43	1.06	1.02	0.78	0.24	0.76	1.44	0.19	1.06	0.74	1.59	0.29	3.56	1.42	-	1.03	0.67
Peptide Alimentation/Enzymatically Hydrolysed Casein Method (EHC)																					
Lemme *et al.* (2004)	-	9.23	-	-	0.22	0.21	0.39	0.38	0.26	0.08	0.24	0.57	0.08	0.45	-	-	0.17	-	-	-	-	-
Ravindran and Hendriks (2004)	-	15.5	12.3	2.48	0.46	0.35	0.52	0.73	0.46	0.19	0.53	0.97	-	0.72	0.52	1.11	0.31	2.09	0.80	1.20	0.93	0.41
Ravindran *et al.* (2004)	35	19.3	16.5	3.08	0.54	0.44	0.88	0.99	1.05	0.25	0.73	1.09	0.20	0.95	0.69	1.43	0.33	3.87	1.43	-	1.02	0.61
Woyengo *et al.* (2010)	21	-	-	1.23	0.20	0.28	0.38	0.37	0.16	0.09	0.15	0.55	-	0.35	0.29	0.54	0.03	1.27	0.25	0.39	0.68	0.14
Ravindran *et al.* (2017)	35	-	6.62	1.31	0.25	0.12	0.25	0.35	0.25	0.11	0.23	0.58	-	0.35	0.35	0.68	0.24	0.90	0.69	0.56	0.52	0.19

AA = amino acids; CP = crude protein; N = nitrogen.

2.4.3.6. Feed formulation based on digestible amino acids

The rationale of correcting AID values for endogenous losses has been a subject of debate over the years and this issue has been discussed previously (Ravindran and Bryden, 1999; Parsons, 2002; Lemme *et al.*, 2004). It must be noted, however, both the apparent and standardised digestible AA systems are superior to the total AA system (Bryden *et al.*, 2009a).

Apparent digestibility of AA of ingredients may not deliver the arithmetically predictable value in a mixed diet (Kong and Adeola, 2013b; Cowieson *et al.*, 2019). Some studies have shown that the determined AID values for most AA in mixed diets for pigs and broilers were numerically higher compared to those predicted from individual ingredients (Angkanaporn *et al.*, 1996; Imbeah *et al.*, 1988).

Previous studies have already documented that the SIDC AA are more additive than the AIDC (Kong and Adeola, 2013b; Cowieson *et al.*, 2019). The underestimation of AIDC of AA in low-protein ingredients like cereals, mainly contributes to the poor additivity of AID (Kong and Adeola, 2013b). Besides, the AID AA undervalues the digestibility of several nutritionally critical AA (Cowieson *et al.*, 2019).

2.5. Poultry feed form: Impact on nutrient digestibility

2.5.1. Feed form (mash vs. pellet)

Commercial feed mills produce various forms of poultry feed such as mash, pellet and crumble. Mash is the form of complete feed that is a mixture of ground ingredients without further processing. Pelleting,

extrusion and expansion are the common hydrothermal processing (HTP) techniques used in the feed industry (Goodarzi Boroojeni *et al.*, 2016). Among these, pelleting is the method most employed in broiler feed manufacture (Dozier *et al.*, 2010). This process involves agglomeration of smaller feed particles into larger particles by the use of mechanical pressure, heat and moisture (Abdollahi *et al.*, 2010a, 2013a). Conditioning by adding steam to mash is the most important step in pelleting. Steam pelleting promotes conversion of fibrous, unpalatable, bulky, and finely-ground blends of feed ingredients into compact, free-flowing pellets. It facilitates easy prehension of feed and has a stimulatory effect on FI of birds (Abdollahi *et al.*, 2014, 2018b).

Feed intake, which is the primary driver of weight gain in broilers, is higher in birds fed pellet diets compared to mash (Svihus *et al.*, 2004; Abdollahi *et al.*, 2013a). Offering agglomerated feed prevents the birds from feed particle selection and ensures birds receive macro and micro ingredients simultaneously (Goodarzi Boroojeni *et al.*, 2016). The significant advantages of pelleting can be summarised as improved feed efficiency, increased palatability, decreased feed wastage, prevention of selective feeding, and reduced time and energy spent for feeding (Abdollahi *et al.*, 2010a, 2013b; Naderinejad *et al.*, 2016; Roza *et al.*, 2018). Destruction of pathogenic organisms by heat treatment, reduction of thermolabile ANFs, and thermal modification of starch and protein are also some of the benefits associated with pelleting process (Peisker, 2006; Amerah *et al.*, 2007).

2.5.2. Effect of pelleting on starch digestibility

Pelleting process results in cell wall breakage in grains and renders greater accessibility of nutrients, encapsulated within endosperm sub-aleurone, to digestive enzymes (Abdollahi *et al.*, 2013a). Starch gelatinisation is the main impact of hydrothermal processing that influences the digestibility of starch (Svihus *et al.*, 2005). Hydrolysis of starch is mostly carried out by the action of pancreatic α-amylase in the duodenum and jejunum. This enzyme hydrolyses most glycosidic linkages in amylose and amylopectin (Lehmann and Robin, 2007). Presence of water and heat breaks down intermolecular bonds of starch granules during starch gelatinisation. It allows hydrogen bonding sites to engage more water that results swelling and loss of crystallinity (Hoover, 1995). The granular structure is exposed to digestive enzymes that increases the susceptibility for amylolytic degradation (Kishida *et al.*, 2001; Singh *et al.*, 2007). Most starches are gelatinised at high water contents in a temperature range between 50 and 70 ºC (Donald, 2001). Since low water content are generally used in most heat processing methods, high temperatures are necessary to initiate starch gelatinisation (Svihus *et al.*, 2005). The majority of starch gelatinisation occurs in pellet die due to high friction heat though part of it can occur during steam-conditioning (Zimonja *et al.*, 2008; Abdollahi *et al.*, 2010b, 2011). However, the extent of starch gelatinisation due to pelleting is limited and, in most cases, has been reported to range between 8-20% (Zaefarian *et al.*, 2015).

Contradictory results have been reported on the effects of pelleting on the AIDC of starch (Svihus, 2001; Abdollahi *et al.*, 2013b). Abdollahi *et al.* (2011) reported a markedly lower AIDC of starch in a wheat-based pelleted diet (0.834) compared to a mash diet (0.959). Lower AIDC of starch in wheat-based pellet diet (0.920) compared to mash (0.966) diet was also recorded by Abdollahi *et al*. (2013b). However, in their study, pelleting had no effect on AIDC of starch in a maize-based diet. Selle *et al.* (2012) did not observe any effect of pelleting on the ileal starch digestibility of broilers fed sorghum-based diets. The reduction of starch digestibility in pelleted wheat-based diets might be due to an increased concentration of high molecular weight soluble NSPs in the gut resulting in increased digesta viscosity (de Vries *et al.*, 2012) and an overload of wheat starch in small intestine (Svihus and Hetland, 2001) due to higher FI, reducing absorption.

2.5.3. Effect of pelleting on protein and amino acid digestibility

High temperature, shear forces, together with pressure and water induce protein denaturation by destruction of three-dimensional structure, formation of new covalent bonds (i.e. disulphide bridges, iso-peptide bonds). The denaturation of protein occurs in two steps. The first step is reversible that involves breakage of hydrogen and Van der Waals bond. The second step is irreversible involving breakage or formation of covalent bonds like disulphide bridges. This process is facilitated by high moisture content (Weijers and Van’t Riet, 1992).

The interaction between proteins and water molecules occurs by the Van der Waals bond. Water may also contribute to conformational stability of protein (Svihus and Zimonja, 2011). When the temperature increases, water molecules destabilise proteins in a concentration dependent manner. For this interaction, protein can tolerate severe heat treatments when the water concentration is lower (Ludikhuyze *et al.*, 2003). Adams (1991) noted that the denaturation starts at a temperature of 60-70 ºC in the case of most proteins. Some proteins are stable at ≥ 80 ºC, whereas some are inactivated even at temperatures > 40 ºC.

Several enzyme inhibitors are found in ingredients, for example, Bowman-Birk and Kunitz factor in soybeans and α-amylase inhibitors in wheat (Prasad *et al.*, 2010). There are also presence of trypsin and chymotrypsin inhibitors in sorghum (Kumar *et al.*, 1978) and trypsin inhibitors in soybeans (Han and parsons, 1991). It has been suggested that pelleting can increase protein digestibility by deactivating inherent protease inhibitors in diet and inducing protein denaturation (Mauron, 1990; Svihus and Zimonja, 2011; Roza *et al.*, 2018). However, the improvement in protein digestibility documented by Mauron (1990) was neither large nor always present. Carré *et al.* (1991) did not observe any improvement in protein digestibility with pelleting.

Protein solubilisation is the first step in protein digestion (Cowieson and Ravindran, 2008). Over-processing of feed can decrease the protein solubility in the GIT leading to depressed AA digestibility, especially of Lys (Goodarzi Boroojeni *et al.*, 2016). The AIDC of

different nutrients at three conditioning temperatures (60, 75 and 90 ºC) were determined by Abdollahi *et al.* (2010a) in wheat-based diets. In their study, increased conditioning temperature resulted in lower AIDC of N and starch. Abdollahi *et al.* (2010b) observed lower AIDC of N by increasing the conditioning temperature from 60 to 90 ºC in a sorghum-based diet. The formation of cross-linkages during processing of some ingredients such as meat and bone meal (MBM) may also reduce digestion of some AA, especially in young birds (Parsons *et al.*, 1997; Huang *et al.*, 2005).

A well-known factor contributing to reduced AA digestibility is Maillard reaction (Muramatsu *et al.*, 2013) that produces "melanoides" by combining free aldehyde groups from reducing sugars (i.e. glucose, fructose, lactose, maltose) with free amino groups from AA, especially the ε-amino group of Lys. Melanoides increase viscosity of the product as well as darken it (Voragen *et al*., 1995; Thomas *et al*., 1998). The Maillard complex with sugars, higher viscosity by NSP disruption, retrogradation and formation of resistant starch complexes may cause losses of Lys and Arg, heat labile nutrients (i.e. vitamins, enzyme, crystalline AA etc.), and available energy (Creswell and Bedford, 2006; Abdollahi *et al.*, 2010a).

However, Cheftel (1986) stated that conditions favourable for the "Maillard reaction" are high temperature (>180 ºC), low moisture (<15%) in combination with shear forces (>100 rpm in an extruder). Pelleting involves conditioning of dry feed where saturated steam is injected into feed and mixed in the conditioner. This process usually

takes less than 60 s, results in a temperature rise to 75 ºC and an increase in moisture level by 3-4%. Then the feed enters the pellet press and forced through cylindrical holes in a die where the pellet is shaped. The friction from the rolls that force the material into the die holes, and the highest temperature achieved is 85 ºC (Svihus *et al.*, 2004). Consequently, the conditions of pelleting are too mild to cause Maillard reaction (Svihus and Zimonja, 2011; Goodarzi Boroojeni *et al.*, 2016). Hussar and Robblee (1962) did not notice any effect on the Lys content in wheat, oats and barley with a pelleting temperature of 72 ºC and concluded that this temperature was not severe to damage Lys.

Significant correlations exist between AA digestion and, the gizzard weight, gizzard pH, FI and feed passage rate. The normal pH in the gizzard content is between 1.9 and 4.5, with a mean value of 3.5 (Svihus, 2011a). Since commercial feeds usually have a pH close to neutral; therefore, higher FI in pellet fed birds can increase the gizzard pH unless the gastric juice secretion is increased accordingly. However, in pellet-fed birds the gizzard pH has been reported to be between 3 and 4 (Svihus, 2014). Several other studies have also reported higher pH values in the gizzard of pellet-fed birds (Frikha *et al.*, 2009; Naderinejad *et al.*, 2016; Abdollahi *et al.*, 2018b) that might account for lower AA digestibility (Goodarzi Boroojeni *et al.*, 2016).

Engberg *et al.* (2002) observed reduced gizzard weight in pellet-feed birds compared to mash fed birds and speculated that the reduction of gizzard weight is largely a consequence of the lack of mechanical

stimulation by the feed. Following disintegration in crop and oesophagus, pellets pass through the proventriculus and gizzard and enter the duodenum. On the other hand, mash diet, particularly coarsely ground mash, stays longer in the gizzard, increases gizzard volume, extensively ground and reduces the gizzard pH (Svihus, 2014). The mechanism is that increased gizzard volume and longer retention time in mash feed stimulate gastric function, and increases secretion of HCl by the proventriculus (Engberg *et al.*, 2002). The proteolytic enzyme pepsinogen is secreted as precursor pepsinogen and released by the chief cells in the proventriculus that autoactivates under low pH (Auer and Glick, 1984). It is anticipated that the reduction of gizzard pH or increased mechanical stimulation of feed in mash diet might significantly affect AA digestibility.

2.6. Influence of bird age on nutrient digestibility

2.6.1. Factors affecting the nutrient digestion at different ages of broiler

Age and physiological state of birds have significant effects on digestion and absorption of dietary nutrients. The first week after hatch is the most critical period in the life of a broiler chicken. When the chick emerges from the egg, its digestive and immune systems are still immature, and the bird is not well prepared to face the challenges confronting it. First, there is the transition from yolk to oral nutrition. Associated with this are the substantial physical and functional development of the digestive tract and organs and the development of active immunity. The capacity to

digest the feed, absorb and transport nutrients is therefore limiting during the early life of broilers.

Plethora of factors might influence the nutrient digestibility, including AA, at different broiler ages as discussed below:

Presence of residual yolk

The yolk is internalised in the abdominal cavity of the chick before hatching as an extension of intestine. At the time of hatching, the residual yolk comprises 20-25% of the chick's BW (Noy and Sklan, 1997). The yolk sac plays a significant role in the function and development of the digestive tract during the first few days of life (Murakami *et al.*, 1992; Noy *et al.*, 1996). The chicks need to acquire the ability to utilise externally derived nutrients in within 4 to 5 d of hatching (Jin *et al.*, 1998). The young chick must switch from a lipid-dense yolk sac to a high carbohydrate diet. The digestibility of nutrients is reported to be low during 5 to 9 d post-hatch (Ward, 2014).

Development of digestive tract

Following hatch, rapid growth occurs in the relative weights of proventriculus, gizzard, and small intestine compared to other organs (Katanbaf *et al.*, 1988). Maximum growth is observed between d 4 to 8, followed by a relative decrease. The relative pancreas weight peaks at d 8 of age (Nitsan *et al.*, 1991). Dror *et al.* (1977) reported that though the relative weights of crop, oesophagus, proventriculus and gizzard did not increase during first 3 d after hatch, an increase of all intestinal segments was observed and peaked at d 8. Gizzard, the pacemaker of normal gut

motility, contributes to the grinding action of feed. Increased gizzard activity assists greater gastric and intestinal refluxes (Singh *et al.*, 2014) that improves digesta mixing with enzymes and facilitates nutrient digestion. At hatch, gizzard is the largest organ associated with GIT. The relative weight of the gizzard continuously decreases with age (Ravindran *et al.*, 2006). The mass of small intestine rises approximately six times by the first week post-hatch.

In a study by Uni *et al.* (1988), the relative weight of small intestine increased four times from hatch to 4 d of broiler age. The relative weights of digestive organs and GIT outstrips the BW gain, and the peak intestinal weight is recorded between d 7 to 14 (Iji *et al.*, 2001a). However, with advancing age, an increase is observed in the length of all segments of small intestine (Noy *et al.*, 2001). According to Nitsan *et al.* (1991), the relative weights of intestinal segments (duodenum, jejunum and ileum) reach at maximum at d 6 that decreased thereafter. A study by Ravindran *et al.* (2006) indicated that the relative weights of duodenum, jejunum and ileum increased over the first two weeks followed by a decline thereafter. The morphological development showed that the height and perimeter of villi increased by 34 to 100% in all small intestinal segments between d 4 and 10 post-hatch (Uni *et al.*, 1996). The rapid growth in gut size coupled with very low feed consumption during week 1 may result in mechanical inefficiency in the passage and mixing of digesta, negatively affecting nutrient digestion (Thomas *et al.*, 2008).

Proteolytic enzymes

Secretion and activity of proteolytic enzymes (trypsin, chymotrypsin, peptidase) in pancreas and small intestine are variable depending on age. Noy and Sklan (1995) determined trypsin, lipase, and amylase secretions from 4 to 21 d using 141Ce and reported lower duodenal secretion of all enzymes at d 4 compared to d 21. The net secretion of trypsin to the duodenum increased by 50-fold from d 4 to 21. According to Nitsan *et al.* (1991), the specific activity of pancreatic trypsin, expressed at units/g, declined at the first 3 to 6 d post-hatch, and increased on d 14 being 10 to 20% higher than those at hatching. Chymotrypsin activity consistently increased post-hatch to d 14 and remained steady until d 21. The activity of all pancreatic enzymes, expressed as units per kg BW, increased with advancing broiler age and reached a maximum at d 11 for trypsin and chymotrypsin. The activity of trypsin in small intestine enhanced by 10-fold from hatch to d 14 and chymotrypsin activity enhanced 3-fold to a maximum at d 20. In another study, the relative activity of aminopeptidase in all intestinal segments declined rapidly after hatching, reached at low point at d 10, followed by an increase at d 15. Similar results were recorded for dipeptidases (Tarvid, 1992). The increased activity and secretion of proteases with advancing age may confer some positive effects on AA digestibility in older birds.

Digesta passage rate

An important determinant of nutrient digestion in the GIT is feed transit time that influences the contact time among nutrients, digestive

enzymes, microbiome, and absorptive surface (Clemens *et al.*, 1975; Mateos *et al.*, 1982). Nutrient absorption is facilitated by longer digesta retention that increases contact time with absorptive cells (Washburn, 1991). Previous studies have reported slower passage rate in older birds (Sklan *et al.*, 1975; Noy and Sklan, 1995). Therefore, in older birds, the digesta are exposed longer to digestive and absorptive processes that may improve AA digestibility.

Activity of gut microbiota

The differences in the profile of microbiota at different broiler ages might influence AA digestibility. The GIT of newly hatched chick is sterile. At post-hatch, chicks receive initial seeding of microbiome from the farm environment (Donaldson *et al.*, 2017). Chicks may also source microbiome through eggshell pores prior to hatching (Roto *et al.*, 2016; Lee *et al.*, 2019). A series of developmental process occurs in the life cycle of microbiome including initial colonisation, species richness, followed by the complexity of population and maturation. The gut microbiota gradually changes with advancing broiler age and stabilises around three weeks post-hatch (Oakley *et al.*, 2014; Johnson *et al.*, 2018). Microbiota in chicken GIT are potential consumers of protein and AA and can compete for nutrients. Hence, the gut microbiome and their stability in chicken GIT may affect AA digestibility estimates in the host (Harrow *et al*., 2007; Yadav and Jha, 2019).

Antinutritional factors

All poultry feed ingredients, except for maize, contain an array of ANFs that negatively influence the digestion, absorption and utilisation of nutrients (Rickard and Thompson, 1997; Cowieson, 2005). Of these, perhaps most relevant in poultry diets is the NSP that are present in viscous grains such as wheat and barley (Choct and Annison, 1992a; Hughes and Choct, 1999). The soluble forms of NSP such as β-glucans, the major NSP in barley, and the pentosans of rye and wheat are viscous and exhibit notable antinutritive effects in poultry (Burnett, 1966; Campbell *et al.*, 1989).

A portion of NSP is of high molecular weight dissolves in the GIT of poultry that leads to an increase in the viscosity of the gut contents (Fengler and Marquardt, 1988; Bedford, 2002). Higher intestinal viscosity has a number of adverse effects on the nutritive values of diets including reduction of digesta passage, increased water intake, deterioration of litter quality, increased bacterial proliferation in the GIT leading to a change in the GIT environment (Choct and Annison, 1992a; Silversides and Bedford, 1999; Bedford, 2002). The NSP impedes the nutrient digestion and absorption mainly by two mechanisms. First, the soluble NSP forms gel-like viscous matter, impairing the interaction between nutrient substrates and endogenous enzymes. Secondly, the insoluble NSP fraction exerts a “cage effect” by encapsulating the nutrients (starch, protein) in endosperm cells, which impairs the contact between nutrients and digestive enzymes, consequently, reducing the nutrient digestibility (Åman and Graham,

1987; Izydorczyk and Dexter, 2008). Heating of diets might potentially increase intestinal viscosity by increasing solubility of NSP (Nissinen, 1994; Silversides and Bedford, 1999) and destroying endogenous enzymes in grains (Silversides and Bedford, 1999).

Sorghum also contains different antinutritive factors like phenolic compounds and phytate (Selle *et al.*, 2018). The phytate content in sorghum is higher than most grains (Selle *et al.*, 2003). The negative effects of antinutrients present in feed ingredients may vary depending on bird age and will potentially affect nutrient digestion. The increased FI, consequently, greater ingestion of antinutrients can exacerbate the effects in older birds (Szczurek *et al.*, 2020). On the contrary, according to Rotter *et al.* (1990), the volume of the GIT in older birds is sufficiently developed to resist the negative impact of digesta viscosity induced by β-glucan. Similarly other studies (Salih *et al.*, 1991; Almirall and Esteve-Garcia, 1994) suggest that the nutrient digestibility of barley diets increases with age and that the viscosity is not a limiting factor in older birds (Almirall *et al*., 1995).

Soybean meal contains some antinutritive factors like α-galactosides including raffinose, verbascose, stachyose. Young broilers are unable to digest α-galactosides (Carre´*et al*., 1995) that might affect nutrient digestibility. The ANFs present in canola meal (CM) are soluble and insoluble NSP and, phytic acid. The higher fibre and phytate contents lower the AA utilisation (Cowieson *et al.*, 2009) owing to poor digestion of protein-phytate complex (Bell and Keith, 1991). As stated by Edwards *et al.* (1989), older birds can utilise phytate-P more than

young birds. The higher endogenous phytase activity in the GIT of older birds increase phytate utilisation (Ravindran *et al.*, 1995), thereby reducing the adverse effects on AA digestion.

Gastric pH

Hydrochloric acid secretion in the GIT is pivotal to maintain a low digesta pH, which is necessary to convert pepsinogen to pepsin. The variation in gizzard pH depending on broiler age may affect AA digestion by influencing the secretion and activity of gastric proteolytic enzymes (Ravindran and Abdollahi, 2021). David *et al*. (2020) measured the gizzard pH at six ages (d 7, 14, 21, 28, 35 and 42) of broilers fed a maize-based diet. Compared to d 7, a reduction in gizzard pH was reported at d 14, which increased afterwards with advancing age. Angel *et al.* (2010) also reported a reduction in gizzard pH in broilers at d 14 following analysis of 15 published studies. As stated by Rynsburger (2009), the gastric acid secretion in the proventriculus increased from d 2 to 15, causing a decrease in pH. Although the digestive secretions and enzyme activities (Nitsan *et al.*, 1991), and concurrent secretion of HCl increase with broiler age, the gizzard pH increases with age due to increased intake of feed with a neutral pH (Ravindran, 2013).

2.6.2. Impact of bird age on the nutrient digestibility in feed ingredients

Age of birds had a significant influence on the metabolisable energy (ME) and, starch and fat digestibility of broilers in a study using three

cereal sources (wheat, maize, sorghum; Thomas *et al.*, 2008). In general, the values for nitrogen corrected apparent metabolisable energy (AME_n) decreased from d 3 to 7 and then increased at d 14. The AME_n for wheat were 13.90, 12.17, 11.06, 12.32, 13.24 (MJ/kg) at d 3, 5, 7, 9, and 14, respectively. Similar patterns were observed for the total tract digestibility coefficients of starch and fat. Starch digestibility values at 3, 7 and 14 d were 0.953, 0.898, 0.968 (wheat); 0.946, 0.920, 0.970 (sorghum); 0.962, 0.936, 0.975 (maize), respectively. The corresponding values for fat digestibility were 0.620, 0.419, 0.759 (wheat); 0.519, 0.426, 0.764 (sorghum); 0.687, 0.651, 0.775 (maize). The apparent total tract digestibility of starch, fat, and AA increased with increasing age of broilers in a study by Batal and Parsons (2002).

Increased MEn (from 12.4 to 14.4 MJ/kg) with maize-SBM based diet was determined between 1 and 14 d of broiler age (Batal and Parsons, 2002). Batal and Parsons (2003) found that 4 d old chicks extracted only 85% of the ME (11.4 MJ/kg DM) from SBM-based diet compared to 96.2% in 21-d-old broilers (12.9 MJ/kg DM). Examining the influence of age in utilisation of carbohydrate sources (i.e. dextrose, conventional maize starch, dextrinised maize starch and sucrose), Batal and Parsons (2004) found that the AME_n generally increased over the first 14 d of age of broilers. However, a decreased AME_n was found in 3 and 4 d of age in the same study. The dietary ME was observed to increase in broilers fed wheat-based diet at d 50

(13.98 MJ/kg DM) compared to d 30 (13.31 MJ/kg DM) by Wallis and Balnave (1984).

Fasina *et al.* (2004) reported that the excreta digestibility of starch and fat in turkey poults improved from d 7 to 14 by 3-8% and 9-15%, respectively. On the contrary, Moss *et al.* (2020) reported a reduction in ileal starch digestibility from 0.935 on d 10 to 0.885 on d 35 in broilers fed a maize-based diet. The corresponding starch digestibility values on d 10 and 35 were reported to be 0.901 and 0.845 for sorghum-based and 0.987 and 0.904 for wheat-based diets, respectively.

David *et al.* (2020) observed a decrease in apparent ileal calcium digestibility with advancing age in broilers from 0.51 on d 7 to 0.36 on d 21, and 0.27 on d 42. As observed by Li *et al.* (2018), the AIDC of calcium was higher in 9-d old broiler chickens than 21 d, regardless of the dietary calcium level. Babatunde and Adeola (2020) observed a decline in both the AIDC and SIDC of phosphorus with advancing broiler age (d 13 vs. 21) following feeding a maize-CM-based diet.

2.6.3. The effects of age on the digestibility of protein and amino acids in feed ingredients for broilers

From the above discussion, it can be hypothesised that the AA digestibility will be variable depending on broiler age. The ability of the bird to extract nutrients is limited during the first few weeks after hatch due to an immature digestive tract and lower activity of digestive enzymes (Nitsan *et al.*, 1991). This period is crucial because chicks

consume only small amount of feed, but have a high AA demand for tissue growth. Therefore, the relevance of using AA digestibility data obtained from older birds to younger birds is questionable. According to previous reports, the digestibility of AA appears to increase with broiler age (Wallis and Balnave, 1984; ten Doeschate *et al.*, 1993; Huang *et al.*, 2005). In contrast, some studies reported a decrease in AA digestibility with advancing broiler age (Fonolla *et al.*, 1981; ten Doeschate *et al.*, 1993). The discrepancy in published data underline the importance of better understanding of AA digestibility trends at different broiler ages.

Most published data on the AIDC AA in feed ingredients have been generated in older birds, mostly 22 to 35-d-old broilers. From previous studies, it is evident that the apparent AA digestibility differed depending on broiler age (Fonolla *et al.*, 1981; Wallis and Balnave, 1984; Noy and Sklan, 1995; Huang *et al.*, 2005).

Wallis and Balnave (1984) observed increased AIDC AA in broilers from d 30 to 50 post-hatch fed finisher diets containing a range of grain and protein sources. Batal and Parsons (2002) reported an increase in the total tract digestibility of AA in a maize-SBM diet from hatch to 10 d of age. Uni *et al.* (1995), in a study with maize-SBM-based diets, reported an increase in N digestion with age in heavy and light strain chicks from 70% on d 4 to 90% on d 14. An increase in ileal N digestibility from 78% at d 4 to about 90% at d 21 has been reported in broilers fed diets based on maize-SBM by Noy and Sklan (1995). It was

concluded that the hydrolysis of proteins was not optimum due to insufficient proteolytic activity during the early post-hatch period.

Huang *et al.* (2005) evaluated the AIDC AA in a range of feed ingredients (wheat, sorghum, maize, SBM, CM, MBM, cotton seed meal and mill run) at three ages (d 14, 28 and 42) of broilers. They summarised that the AIDC AA increased in older birds though the age effect was variable depending on the ingredient and AA. The AIDC AA in maize, CM, SBM and, MBM were higher at d 28 and 42 than d 14. In sorghum, the AIDC AA was higher at d 42 than d 28, but similar to that at d 14. On the contrary, the AIDC of most AA in wheat was found to be higher at d 14 than at d 28 and 42. No age effect was observed in the case of cottonseed meal.

Adedokun *et al.* (2008) reported the ileal AA digestibility of five plant-based feed ingredients (light and dark distiller's dried grains with solubles, CM, SBM and maize) in broilers at d 5 and 21 of age. The AIDC AA increased in all ingredients as the birds grew older. A reduction in the AIDC of total AA in MBM for broilers from 0.705 on d 5 to 0.595 on d 21 was recorded by Adedokun *et al.* (2007a).

Toghyani *et al.* (2015) reported a decrease in the AIDC of crude protein and most AA in expeller extracted CM processed at different temperatures (90, 95 and 100 ºC) at d 10 compared to d 24 broilers.

In contrast to the above findings, some studies reported lower digestibility of protein and AA with advancing age (Hakansson and Erikson, 1974; Fonolla *et al.*, 1981; Zuprizal *et al.*, 1992). Fonolla *et*

al. (1981) reported a reduction in apparent protein digestibility of broilers with advancing age (d 21 vs. d 52) by feeding four diets (starter, finisher, finisher diet with 1.39% urea, finisher diet with 0.25% Lys, 0.10% Met and 0.22% Gly). These researchers suggested that this reduction was caused by increased excretion of metabolic N. They also indicated that the result may be due to error in the estimation of faecal component of protein. Zuprizal *et al.* (1992) reported that the true digestibility of protein and AA in SBM or rapeseed meal for broilers decreased from week 3 to 6. ten Doeschate *et al.* (1993) also observed decreased protein digestibility coefficients with increasing broiler age, with values of 0.849 (d 13 to 15), 0.830 (d 27 to 29) and 0.840 (d 41 to 43). However, it must be highlighted that, as discussed previously, the early studies based on total tract digestibility are not comparable to ileal digestibility findings.

A compilation of SIDC AA values in broilers at different ages for a range of feed ingredients is presented in Tables 2.2 and 2.3. Except for some (Garcia *et al.*, 2007; Adedokun *et al.*, 2007a, 2008; Toghyani *et al.*, 2015; Szczurek, 2010; Szczurek *et al.*, 2020), all studies were conducted with broilers over 20 d of age.

Table 2.2. Standardised ileal digestibility coefficients of digestible crude protein (CP) and amino acids in grains at different broiler ages

				Indispensable AA										Dispensable AA							
References	Age (d)	GT[1]	CP	Arg	His	Ile	Leu	Lys	Met	Phe	Thr	Trp	Val	Ala	Asp	Cys[2]	Glu	Gly[2]	Pro	Ser	Tyr
Garcia *et al.* (2007)	7	Maize	-	0.863	0.883	0.676	0.919	0.804	0.841	0.872	0.749	-	0.839	0.862	0.764	0.829	0.876	-	-	0.779	0.846
	21		-	0.899	0.922	0.794	0.945	0.874	0.889	0.916	0.840	-	0.905	0.885	0.782	0.847	0.898	-	-	0.789	0.865
Adedokun *et al.* (2008)	5		-	0.820	0.765	0.812	0.852	0.732	0.788	0.820	0.695	0.848	0.768	0.843	0.775	0.801	0.867	0.737	0.755	0.783	0.827
Adedokun *et al.* (2008, 2009a)	21		-	0.922	0.883	0.913	0.938	0.863	0.952	0.923	0.840	-	0.890	0.930	0.882	0.895	0.942	0.857	0.910	0.873	0.913
Szczurek (2009)	30		-	0.935	0.913	0.889	0.908	0.859	0.892	0.897	0.752	0.946	0.906	-	-	0.753	-	0.805	-	0.859	0.848
Szczurek (2010)	14		-	0.850	0.840	0.820	0.830	0.750	0.840	0.830	0.720	0.840	0.800	0.830	0.780	0.720	0.870	0.750	0.820	0.760	0.750
Kim *et al.* (2012a)	21		-	0.870	0.853	0.856	0.912	0.744	0.891	0.894	0.765	-	0.796	0.898	0.823	0.880	0.910	-	0.906	0.846	0.870
Kong and Adeola (2013b)	26		0.880	0.925	0.895	0.914	0.926	0.873	0.933	0.912	0.858	0.917	0.885	0.915	0.901	0.899	0.934	0.849	0.911	0.898	0.886
Ullah *et al.* (2016)	21		-	0.933	0.915	0.978	0.947	0.910	0.949	0.942	0.866	-	0.926	0.901	0.834	0.886	0.913	0.794	-	0.823	-
Ravindran *et al.* (2017)	28		0.865	0.924	0.877	0.893	0.930	0.842	0.923	0.902	0.816	0.849	0.878	0.910	0.871	0.885	0.823	0.932	0.908	0.899	-
Cowieson *et al.* (2019)	28		-	0.868	0.854	0.818	0.897	0.699	0.864	0.872	0.714	-	0.814	0.868	0.773	0.868	0.890	0.759	0.863	0.809	0.842
Kadim *et al.* (2002)	35	Sorghum	-	0.983	0.918	0.981	0.953	0.983	0.990	0.998	0.916	-	0.991	0.945	0.982	-	0.950	0.869	0.949	0.957	0.975
Kadim *et al.* (2002)	35	Wheat	-	0.819	0.788	0.883	0.803	0.906	0.921	0.858	0.913	-	0.829	0.594	0.702	-	0.921	0.679	0.925	0.875	0.594
Garcia *et al.* (2007)	7		-	0.766	0.784	0.603	0.801	0.576	0.651	0.809	0.550	-	0.697	0.580	0.497	0.777	0.897	-	-	0.708	0.773
	21			0.786	0.882	0.774	0.914	0.767	0.784	0.912	0.808	-	0.859	0.740	0.622	0.862	0.933	-	-	0.799	0.837
Bandegan *et al.* (2009)	21		0.884	0.839	0.863	0.911	0.908	0.821	0.924	0.993	0.851	-	0.870	0.834	0.825	0.916	0.960	0.838	0.955	0.887	0.834
Szczurek (2009)	30		-	0.826	0.879	0.861	0.875	0.783	0.815	0.856	0.767	0.865	0.861	-	-	0.800	-	0.798	-	0.861	-
Szczurek (2010)	14		-	0.830	0.870	0.870	0.890	0.810	0.860	0.870	0.760	0.850	0.850	0.830	0.820	0.850	0.940	0.820	0.940	0.840	0.790
Bandegan *et al.* (2011)	21		0.872	0.852	0.870	0.904	0.905	0.837	0.914	0.938	0.854	-	0.877	0.838	0.838	0.908	0.966	0.841	0.954	0.891	-
Szczurek *et al.* (2020)	14		-	0.890	0.900	0.910	0.920	0.850	0.920	0.900	0.790	0.900	0.860	0.830	0.870	0.880	0.960	0.830	0.950	0.870	0.890
	28		-	0.870	0.890	0.890	0.880	0.820	0.900	0.890	0.790	0.890	0.880	0.790	0.760	0.840	0.950	0.790	0.960	0.860	0.910
Szczurek (2009)	30	Barley	-	0.793	0.902	0.850	0.809	0.792	0.777	0.850	0.782	0.826	0.835	-	-	0.739	-	0.763	-	0.803	-
Bandegan *et al.* (2011)	21		0.797	0.804	0.807	0.839	0.848	0.805	0.883	0.909	0.806	-	0.825	0.781	0.781	0.839	0.876	0.767	0.866	0.822	-
Szczurek *et al.* (2020)	14		-	0.770	0.760	0.810	0.830	0.780	0.760	0.800	0.750	0.860	0.790	0.760	0.750	0.750	0.850	0.720	0.860	0.760	0.760
	28		-	0.820	0.910	0.920	0.880	0.820	0.820	0.850	0.870	0.920	0.860	0.790	0.810	0.790	0.910	0.850	0.910	0.830	0.870

[1]Grain Type (GT); [2]Semi-indispensable amino acids for poultry.

Table 2.3. Standardised ileal digestibility coefficients of crude protein (CP) and amino acids in protein sources at different broiler ages

				Indispensable AA										Dispensable AA							
References	Age (d)	PS[1]	CP	Arg	His	Ile	Leu	Lys	Met	Phe	Thr	Trp	Val	Ala	Asp	Cys[2]	Glu	Gly[2]	Pro	Ser	Tyr
Kadim *et al.* (2002)	35	MBM	-	0.841	0.806	0.798	0.830	0.883	0.959	0.800	0.855	-	0.836	0.845	0.711	-	0.765	0.919	0.834	0.821	0.736
Adedokun *et al.* (2007a)	5		-	0.735	0.533	0.485	0.578	0.598	0.545	0.603	0.483	-	0.520	0.683	0.425	0.030	0.605	0.690	0.628	0.520	0.510
	21		-	0.838	0.720	0.712	0.765	0.758	0.763	0.782	0.705	-	0.745	0.817	0.617	0.332	0.770	0.807	0.767	0.700	0.723
Szczurek (2009)	30		-	0.886	0.871	0.865	0.863	0.872	0.913	0.872	0.847	0.883	0.857	-	-	0.843	-	0.864	-	0.852	0.870
Adedokun *et al.* (2009a)	21		-	0.755	0.718	0.725	0.750	0.550	0.753	0.748	0.692	0.652	0.728	0.725	0.540	0.392	0.722	0.647	0.627	0.650	0.718
Kim *et al.* (2012b)	21		-	0.848	0.735	0.736	0.777	0.761	0.740	78.4	0.754	-	0.763	0.847	0.713	0.639	0.796	-	0.835	0.807	0.737
Adedokun *et al.* (2014)	21		-	0.758	0.664	0.705	0.735	0.704	0.718	0.780	0.663	-	0.717	0.741	0.560	0.385	0.701	0.688	0.618	0.650	-
Kadim *et al.* (2002)	35	SBM	-	0.960	0.931	0.962	0.909	0.957	0.980	0.915	0.947	-	0.927	0.908	0.918	-	0.918	0.897	0.921	0.947	0.917
Garcia *et al.* (2007)	7		-	0.781	0.817	0.663	0.757	0.804	0.721	0.790	0.674	-	0.754	0.717	0.670	0.578	0.815	-	-	0.680	-
	21		-	0.857	0.807	0.776	0.796	0.830	0.760	0.777	0.705	-	0.758	0.728	0.780	0.655	0.778	-	-	0.787	-
Adedokun *et al.* (2008)	5		-	0.897	0.875	0.852	0.857	0.873	0.882	0.857	0.805	0.925	0.848	0.850	0.827	0.761	0.880	0.814	0.813	0.858	0.868
Adedokun *et al.* (2008, 2009a)	21		-	0.915	0.892	0.878	0.870	0.895	0.915	0.875	0.837	-	0.870	0.868	0.858	0.818	89.5	85.0	87.5	85.7	88.0
Szczurek (2009)	30		-	0.886	0.871	0.865	0.863	0.872	0.913	-	0.847	0.883	0.857	-	-	0.843	-	0.864	-	0.852	0.870
Szczurek (2010)	14		-	0.880	0.880	0.840	0.870	0.860	0.920	0.880	0.820	0.930	0.820	0.830	0.860	0.780	0.930	0.850	0.850	0.850	0.880
Kim *et al.* (2012b)	21		-	0.917	0.894	0.870	0.875	0.891	0.904	0.885	0.851	-	0.858	0.873	0.863	0.830	0.903	-	0.884	0.889	0.889
Kong and Adeola (2013b)	26		0.855	0.898	0.885	0.857	0.850	0.867	0.902	0.861	0.836	0.883	0.857	0.854	0.831	0.789	0.869	0.838	0.860	0.844	0.858
Ravindran *et al.* (2014b)	34		0.850	0.900	0.860	0.850	0.850	0.880	0.880	0.860	0.810		0.840	0.850	0.850	0.730	0.880	0.830	0.850	0.860	0.870
Adedokun *et al.* (2014)	21		0.890	0.928	0.910	0.896	0.898	0.907	0.923	0.927	0.874	-	0.896	0.893	0.882	0.854	0.918	0.879	0.890	0.895	-
Ullah *et al.* (2016)	21		-	0.915	0.879	0.878	0.871	0.896	0.898	0.854	0.837	-	0.865	0.848	0.821	0.772	0.824	0.876	-	0.830	-
Cowieson *et al.* (2019)	28		-	0.868	0.854	0.818	0.897	0.699	0.864	0.872	0.714	-	0.814	0.868	0.773	0.868	0.890	0.759	0.863	0.809	0.842
Adedokun *et al.* (2008)	5	CM	-	0.862	0.843	0.793	0.825	0.800	0.842	0.823	0.735	0.950	0.792	0.813	0.783	0.772	0.873	0.768	0.733	0.782	0.810
Adedokun *et al.* (2008, 2009a)	21		-	0.857	0.825	0.780	0.802	0.792	0.853	0.802	0.738	-	0.775	0.800	0.768	0.780	0.872	0.788	0.773	0.758	0.783
Kim *et al.* (2012b)	21		-	0.846	0.820	0.768	0.786	0.769	0.819	0.802	0.734	-	0.757	0.781	0.768	0.770	0.853	-	0.788	0.779	0.775
Woyengo *et al.* (2010)	21	SECM[3]	-	0.795	0.840	0.779	0.756	0.773	0.864	0.764	0.757	-	0.789	0.770	0.691	0.681	0.834	0.792	0.770	0.758	0.747
	21	EECM[4]	-	0.837	0.849	0.833	0.795	0.787	0.837	0.804	0.833	-	0.836	0.797	0.775	0.742	0.865	0.827	0.726	0.828	0.795
Toghyani *et al.* (2015)	10	EECM[4]	0.777	0.842	0.816	0.750	0.793	0.764	0.809	0.788	0.717	-	0.740	0.764	0.740	0.739	0.863	0.776	0.708	0.745	-
	24	EECM[4]	0.783	0.861	0.846	0.797	0.838	0.761	0.867	0.800	0.755	-	0.791	0.812	0.770	0.754	0.851	0.786	0.783	0.775	-

[1]Protein Sources (PS); [2]Semi-indispensable amino acids for poultry; [3]Solvent extracted canola meal (SECM); [4]Expeller extracted canola meal (EECM).

CM = canola meal; MBM = meat and bone meal; SBM = soybean meal.

The limitation of previous age-related standardised AA digestibility studies is that they were restricted to either two or three specific broiler ages. Apart from few (Adedokun *et al.*, 2007a, 2008; Szczurek *et al.*, 2020; Toghyani *et al.*, 2015), these SIDC AA studies used a single EAA flow value, derived from older birds.

Szczurek *et al.* (2020) investigated the ileal AA digestibility in wheat, triticale and barley at two ages (d 14 and 28) of broilers and reported no age effect on the SIDC of AA in wheat. In contrast, the SIDC AA of all AA in barley and most AA in triticale increased by the age of broilers. Garcia *et al.* (2007) determined the SIDC AA in maize and SBM at 7 and 21-d-old broilers and did not observe any difference between the two ages for most IAA.

In a study by Adedokun *et al.* (2008), lower SIDC AA was recorded at d 5 compared to d 21 post-hatch in case of maize and, light and dark distiller's dried grains with solubles. The SIDC in SBM and CM was not influenced by age. Toghyani *et al.* (2015) reported higher SIDC AA in older birds (d 10 vs. 24) in expeller extracted CM. However, Adedokun *et al.* (2007a) recorded decreased SIDC AA with age (0.760 on d 5 vs. 0.632 on d 21) in MBM.

The specific reasons for the age influence on AA digestibility are complex and complicated by the interactions among myriad of factors including digestion and absorption of nutrients, antinutritive factors in the ingredients, FI, digesta passage rate and stability of intestinal

microbiota. The variation in EAA flow at different broiler ages is one prime contributing factor contributing to variable SIDC AA.

2.7. Research gaps and conclusions

The AA digestibility estimates from broilers fed pelleted diets may differ from assays where birds were provided unprocessed mash diets. Diverse factors associated with pelleting (as discussed in section 2.5.3.) may influence the AA digestibility. Pellet is the most prevalent FF used in the broiler industry and the application of AA digestibility data generated in mash diet to feed formulations could be questioned. All previous studies comparing nutrient digestibility in mash vs. pellet have used complete diets. No studies to date have investigated the influence of FF (mash vs. pellet) on the SIDC AA in specific feed ingredients of broilers. Therefore, the first two experiments of this thesis research examined the possible effect of FF on the SIDC AA in grains and protein sources for broilers. The aim was to identify the FF to be used in the succeeding research phase on age-related SIDC AA.

The digestibility of AA is generally determined using older birds (3 to 5 weeks post-hatch) and the measured values are used across the whole growth period. As the digestive enzyme production and absorptive capacity increase with age, the ability of birds to extract nutrients from feeds may potentially change with age. Therefore, the application of a single value of digestible AA for all growth stages of broilers is debateable and highlights the need for age-dependent digestible AA estimates for use in feed formulations. To date, no studies have

investigated the influence of age on the SIDC AA from hatch to the market age of broilers (d 42 post-hatch). Another important issue is that the basal EAA flow may vary depending on broiler age. To the author's knowledge, no studies reported the EAA flows from hatching to the end of growth cycle of broilers. Considering these limitations, the second objective of this thesis research was to evaluate the influence of age on the SIDC AA in commonly used feed ingredients at six broiler ages (d 7, 14, 21, 28, 35 and 42). To correct the AIDC AA estimates, the EAA flows were also determined at these six ages. Throughout the whole thesis research, the AIDC AA data were standardised using age-specific EAA flows.

CHAPTER 3 Influence of feed form on the standardised ileal amino acid digestibility of common grains for broiler chickens[1]

3.1. Abstract

The present experiment was carried out to determine the influence of feed form (FF) on the apparent ileal digestibility coefficients (AIDC) of dry matter (DM), starch and, standardised ileal digestibility coefficients (SIDC) of nitrogen (N) and amino acids (AA) in different grain types (GT). Eight experimental diets were developed in a 4 × 2 factorial arrangement of treatments that included four GT (maize, sorghum, wheat and barley) and two FF (mash vs. pellet). A N-free diet was also used to determine the endogenous N and AA losses for the calculation of SIDC values. Titanium dioxide was incorporated in all diets as an indigestible indicator. Ileal nutrient digestibility was determined using the direct method. The four assay diets were formulated to contain 938 g/kg grain as the sole source of AA and starch in the diet. The assay diets and N-free diet were randomly allotted to six replicate cages (eight birds per cage) and fed from 19 to 23 d post-hatch and the ileal digesta was collected on d 23. Pelleting reduced ($P < 0.05$) the AIDC of DM in all test grains. A significant ($P < 0.001$) GT × FF interaction was observed for AIDC of starch. Starch digestibility was unaffected by FF in maize- and sorghum-based diets, but was reduced in pelleted wheat- and barley-based diets. The GT had

[1]*Animal Feed Science and Technology* (2021), *272*, 114743.

a significant ($P < 0.05$ to 0.001) effect on the SIDC of N and AA. In general, the average SID AA values were higher in maize followed by sorghum or wheat and barley. The FF showed no significant ($P > 0.05$) effect on the SIDC of N and AA except Cys and Pro, in which the SIDC values were higher ($P < 0.05$) in mash than the pelleted diet. Overall, the present data suggest that the physical form of the feed can impact the digestibility of DM, starch and AA. Because the FF effect on nutrient digestibility can vary depending on the grain type and nutrient, the FF effect should be taken into account in feed evaluation assays to achieve the optimum use of nutrients especially AA.

3.2. Introduction

Knowledge on the digestibility of nutrients in different feed ingredients used in poultry diets is critical for precise feed formulation and for more efficient and sustainable use of feed ingredients. It is well known that the digestible amino acid (AA) contents of feed ingredients are superior to total AA contents in expressing the amounts that actually become available for maintenance and production purposes (Ravindran *et al.*, 1999). Generally, AA digestibility is determined by sampling and analysis of ileal digesta or excreta from intact or caecectomised birds. The ileal digestibility assay has advantages over the excreta collection method as the modifying action of hindgut microflora on AA profile is avoided and there is no complication of combined voiding of faecal and urinary AA (Kadim *et al.*, 2002). Ileal AA digestibility values can be referred to as either apparent or standardised/true. Standardised AA digestibility differs from the apparent digestibility as it involves a

correction for endogenous AA (EAA) losses arising from various digestive secretions, mucoproteins and desquamated epithelial cells lining the gut (Ravindran and Hendriks, 2004). The standardised ileal digestibility (SID) values for different feed ingredients are more additive than the apparent values. The application of SID of AA in practical feed formulation would benefit poultry production by improving the accuracy of feed formulation and reducing the diet cost and nitrogen pollution into the environment (Kong and Adeola, 2013b; Cowieson *et al.*, 2019).

Starch is the principal source of energy in grain-based poultry diets and contributes to more than half of the metabolisable energy intake (Svihus, 2011b). Therefore, the digestibility of starch renders a great influence on the energy value in poultry diets. Several studies have reported the effect of pelleting on the starch digestibility of different grain-based diets. Abdollahi *et al.* (2011) reported a markedly lower apparent ileal digestibility coefficients (AIDC) of starch in a wheat-based pelleted diet (0.834) compared to a mash diet (0.959). Pelleting did not have any effect on the AIDC of starch in maize-based diets (Abdollahi *et al.*, 2013b). Inferior starch digestibility in a sorghum-based pelleted diet (0.744) compared to mash (0.816) was observed by Selle *et al.* (2013).

A large volume of published data (Ravindran *et al.*, 1998; Lemme *et al.*, 2004; Bryden *et al.*, 2009a) on AA digestibility in a range of feed ingredients for broilers are now available. Different methodologies (i.e. direct, difference, regression) have been used to generate the data.

Regardless of the methodology, available values on digestible AA for broilers have been determined using mash diets because of its simplicity and the influence of pelleting process and feed form (FF) has been overlooked in AA digestibility assays. However, the majority of feeds used in broiler production is fed in pelleted or crumbled forms. Feed processing, texture and conditions associated with the pelleting process such as temperature, pressure and moisture could have a substantial impact on nutrient digestion (Svihus, 2001; Abdollahi *et al.*, 2013a). Therefore, applying AA digestibility data generated with mash diets to practical situations, where the most of feed is pelleted, could be questioned. It has been speculated that the pelleting process might enhance protein digestibility by increasing protein denaturation and dissociation of protein macrostructure into smaller subunits (Camire, 1991; Thomas *et al.*, 1998; Ludikhuyze *et al.*, 2003). On the other hand, steam-pelleting of diets might decrease protein solubility and compromise its availability (Svihus and Zimunja, 2011). Gelatinisation of starch during pelleting renders it more available for enzymatic degradation. Hence, digestibility of starch may be expected to increase after pelleting (Abdollahi *et al.*, 2013b). Where gelatinisation minimal, this effect of pelleting on starch utilisation is only of modest importance (Svihus *et al.*, 2004). Recent evidence suggests that the nature of digestibility response to pelleting is dependent on the ingredient and the specific nutrient (Abdollahi *et al.*, 2013b, 2014; Naderinejad *et al.*, 2016) and that pelleting has no positive impact on the digestibility of

major nutrients, including nitrogen (N) and starch, in poultry diets (Abdollahi *et al.*, 2013a).

The relevance of the current study is that all previous studies comparing nutrient digestibility in mash vs. pellets have used complete diets. Despite the potential effect of FF on nutrient digestibility of grains in poultry, to the author's knowledge, no studies to date have investigated this aspect. It was hypothesised that the pelleting process will impact the nutrient digestibility of grains and grain type (GT) and FF will interact on nutrient digestibility. Therefore, the present study was designed to determine the impact of FF (mash vs. pellet) on the standardised ileal digestibility coefficients (SIDC) of N and AA, and AIDC of starch and dry matter (DM) in four common grains (maize, sorghum, wheat, and barley) used in broiler diets.

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. Diets and experimental design

Four grains (maize, sorghum, wheat and barley) and two FF (mash and pellet) were used to generate a 4 × 2 factorial arrangement of eight dietary treatments. The grains were obtained from a commercial supplier and ground in a hammer mill to pass through a screen size of 3.0 mm. The nutritional evaluation of grain samples was carried out in

two phases namely, (i) laboratory evaluation, and (ii) standardised ileal AA digestibility assay. A representative sample of each grain was analysed, in duplicate, for DM, N, starch, crude fat, neutral detergent fibre (NDF), gross energy (GE), AA, calcium (Ca), phosphorus and ash, as described below.

The SIDC of N and AA in the grains was determined using the direct method, as it reflects the AA digestibility in the single ingredient. Four assay diets, containing 938 g/kg of grain as the only source of AA and starch in the diet, were developed (Table 3.1). Each diet was then divided into two equal batches, and one was offered as mash and the other was pelleted.

Table 3.1. Composition of the basal diets and nitrogen-free diet (g/kg, as fed basis) used in the ileal amino acid digestibility assay

Ingredients	Maize	Sorghum	Wheat	Barley	NFD
Test ingredient	938	938	938	938	-
Soybean oil	20	20	20	20	50
Titanium dioxide	5.0	5.0	5.0	5.0	5.0
Dicalcium phosphate	18	18	18	18	19
Limestone	13	13	13	13	13
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 premix[1]	1.0	1.0	1.0	1.0	3.0
Vitamin premix[1]	1.0	1.0	1.0	1.0	2.0
Maize starch	-	-	-	-	842
Cellulose[2]	-	-	-	-	50
K_2HPO_4	-	-	-	-	12

[1]Supplied per kilogram of diet: antioxidant (ethoxyquin), 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.

[2]Ceolus, Microcrystalline Cellulose, Asahi Kasei Corporation, Tokyo, Japan.

For pelleting, all diets were steam-conditioned at 70 ºC for 30 s and pelleted through a pellet mill (Model Orbit 15; Richard Sizer Ltd.,

Kingston-upon-Hull, UK) capable of manufacturing 180 kg of feed/h and equipped with a die ring with 3 mm apertures and a depth of 35 mm.

Basal endogenous N and AA flow was determined in a cohort assay by offering a N-free diet (NFD) to birds each from 19 to 23 d of age, according to the procedures described by Ravindran *et al.* (2009) (Table 3.1). The diets (test diets and NFD) contained titanium dioxide (5 g/kg; Merck KGaA, Darmstadt, Germany) as an indigestible marker.

3.3.2. Birds and housing

A total of 432, day-old male Ross 308 broilers, obtained from a commercial hatchery, were raised in floor pens and fed a commercial broiler starter diet (12.60 MJ/kg metabolisable energy; 230 g/kg crude protein) until d 18.

On d 18, the birds were individually weighed and allocated to 54 cages (48 cages for dietary treatments and six cages for N-free diet; eight birds per cage), so that the average body weight per cage was similar. The experimental diets were offered for 4 d from 19 to 23 d post-hatch. The diets, in mash or pellet forms, were offered *ad libitum* and water was available at all times. The floor pens and grower cages were housed in an environmentally controlled room with 20 h of fluorescent illumination per day. The average temperature was 32 ± 1 ºC during first week and was gradually reduced to 23 ºC by the end of the third week.

3.3.3. Feed intake and excreta scoring

Feed intake (FI) was recorded from d 19 to 23 post-hatch on a cage basis and mortality was recorded daily. Freshly voided excreta in different cages were also scored daily for wetness on a scale of 1 to 5 (with 1 representing normally formed excreta and 5 representing very watery excreta) (Ravindran *et al.*, 2008).

3.3.4. Determination of the coefficient of apparent ileal digestibility

On d 23, all birds were euthanised by intravenous injection (1 mL per 2 kg body weight) of sodium pentobarbitone solution (Provet NZ Pty. Ltd., Auckland, New Zealand) and the digesta were collected from the lower half of the ileum, as described by Ravindran *et al.* (2005). The ileum was defined as that portion of the small intestine extending from the Meckel's diverticulum to a point ~40 mm proximal to the ileocaecal junction. Briefly, the ileum was excised and divided into halves (anterior and posterior ileum) and the digesta samples were collected from the lower half towards the ileocaecal junction after gently flushing with distilled water into plastic containers. Digesta from birds within a cage were pooled, frozen immediately after collection and subsequently lyophilised (Model 0610, Cuddon Engineering, Blenheim, New Zealand). Diet and lyophilised digesta samples were ground to pass through a 0.5-mm sieve and stored in airtight plastic containers at 4 ºC until laboratory analysis of DM, starch, titanium (Ti), N and AA.

3.3.5. Chemical analysis

Dry matter content was measured using the standard procedure (Method 930.15; AOAC International, 2016). Titanium was determined on a UV spectrophotometer (Berthold Technologies GmbH and Co. KG, Bad Wildbad, Germany) following the method described by Short *et al.* (1996). Gross energy was measured by adiabatic bomb calorimeter (Gallenkamp autobomb, Weiss Gallenkamp Ltd., Loughborough, UK) standardised with benzoic acid. Starch was measured using the Megazyme Total Starch Assay kit (Megazyme International Ireland Ltd., Wicklow, Ireland) based on thermostable α-amylase and amyloglucosidase (McCleary *et al.*, 1997; AOAC International, 2016). Nitrogen was determined by combustion (Method 968.06; AOAC International, 2016) using a carbon nanosphere-200 carbon, N and sulphur auto analyser (LECO Corporation, St. Joseph, MI). The crude protein (CP) content was calculated as N × 6.25. The fat content was measured using the Soxhlet extraction procedure (Method 2003.06; AOAC International, 2016) and the neutral detergent fibre (NDF) content was measured (Method 2002.04; AOAC International, 2016) by using Tecator Fibertec^{TM} (FOSS Analytical AB, Höganäs, Sweden). Ash content was measured by a standard procedure (Method 942.05; AOAC International, 2016) using a muffle furnace at 550 ºC for 16 h. Calcium and phosphorus concentrations were measured by Inductively Coupled Plasma-Optical Emission Spectroscopy (ICP-OES) using a Thermo Jarrell Ash IRIS instrument (Thermo Jarrell Ash Corporation, Franklin, MA).

Amino acids were determined following standard procedures (Method 994.12; AOAC International, 2011). The samples were hydrolysed with 6 N hydrochloric acid (HCl) containing phenol for 24 h at 110 ± 2 ºC in glass tubes in an oven. The AA were determined using AA analyser (ion exchange) with ninhydrin post column derivatisation. The chromatograms detected at 570nm and 440nm were integrated using dedicated software (Agilent Open Lab software, Waldbronn, Baden-Württemberg, Germany). Cysteine and Met, the sulphur containing AA, were analysed as cysteic acid and methionine sulphone, respectively by oxidation with performic acid-phenol for 16 h at 0 ºC prior to hydrolysis.

In the case of Trp analysis, the samples were saponified under alkaline conditions with barium hydroxide solution in the absence of air at 110 ºC for 20 h in an autoclave. Following hydrolysis, the α-methyl Trp as internal standard, was added to the mixture. After adjusting the hydrolysate to pH 3.0 and diluting with 30% methanol, Trp and the internal standard were separated by reverse phase chromatography (RP-18) on a HPLC column (CORTECS C18 Column; 2.7 µm, Waters Corporation, Dublin, Ireland). Detection was selectively done by means of a fluorescence detector to prevent interference by other AA and other constituents.

3.3.6. Calculations

All data were expressed on a DM basis for calculations. The AIDC of nutrients were calculated from the dietary ratio of nutrient to Ti relative

to the corresponding ratio in the ileal digesta using the following formula.

AIDC of nutrient = $[(\text{nutrient}/\text{Ti})_d - (\text{nutrient}/\text{Ti})_i]/(\text{nutrient}/\text{Ti})_d$

Where, $(\text{nutrient} / \text{Ti})_d$ = ratio of nutrient to Ti in the diet, and

$(\text{nutrient} / \text{Ti})_i$ = ratio of nutrient to Ti in the ileal digesta.

The basal ileal endogenous AA (EAA) flow at the terminal ileum of the birds fed the N-free diet was calculated as grams lost per kilogram of DM intake (DMI) using the following formula (Moughan *et al*., 1992).

Basal EAA flow (g/kg DMI) = AA concentration in ileal digesta (g/kg) × [Diet Ti (g/kg) /Ileal digesta Ti (g/kg)]

Apparent digestibility data for N and AA were then converted to SIDC, using endogenous N and AA values determined from birds fed the N-free diet (Ravindran *et al*., 2014b).

SIDC = AIDC + [Basal EAA (g/kg DMI)/Ing. AA (g/kg DM)]

Where, SIDC = standardised ileal digestibility coefficients of the AA,

AIDC = apparent ileal digestibility coefficients of the AA,

Basal EAA = basal endogenous AA loss, and

Ing. AA = concentration of the AA in the ingredient.

3.3.7. Data Analysis

Cage was considered as the experimental unit. The data were subjected to two-way analysis of variance to determine the main effects (GT and FF) and their interaction using the General Linear Model (GLM) procedure of the SAS Institute Inc. (version 9.4; 2015; SAS Institute

Inc., Cary, NC.). Differences were considered to be significant at $P < 0.05$ and significant differences between means were separated by Least Significant Difference test when a significant F-test was detected.

3.4. Results

3.4.1. Proximate and nutrient compositions

The proximate and nutrient compositions of the grains are represented in Table 3.2.

Table 3.2. Proximate, carbohydrate and amino acid composition of grains (g/kg, as-received basis)

	Maize	Sorghum	Wheat	Barley
DM	875	881	869	880
Nitrogen (N)	12.9	17.0	19.7	17.0
CP (N×6.25)	80.6	106	123	106
Starch	590	606	532	541
Fat	32.4	32.6	18.5	20.9
NDF	83.1	62.2	104	110
GE (MJ/kg)	16.3	16.7	16.2	16.3
Ash	20.5	15.5	18.4	18.6
Calcium	0.17	0.10	0.21	0.14
Phosphorus	2.47	2.89	3.51	3.02
Indispensable amino acids (IAA)				
Arg	4.09	4.20	5.96	5.50
His	2.15	2.42	2.77	2.39
Ile	2.60	4.14	4.03	3.86
Leu	8.29	14.4	8.04	7.51
Lys	2.89	2.38	3.50	4.19
Met	1.48	1.70	1.88	1.81
Thr	2.81	3.39	3.39	3.56
Trp	0.68	1.26	1.44	1.39
Val	3.69	5.25	5.26	5.62
Total IAA	28.7	39.1	36.3	35.8
Dispensable amino acids (DAA)				
Ala	5.22	9.93	4.30	4.51
Asp	5.58	7.19	6.17	6.58
Cys[1]	1.75	1.92	2.74	2.48
Glu	13.7	22.6	35.2	25.3
Gly[1]	3.26	3.32	5.14	4.41
Pro	6.95	9.81	12.9	12.9
Ser	3.58	4.84	5.47	4.45
Total DAA	40.0	60	72	61
Total AA[2]	68.7	98.7	108	96.5

CP = crude protein; DM = dry matter; GE = gross energy; NDF = neutral detergent fibre.
[1]Semi-indispensable amino acids for poultry. [2]Total AA = IAA + DAA.

The CP content (106 g/kg) for both sorghum and barley was similar. The highest and lowest values were observed in wheat (123 g/kg) and

maize (80.6 g/kg), respectively. Starch contents were higher in maize (590 g/kg) and sorghum (606 g/kg) than wheat (532 g/kg) and barley (541 g/kg). The total AA (TAA) content was higher in wheat (108 g/kg) followed by sorghum (98.7 g/kg), barley (96.5 g/kg), and maize (68.7 g/kg). Among the indispensable amino acids (IAA), and in general overall, the lowest contents were recorded for Trp and Met and the highest content for Leu followed by Arg and Val. Cysteine showed the lowest content among all dispensable AA (DAA), regardless of the grain. Glutamic acid, Pro and Asp were the DAA with the highest contents.

3.4.2. Feed intake, dry matter and starch digestibility, and excreta score

Mortality during the experiment was negligible with only one death out of 432 birds.

Influence of GT and FF on FI, AIDC of DM and starch, and excreta score is presented in Table 3.3. A significant ($P < 0.001$) GT × FF interaction was observed for FI during the 4-d assay period, with greater response to pelleting in maize and barley than wheat and sorghum. The highest FI was recorded in pelleted barley diets and the lowest in maize- and sorghum-based diets in mash form.

No interaction ($P > 0.05$) between GT and FF was observed for the AIDC of DM. The main effect of GT was significant ($P < 0.001$) for the AIDC of DM. Birds fed maize- and sorghum-based diets had

similar, but higher DM digestibility than the diets based on either wheat or barley. Pelleting decreased (P < 0.05) AIDC of DM by 6.8%.

Table 3.3. Influence of grain type (GT) and feed form (FF) on feed intake (g/bird/4d), apparent ileal digestibility coefficients (AIDC) of dry matter (DM) and starch, and excreta score in broilers[1]

			AIDC		
GT	FF	Feed intake	DM	Starch	Excreta score[2]
Maize	Mash	317[e]	0.728	0.989[a]	3.7[bc]
	Pellet	459[b]	0.717	0.981[ab]	3.7[bc]
Sorghum	Mash	337[e]	0.736	0.966[bcd]	3.5[c]
	Pellet	400[c]	0.713	0.949[d]	4.1[ab]
Wheat	Mash	372[d]	0.624	0.971[abc]	3.5[c]
	Pellet	450[b]	0.546	0.909[e]	4.3[a]
Barley	Mash	399[c]	0.631	0.982[ab]	2.7[d]
	Pellet	517[a]	0.557	0.955[cd]	3.8[bc]
Pooled SEM		7.6	0.0292	0.0069	0.13
Main effects					
GT					
Maize		388	0.722[a]	0.985	3.7
Sorghum		369	0.724[a]	0.958	3.8
Wheat		411	0.585[b]	0.941	3.9
Barley		458	0.593[b]	0.968	3.2
Pooled SEM		5.4	0.0207	0.0049	0.10
FF					
	Mash	356	0.679[a]	0.977	3.3
	Pellet	457	0.633[b]	0.949	4.0
Pooled SEM		3.8	0.0146	0.0034	0.07
GT		0.001	0.001	0.001	0.001
FF		0.001	0.031	0.001	0.001
GT × FF		0.001	0.555	0.002	0.002

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

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

[2]Excreta scoring was done daily on a scale of 1 to 5 (1 represents normally formed excreta and 5 represents very watery excreta).

There was a significant (P < 0.01) GT × FF interaction for the AIDC of starch. Starch digestibility was not influenced (P > 0.05) by

FF in maize- and sorghum-based diets, but was compromised ($P < 0.05$) in diets made of wheat and barley.

Feeding pelleted diets had no effect ($P > 0.05$) on the excreta score in maize-based diets, but increased ($P < 0.05$) the scores in diets made of sorghum, wheat and barley, resulting in a significant ($P < 0.01$) GT × FF interaction.

3.4.3. Standardised ileal digestibility of nitrogen and amino acids

The influence of GT and FF on the SIDC of N and AA, and standardised digestible CP and AA contents is shown in Tables 3.4 and 3.5, respectively.

There was no significant ($P > 0.05$) GT × FF interaction for SIDC of N and any of 16 AA (Table 3.4). A significant ($P < 0.05$ to 0.001) effect of GT on the SIDC of N and AA was noticed. In general, the highest SIDC values were observed in maize and sorghum followed by wheat and barley. Maize showed similar ($P > 0.05$) average SIDC values for IAA to sorghum, but higher ($P < 0.05$) than wheat and barley. Average SIDC values for DAA and TAA in maize and sorghum were similar to wheat and higher than the barley. With the exception of Cys and Pro, there was no significant effect of FF on SIDC of N and any other AA. Feeding pelleted diets reduced the SIDC of Cys and Pro and tended ($P = 0.063$) to decrease the SIDC of Glu.

The standardised digestible CP and AA contents were significantly ($P < 0.05$ to 0.001) influenced by GT (Table 3.5). The highest standardised digestible CP content was observed in wheat, followed by sorghum,

barley and maize. Sorghum and maize showed the highest and lowest standardised digestible contents for total IAA, respectively, with wheat and barley being intermediate. Total standardised digestible contents of DAA and TAA, however, were higher in wheat followed by sorghum, barley and maize, respectively. Similar to SIDC values, pelleting significantly ($P < 0.05$) reduced the SID content of Cys and Pro and tended ($P = 0.077$) to reduce the SID content of Glu regardless of GT. No significant ($P > 0.05$) GT × FF interaction was observed for the standardised digestible CP and AA contents.

Table 3.4. Influence of grain type (GT) and feed form (FF) on the standardised ileal digestibility coefficients[1] of nitrogen (N) and amino acids[2]

			Indispensable amino acids										Dispensable amino acids								
GT	FF	N	Arg	His	Ile	Leu	Lys	Met	Thr	Trp	Val	IAA	Ala	Asp	Cys[3]	Glu	Gly[3]	Pro	Ser	DAA	TAA
Maize	Mash	0.844	0.881	0.862	0.843	0.910	0.786	0.899	0.822	0.751	0.847	0.845	0.892	0.844	0.882	0.909	0.769	0.881	0.879	0.865	0.856
	Pellet	0.815	0.866	0.820	0.807	0.886	0.747	0.881	0.795	0.688	0.820	0.812	0.865	0.793	0.831	0.879	0.721	0.847	0.836	0.825	0.819
Sorghum	Mash	0.812	0.818	0.746	0.813	0.853	0.723	0.839	0.790	0.802	0.808	0.799	0.852	0.814	0.796	0.856	0.706	0.812	0.832	0.809	0.805
	Pellet	0.805	0.864	0.728	0.806	0.832	0.793	0.853	0.797	0.797	0.807	0.809	0.833	0.815	0.766	0.838	0.720	0.781	0.829	0.798	0.804
Wheat	Mash	0.798	0.722	0.773	0.767	0.801	0.627	0.811	0.726	0.762	0.747	0.749	0.686	0.687	0.877	0.916	0.729	0.917	0.827	0.806	0.778
	Pellet	0.797	0.769	0.777	0.769	0.809	0.643	0.816	0.728	0.733	0.763	0.757	0.697	0.677	0.845	0.913	0.733	0.906	0.822	0.799	0.779
Barley	Mash	0.724	0.718	0.725	0.706	0.753	0.661	0.778	0.709	0.685	0.735	0.719	0.686	0.695	0.844	0.827	0.664	0.834	0.759	0.758	0.741
	Pellet	0.697	0.711	0.702	0.662	0.719	0.618	0.742	0.692	0.649	0.709	0.689	0.655	0.654	0.786	0.782	0.639	0.782	0.713	0.716	0.705
Pooled SEM		0.0298	0.0317	0.0276	0.0335	0.0253	0.0521	0.0293	0.0401	0.0350	0.0293	0.0331	0.0296	0.0351	0.0254	0.0179	0.0339	0.0177	0.0288	0.0261	0.0296
Main effects																					
GT																					
Maize		0.829a	0.873a	0.841a	0.825a	0.898a	0.767a	0.890a	0.809a	0.719bc	0.833a	0.828a	0.878a	0.818a	0.857a	0.895a	0.745a	0.864b	0.858a	0.845a	0.838a
Sorghum		0.808a	0.841a	0.737bc	0.809a	0.842b	0.758a	0.846ab	0.794ab	0.799a	0.808ab	0.804ab	0.843a	0.814a	0.781b	0.847b	0.713ab	0.797c	0.831a	0.804a	0.804a
Wheat		0.797a	0.746b	0.775b	0.769a	0.805b	0.635b	0.813bc	0.727bc	0.747ab	0.755bc	0.753bc	0.692b	0.682b	0.861a	0.914a	0.731a	0.912a	0.824a	0.802a	0.778ab
Barley		0.711b	0.714b	0.714c	0.684b	0.736c	0.639b	0.760c	0.701c	0.667c	0.722c	0.704c	0.671b	0.674b	0.815ab	0.804c	0.652b	0.808c	0.736b	0.737b	0.723b
Pooled SEM		0.0211	0.0224	0.0195	0.0237	0.0179	0.0369	0.0208	0.0283	0.0248	0.0207	0.0234	0.0209	0.0248	0.0179	0.0127	0.0239	0.0125	0.0204	0.0185	0.0209
FF																					
	Mash	0.795	0.785	0.777	0.782	0.829	0.699	0.832	0.762	0.749	0.784	0.778	0.779	0.759	0.849a	0.877	0.717	0.861a	0.824	0.809	0.795
	Pellet	0.778	0.802	0.757	0.761	0.812	0.700	0.823	0.753	0.716	0.775	0.767	0.762	0.734	0.807b	0.853	0.703	0.829b	0.800	0.784	0.777
Pooled SEM		0.0149	0.0159	0.0138	0.0167	0.0127	0.0261	0.0147	0.0200	0.0175	0.0146	0.0166	0.0148	0.0175	0.0127	0.0089	0.0169	0.0089	0.0144	0.0131	0.0148
Probabilities, P ≤																					
GT		0.001	0.001	0.001	0.001	0.001	0.017	0.001	0.028	0.005	0.002	0.003	0.001	0.001	0.009	0.001	0.044	0.001	0.001	0.002	0.003
FF		0.445	0.439	0.316	0.378	0.334	0.981	0.680	0.747	0.186	0.665	0.637	0.430	0.311	0.022	0.063	0.567	0.017	0.243	0.175	0.394
GT × FF		0.953	0.649	0.878	0.878	0.844	0.669	0.814	0.971	0.868	0.861	0.866	0.888	0.866	0.933	0.697	0.799	0.717	0.802	0.858	0.863

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

[1]Apparent digestibility values were standardised using the following basal ileal endogenous flow values (g/kg DM intake), determined by the feeding protein-free diet: crude protein, 17.3; Arg, 0.59; His, 0.27; Ile, 0.54; Leu, 0.87; Lys, 0.57; Met, 0.22; Thr, 1.04; Trp, 0.19; Val, 0.72; Ala, 0.65; Asp, 1.15; Cys, 0.42; Glu, 1.42; Gly, 0.68; Pro, 0.83; and Ser, 0.86.

[2]Each value represents the mean of six replicates (eight birds per replicate). [3]Semi-indispensable amino acids for poultry.

DAA = Average digestibility of dispensable amino acids; IAA = Average digestibility of indispensable amino acids; TAA = Average digestibility of all amino acids.

Table 3.5. Influence of grain type (GT) and feed form (FF) on the standardised digestible protein (CP) and amino acid contents[1] (g/kg), as-received basis

			Indispensable amino acids										Dispensable amino acids								
GT	FF	CP	Arg	His	Ile	Leu	Lys	Met	Thr	Trp	Val	IAA	Ala	Asp	Cys[2]	Glu	Gly[2]	Pro	Ser	DAA	Total AA
Maize	Mash	68.2	3.60	1.85	2.19	7.55	2.27	1.33	2.31	0.51	3.13	24.8	4.66	4.71	1.54	12.4	2.51	6.12	3.15	35.1	59.9
	Pellet	65.8	3.54	1.76	2.09	7.35	2.16	1.30	2.23	0.47	3.03	23.9	4.51	4.43	1.46	12.3	2.35	5.89	2.99	33.7	57.6
Sorghum	Mash	90.1	3.44	1.81	3.37	12.3	1.72	1.43	2.68	1.01	4.24	31.9	8.46	5.85	1.53	19.4	2.35	7.96	4.03	49.5	81.5
	Pellet	89.3	3.63	1.76	3.34	11.9	1.89	1.45	2.70	1.01	4.24	31.9	8.27	5.85	1.47	18.9	2.39	7.67	4.02	48.6	80.6
Wheat	Mash	100.6	4.31	2.14	3.09	6.44	2.19	1.52	2.46	1.09	3.93	27.2	2.95	4.24	2.41	32.3	3.75	11.8	4.52	61.9	89.1
	Pellet	100.5	4.59	2.15	3.10	6.51	2.25	1.54	2.47	1.06	4.01	27.7	2.99	4.17	2.32	32.2	3.77	11.7	4.49	61.6	89.3
Barley	Mash	83.1	3.95	1.73	2.73	5.66	2.77	1.41	2.53	0.96	4.13	25.9	3.09	4.57	2.09	20.9	2.93	10.8	3.38	47.8	73.6
	Pellet	79.9	3.91	1.68	2.56	5.39	2.59	1.34	2.46	0.92	3.99	24.8	2.95	4.30	1.95	19.8	2.82	10.1	3.17	45.1	69.9
Pooled SEM		3.26	0.159	0.067	0.124	0.232	0.174	0.051	0.135	0.042	0.149	1.11	0.163	0.226	0.055	0.41	0.136	0.19	0.131	1.28	2.37
Main effects																					
GT																					
Maize		67.0^{d}	3.57^{c}	1.81^{b}	2.14^{d}	7.44^{b}	2.22^{b}	1.32^{c}	2.27^{b}	0.49^{c}	3.08^{b}	24.3^{c}	4.59^{b}	4.57^{b}	1.49^{c}	12.2^{d}	2.43^{c}	6.01^{d}	3.07^{c}	34.4^{d}	58.7^{d}
Sorghum		89.6^{b}	3.53^{c}	1.78^{b}	3.35^{a}	12.1^{a}	1.81^{c}	1.44^{ab}	2.69^{a}	1.01^{ab}	4.24^{a}	31.9^{a}	8.37^{a}	5.85^{a}	1.49^{c}	19.1^{c}	2.37^{c}	7.82^{c}	4.02^{b}	49.1^{b}	81.0^{b}
Wheat		100.6^{a}	4.45^{a}	2.15^{a}	3.09^{b}	6.48^{c}	2.22^{b}	1.53^{a}	2.47^{ab}	1.08^{a}	3.97^{a}	27.4^{b}	2.98^{c}	4.21^{b}	2.36^{a}	32.2^{a}	3.76^{a}	11.8^{a}	4.50^{a}	61.8^{a}	89.2^{a}
Barley		81.5^{c}	3.93^{b}	1.71^{b}	2.64^{c}	5.53^{d}	2.68^{a}	1.38^{bc}	2.49^{ab}	0.93^{b}	4.06^{a}	25.3^{bc}	3.03^{c}	4.44^{b}	2.02^{b}	20.4^{b}	2.87^{b}	10.4^{b}	3.28^{c}	46.4^{c}	71.8^{c}
Pooled SEM		2.31	0.113	0.047	0.087	0.164	0.123	0.036	0.095	0.029	0.106	0.79	0.115	0.159	0.039	0.29	0.096	0.134	0.093	0.91	1.68
FF																					
	Mash	85.5	3.83	1.88	2.84	7.98	2.24	1.42	2.49	0.89	3.86	27.4	4.79	4.84	1.89^{a}	21.3	2.88	9.17^{a}	3.77	48.6	76.0
	Pellet	83.9	3.92	1.84	2.77	7.81	2.22	1.41	2.47	0.86	3.82	27.1	4.69	4.69	1.79^{b}	20.7	2.83	8.84^{b}	3.67	47.2	74.3
Pooled SEM		1.63	0.079	0.034	0.062	0.116	0.087	0.026	0.068	0.021	0.075	0.56	0.082	0.113	0.028	0.20	0.068	0.095	0.066	0.64	1.19
Probabilities, P ≤																					
GT		0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.033	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001
FF		0.489	0.418	0.351	0.426	0.306	0.886	0.693	0.771	0.236	0.713	0.679	0.360	0.342	0.019	0.077	0.601	0.018	0.289	0.142	0.321
GT × FF		0.965	0.639	0.904	0.892	0.856	0.744	0.809	0.978	0.949	0.876	0.893	0.889	0.891	0.893	0.641	0.856	0.534	0.854	0.829	0.865

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

Standardised digestible amino acid content (g/kg) = [ingredient amino acid content (g/kg) × standardised ileal digestibility (%)]/100.

[1]Each value represents the mean of six replicates (eight birds per replicate). [2]Semi-indispensable amino acids for poultry.

DAA = Total digestible dispensable amino acid contents; IAA = Total digestible indispensable amino acid contents; Total AA = Total digestible content of all amino acids.

3.5. Discussion

The results of proximate analysis and nutrient composition of the grains, in general, were within the range reported in the previous studies (Huang *et al.*, 2005; Bandegan *et al.*, 2011; Ullah *et al.*, 2016). Starch was the major chemical component in all grains and within the range reported in the literature (Abdollahi *et al.*, 2013a; Mateos *et al.*, 2019). Higher content of NDF was observed in barley and wheat followed by maize, while sorghum contained the lowest NDF. The NDF represents the structural components (lignin, cellulose and hemicellulose) in plant cells. The major non-starch polysaccharides (NSPs) found in grains are cellulose, hemicellulose and pectin. Ward (2014) recorded higher total NSPs in wheat (100 g/kg) and barley (169 g/kg) compared to maize (83 g/kg) and sorghum (55 g/kg) that is in coherence with the present findings of lower NDF in maize and sorghum. The high molecular weight NSPs, present especially in wheat and barley, can be solubilised in the intestinal tract. This leads to increased viscosity of gut contents and impeded absorption of nutrients (Choct *et al.*, 1999).

The CP content in maize (80.6 g/kg) was similar to the values (80-81 g/kg) reported by INRA (2002), Premier Atlas (2014) and Feedipedia (2017), and lower than the values reported by NRC (1994) and RPRI (2014) at 86 and 85 g/kg, respectively. The CP content of sorghum grain (106 g/kg) was close to the values reported by WPSA (1989) and RPRI (2014). Values for CP content of wheat (123 g/kg) and barley (106 g/kg) samples in the present study were also within the range of previous studies for wheat (100-180 g/kg) and barley (110-160 g/kg)

(Leeson and Summers, 2005; Bandegan *et al.*, 2011). The variability of nutrient composition within each grain reported in different studies might be explained by the variation in genotype/cultivar, geographical locations, growing season, agronomic practices and techniques used to analyse different nutrient components (Jeroch and Danicke, 1995; Gutierrez-Alamo *et al.*, 2008; Mateos *et al.*, 2019). Analysed AA contents in different grains were close to the values reported in the literature (Huang *et al.*, 2005; Ravindran *et al.*, 1996, 2017; Bandegan *et al.*, 2011).

Increased FI is the paramount motive for offering pelleted diets to broilers (Abdollahi *et al.*, 2014, 2018a). Higher bulk density in pelleted diets facilitates easy ingestion by the bird and contributes to higher FI (Hamilton and Proudfoot, 1995). Massuquetto *et al.* (2019) reported that broilers fed a pelleted diet *ad libitum* consumed 11% more feed and gained 17% higher body weight compared to their mash-fed counterparts. However, all the advantages of pelleting on growth performance disappeared when the intake of pelleted diet was restricted to the same amount as the mash diet. In the present study, offering pelleted diets, regardless of GT, increased the FI of birds by an average of 101 g/bird (457 vs. 356). However, the FI responses to pelleting were markedly higher in maize (44.8%) and barley (29.6%), compared to wheat (21.0%) and sorghum (18.7%). These findings are in agreement with the findings of previous studies (Abdollahi *et al.*, 2011, 2014; Yang *et al.*, 2017; Roza *et al.*, 2018) who reported increased FI in pellet fed birds.

Maize and sorghum showed higher DM digestibility, by an average of 22.7%, than wheat and barley. Viscous grains, such as wheat and barley, contain high levels of NSPs (i.e. xylans in wheat and β-glucans in barley) than non-viscous grains (i.e. maize and sorghum) and their antinutritive characteristics account for the lower nutrient digestibility in birds fed diets based on viscous grains (Hughes and Choct, 1999; Thomas *et al.*, 2008; Mateos *et al.*, 2019). The lower DM digestibility in wheat and barley may account for the higher NDF in these grains compared to maize and sorghum in current study.

Reduction in AIDC of DM in pelleted diets is not without precedent. It has been shown that nutrient digestibility is affected in pelleted grain-based diets with the magnitude of the effect being dependent on the specific nutrient and the type of grain used (Abdollahi *et al.*, 2013b, 2014). The negative effect of pelleting on nutrient digestibility, however, has been reported to be more pronounced in viscous grains such as wheat and barley (Abdollahi *et al.*, 2013a). In contrast, steam-cooking of maize followed by flaking was reported to increase the DM digestibility of the diet (Jimenez-Moreno *et al.*, 2009). Abdollahi *et al.* (2013b) found that the AIDC of nutrients was negatively influenced in pelleted wheat-based diets. Pelleting increased AIDC of fat, Ca and P, but not N and starch, in a maize-based diet. Ileal digestibility of N, starch, fat and Ca was lower in sorghum-based pellets compared to mash in another study by Abdollahi *et al.* (2014).

The starch digestibility responses to pelleting were inconsistent across the grains in the present study, as evidenced by the significant GT x FF

interaction. Pelleted diets supported a similar AIDC of starch as mash diets in maize and sorghum, but resulted in lower starch digestibility by 6.4% (0.909 vs. 0.971) and 2.7% (0.955 vs. 0.982) in wheat and barley, respectively. This finding indicates that pellet-feeding does not improve the starch digestibility in grains and can depress starch digestibility in viscous grains, corresponding with several previous findings (Svihus, 2001; Abdollahi *et al.*, 2011, 2013b, 2018b). Selle *et al.* (2012) did not observe any effect of pelleting on the ileal starch digestibility of broilers fed sorghum-based diets. The reduction of starch digestibility in pelleted wheat-based diets might be due to an increased concentration of high molecular weight soluble NSPs in the gut resulting in increased digesta viscosity (de Vries *et al.*, 2012) and an overload of wheat starch in small intestine (Svihus and Hetland, 2001). Ileal starch digestibility was lower in a wheat-based pelleted diet (0.920) compared to a mash diet (0.966) (Abdollahi *et al.*, 2013b). However, in their study, pelleting had no effect on AIDC of starch in a maize-based diet. In contrast to present findings, steam-pelleting of normal and waxy barley-based broiler diets at 75 ºC improved starch digestibility coefficients by an average of 50% (proximal ileum) and 32.3% (distal ileum), respectively (Ankrah *et al.*, 1999). However, neither cold-pelleting nor steam-pelleting at 75 ºC revealed any effect on starch digestibility in a wheat-based diet (Zimonja and Svihus, 2009).

Because starch gelatinisation renders the starch molecules more accessible to α-amylase and enzymatic degradation, the digestibility of

starch is expected to increase in pelleted diets. Moreover, the thermal effect during feed processing is believed to denature α-amylase inhibitors and increase starch digestibility (Zaefarian *et al.*, 2015). However, the extent of starch gelatinisation during pelleting is minimal and of modest importance in terms of starch digestion (Svihus *et al.*, 2004; Zimonja *et al.*, 2008). The pelleting-induced reduction of starch (observed in wheat- and barley-based diets) and DM (in all the grains) digestibility in present study may also, at least in part, be explained with the increased FI in pelleted diets. Over-consumption of feed that results in starch overload, faster passage rate and elevated gizzard pH associated with feeding pelleted diets, have been suggested to lower the starch digestibility in pelleted diets (Svihus, 2001; Abdollahi *et al.*, 2013b, 2018b). Svihus and Hetland (2001) stated that the higher intake of wheat-based pelleted diets increased the starch load in the gut, lowering the digestibility of starch. Higher consumption of feed in wheat- and barley-based pellets (by 78 and 118 g/bird, respectively) compared to mash in current experiment may account for the lower starch digestibility in these grains. Present study indicates that the FI effect on starch digestibility was more pronounced in viscous grains. There was also an increase in FI in the case of maize- and sorghum-based pelleted diets, but no effect was observed in starch digestibility.

Cowieson *et al.* (2005) concluded that the dietary viscosity is increased by heat processing in viscous grains with high NSP contents, which can, consequently, compromise the nutrient digestibility. They recorded higher dietary viscosity for pelleted wheat-based diets than

that of mash diets. In the case of starter diets, the dietary viscosity in pelleted diets was 53, 116 and 80 % higher compared to mash diets at pelleting temperatures of 80, 85 and 90 ºC, respectively. For finisher diets, the magnitudes of increase were 57, 83 and 76 %, respectively, at these three temperatures. The digesta viscosity may also increase as a result of solubilisation of NSP in intestinal tract, impeding the absorption of nutrients (Choct *et al.*, 1999; Abdollahi *et al.*, 2010a). The digestibility of starch may also be reduced in pelleted diets if significant amount of resistant starch is formed during thermal processing (Htoon *et al.*, 2009). Abdollahi *et al.* (2011) reported higher resistant starch content in the pelleted diet conditioned at either 20 or 90 ºC compared to a diet conditioned at 60 ºC, but similar to a diet conditioned at 75 ºC.

Feeding diets in pelleted form increased the excreta score in wheat-, sorghum- and barley-based diets compared to their mash counterparts. Reduced length of caeca (Mirghelenj and Golian, 2009) and a lower caeca weight (Abdollahi *et al.*, 2011) in pellet-fed compared to mash-fed birds, might partly explain the current findings. As recycling and reabsorption of water occurs largely in the caeca and colon, the poor caecal development in birds fed pelleted diets has implications for water loss and conservation (Svihus *et al.*, 2013). A lower weight of caeca has been proposed for a higher amount of water being excreted relative to FI (Maisonnier *et al.*, 2001).

Grain type significantly influenced the SIDC of N and all AA. Although the N and total AA contents of barley were higher than maize

and similar to sorghum (Table 3.2), barley was determined to have the lowest SIDC of N among the four grain types, confirming the poor N digestibility in this grain. Average SIDC of IAA in maize (0.828) was similar to that of sorghum (0.804), but greater than those of wheat (0.753) and barley (0.704). The average SIDC for DAA and TAA were similar among maize, sorghum and wheat, and the lowest values were observed in barley. In general, the trends of higher AA digestibility in maize followed by sorghum, wheat, and barley are in agreement with previous studies (Lemme *et al.*, 2004; Huang *et al.*, 2005, 2007; Szczurek, 2009). Lower SIDC values for N and TAA (though not statistically significant) for barley compared to wheat correspond with Bandegan *et al.* (2011), who compared SIDC of six wheat and seven barley samples and reported lower SIDC values for CP and AA in barley compared with wheat. The lower AA digestibility values in wheat (for some AA) and barley, in comparison with maize and sorghum, might be partly explained by the higher content of NSP in these grains (Annison and Choct, 1991; Szczurek, 2009). The SIDC values of IAA recorded for wheat (0.727 to 0.805) in the present study were lower than the values (0.837 to 0.938) reported by Bandegan *et al.* (2011) and similar to the findings (0.657 to 0.839) of Short *et al.* (1999).

Several published data are available on the AA digestibility of different grains for broilers (Lemme *et al.*, 2004; Huang *et al.*, 2005; Ravindran *et al.*, 2014a, 2017). However, the AA digestibility values for grains are variable due to differences in methodology used (apparent vs.

standardised), site of measurement (excreta vs. ileal digesta), age of birds, and the method for determination of endogenous AA losses. Moreover, no data, to the author's knowledge, are available on SIDC of N and AA in grains using pelleted diets in digestibility assays. Therefore, applying SIDC of AA values generated in assays with mash diets to commercial situations, where the majority of the broiler feed is pelleted, clearly overlooks the possible effect of processing and FF on AA digestibility, and highlights the need for pellet-based AA digestibility estimates for use in formulation of pelleted diets.

The lack of FF effect on SIDC of N in this experiment is in line with the study by Abdollahi *et al.* (2013b) in which no effect of pelleting on ileal N digestibility was observed in a maize-based diet. Selle *et al.* (2012) also reported that pelleting and re-grinding of a sorghum-based diet did not have any effect on AIDC of N. In contrast, Abdollahi *et al*. (2011) recorded lower AIDC of N in wheat-based pelleted diets compared to the diets in mash form. Whilst the SIDC of Cys and Pro deteriorated in pelleted diets in the current work, SIDC of other AA was unaffected by FF. It has been suggested that the thermo-mechanical forces during feed processing may cause "Maillard reaction" where free aldehyde from reducing sugars (i.e. glucose, lactose) combine with amino groups from AA and could negatively impact AA, especially Lys utilisation (Thomas *et al.*, 1998; Martins *et al.*, 2000). Cheftel (1986) stated that conditions favourable for the "Maillard reaction" are high temperature (> 180 ºC) and low moisture (< 15%) in combination with shear (> 100 rpm in an extruder). Pelleting involves conditioning of dry

feed where saturated steam is injected into feed and mixed in a paddle mixer. This process usually takes less than 1 min, results in a temperature rise to 75 ºC and an increase in moisture level by 3-4%. Then the feed enters into the pellet press and forced through cylindrical holes in a die where the pellet shaped is achieved. The friction from the rolls that force the material into the holes, and the friction in these holes cause the highest temperature achieved of 85 ºC (Svihus *et al.*, 2004). Therefore, the conditions of pelleting seem to be too mild to cause Maillard reactions (Svihus and Zimonja, 2011). According to Goodarzi Boroojeni *et al.* (2016), hydrothermal processing such as steam-pelleting has only negligible effect on protein or AA availability in poultry. On the other hand, high temperature heat treatment can reduce the digestibility of heat sensitive AA (Abdollahi *et al.*, 2011; Muramatsu *et al.*, 2013). In the present study, pelleting reduced the SIDC of Cys and Pro by 4.9 and 3.7%, respectively. Cysteine is the most heat labile of all AA and its digestibility is affected during hydrothermal treatment (Goodarzi Boroojeni *et al.*, 2016), due probably to formation of disulphide bonds (Wall, 1971; Ravindran *et al.*, 2005, 2014b).

For accurate feed formulation, the digestible AA contents in a complete feed need to be predicted from the digestible AA contents for individual feed ingredients (Angkanaporn *et al.*, 1996; Bryden and Li, 2003). The SID contents of CP, total of DAA and TAA were higher in wheat followed by sorghum, barley and maize. In the case of IAA, highest SID content was recorded for sorghum, due to very high contents of Ile

and Leu. In general, SID contents of IAA in different grains demonstrated comparatively higher values for Arg, Leu, Val and lower values for His, Met, and Trp. Among the DAA, higher values were recorded for Glu, Pro and the lowest values for Cys. A number of factors, such as plant breeding programmes, agronomic conditions, presence of antinutritive factors, processing methods and digestibility assay procedure, can affect the content and digestibility of AA (Bryden *et al.*, 2009a), and consequently the digestible AA content of different grains.

Despite the lack of significant effect of FF on SID content of most AA in the grains investigated in the present study, the SID content of Cys, Pro and Glu (only a tendency) was lower in pelleted diets than the mash diets. This finding underlines the need for consideration of FF in digestible AA assays, and the fact that the physical form of diet in these assays should resemble the form of diets commonly used in the broiler industry.

3.6. Conclusions

Overall, pelleting reduced the digestibility of starch in wheat and barley, and compromised the SIDC of Cys, Pro and Glu (a tendency) in all grains. These findings suggest that the effect of processing and FF need to be taken into account in feed evaluation assays used for the determination of digestible nutrient content of grains. Moreover, existence of FF form effect on SIDC of some IAA in grains in the current study indicates that there might be some significant effect of FF

on SIDC of AA in protein sources with higher N and AA contents. Future studies are warranted to evaluate the influence of FF on the AA digestibility of protein source ingredients.

CHAPTER 4 Standardised ileal amino acid digestibility of protein sources for broiler chickens is influenced by feed form[1]

4.1. Abstract

The aim of the present study was to determine the influence of feed form (FF) on apparent ileal digestibility coefficients (AIDC) of dry matter (DM) and, standardised ileal digestibility coefficients (SIDC) of nitrogen (N) and amino acids (AA) in three protein sources (PS) for broilers. A completely randomised design was used to develop six assay diets in a 3 × 2 factorial arrangement of treatments involving three PS (meat and bone meal [MBM], soybean meal [SBM], and canola meal [CM]) in mash and pelleted forms. Three assay diets, containing different inclusions of each PS, as the only source of AA in the diet, and maize starch were developed to supply about 180 g/kg dietary protein. The basal endogenous N and AA losses were determined by offering a N-free diet in mash form. From d 1 to 18, the birds were offered a common broiler starter diet. The assay diets and the N-free diet were randomly assigned to six replicate cages (eight birds per cage) and fed from d 19 to 23. The ileal digesta were collected on d 23. The AIDC of DM was lower ($P < 0.05$) in CM compared to MBM and SBM with no influence of FF ($P > 0.05$). The SIDC of N was higher ($P < 0.05$) in SBM followed by MBM and CM. The average SIDC values for indispensable AA (IAA) were similar in SBM and

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MBM and greater ($P < 0.05$) than CM. Average SIDC value for dispensable AA (DAA) was higher ($P < 0.05$) in SBM than MBM and CM. Feed form had no influence on the SIDC of IAA, the only exception being that of His, which was reduced ($P < 0.05$) by pelleting. Pelleting, however, resulted in significant ($P < 0.001$) reduction in the SIDC of all DAA and average of all AA. The most affected AA by pelleting was Cys, with a 15.4% decrease in SIDC in pelleted diets compared to mash. The standardised ileal digestible content of protein and average of IAA and DAA were higher in MBM than in SBM, and CM being the lowest. Pelleting decreased ($P < 0.05$) the digestible protein and total digestible AA content. Overall, present data showed that FF can influence the measurement of AA digestibility of protein ingredients and it, therefore, should be considered in feed evaluation assays of ingredients with high protein and AA contents.

4.2. Introduction

Intensive selection and breeding programmes have resulted in contemporary broilers that are 400% more efficient compared to the breeds grown in 1956 (Zuidhof *et al.*, 2014). Today, broilers can reach a body weight (BW) of 2.5 kg in 35 d, with a feed conversion ratio of 1.5. Feed is the greatest single cost item in modern broiler production representing about 70% of the total production cost (Nolan *et al.*, 2010). Knowledge of the digestibility of amino acids (AA) in ingredients is crucial for the formulation of well-balanced and economical diets. Determination of digestible AA values is considered to be the best way to measure the protein value of ingredients (Rostagno *et al.*, 1995). The

use of excreta-based digestibility measurements for determining nitrogen (N) and AA availability is questionable due to the variable effects of caecal microflora on protein utilisation and the contribution of microbial proteins to AA excretion in the excreta. In the ileal digestibility assay, these confounding issues are avoided. Ileal AA digestibility values can be referred to as either apparent or standardised/true. Standardised ileal digestibility (SID) involves a correction for the inevitable basal endogenous AA losses from the gastrointestinal tract (Lemme *et al.*, 2004). It has been documented that the SID AA values are more additive than the apparent ileal digestibility (AID) values in terms of accuracy of feed formulation (Kong and Adeola, 2013b; Cowieson *et al.*, 2019).

Among plant-based protein sources (PS), soybean meal (SBM) is the preferred one in poultry feed formulations as it contains about 485 g/kg crude protein (CP; NRC, 1994), more consistent AA profile and high AA digestibility (Ravindran *et al.*, 2014b). Antinutritional factors (ANFs) such as trypsin inhibitor are present in soybeans but can be easily inactivated by heat treatment (Liener, 1994). Several factors including the season and location of cultivation, level of ANFs and, processing techniques may affect the AA digestibility of SBM (Parsons, 1991; Adedokun *et al.*, 2014) and if the thermal treatment is not optimal, the digestibility could be negatively affected (Frikha *et al.*, 2012). Canola meal (CM) is another important source of plant protein that is commonly used in poultry diets and contains about 380 g/kg CP

(NRC, 1994). Its high fibre content, however, limits the inclusion in poultry diets (Zuprizal *et al.*, 1992; Bell, 1993).

Animal-based PS are also used in poultry feed as they contain higher protein and often high phosphorus (P) and calcium (Ca) contents. Meat and bone meal (MBM) is an important animal-based PS that is produced from the bones, lungs, gastrointestinal tract, tendons, ligaments, and some skeletal muscles from mammalian species where the finished product contains less than 550 g/kg CP and more than 44 g/kg P (Ravindran *et al.*, 2002). The animal species, type and proportion of raw materials and processing condition result in wide variations in protein quality in terms of AA composition and digestibility (Skurray, 1974; Parsons *et al.*, 1997).

Anecdotal evidence suggests that pelleting influences protein and AA digestibility in broiler chickens (Svihus and Zimonja, 2011; Abdollahi *et al.*, 2010a, 2011, 2013b). Hydrothermal processing (HTP) may improve protein digestibility by protein denaturation and dissociation of the protein macrostructure into smaller subunits (Camire, 1991; Thomas *et al.*, 1998; Ludikhuyze *et al.*, 2003). On the contrary, protein denaturation can also delay digestion rate of protein through reduction in protein solubility (Camire, 1991) because solubilisation in the gastrointestinal tract is an essential first step of protein digestion (Cowieson and Ravindran, 2008). A combination of high temperature, shear forces and moisture during HTP favours Maillard reactions (Mauron, 1981; Cheftel, 1986), which occur between free amino groups of AA, mainly ε- amino group of Lys and free aldehyde groups

of reducing sugars such as starch, glucose and lactose (Thomas *et al.*, 1998; Martins *et al.*, 2000). Maillard reactions result in enzymatically undegradable end products, leading to a reduction in protein and AA availability (Camire *et al.*, 1990; Hendriks *et al.*, 1994). Reports indicate that HTP may also degrade heat labile AA including Cys, Ly, Arg and Thr (Camire *et al.*, 1990; Marsman *et al.*, 1995).

A large volume of published data exists on the ileal digestible AA content of feed ingredients for broilers (Lemme *et al.*, 2004; Bryden *et al.*, 2009a). These evaluations have been accomplished using mash diet because of its simplicity. Commercially, however, broilers are fed pelleted diets and the applicability of data generated using mash diets to pelleted diets could be questioned. Besides, available data on the effects of pelleting on protein digestibility were derived using complete diets. Based on the above, it was hypothesised that broilers fed the same ingredient, but in a different feed form (FF), may show different AA digestibility values. Consequently, the current experiment was designed to evaluate the influence of FF (mash vs. pellet) on the standardised ileal digestibility coefficients (SIDC) of N and AA of SBM, CM and MBM in broilers.

4.3. Materials and methods

The experimental procedure was in accordance with the New Zealand Revised Code of Ethical Conduct for the use of live animals for research, testing and teaching, approved by Massey University Animal Ethics Committee guidelines.

4.3.1. Diets and experimental design

The experimental design was a 3 × 2 factorial arrangement of treatments involving three PS (SBM, MBM, and CM) in two FF (mash and pellet). The PS ingredients were obtained from a commercial supplier. Three assay diets, containing different inclusions of each PS, as the only source of AA in the diet, and maize starch were developed to supply about 180 g/kg dietary protein (Table 4.1). Following the mixing of assay diets, each diet was divided into two equal batches. One batch was offered as mash and the other was pelleted. For pelleting, the diets were steam-conditioned at 70 ºC for 30 s using a pellet mill (Richard Size Limited Engineers, Orbit 15, Kingston-upon-Hull, UK) that is able to manufacture 180 kg of feed/h and equipped with a die ring of 3-mm holes and 35-mm thickness.

Basal endogenous AA (EAA) losses were measured using a N-free diet (NFD) in mash form for the calculation of SID values (Table 4.1). Titanium dioxide (5 g/kg; Merck KGaA, Darmstadt, Germany) was added to all diets as an indigestible marker.

4.3.2. Birds and housing

A total of 336, day-old male broilers (Ross 308) were obtained from a commercial hatchery, and raised in floor pens and fed a commercial broiler starter crumble (12.60 MJ/kg metabolisable energy; 230 g/kg CP) until d 18. On d 18, all birds were weighed and randomly assigned to 42 cages (36 cages for six dietary treatments having six replicate cages and six cages for the NFD), each housing eight birds in such a

way that the average bird weight per cage was similar. The diets were offered to birds *ad libitum* from d 19 to 23 and water was freely available throughout the assay period.

The floor pens and grower cages, with wire floor, were housed in an environmentally controlled rooms with 20 h of fluorescent illumination per day. The average temperature was 31 ºC during first week, and was gradually reduced to 23 ºC by 21 d of age.

Table 4.1. Composition of the basal diets (g/kg, as fed basis) used in the ileal amino acid digestibility assay

Ingredients	MBM	SBM	CM	NFD
Maize starch	546	517	377	842
Test ingredient	383	413	553	-
Soybean oil	30	30	30	50
Cellulose[1]	30	-	-	50
Dicalcium phosphate	-	19	19	19
Limestone	-	10	10	13
Dipotassium phosphate	-	-	-	12
Titanium dioxide	5.0	5.0	5.0	5.0
Sodium chloride	2.0	2.0	2.0	2.0
Sodium bicarbonate	2.0	2.0	2.0	2.0
Trace mineral premix[2]	1.0	1.0	1.0	3.0
Vitamin premix[2]	1.0	1.0	1.0	2.0

[1]Ceolus, Microcrystalline Cellulose, Asahi Kasei Corporation, Tokyo, Japan.

[2]Supplied per kilogram of 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.

CM = canola meal; MBM = meat and bone meal; NFD = nitrogen-free diet; SBM = soybean meal.

4.3.3. Feed intake and excreta scoring

Feed intake (FI) was recorded on a cage basis from d 19 to 23. Mortality was recorded daily.

Scoring of freshly voided excreta was done daily between d 19 to 23. The scores were based on a scale of 1 to 5, with 1 representing normally formed excreta and 5 representing very watery excreta (Ravindran *et al.*, 2008).

4.3.4. Determination of ileal nutrient digestibility

All birds were euthanised by intravenous injection (1 mL per 2 kg BW) of sodium pentobarbitone solution (Provet NZ Pty. Ltd., Auckland, New Zealand) on d 23. Digesta were collected from the lower half of the ileum, as described by Ravindran *et al.* (2005). The ileum was considered as the portion of the small intestine from Meckel's diverticulum to a point about ~40 mm proximal to the ileocaecal junction. The ileal digesta were collected from all birds within a cage into plastic containers by gentle flushing by distilled water, pooled, immediately frozen, and subsequently freeze dried (Model 0610, Cuddon Engineering, Blenheim, New Zealand). The diet and freeze-died digesta samples were ground to pass through a 0.5-mm sieve. The samples were stored in air-tight plastic containers at 4 ºC till analysis of dry matter (DM), titanium (Ti), N and AA.

4.3.5. Chemical analysis

The DM was determined using standard procedure (Method 930.15; AOAC International, 2016). Titanium was measured on a UV spectrophotometer (Berthold Technologies GmbH and Co. KG, Bad Wildbad, Germany) following the method of Short *et al.* (1996). Nitrogen content was analysed by combustion (Method 968.06; AOAC

International, 2016) using a CNS-200 carbon, N, sulphur analyser (LECO Corporation, St. Joseph, MI). The CP content was calculated as N × 6.25. The neutral detergent fibre (NDF) was measured (Method 2002.04; AOAC International, 2016) by using Tecator Fibertec™ (FOSS Analytical AB, Höganäs, Sweden). Gross energy was determined by adiabatic bomb calorimeter (Gallenkamp Autobomb, Weiss Gallenkamp Ltd., Loughborough, UK) standardised with benzoic acid. Fat was measured using Soxhlet extraction procedure (Method 2003.06; AOAC International, 2016). Ash was measured by a standard procedure (Method 942.05; AOAC International, 2016) using a muffle furnace at 550 ºC for 16 h. For mineral analysis, the samples were wet digested in a mixture of nitric and perchloric acid. The concentrations of Ca and P were measured by Inductively Coupled Plasma-Optical Emission Spectroscopy (ICP-OES) using a Thermo Jarrell Ash IRIS instrument (Thermo Jarrell Ash Corporation, Franklin, MA).

Amino acids were determined following standard procedures (Method 994.12; AOAC International, 2011). The samples were hydrolysed with 6 N hydrochloric acid (containing phenol) for 24 h at 110 ± 2 ºC in glass tubes in an oven. The AA were determined using AA analyser (ion exchange) with ninhydrin post column derivatisation. The chromatograms detected at 570nm and 440nm were integrated using dedicated software (Agilent Open Lab software, Waldbronn, Baden-Württemberg, Germany). The sulphur containing AA, Cys and Met were determined as cysteic acid and methionine sulphone, respectively,

by oxidation with performic acid for 16 h at 0 ºC and neutralisation with hydrobromic acid prior to hydrolysis.

For Trp analysis, the samples were saponified under alkaline conditions with barium hydroxide solution in the absence of air at 110 ºC for 20 h in an autoclave. The α-methyl Trp as internal standard, was added to the mixture following hydrolysis. After adjusting the hydrolysate to pH 3.0 and diluting with 30% methanol, Trp and the internal standard were separated by reverse phase chromatography (RP-18) on a HPLC column (CORTECS C18 Column; 2.7 µm, Waters Corporation, Dublin, Ireland). Detection was selectively done by means of a fluorescence detector to prevent interference by other AA and other constituents.

4.3.6. Calculations

The data were expressed on a DM basis for calculations. The apparent ileal digestibility coefficients (AIDC) of nutrients were calculated from the dietary ratio of nutrient to Ti relative to the corresponding ratio in the ileal digesta using the following formula.

$$\text{AIDC of nutrient} = [(\text{nutrient/Ti})_d - (\text{nutrient/Ti})_i]/(\text{nutrient/Ti})_d$$

Where, $(\text{nutrient/ Ti})_d$ = ratio of nutrient to Ti in the diet, and

$(\text{nutrient / Ti})_i$ = ratio of nutrient to Ti in the ileal digesta.

The basal ileal EAA losses from birds fed the NFD was calculated as grams of AA flow per kilogram of DM intake (DMI; Moughan *et al*., 1992).

Basal EAA flow (g/kg DMI) =

AA concentration in ileal digesta (g/kg) × [Diet Ti (g/kg)/Ileal digesta Ti (g/kg)]

The apparent digestibility data for N and AA were standardised by using the basal EAA flow.

SIDC = AIDC + [Basal EAA (mg/kg DMI)/Ing. AA (mg/kg DM)]

Where, SIDC = standardised ileal digestibility coefficients of the AA,

AIDC = apparent ileal digestibility coefficients of the AA,

Basal EAA = basal endogenous AA loss, and

Ing. AA = concentration of the AA in the ingredient.

4.3.7. Data analysis

Data were analysed by two-way analysis of variance using the General Linear Model procedure of SAS (version 9.4; 2015; SAS Institute, Cary, NC.) to determine the main effects (PS and FF) and their interaction. The cages were the experimental units. Differences were deemed significant based on P-value of equal to or < 0.05, and a P-value between 0.05 and 0.10 was considered as a trend. The Least Significant Difference test was used to separate significant differences between means.

4.4. Results

4.4.1. Proximate and nutrient compositions

The chemical composition of the three PS is presented in Table 4.2.

Table 4.2. Proximate and amino acid composition of the three protein sources (meat and bone meal, soybean meal, canola meal; g/kg, as-received basis)

	MBM	SBM	CM
DM	945	893	891
Nitrogen (N)	103	77.4	56.9
CP (N×6.25)	644	484	356
Fat	142	12.3	48.5
NDF	154	84.6	250
GE (MJ/kg)	21.2	17.7	17.8
Ash	149	65.4	72.5
Calcium	41.3	3.26	5.78
Phosphorus	23.3	6.61	10.5
Indispensable amino acids (IAA)			
Arg	40.3	33.8	20.4
His	13.3	11.9	8.88
Ile	21.8	20.3	13.5
Leu	44.1	35.2	23.7
Lys	38.7	28.9	19.2
Met	11.8	6.36	6.83
Thr	22.8	18.1	14.6
Trp	6.30	6.36	4.81
Val	30.6	21.9	17.9
Total IAA	230	183	130
Dispensable amino acids (DAA)			
Ala	41.2	20.2	15.1
Asp	49.5	52.7	24.3
Cys[1]	7.0	6.94	7.86
Glu	77.9	83.9	58.9
Gly[1]	61.9	19.5	17.2
Pro	45.4	25.9	22.6
Ser	23.7	23.4	13.9
Total DAA	307	233	160
Total AA[2]	537	416	290

CM = canola meal; CP = crude protein; DM = dry matter; GE = gross energy; MBM = meat and bone meal; NDF = neutral detergent fibre; SBM = soybean meal.

[1]Semi-indispensable amino acids for poultry.

[2]Total AA = IAA + DAA.

The CP content was higher in MBM (644 g/kg), followed by SBM (484 g/kg) and CM (356 g/kg). The CM contained the highest content (250 g/kg) of NDF and, the SBM the lowest (84.6 g/kg).

The total AA (TAA) content was higher in MBM (537 g/kg) followed by SBM (416 g/kg) and CM (290 g/kg). The contents of total indispensable AA (IAA) and total dispensable AA (DAA) followed the same pattern as TAA with comparatively higher content in MBM. Among the IAA, Arg, Leu, Lys, and Val were the major AA and His, Met, and Trp were the AA with the lower contents, regardless of PS. In general, Glu was the major DAA followed by Gly, Asp and Pro. The lowest content was recorded for Cys.

4.4.2. Feed intake, dry matter digestibility and excreta score

Influence of PS and FF on FI, AIDC of DM and excreta score is shown in Table 4.3.

Pelleting increased the FI of birds during the 4-d assay period by an average of 198 g/bird (527 vs. 329). However, the magnitude of the response to pelleting was greater in SBM (86.3%) than in CM (48.8%) and MBM (50.2%), resulting in a significant ($P < 0.001$) PS × FF interaction. The highest FI was recorded in the pelleted CM diet and the lowest in SBM- and MBM-based mash diets.

There was a significant ($P < 0.001$) main effect of PS on the AIDC of DM. Birds fed MBM- and SBM-based diets showed higher DM digestibility than diets based on the CM. Neither the main effect of FF nor the interaction between PS and FF was significant for the AIDC of DM.

The PS × FF interaction was significant (P < 0.001) for excreta score. Higher scores were recorded in pelleted SBM compared to mash SBM, but FF had no effect on the excreta score in MBM- and CM-based diets.

Table 4.3. Influence of protein source (PS) and feed form (FF) on feed intake (g/bird/4d), apparent ileal digestibility coefficient (AIDC) of dry matter (DM) and excreta score in broilers[1]

PS	FF	Feed intake	AIDC of DM	Excreta score[2]
Meat and bone meal	Mash	283[d]	0.745	4.9[a]
	Pellet	425[c]	0.707	4.9[a]
Soybean meal	Mash	293[d]	0.709	4.1[b]
	Pellet	546[b]	0.698	4.9[a]
Canola meal	Mash	410[c]	0.507	3.5[c]
	Pellet	610[a]	0.486	3.3[c]
Pooled SEM		10.1	0.0271	0.08
Main effects				
PS				
Meat and bone meal		355	0.726[a]	4.9
Soybean meal		419	0.704[a]	4.5
Canola meal		510	0.496[b]	3.4
Pooled SEM		7.1	0.0191	0.06
FF				
	Mash	329	0.654	4.2
	Pellet	527	0.630	4.4
Pooled SEM		5.8	0.0156	0.05
Probabilities, P ≤				
PS		0.001	0.001	0.001
FF		0.001	0.292	0.007
PS × FF		0.001	0.875	0.001

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]Excreta scoring was done daily on a scale of 1 to 5 (1 represents normally formed excreta and 5 represents very watery excreta).

4.4.3. Standardised ileal digestibility of nitrogen and amino acids

The influence of PS and FF on the SIDC of N and AA and, digestible protein and AA contents is summarised in Tables 4.4 and 4.5, respectively.

No interactions (P > 0.05) between PS and FF were observed for the SIDC of N and AA (Table 4.4). There was a significant (P < 0.01 to 0.001) effect of PS on the SIDC of N and all AA, except Met and Cys. The SIDC of N was significantly higher in SBM (0.799) than the MBM (0.732) and CM (0.640).

Soybean meal and MBM had similar (P > 0.05) average SIDC values for IAA, which were greater (P < 0.05) than CM. Average SIDC values for DAA was higher (P < 0.05) in SBM than MBM and CM. Average digestibility values for all AA (TAA; IAA + DAA) were similar in SBM and MBM and higher (P < 0.05) than CM.

Histidine was the only IAA influenced (P < 0.05) by FF, but there were tendencies for the SIDC of N (P = 0.060), Thr (P = 0.054), Trp (P = 0.073), Val (P = 0.079) and average of IAA (P = 0.089) to be reduced by pelleting. Pelleting, however, resulted in reductions (P < 0.05) in the SIDC of all individual DAA, average digestibility of DAA and TAA.

There was no significant (P > 0.05) PS × FF interaction for the contents of digestible protein and AA, except Gly (P < 0.05; Table 4.5). Pelleting reduced (P < 0.05) the digestible Gly content in MBM compared to mash, but had no effect in SBM and CM.

The PS had a significant (P < 0.001) effect on the digestible protein and AA contents, except Cys. The contents of digestible protein, individual IAA (except Ile and Trp), total of IAA and, Ala, Pro and total of DAA were higher (P < 0.05) in MBM than SBM. The digestible contents of Asp, Glu and Ser were greater (P < 0.05) in SBM than MBM. The CM

sample had the lowest ($P < 0.05$) digestible content for protein, IAA and DAA. The highest and lowest contents of total digestible AA were observed in MBM (397 g/kg) and CM (196 g/kg), with the SBM (333 g/kg) being intermediate.

Pelleting significantly ($P < 0.05$) reduced the digestible contents of protein, His and Thr, and tended ($P = 0.051$ to 0.085) to reduce the digestible content of other IAA (except Lys) and total digestible content of IAA ($P = 0.061$). However, FF significantly ($P < 0.05$ to 0.001) influenced the digestible contents of all individual DAA, total of DAA and total AA, regardless of PS, with higher values in mash diets.

Table 4.4. Influence of protein source (PS) and feed form (FF) on the standardised ileal digestibility coefficients[1] of nitrogen (N) and amino acids[2]

			Indispensable amino acids										Dispensable amino acids								
PS	FF	N	Arg	His	Ile	Leu	Lys	Met	Thr	Trp	Val	IAA	Ala	Asp	Cys[3]	Glu	Gly[3]	Pro	Ser	DAA	TAA
Meat and bone meal	Mash	0.760	0.830	0.788	0.779	0.807	0.817	0.812	0.762	0.749	0.785	0.792	0.779	0.725	0.659	0.791	0.716	0.731	0.755	0.737	0.767
	Pellet	0.703	0.778	0.757	0.749	0.781	0.785	0.784	0.718	0.721	0.747	0.758	0.713	0.659	0.568	0.738	0.596	0.627	0.699	0.657	0.713
Soybean meal	Mash	0.825	0.887	0.846	0.825	0.832	0.843	0.854	0.773	0.807	0.820	0.832	0.819	0.805	0.746	0.865	0.779	0.832	0.832	0.811	0.821
	Pellet	0.773	0.857	0.786	0.768	0.781	0.818	0.799	0.689	0.728	0.760	0.776	0.756	0.727	0.589	0.812	0.699	0.760	0.762	0.729	0.752
Canola meal	Mash	0.648	0.771	0.738	0.634	0.700	0.643	0.774	0.585	0.639	0.642	0.681	0.683	0.607	0.637	0.791	0.639	0.638	0.634	0.661	0.674
	Pellet	0.633	0.782	0.714	0.621	0.682	0.632	0.763	0.561	0.627	0.628	0.668	0.661	0.571	0.572	0.771	0.604	0.613	0.601	0.628	0.651
Pooled SEM		0.0259	0.0193	0.213	0.0254	0.0233	0.0239	0.0226	0.0309	0.0259	0.0253	0.0238	0.0271	0.0276	0.0346	0.0185	0.0308	0.0272	0.0268	0.0270	0.0252
Main effects																					
PS																					
Meat and bone meal		0.732[b]	0.804[b]	0.772[b]	0.765[a]	0.794[a]	0.801[a]	0.798	0.739[a]	0.735[a]	0.766[a]	0.775[a]	0.746[a]	0.692[b]	0.614	0.765[b]	0.656[b]	0.679[b]	0.727[b]	0.697[b]	0.740[a]
Soybean meal		0.799[a]	0.872[a]	0.816[a]	0.797[a]	0.807[a]	0.831[a]	0.826	0.731[a]	0.768[a]	0.790[a]	0.804[a]	0.788[a]	0.766[a]	0.668	0.839[a]	0.739[a]	0.796[a]	0.797[a]	0.770[a]	0.787[a]
Canola meal		0.640[c]	0.777[b]	0.729[b]	0.628[b]	0.691[b]	0.638[b]	0.768	0.573[b]	0.633[b]	0.635[b]	0.675[b]	0.672[b]	0.588[c]	0.604	0.781[b]	0.622[b]	0.626[b]	0.618[c]	0.644[b]	0.663[b]
Pooled SEM		0.0183	0.0137	0.0151	0.0179	0.0165	0.0169	0.0159	0.0219	0.0183	0.0179	0.0168	0.0191	0.0195	0.0244	0.0131	0.0218	0.0192	0.0189	0.0191	0.0178
FF																					
	Mash	0.744	0.829	0.791[a]	0.746	0.779	0.768	0.813	0.707	0.731	0.749	0.768	0.760[a]	0.712[a]	0.681[a]	0.816[a]	0.712[a]	0.734[a]	0.739[a]	0.736[a]	0.754[a]
	Pellet	0.703	0.806	0.754[b]	0.713	0.748	0.745	0.782	0.656	0.692	0.712	0.734	0.709[b]	0.652[b]	0.576[b]	0.774[b]	0.633[b]	0.667[b]	0.688[b]	0.671[b]	0.705[b]
Pooled SEM		0.0149	0.0111	0.0123	0.0147	0.0135	0.0139	0.0130	0.0179	0.0149	0.0146	0.0137	0.0156	0.0159	0.0199	0.0107	0.0178	0.0157	0.0155	0.0156	0.0145
Probabilities, P ≤																					
PS		0.001	0.001	0.001	0.001	0.001	0.001	0.051	0.001	0.001	0.001	0.001	0.001	0.001	0.156	0.001	0.002	0.001	0.001	0.001	0.001
FF		0.060	0.148	0.044	0.117	0.104	0.255	0.101	0.054	0.073	0.079	0.089	0.030	0.013	0.001	0.009	0.004	0.005	0.023	0.006	0.025
PS × FF		0.681	0.269	0.606	0.703	0.773	0.906	0.621	0.634	0.421	0.672	0.669	0.664	0.741	0.408	0.589	0.399	0.356	0.797	0.612	0.648

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

[1]Apparent digestibility values were standardised using the following basal ileal endogenous flow values (g/kg DM intake), determined by the feeding N-free diet: crude protein, 17.3; Arg, 0.59; His, 0.27; Ile, 0.54; Leu, 0.87; Lys, 0.57; Met, 0.22; Thr, 1.04; Trp, 0.19; Val, 0.72; Ala, 0.65; Asp, 1.15; Cys, 0.42; Glu, 1.42; Gly, 0.68; Pro, 0.83; and Ser, 0.86.

[2]Each value represents the mean of six replicates (eight birds per replicate). [3]Semi-indispensable amino acids for poultry.

DAA = Average digestibility of dispensable amino acids; IAA = Average digestibility of indispensable amino acids; TAA = Average digestibility of all amino acids.

Table 4.5. Influence of protein source (PS) and feed from (FF) on the standardised digestible protein (CP) and amino acid contents[1] (g/kg), as-received basis

			Indispensable amino acids										Dispensable amino acids								
PS	FF	CP	Arg	His	Ile	Leu	Lys	Met	Thr	Trp	Val	IAA	Ala	Asp	Cys[2]	Glu	Gly[2]	Pro	Ser	DAA	Total AA
Meat and bone meal	Mash	484	33.4	10.4	16.9	35.6	31.6	9.57	17.4	4.68	24.0	184	32.1	35.9	4.59	61.7	44.4^{a}	33.2	17.9	230	413
	Pellet	448	31.3	10.0	16.3	34.4	30.4	9.24	16.4	4.51	22.9	175	29.4	32.6	3.96	57.6	36.9^{b}	28.5	16.6	206	381
Soybean meal	Mash	393	29.9	10.1	16.7	29.2	24.3	5.43	14.0	5.13	17.9	153	16.5	42.4	5.18	72.6	15.2^{c}	21.6	19.5	193	346
	Pellet	368	28.9	9.33	15.6	27.5	23.6	5.08	12.5	4.63	16.6	144	15.3	38.3	4.09	68.1	13.6^{cd}	19.8	17.9	177	321
Canola meal	Mash	229	15.8	6.55	8.58	16.6	12.3	5.28	8.51	3.07	11.5	88.1	10.3	14.8	5.01	46.6	10.9^{d}	14.4	8.86	111	199
	Pellet	224	15.9	6.39	8.39	16.2	12.1	5.21	8.16	3.02	11.2	86.6	9.99	13.9	4.49	45.4	10.4^{d}	13.9	8.39	106	193
Pooled SEM		12.08	0.59	0.235	0.439	0.74	0.60	0.173	0.539	0.147	0.55	3.94	0.684	1.09	0.251	1.36	1.25	0.90	0.515	5.81	9.66
Main effects																					
PS																					
Meat and bone meal		466^{a}	32.4^{a}	10.2^{a}	16.7^{a}	35.0^{a}	30.9^{a}	9.41^{a}	16.9^{a}	4.59^{a}	23.5^{a}	180^{a}	30.7^{a}	34.2^{b}	4.28	59.6^{b}	40.6	30.8^{a}	17.3^{b}	218^{a}	397^{a}
Soybean meal		380^{b}	29.5^{b}	9.69^{b}	16.2^{a}	28.3^{b}	23.9^{b}	5.26^{b}	13.3^{b}	4.88^{a}	17.3^{b}	148^{b}	15.9^{b}	40.3^{a}	4.64	70.3^{a}	14.4	20.7^{b}	18.7^{a}	185^{b}	333^{b}
Canola meal		226^{c}	15.9^{c}	6.47^{c}	8.48^{b}	16.4^{c}	12.2^{c}	5.25^{b}	8.33^{c}	3.04^{b}	11.3^{c}	87.4^{c}	10.2^{c}	14.3^{c}	4.75	45.9^{c}	10.7	14.2^{c}	8.62^{c}	109^{c}	196^{c}
Pooled SEM		8.54	0.42	0.166	0.310	0.52	0.43	0.122	0.381	0.104	0.39	2.79	0.48	0.78	0.178	0.96	0.89	0.63	0.364	4.11	6.83
FF																					
	Mash	368^{a}	26.4	9.01^{a}	14.1	27.2	22.8	6.76	13.3^{a}	4.29	17.8	142	19.6^{a}	30.9^{a}	4.93^{a}	60.3^{a}	23.5	23.1^{a}	15.4^{a}	178^{a}	319^{a}
	Pellet	346^{b}	25.4	8.58^{b}	13.4	26.0	22.1	6.51	12.3^{b}	4.05	16.9	135	18.2^{b}	28.3^{b}	4.18^{b}	57.0^{b}	20.3	20.7^{b}	14.3^{b}	163^{b}	298^{b}
Pooled SEM		6.97	0.34	0.136	0.25	0.43	0.35	0.099	0.31	0.085	0.32	2.28	0.39	0.63	0.145	0.78	0.72	0.52	0.29	3.36	5.58
Probabilities, P ≤																					
PS		0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.167	0.001	0.001	0.001	0.001	0.001	0.001
FF		0.033	0.059	0.032	0.074	0.070	0.151	0.085	0.037	0.051	0.053	0.061	0.015	0.005	0.001	0.006	0.004	0.003	0.012	0.004	0.012
PS × FF		0.443	0.167	0.505	0.558	0.662	0.693	0.670	0.567	0.313	0.593	0.582	0.225	0.329	0.495	0.419	0.022	0.078	0.517	0.252	0.382

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

Standardised digestible amino acid content (g/kg) = [ingredient amino acid content (g/kg) × standardised ileal digestibility (%)]/100.

[1]Each value represents the mean of six replicates (eight birds per replicate). [2]Semi-indispensable amino acids for poultry.

DAA = Total digestible dispensable amino acid contents; IAA = Total digestible indispensable amino acid contents; Total AA = Total digestible content of all amino acids.

4.5. Discussion

In general, the DM, fat, NDF, ash, Ca and P contents of MBM, SBM and CM were within the range reported in published literature (NRC, 1994; Adedokun *et al.*, 2008, 2009a; Woyengo *et al.*, 2010; Ravindran *et al.*, 2014b).

The CP content of MBM (644 g/kg) was slightly higher than the previous reports (488-600 g/kg; Angkanaporn *et al.*, 1996; Ravindran *et al.*, 2002; Adedokun *et al.*, 2007a). The wide variability of in the CP content of MBM may be attributed to the variation in the source of raw materials (Karakas *et al.*, 2001; Adedokun *et al.*, 2007a). The variation in CP and AA contents among different batches of animal-based PS is well known (Ravindran and Bryden, 1999). The CP content of SBM (484 g/kg) was similar to the value of 485 g/kg documented by NRC (1994) and close to the range (464-482 g/kg) reported by Ravindran *et al.* (2014b). Number of factors including crushing and oil extraction conditions, hull inclusion and location of bean production may influence the nutrient content of SBM (Mateos *et al.*, 2019). In the case of CM, the CP content (356 g/kg) was comparable to the value of 365 g/kg reported by Khajali and Slominski (2012).

The contents of IAA, DAA and TAA in the three PS assayed in the current study approximated published values (Ravindran *et al.*, 2005, 2014b; Adedokun *et al.*, 2009a; Woyengo *et al.*, 2010; Kim *et al.*, 2012b; Ullah *et al.*, 2016). The AA composition of ingredients of plant origin can be influenced by the country of origin, variation in planting

season, cultivar and harvesting practices (Evers *et al.*, 1999; Jondreville *et al.*, 2001; Adedokun *et al.*, 2008).

To achieve the genetic potential of modern broilers, it is essential to maximise their FI. Feeding pelleted diets increases FI, which is the major driver of weight gain in broilers (Svihus *et al.*, 2004; Abdollahi *et al.*, 2018a,b). However, a wide variation in FI increases, from 2.8% (Serrano *et al.*, 2012) to 64% (Amerah *et al.*, 2007) due to pelleting, has been reported. Predictably, in the current study, birds offered pelleted diets showed different extents of increased FI in three PS compared to those fed mash diets, with a significant interaction between PS and FF. Offering pelleted diet increased the FI in broilers fed a maize-SBM-based diet (Roza *et al.*, 2018). Abdollahi *et al.* (2018b) observed an interaction between nutrient density and FF in the performance of broilers fed a complete diet that comprised of maize, SBM and CM and observed that the pellet-associated benefit on FI was more pronounced at the lowest nutrient density. Pelleted diets increased the FI, but the magnitude of response eroded from 23.3% in very low nutrient density diets to 9.8% in very high nutrient density diets.

Pelleting increased the excreta scores only in SBM-based diets. Viera and Lima (2005) similarly found that the dietary inclusion of SBM increased the water intake and excreta moisture in broilers. Number of dietary factors, including CP content, electrolyte balance, ionophores, cereal type, fibre content, legume content and non-starch polysaccharides can impact excreta moisture and quality (Smith *et al.*,

2000; Murakami *et al.*, 2001; Ravindran *et al.*, 2008) and interact with FF.

The higher SIDC of N in SBM than MBM and CM in the current study is in agreement with the findings of Lemme *et al.* (2004), who reported higher SIDC of CP in SBM (0.90) than CM (0.76) and MBM (0.65) in broilers. The average SIDC of AA for SBM (0.787) in the current study was similar to previous findings (0.82-0.84; Kong and Adeola, 2013b; Ravindran *et al.*, 2014b; Ullah *et al.*, 2016) and the value for CM (0.663) was lower than that (0.79) reported by Adedokun *et al.* (2008) and Kim *et al.* (2012b). In the current study, the average SIDC of DAA was higher in SBM (0.770) compared to MBM (0.697) and CM (0.644). However, the average SIDC of IAA was similar in MBM (0.775) and SBM (0.804), and higher than CM (0.675). The same pattern was observed for average digestibility of TAA, with the SIDC being similar in MBM (0.740) and SBM (0.787) but higher than CM (0.663). Adedokun *et al.* (2008), in a study with 21-d-old broilers, reported higher SIDC AA for SBM (0.873) than CM (0.798). In a study by Ravindran *et al.* (2005), a higher AIDC was reported for SBM (0.820), with a lower value for CM (0.780).

The lower AA digestibility in CM can be attributed to several reasons and the primary factor is its high fibre content (Jansen and Carre´, 1985; Bell, 1993). The content of fibre in canola seeds is higher compared to soybean seeds (159 vs. 60 g/kg; Sauer *et al.*, 1982) and this is reflected in the fibre content of CM (Newkirk, 2009). The concentration of NDF

in CM ranges from 220 to 300 g/kg (Maison, 2013). The CM used in present study had similar NDF (250 g/kg) content.

Canola meal also contains a higher content (6.4 g/kg) of phytate-P compared to SBM (3.8 g/kg). The negative influence of phytate on protein digestion and absorption is now well documented (Selle and Ravindran, 2007). Phytate can influence the gut-capacity for transportation of Na-dependant nutrients including glucose and peptides by changing Na partitioning (Cowieson *et al.*, 2009). Canola meal also contains a higher content of lignin with associated polyphenols (Khajali and Slominski, 2012), which can bind with proteins and lower digestibility. According to Newkirk and Classen (2002), the removal of some AA by desolventisation and toasting stage during prepress solvent extraction of canola seeds may also have partly contributed to the low digestibility (Newkirk and Classen, 2002).

The MBM evaluated in the current work was rendered from slaughter by-products of cattle and sheep, but the average SIDC of AA for MBM (0.740) of this sample was markedly higher than the value (0.632) reported by Adedokun *et al.* (2007a) evaluating cattle-originated MBM. This finding is consistent with the wide variation (0.62-0.82) has been reported for SID AA of MBM in the literature (Kadim e*t al.*, 2002; Adedokun *et al.*, 2009a, 2014). A previous experiment on the AID of MBM in broilers showed that the coefficient of variation for the digestibility of most AA ranged from 0.13 to 0.16 (Ravindran *et al.*, 2002). The wide variability is a reflection of the differences in the

source of raw materials and processing conditions (Parsons *et al.*, 1997; Adedokun *et al.*, 2014).

High conditioning temperatures during pelleting process has been shown to reduce the digestibility of CP in maize-SBM- (Abdollahi *et al.*, 2013b) and wheat-SBM- (Abdollahi *et al.*, 2011) based diets. Loar II *et al.* (2014) reported 3 to 5% reduction in the digestibility of some AA in a maize-SBM-based diet when the pelleting temperature was increased from 74 to 85 and 96 ºC. However, despite the application of moderate conditioning temperature (70 ºC) in the current study, among the IAA, feeding pelleted diets reduced the SIDC of His and tended to reduce those of N, Thr, Trp, Val and average of IAA. Pelleting also resulted in lower SIDC of all DAA, average DAA (8.8%) and average TAA (6.5%) compared to mash. Cysteine was the most affected DAA with 15.4% reduction in SIDC due to pelleting, followed by 11.1% in Gly, 9.1% in Pro, 8.4% in Asp, 6.9% in Ser and 5.1% in Glu. Papadopulos (1989) found that the most heat labile AA was Cys followed by Lys, Arg, Thr and Ser after autoclaving at 110 to 130 ºC. Heat susceptibility of Cys has long been known (Evans and McGinnis, 1948) and the present findings confirm that Cys is the most heat labile AA and its digestibility can deteriorate during the pelleting process (Wall 1971; Ravindran *et al.*, 2014b). Shirley and Parsons (2000) found that the processing pressure that was applied after or during the rendering process of raw materials caused the greatest negative influence on the digestibility of Cys, compared to other AA, in MBM. It has been documented that in the absence of reducing substances,

degradation of Cys during heat processing can form dehydroalanine by the fission of disulphide bond. Dehydroalanine can form either lanthionine by reacting with rest of Cys or form lysoalanine by reacting with ε-amino groups of Lys (Bohak, 1964; Papadopoulos, 1989). Amino acid derivatives such as lysoalanine and lanthionine formed during heating process have been reported to be poorly digestible (Gilani *et al.*, 2005).

The moisture content plays an important role in protein denaturation. According to Adams (1991), most proteins start to denature at a temperature of 60-70 ºC in the presence of excess moisture, while some proteins may be denatured at temperatures of more than 40 ºC and others remain stable even at 80 ºC. Proteins remain stable even during severe heat treatments (80 to even 190 ºC) when the moisture content is very low (Ludikhuyze *et al.*, 2003). At excess moisture (the moisture level beyond standard pelleting requirements which is about 3-4%; Svihus *et al.*, 2004), the temperature of denaturation of two main storage protein of SBM such as conglycinin and glycinin were recorded as 76.5 ºC and 93.3 ºC. When the moisture content was reduced to 290 g/kg, the denaturation temperature for conglycinin increased to over 180 ºC and in case of glycinin, this denaturation temperature could not even be detected (Kitabatake and Doi, 1992). At moisture levels of 0 to 300 g/kg, the denaturation temperature of sunflower globulin was in between 120 to 190 ºC (Rouilly *et al.*, 2003). Zimonja *et al.* (2007) stated that at the time pelleted diet leaves the pellet press the temperature of pellet varies from 80 to 90 ºC and the moisture is about

150 to 170 g/kg. This temperature is reduced to around 8 ºC above ambient temperature during cooling process while the moisture is 100 to 120 g/kg. Therefore, it can be speculated that the moisture and medium temperature level applied in pelleting process in current study was probably unable to cause significant protein denaturation.

The lower SIDC of AA in pelleted diets in present study might also be associated with the higher FI. Over-consumption and overload of starch in birds fed pelleted diets have been shown to reduce the digestibility of starch (Abdollahi *et al.*, 2013b, 2018b) through a faster passage rate; this might also apply to AA in high-protein ingredients. Engberg *et al.* (2002) observed lower gizzard and pancreas weights in pellet-fed birds than those fed the mash diet. Lack of mechanical stimulation was suggested as the possible reason for the reduced gizzard weights. The same study documented lower activities (units/100g BW) of amylase, lipase, trypsin and chymotrypsin in pellet-fed birds. Decreased SIDC AA in pellet-fed birds might be explained by the reduced activities of proteolytic enzymes. In addition, the lower digestive organ weights induced by feeding pelleted diets might shorten digesta retention time, compromising the digestion and absorption of nutrients (Frikha *et al.*, 2009; Naderinejad *et al.*, 2016; Abdollahi *et al.*, 2018b).

The pH of gastric juice secreted from the proventriculus is around 2 (Duke, 1986). The average gizzard pH in broiler chicken was reported to be between 3 and 4 when pelleted diets were fed. The pH in gizzard or proventriculus is variable depending on the FI, retention time and the chemical characteristics of feed (Svihus, 2011a). The lower gizzard

pH in mash diets will stimulate the gastric function facilitating digestive processes (Engberg *et al.*, 2002). The proteolytic enzyme pepsin is secreted as a precursor called pepsinogen by the chief cells in the proventriculus. Pepsin is auto-activated in the acidic environment and then initiates the break-down of dietary proteins into peptides (Baudys and Kostka, 1983). Since feed has a pH close to neutral, higher FI may result in elevation of gizzard pH if the gastric juice is not secreted in accordance. Previous studies have documented higher gizzard pH with pelleted diet in comparison with mash diet (Engberg *et al.*, 2002; Frikha *et al.*, 2009; Naderinejad *et al.*, 2016) with potential deleterious effects on protein digestion.

The highest digestible content of IAA, DAA and TAA was found in the MBM, followed by SBM and CM. This was to be expected because CP and AA contents were higher in MBM compared to those in SBM and CM.

Pelleting reduced the digestible CP contents by 6.0% (346 vs. 368 g/kg) compared to mash. Protein solubilisation in the gastrointestinal tract is the initial step in protein digestion (Cowieson and Ravindran, 2008). Generally, the intact protein in feed ingredients is in stable form with a three-dimensional configuration (Davis and Williams, 1998). Though the HTP induces some degree of protein denaturation and may enhance protein digestibility, the conditions of HTP such as pressure, moisture added during thermal treatment, temperature and time of processing determines the eventual impact (Svihus and Zimonja, 2011). The hydrophobic AA that are inward oriented in their native form are turned

outward during the denaturation, which might reduce protein solubilisation (Araba and Dale, 1990; Camire, 1991) and compromise protein digestion. Several factors are associated with the reduction of digestible AA contents in the pelleted diet. Maillard reaction during feed processing might be a possible factor contributing to reduced AA availability. As discussed by Cheftel (1986), the favourable conditions for Maillard reaction are high temperature (> 180 ºC) and low moisture (< 15%) combined with > 100 rpm shear. However, pelleting involves conditioning of dry feed by adding steam that increases the moisture level by up to 3-4% units and temperature rise to 85-90 ºC. Subsequently the feed is passed through die holes and, friction in these holes and the friction by rolls which force the material into the holes produce a further rise of temperature (Svihus *et al.*, 2004). Svihus and Zimonja (2011), however, speculated that the conditions of commercial pelleting are too mild to cause Maillard reactions (Svihus and Zimonja, 2011). Lysine is the AA mostly affected by the Maillard reaction, but the SIDC of Lys was unaffected by pelleting in present study.

Hydrothermal treatment of feed has also been suggested to degrade heat labile Cys, Lys, Arg, Thr and Ser, reducing their AA digestibility (Camire *et al.*, 1990; Marsman *et al.*, 1995). These observations are in line with the present findings of reductions or tendency to reduce the digestible contents of most IAA (except Lys), all DAA and total AA in pelleted ingredients compared to mash.

4.6. Conclusions

This is the first report demonstrating that the FF has a substantial impact on digestibility estimates of AA, particularly DAA, in high-protein feed ingredients for broiler chickens, and therefore, it should be considered in AA digestibility assays. The reduction of AA digestibility in pelleted assay diets may be due partly to the impact of hydrothermal processing. However, other factors induced by pellet-feeding such as feed overconsumption, AA overload, shorter digesta retention time, and an elevated gizzard pH might be even more influential. The present findings question the validity of using AA digestibility data that have been derived using mash diets for commercial broiler chickens' formulations where feed is generally offered in the pelleted form. Application of AA digestibility data generated using mash diets can overestimate the availability of AA in PS for broiler chickens, considerably affecting the precision of feed formulation and consequent growth performance.

CHAPTER 5 Basal ileal endogenous amino acid flow in broiler chickens as influenced by age[1]

5.1. Abstract

The current study was carried out to measure the basal ileal endogenous amino acid (EAA) flow in male broilers (Ross 308) at different ages (d 7, 14, 21, 28, 35, and 42), following the feeding of a nitrogen-free diet. Titanium dioxide (5 g/kg) was included as an indigestible marker. The nitrogen-free diet was offered for 4 d prior to ileal digesta collection to six replicate cages housing 14 (d 3-7), 12 (d 10-14), 10 (d 17-21), 8 (d 24-28), 8 (d 31-35), and 6 (d 38-42) birds per cage. The basal EAA flow was calculated as g/kg dry matter intake. The amino acid (AA) profile of endogenous protein, expressed as g/100 g protein, was also calculated. The basal endogenous flow of nitrogen and all individual and total AA decreased quadratically ($P < 0.05$ to 0.001), with flows being higher on d 7, then decreasing on d 14, plateauing until d 35 and decreasing further on d 42. The concentrations of Trp, Cys, and Gly in the endogenous protein increased linearly ($P < 0.01$ to 0.001) with advancing age, whereas a linear decrease ($P < 0.001$) was noted for Lys. A quadratic influence ($P < 0.05$ to 0.001) was observed for the concentrations of Ile, Leu, Met, Val, and Asp. These changes in the endogenous protein profile may be attributed to variations in the contribution of endogenous sources with age but delineating the exact

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contribution of different sources is complicated. Overall, the current findings suggest that the basal ileal EAA flow is influenced by broiler age and age-specific EAA flows may need to be considered to standardise the AA digestibility.

5.2. Introduction

Continuous flow of significant quantities of endogenous protein occurs during the process of digestion of ingested feed in poultry (Ravindran, 2021). The sources of endogenous protein are various digestive secretions (bile, gastric, pancreatic, and intestinal secretions), mucoproteins and desquamated epithelial cells lining the gastrointestinal tract (GIT). Accurate quantification of the flow of endogenous amino acids (EAA) is necessary to calculate the standardised amino acid (AA) digestibility of feed ingredients. These inevitable flows also contribute to the metabolic costs associated with protein synthesis and turnover in the GIT, and to the determination of protein and AA requirements by the factorial method. The measurement and a better understanding of the factors influencing the EAA flow is relevant in improving protein or AA utilisation, and thereby reducing nitrogen (N) excretion into the environment (Cowieson *et al.*, 2009).

Endogenous AA losses are influenced primarily by dry matter intake (DMI) and secondarily by the composition of the feed ingredient or diet. These two fractions are categorised as basal and specific EAA losses, respectively (Ravindran, 2016). Basal endogenous losses are defined as those closely associated with the metabolic functions of the

animal and are independent of the diet type. These losses represent the minimum losses that can be expected under any feeding situation. Several methods are available to determine the basal EAA flows in poultry including the regression method, feeding a N-free diet (NFD) or a diet containing highly digestible protein, the peptide alimentation (enzyme hydrolysed casein) method, or the use of fasted birds. Each of these approaches has its advantages and limitations (Ravindran and Bryden, 1999; Blok *et al.*, 2017; Parsons, 2020). Of these, basal ileal EAA estimates determined following the feeding of an NFD is now accepted as being valid for the correction of apparent AA digestibility values (Stein *et al.*, 2007; Ravindran *et al.*, 2017; Parsons, 2020).

The NFD is generally composed of starch or sugar (>80%), fortified with insoluble fibre (e.g., cellulose), minerals and vitamins. The assumption is that, since no protein is fed, all N and AA in the ileal digesta are of endogenous origin and represent the basal flows. Feeding an NFD is a simple method despite suffering from limitations of underestimation and nonphysiological feeding (Donkoh and Moughan, 1999; Stein *et al.*, 2007).

A wide range of factors including the class of birds (broilers, layers, roosters; Ravindran and Hendriks, 2004), protein status, body weight (BW), health status, and DMI are reported to influence the basal EAA estimates (Lemme *et al.*, 2004; Adedokun *et al.*, 2007b). Possible age effect may be an important factor. However, no previous studies have compared the EAA flows throughout the growth cycle of broilers. To the author's knowledge, except those by Adedokun *et al.* (2007b,c), all

previous studies (Ravindran *et al.*, 2004; Golian *et al.*, 2008; Soleimani *et al.*, 2010; Kong and Adeola, 2013a) have the measured EAA flow at a single age using broilers older than 21 d. The applicability of EAA data derived from one single age to all broiler ages is questionable and, therefore, the aim of present study was to determine the basal ileal endogenous N and AA flows at different ages of broilers.

5.3. Materials and Methods

The experimental procedures were in accordance with the New Zealand Revised Code of Ethical Conduct for the use of live animals for research, testing, and teaching, approved by Massey University Animal Ethics Committee.

5.3.1. Diets

The composition of the NFD is shown in Table 5.1. Titanium dioxide was included as an indigestible marker.

Table 5.1. Composition of the nitrogen-free diet (g/kg, as fed basis)

Ingredients	g/kg
Maize starch	842
Cellulose[1]	50
Soybean oil	50
Dicalcium phosphate	19
Limestone	13
Dipotassium phosphate	12
Titanium dioxide[2]	5.0
Trace mineral premix[3]	3.0
Vitamin premix[3]	2.0
Sodium bicarbonate	2.0
Sodium chloride	2.0

[1]Ceolus, Microcrystalline Cellulose, Asahi Kasei Corporation, Tokyo, Japan.

[2]Merck KGaA, Darmstadt, Germany.

[3]Provided per kilogram of diet: antioxidant, 100 mg; biotin, 0.2 mg; calcium pantothenate, 12.8 mg; cholecalciferol, 0.06 mg; 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, 0.2 mg; Zn, 60 mg.

5.3.2. Birds and housing

A total of 348, day-old male broilers (Ross 308) were obtained from a commercial hatchery, raised in floor pens and fed a commercial crumbled broiler starter diet (12.14 MJ/kg apparent metabolisable energy; 225 g/kg crude protein; CP) from d 1 to 21 and a broiler finisher diet (12.69 MJ/kg apparent metabolisable energy; 190 g/kg CP) in pelleted form from d 22 to 38 (Table 5.2).

Table 5.2. Composition and calculated analysis (g/kg, as fed basis) of broiler starter and finisher diets

Ingredients	Starter diet (0-21 d)	Finisher diet (22-38 d)
Maize	574.2	660
Soybean meal, 460 g/kg	381.4	295.6
Soybean oil	8.8	13.6
Limestone	11.3	9.9
Dicalcium phosphate	10.7	8.2
DL-methionine	3.3	3.0
L-lysine HCl	2.0	1.9
L-threonine	1.0	0.7
Sodium bicarbonate	2.7	2.5
Sodium chloride	2.5	2.5
Trace mineral premix[1]	1.0	1.0
Vitamin premix[1]	1.0	1.0
Phytase	0.1	0.1
Calculated analysis		
Apparent metabolisable energy (MJ/kg)	12.14	12.69
Crude protein	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]Supplied per kilogram of diet: antioxidant (ethoxyquin), 100 mg; biotin, 0.2 mg; calcium pantothenate, 12.8 mg; cholecalciferol, 0.06 mg; 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, 0.2 mg; Zn, 60 mg.

The starter, finisher and NFD diets, in mash form, were offered *ad libitum* and fresh drinking water was available at all times. During the first week, the average temperature was 32 ± 1 ºC and gradually reduced to 23 ºC by the end of the third week.

There were six groups of different ages (d 3-7, 10-14, 17-21, 24-28, 31-35, and 38-42) of broilers. On d 3, 84 birds were weighted individually and allocated to six battery brooders (n = 14 chicks per replicate) in such a way that the average bird weight per cage was similar. The remaining chicks (n = 264) were raised in floor pens until they were weighed and allocated to six replicate grower cages per age group on d 10 (n = 12 birds per cage), d 17 (n = 10 birds per cage), d 24 (n = 8 birds per cage), d 31 (n = 8 birds per cage), and d 38 (n = 6 birds per cage), respectively. In each age group, the NFD was fed for 4 d (d 3-7, 10-14, 17-21, 24-28, 31-35, and 38-42) prior to the ileal digesta collection.

The floor pens, battery brooder and grower cages were housed in an environmentally controlled room with 20 h of fluorescent illumination per day.

5.3.3. Determination of ileal nutrient digestibility

The birds were euthanised by intravenous injection (1 mL per 2 kg BW) of sodium pentobarbitone solution (Provet NZ Pty. Ltd., Auckland, New Zealand) on d 7, 14, 21, 28, 35, and 42. Digesta were collected from the lower half of the ileum, as described by Ravindran *et al.* (2005). The ileum was considered as the portion of the small intestine from Meckel's diverticulum to a point about ~40 mm proximal to the

ileocaecal junction. The ileal digesta were collected from all birds into plastic containers by gentle flushing by distilled water, pooled within a cage, immediately frozen, and subsequently freeze dried (Model 0610, Cuddon Engineering, Blenheim, New Zealand). The diet and freeze-died digesta samples were ground to pass through a 0.5-mm sieve. The samples were stored in air-tight plastic containers at 4 ºC until the analysis of dry matter (DM), titanium (Ti), N and AA.

5.3.4. Chemical analysis

The DM was determined using the standard procedure (Method 930.15; AOAC International, 2016). Titanium was measured on an ultraviolet spectrophotometer (Berthold Technologies GmbH and Co. KG, Bad Wildbad, Germany) following the method of Short *et al.* (1996). Nitrogen was analysed by combustion (Method 968.06; AOAC International, 2016) using a CNS-200 carbon, N, sulphur analyser (LECO Corporation, St. Joseph, MI). These analyses were done in duplicate.

Amino acids were analysed following the standard procedures (Method 994.12; AOAC International, 2011). In brief, the samples were hydrolysed with 6 N hydrochloric acid (containing phenol) for 24 h at 110 ± 2 ºC in glass tubes in an oven. The AA was measured using AA analyser (ion exchange) with ninhydrin postcolumn derivatisation. The chromatograms were integrated using dedicated software (Agilent Open Lab software, Waldbronn, Baden-Württemberg, Germany) with AA simultaneously detected at 570 and 440 nm. Cysteine and Met were determined as cysteic acid and methionine sulphone, respectively, by

oxidation with performic acid for 16 h at 0 ºC and neutralisation with hydrobromic acid prior to hydrolysis.

For Trp analysis, the samples were saponified under alkaline conditions with barium hydroxide solution in the absence of air at 110 ºC for 20 h in an autoclave. Following alkaline hydrolysis, the internal standard α-methyl Trp was added to the mixture. After adjusting the hydrolysate to pH 3.0 and diluting with 30% methanol, Trp and the internal standard were separated by reverse phase chromatography (RP-18) on a HPLC column (CORTECS C18 Column; 2.7 µm, Waters Corporation, Dublin, Ireland). Finally, detection was selectively performed by means of a fluorescence detector to prevent interference by other AA and constituents.

5.3.5. Calculations

All data were expressed on a DM basis for calculations. The basal EAA flow at the terminal ileum was calculated as grams of AA flow per kilogram of DMI using the following formula (Moughan *et al*., 1992).

Basal EAA flow (g/kg DMI) =

AA concentration in ileal digesta (g/kg) × [Diet Ti (g/kg) /Ileal digesta Ti (g/kg)]

The AA profile of endogenous protein was calculated by expressing each AA as g per 100 g of endogenous protein (N × 6.25), as indicated below.

AA composition of endogenous protein = (Endogenous AA/Endogenous CP) × 100

5.3.6. Data analysis

Data were analysed by the General Linear Model procedure of SAS (version 9.4; 2015; SAS Institute, Cary, NC.). Orthogonal polynomial contrasts were performed to determine the linear and quadratic effects of broiler age. Cage served as the experimental unit. Statistical significance was declared at $P < 0.05$.

5.4. Results

No evidence of intestinal abnormalities was observed when the abdominal cavity was opened following euthanasia.

5.4.1. Feed intake

Feed intake (FI) increased (quadratic, $P < 0.001$) with advancing age of birds. The average daily FI of birds were 11.4 (d 7), 27.3 (d 14), 49.1 (d 21), 79.6 (d 28), 80.9 (d 35), 79.1 (d 42) g/bird, respectively.

5.4.2. Basal endogenous flow of nitrogen and amino acids

The basal ileal endogenous flow of N and AA at different ages (d 7, 14, 21, 28, 35 and 42) of broiler, expressed as g/kg DMI, is presented in Table 5.3.

A quadratic influence ($P < 0.05$ to 0.001) was observed on the basal endogenous flow of N and all AA. The flows were higher on d 7, then decreased on d 14 and plateaued until d 35. After d 35, a further decrease to d 42 was observed. The highest and lowest values were recorded on d 7 and 42, respectively (Figures 5.1 and 5.2).

5.4.3. Ileal digesta concentrations of nitrogen and amino acids

The N and AA concentrations in the ileal digesta of birds fed the NFD, expressed as g/100g digesta, are summarised in Table 5.4. The concentrations of N and all AA, except Trp and Cys, linearly decreased ($P < 0.05$ to 0.001) as the broilers grew older.

5.4.4. Amino acid profile of endogenous protein

The AA profile of ileal endogenous protein, expressed as g/100 g protein, in broilers of different ages is shown in Table 5.5. The most abundant AA in the endogenous protein was Glu, Asp, Thr, Ser, Leu, Pro, Val and Ala. The lowest concentration among all AA was recorded for Trp, followed by Met.

The concentration of Trp, Cys and Gly linearly ($P < 0.01$ to 0.001) increased with advancing age, whereas that for Lys linearly ($P < 0.001$) decreased. Quadratic age effects ($P < 0.05$ to 0.001) were observed for the concentration of Ile, Leu, Met, Val, and Asp, but the responses at different ages were variable and inconsistent. No age effect was observed ($P > 0.05$) for the concentrations of other AA.

Table 5.3. Basal ileal endogenous nitrogen (N) and amino acid flows[1] (g/kg dry matter intake) at different ages of broilers

	Age (d)							Orthogonal polynomial contrasts	
Parameter	7	14	21	28	35	42	Pooled SEM	Linear	Quadratic
N	3.599	1.866	1.793	1.823	1.808	1.295	0.1943	0.001	0.001
Indispensable amino acids									
Arg	0.678	0.303	0.313	0.358	0.320	0.203	0.0411	0.001	0.007
His	0.294	0.160	0.148	0.160	0.150	0.100	0.0176	0.001	0.012
Ile	0.626	0.325	0.323	0.343	0.301	0.201	0.0352	0.001	0.013
Leu	0.974	0.485	0.490	0.545	0.485	0.314	0.0574	0.001	0.017
Lys	0.644	0.301	0.283	0.321	0.280	0.175	0.0388	0.001	0.004
Met	0.266	0.123	0.124	0.140	0.118	0.069	0.0157	0.001	0.013
Thr	1.352	0.733	0.713	0.667	0.712	0.523	0.0751	0.001	0.002
Trp	0.206	0.116	0.121	0.127	0.121	0.088	0.0129	0.001	0.048
Val	0.811	0.431	0.433	0.453	0.420	0.299	0.0458	0.001	0.009
IAA	5.852	2.975	2.948	3.114	2.906	1.973	0.3365	0.001	0.007
Dispensable amino acids									
Ala	0.745	0.366	0.361	0.408	0.370	0.248	0.0437	0.001	0.008
Asp	1.407	0.733	0.721	0.744	0.721	0.497	0.0785	0.001	0.005
Cys[2]	0.474	0.270	0.257	0.249	0.263	0.206	0.0251	0.001	0.001
Glu	1.707	0.799	0.804	0.890	0.809	0.531	0.0985	0.001	0.004
Gly[2]	0.776	0.397	0.399	0.425	0.399	0.284	0.0437	0.001	0.005
Pro	0.911	0.479	0.481	0.476	0.473	0.341	0.0509	0.001	0.004
Ser	1.061	0.554	0.559	0.536	0.563	0.401	0.0579	0.001	0.002
DAA	7.081	3.599	3.583	3.729	3.559	2.508	0.3966	0.001	0.004
TAA	12.93	6.574	6.531	6.842	6.505	4.481	0.7328	0.001	0.005

[1]Each value represents the mean of six replicates (14, 12 and 10 birds per replicate for 7, 14 and 21-d old birds, respectively; eight birds per replicate for 28, 35-d old birds; and six birds per replicate for 42-d old birds).

[2]Semi-indispensable amino acids for poultry.

DAA = Total endogenous flow of dispensable amino acids; IAA = Total endogenous flow of indispensable amino acids; TAA = Total endogenous flow of all amino acids.

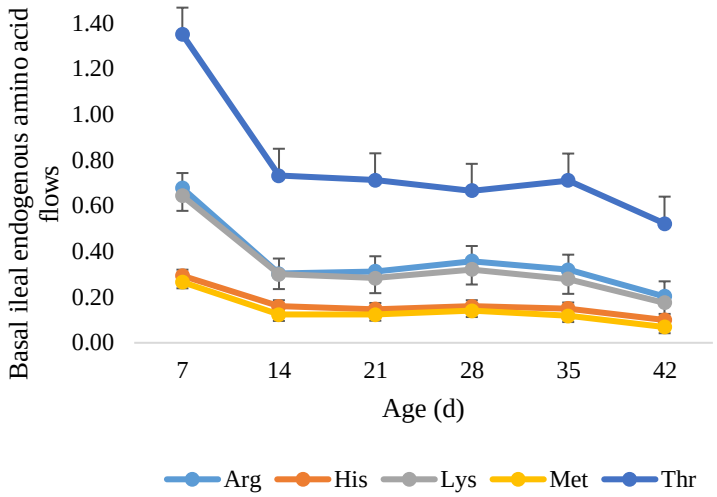

Figure 5.1. Basal ileal endogenous amino acid flow of selected indispensable amino acids (bars represent means ± standard error) as influenced by broiler age (Quadratic effects, P < 0.05 to 0.01).

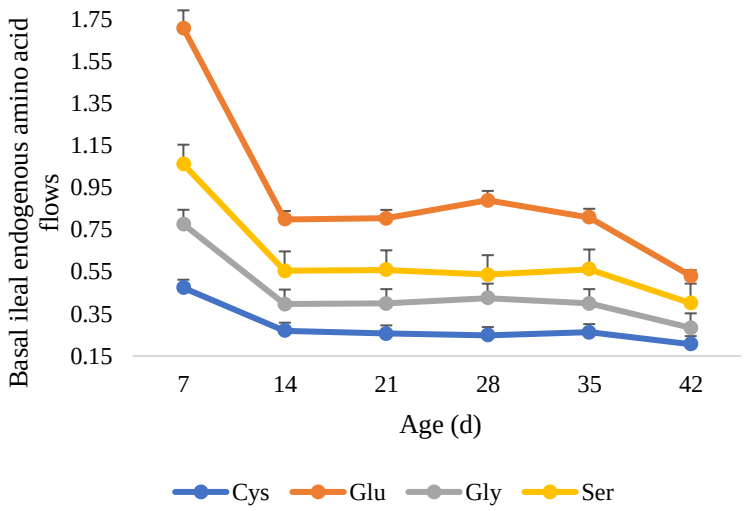

Figure 5.2. Basal ileal endogenous amino acid flow of selected dispensable amino acids (bars represent means ± standard error) as influenced by broiler age (Quadratic effects, P < 0.05 to 0.01).

Table 5.4. Amino acid concentrations[1] (g/100g of digesta) in the ileal digesta of broilers fed NFD at different ages

	Age (d)							Orthogonal polynomial contrasts	
Parameter	7	14	21	28	35	42	Pooled SEM	Linear	Quadratic
N	1.072	0.884	0.957	1.001	0.848	0.725	0.0663	0.003	0.344
Indispensable amino acids									
Arg	0.202	0.145	0.168	0.197	0.149	0.114	0.0157	0.005	0.234
His	0.088	0.076	0.079	0.088	0.069	0.056	0.0072	0.009	0.152
Ile	0.187	0.155	0.174	0.189	0.141	0.113	0.0144	0.003	0.066
Leu	0.289	0.233	0.263	0.299	0.227	0.176	0.0227	0.007	0.075
Lys	0.192	0.143	0.152	0.176	0.129	0.098	0.0146	0.001	0.326
Met	0.079	0.058	0.067	0.077	0.055	0.039	0.0059	0.001	0.078
Thr	0.404	0.349	0.381	0.366	0.335	0.294	0.0294	0.019	0.487
Trp	0.061	0.055	0.066	0.069	0.057	0.049	0.0055	0.279	0.059
Val	0.242	0.205	0.233	0.249	0.198	0.167	0.0183	0.019	0.100
IAA	1.743	1.418	1.583	1.711	1.359	1.105	0.1313	0.006	0.165
Dispensable amino acids									
Ala	0.222	0.174	0.194	0.224	0.172	0.139	0.0159	0.007	0.154
Asp	0.419	0.349	0.386	0.409	0.338	0.278	0.0293	0.007	0.166
Cys[2]	0.141	0.128	0.137	0.137	0.124	0.115	0.0091	0.074	0.444
Glu	0.508	0.381	0.431	0.489	0.377	0.297	0.0368	0.003	0.229
Gly[2]	0.231	0.189	0.214	0.233	0.188	0.159	0.0166	0.018	0.160
Pro	0.271	0.229	0.258	0.261	0.223	0.191	0.0204	0.021	0.255
Ser	0.317	0.264	0.299	0.295	0.264	0.225	0.0221	0.018	0.338
DAA	2.109	1.714	1.919	2.049	1.688	1.404	1.4881	0.009	0.220
TAA	3.853	3.132	3.502	3.760	3.047	2.509	0.2799	0.008	0.193

[1]Each value represents the mean of six replicates (14, 12 and 10 birds per replicate for 7, 14 and 21-d old birds, respectively; eight birds per replicate for 28, 35-d old birds; and six birds per replicate for 42-d old birds).

[2]Semi-indispensable amino acids for poultry.

DAA = Total endogenous flow of dispensable amino acids; IAA = Total endogenous flow of indispensable amino acids; TAA = Total endogenous flow of all amino acids.

Table 5.5. Amino acid composition of endogenous protein[1] (g per 100 g crude protein) at different ages of broilers

	Age (d)							Orthogonal polynomial contrasts	
Parameter	7	14	21	28	35	42	Pooled SEM	Linear	Quadratic
Indispensable amino acids									
Arg	3.031	2.439	2.679	2.992	2.883	2.551	0.1069	0.415	0.922
His	1.304	1.359	1.243	1.367	1.348	1.232	0.0422	0.462	0.253
Ile	2.780	2.658	2.763	2.942	2.712	2.479	0.0401	0.004	0.001
Leu	4.335	3.971	4.178	4.622	4.367	3.910	0.1028	0.582	0.031
Lys	2.869	2.429	2.366	2.695	2.532	2.217	0.1139	0.018	0.832
Met	1.189	0.985	1.045	1.200	1.078	0.859	0.0357	0.001	0.031
Thr	5.952	6.284	6.234	5.874	6.287	6.353	0.1724	0.274	0.747
Trp	0.923	0.967	1.039	1.096	1.074	1.086	0.0343	0.001	0.115
Val	3.599	3.583	3.751	3.927	3.764	3.683	0.0569	0.035	0.008
Dispensable amino acids									
Ala	3.312	3.028	3.124	3.569	3.319	3.106	0.0803	0.676	0.189
Asp	6.244	6.093	6.331	6.489	6.428	6.169	0.0861	0.297	0.049
Cys[2]	2.092	2.345	2.314	2.192	2.310	2.540	0.0572	0.001	0.379
Glu	7.616	6.504	6.889	7.547	7.279	6.613	0.1668	0.171	0.808
Gly[2]	3.443	3.269	3.497	3.697	3.571	3.527	0.0455	0.002	0.091
Pro	4.043	4.068	4.227	4.111	4.212	4.179	0.1046	0.273	0.599
Ser	4.684	4.689	4.919	4.699	4.916	4.909	0.1097	0.109	0.914

[1]Each value represents the mean of six replicates (14, 12 and 10 birds per replicate for 7, 14 and 21-d old birds, respectively; eight birds per replicate for 28, 35-d old birds; and six birds per replicate for 42-d old birds).

[2]Semi-indispensable amino acids for poultry.

5.5. Discussion

The aim of the current study was to investigate whether the basal ileal endogenous flows of N and AA are influenced by the age of broilers. The results showed that the basal ileal endogenous N and AA flows were markedly higher on d 7 and, then declined on d 14 and plateaued until d 35. A further decrease was observed on d 42. Compared to d 7, the basal endogenous N flow declined by 48 and 64% on d 14 and d 42, respectively. The endogenous flow of all AA on d 7 was almost twice of the values from d 14 to 35, and three times higher than the value on d 42. In agreement with the current findings, following the feeding of an NFD to broilers, Adedokun *et al.* (2007b) recorded approximately two times higher ileal EAA flow on d 5 (8.69 g/kg DMI) compared to

d 15 (3.73 g/kg DMI) and 21 (3.95 g/kg DMI), with no significant differences between the flows on d 15 and 21.

Possible explanations for the higher ileal EAA flows determined on d 7 and reduced flows with advancing age are intricate. A proportion of secreted endogenous proteins is digested, along with ingested dietary proteins, in the GIT and absorbed. The EAA values determined at the ileum therefore relate to the algebraic differences between that secreted and absorbed (Moughan, 2003; Ravindran *et al.*, 2004). The source of endogenous proteins and the entry point into the GIT further complicates this dynamic (Ravindran, 2021). When digestive enzymes dominate the endogenous flow, the proteins pass through the duodenum and jejunum where there is greater opportunity for digestion and absorption. In contrast, if mucus secretion or desquamation is significant, particularly if they occur distal to the duodenum, then the opportunity for digestion is lower and there will be relatively higher endogenous losses at the ileal level. Owing to this complicated dynamic, it is difficult to differentiate the true contribution of each endogenous source.

Nevertheless, the ramification is that the higher EAA losses on d 7 may reflect increased secretion, reduced absorption, or both. The first week after hatch is the most critical period in the life of a broiler chicken. At hatching, the digestive system of the chick is still immature and there is the transition from yolk to oral nutrition. Substantial physical and functional development of the GIT and digestive organs take place during the first week. It is therefore clear that the capacity to digest the

feed and, absorb and transport nutrients is limited during this period. Thus, reduced absorption, rather than increased secretion, is the likely reason for the greater ileal EAA losses determined at d 7. Against this background, several possibilities may be considered as discussed below.

First, the GIT of the newly hatched chick is immature and the bird places high priority on intestinal growth to ensure the development of nutrient supply functions, as evidenced by dramatic growth during the first week (Sklan, 2001). In the days following hatching, weights of proventriculus, gizzard, and small intestine increase more rapidly in relation to BW than other organs and tissues (Katanbaf *et al.*, 1988; Sell *et al.*, 1991). This enhanced growth is maximal in chicks between 4 and 8 d of age and thereafter there is a relative decline. The mass of the small intestine increases almost 600% within the first 7 d (Noy *et al.*, 2001). The length of the small intestine and its individual component regions also increase with age. According to Iji *et al.* (2001a), the relative weight of small intestine peaked between d 7 and 14. The rapid growth in intestinal size during the first week may lead to mechanical inefficiency in the passage and mixing of digesta, reducing nutrient digestion, and increasing the EAA flow.

Second, the function of the GIT is strictly related to its microscopic structure. The architecture of GIT is not well developed during the first week of life but rapidly develops with age. The dramatic post-hatch increases observed in the weight and length of small intestine is reported to be minor relative to the growth of gut mucosa (Dibner *et*

al., 1996). Uni *et al.* (1995) found that the villus height and area in the chick increase rapidly at different rates in different intestinal segments, particularly in the jejunum and ileum from 4 to 10 d post-hatch. Crypt depth, which reflects enterocyte maturation rate, increased linearly in both the duodenum and jejunum until 10 d. A study of morphological development of GIT in broiler chicks by Uni *et al.* (1996) showed that the height and perimeter of villi increased by 34 to 100% in all small intestinal segments between 4 and 10 d after hatching. In addition, the crypt depth, the enterocytes number per longitudinal section of villi increased with increasing age. The poorly developed intestinal architecture will also lead to lower digestion during week 1.

Third, the secretion of digestive enzymes by pancreas and the brush border of the small intestine is low at the time of hatch (Sell, 1996), but increase after hatch although the rate of increase was different for different enzymes (Tarvid, 1995; Noy and Sklan, 1997). The relative activity of aminopeptidases in all intestinal segments reduces after hatching, reaches a low point on d 10 and increase on d 15 (Tarvid, 1992). The greatest activity of dipeptidase was found at hatching and decrease within 7 days by 25% (Tarvid, 1990). The specific activity of pancreatic trypsin is lower during the first 3 to 6 d post-hatch, increases afterwards by up to 20% on d 14 (Nitsan *et al.*, 1991). Both the relative trypsin and chymotrypsin activities (unit per kg BW) in pancreas increases with age, reaching a maximum level on d 11. The activity of trypsin in small intestine contents increases 10-fold from hatching to d 14. In case of chymotrypsin, it increases 3-fold to a maximum at 20 d

of age, suggesting that lower protease activities during the first week after hatch could limit the AA digestibility. However, after week 1, the trypsin activity increases up to 21 d, with no noticeable changes afterward (Nitsan *et al.*, 1991).

In summary, during week 1, the GIT is in a maturation phase in terms of growth, morphology and, secreted amounts and activities of proteolytic enzymes. Taken together, these imply a compromised protein digestion increasing the endogenous AA recovery at the ileal level.

Some other relevant factors potentially contributing to the higher EAA flow at d 7 may be worth considering. Cell proliferation and cell turnover are greater during week 1 (Iji *et al.*, 2001a) and desquamated cells may therefore be important contributors to the EAA flow at d 7. There is some evidence that, when an NFD is fed, the source of EAA is largely mucoproteins (Adedokun *et al.*, 2007b). The intestinal mucous layer is a protective barrier against harmful intraluminal components and microflora (Gork *et al.*, 1999). Mucin plays a significant role in filtering nutrients in GIT and thus affecting the absorption of nutrients (Smirnov *et al.*, 2006). Mucin glycoproteins are rich in Thr, Ser, Pro, Glu, and Asp (Souffrant, 1991; Lien *et al.*, 1997). Adedokun *et al.* (2007c) reported higher Thr and Ser contents in the ileal digesta of 5-d-old broiler chickens compared to d 15 and 21 following feeding an NFD, reflecting a higher concentration of intestinal mucin at early ages. Similarly, in the present study, higher mucin production in the newly hatched chick was manifested by the

higher endogenous flows of Thr (102%), Asp (106%), Glu (123%), Pro (103%), and Ser (103%) on d 7 than the average of other ages (d 14-42). Intestinal cells and mucin are poorly digested, and this may explain the higher EAA recovery at d 7. Lower relative mucin secretion and, increased endogenous protein digestion and absorption with age (Nasset, 1972; Ravindran and Bryden, 1999) may account for the lower endogenous N and AA secretion in older birds.

Food transit time through the digestive tract is an important factor influencing the nutrient digestion by determining the available time for contact among nutrients, digestive enzymes, absorptive surfaces, and microbiome (Clemens *et al.*, 1975; Mateos *et al.*, 1982; Vergara *et al.*, 1989). Longer digesta retention increases the absorption of nutrients by enhancing contact time with absorptive cells (Washburn, 1991). Several studies have recorded slower intestinal passage rate in adult birds (Hurwitz and Bar, 1966; Sklan *et al.*, 1975; Noy and Sklan, 1995). Decreased passage rate with age allows the digesta to be exposed to the digestive and absorptive processes for a longer time that may improve nutrient digestibility, including AA, in older birds.

Since all N in the ileal digesta in birds fed the NFD diet come only from endogenous proteins, one may argue that the AA concentration in the ileal digesta, expressed as g/kg and not corrected for DMI, may give some representation of the dynamic in EAA flow with age. When DMI was excluded, the concentration of almost all AA in the ileal digesta decreased with advancing broiler age. Since the AA originated from endogenous sources, this observation may suggest increased re-

absorption of endogenous protein with age. In present study, FI increased gradually from 11.4 g/bird on d 7 to 79.1 g/bird on d 42, which might account, to some extent, for the observed differences in basal EAA flows, expressed as g/kg DMI, among broiler ages. It must be noted, however, the primary aim of measuring EAA losses is to standardise the apparent digestibility values and this correction will not be possible when EAA values are not linked to the DMI.

The concentrations of EAA in the ileal digesta are within the range reported in the literature (Ravindran, 2021). The higher proportions of Glu, Asp, Thr, and Ser determined in the endogenous protein were as expected. Asp, Leu, Ser, annd Glu are the major AA in the ileal endogenous secretions in chickens (Bielorai and Iosif, 1987; Siriwan *et al.*, 1994; Adedokun *et al.*, 2007c). Ravindran *et al.* (2004) reported that the major AA in endogenous protein in the ileal digesta of 35-d-old chickens fed an NFD were Thr, Asp, and Glu. Tarvener *et al.* (1981) speculated that the high proportions of Glu, Asp, Thr, Ser, and Leu in the endogenous protein may be due to their slower rate of re-absorption compared with other AA. The lower concentrations of Met and His can be attributed to the fact that these AA are absorbed in greatest proportions compared to others in the GIT (Webb, 1990).

It is generally assumed that the AA profile of endogenous protein is somewhat constant, although it is known that individual sources of endogenous protein have different AA composition (Ravindran, 2021). The current findings suggest that the composition of the AA flow was altered by broiler age, ostensibly reflecting changes in the contribution

of endogenous protein components with age. The concentration of Cys, Gly, and Trp was linearly increased with age. Cysteine is found in relatively higher concentrations in the mucin (Lien *et al.*, 1997). The Gly was previously assumed to be originating from biliary secretions (Ravindran and Bryden, 1999; Ravindran and Hendriks, 2004; Adedokun *et al.*, 2011), but current evidence indicates that the bile acid conjugation in poultry is exclusively with taurine and not with Gly as in pigs (Ravindran, 2021). The inconsistent effects of age on Ile, Leu, Met, Val, and Asp are difficult to explain and, as mentioned earlier, underline the complexity of identifying the exact contribution of different endogenous components.

To explore the possibility of developing a consensus basal EAA value for the standardisation of ileal AA digestibility, data generated at Massey University during the past 7 years on ileal EAA flows (g/kg DMI) in broilers fed NFD were compiled and the results are summarised in Table 5.6 (See Appendix A for detailed data). The range of basal endogenous flow varied from 0.74 to 1.97 g/kg of DMI for N and 3.83 to 8.66 g/kg of DMI for total AA and the coefficient of variations were 25.8 and 22.1%, respectively. Since the NFD methodology and standard assay procedures (housing, laboratory analysis etc.) were employed in all assays, the substantial variability observed was surprising. Number of factors may have contributed to this variability. Broilers of 21 to 37 d old were used in these assays and, based on present findings, age can be one contributing factor. Other contributing factors may include differences in BW, health status, DMI, mucin secretion (Lemme *et al.*,

2004; Adedokun *et al.*, 2007b) and efficacy of endogenous protein utilisation. It is noteworthy, however, that the average EAA flows over the years were remarkably close to the EAA values determined for birds of 21 to 35 d old in the current study.

Table 5.6. Basal endogenous amino acid flow values (g/kg dry matter intake) in broilers following the feeding of NFD (from Massey University studies, 2014-2020)

Parameter	Mean ± SD	CV (%)	Range
N	1.36±0.351	25.8	0.74-1.97
Indispensable amino acids			
Arg	0.32±0.095	29.5	0.19-0.49
His	0.13±0.038	28.9	0.07-0.20
Ile	0.29±0.073	25.1	0.16-0.42
Leu	0.46±0.122	26.4	0.25-0.69
Lys	0.29±0.097	33.1	0.17-0.52
Met	0.11±0.032	29.3	0.05-0.16
Phe	0.28±0.095	33.9	0.15-0.49
Thr	0.52±0.099	19.1	0.37-0.77
Trp	0.11±0.038	34.7	0.06-0.16
Val	0.40±0.090	22.7	0.24-0.55
Dispensable amino acids			
Ala	0.33±0.078	23.9	0.20-0.48
Asp	0.62±0.157	25.3	0.38-0.96
Cys[1]	0.20±0.051	24.8	0.15-0.34
Glu	0.79±0.199	25.3	0.43-1.11
Gly[1]	0.39±0.103	26.4	0.23-0.50
Pro	0.45±0.176	38.9	0.28-1.07
Ser	0.46±0.094	20.2	0.34-0.63
Tyr	0.25±0.056	22.6	0.15-0.34
TAA	6.23±1.379	22.1	3.83-8.66

[1]Semi-indispensable amino acids for poultry.

CV = Coefficient of variation; N = nitrogen; SD = Standard deviation; TAA = Total endogenous flow of all amino acids.

No. of observation, 18; Age range: 21-37 d.

5.6. Conclusions

The current findings, for the first time, provides information on the basal endogenous AA flows in broilers from hatching to 42 d of age. The flow was higher on d 7, reduced on d 14 and remained constant until d 35. A further reduction was noticed on d 42. The higher EAA flow at d 7 is ostensibly reflective of the immature digestive system and

the resultant low digestive capacity in the newly hatched broiler chick. Factors like longer digesta retention, improved AA digestibility and, relatively lower production and increased utilisation of mucin may account for the lower basal EAA flows in older birds. Therefore, the use of a single EAA value for birds of all ages will underestimate the standardised ileal AA digestibility in early life and overestimate the digestibility in older birds. Application of age-specific values for EAA flow may benefit accurate standardisation of AA digestibility and improve the precision of feed formulations.

CHAPTER 6 An investigation into the influence of age on the standardised amino acid digestibility of wheat and sorghum in broilers[1]

6.1. Abstract

Standardised ileal digestibility coefficients (SIDC) of nitrogen (N) and amino acids (AA) in wheat and sorghum at six different ages (d 7, 14, 21, 28, 35, and 42) of broilers were determined. Two assay diets were formulated to contain 938 g/kg of each grain as the sole source of AA in the diet. Titanium dioxide (5 g/kg) was added as an indigestible marker. Each assay diet was fed to six replicate cages housing 14 (d 7), 12 (d 14), 10 (d 21), 8 (d 28), 8 (d 35), and 6 (d 42) birds per cage for 4 d prior to ileal digesta collection. The apparent ileal digestibility coefficients (AIDC) were calculated and standardised by using the age-appropriate basal endogenous AA losses determined in a previous study (Chapter 5). In the case of wheat, AIDC of N and all AA increased (linear or quadratic, $P < 0.05$ to 0.001) with advancing age. No age effect was noticed on the SIDC of N, average of indispensable (IAA) and dispensable AA (DAA), though the average of total AA (TAA) tended (linear, $P = 0.09$) to increase as the birds grew older. In sorghum, the AIDC of N, average of IAA and DAA were unaffected ($P > 0.05$) by age. The SIDC of N, average SIDC of IAA, DAA and TAA were higher at d 7, reduced at d 14 and then plateaued. Among the IAA, the SIDC of Arg, His, Ile, Leu, Lys, Thr, Val, and the SIDC of all

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individual DAA (except Cys) decreased with age (linear or quadratic, $P < 0.05$ to 0.001) with higher values at d 7. The higher SIDC values determined at d 7 were due to higher EAA losses during week 1. The results showed that broiler age influences AA digestibility and this may need be considered in practical feed formulations. The age effect is variable depending on the grain type and specific AA.

6.2. Introduction

Historically, amino acid (AA) digestibility in poultry has been measured at the excreta level (Fonolla *et al.*, 1981; ten Doeschate *et al.*, 1993; Angkanaporn *et al.*, 1997) but this approach suffers from several limitations, including the modifying effects of hindgut microbiome, contamination with urine AA and contribution of microbial proteins to excreta AA (Ravindran and Bryden, 1999). These confounding issues are avoided when the digestibility is measured at the terminal ileal level. The ileal AA digestibility is expressed as either apparent or standardised. The calculation of standardised ileal digestibility (SID) involves the correction of apparent ileal digestibility (AID) values for basal endogenous AA (EAA) losses (Moughan *et al.*, 1992; Lemme *et al.*, 2004). The SID of AA is more additive than AID in feed formulations (Cowieson *et al.*, 2019; An *et al.*, 2020) and increasingly being used by the poultry industry.

The first week after hatch is the most challenging period in broilers because of the immaturity of the digestive tract and, higher protein

demand for organ and muscle development. The intestinal development in the neonatal chick is dynamic. During the first few weeks of life, significant morphological and developmental changes occur. The secretion and activity of different proteases (chymotrypsin, trypsin), intestinal peptidases and dipeptidases are low in the hatchling (Jin *et al.*, 1998). Despite possible age effects on nutrient utilisation (Zelenka, 1968; Murakami *et al.*, 1992; Tarvid, 1995; Batal and Parsons, 2002), there are only sporadic data on the influence of age on the AID or SID of AA of ingredients in broilers (Fonolla *et al.*, 1981; Wallis and Balnave, 1984; Huang *et al.*, 2005; Adedokun *et al.*, 2007a, 2008; Szczurek *et al.*, 2020) and the results are contradictory. Some studies have recorded improved protein or AA digestibility with advancing age (Wallis and Balnave, 1984; Huang *et al.*, 2005), while others have reported reduced AA digestibility (Fonolla *et al.*, 1981; Zuprizal *et al.*, 1992).

Grains, because of their high inclusion levels in broiler feed formulations, contribute up to 40% of the dietary protein supply. Wheat and sorghum are two common grains used in broiler diets in New Zealand, Australia, Canada, and parts of Europe. The digestibility of nutrients in wheat and sorghum depends, *inter alia*, on the antinutritive factors present in these grains. Wheat contains high levels of soluble non-starch polysaccharides (NSP) that are not hydrolysed in the intestinal tract of poultry (Choct *et al.*, 1999; Bach Knudsen, 2014). The water-soluble fractions of NSP are viscous and have a negative impact on the digestion and absorption of nutrients (Choct and

Annison, 1992a). Sorghum, a non-viscous grain, contains several antinutritive factors like kafirin, phenolic compounds and phytate (Selle *et al.*, 2018). The phytate content of sorghum is greater than other grains (Selle *et al.*, 2003). Kafirin, a dominant protein fraction in sorghum endosperm, is present as discrete protein bodies in association with starch granules, may reduce the nutrient utilisation because of starch-protein interactions (Rooney and Pflugfelder, 1986). The effects of antinutrients present in wheat and sorghum may vary with the age of birds and potentially influence AA digestion. Furthermore, the increased feed consumption and the resultant ingestion of greater amounts of antinutrients with advancing age can lower AA digestion (Szczurek *et al.*, 2020).

Almost all published estimates of SID AA for feed ingredients have been derived using older broilers (22 to 35 d) and used to formulate diets for different growth stages of broiler chickens. To the author's knowledge, only scattered data are available on the age effect on AA digestibility of grains, with no studies investigating the SID AA from hatch to the end of broiler growth cycle. The present study was, therefore, aimed to compare the standardised ileal digestibility coefficients (SIDC) of AA in wheat and sorghum at six different ages (d 7, 14, 21, 28, 35 and, 42) of broilers.

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. Diets and experimental design

Two experimental diets with similar inclusions (938 g/kg) of either wheat or sorghum, as the sole source of AA in the diet, were developed (Table 6.1). The diets contained titanium dioxide (5 g/kg; Merck KGaA, Darmstadt, Germany) as an indigestible marker. Wheat and sorghum were of Australian origin, obtained from a commercial supplier and ground in a hammer mill to pass through a screen size of 3.0 mm. The diets were steam-conditioned at 70 ºC for 30 s and pelleted using a pellet mill (Model Orbit 15; Richard Size Limited Engineers, Kingston-upon-Hull, UK) capable of manufacturing 180 kg of feed/h and equipped with a die ring (3 mm apertures and 35 mm thickness). The pelleted diets were crumbled for feeding during the first two weeks of the study.

Table 6.1. Composition of the basal diets (g/kg, as fed basis) used in the ileal amino acid digestibility assay at different ages.

Ingredients	Wheat	Sorghum
Wheat	938	-
Sorghum	-	938
Soybean oil	20	20
Dicalcium phosphate	18	18
Limestone	13	13
Titanium dioxide[1]	5.0	5.0
Sodium chloride	2.0	2.0
Sodium bicarbonate	2.0	2.0
Trace mineral premix[2]	1.0	1.0
Vitamin premix[2]	1.0	1.0

[1] Merck KGaA, Darmstadt, Germany.

[2]Supplied per kilogram of diet: antioxidant (ethoxyquin), 100 mg; biotin, 0.2 mg; calcium pantothenate, 12.8 mg; cholecalciferol, 0.06 mg; 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, 0.2 mg; Zn, 60 mg.

Representative samples of each grain were analysed, in duplicate, for dry matter (DM), N, titanium (Ti), crude fat, crude fibre, neutral detergent fibre (NDF), gross energy (GE), AA, calcium (Ca), phosphorus and ash. The apparent ileal digestibility coefficients (AIDC) of N and AA in the grains were determined using the direct method.

6.3.2. Birds and housing

Day-old male Ross 308 broilers were obtained from a commercial hatchery, raised in floor pens and fed a commercial crumbled broiler starter diet (12.14 MJ/kg apparent metabolisable energy; 225 g/kg crude protein; CP) from d 1 to 21 and a broiler finisher diet (12.69 MJ/kg apparent metabolisable energy; 190 g/kg CP) from d 22 until d 38 in pelleted forms (Table 6.2).

A total of 696 birds were used in this experiment. On d 1, 168 chicks were individually weighed and allocated to 12 battery brooders (n = 14 chicks per replicate) so that the average body weight (BW) per replicate was similar. The remaining chicks were raised in floor pens and fed the standard diet (Table 6.2) until they were individually weighed and allocated to 12 replicate cages per age group on d 7 (n = 12 birds per replicate cage), d 14 (n = 10 birds per cage), d 21 (n = 8 birds per cage), d 28 (n = 8 birds per cage), and d 35 (n = 6 birds per cage). Once assigned to cages, the birds were fed broiler starter or finisher diets and the first 3 d were considered as the adaptation period. In each age group, the test diets were then fed for 4 d (d 3-7 and 10-14 [crumbled]; d 17-

21, 24-28, 31-35, 38-42 [pelleted]) prior to ileal digesta collection on d 7, 14, 21, 28, 35, and 42 post-hatch.

Table 6.2. Composition and calculated analysis (g/kg, as fed basis) of broiler starter and finisher diets

Ingredients	Starter diet (0-21 d)	Finisher diet (22-38 d)
Maize	574.2	660
Soybean meal, 460 g/kg	381.4	295.6
Soybean oil	8.8	13.6
Limestone	11.3	9.9
Dicalcium phosphate	10.7	8.2
DL-methionine	3.3	3.0
L-lysine HCl	2.0	1.9
L-threonine	1.0	0.7
Sodium bicarbonate	2.7	2.5
Sodium chloride	2.5	2.5
Trace mineral premix[1]	1.0	1.0
Vitamin premix[1]	1.0	1.0
Phytase	0.1	0.1
Calculated analysis		
Apparent metabolisable energy (MJ/kg)	12.14	12.69
Crude protein	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]Supplied per kilogram of diet: antioxidant (ethoxyquin), 100 mg; biotin, 0.2 mg; calcium pantothenate, 12.8 mg; cholecalciferol, 0.06 mg; 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, 0.2 mg; Zn, 60 mg.

The birds had *ad libitum* access to feed and water at all times. During the first week, the average room temperature was 32 ± 1 ºC and gradually reduced to 23 ºC by the end of the third week. The floor pens, battery brooders and grower cages were housed in an environmentally controlled room with 20 h of fluorescent illumination per day.

6.3.3. Growth performance

Feed intake (FI) and BW were recorded on a cage basis during the 4-d experimental period in each week. Mortality was recorded daily.

6.3.4. Determination of the coefficient of apparent ileal digestibility

The birds were euthanised by intravenous injection (1 mL per 2 kg BW) of sodium pentobarbitone solution (Provet NZ Pty. Ltd., Auckland, New Zealand) at the end of respective experimental periods (d 7, 14, 21, 28, 35, and 42). Digesta were collected from the lower half of the ileum and processed as described by Ravindran *et al.* (2005). The ileum was defined as that portion of the small intestine extending from the Meckel's diverticulum to a point ~40 mm proximal to the ileocaecal junction. Briefly, the ileum was excised and divided into halves (proximal and distal ileum) and the digesta samples were collected from the lower half towards the ileocaecal junction after gently flushing with distilled water into plastic containers. After the collection, the digesta from birds were pooled within a cage, frozen immediately and subsequently lyophilised (Model 0610, Cuddon Engineering, Blenheim, New Zealand). Diets and lyophilised samples were ground to pass through a 0.5-mm sieve and stored in airtight plastic containers at 4 ºC until laboratory analysis.

6.3.5. Gizzard pH and jejunal digesta viscosity

Two birds from each replicate cage, euthanised for ileal digesta collection, were used for the measurement of gizzard pH by a digital pH meter (pH spear, Oakton Instruments, Vernon Hill, IL). Briefly, the

glass probe was inserted through an opening made in the gizzard, directly placed in the digesta and three values were taken from the proximal, middle, and distal regions. The average value was considered as the final pH value. The jejunal digesta viscosity was also measured in the same birds. Digesta was collected from the distal jejunum and centrifuged at 3000 × g at 20 ºC for 15 min. A 0.5 mL aliquot of the supernatant was used in a viscometer (Brookfield digital viscometer, Model DV2TLV; Brookfield Engineering Laboratories Inc., Stoughton, MA) fitted with CP-40 cone spindle with shear rates of 5-500/s to determine the viscosity.

6.3.6. Chemical analysis

Dry matter was determined using the standard procedure (Method 930.15; AOAC International, 2016). Titanium was determined on a UV spectrophotometer (Berthold Technologies GmbH and Co. KG, Bad Wildbad, Germany) following the method described by Short *et al.* (1996). Gross energy was measured by adiabatic bomb calorimeter (Gallenkamp Autobomb, Weiss Gallenkamp Ltd., Loughborough, UK) standardised with benzoic acid. Starch was measured using the Megazyme Total Starch Assay kit (Megazyme International Ireland Ltd., Wicklow, Ireland) based on thermostable α-amylase and amyloglucosidase (McCleary *et al.*, 1997; AOAC International, 2016). Nitrogen was determined by combustion (Method 968.06; AOAC International, 2016) using a carbon nanosphere-200 carbon, N and sulphur auto analyser (LECO Corporation, St. Joseph, MI). The CP content was calculated as N × 6.25. Crude fat was measured using the

Soxhlet extraction procedure (Method 2003.06; AOAC International, 2016). Neutral detergent fibre was measured (Method 2002.04; AOAC International, 2016) using Tecator Fibretec™ (FOSS Analytical AB, Höganäs, Sweden). Ash was determined by ashing in a muffle furnace at 550 ºC for 16 h (Method 942.05; AOAC International, 2016). Calcium and phosphorus concentrations were measured by Inductively Coupled Plasma-Optical Emission Spectroscopy (ICP-OES) using a Thermo Jarrell Ash IRIS instrument (Thermo Jarrell Ash Corporation, Franklin, MA).

Amino acids were analysed following standard procedures (Method 994.12; AOAC International, 2011). The samples were hydrolysed with 6 N hydrochloric acid (HCl) containing phenol for 24 h at 110 ± 2 ºC in glass tubes in an oven. The AA were determined using AA analyser (ion exchange) with ninhydrin post column derivatisation. The chromatograms were integrated using a dedicated software (Agilent Open Lab software, Waldbronn, Baden-Württemberg, Germany) with AA simultaneously detected at 570 and 440 nm. For the sulphur containing AA, Cys was analysed as cysteic acid and Met as methionine sulphone by oxidation with performic acid for 16 h at 0 ºC and neutralisation with hydrobromic acid prior to hydrolysis.

For Trp analysis, the samples were saponified under alkaline conditions with barium hydroxide solution in the absence of air at 110 ºC for 20 h in an autoclave. Following hydrolysis, α-methyl Trp as internal standard, was added to the mixture. After adjusting the hydrolysate to pH 3.0 and diluting with 30% methanol, Trp and the internal standard

were separated by reverse phase chromatography (RP-18) on a HPLC column (CORTECS C18 Column; 2.7 µm, Waters Corporation, Dublin, Ireland). Detection was selectively done by means of a fluorescence detector to prevent interference by other AA and constituents.

6.3.7. Calculations

All data were expressed on a DM basis. The AIDC of AA was calculated from the dietary ratio of AA to Ti relative to the corresponding ratio in the ileal digesta using the following formula.

$$\text{AIDC of AA} = [(AA / Ti)_d - (AA / Ti)_i]/(AA / Ti)_d$$

Where, $(AA / Ti)_d$ = ratio of AA to Ti in the diet, and $(AA / Ti)_i$ = ratio of AA to Ti in the ileal digesta.

Apparent digestibility data for N and AA were then converted to SIDC, using the age-appropriate basal endogenous N and AA estimates (grams of AA flow per kilogram of DM intake [DMI]) determined at different ages (d 7, 14, 21, 28, 35 and 42) in a previous study (Chapter 5).

$$\text{SIDC} = \text{AIDC} + [\text{Basal EAA (g/kg DMI)}/\text{Ing. AA (g/kg DM)}]$$

Where, SIDC = standardised ileal digestibility coefficient of the AA,

AIDC = apparent ileal digestibility coefficient of the AA,

Basal EAA = basal endogenous AA loss, and

Ing. AA = concentration of the AA in the ingredient.

6.3.8. Data Analysis

Cage was considered as the experimental unit. For each grain, data were analysed by the General Linear Model procedure of SAS (version 9.4; 2015; SAS Institute, Cary, NC.). Orthogonal polynomial contrasts were performed to determine the linear and quadratic effects of broiler age. Statistical significance was declared at $P < 0.05$. The relationship between AA digestibility and other parameters were analysed by Pearson correlation.

6.4. Results

6.4.1. Proximate and nutrient composition

The proximate and nutrient composition of the grains are presented in Table 6.3.

The CP content in wheat and sorghum were 94.3 g/kg and 106 g/kg, respectively. The starch content in sorghum (606 g/kg) was higher than that in wheat (579 g/kg) whereas wheat had higher NDF content (83.8 g/kg) than sorghum (62.2 g/kg). Among the indispensable AA (IAA), the contents of Leu, Arg, Val and Ile were the highest in both wheat and sorghum. Lower IAA contents were recorded for Trp, followed by Met, in both grains. In the case of dispensable AA (DAA), higher contents were recorded for Glu, followed by Pro and Asp in both grains. The total IAA and total DAA contents in wheat were 29.2 and 53.7 g/kg, respectively. In sorghum, the total IAA and DAA contents were 39.1 and 60 g/kg, respectively. The total AA (TAA) content was lower in wheat (82.8 g/kg) than sorghum (98.7 g/kg).

Table 6.3. Proximate, carbohydrate and amino acid composition of wheat and sorghum grains (g/kg, as-received basis)

	Wheat	Sorghum
Dry matter	899	881
Crude protein (N×6.25)	94.3	106
Starch	579	606
Fat	24.5	32.6
Neutral detergent fibre	83.8	62.2
Gross energy (MJ/kg)	16.1	16.7
Ash	12.2	15.5
Calcium	0.22	0.10
Phosphorus	2.12	2.89
Indispensable amino acids (IAA)		
Arg	4.68	4.20
His	2.17	2.42
Ile	3.22	4.14
Leu	6.31	14.4
Lys	2.85	2.38
Met	1.68	1.70
Thr	2.89	3.39
Trp	1.25	1.26
Val	4.12	5.25
Total IAA	29.2	39.1
Dispensable amino acids (DAA)		
Ala	3.54	9.93
Asp	4.99	7.19
Cys[1]	2.13	1.92
Glu	25.8	22.6
Gly[1]	4.11	3.32
Pro	8.79	9.81
Ser	4.32	4.84
Total DAA	53.7	60.0
Total AA[2]	82.8	98.7

[1]Semi-indispensable amino acids for poultry. [2]Total AA = IAA + DAA.

6.4.2. Growth performance, gizzard pH and jejunal digesta viscosity

Table 6.4 presents weekly data on the performance, gizzard pH and jejunal digesta viscosity of bird fed wheat- or sorghum-based experimental diets.

Mortality was negligible during the experiment. Only two out of 696 birds died, and the deaths were not related to any specific treatment.

Bird age had a significant (P < 0.001) effect on performance parameters. The daily feed intake (DFI) and daily weight gain (DWG) increased quadratically (P < 0.001) with advancing age in both wheat- and sorghum-based diets.

Gizzard pH increased linearly (P < 0.001) in wheat as the birds grew older. A quadratic (P < 0.001) effect was observed on the jejunal digesta viscosity with advancing age. The viscosity increased from d 7 to 35 and then decreased.

In the case of sorghum, both the gizzard pH and the jejunal digesta viscosity was affected quadratically (P < 0.001) by bird age. The gizzard pH decreased from d 7 to d 21 and then increased. The viscosity increased until d 21, plateaued to d 28 and then decreased.

Table 6.4. Daily feed intake (DFI; g/bird/d), daily weight gain (DWG; g/bird/d), gizzard pH and viscosity (cP) in jejunal digesta of broilers fed wheat- and sorghum-based diets at different ages[1]

	Age (d)							Orthogonal polynomials contrasts	
Parameter	7	14	21	28	35	42	Pooled SEM	Linear	Quadratic
					Wheat				
DFI[2]	14.2	49.4	88.8	127	160	178	3.4	0.001	0.001
DWG[2]	13.6	32.2	41.7	58.1	62.6	63.5	1.88	0.001	0.001
Gizzard pH[3]	2.68	2.39	3.54	3.20	3.40	3.61	0.105	0.001	0.174
Viscosity[3]	3.31	5.60	6.38	8.29	8.01	7.77	0.470	0.001	0.001
					Sorghum				
DFI[2]	12.0	36.5	72.5	111	138	142	2.51	0.001	0.001
DWG[2]	9.41	23.0	37.6	47.0	54.1	56.4	1.35	0.001	0.001
Gizzard pH[3]	2.56	2.01	2.14	2.80	3.54	3.66	0.121	0.001	0.001
Viscosity[3]	1.86	2.32	3.68	3.64	2.87	2.55	0.142	0.001	0.001

[1]Each value represents the mean of six replicates (14, 12 and 10 birds per replicate for 7, 14 and 21-d old birds, respectively; eight birds per replicate for 28 and 35-d old birds; and six birds per replicate for 42-d old birds).
[2]Measured during 4-d feeding of experimental diets.
[3]Calculated as the mean of six replicates (two birds per replicate).

6.4.3. Ileal digestibility coefficients of N and AA in wheat

The influence of broiler age on AIDC, SIDC and SID content of N and AA in wheat is summarised in Tables 6.5, 6.6 and 6.7, respectively.

Age quadratically influenced the AIDC of N ($P < 0.001$) and average digestibility of IAA, DAA and TAA of wheat ($P < 0.01$; Table 6.5). The average AIDC of IAA, DAA and TAA increased from d 7 to 14, then plateaued up to d 21, and then increased to d 42. The AIDC of Arg, Glu and Gly increased linearly ($P < 0.001$) with age, while the increases in those of other AA were quadratic ($P < 0.05$ to < 0.001).

No age effect was observed on SIDC of N and, average digestibility of IAA and DAA in wheat ($P > 0.05$; Table 6.6). The broiler age, however, tended ($P = 0.092$) to linearly influence the average of TAA. Among IAA, the SIDC of Met (quadratic; $P < 0.05$) was higher on d 7, increased from d 14 to 21, and then plateaued. The SIDC of Trp increased linearly ($P < 0.001$) with advancing age. Among the DAA, a linear effect of age was observed for SIDC of Asp ($P < 0.001$), Cys ($P < 0.001$) and Glu ($P < 0.05$).

Table 6.5. Apparent ileal digestibility coefficients[1] of nitrogen (N) and amino acids of wheat at different ages of broilers[1]

	Age (d)							Orthogonal polynomial contrasts	
Parameter	7	14	21	28	35	42	Pooled SEM	Linear	Quadratic
N	0.737	0.793	0.825	0.841	0.840	0.856	0.0088	0.001	0.001
Indispensable amino acids									
Arg	0.823	0.844	0.854	0.864	0.867	0.875	0.0090	0.001	0.275
His	0.784	0.831	0.838	0.852	0.858	0.872	0.0085	0.001	0.033
Ile	0.755	0.809	0.817	0.844	0.846	0.865	0.0105	0.001	0.045
Leu	0.796	0.842	0.851	0.876	0.876	0.892	0.0088	0.001	0.032
Lys	0.638	0.730	0.751	0.784	0.771	0.808	0.0186	0.001	0.022
Met	0.745	0.803	0.855	0.875	0.870	0.886	0.0103	0.001	0.001
Thr	0.577	0.664	0.690	0.699	0.711	0.739	0.0159	0.001	0.021
Trp	0.705	0.777	0.812	0.825	0.831	0.848	0.0127	0.001	0.002
Val	0.723	0.782	0.800	0.818	0.823	0.840	0.0107	0.001	0.012
IAA	0.727	0.787	0.808	0.826	0.828	0.847	0.0108	0.001	0.008
Dispensable amino acids									
Ala	0.694	0.765	0.773	0.784	0.779	0.801	0.0131	0.001	0.017
Asp	0.561	0.649	0.727	0.752	0.753	0.775	0.0162	0.001	0.001
Cys[2]	0.697	0.765	0.809	0.823	0.828	0.839	0.0086	0.001	0.001
Glu	0.922	0.942	0.935	0.943	0.941	0.948	0.0039	0.001	0.232
Gly[2]	0.725	0.766	0.781	0.799	0.802	0.819	0.0104	0.001	0.090
Pro	0.879	0.902	0.908	0.917	0.918	0.922	0.0043	0.001	0.007
Ser	0.728	0.789	0.809	0.820	0.826	0.845	0.0102	0.001	0.007
DAA	0.744	0.797	0.820	0.834	0.835	0.849	0.0092	0.001	0.002
TAA	0.734	0.791	0.813	0.829	0.831	0.848	0.0101	0.001	0.005

[1]Each value represents the mean of six replicates (14, 12 and 10 birds per replicate for 7, 14 and 21-d old birds, respectively; eight birds per replicate for 28 and 35-d old birds; and six birds per replicate for 42-d old birds).

[2]Semi-indispensable amino acids for poultry.

DAA = Average digestibility of dispensable amino acids; IAA = Average digestibility of indispensable amino acids; TAA = Average digestibility of all amino acids.

Table 6.6. Standardised ileal digestibility coefficients[1] of nitrogen (N) and amino acids of wheat at different ages of broilers[2]

	Age (d)							Orthogonal polynomial contrasts	
Parameter	7	14	21	28	35	42	Pooled SEM	Linear	Quadratic
N	0.950	0.904	0.931	0.949	0.947	0.933	0.0088	0.393	0.586
Indispensable amino acids									
Arg	0.953	0.909	0.914	0.933	0.928	0.914	0.0089	0.197	0.162
His	0.904	0.896	0.899	0.919	0.919	0.913	0.0085	0.079	0.975
Ile	0.929	0.899	0.907	0.939	0.929	0.921	0.0105	0.333	0.718
Leu	0.934	0.910	0.920	0.953	0.944	0.937	0.0088	0.051	0.938
Lys	0.839	0.824	0.840	0.884	0.859	0.863	0.0186	0.097	0.705
Met	0.886	0.868	0.921	0.949	0.933	0.923	0.0103	0.001	0.017
Thr	0.965	0.891	0.911	0.905	0.931	0.900	0.0139	0.083	0.066
Trp	0.852	0.859	0.898	0.915	0.918	0.911	0.0127	0.001	0.070
Val	0.899	0.876	0.894	0.917	0.914	0.905	0.0108	0.075	0.903
IAA	0.910	0.881	0.900	0.924	0.919	0.909	0.0108	0.141	0.987
Dispensable amino acids									
Ala	0.881	0.857	0.865	0.887	0.873	0.863	0.0131	0.862	0.922
Asp	0.813	0.779	0.855	0.885	0.882	0.864	0.0162	0.001	0.119
Cys[3]	0.896	0.879	0.917	0.928	0.939	0.926	0.0085	0.001	0.282
Glu	0.981	0.969	0.963	0.974	0.969	0.967	0.0039	0.047	0.144
Gly[3]	0.894	0.853	0.868	0.892	0.889	0.881	0.0104	0.448	0.334
Pro	0.972	0.949	0.957	0.966	0.966	0.957	0.0043	0.712	0.372
Ser	0.948	0.905	0.926	0.931	0.943	0.928	0.0101	0.819	0.273
DAA	0.912	0.885	0.907	0.923	0.923	0.912	0.0092	0.101	0.950
TAA	0.909	0.882	0.903	0.923	0.921	0.911	0.0099	0.092	0.902

[1]Apparent digestibility values were standardised using the following basal ileal endogenous flow values (g/kg DM intake), determined by the feeding N-free diet at different ages (Chapter 5):
D7: N, 3.59; Arg, 0.68; His, 0.29; Ile, 0.63; Leu, 0.97; Lys, 0.64; Met, 0.27; Thr, 1.35; Trp, 0.21; Val, 0.81; Ala, 0.75; Asp, 1.41; Cys, 0.47; Glu, 1.71; Gly, 0.78; Pro, 0.91; and Ser, 1.06.
D14: N, 1.87; Arg, 0.30; His, 0.16; Ile, 0.33; Leu, 0.49; Lys, 0.30; Met, 0.12; Thr, 0.73; Trp, 0.12; Val, 0.43; Ala, 0.37; Asp, 0.73; Cys, 0.27; Glu, 0.80; Gly, 0.39; Pro, 0.48; and Ser, 0.55.
D21: N, 1.79; Arg, 0.31; His, 0.15; Ile, 0.32; Leu, 0.49; Lys, 0.28; Met, 0.12; Thr, 0.71; Trp, 0.12; Val, 0.43; Ala, 0.36; Asp, 0.72; Cys, 0.26; Glu, 0.80; Gly, 0.40; Pro, 0.48; and Ser, 0.56.
D28: N, 1.82; Arg, 0.36; His, 0.16; Ile, 0.34; Leu, 0.55; Lys, 0.32; Met, 0.14; Thr, 0.67; Trp, 0.13; Val, 0.45; Ala, 0.41; Asp, 0.74; Cys, 0.25; Glu, 0.89; Gly, 0.43; Pro, 0.48; and Ser, 0.54.
D35: N, 1.81; Arg, 0.32; His, 0.15; Ile, 0.30; Leu, 0.49; Lys, 0.28; Met, 0.12; Thr, 0.71; Trp, 0.12; Val, 0.42; Ala, 0.37; Asp, 0.72; Cys, 0.26; Glu, 0.81; Gly, 0.40; Pro, 0.47; and Ser, 0.56.
D42: N, 1.29; Arg, 0.20; His, 0.10; Ile, 0.20; Leu, 0.31; Lys, 0.18; Met, 0.07; Thr, 0.52; Trp, 0.09; Val, 0.29; Ala, 0.25; Asp, 0.49; Cys, 0.21; Glu, 0.53; Gly, 0.28; Pro, 0.34; and Ser, 0.40.

[2]Each value represents the mean of six replicates (14, 12 and 10 birds per replicate for 7, 14 and 21-d old birds, respectively; eight birds per replicate for 28 and 35-d old birds; and six birds per replicate for 42-d old birds).

[3]Semi-indispensable amino acids for poultry.

DAA = Average digestibility of dispensable amino acids; IAA = Average digestibility of indispensable amino acids; TAA = Average digestibility of all amino acids.

No age effects (P > 0.05) were observed for the SID contents of protein, total IAA, total DAA, and TAA of wheat (Table 6.7). Among the IAA, the SID contents of Leu and Met increased (P < 0.05) by broiler age in linear and quadratic manner, respectively. However, the SID content of Thr showed quadratic decline (P < 0.05) as birds grew older. The SID Trp content increased linearly from 1.06 g/kg at d 7 to 1.14 g/kg at d 42. Among the DAA, the SID content of Asp and Cys increased linearly

(P < 0.001) with age. A linear effect (P = 0.045) of bird age was observed for the SID content of Glu.

Table 6.7. Influence of age on standardised ileal digestible protein (CP) and amino acid contents[1] of wheat (g/kg; as-received basis)

	Age (d)							Orthogonal polynomial contrasts	
Parameter	7	14	21	28	35	42	Pooled SEM	Linear	Quadratic
CP	89.6	85.2	87.8	89.5	89.3	87.9	0.83	0.393	0.584
Indispensable amino acids									
Arg	4.46	4.22	4.28	4.37	4.34	4.28	0.043	0.213	0.169
His	1.96	1.95	1.95	1.99	1.99	1.98	0.018	0.074	0.984
Ile	2.98	2.89	2.92	3.02	2.99	2.97	0.034	0.306	0.722
Leu	5.89	5.74	5.81	6.02	5.96	5.91	0.055	0.048	0.974
Lys	2.39	2.35	2.39	2.52	2.45	2.46	0.053	0.097	0.715
Met	1.49	1.46	1.55	1.59	1.57	1.55	0.018	0.001	0.021
Thr	2.88	2.57	2.63	2.62	2.69	2.60	0.046	0.013	0.012
Trp	1.06	1.07	1.12	1.14	1.15	1.14	0.016	0.001	0.069
Val	3.70	3.61	3.68	3.78	3.77	3.73	0.044	0.069	0.913
IAA	26.7	25.9	26.3	27.0	26.9	26.6	0.30	0.221	0.862
Dispensable amino acids									
Ala	3.12	3.04	3.06	3.14	3.09	3.05	0.046	0.814	0.904
Asp	4.06	3.89	4.27	4.41	4.40	4.31	0.081	0.001	0.123
Cys[2]	1.91	1.87	1.95	1.98	1.99	1.98	0.018	0.001	0.334
Glu	25.3	24.9	24.8	25.1	24.9	24.9	0.10	0.045	0.144
Gly[2]	3.67	3.50	3.57	3.67	3.65	3.62	0.043	0.461	0.341
Pro	8.54	8.34	8.41	8.49	8.49	8.41	0.038	0.722	0.342
Ser	4.09	3.91	3.99	4.02	4.07	4.01	0.044	0.773	0.277
DAA	50.7	49.5	50.1	50.8	50.7	50.3	0.36	0.488	0.735
Total AA	77.4	75.4	76.4	77.8	77.6	76.9	0.66	0.347	0.790

Standardised digestible amino acid content (g/kg) = [ingredient amino acid content (g/kg) × standardised ileal digestibility (%)]/100.

[1]Each value represents the mean of six replicates (14, 12 and 10 birds per replicate for 7, 14 and 21-d old birds, respectively; eight birds per replicate for 28 and 35-d old birds; and six birds per replicate for 42-d old birds).

[2]Semi-indispensable amino acids for poultry.

DAA = Total digestible dispensable amino acid contents; IAA = Total digestible indispensable amino acid contents; Total AA = Total digestible content of all amino acids.

6.4.4. Ileal digestibility coefficients of N and AA in sorghum

The influence of broiler age on AIDC, SIDC and SID content of N and AA in sorghum is summarised in Tables 6.8, 6.9, and 6.10, respectively.

No effect of age (P > 0.05) was recorded for the AIDC of N and, average digestibility of IAA, DAA and of TAA (Table 6.8). Among the IAA, the AIDC of Met (P < 0.001) and Trp (P < 0.05) increased linearly with

advancing age. The AIDC of Cys increased linearly (P < 0.001) from 0.580 at d 7 to 0.681 at d 42. A linear effect of bird age was also observed for Ala (P < 0.05) and Glu (P < 0.01). The AIDC of Asp tended (P = 0.066) to linearly increase from 0.739 at d 7 to 0.781 at d 42.

Table 6.8. Apparent ileal digestibility coefficients[1] of nitrogen (N) and amino acids of sorghum at different ages of broilers[1]

	Age (d)							Orthogonal polynomial contrasts	
Parameter	7	14	21	28	35	42	Pooled SEM	Linear	Quadratic
N	0.745	0.762	0.748	0.776	0.753	0.777	0.0164	0.242	0.990
Indispensable amino acids									
Arg	0.815	0.805	0.789	0.837	0.817	0.835	0.0173	0.216	0.436
His	0.727	0.733	0.693	0.735	0.701	0.733	0.0156	0.850	0.286
Ile	0.783	0.807	0.769	0.797	0.772	0.801	0.0169	0.917	0.617
Leu	0.845	0.875	0.842	0.844	0.819	0.846	0.0115	0.098	0.862
Lys	0.677	0.727	0.649	0.748	0.696	0.744	0.0335	0.227	0.762
Met	0.737	0.779	0.791	0.823	0.792	0.824	0.0164	0.001	0.152
Thr	0.637	0.627	0.607	0.650	0.633	0.664	0.0252	0.359	0.355
Trp	0.719	0.764	0.733	0.770	0.754	0.786	0.0192	0.045	0.978
Val	0.753	0.766	0.737	0.772	0.749	0.775	0.0182	0.550	0.586
IAA	0.744	0.765	0.734	0.775	0.748	0.778	0.0182	0.289	0.716
Dispensable amino acids									
Ala	0.845	0.879	0.838	0.835	0.811	0.836	0.0115	0.014	0.816
Asp	0.739	0.747	0.754	0.780	0.759	0.781	0.0173	0.066	0.789
Cys[2]	0.580	0.501	0.605	0.679	0.658	0.681	0.0318	0.001	0.975
Glu	0.858	0.892	0.841	0.842	0.816	0.841	0.0114	0.002	0.608
Gly[2]	0.702	0.688	0.615	0.683	0.657	0.679	0.0234	0.493	0.094
Pro	0.781	0.788	0.753	0.777	0.743	0.764	0.0146	0.126	0.596
Ser	0.748	0.736	0.724	0.758	0.739	0.767	0.0209	0.438	0.386
DAA	0.750	0.747	0.733	0.765	0.741	0.764	0.0176	0.586	0.566
TAA	0.747	0.758	0.735	0.771	0.745	0.772	0.0177	0.385	0.664

[1]Each value represents the mean of six replicates (14, 12 and 10 birds per replicate for 7, 14 and 21-d old birds, respectively; eight birds per replicate for 28 and 35-d old birds; and six birds per replicate for 42-d old birds).

[2]Semi-indispensable amino acids for poultry.

DAA = Average digestibility of dispensable amino acids; IAA = Average digestibility of indispensable amino acids; TAA = Average digestibility of all amino acids.

The highest SIDC of N, average SIDC of IAA, DAA and TAA were determined at d 7, declining at d 14 and then plateauing (Table 6.9). Among the IAA, a quadratic decline (P < 0.05 to 0.01) was observed for the SIDC of Arg, His, Thr, and Val with advancing age. The SIDC of Ile, Leu, Lys, and the average of IAA were linearly decreased (P < 0.05 to 0.001) with advancing age, with the highest SIDC values being

recorded on d 7. The SIDC of all individual DAA, except Cys, and the average of DAA was influenced by bird age either linearly or quadratically (P < 0.05 to 0.001; Table 6.9). A linear decrease (P < 0.05 to 0.001) was observed in SIDC of Ala, Asp, Glu, and Pro as birds grew older. The SIDC of Gly, Ser and average DAA reduced with advancing age, but the decline was greater between d 7 and 14 resulting in a quadratic effect (P < 0.05 to 0.01). The average SIDC of TAA declined linearly (P < 0.01) with advancing age, from 0.903 at d 7 to 0.828 at d 42.

Table 6.9. Standardised ileal digestibility coefficients[1] of nitrogen (N) and amino acids of sorghum at different ages of broilers[2]

	Age (d)							Orthogonal polynomial contrasts	
Parameter	7	14	21	28	35	42	Pooled SEM	Linear	Quadratic
N	0.933	0.860	0.842	0.872	0.848	0.845	0.0164	0.003	0.037
Indispensable amino acids									
Arg	0.961	0.869	0.857	0.913	0.885	0.879	0.0173	0.043	0.031
His	0.837	0.793	0.748	0.795	0.757	0.771	0.0156	0.005	0.034
Ile	0.915	0.875	0.837	0.869	0.836	0.843	0.0170	0.004	0.109
Leu	0.906	0.905	0.872	0.878	0.849	0.865	0.0115	0.001	0.345
Lys	0.934	0.847	0.841	0.877	0.808	0.814	0.0252	0.003	0.353
Met	0.874	0.842	0.855	0.895	0.852	0.859	0.0164	0.988	0.866
Thr	0.967	0.817	0.792	0.824	0.818	0.799	0.0246	0.001	0.003
Trp	0.869	0.848	0.821	0.862	0.842	0.849	0.0192	0.637	0.339
Val	0.891	0.839	0.810	0.849	0.819	0.826	0.0183	0.031	0.009
IAA	0.906	0.849	0.826	0.862	0.829	0.834	0.0167	0.011	0.089
Dispensable amino acids									
Ala	0.912	0.913	0.870	0.872	0.844	0.859	0.0115	0.001	0.232
Asp	0.916	0.839	0.845	0.874	0.851	0.844	0.0173	0.048	0.149
Cys[3]	0.810	0.725	0.729	0.800	0.786	0.781	0.0207	0.554	0.095
Glu	0.927	0.924	0.874	0.878	0.848	0.863	0.0113	0.001	0.116
Gly[3]	0.911	0.795	0.723	0.797	0.765	0.756	0.0234	0.001	0.003
Pro	0.873	0.837	0.802	0.825	0.792	0.799	0.0146	0.001	0.105
Ser	0.943	0.838	0.827	0.857	0.843	0.841	0.0209	0.012	0.014
DAA	0.899	0.826	0.810	0.843	0.819	0.820	0.0176	0.014	0.045
TAA	0.903	0.844	0.819	0.854	0.825	0.828	0.0164	0.007	0.059

[1]Apparent digestibility values were standardised using the following basal ileal endogenous flow values (g/kg DM intake), determined by the feeding N-free diet at different ages (Chapter 5); see Table 6.6.

[2]Each value represents the mean of six replicates (14, 12 and 10 birds per replicate for 7, 14 and 21-d old birds, respectively; eight birds per replicate for 28 and 35-d old birds; and six birds per replicate for 42-d old birds).

[3]Semi-indispensable amino acids for poultry.

DAA = Average digestibility of dispensable amino acids; IAA = Average digestibility of indispensable amino acids; TAA = Average digestibility of all amino acids.

The SID protein content of sorghum declined quadratically (P < 0.05) from 97.8 g/kg on d 7 to 88.6 g/kg on d 42 (Table 6.10). With the exception of Met and Trp, the SID contents of individual IAA declined either in quadratic (Arg and Thr; P < 0.05 to 0.01) or linear (His, Ile, Leu, Lys and Val; P < 0.01 to 0.001) manner with age, with the highest values recorded on d 7. Except Asp and Cys, the SID contents of all individual DAA and TAA of sorghum declined either linearly or quadratically (P < 0.01 to 0.001) with advancing age. Similar to IAA, the greatest SID AA contents were observed on d 7.

Table 6.10. Influence of age on standardised ileal digestible protein (CP) and amino acid contents[1] of sorghum (g/kg; as-received basis)

	Age (d)							Orthogonal polynomial contrasts	
Parameter	7	14	21	28	35	42	Pooled SEM	Linear	Quadratic
CP	97.8	94.5	88.2	91.4	88.8	88.6	1.58	0.001	0.048
Indispensable amino acids									
Arg	3.94	3.69	3.51	3.75	3.63	3.60	0.060	0.003	0.021
His	1.96	2.09	1.75	1.86	1.77	1.80	0.036	0.001	0.138
Ile	3.82	3.76	3.49	3.63	3.49	3.53	0.066	0.001	0.119
Leu	12.8	12.8	12.3	12.4	11.9	12.2	0.16	0.001	0.354
Lys	2.06	1.81	1.85	1.93	1.78	1.79	0.054	0.006	0.297
Met	1.49	1.48	1.46	1.53	1.46	1.47	0.028	0.558	0.732
Thr	3.27	3.01	2.68	2.78	2.77	2.70	0.073	0.001	0.002
Trp	1.05	1.04	0.99	1.04	1.02	1.03	0.023	0.492	0.375
Val	4.59	4.52	4.18	4.38	4.23	4.26	0.084	0.002	0.105
IAA	34.9	34.2	32.2	33.3	32.1	32.4	0.55	0.001	0.106
Dispensable amino acids									
Ala	8.87	8.34	8.47	8.48	8.22	8.36	0.111	0.004	0.091
Asp	6.39	5.44	5.89	6.09	5.93	5.88	0.114	0.391	0.060
Cys[2]	1.47	1.57	1.32	1.45	1.42	1.42	0.038	0.079	0.332
Glu	20.1	21.0	18.9	19.1	18.4	18.7	0.24	0.001	0.241
Gly[2]	2.97	2.78	2.36	2.59	2.49	2.47	0.067	0.001	0.002
Pro	7.54	8.19	6.92	7.13	6.83	6.89	0.115	0.001	0.362
Ser	4.50	4.31	3.94	4.09	4.02	4.01	0.078	0.001	0.006
DAA	51.9	51.7	47.9	48.9	47.3	47.7	0.74	0.001	0.090
Total AA	86.9	85.9	79.9	82.2	79.4	80.1	1.32	0.001	0.088

Standardised digestible amino acid content (g/kg) = [ingredient amino acid content (g/kg) × standardised ileal digestibility (%)]/100.

[1]Each value represents the mean of six replicates (14, 12 and 10 birds per replicate for 7, 14 and 21-d old birds, respectively; eight birds per replicate for 28 and 35-d old birds; and six birds per replicate for 42-d old birds).

[2]Semi-indispensable amino acids for poultry.

DAA = Total digestible dispensable amino acid contents; IAA = Total digestible indispensable amino acid contents; Total AA = Total digestible content of all amino acids.

6.4.5. Uplift in digestibility coefficients due to correction for endogenous amino acid losses as influenced by age

The percentage increase in the digestibility coefficients of N and AA in wheat and sorghum after correction of AIDC for endogenous N and AA losses is shown in Table 6.11.

Although the correction of AIDC for endogenous losses increased the SIDC of N and AA at all ages, the magnitude of increase was remarkably higher on d 7 and declined with advancing age. Standardisation of AIDC estimates for EAA losses increased the average TAA digestibility coefficients in wheat by 23.8% (d 7), 11.5% (d 14), 11.1% (d 21), 11.3% (d 28), 10.8% (d 35), and 7.43% (d 42), respectively. The corresponding uplifts in digestibility coefficients in sorghum were 20.9% (d 7), 11.3% (d 14), 11.4% (d 21), 10.8% (d 28), 10.7% (d 35), and 7.25% (d 42), respectively.

Table 6.11. Percentage increase in digestibility coefficients of nitrogen (N) and amino acids in wheat and sorghum after correction of apparent ileal digestibility coefficients for endogenous amino acid losses at different ages of broilers

	Wheat						Sorghum					
	Age (d)						Age (d)					
Parameter	7	14	21	28	35	42	7	14	21	28	35	42
N	28.9	13.9	12.9	12.8	12.7	8.99	25.2	12.9	12.6	12.4	12.6	8.75
Indispensable amino acids												
Arg	15.8	7.70	7.03	7.99	7.04	4.46	17.9	7.95	8.62	9.08	8.32	5.27
His	15.3	7.82	7.28	7.86	7.11	4.70	15.1	8.19	7.94	8.16	7.99	5.18
Ile	23.0	11.1	11.0	11.3	9.81	6.47	16.9	8.43	8.84	9.03	8.29	5.24
Leu	17.3	8.08	8.11	8.79	7.76	5.04	7.22	3.43	3.56	4.03	3.66	2.25
Lys	31.5	12.9	11.9	12.8	11.4	6.81	37.9	16.5	29.6	17.3	16.1	9.41
Met	18.9	8.09	7.72	8.46	7.24	4.18	18.6	8.09	8.09	8.75	7.58	4.25
Thr	67.2	34.2	32.0	29.5	30.9	21.8	51.8	30.3	30.5	26.8	29.2	20.3
Trp	20.9	10.6	10.6	10.9	10.5	7.43	20.9	11.0	12.0	11.9	11.7	8.02
Val	24.3	12.0	11.8	12.1	11.1	7.74	18.3	9.53	9.91	9.97	9.35	6.58
IAA	25.2	11.9	11.4	11.9	10.9	7.32	21.8	11.0	12.5	11.2	10.8	7.19
Dispensable amino acids												
Ala	26.9	12.0	11.9	13.1	12.1	7.74	7.93	3.87	3.82	4.40	4.07	2.75
Asp	44.9	18.6	17.6	17.7	17.1	11.5	24.0	12.3	12.1	12.15	12.1	8.07
Cys[1]	28.6	14.9	13.4	12.8	13.4	10.4	39.7	44.7	20.5	17.8	19.5	14.7
Glu	6.39	2.87	2.99	3.29	2.98	2.00	8.04	3.59	3.92	4.28	3.92	2.62
Gly[1]	23.3	11.4	11.1	11.6	10.9	7.57	29.8	15.6	17.6	16.7	16.4	11.3
Pro	10.6	5.21	5.40	5.34	5.23	3.79	11.8	6.22	6.51	6.18	6.59	4.58
Ser	30.2	14.7	14.5	13.5	14.2	9.82	26.1	13.9	14.2	13.1	14.1	9.65
DAA	22.6	11.0	10.6	10.7	10.5	7.42	19.9	10.6	10.5	10.2	10.5	7.33
TAA	23.8	11.5	11.1	11.3	10.8	7.43	20.9	11.3	11.4	10.8	10.7	7.25

[1]Semi-indispensable amino acids for poultry.

DAA = Average digestibility of dispensable amino acids; IAA = Average digestibility of indispensable amino acids; TAA = Average digestibility of all amino acids.

6.5. Discussion

The main aim of this study was to investigate whether the age affects the SIDC of AA in wheat and sorghum in broilers. Whilst there are sporadic data on age-related digestibility of AA in different ingredients for broilers (Fonolla *et al.*, 1981; Wallis and Balnave, 1984; Huang *et al.*, 2005; Adedokun *et al.*, 2007a; Szczurek *et al.*, 2020), these studies determined the digestibility only at two or three specific ages of broilers using assay diets fed in mash form. Previous attempts to establish a link between the age and AA digestibility have been inconclusive (Fonolla *et al.*, 1981; Wallis and Balnave, 1984; Zelenka and Liska, 1986; Huang

et al., 2005). The unique features of the present experiment are that 1) this is the first study reporting the SIDC of AA in wheat and sorghum from hatch to the end of growth cycle, 2) age-appropriate EAA losses (Chapter 5) were used to standardise the AIDC and 3) the assay diets were pelleted so the physical form of diet resembled the industry practice.

Nutrient composition

The proximate and AA concentrations of test cereals were, in general, close to the range reported in the literature (Huang *et al.*, 2005; Leeson and Summers, 2005; Ravindran *et al.*, 2005; Bryden *et al.*, 2009a,b; Selle *et al.*, 2012). The CP contents in wheat (94.3 g/kg) was slightly lower than the range (100-180 g/kg) stated by Leeson and Summers (2005). In sorghum, the analysed CP content (106 g/kg) was within the range (66.1-114.2 g/kg) recorded by Bryden *et al.* (2009b) but lower than those reported (125-128 g/kg) by Selle *et al.* (2012). The AA contents in wheat and sorghum were comparable with those reported in previous studies (Ravindran *et al.*, 2005; Bryden *et al.*, 2009a,b). Some variation in ingredient nutrient composition is to be expected due to differences in cultivar, geographical location, agronomy, growing season, and analytical techniques (Gutierrez-Alamo *et al.*, 2008; Mateos *et al.*, 2019).

Performance, gizzard pH and jejunal digesta viscosity

As could be expected, the DFI and DWG values increased as the birds grew older, regardless of the grain type. The gizzard pH in birds fed

wheat- (2.68) and sorghum-based diets (2.56) on d 7 in the current study was close to the findings of Angel *et al.* (2010) that analysed 15 published studies and Morgan *et al.* (2014) in a study with maize-, wheat- and sorghum- based diets, reporting gizzard pH of 2.37 and 2.42 on d 7, respectively. Gizzard pH increased from 2.68 in d 7 to 3.61 in d 42 in wheat-based diets and from 2.56 in d 7 to 3.66 in d 42 in sorghum-based diets. Although the digestive secretions and enzyme activities (Nitsan *et al.*, 1991), and concurrent secretion of HCl, increase with broiler age, the increase in gizzard pH with age in present study could be, partially, due to increased consumption of feed with neutral pH (Ravindran, 2013), which could dilute the HCl concentration and increase the pH.

Protein digestion in birds starts in the proventriculus with the secretion of pepsinogen and HCl. Hydrochloric acid in necessary to maintain the acidity of digesta for the conversion of pepsinogen to pepsin, the protease initiating protein digestion (Rynsburger, 2009). It may be logical to assume that lower the gizzard pH better will be the digestion of protein. Gizzard pH, however, was not correlated with the average SIDC of TAA of wheat ($r = 0.617$; $P > 0.05$) and sorghum ($r = -0.236$; $P > 0.05$) in the current study.

In birds fed wheat-based diets, the jejunal digesta viscosity increased with advancing age until d 35, with lower viscosities recorded on d 7 and 14. The positive relationship between the soluble NSP content and digesta viscosity is known (Choct and Annison, 1992b). Higher viscosity with advancing age could be a reflection of increased FI and

intake of soluble NSP. The measured viscosities, however, were lower in birds fed sorghum diets, which is an expected result for a grain with low soluble NSP contents (Bryden *et al.*, 2009b). Viscosity is correlated with the antinutritive effects of NSP on nutrient digestion and absorption (Fengler and Marquardt, 1988; Choct *et al.*, 1999). In the present study, however, despite increased viscosity, the AIDC of AA in wheat increased with age.

The higher AA digestibility in older birds in case of wheat might be due to better tolerance of older birds to viscous NSP due to higher stability of the intestinal microbiota (Choct and Annison, 1992a). An increase in the relative size of gastrointestinal tract of broilers was observed in broilers fed diets based on viscous grains like wheat and barley (Brenes *et al.*, 1993) or barley (Viveros *et al.*, 1994). Viscous NSP has been shown to increase pancreas weight (Almirall *et al.*, 1995), small intestinal length and enterocyte division rate (Viveros *et al.*, 1994) in broilers. Higher FI and increased ingestion of NSP may be associated with the increased intestinal length, potentially increasing the digestion and absorption of AA. The viscosity and average SIDC of TAA in wheat, however, were not correlated ($r = 0.503$; $P > 0.05$) in present study. In sorghum, the jejunal digesta viscosity was variable at different ages with no correlation between the viscosity and average SIDC of TAA ($r = -0.589$; $P > 0.05$).

Ileal digestibility coefficients of nitrogen and amino acids

It is widely accepted that poultry feed formulations based on digestible AA are superior to those based on total AA (Rostagno *et al.*, 1995; Ravindran and Bryden, 1999). To ensure adequate supply of available AA, therefore, better understanding of AA digestibility to birds at different ages of growth is fundamental.

In the present study, the AIDC of N, IAA, DAA, and TAA of wheat increased with advancing age in broilers. Several earlier studies have found that the AIDC of AA differs between broilers of different ages (Fonolla *et al.*, 1981; Wallis and Balnave, 1984; Noy and Sklan, 1995; Uni *et al.*, 1995; Huang *et al.*, 2005). Wallis and Balnave (1984) reported an increased ileal AA digestibility in broilers from d 30 to 50 post-hatch fed finisher diets containing a range of grain and protein sources. Batal and Parsons (2002) reported an increase in the total tract digestibility of AA in a maize-soybean diet from 0 to 10 d of age. Huang *et al.* (2005) determined AIDC AA of eight feed ingredients (wheat, sorghum, maize, soybean meal, canola meal, cotton seed meal, mill run and, meat and bone meal) at three different ages (d 14, 28 and 42) of broiler and, observed variable age effects depending on the ingredient. They concluded a general increase in digestibility with age. The AIDC of AA in maize, canola meal, soybean meal and, meat and bone meal were higher at d 28 and 42 than d 14. In contrast, the AIDC of most AA in wheat was found to be higher at d 14 than at d 28 and 42. No age effect was observed in the case of cottonseed meal. The ileal N digestibility of a maize-soybean meal diet was reported to be low in

broilers at d 4 post-hatch and increased at d 14 or 21 (Noy and Sklan, 1995; Uni *et al.*, 1995). Adedokun *et al.* (2008) reported the ileal AA digestibility of five plant-based feed ingredients (light and dark distiller's dried grains with solubles, canola meal, soybean meal and maize) in broilers at d 5 and 21 of age. Though the AID AA increased with age in all feed ingredients, the SID AA increased only in distiller's dried grains with solubles and maize. In the case of soybean meal and canola meal, there was no differences in SID AA between the two age groups.

Increased apparent AA digestibility of wheat with bird age may be, partly, explained by the age effect on feed passage rate. Food transit time through the gastrointestinal tract plays an important role in the digestion of nutrients by determining the contact time among nutrients, absorptive surfaces, digestive enzymes and microbiome (Clemens *et al.*, 1975; Mateos *et al.*, 1982). The nutrient absorption is facilitated by increased digesta retention time as the contact time between the digesta and absorptive surface is increased (Washburn, 1991). It has been reported that feed passage rates decrease with bird age (Wilson *et al.*, 1980). Hence, the digesta is exposed longer to digestive and absorptive processes, thus increasing nutrient digestibility (Wallis and Balnave, 1984). Besides, the gizzard of older birds is more developed than the young birds with heavy musculature. Increased digesta retention time in a well-developed gizzard enhances nutrient digestibility, including AA, by increasing intestinal refluxes and re-exposing the digesta to pepsin (Abdollahi and Ravindran, 2013).

Intestinal microbiome is known to play a key role in the digestive process by competing for nutrients (Stanley *et al.*, 2013; Mancabelli *et al.*, 2016). The colonisation of microbiota in avian gut occurs naturally either at hatching or even before hatching by passing the microbes through the eggshell pores (Roto *et al.*, 2016; Lee *et al.*, 2019). Strict hygienic practices in the hatchery prevent any colonisation and the gastrointestinal tract of newly hatched chick is sterile. Instead of natural maternal source, the hatchlings receive the initial microbial load from the farm environment (Donaldson *et al.*, 2017). Initial colonisation, followed by species richness and complexity of population structure, and finally maturation and stabilisation of microbiota is noticed as the birds grow older. This process of development and stabilisation of gut microbiota normally takes about three weeks in commercial broilers (Oakley *et al.*, 2014; Johnson *et al.*, 2018; Lee *et al.*, 2019). Gut bacteria are consumers of protein and AA, potentially influencing the digestibility estimates in the host (Yadav and Jha, 2019) and may partly contribute to the age effects.

While the AIDC of N and most AA in wheat increased with advancing age, only the SIDC of Met, Trp, Asp, Cys and Glu were influenced. The SIDC of Met, Trp, Asp and Cys followed the same pattern as AIDC and increased with broiler age. In contrast, Szczurek *et al.* (2020) measured the ileal AA digestibility in wheat, triticale and barley at two ages (d 14 and 28) of broilers and reported no age effect on the SIDC of AA in wheat.

In case of sorghum, only the AIDC of Met, Trp and Cys increased with broiler age. Broiler age had no effect on the average AIDC of IAA, DAA and TAA. Huang *et al.* (2005) reported that the AIDC AA in sorghum was higher at d 42 than d 28, but similar to that at d 14. In contrast to the trends observed for AIDC, the highest SIDC of N, average of IAA, DAA and TAA were determined at d 7, which declined at d 14 and then plateaued. The SIDC of TAA in sorghum at d 7 was 8.3% higher than those from average SIDC of d 14 to 42. Among the IAA, higher values were recorded on d 7 for the SIDC of Arg, His, Thr and Val. The SIDC of most individual IAA and all individual DAA, except that of Cys, reduced with advancing age.

A number of early studies (Hakansson and Eriksson, 1974; Fonolla *et al.*, 1981; Hassan and Delpech, 1986; Zelenka and Liska, 1986; Carré *et al.*, 1991; ten Doeschate *et al.*, 1993) have investigated the influence of age on the AA digestibility in poultry and, found that the protein and AA digestibility is lower in older birds. These studies, however, determined the digestibility over the total tract. Carré *et al.* (1991) reported that the apparent protein digestibility of field pea was higher in 3-week-old broilers than in adult roosters. Fonolla *et al.* (1981) observed a reduction in apparent protein digestibility of broilers with advancing age (d 21 vs. d 52) by feeding four diets (starter, finisher, finisher diet with 1.39% urea, finisher diet with 0.25% Lys, 0.10% Met and 0.22% Gly). These researchers suggested that this reduction was caused by increased excretion of metabolic N. ten Doeschate *et al.* (1993) also observed decreased protein digestibility coefficients with

increasing broiler age, with the values of 0.849 (d 13 to 15), 0.830 (d 27 to 29), 0.840 (d 41 to 43). Zuprizal *et al.* (1992) reported that the true digestibility of protein and AA in soybean meal or rapeseed meal for broilers decreased from week 3 to 6. The total tract digestibility findings, however, are not comparable to the ileal digestibility values determined in the current study, because of the possible microbial fermentation and alteration of AA composition in the hindgut (Salter and Coats, 1971; Ravindran and Bryden, 1999), and AA contamination from urine (Sykes, 1971; Terpstra, 1978).

Some reports suggest that the ileal digestibility of major nutrients, including AA, in feed ingredients declines with advancing age. Adedokun *et al.* (2007a) reported a reduction in the AIDC of TAA in meat and bone meal for broilers from 0.705 on d 5 to 0.595 on d 21. Decreased digestibility with age was observed even after the standardisation of AIDC using EAA loss determined by feeding a N-free diet (0.760 on d 5 vs. 0.632 on d 21). Reductions in the digestibility of other nutrients with bird age has also been reported. Moss *et al.* (2020) observed a reduction in starch digestibility from 0.935 on d 10 to 0.885 on d 35 in broilers fed a maize-based diet. The corresponding starch digestibility values on d 10 and 35 reported to be 0.901 and 0.845 for red sorghum, and 0.987 and 0.904 for wheat, respectively. David *et al.* (2020) reported a decrease in apparent ileal Ca digestibility with advancing age in broilers from 0.51 on d 7 to 0.36 on d 21, and 0.27 on d 42. According to Li *et al.* (2018), the AIDC of Ca was higher in 9-d old broiler chickens than 21 d, regardless of the dietary Ca level.

Currently broiler feeds are being formulated based on SIDC AA because of the recognition that SIDC values for individual feed ingredients are additive when mixed into a complete diet. On the other hand, the AIDC AA undervalues the digestibility of several nutritionally critical AA and less additive in feed formulations than SIDC (Cowieson *et al.*, 2019). The only difference between AIDC and SIDC values is the correction for basal EAA flow (Ravindran, 2021). Despite the shortcomings of apparent digestibility estimates, the AIDC data are also presented in this chapter to understand what proportion of the age effect on SIDC could be attributed to EAA losses. The data demonstrated that the observed differences in patterns between AIDC and SIDC (Figures 6.1 and 6.2) was due the use of age-appropriate EAA flows which were substantially higher at d 7, almost similar from d 14 to 35, and much lower at d 42. The basal ileal endogenous flow of TAA, used for standardisation of AIDC values (Chapter 5), in d 7 (12.93 g/kg DMI) was 96% higher than the average endogenous loss of TAA from d 14 to 35 (6.61 g/kg DMI), and 189% higher than the endogenous loss of TAA in d 42 (4.48 g/kg DMI). Once corrected for age-related endogenous AA flows, substantial differences were observed for the percentage increase in SIDC of AA over AIDC values at different ages (Table 6.11). Though a large number of studies (Chapter 3, 4; Lemme *et al.*, 2004; Szczurek, 2009; Bandegan *et al.*, 2011; Kim *et al.*, 2012a; Ravindran *et al.*, 2014b, 2017) exist on the SIDC of AA of a wide range of poultry feed ingredients, only few studies (Garcia *et al.*, 2007; Adedokun *et al.*, 2007a, 2008; Szczurek *et*

al., 2020) have determined the SIDC at different broiler ages. To the author's knowledge, apart from some (Adedokun *et al.* 2007a, 2008; Szczurek *et al.*, 2020), all previous studies have used a single EAA flow value, derived from older birds. As the EAA losses is highest in d 7 and decreases with advancing age (Chapter 5), the use of a single EAA value for correction at different ages underestimates the SIDC in early life and overestimates the SIDC in birds older than d 35.

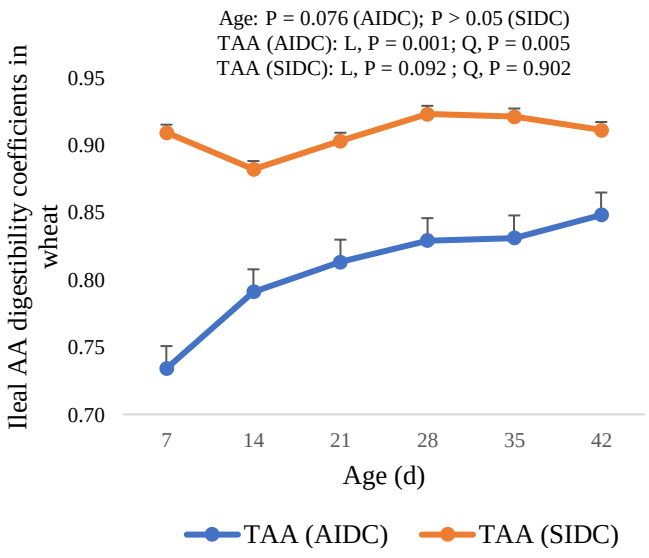

Figure 6.1. Apparent and standardised ileal digestibility coefficients of total amino acids (TAA) in wheat (bars represent mean ± SE) as influenced by broiler age. L, Linear; Q, Quadratic.

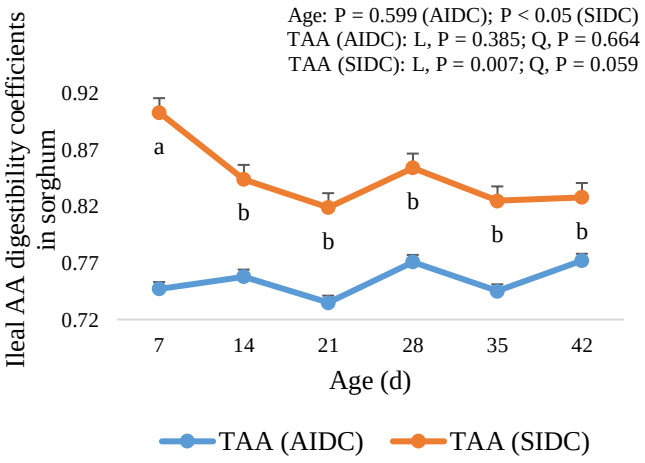

Figure 6.2. Apparent and standardised ileal digestibility coefficients of total amino acids (TAA) in sorghum (bars represent mean ± SE) as influenced by broiler age. [a,b]Values with different superscripts differ significantly (P < 0.05). L, Linear; Q, Quadratic.

Correction of AIDC values using age-appropriate EAA losses resulted in an increase of 23.8% in the SIDC of average TAA in wheat in d 7, which was almost twice than the increases in d 14 to 35 (10.8-11.5%), three times more than d 42 (7.43%). Similar trends were recorded for the average SIDC of TAA in sorghum at d 7 being 20.9% higher than the average AIDC of TAA. The magnitude of uplift in the average SIDC of TAA at d 14 to 35 and d 42 were 10.7 to 11.4% and 7.25%, respectively. In essence, the trends in age effects on SIDC were reflective of those on EAA flow.

Overall, the exact reasons for the observed age effects on AA digestibility are intricate and complicated by the interactions among plethora of factors including digestion, absorption, transport, endogenous losses, FI, digesta passage rate and intestinal microbiome.

The present data also showed that the EAA flow is a prime contributing factor contributing to SIDC, especially during week 1.

6.6. Conclusions

The present study, for the first time, provides information on the SIDC of AA in wheat and sorghum from hatching to the end of growth cycle of broilers. These results suggest that the age effect on AA digestibility is variable depending on the grain type and specific AA. Though the apparent AA digestibility in wheat increased by age, the SIDC was not affected in most of the AA. In the case of sorghum, except for a few AA, the AIDC of most AA was unaffected by age. However, the highest SIDC AA of sorghum were recorded at d 7, reduced at d 14, and then plateaued. The correction of apparent AA digestibility values using age-appropriate EAA flows resulted in substantial increases in SIDC over AIDC at different ages in both wheat and sorghum. The EAA value used for correction of apparent values in this study was notably higher at d 7 compared to other ages. Because the use of a single EAA value for birds of different ages might underestimate the SIDC in early life and overestimate the SIDC in older birds, application of age-appropriate EAA values for standardisation should be considered to improve the precision of feed formulations.

CHAPTER 7 Influence of age on the standardised ileal amino acid digestibility of maize and barley in broilers[1]

7.1. Abstract

The aim of the current study was to determine the influence of broiler age on the standardised ileal digestibility coefficients (SIDC) of nitrogen (N) and amino acids (AA) in maize and barley. The digestibility coefficients were determined in broilers at six different ages (d 7, 14, 21, 28, 35, and 42). Two assay diets were formulated to contain 938 g/kg of either maize or barley as the sole source of AA in the diet. Titanium dioxide (5 g/kg) was incorporated as an indigestible marker. The assay diets, in pellet form, were fed to six replicate cages housing 14 (d 7), 12 (d 14), 10 (d 21), 8 (d 28), 8 (d 35), and 6 (d 42) birds per cage for 4 d prior to digesta collection from the terminal ileum. The apparent ileal digestibility coefficients (AIDC) were standardised using age-appropriate basal endogenous AA (EAA) losses determined in a previous study from birds fed an N-free diet (Chapter 5). As the birds grew older, an increase (quadratic; $P < 0.001$) in the AIDC of N, average AIDC of indispensable (IAA), dispensable (DAA) and total AA (TAA) was observed in maize. The digestibility increased from d 7 to 21, and then plateaued until d 42. Except Cys ($P > 0.05$), an increase (quadratic, $P < 0.001$) in the AIDC of all individual AA was also recorded with advancing broiler age. Correction of AIDC with age-

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appropriate EAA losses resulted marked differences in SIDC estimates. No age effect (P > 0.05) was noted for the SIDC of N in maize. But the average SIDC of indispensable (IAA) and total AA (TAA) was influenced in a quadratic manner (P < 0.05) with the values being higher at d 7 that decreased at d 14, increased and plateaued between d 21 to 35 with a further decrease at d 42. The average SIDC of dispensable AA (DAA) was influenced linearly (P < 0.05) with higher values at d 7. In barley, the SIDC of N, average IAA, DAA and TAA was affected (quadratic; P < 0.001) by age. The digestibility increased from d 7 to 21, and then plateaued upto d 42. With the exception of Cys (P > 0.05), the SIDC of all individual AA increased (quadratic, P < 0.05 to 0.001) by age. The values were reported to be lower at d 7, that increased until d 21, and plateaued from d 21 to 42. Overall, the age influence on AA digestibility in cereals is grain- and AA-dependent. The present findings suggest that the SIDC of AA in maize and barley are influenced by broiler age and that the age effect on AA digestibility may need to be considered for precise feed formulation.

7.2. Introduction

To improve broiler performance and lower production economics, accurate feed formulation that closely matches nutrient requirements is crucial. Determination of amino acid (AA) digestibility in feed ingredients is an important way to reach the goal of meeting protein requirements. Poultry feed formulations based on digestible AA are superior to those based total AA due to the fact that digestible AA is literally utilised by the birds for maintenance and production (Rostagno

et al., 1995; Parsons, 2020). Better understanding of the factors influencing AA digestibility is crucial to supply available AA at optimum level that is important in terms of accuracy of feed formulation, reduction of diet cost and nitrogenous pollution in the environment (Cowieson *et al.*, 2019).

The AA digestibility in poultry was measured at the excreta level in the past (Fonolla *et al.*, 1981; ten Doeschate *et al.*, 1993), but currently measured at the terminal ileal level. The excreta digestibility assay has several shortcomings including possible modifying action of hindgut microflora on excreta AA profile and contamination with nitrogen (N) and AA from urine (Ravindran *et al.*, 1999; Kadim *et al.*, 2002). Ileal AA digestibility can be categorised as either apparent or standardised/true. For the calculation of standardised ileal digestibility (SID), the apparent ileal digestibility (AID) values are corrected for basal endogenous AA (EAA) losses originating from various digestive, pancreatic and enzymatic secretions (Lemme *et al.*, 2004). The SID is more additive than AID in broiler feed formulations (Kong and Adeola, 2013b; Cowieson *et al.*, 2019; An *et al.*, 2020).

Grains are the major energy sources in broiler diets. However, they also supply about 40% of the total dietary protein and contribute significantly to the provision of some indispensable AA (IAA; Szczurek *et al.*, 2020). Maize (*Zea mays* L.) is used extensively worldwide in poultry diets because of its high palatability, low fibre, high energy and essential fatty acids. Despite the low protein content in maize, owing to its higher inclusion levels (50-70%), it may contribute

approximately up to one third of the protein requirement of broilers (NRC, 1994; Lasek *et al.*, 2012). Barley (*Hordeum vulgare* L.) is another grain used in the European Union, Western Canada, New Zealand and Australia. Nevertheless, the inclusion of barley in poultry diets remains limited because of relatively low metabolisable energy, high content of fibre (220 g/kg), and high soluble (45 g/kg) and insoluble (122 g/kg) non-starch polysaccharide (NSP) content (Englyst, 1989; Jacob and Pescatore, 2012). Because of their viscous nature, the water-soluble fractions of barley exert a negative impact on the digestion and absorption of nutrients, including AA (Choct and Annison, 1992a).

Several datasets are available on the AID AA digestibility in feed ingredients for broilers (Ravindran *et al.*, 2005; Bryden *et al.*, 2009a). However, the AA digestibility varies depending on ingredient type (Bryden *et al.*, 2009a), class of bird (rooster, broiler, layer; Huang *et al.*, 2006) and, feed form (mash vs. pellet; Chapters 3, 4). Despite the potential effects of age (Chapter 6; Fonolla *et al.*, 1981; Huang *et al.*, 2005), only sporadic and inconsistent data exist on the age influence on AA digestibility of ingredients in broilers (Wallis and Balnave, 1984; Fonolla *et al.*, 1981; Carré *et al.*, 1991; Zuprizal *et al.*, 1992; ten Doeschate *et al.*, 1993; Huang *et al.*, 2005). Although a number of studies (Chapter 3, 4; Lemme *et al.*, 2004; Bandegan *et al.*, 2011; Ravindran *et al.*, 2017) have reported the SID AA in a range of feed ingredients, only a few (Garcia *et al.*, 2007; Adedokun *et al.*, 2007a, 2008; Szczurek *et al.*, 2020) exist on the age-related SID AA and the data

are limited to two or three specific broiler ages. To the author's knowledge, no studies have investigated the SIDC AA of ingredients from hatching to the end of growth cycle of commercial broilers. The current study was designed to determine the standard ileal digestibility coefficients (SIDC) of AA in maize and barley at six different ages (d 7, 14, 21, 28, 35 and, 42 post-hatch) of broilers.

7.3. Materials and methods

The experimental procedure was complied with 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. Diets and experimental design

Maize and barley were obtained from a commercial supplier and ground in a hammer mill to pass through a screen size of 3.0 mm. Two experimental diets were developed with similar inclusions (938 g/kg) of either maize or barley as the only source of AA in the diet (Table 7.1). Titanium dioxide (5 g/kg; Merck KGaA, Darmstadt, Germany) was incorporated in both diets as an indigestible marker. The diets were steam-conditioned at 70 ºC for 30 s and pelleted using a pellet mill (Model Orbit 15; Richard Size Limited Engineers, 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. Pelleted diets were crumbled for the feeding of young chicks during the first two weeks of the experiment.

Table 7.1. Composition of the experimental diets (g/kg, as fed basis)

Ingredient	Maize	Barley
Maize	938	-
Barley	-	938
Soybean oil	20	20
Dicalcium phosphate	18	18
Limestone	13	13
Titanium dioxide[1]	5.0	5.0
Sodium chloride	2.0	2.0
Sodium bicarbonate	2.0	2.0
Trace mineral premix[2]	1.0	1.0
Vitamin premix[2]	1.0	1.0

[1]Merck KGaA, Darmstadt, Germany.

[2]Supplied per kilogram of diet: antioxidant (ethoxyquin), 100 mg; biotin, 0.2 mg; calcium pantothenate, 12.8 mg; cholecalciferol, 0.06 mg; 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, 0.2 mg; Zn, 60 mg.

Representative grain samples were analysed for dry matter (DM), N, starch, crude fat, crude fibre, neutral detergent fibre (NDF), gross energy (GE), AA, calcium, phosphorus (P) and ash. The apparent ileal digestibility coefficients (AIDC) of N and AA in each grain was determined using the direct method. The AIDC were then standardised using the age-dependent (d 7, 14, 21, 28, 35 and 42) basal endogenous N and AA losses measured in a previous experiment (Chapter 5).

7.3.2. Birds and housing

A total of 696, day-old male broilers (Ross 308), obtained from a commercial hatchery, were used in this study. The birds were raised in floor pens and fed a commercial broiler starter diet (12.14 MJ/kg apparent metabolisable energy; 225 g/kg crude protein; CP) from d 1 to 21 and a commercial broiler finisher diet (12.69 MJ/kg apparent metabolisable energy; 190 g/kg CP) from d 22 until d 38 in pelleted form (Table 7.2).

Table 7.2. Composition and calculated analysis (g/kg, as fed basis) of broiler starter and finisher diets

Ingredients	Starter diet (0-21 d)	Finisher diet (22-38 d)
Maize	574.2	660
Soybean meal, 460 g/kg	381.4	295.6
Soybean oil	8.8	13.6
Limestone	11.3	9.9
Dicalcium phosphate	10.7	8.2
DL-methionine	3.3	3.0
L-lysine HCl	2.0	1.9
L-threonine	1.0	0.7
Sodium bicarbonate	2.7	2.5
Sodium chloride	2.5	2.5
Trace mineral premix[1]	1.0	1.0
Vitamin premix[1]	1.0	1.0
Phytase	0.1	0.1
Calculated analysis		
Apparent metabolisable energy (MJ/kg)	12.14	12.69
Crude protein	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]Supplied per kilogram of diet: antioxidant (ethoxyquin), 100 mg; biotin, 0.2 mg; calcium pantothenate, 12.8 mg; cholecalciferol, 0.06 mg; 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, 0.2 mg; Zn, 60 mg.

On d 1, 168 chicks were individually weighed, and allocated to 12 cages (14 chicks per cage) in such a way group mean body weight (BW) per replicate was identical. The remaining chicks were allocated to 12 cages at five different ages, namely d 7 (12 birds per cage), d 14 (10 birds per cage), d 21 (8 birds per cage), d 28 (8 birds per cage), and d 35 (6 birds per cage). The test diets were offered for 4 d [d 3-7 and 10-14 (crumbled); d 17-21, 24-28, 31-35, and 38-42 (pelleted)] before collecting ileal digesta on d 7, 14, 21, 28, 35 and 42 post-hatch, respectively.

The birds were offered *ad libitum* feed and water was freely available throughout the whole experimental period. The room temperature was 32 ± 1 ºC in the first week that was gradually reduced to 23 ºC by the end of the third week. The floor pens, battery brooders and grower cages were housed in an environmentally controlled room with 20 h of fluorescent illumination per day.

7.3.3. Growth performance data

During the 4-d study period, feed intake (FI) and BW were recorded on a cage basis in each week.

7.3.4. Determination of the coefficient of apparent ileal digestibility

At the end of each experimental period (d 7, 14, 21, 28, 35 and 42), all birds were euthanised by intravenous injection (0.5 mL per kg BW) of sodium pentobarbitone solution (Provet NZ Pty. Ltd., Auckland, New Zealand). The digesta were collected from the lower half of the ileum, and processed as described by Ravindran *et al.* (2005). The ileum was marked as that portion of the small intestine extending from the Meckel's diverticulum to a point ~40 mm proximal to the ileocaecal junction. In brief, the ileum was excised and divided into halves (proximal and distal ileum) and the digesta samples were collected from the lower half towards the ileocaecal junction after gently flushing with distilled water into plastic containers. The ileal digesta from birds within a cage were pooled after collection, frozen immediately and then lyophilised (Model 0610, Cuddon Engineering, Blenheim, New

Zealand). Diet and lyophilised digesta samples were ground to pass through a 0.5-mm sieve and stored in airtight plastic containers at 4 ºC pending analysis.

7.3.5. Gizzard pH and jejunal digesta viscosity

From the birds euthanised for ileal digesta collection, two birds from each replicate cage were used for the measurement of gizzard pH by a digital pH meter (pH spear, Oakton Instruments, Vernon Hill, IL). The glass probe was inserted through an opening made in the gizzard and was placed directly in the digesta. Three values were taken from the proximal, middle, and distal regions and the average value was considered as the final pH value. The jejunal digesta viscosity was also determined from these birds. The digesta was collected from the distal jejunum, followed by centrifugation at 3000 × g at 20 ºC for 15 min. A 0.5 mL aliquot of the supernatant was used in a viscometer (Brookfield digital viscometer, Model DV2TLV; Brookfield Engineering Laboratories Inc., Stoughton, MA) fitted with CP-40 cone spindle with shear rates of 5-500/s to measure the digesta viscosity.

7.3.6. Chemical analysis

Except for AA, all chemical analyses were accomplished in duplicates. Single analysis per sample was conducted for AA. Dry matter was measured using the standard procedure (Method 930.15; AOAC International, 2016). Titanium was analysed on a UV spectrophotometer (Berthold Technologies GmbH and Co. KG, Bad Wildbad, Germany) following the method described by Short *et al.*

(1996). Gross energy was determined by adiabatic bomb calorimeter (Gallenkamp autobomb, Weiss Gallenkamp Ltd., Loughborough, UK) standardised with benzoic acid. Starch was analysed using the Megazyme Total Starch Assay kit (Megazyme International Ireland Ltd., Wicklow, Ireland) based on thermostable α-amylase and amyloglucosidase (McCleary *et al.*, 1997; AOAC International, 2016). Nitrogen was determined by combustion (Method 968.06; AOAC International, 2016) using a carbon nanosphere-200 carbon, N and sulphur auto analyser (LECO Corporation, St. Joseph, MI). The CP content was calculated as N × 6.25. Fat was determined using the Soxhlet extraction procedure (Method 2003.06; AOAC International, 2016). Neutral detergent fibre was determined (Method 2002.04; AOAC International, 2016) using Tecator Fibertec™ (FOSS Analytical AB, Höganäs, Sweden). Samples were measured for ash by ashing in a muffle furnace at 550 ºC for 16 h (Method 942.05; AOAC International, 2016). Calcium and P concentrations were measured by Inductively Coupled Plasma-Optical Emission Spectroscopy (ICP-OES) using a Thermo Jarrell Ash IRIS instrument (Thermo Jarrell Ash Corporation, Franklin, MA).

Amino acids were analysed following standard procedures (Method 994.12; AOAC International, 2011). Briefly, the samples were hydrolysed with 6 N hydrochloric acid (HCl) containing phenol for 24 h at 110 ± 2 ºC in glass tubes in an oven. Amino acids were measured using AA analyser (ion exchange) with ninhydrin post column derivatisation. The chromatograms detected at 570 and 440 nm were

integrated using dedicated software (Agilent Open Lab software, Waldbronn, Baden-Württemberg, Germany). For the sulphur containing AA, Cys and Met were analysed as cysteic acid and methionine sulphone, respectively by oxidation with performic acid-phenol for 16 h at 0 ºC prior to hydrolysis.

For the measurement of Trp, the samples were saponified under alkaline conditions with barium hydroxide solution in the absence of air at 110 ºC for 20 h in an autoclave. The α-methyl Trp as internal standard, was added to the mixture following hydrolysis. After adjusting the hydrolysate to pH 3.0 and diluting with 30% methanol, Trp and the internal standard were separated by reverse phase chromatography (RP-18) on a HPLC column (CORTECS C18 Column; 2.7 µm, Waters Corporation, Dublin, Ireland). Detection was selectively done by means of a fluorescence detector to prevent interference by other AA and constituents.

7.3.7. Calculations

Data were expressed on a DM basis. The AIDC of AA were calculated from the dietary ratio of AA to Ti relative to the corresponding ratio in the ileal digesta using the following formula.

$$\text{AIDC of AA} = [(AA / Ti)_d - (AA / Ti)_i]/(AA / Ti)_d$$

Where, $(AA / Ti)_d$ = ratio of AA to Ti in the diet, and $(AA / Ti)_i$ = ratio of AA to Ti in the ileal digesta.

Apparent digestibility data for N and AA were then standardised using the age-appropriate basal endogenous N and AA estimates (EAA;

grams per kilogram of DM intake [DMI]) analysed at different ages (d 7, 14, 21, 28, 35 and 42) in a previous experiment (Chapter 5).

SIDC = AIDC + [Basal EAA (g/kg DMI)/Ing. AA (g/kg DM)]

Where, SIDC = standardised ileal digestibility coefficient of the AA, AIDC = apparent ileal digestibility coefficient of the AA, Basal EAA = basal endogenous AA loss, and Ing. AA = concentration of the AA in the ingredient.

7.3.8. Data Analysis

Cage was considered as the experimental unit. Data were analysed by the General Linear Model procedure of SAS (version 9.4; 2015; SAS Institute, Cary, NC.) for each grain. Differences were considered significant at $P < 0.05$. Orthogonal polynomial contrasts were performed to determine the linear and quadratic effects of age. The relationships between SIDC AA and other parameters were analysed by Pearson correlation.

7.4. Results

7.4.1. Proximate and nutrient composition

The proximate and nutrient composition of the grains are summarised in Table 7.3. The results are presented ‘as received’ basis.

In both grains, starch was the main component followed by NDF in maize, and CP in barley. The starch content in maize and barley were 590 g/kg and 541 g/kg, respectively. The CP content in barley (115 g/kg) was higher than maize (67.8 g/kg). The NDF in maize was

determined to be 83.1 g/kg that was lower than that in barley (110 g/kg). The contents of Ca in both maize (0.17 g/kg) and barley (0.14 g/kg) were negligible.

Among the IAA, the content of Leu was the highest followed by Val, Arg, Ile and Thr in both grains, whereas lower contents were determined for Trp and Met. The Glu was the major dispensable AA (DAA) followed by Pro in both maize and barley. In maize, the contents of total IAA and total DAA were 26.4 g/kg and 35.4 g/kg, respectively. The total IAA and total DAA contents in barley were 35.1 g/kg and 57.5 g/kg, respectively. The variations in CP contents between the two grains were reflected in total AA contents with higher value in barley (92.5 g/kg) compared to maize (61.8 g/kg).

Table 7.3. Proximate, carbohydrate and amino acid composition of grains (g/kg, as-received basis)

	Maize	Barley
Dry matter	859	875
Nitrogen (N)	10.8	18.4
Crude protein (N×6.25)	67.8	115
Starch	590	541
Fat	32.4	21.0
Neutral detergent fibre	83.1	110
Gross energy (MJ/kg)	16.3	16.3
Ash	20.5	18.6
Calcium	0.17	0.14
Phosphorus	2.47	3.02
Indispensable amino acids (IAA)		
Arg	3.43	5.28
His	2.07	2.37
Ile	2.49	3.98
Leu	8.14	7.53
Lys	2.33	3.72
Met	1.31	1.77
Thr	2.59	3.54
Trp	0.58	1.35
Val	3.44	5.52
Total IAA	26.4	35.1
Dispensable amino acids (DAA)		
Ala	5.14	4.54
Asp	4.77	6.78
Cys[1]	1.37	2.23
Glu	12.4	24.3
Gly[1]	2.85	4.44
Pro	5.60	10.8
Ser	3.29	4.41
Total DAA	35.4	57.5
Total AA[2]	61.8	92.5

[1]Semi-indispensable amino acids for poultry. [2]Total AA = IAA + DAA.

7.4.2. Growth performance, gizzard pH and jejunal digesta viscosity

Weekly data on the performance, gizzard pH and jejunal digesta viscosity of birds fed maize- or barley-based diets are presented in Table 7.4.

Mortality during the experiment was negligible. Out of 696, only four birds died, and the deaths were not related to any specific treatment. The daily feed intake (DFI) and daily weight gain (DWG) increased

(quadratic; P < 0.001) in both maize- and barley-based diets as birds grew older.

Gizzard pH increased in a quadratic manner (P < 0.001) with advancing age, regardless of grain type. A decline in gizzard pH was observed from d 7 to d 14, but increased beyond d 21.

The jejunal viscosity in maize was unaffected (P > 0.05) by age. In the case of barley, however, the jejunal digesta viscosity was influenced quadratically (P < 0.001) by age. Higher viscosity was observed on d 7 and d 42 (2.94 cP). After d 7, a reduction in viscosity was observed at d 14 that plateaued until d 35. A further increase was observed at d 42.

Table 7.4. Daily feed intake (DFI; g/bird/d), daily weight gain (DWG; g/bird/d), gizzard pH and viscosity (cP) in jejunal digesta of broilers fed maize- and barley-based diets at different ages[1]

	Age (d)							Orthogonal polynomials contrasts	
Parameter	7	14	21	28	35	42	Pooled SEM	Linear	Quadratic
					Maize				
DFI[2]	12.1	34.8	77.9	123	135	165	1.2	0.001	0.001
DWG[2]	10.5	28.4	35.8	54.3	58.9	59.7	0.48	0.001	0.001
Gizzard pH[3]	2.53	2.07	2.24	2.59	3.19	3.61	0.108	0.001	0.001
Viscosity[3]	2.03	2.33	2.23	2.25	2.11	2.34	0.079	0.168	0.480
					Barley				
DFI[2]	13.6	37.3	84.8	129	142	170	1.2	0.001	0.001
DWG[2]	12.5	31.4	38.8	56.2	61.1	62.9	0.86	0.001	0.001
Gizzard pH[3]	2.14	2.08	2.75	3.24	3.38	3.05	0.105	0.001	0.001
Viscosity[3]	2.94	2.70	2.69	2.80	2.75	2.94	0.093	0.745	0.024

[1]Each value represents the mean of six replicates (14, 12 and 10 birds per replicate for 7, 14 and 21-d old birds, respectively; eight birds per replicate for 28 and 35-d old birds; and six birds per replicate for 42-d old birds).

[2]Measured during 4-d feeding of experimental diets.

[3]Calculated as the mean of six replicates (two birds per replicate).

7.4.3. Ileal digestibility coefficients of N and AA in maize

The influence of broiler age on AIDC, SIDC and SID content of N and AA in maize is presented in Tables 7.5, 7.6 and 7.7, respectively.

A quadratic increase (P < 0.001) was observed for AIDC of N, average digestibility of IAA, DAA and TAA of maize with advancing age of

broilers (Table 7.5). The AIDC of N, average AIDC of IAA, DAA and TAA increased from d 7 to 21, then plateaued up to d 42. The AIDC of all individual IAA increased in a quadratic manner (P < 0.001) with age. With the exception of Cys, an increase (quadratic; P < 0.001) was observed on the AIDC of all individual DAA.

Table 7.5. Apparent ileal digestibility coefficients[1] of nitrogen (N) and amino acids of maize at different ages of broilers[1]

	Age (d)							Orthogonal polynomial contrasts	
Parameter	7	14	21	28	35	42	Pooled SEM	Linear	Quadratic
N	0.651	0.669	0.779	0.766	0.764	0.766	0.0089	0.001	0.001
Indispensable amino acids									
Arg	0.745	0.752	0.864	0.849	0.850	0.859	0.0056	0.001	0.001
His	0.718	0.744	0.805	0.795	0.792	0.802	0.0080	0.001	0.001
Ile	0.628	0.645	0.805	0.779	0.771	0.789	0.0100	0.001	0.001
Leu	0.799	0.817	0.901	0.882	0.872	0.882	0.0059	0.001	0.001
Lys	0.467	0.473	0.753	0.709	0.701	0.712	0.0142	0.001	0.001
Met	0.724	0.721	0.882	0.856	0.850	0.859	0.0098	0.001	0.001
Thr	0.464	0.435	0.636	0.635	0.635	0.648	0.0138	0.001	0.001
Trp	0.464	0.469	0.648	0.635	0.654	0.662	0.0143	0.001	0.001
Val	0.604	0.616	0.794	0.779	0.768	0.786	0.0100	0.001	0.001
IAA	0.624	0.630	0.788	0.769	0.766	0.778	0.0093	0.001	0.001
Dispensable amino acids									
Ala	0.771	0.779	0.874	0.854	0.839	0.846	0.0072	0.001	0.001
Asp	0.609	0.601	0.773	0.753	0.739	0.753	0.0100	0.001	0.001
Cys[2]	0.709	0.737	0.747	0.738	0.730	0.747	0.0127	0.077	0.209
Glu	0.794	0.798	0.893	0.869	0.862	0.866	0.0067	0.001	0.001
Gly[2]	0.551	0.534	0.724	0.708	0.703	0.721	0.0121	0.001	0.001
Pro	0.706	0.725	0.811	0.801	0.798	0.813	0.0079	0.001	0.001
Ser	0.625	0.589	0.769	0.757	0.761	0.772	0.0119	0.001	0.001
DAA	0.681	0.680	0.799	0.783	0.776	0.788	0.0089	0.001	0.001
TAA	0.649	0.652	0.792	0.775	0.770	0.782	0.0087	0.001	0.001

[1]Each value represents the mean of six replicates (14, 12 and 10 birds per replicate for 7, 14 and 21-d old birds, respectively; eight birds per replicate for 28 and 35-d old birds; and six birds per replicate for 42-d old birds).

[2]Semi-indispensable amino acids for poultry.

DAA = Average digestibility of dispensable amino acids; IAA = Average digestibility of indispensable amino acids; TAA = Average digestibility of all amino acids.

The SIDC of N in maize was unaffected (P > 0.05; Table 7.6) by age. Bird age, however, quadratically influenced the average SIDC of IAA (P < 0.002) and TAA (P < 0.05). The higher values were recorded on d 7 than d 14, and the SIDC values increased to d 21 and plateaued to d 35, followed by a decrease on d 42. The SIDC of average DAA was influenced in a linear (P < 0.05) manner with higher value on d 7 (0.881)

than d 14 (0.788). Afterwards, an increase in the SIDC was observed on d 21 that plateaued until d 35, a further decrease was observed on d 42. Except Thr (P > 0.05), the SIDC of all individual IAA and DAA was influenced (linear or quadratic; P < 0.05 to < 0.001) by broiler age.

Table 7.6. Standardised ileal digestibility coefficients[1] of nitrogen (N) and amino acids of maize at different ages of broilers[2]

	Age (d)							Orthogonal polynomial contrasts	
Parameter	7	14	21	28	35	42	Pooled SEM	Linear	Quadratic
N	0.936	0.817	0.922	0.911	0.907	0.868	0.0089	0.295	0.684
Indispensable amino acids									
Arg	0.914	0.828	0.943	0.939	0.930	0.909	0.0056	0.001	0.003
His	0.839	0.810	0.866	0.862	0.854	0.844	0.0080	0.034	0.039
Ile	0.844	0.757	0.917	0.898	0.875	0.859	0.0100	0.001	0.001
Leu	0.902	0.868	0.952	0.939	0.923	0.915	0.0059	0.001	0.001
Lys	0.704	0.583	0.858	0.827	0.805	0.776	0.0142	0.001	0.001
Met	0.899	0.801	0.963	0.948	0.927	0.904	0.0097	0.001	0.001
Thr	0.912	0.678	0.872	0.856	0.871	0.821	0.0138	0.348	0.119
Trp	0.769	0.640	0.828	0.823	0.834	0.792	0.0143	0.001	0.048
Val	0.807	0.723	0.902	0.892	0.873	0.861	0.0100	0.001	0.001
IAA	0.843	0.743	0.900	0.887	0.877	0.854	0.0091	0.001	0.002
Dispensable amino acids									
Ala	0.896	0.841	0.934	0.923	0.901	0.887	0.0072	0.043	0.001
Asp	0.863	0.733	0.902	0.887	0.868	0.843	0.0100	0.002	0.017
Cys[3]	0.968	0.906	0.908	0.894	0.895	0.877	0.0089	0.001	0.013
Glu	0.912	0.853	0.948	0.931	0.918	0.903	0.0067	0.026	0.002
Gly[3]	0.784	0.654	0.845	0.836	0.823	0.807	0.0121	0.001	0.034
Pro	0.845	0.799	0.885	0.874	0.871	0.866	0.0079	0.001	0.048
Ser	0.902	0.733	0.915	0.897	0.907	0.876	0.0119	0.001	0.996
DAA	0.881	0.788	0.905	0.892	0.883	0.865	0.0083	0.010	0.109
TAA	0.860	0.763	0.902	0.889	0.879	0.859	0.0087	0.001	0.011

[1]Apparent digestibility values were standardised using the following basal ileal endogenous flow values (g/kg DM intake), determined by the feeding N-free diet at different ages (Chapter 5):
D7: N, 3.59; Arg, 0.68; His, 0.29; Ile, 0.63; Leu, 0.97; Lys, 0.64; Met, 0.27; Thr, 1.35; Trp, 0.21; Val, 0.81; Ala, 0.75; Asp, 1.41; Cys, 0.47; Glu, 1.71; Gly, 0.78; Pro, 0.91; and Ser, 1.06.
D14: N, 1.87; Arg, 0.30; His, 0.16; Ile, 0.33; Leu, 0.49; Lys, 0.30; Met, 0.12; Thr, 0.73; Trp, 0.12; Val, 0.43; Ala, 0.37; Asp, 0.73; Cys, 0.27; Glu, 0.80; Gly, 0.39; Pro, 0.48; and Ser, 0.55.
D21: N, 1.79; Arg, 0.31; His, 0.15; Ile, 0.32; Leu, 0.49; Lys, 0.28; Met, 0.12; Thr, 0.71; Trp, 0.12; Val, 0.43; Ala, 0.36; Asp, 0.72; Cys, 0.26; Glu, 0.80; Gly, 0.40; Pro, 0.48; and Ser, 0.56.
D28: N, 1.82; Arg, 0.36; His, 0.16; Ile, 0.34; Leu, 0.55; Lys, 0.32; Met, 0.14; Thr, 0.67; Trp, 0.13; Val, 0.45; Ala, 0.41; Asp, 0.74; Cys, 0.25; Glu, 0.89; Gly, 0.43; Pro, 0.48; and Ser, 0.54.
D35: N, 1.81; Arg, 0.32; His, 0.15; Ile, 0.30; Leu, 0.49; Lys, 0.28; Met, 0.12; Thr, 0.71; Trp, 0.12; Val, 0.42; Ala, 0.37; Asp, 0.72; Cys, 0.26; Glu, 0.81; Gly, 0.40; Pro, 0.47; and Ser, 0.56.
D42: N, 1.29; Arg, 0.20; His, 0.10; Ile, 0.20; Leu, 0.31; Lys, 0.18; Met, 0.07; Thr, 0.52; Trp, 0.09; Val, 0.29; Ala, 0.25; Asp, 0.49; Cys, 0.21; Glu, 0.53; Gly, 0.28; Pro, 0.34; and Ser, 0.40.

[2]Each value represents the mean of six replicates (14, 12 and 10 birds per replicate for 7, 14 and 21-d old birds, respectively; eight birds per replicate for 28 and 35-d old birds; and six birds per replicate for 42-d old birds).

[3]Semi-indispensable amino acids for poultry.

DAA = Average digestibility of dispensable amino acids; IAA = Average digestibility of indispensable amino acids; TAA = Average digestibility of all amino acids.

No age influence (P > 0.05) was recorded for the SID protein content of maize (P > 0.05; Table 7.7). The SID contents of total IAA, DAA and total AA was influenced by age (quadratic; P < 0.05 to < 0.001). The SID content of total AA was higher at d 7 (54.1 g/kg). The SID contents increased from d 14 to 21 and plateaued until d 42. The SID contents of all individual AA, except Thr and Ser, were influenced quadratically (P < 0.05 to < 0.001) by bird age. A linear pattern (P < 0.001) was observed for age effect on the SID content of Ser.

Table 7.7. Influence of age on standardised digestible protein (CP) and amino acid contents[1] (g/kg) of maize, as-received basis

	Age (d)							Orthogonal polynomial contrasts	
Parameter	7	14	21	28	35	42	Pooled SEM	Linear	Quadratic
CP	63.4	55.4	62.5	61.7	61.5	58.9	0.61	0.295	0.685
Indispensable amino acids									
Arg	3.14	2.84	3.23	3.22	3.19	3.12	0.019	0.001	0.003
His	1.74	1.68	1.79	1.78	1.77	1.75	0.017	0.034	0.039
Ile	2.10	1.88	2.28	2.24	2.18	2.14	0.025	0.001	0.001
Leu	7.34	7.06	7.75	7.65	7.51	7.45	0.048	0.001	0.001
Lys	1.64	1.36	1.99	1.93	1.88	1.81	0.033	0.001	0.001
Met	1.18	1.05	1.26	1.24	1.21	1.18	0.013	0.001	0.001
Thr	2.36	1.75	2.26	2.22	2.26	2.13	0.036	0.348	0.119
Trp	0.45	0.37	0.48	0.48	0.48	0.46	0.008	0.001	0.048
Val	2.78	2.49	3.10	3.07	3.00	2.96	0.034	0.001	0.001
IAA	22.7	20.5	24.2	23.8	23.5	22.9	0.22	0.001	0.001
Dispensable amino acids									
Ala	4.60	4.32	4.80	4.74	4.63	4.56	0.037	0.043	0.001
Asp	4.11	3.49	4.30	4.23	4.14	4.02	0.048	0.002	0.017
Cys[2]	1.33	1.24	1.24	1.22	1.23	1.20	0.012	0.001	0.013
Glu	11.3	10.6	11.8	11.5	11.4	11.2	0.083	0.026	0.002
Gly[2]	2.24	1.86	2.41	2.38	2.35	2.29	0.034	0.001	0.034
Pro	4.73	4.47	4.96	4.89	4.88	4.85	0.044	0.001	0.047
Ser	2.97	2.41	3.01	2.95	2.99	2.88	0.039	0.001	0.996
DAA	31.2	28.4	32.5	31.9	31.6	31.0	0.28	0.002	0.018
Total AA	54.1	48.9	56.6	55.8	55.1	53.9	0.49	0.001	0.005

Standardised digestible amino acid content (g/kg) = [ingredient amino acid content (g/kg) × standardised ileal digestibility (%)]/100.

[1]Each value represents the mean of six replicates (14, 12 and 10 birds per replicate for 7, 14 and 21-d old birds, respectively; eight birds per replicate for 28 and 35-d old birds; and six birds per replicate for 42-d old birds).

[2]Semi-indispensable amino acids for poultry.

DAA = Total digestible dispensable amino acid contents; IAA = Total digestible indispensable amino acid contents; Total AA = Total digestible content of all amino acids.

7.4.4. Ileal digestibility coefficients of N and AA in barley

The impact of broiler age on AIDC, SIDC and SID content of N and AA in barley is presented in Tables 7.8, 7.9 and 7.10, respectively.

The AIDC of N, average digestibility of IAA, DAA and TAA increased (quadratic; P < 0.001) from d 7 to 21, and then plateaued from d 21 to 42 (Table 7.8). The AIDC of all individual AA in barley increased in a quadratic manner (P < 0.001) as the birds grew older.

The SIDC of N, average SIDC of IAA, DAA and TAA increased quadratically (P < 0.001) with advancing age of broiler (Table 7.9). The values increased from d 7 to 21, and then plateaued until d 42. The lower SIDC of TAA was recorded at d 7 (0.617), followed by d 14 (0.738). The SIDC of every single AA in barley increased (quadratic; P < 0.05 to 0.001) with bird age with lower values on d 7.

Table 7.8. Apparent ileal digestibility coefficients[1] of nitrogen (N) and amino acids of barley at different ages of broilers[1]

	Age (d)							Orthogonal polynomial contrasts	
Parameter	7	14	21	28	35	42	Pooled SEM	Linear	Quadratic
N	0.504	0.670	0.731	0.713	0.730	0.720	0.0142	0.001	0.001
Indispensable amino acids									
Arg	0.611	0.705	0.803	0.772	0.802	0.791	0.0135	0.001	0.001
His	0.544	0.703	0.726	0.702	0.729	0.719	0.0142	0.001	0.001
Ile	0.436	0.660	0.749	0.725	0.747	0.749	0.0155	0.001	0.001
Leu	0.537	0.717	0.794	0.766	0.788	0.783	0.0133	0.001	0.001
Lys	0.230	0.545	0.739	0.682	0.717	0.717	0.0211	0.001	0.001
Met	0.479	0.692	0.812	0.777	0.796	0.789	0.0186	0.001	0.001
Thr	0.261	0.524	0.646	0.633	0.669	0.655	0.0196	0.001	0.001
Trp	0.430	0.646	0.709	0.694	0.724	0.713	0.0163	0.001	0.001
Val	0.449	0.652	0.756	0.737	0.753	0.752	0.0144	0.001	0.001
IAA	0.442	0.649	0.748	0.721	0.747	0.741	0.0155	0.001	0.001
Dispensable amino acids									
Ala	0.423	0.633	0.734	0.699	0.722	0.717	0.0158	0.001	0.001
Asp	0.328	0.569	0.703	0.680	0.707	0.699	0.0179	0.001	0.001
Cys[2]	0.620	0.764	0.743	0.731	0.739	0.728	0.0171	0.004	0.001
Glu	0.709	0.812	0.845	0.822	0.832	0.825	0.0107	0.001	0.001
Gly[2]	0.360	0.561	0.675	0.658	0.676	0.673	0.0158	0.001	0.001
Pro	0.681	0.782	0.805	0.793	0.803	0.799	0.0112	0.001	0.001
Ser	0.413	0.622	0.727	0.708	0.741	0.728	0.0159	0.001	0.001
DAA	0.505	0.678	0.747	0.727	0.746	0.738	0.0140	0.001	0.001
TAA	0.469	0.662	0.748	0.724	0.747	0.739	0.0148	0.001	0.001

[1]Each value represents the mean of six replicates (14, 12 and 10 birds per replicate for 7, 14 and 21-d old birds, respectively; eight birds per replicate for 28 and 35-d old birds; and six birds per replicate for 42-d old birds).

[2]Semi-indispensable amino acids for poultry.

DAA = Average digestibility of dispensable amino acids; IAA = Average digestibility of indispensable amino acids; TAA = Average digestibility of all amino acids.

Table 7.9. Standardised ileal digestibility coefficients[1] of nitrogen (N) and amino acids of barley at different ages of broilers[2]

	Age (d)							Orthogonal polynomial contrasts	
Parameter	7	14	21	28	35	42	Pooled SEM	Linear	Quadratic
N	0.674	0.759	0.816	0.799	0.816	0.782	0.0143	0.001	0.001
Indispensable amino acids									
Arg	0.724	0.755	0.855	0.831	0.854	0.824	0.0135	0.001	0.001
His	0.652	0.762	0.780	0.762	0.783	0.756	0.0143	0.001	0.001
Ile	0.574	0.732	0.821	0.801	0.813	0.793	0.0155	0.001	0.001
Leu	0.650	0.773	0.851	0.829	0.844	0.819	0.0133	0.001	0.001
Lys	0.382	0.616	0.806	0.757	0.783	0.758	0.0211	0.001	0.001
Met	0.611	0.752	0.874	0.847	0.854	0.823	0.0186	0.001	0.001
Thr	0.595	0.705	0.822	0.798	0.845	0.784	0.0196	0.001	0.001
Trp	0.564	0.721	0.787	0.776	0.802	0.769	0.0163	0.001	0.001
Val	0.578	0.719	0.825	0.809	0.819	0.799	0.0144	0.001	0.001
IAA	0.592	0.726	0.825	0.801	0.822	0.792	0.0155	0.001	0.001
Dispensable amino acids									
Ala	0.566	0.703	0.803	0.778	0.793	0.764	0.0158	0.001	0.001
Asp	0.509	0.664	0.796	0.776	0.800	0.763	0.0179	0.001	0.001
Cys[3]	0.806	0.870	0.844	0.829	0.843	0.809	0.0171	0.553	0.045
Glu	0.771	0.841	0.874	0.854	0.861	0.844	0.0107	0.001	0.001
Gly[3]	0.513	0.639	0.753	0.742	0.755	0.729	0.0158	0.001	0.001
Pro	0.755	0.821	0.844	0.831	0.842	0.827	0.0112	0.001	0.001
Ser	0.623	0.732	0.838	0.815	0.852	0.808	0.0159	0.001	0.001
DAA	0.649	0.753	0.822	0.804	0.821	0.792	0.0140	0.001	0.001
TAA	0.617	0.738	0.823	0.802	0.822	0.792	0.0148	0.001	0.001

[1]Apparent digestibility values were standardised using the following basal ileal endogenous flow values (g/kg DM intake), determined by the feeding N-free diet at different ages (Chapter 5); see Table 7.6.

[2]Each value represents the mean of six replicates (14, 12 and 10 birds per replicate for 7, 14 and 21-d old birds, respectively; eight birds per replicate for 28 and 35-d old birds; and six birds per replicate for 42-d old birds).

[3]Semi-indispensable amino acids for poultry.

DAA = Average digestibility of dispensable amino acids; IAA = Average digestibility of indispensable amino acids; TAA = Average digestibility of all amino acids.

The SID protein content of barley increased (quadratic; P < 0.001) from 77.7 g/kg on d 7 to 94.1 g/kg on d 21, and then plateaued from d 21 to 42 (Table 7.10). The SID contents of total IAA, DAA and total AA increased (quadratic; P < 0.001) from d 7 to 21 and then plateaued to d 42. The SID contents of all individual AA increased in a quadratic manner (P < 0.05 to 0.001) with lower values on d 7, that increased either at d 14 or 21 and then plateaued until d 42.

Table 7.10. Influence of age on standardised digestible protein (CP) and amino acid contents[1] (g/kg) of barley, as-received basis

	Age (d)							Orthogonal polynomial contrasts	
Parameter	7	14	21	28	35	42	Pooled SEM	Linear	Quadratic
CP	77.7	87.5	94.1	92.1	92.9	90.1	1.57	0.001	0.001
Indispensable amino acids									
Arg	3.82	3.99	4.51	4.39	4.51	4.35	0.071	0.001	0.001
His	1.55	1.81	1.85	1.81	1.86	1.79	0.034	0.001	0.001
Ile	2.28	2.91	3.27	3.19	3.24	3.16	0.062	0.001	0.001
Leu	4.89	5.82	6.41	6.25	6.36	6.17	0.100	0.001	0.001
Lys	1.42	2.29	2.99	2.82	2.91	2.82	0.079	0.001	0.001
Met	1.08	1.33	1.55	1.49	1.51	1.46	0.033	0.001	0.001
Thr	2.10	2.49	2.91	2.82	2.99	2.78	0.069	0.001	0.001
Trp	0.76	0.97	1.06	1.05	1.08	1.04	0.021	0.001	0.001
Val	3.19	3.97	4.55	4.46	4.53	4.41	0.079	0.001	0.001
IAA	21.1	25.6	29.1	28.3	28.9	27.9	0.53	0.001	0.001
Dispensable amino acids									
Ala	2.57	3.19	3.65	3.53	3.60	3.47	0.072	0.001	0.001
Asp	3.45	4.50	5.39	5.26	5.43	5.17	0.122	0.001	0.001
Cys[2]	1.79	1.94	1.88	1.85	1.88	1.80	0.038	0.553	0.044
Glu	18.8	20.5	21.2	20.8	20.9	20.5	0.259	0.001	0.001
Gly[2]	2.28	2.84	3.34	3.29	3.35	3.24	0.070	0.001	0.001
Pro	8.12	8.84	9.08	8.94	9.06	8.89	0.121	0.001	0.001
Ser	2.75	3.23	3.69	3.59	3.76	3.56	0.069	0.001	0.001
DAA	38.1	43.3	46.6	45.6	48.0	45.0	0.70	0.001	0.001
Total AA	60.8	70.6	77.4	75.5	76.9	74.6	1.25	0.001	0.001

Standardised digestible amino acid content (g/kg) = [ingredient amino acid content (g/kg) × standardised ileal digestibility (%)]/100.

[1]Each value represents the mean of six replicates (14, 12 and 10 birds per replicate for 7, 14 and 21-d old birds, respectively; eight birds per replicate for 28 and 35-d old birds; and six birds per replicate for 42-d old birds).

[2]Semi-indispensable amino acids for poultry.

DAA = Total digestible dispensable amino acid contents; IAA = Average digestibility of indispensable amino acids; Total AA = Total digestible content of all amino acids.

7.4.5. Uplift in digestibility coefficients due to correction for endogenous amino acid losses as influenced by age

The percentage increase in the digestibility coefficients of N and AA after standardisation of apparent values for basal endogenous N and AA losses is shown in Table 7.11.

The correction of AIDC for age-appropriate endogenous N and AA losses resulted in an increase in the SIDC regardless of age, though the extent of increase reduced as the birds grew older. After standardisation of the AIDC estimates, the average TAA digestibility coefficients

increased in maize by 32.5% (d 7), 17% (d 14), 13.9% (d 21), 14.7% (d 28), 14.2% (d 35), and 9.85% (d 42). In case of barley, the corresponding increases were recorded as 31.6% (d 7), 11.5% (d 14), 10.0% (d 21), 10.8% (d 28), 10.0% (d 35), and 7.17% (d 42).

Table 7.11. Percentage increase in digestibility coefficients of nitrogen (N) and amino acids in maize and barley after correction of apparent ileal digestibility coefficients for age-appropriate endogenous amino acid losses of broilers

	Maize						Barley					
	Age (d)						Age (d)					
Parameter	7	14	21	28	35	42	7	14	21	28	35	42
N	43.8	22.1	18.4	18.9	18.7	13.3	33.7	13.3	11.6	12.1	11.8	8.61
Indispensable amino acids												
Arg	22.7	10.1	9.14	10.6	9.41	5.82	18.5	7.09	6.48	7.64	6.48	4.17
His	16.9	8.87	7.58	8.43	7.83	5.24	19.9	8.39	7.44	8.55	7.41	5.15
Ile	34.4	17.4	13.9	15.3	13.5	8.87	31.7	10.9	9.61	10.5	8.84	5.87
Leu	12.9	6.24	5.66	6.46	5.85	3.74	21.0	7.81	7.18	8.22	7.11	4.60
Lys	50.7	23.3	13.9	16.6	14.8	8.99	66.1	13.0	9.07	11.0	9.21	5.72
Met	24.2	11.1	9.18	10.8	9.06	5.24	27.6	8.67	7.64	9.01	7.29	4.31
Thr	96.6	55.9	37.1	34.8	37.2	26.7	128	34.5	27.2	26.1	26.3	19.7
Trp	65.7	36.5	27.8	29.6	27.5	19.6	31.2	11.6	11.0	11.8	10.8	7.85
Val	33.6	17.4	13.6	14.5	13.7	9.54	28.7	10.3	9.13	9.77	8.76	6.25
IAA	35.1	17.9	14.2	14.0	14.5	9.77	33.9	11.9	10.3	11.1	10.0	6.88
Dispensable amino acids												
Ala	16.2	7.96	6.90	8.08	7.39	4.85	33.8	11.1	9.40	11.3	9.83	6.56
Asp	41.7	21.9	16.7	17.8	17.5	11.9	55.2	16.7	13.2	14.1	13.2	9.16
Cys[1]	36.5	22.9	21.6	21.1	22.6	17.4	30.0	13.9	13.6	13.4	14.1	11.1
Glu	14.9	6.89	6.16	7.13	6.50	4.27	8.74	3.57	3.43	3.89	3.49	2.30
Gly[1]	42.3	22.5	16.7	18.1	17.1	11.9	42.5	13.9	11.6	12.8	11.7	8.32
Pro	19.7	10.2	9.12	9.11	9.15	6.52	10.9	4.99	4.84	4.79	4.86	3.50
Ser	44.3	24.5	18.9	18.5	19.2	13.5	50.8	17.7	15.3	15.1	14.9	10.9
DAA	29.4	15.9	13.3	13.9	13.8	9.77	28.5	11.1	10.0	10.6	10.1	7.32
TAA	32.5	17.0	13.9	14.7	14.2	9.85	31.6	11.5	10.0	10.8	10.0	7.17

[1]Semi-indispensable amino acids for poultry.

DAA = Average digestibility of dispensable amino acids; IAA = Average digestibility of indispensable amino acids; TAA = Average digestibility of all amino acids.

7.5. Discussion

Several reports exist on age-related AA digestibility in ingredients for poultry, but the results are contradictory and inconclusive. Some studies have documented reductions in protein or AA digestibility (Hakansson and Eriksson, 1974; Fonolla *et al.*, 1981; Zelenka and Liska, 1986) with advancing age, while others reported increases in digestibility estimates

(Wallis and Balnave, 1984; ten Doeschate *et al*., 1993; Huang *et al.*, 2005). The present experiment aimed at identifying whether the broiler age has an impact on the SIDC of AA in maize and barley.

Although it may be intuitively expected that the AA digestibility in broilers will vary depending on broiler age, studies comparing the SID of AA corrected using age-appropriate EAA are limited (Adedokun *et al.*, 2007a, 2008; Szczurek *et al.*, 2020). An extensive study (Chapter 6) has been conducted determining the SIDC in wheat and sorghum at six different ages (d 7, 14, 21, 28, 35 and 42) of broilers. Most available data on the AID and SID AA of ingredients have been determined using older broilers (22 to 35 d of age) and the estimates are applied in feed formulations regardless of broiler age. In the current research, the AIDC AA and SIDC AA in maize and barley were determined from hatching to the end of growth cycle of broilers, and the AIDC values were standardised using age-appropriate basal EAA losses.

Nutrient composition

The proximate nutrient contents of maize and barley were comparable to the values reported previously (Chapter 3; Abdollahi *et al.*, 2010a; Bandegan *et al.*, 2011; Lasek *et al.*, 2012; Perera *et al.*, 2019). The higher starch content in maize (590 g/kg) than barley (541 g/kg) was expected. Due to high starch (620 to 720 g/kg) and crude fat (34 to 52 g/kg) contents in maize, it contains higher energy than any other grain. In this study, both grains had a higher GE content (16.3 g/kg). However, the energy content may vary depending on the amylose level

in starch (Svihus *et al.*, 2005; Lasek *et al.*, 2012), factors like starch encapsulation, ratio of amylose and amylopectin, antinutritional factors, especially enzyme inhibitors and resistant starch (Cowieson, 2005). The analysed CP content in maize (67.8 g/kg) was lower than the range (71 to 94 g/kg) reported by Cowieson (2005).

The CP content (115 g/kg) in barley was marginally higher than the value (101 g/kg) reported by Perera *et al.* (2019) and lower than range (121 to 180 g/kg) of Bandegan *et al.* (2011). The higher CP content in barley compared to maize was in agreement with previous studies (Bryden *et al.*, 2009a; Rodehutscord *et al*., 2016). The AA contents of maize and barley were identical or close to those reported in previous studies (Chapter 3; Ravindran *et al.*, 2007; Bryden *et al.*, 2009a; Bandegan *et al.*, 2011; Rodehutscord *et al*., 2016; Perera *et al.*, 2019).

Performance, gizzard pH and jejunal digesta viscosity

As anticipated, regardless of the grain type, an increase in both the DFI and DWG was observed with advancing age. The gizzard pH on d 7 fed maize (2.53) and barley diets (2.14) was close to the values of 2.39 and 2.33 observed by David *et al.* (2020) and Morgan *et al.* (2014), respectively, for broilers of similar age. The reduction of gizzard pH at d 14 compared to d 7 was in line with the findings of David *et al*. (2020) fed a maize-based diet. Following analysis of 15 published studies, Angel *et al.* (2010) also reported a reduction in gizzard pH in broilers at d 14. According to Rynsburger (2009), the secretion of gastric acid

in the proventriculus increased from d 2 to 15, causing a decrease in pH.

As observed by Nitsan *et al.* (1991), the secretion and activity of digestive enzymes, and HCl secretion from proventriculus increases with broiler age. However, the observed increase in gizzard pH with bird age in both diets could be, at least in part, explained by increasing intake of feed with neutral pH with advancing age (Ravindran, 2013). An increased feed load can dilute the HCl secreted and consequently increase the gizzard pH. The secretion of HCl is fundamental to sustain an acidic environment and to convert pepsinogen to pepsin, the first step in protein digestion (Rynsburger, 2009). Thus, the implication is that a high gizzard pH would compromise the protein digestion. In this experiment, however, gizzard pH was not correlated ($r = 0.242$; $P > 0.05$) with the average SIDC of TAA in maize. On the contrary, in the case of barley, there was a moderate positive correlation ($r = 0.644$; $P < 0.001$) between the gizzard pH and the average SIDC of TAA. Therefore, it could be speculated that, though the gizzard pH was elevated by age in barley, contrary to expectations, AA digestibility also increased. Besides the pH, there are several other factors, to be discussed later, might potentially influence the AA digestibility in barley.

The jejunal digesta viscosity in maize was not influenced by age and no correlation ($r = -0.292$; $P > 0.05$) existed between the digesta viscosity and the average SIDC of TAA. In barley, the range (2.69-2.94) of jejunal digesta viscosity in barley at different ages was notably higher

than that of maize (2.03-2.34), with viscosity being higher on d 7 and 42. Yu *et al.* (1998) reported the duodenal digesta viscosity in 3- and 6-weeks old broilers at different replacement levels of maize with barley, and observed elevated viscosity with increasing barley inclusions. A positive relationship exists between the soluble NSP content and digesta viscosity (Choct and Annison, 1992a). Maize contains negligible amounts of soluble NSP compared to wheat and barley (Choct, 2015). The NSP in plant cell walls such as β-glucans, the major NSP in barley, and the pentosans of rye and wheat exhibit significant antinutritive effects in poultry (Burnett, 1966; Antoniou *et al.*, 1981; Campbell *et al.*, 1989). A portion of NSP of high molecular weight dissolves in the gastrointestinal tract, increasing the viscosity of gut contents that impedes the digestion and absorption of nutrients (Fengler and Marquardt, 1988; Choct *et al.*, 1999). In the case of barley, however, the jejunal digesta viscosity tended ($r = -0.292$; $P = 0.084$) to be negatively correlated with the average SIDC of TAA.

Besides the soluble NSP content, a myriad of factors like growing location (Campbell *et al*., 1989), storage time (Fuente *et al*., 1998), ingredient inclusion level (Fuente *et al*., 1995; Yu *et al*., 1998), age of bird (Salih *et al*., 1991), heat processing (Gracia *et al*., 2003) and pelleting temperature (Samarasinghe *et al*., 2000), have been shown to influence the digesta viscosity in barley-containing diets. It is difficult to explain high viscosity at d 42 observed in the current work, since previous studies (Salih *et al*., 1991; Petersen *et al*., 1999; Gracia *et al*., 2003) have reported decreased intestinal viscosity in older birds feeding

barley-based diets. During the evaluation of a high viscosity hull-less barley, Salih *et al*. (1991) recorded a drop in the digesta viscosity in broilers from two weeks (2.59 cP) to eight weeks of age (1.74 cP). These findings support the evaluation of mechanisms of viscosity as a function of grain type and age of birds (Bedford, 2018).

Ileal digestibility coefficients of nitrogen and amino acids

The first week is the most critical period in bird's life when they consume only small amounts of feed and depend mostly on the nutrients from residual yolk (Noy *et al.*, 1996; Noy and Sklan, 1997). Notable changes occur in the morphology and development of the gastrointestinal tract during the first few weeks of life that contribute to improved digestion and absorption of nutrients. During the first few weeks, there is high protein demand for the development of supply organs and muscle. The secretion and activity of different proteolytic enzymes like trypsin, chymotrypsin, intestinal peptidase and dipeptidase are also generally increase with age (Marchim and Kulka, 1967; Nitsan *et al.*, 1991; Jin *et al.*, 1998).

From previous studies, it is evident that the AIDC of AA is variable depending on broiler age (Fonolla *et al.*, 1981; Wallis and Balnave, 1984; Noy and Sklan, 1995; Batal and Parsons, 2002; Huang *et al.*, 2005). In the current experiment, with advancing age, an increase in the AIDC of N, all individual AA (except Cys) and average of TAA was observed in broilers fed maize-based diets. Compared to d 7, the

average AIDC of all AA increased by 22.0, 19.4, 18.6 and 20.5% at d 21, 28, 35 and 42, respectively.

The increased AIDC AA with age is in agreement with some previous findings (Batal and Parsons, 2002; Huang *et al.*, 2005; Adedokun *et al.*, 2008). A lower ileal N digestibility at d 4 compared to d 14 and 21 has been reported in maize-soybean meal-based diets (Noy and Sklan, 1995; Uni *et al.*, 1995). An increase in the total tract AIDC AA from 1 to 10 days of age was reported with a maize-soybean meal diet by Batal and Parsons (2002). Wallis and Balnave (1984), recorded an increase in AIDC AA from d 30 to 50 post-hatch feeding a conventional finisher diet combined with wide range of feed ingredients (wheat, sorghum, soyabean meal, cottonseed meal, meat and bone meal, poultry tallow, poultry offal meal, feather meal). Huang *et al.* (2005) determined the AIDC AA of eight ingredients (maize, wheat, sorghum, soybean meal, canola meal, meat and bone meal, cotton seed meal and, mill run) at three broiler ages (d 14, 28 and 42). Combining all the results it was concluded that, in general, the digestibility increased with advancing age. The trends, however, were variable depending on the AA and ingredient type. In their study, higher AIDC was determined at d 28 and 42 compared to d 14 in the case of maize, soybean meal, canola meal, meat and bone meal. The AIDC in millrun at d 42 was higher than those at 14 and 28 d. Whilst there was no age influence in the AIDC of most AA in cottonseed meal, Lys and Arg digestibility increased with age. On the contrary, in wheat, the AIDC of most individual AA was recorded to be higher at d 14 than at d 28 and 42. In sorghum, the AIDC

AA was higher at d 42 compared to d 28, but similar to those at d 14 with the exception of His, Lys, Ser and Gly which were higher at d 42.

In the present study, with the exception of Thr, linear or quadratic responses to broiler age were observed for the SIDC of all individual AA in maize. Unlike the AIDC, the pattern of increase in SIDC AA in maize was not gradual with increasing age. Rather, a decline was observed from d 7 to d 14 followed by an increase from d 14 to 21, plateau between d 21 and 35, and a decline at d 42.

Differing age-related trends between the AIDC and SIDC estimates have also been observed by Adedokun *et al.* (2008). These researchers, comparing AA digestibility between d 5 and 21 of broilers in five ingredients (maize, light and dark distiller's dried grains with solubles, canola meal and soybean meal), reported an increased AID AA with age in all test ingredients. However, increasing broiler age elevated the SID AA only in maize and distiller's dried grains with solubles, and had no influence on the SID AA in soybean meal and canola meal.

Apart from age effect, another notable observation was made on the AA digestibility in maize in the current study. Though maize contained lower CP and TAA contents compared to barley (Table 7.3), the average SIDC in maize was 39.4% (d 7), 3.4% (d 14), 9.6% (d 21), 10.8% (d 28), 6.9% (d 35), and 8.5% (d 42) higher than barley. Compared to other cereals, maize contains low levels of soluble NSP and highly digestible nutrients for broilers (Cowieson, 2005). Barley contains high amount of soluble NSP compared to maize (Choct, 2015).

According to Andriotis *et al.* (2016), the barley endosperm is composed of β-glucans (70%) and arabinoxylans (20%). The high content of soluble β-glucan is the major antinutritional factor in barley and, to assess the potential feed value of barley for poultry, determination of the content and properties of β-glucan is crucial (Burnett, 1966). When birds are fed diets with high inclusions of barley, the NSP impedes the nutrient digestion and absorption by two mechanisms. First, the soluble NSP forms gel-like viscous matter, which impairs the interaction between nutrient substrates and endogenous enzymes (Burnett, 1966; Svihus *et al.*, 2000). Second, the insoluble NSP fraction exerts a "cage effect" by encapsulating the nutrients (starch, protein) in endosperm cells. The nutrients are enclosed in endosperm cells by intact cell wall structures that act as a physical barrier impeding the contact between nutrients and digestive enzymes. High levels of β-glucans result in thicker cell walls than low levels of ß-glucans (Åman and Graham, 1987; Oscarsson *et al*., 1997; Izydorczyk and Dexter, 2008).

In the present study, an increase in the AIDC of N, average AIDC of IAA, DAA and TAA with age was observed in barley. The AIDC of all AA increased from d 7 to 21 and then plateaued until d 42. Similar patterns of increase were observed in the SIDC of N, SIDC of all individual AA, average SIDC of IAA, DAA and TAA with lower values on d 7, then an increase to d 21, that plateaued from d 21 to 42. The average SIDC of TAA in barley at d 7 was 16.4% lower than d 14, 25.0% lower than d 21, and 23.4% lower than the average from d 28 to 42. Szczurek *et al.* (2020) determined the AA digestibility in three grain

sources (barley, triticale and wheat) in broilers at d 14 vs. 28 post-hatch. In barley and triticale, the average AIDC of IAA was notably higher at d 28 than 14. The average AIDC of IAA in barley was reported to be 0.730 on d 14 and 0.780 on d 28. The AIDC of most individual IAA and DAA increased at d 28 compared to d 14. The SIDC of all AA in barley and most AA in triticale was also higher at d 28. In the case of wheat, however, no difference was recorded for the average AIDC of IAA between the two ages. The average SIDC of IAA was higher at d 14 (0.880) was higher than d 28 (0.870). The effect of age on the AA digestibility in wheat and sorghum at six different ages (d 7, 14, 21, 28, 35 and 42) of broilers has already been documented (Chapter 6). The AIDC of all individual AA in wheat increased as the birds grew older. Although the average SIDC of TAA was unaffected by age, the digestibility of some individual AA (Met, Trp, Asp and Cys) was higher in the older birds. As observed by Rotter *et al.* (1990), the GIT of older birds is developed sufficiently enough to counteract any adverse effects of digesta viscosity induced by β-glucan. Similar observations were made in other studies (Salih *et al.*, 1991; Almirall and Esteve-Garcia, 1994), indicating that digestibility of barley diets improves with age and that viscosity is not a limiting factor in older birds (Almirall *et al.*, 1995).

Several factors may explain the increased AIDC AA with advancing age for maize and barley. Lower activity of pancreatic enzymes is a constraint for nutrient digestion in young chicks (Iji *et al.*, 2001b). Nitsan *et al.* (1991) measured the activities (units/kg BW) of trypsin

and chymotrypsin in the pancreas and small intestine from hatching to 23 d of age. The activity of trypsin was low at d 3 to 6 post-hatch, and increased on d 14. A gradual increase in chymotrypsin activity was also observed from hatch to d 14 and this remained constant afterwards. Maximum activities of trypsin and chymotrypsin were observed on d 11. In the intestinal contents, the activity of trypsin increased 10-folds from hatching to d 14 and that of chymotrypsin increased 3-folds by d 20. As stated by Tarvid (1995), the total intestinal peptidase (aminopeptidase and dipeptidase) activity increases with advancing age of broilers. Tarvid (1992) reported that the relative activity of aminopeptidase in all intestinal segments reduces after hatch, reaching the lowest point on d 10, and then increasing to d 15. In young birds, there is limited production of pancreatic enzymes, which is exacerbated by the high intestinal viscosity in barley (Almirall *et al.*, 1995), leading to reduced AA digestibility.

Another possible factor contributing to the increased apparent AA digestibility with age may be the size of digestive organs. In a study by Nitsan *et al.* (1991), the weight of pancreas increased by 10- and 30-folds from hatch to d 8 and d 23, respectively. Likewise, the weight of small intestine increased by 10-folds at d 8 and 20-folds at d 23. Insoluble NSP is considered to facilitate the development of gizzard (Svihus, 2011a). As barley contains high insoluble NSP (142 g/kg), it is likely that more developed gizzard in barley will facilitate better digestion of nutrients by mechanical breakdown of digesta. Well-

developed GIT in older birds can overcome the negative viscosity effects of β-glucans (Almirall and Esteve-garcia, 1994).

Digesta retention time in the GIT plays a vital role in nutrient digestion because it determines the actual contact time among nutrients, digestive enzymes and microbiota (Clemens *et al.*, 1975; Mateos *et al.*, 1982). An increase in digesta retention enhances absorption of nutrients by increasing the exposure time between the digesta and intestinal absorptive surface (Washburn, 1991). Feed passage time through the GIT is reduced with bird age (Wilson *et al.*, 1980; Shires *et al.*, 1987). Therefore, as birds grow older, digestion and absorption are facilitated by the longer exposure of digesta. Increased digesta retention, in combination with a well-developed digestive tract, especially foregut, in older birds may also increase protein and AA digestibility by enhancing intestinal refluxes, subsequently re-exposing the digesta to pepsin (Abdollahi and Ravindran, 2013).

Higher AA digestibility in barley with advancing age might be due to better tolerance of older birds to high NSP and viscosity owing to the increased stability of intestinal microbiota with advancing age (Choct and Annison, 1992a). Gut microbiota, a major consumer of AA, may potentially influence the digestion by competing for nutrients (Stanley *et al.*, 2013; Mancabelli *et al.*, 2016). The GIT of the newly hatched chick is sterile. The primary source of initial microbiota is the farm environment where the hatchlings are reared (Donaldson *et al.*, 2017). With increasing age, a cascade of changes occurs in microbiota including species diversity, followed by complexity of population

structure, and finally maturation and stabilisation. This process continues with age in commercial broilers and the microbiota profile is stabilised by week 3 (Oakley *et al.*, 2014; Johnson *et al.*, 2018). In birds fed barley-based diets, an increased gut microbial numbers can irritate and thicken the gut lining, damage the microvilli and reduce absorption of nutrients (Visek, 1978). According to Choct *et al.* (1995), the NSP can promote microbial growth and fermentation. Increased degradation of soluble NSP and the resultant reduction in the viscosity with age limit the bacterial growth and impede the adverse effects caused by excessive population of microbiota.

In contrast to the present findings, earlier age-related studies measuring the total tract AA digestibility in poultry (Hakansson and Eriksson, 1974; Fonolla *et al.*, 1981; Hassan and Delpech, 1986; Zelenka and Liska, 1986; Carré *et al.*, 1991; Zuprizal *et al.*, 1992; ten Doeschate *et al.*, 1993) indicated higher protein and AA digestibility in younger birds. According to Carré *et al.* (1991), the apparent protein digestibility in pea was lower in adult roosters than in 3-week-old broilers. Fonolla *et al.* (1981) reported a decline in the apparent digestibility of protein in broilers with advancing age (d 21 vs. d 52), which was attributed to an increased excretion of metabolic N. Similar reductions in protein or AA digestibility with advancing age of broilers were also reported by Zuprizal *et al.* (1992) and ten Doeschate *et al.* (1993). However, these earlier studies based on total tract digestibility are not comparable with current findings due to possible microbial fermentation in the hindgut and, consequently, modifications in the AA

constituents (Salter and Coats, 1971; Ravindran and Bryden, 1999), and the contamination of AA from urine (Sykes, 1971; Terpstra, 1978).

The reduction in ileal digestibility of other major nutrients in feed ingredients with broiler age has also been reported. Adedokun *et al.* (2007a) reported 18.5% higher AIDC for TAA in meat and bone meal in 5-d old broilers (0.705) compared to that of 21-d broilers (0.595). This pattern remained unchanged even after the standardisation of AIDC, with SIDC values of 0.760 and 0.632 at d 5 and d 21, respectively. In a previous experiment (Chapter 6), though there was no age influence on the average AIDC of TAA in sorghum, the average SIDC of TAA on d 7 (0.903) was 6.9% higher than on d 14 (0.844) and 8.5% higher than the average from d 21 to d 42 (0.819-0.854). Moss *et al.* (2020) reported that the starch digestibility decreased in broilers fed a maize-based diet from 0.935 on d 10 to 0.885 on d 35. For wheat, the corresponding values were recorded as 0.987 and 0.904 on d 10 and d 35, respectively. Li *et al.* (2018) reported higher AIDC of Ca in 9-d old broilers (0.53-0.77) compared to 21 d (0.44-0.59) at varying dietary Ca levels (6.5, 8.0 and 9.5 g/kg). A linear decrease in the AIDC of Ca with age was recorded by David *et al.* (2020). The digestibility coefficients declined from 0.51 at d 7 to 0.36 at d 21, and 0.27 at d 42. Following the feeding a maize-canola meal-based diet, Babatunde and Adeola (2020) observed a decline in both the AID and SID of phosphorus with advancing age (d 13 vs. 21) of broilers. It is not straightforward to make comparison between published age-related SID digestibility estimates because of differences in ingredient type (Lemme *et al.*, 2004; Huang

et al., 2005), assumed endogenous losses (Chapter 5), secretion and activity of nutrient digestive enzymes (Jin *et al.*, 1998), development and activity of gastrointestinal microbiota (Oakley *et al.*, 2014), environment where the birds are kept, chemical nature of nutrients, and methodology.

In the SIDC calculations, the AIDC were corrected for the basal endogenous AA losses from various digestive, pancreatic and enzymatic secretions (Ravindran, 2021) and unsurprisingly these two values were different with SIDC being higher. In practical feed formulations, the SIDC AA of ingredients is preferred because it is more additive especially when mixed into a complete diet. Moreover, standardisation eliminates the underestimation of some nutritionally critical and less additive AA in poultry diets (Cowieson *et al.*, 2019; An *et al.*, 2020). Though there are some constraints in AIDC estimates, it is reported in this study for better understanding the magnitude of age impact on the SIDC due to correction for age-dependent EAA losses. The differences between the AIDC and SIDC illustrated in Figures 7.1 and 7.2 were due to the correction of apparent values by age-appropriate EAA losses determined in a previous study in our laboratory (Chapter 5). Although data (Chapters 3 and 4; Lemme *et al.*, 2004; Bandegan *et al.*, 2011; Kim *et al.*, 2012a; Ravindran *et al.*, 2014b, 2017; Blok and Dekker, 2017) exist on the SID AA of different poultry feed ingredients, only few studies (Garcia *et al.*, 2007; Adedokun *et al.*, 2007a, 2008; Szczurek *et al.*, 2020) have determined the SID AA at different broiler ages. Except for some (Chapter 6; Adedokun *et al.*,

2007a, 2008; Szczurek *et al.*, 2020), all previous studies have used a single EAA flow value, derived from older birds, to standardise the AIDC values at different ages. From our previous findings (Chapter 5), the basal EAA losses of TAA at d 7 (12.93 g/kg DMI) was twice than that of the average of d 14 to 35 (6.19 g/kg DMI), and almost three times higher than d 42 (4.48 g/kg DMI). These resulted in substantial differences in the percentage of increase in SIDC over AIDC (Table 7.11) after correction of apparent data when age-appropriate EAA losses are used. Hence, correcting AIDC AA using a single EAA flow value across ages underestimates the SIDC AA of feed ingredients in young birds and overestimates in older birds.

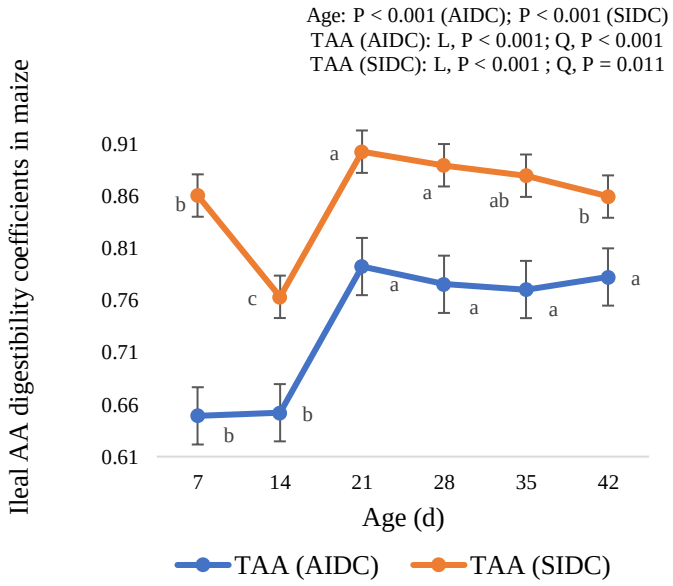

Figure 7.1. Apparent and standardised ileal digestibility coefficients of total amino acids (TAA) in maize (bars represent means ± SE) as influenced by broiler age. [a,b]Values with different superscripts differ significantly ($P < 0.05$). L, Linear; Q, Quadratic.

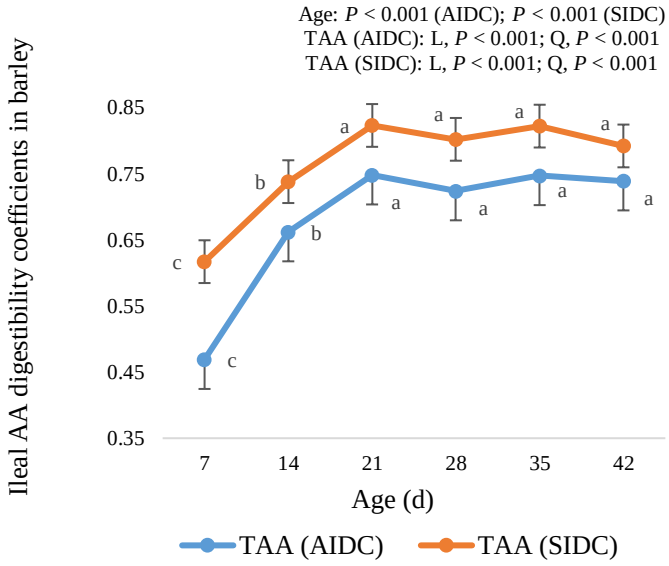

Figure 7.2. Apparent and standardised ileal digestibility coefficients of total amino acids in barley (bars represent means ± SE) as influenced by broiler age. [a,b,c]Values with different superscripts differ significantly ($P < 0.05$). L, Linear; Q, Quadratic.

In general, the magnitude of increase in SIDC AA of maize and barley after correction for age-appropriate EAA decreased with advancing broiler age. After correcting for age-appropriate EAA, an increase of 32.5% in the average SIDC of TAA at d 7 was observed in maize that was almost two times higher compared to that from d 14 to 35 (13.9-17.0%), and three times more than the increase at d 42 (9.85%). Similar to maize, the average SIDC of TAA in barley at d 7 was 31.5% higher than the average AIDC of TAA, which decreased to 7.17% at d 42.

To summarise, several factors such as composition of grains, antinutritive factors, digestive tract development, secretion of enzymes, nutrient load, digesta passage rate and gut microbiota contribute to the

age effect on the AA digestibility in broilers. The endogenous AA losses is a key factor with the greatest impact on the SIDC being observed in young broilers.

7.6. Conclusions

The present study, for the first time, gives information on the SIDC of AA in maize and barley from hatching to the end of broiler growth cycle. The findings suggest that the age influence on AA digestibility is dependent on cereal type and AA. The AIDC AA in maize increased with advancing age. The SIDC AA was higher at d 7 that decreased at d 14, increased and plateaued between d 21 and 35. A further decrease was observed at d 42. In the case of barley, both the AIDC and SIDC of AA increased as the birds grew older. Standardisation of AIDC AA with age-appropriate EAA flows made a marked difference in the SIDC, especially in case of maize. Application of single EAA value for correction of AIDC for broilers of different ages will result in the underestimation of the SIDC AA in young birds and overestimation in older birds. It is concluded that the precision of feed formulations can be improved by considering age-specific EAA values for the standardisation of AIDC AA values.

CHAPTER 8 Influence of age on the standardised ileal amino acid digestibility of soybean meal and canola meal in broilers

8.1. Abstract

The influence of broiler age on the standardised ileal digestibility coefficients (SIDC) of nitrogen (N) and amino acids (AA) in two protein sources (PS) [soybean meal (SBM) and canola meal (CM)] was investigated. Six age groups (d 7, 14, 21, 28, 35, and 42) were utilised. Two assay diets were formulated to contain either SBM (413 g/kg) or CM (553 g/kg), as the sole source of dietary AA. Titanium dioxide (5 g/kg) was added as an indigestible marker. Each assay diet, in pelleted form, was offered to birds for 4 d during the six periods namely d 3-7, 10-14, 17-21, 24-28 and 38-42. The ileal digesta were collected on d 7 (14 birds per cage), 14 (12 birds per cage), 21 (10 birds per cage), 28 (8 birds per cage), 35 (8 birds per cage) and 42 (6 birds per cage), respectively. The apparent ileal digestibility coefficients (AIDC) were standardised using previously determined age-appropriate basal endogenous AA (EAA) flows. In the SBM, the AIDC of N, average AIDC of indispensable (IAA), dispensable (DAA) and total AA (TAA) increased linearly ($P < 0.01$) with broiler age. The AIDC of all individual AA, except Leu, Asp and Glu ($P > 0.05$), was influenced (linear or quadratic; $P < 0.05$ to 0.001) by age. Though the SIDC of N tended to be influenced (quadratic; $P = 0.075$) by age, the average SIDC of IAA and TAA were unaffected ($P > 0.05$). An age effect (quadratic; $P < 0.05$) was observed in the average SIDC of DAA in SBM with the

highest value at d 7, followed by a decrease from d 14 to 28, that increased beyond d 35. The SIDC of some individual AA (Arg, Thr, Trp, Cys, Pro) were affected ($P < 0.05$ to 0.001) in a quadratic manner by age. In the CM, the AIDC of N (quadratic; $P < 0.05$), average AIDC of IAA (linear; $P < 0.001$), DAA and TAA (quadratic; $P < 0.05$), were low between d 7 to 28 and increased thereafter. The SIDC of N, average SIDC of IAA, DAA and TAA in CM were influenced (quadratic; $P < 0.05$ to 0.001) by age. The SIDC of N and average SIDC of DAA and TAA were higher from d 7 to 14, declined at d 21 and then increased beyond d 28. The average SIDC of IAA was low between d 7 and 28, and increased thereafter. The SIDC of individual AA were affected (linear or quadratic; $P < 0.05$ to 0.001) by different magnitudes by age. In general, the AIDC AA in SBM and CM increased with advancing age. The age influence on the SIDC AA was variable, depending on the PS and AA. The results demonstrate that age-appropriate SIDC AA data might need consideration in broiler feed formulations.

8.2. Introduction

Soybean meal (SBM) is the dominant protein source (PS) used in poultry diets worldwide. Its universal acceptability, compared to other oilseed meals, is due to favourable attributes such as high crude protein (CP) content, an excellent amino acid (AA) profile that complements cereals and high AA digestibility (Ravindran *et al.*, 2014b). In a typical maize-SBM broiler diet, SBM contributes up to 70% of dietary CP.

The ever-increasing demand for SBM, fluctuating supply, market volatility and rising prices have encouraged the poultry industry to explore the use of alternative PS. Canola meal (CM) is relatively high in protein and offers an AA profile close to that of SBM and, a potential PS for poultry. However, the use of CM in poultry diets is restricted because of its higher fibre content and lower AA digestibility than SBM because of the desolventisation and toasting during steps in the prepress solvent extraction of canola (Newkirk *et al.*, 2000; Kim *et al.*, 2012b). The antinutritional factors, glucosinolates and erucic acid, have also limited the use of CM in the past, but their concentrations in current canola cultivars are reduced to almost zero by plant breeding. Studies have shown that CM could be included at levels up to 167 g/kg in balanced broiler diets based on digestible AA without any negative effects on performance (Gopinger *et al.*, 2014).

Formulation of poultry diets to meet the digestible AA requirements is pivotal for reductions in both the feed cost and excess nitrogen (N) excretion into the environment (Cowieson *et al.*, 2019). It is now recognised that the use of digestible AA in diet formulations is superior to total AA as it closely represents the amount utilised by the birds for maintenance and production purposes (Rostagno *et al.*, 1995; Lemme *et al.*, 2004). The superiority of measuring AA digestibility at the ileal level is now accepted unquestionably by the poultry industry (Ravindran *et al.*, 1999; Kadim *et al.*, 2002). The ileal AA digestibility can be expressed either as apparent ileal digestibility (AID) or standardised ileal digestibility (SID). In the calculation of SID, the AID

values are corrected for the basal endogenous AA (EAA) flows from various digestive secretions (bile, pancreatic, enzymatic and intestinal secretions), serum albumin, mucoproteins, and sloughed intestinal epithelial cells (Ravindran, 2021). Use of SID AA has now become commonplace because it is more additive than the AID in practical feed formulations (Kong and Adeola, 2013b; Cowieson *et al.*, 2019; An *et al.*, 2020).

Number of reports (Lemme *et al.*, 2004; Bryden *et al.*, 2009a; Blok and Dekker, 2017; Chapters 3, 4, 6 and 7) are available on the AID and SID coefficients (SIDC) of AA in feed ingredients. However, studies examining the age effects on SIDC AA are limited (Garcia *et al.*, 2007; Adedokun *et al.*, 2007a, 2008; Szczurek *et al.*, 2020) and confined to two or three specific ages. Two previous studies (Chapters 6 and 7) reported the broiler age effect on the AA digestibility in different grain sources (wheat, sorghum, maize and barley) from hatch to the end of growth cycle. The present study aimed to determine SIDC AA in two commonly used PS (SBM and CM) at six broiler ages (d 7, 14, 21, 28, 35 and, 42).

8.3. Materials and methods

The experimental protocol for this study was approved by the Massey University Animal Ethics Committee and conducted in accordance with the New Zealand Revised Code of Ethical Conduct for the use of live animals for scientific purpose.

8.3.1. Diets and experimental design

Soybean meal and CM, obtained from a commercial supplier, were 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. Two experimental diets, based on SBM and CM as the only source of AA, were developed to contain about 180 g/kg dietary protein (Table 8.1). Titanium dioxide (5 g/kg; Merck KGaA, Darmstadt, Germany) was added to all experimental diets as an indigestible marker.

The assay diets were steam-conditioned at 70 ºC for 30 s and pelleted using a pellet mill (Model Orbit 15; Richard Size Limited Engineers, Kingston-upon-Hull, UK) capable to produce 180 kg of feed/h and equipped with a die ring with 3-mm holes and 35-mm thickness. The pelleted diets were crumbled for feeding young birds during the first two weeks of study. The SBM and CM samples were analysed for dry matter (DM), N, crude fat (CF), CP, neutral detergent fibre (NDF), gross energy (GE), AA, calcium (Ca), phosphorus (P) and ash.

Table 8.1. Composition of the experimental diets (g/kg, as fed basis)

Ingredients	Soybean meal	Canola meal
Soybean meal	413	-
Canola meal	-	553
Maize starch	517	377
Soybean oil	30	30
Dicalcium phosphate	19	19
Limestone	10	10
Titanium dioxide[1]	5.0	5.0
Sodium chloride	2.0	2.0
Sodium bicarbonate	2.0	2.0
Trace mineral premix[2]	1.0	1.0
Vitamin premix[2]	1.0	1.0

[1]Merck KGaA, Darmstadt, Germany.

[2]Supplied per kilogram of diet: antioxidant (ethoxyquin), 100 mg; biotin, 0.2 mg; calcium pantothenate, 12.8 mg; cholecalciferol, 0.06 mg; 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, 0.2 mg; Zn, 60 mg.

8.3.2. Birds and housing

A total of 696, day-old male broilers (Ross 308) were obtained from a local hatchery, raised in floor pens and fed a commercial broiler starter diet (12.14 MJ/kg apparent metabolisable energy; 225 g/kg CP) from d 1 to 21 followed by a commercial broiler finisher diet (12.69 MJ/kg apparent metabolisable energy; 190 g/kg CP) from d 22 till d 38 (Table 8.2) in pelleted forms.

Table 8.2. Composition and calculated analysis (g/kg, as fed basis) of broiler starter and finisher diets

Ingredients	Starter diet (0-21 d)	Finisher diet (22-38 d)
Maize	574.2	660
Soybean meal, 460 g/kg	381.4	295.6
Soybean oil	8.8	13.6
Limestone	11.3	9.9
Dicalcium phosphate	10.7	8.2
DL-methionine	3.3	3.0
L-lysine HCl	2.0	1.9
L-threonine	1.0	0.7
Sodium bicarbonate	2.7	2.5
Sodium chloride	2.5	2.5
Trace mineral premix[1]	1.0	1.0
Vitamin premix[1]	1.0	1.0
Phytase	0.1	0.1
Calculated analysis		
Apparent metabolisable energy (MJ/kg)	12.14	12.69
Crude protein	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]Supplied per kilogram of diet: antioxidant (ethoxyquin), 100 mg; biotin, 0.2 mg; calcium pantothenate, 12.8 mg; cholecalciferol, 0.06 mg; 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, 0.2 mg; Zn, 60 mg.

On d 1, 168 chicks were weighed and allotted to 12 battery brooders (14 chicks per replicate) so that the average body weight (BW) per replicate was similar. The rest of the chicks were raised in floor pens and fed starter and/or finisher diets until the introduction of the experimental diet. The birds were then allotted to total 12 cages (6 cages for each PS) at five different ages, namely, d 7 (12 birds per cage), d 14 (10 birds per cage), d 21 (8 birds per cage), d 28 (8 birds per cage), and

d 35 (6 birds per cage), respectively. After 3 d of adaptation period, the experimental diets were offered for 4 d namely d 3-7 and 10-14 (crumbled); d 17-21, 24-28, 31-35, 38-42 (pelleted). Ileal digesta were collected on d 7, 14, 21, 28, 35 and 42 post-hatch.

The birds had *ad libitum* access to feed and water was freely accessible throughout the whole experimental period. The room temperature was maintained as 32 ± 1 ºC on d 1 and gradually reduced to 23 ºC by the end of the third week. A lighting schedule of 20 h of fluorescent illumination per day was supplied.

8.3.3. Performance data

During the 4-d experimental period in each week, BW of birds and feed consumption were recorded on a cage basis and mortality was recorded daily.

8.3.4. Determination of the coefficients of ileal digestibility

At the termination of respective experimental periods (d 7, 14, 21, 28, 35 and 42), all birds were euthanised by intravenous administration (1 mL per 2 kg BW) of sodium pentobarbitone solution (Provet NZ Pty. Ltd., Auckland, New Zealand). Then, the digesta were collected from the lower half of the ileum by gently flushing with distilled water into plastic containers, and processed as discussed by Ravindran *et al.* (2005). The ileum was defined as that portion of the small intestine extending from the Meckel's diverticulum to a point ~40 mm proximal to the ileocaecal junction. Briefly, the ileum was divided into halves (proximal and distal ileum) and the digesta samples were collected

from the lower half towards the ileocaecal junction. Digesta from birds within a cage were pooled after collection, frozen immediately and subsequently lyophilised (Model 0610, Cuddon Engineering, Blenheim, New Zealand). Diet and lyophilised digesta samples were ground to pass through a 0.5-mm sieve and stored in airtight plastic containers at 4 ºC until laboratory analysis. The digesta samples were analysed for DM, Titanium (Ti), N and AA.

8.3.5. Gizzard pH and jejunal digesta viscosity

Two birds from each replicate cage, euthanised for ileal digesta collection, were used for the measurement of gizzard pH by a digital pH meter (pH spear, Oakton Instruments, Vernon Hill, IL). In brief, the glass probe was inserted through an opening made in the gizzard and placed directly in the digesta. Three values were recorded from the proximal, middle, and distal regions and the mean value was considered as the final pH value. The same birds were used to measure the jejunal digesta viscosity. The digesta collected from the distal jejunum were centrifuged at 3000 × g at 20 ºC for 15 min. A 0.5 mL aliquot of the supernatant was used in a viscometer (Brookfield digital viscometer, Model DV2TLV; Brookfield Engineering Laboratories Inc., Stoughton, MA) fitted with CP-40 cone spindle with shear rates of 5-500/s for determining the digesta viscosity.

8.3.6. Chemical analysis

All chemical analyses were conducted in duplicate, except for AA. Single analysis per sample was conducted for AA. Dry matter was

determined using the standard procedure (Method 930.15; AOAC International, 2016). Titanium was analysed on a UV spectrophotometer (Berthold Technologies GmbH and Co. KG, Bad Wildbad, Germany) following the method as stated by Short *et al.* (1996). An adiabatic bomb calorimeter (Gallenkamp Autobomb, Weiss Gallenkamp Ltd., Loughborough, UK) standardised with benzoic acid was used for GE analysis. Nitrogen was determined by combustion (Method 968.06; AOAC International, 2016) using a carbon nanosphere-200 carbon, N and sulphur auto analyser (LECO Corporation, St. Joseph, MI). The CP content was calculated as N × 6.25. Fat was measured using the Soxhlet extraction procedure (Method 2003.06; AOAC International, 2016). Neutral detergent fibre was determined (Method 2002.04; AOAC International, 2016) using Tecator Fibertec™ (FOSS Analytical AB, Höganäs, Sweden). Samples were measured for ash by ashing in a muffle furnace at 550 ºC for 16 h (Method 942.05; AOAC International, 2016). Calcium and P concentrations were determined by Inductively Coupled Plasma-Optical Emission Spectroscopy (ICP-OES) using a Thermo Jarrell Ash IRIS instrument (Thermo Jarrell Ash Corporation, Franklin, MA).

Amino acids were determined following standard procedures (Method 994.12; AOAC International, 2011). The samples were hydrolysed with 6 N hydrochloric acid (HCl) containing phenol for 24 h at 110 ± 2 ºC in glass bottles in an oven. The AA were measured using AA analyser (ion exchange) with ninhydrin post column derivatisation. The chromatograms detected at 570 and 440 nm were integrated using

dedicated software (Agilent Open Lab software, Waldbronn, Baden-Württemberg, Germany). Cysteine and Met, the sulphur containing AA, were analysed as cysteic acid and methionine sulphone, respectively by oxidation with performic acid-phenol for 16 h at 0 ºC prior to hydrolysis.

In the case of Trp analysis, the samples were saponified under alkaline conditions with barium hydroxide solution in the absence of air at 110 ºC for 20 h in an autoclave. Following hydrolysis, the α-methyl Trp as internal standard, was added to the mixture. After adjusting the hydrolysate to pH 3.0 and diluting with 30% methanol, Trp and the internal standard were separated by reverse phase chromatography (RP-18) on a HPLC column (CORTECS C18 Column; 2.7 µm, Waters Corporation, Dublin, Ireland). Detection was selectively done by means of a fluorescence detector to prevent interference by other AA and other constituents.

8.3.7. Calculations

Data were expressed on a DM basis. The AID coefficients (AIDC) of AA were calculated from the dietary ratio of AA to Ti relative to the corresponding ratio in the ileal digesta using the following formula.

$$\text{AIDC of AA} = [(AA / Ti)_d - (AA / Ti)_i]/(AA / Ti)_d$$

Where, $(AA / Ti)_d$ = ratio of AA to Ti in the diet, and $(AA / Ti)_i$ = ratio of AA to Ti in the ileal digesta.

Apparent digestibility data for N and AA were then converted to SIDC, using the age-appropriate basal endogenous N and AA estimates (EAA;

grams per kilogram of DM intake [DMI]) measured at different ages (d 7, 14, 21, 28, 35 and 42) in a previous experiment (Chapter 5).

$$SIDC = AIDC + [\text{Basal EAA (g/kg DMI)}/\text{Ing. AA (g/kg DM)}]$$

Where, SIDC = standardised ileal digestibility coefficient of the AA

AIDC = apparent ileal digestibility coefficient of the AA,

Basal EAA = basal endogenous AA loss, and

Ing. AA = concentration of the AA in the ingredient.

8.3.8. Data Analysis

The data for each PS were analysed by the General Linear Model procedure of SAS (version 9.4; 2015; SAS Institute Inc., Cary, NC.). Cages served as the experimental unit. Differences were considered significant at $P < 0.05$. Orthogonal polynomial contrasts were performed to analyse the linear and quadratic effects of broiler age. The relationships between SIDC AA and other parameters were analysed by Pearson correlation.

8.4. Results

8.4.1. Proximate and nutrient composition

The proximate and AA composition of SBM and CM are shown in Table 8.3.

Table 8.3. Proximate and amino acid composition of protein sources (g/kg, as-received basis)

	Soybean meal	Canola meal
DM	893	891
Nitrogen (N)	77.4	56.9
CP (N×6.25)	484	356
Crude fat	12.3	48.5
NDF	84.6	250
GE (MJ/kg)	17.7	17.8
Ash	65.4	72.5
Calcium	3.26	5.78
Phosphorus	6.61	10.5
Indispensable amino acids (IAA)		
Arg	33.8	20.4
His	11.9	8.88
Ile	20.3	13.5
Leu	35.2	23.7
Lys	28.9	19.2
Met	6.36	6.83
Thr	18.1	14.6
Trp	6.36	4.81
Val	21.9	17.9
Total IAA	183	130
Dispensable amino acids (DAA)		
Ala	20.2	15.1
Asp	52.7	24.3
Cys[1]	6.94	7.86
Glu	83.9	58.9
Gly[1]	19.5	17.2
Pro	25.9	22.6
Ser	23.4	13.9
Total DAA	233	160
Total AA[2]	416	290

CP = crude protein; GE = gross energy; NDF = neutral detergent fibre.

[1]Semi-indispensable amino acids for poultry. [2]Total AA = IAA + DAA.

The CP content was higher in SBM (484 g/kg) compared to CM (356 g/kg). The CM contained higher CF (48.5 vs. 12.3 g/kg) and NDF (250 vs. 84.6 g/kg) contents than SBM. The Ca and P contents were higher in CM than SBM.

The SBM contained a higher content of total AA (TAA; 416 g/kg) than CM (290 g/kg). In both SBM and CM, the major indispensable AA

(IAA) contents were recorded for Leu followed by Arg, Lys, Val, Ile or Thr. The lowest IAA contents were observed for Trp, Met and His. Among the dispensable AA (DAA), higher contents were recorded for Glu, followed by Asp, and Pro in both PS. The concentrations of Cys in both SBM (6.94 g/kg) and CM (7.86 g/kg) were the lowest.

8.4.2. Growth performance, gizzard pH and jejunal digesta viscosity

The data on the performance, gizzard pH and jejunal digesta viscosity of birds fed SBM- or CM-based diets are summarised in Table 8.4.

Mortality during the experiment was negligible. Only three out of the 696 birds died, and the deaths were not connected to any specific treatment. As the birds grew older, the daily feed intake (DFI) in SBM and CM increased ($P < 0.001$) in quadratic and linear manners, respectively. The daily weight gain (DWG) increased (quadratic; $P < 0.001$) in both SBM and CM with advancing broiler age.

In both PS, a quadratic ($P < 0.001$) age effect on the gizzard pH was observed. Gizzard pH was higher at d 7, declined at d 14, and increased afterwards. The jejunal viscosity in SBM tended to be quadratically ($P = 0.060$) influenced by age. In CM, a linear increase ($P < 0.001$) was observed in jejunal viscosity with advancing age.

Table 8.4. Daily feed intake (DFI; g/bird/d), daily weight gain (DWG; g/bird/d), gizzard pH and viscosity (cP) in jejunal digesta of broilers fed soybean meal- and canola meal-based diets at different ages[1]

	Age (d)							Orthogonal polynomials contrasts	
Parameter	7	14	21	28	35	42	Pooled SEM	Linear	Quadratic
					soybean meal				
DFI[2]	18.2	48.9	89.8	115	172	202	1.19	0.001	0.001
DWG[2]	16.6	38.2	69.9	70.8	85.0	88.2	1.60	0.001	0.001
Gizzard	3.17	2.92	3.30	3.43	3.87	4.31	0.108	0.001	0.001
Viscosity[3]	2.85	3.37	3.58	2.80	2.90	3.12	0.109	0.368	0.060
					canola meal				
DFI[2]	20.3	53.0	92.4	121	176	198	1.49	0.001	0.580
DWG[2]	17.6	41.9	72.8	73.5	90.1	97.5	0.99	0.001	0.001
Gizzard	3.16	2.70	3.16	3.21	3.76	3.78	0.095	0.001	0.002
Viscosity[3]	2.02	2.25	2.47	2.29	2.13	2.58	0.056	0.001	0.424

[1]Each value represents the mean of six replicates (14, 12 and 10 birds per replicate for 7, 14 and 21-d old birds, respectively; eight birds per replicate for 28 and 35-d old birds; and six birds per replicate for 42-d old birds).

[2]Measured during 4-d feeding of experimental diets.

[3]Calculated as the mean of six replicates (two birds per replicate).

8.4.3. Ileal digestibility coefficients of N and AA in soybean meal

The impact of broiler age on AIDC, SIDC and SID content of N and AA in SBM during the 42-d experimental period is shown in Tables 8.5, 8.6 and 8.7, respectively.

With advancing bird age, a linear increase was observed for the AIDC of N ($P < 0.01$), average digestibility of IAA ($P < 0.01$), DAA ($P < 0.01$) and TAA ($P < 0.01$) in SBM (Table 8.5). The AIDC of N, average AIDC of IAA was lower at d 7, increased and evened out from d 14 to 28. A further increase was observed beyond d 35. The average AIDC of DAA and TAA plateaued between d 7 and 28, and increased from d 35 onwards.

In the case of IAA, apart from Leu, the AIDC of all individual IAA in SBM increased (linear or quadratic; $P < 0.05$ to 0.001) by broiler age. A tendency of age effect was observed on AIDC of Leu (quadratic; $P = 0.088$). The AIDC of Lys was influenced in a quadratic manner ($P <$

0.05) while a linear effect (P < 0.05 to 0.001) was observed for the AIDC of all other IAA. The AIDC of His, Ile, Thr and Trp increased (linear; P < 0.05 to 0.001) at d 35 with no further increase to d 42.

Among the DAA, with the exception of Asp and Glu (P > 0.05), the AIDC of all individual AA was influenced (linear or quadratic; P < 0.05 to 0.001) by age. A quadratic effect (P < 0.05) was observed for the AIDC of Ala and Cys that increased beyond d 14 and d 35, respectively. The AIDC of Gly, Pro and Ser increased linearly (P < 0.05 to 0.001) by age.

Table 8.5. Apparent ileal digestibility coefficients[1] of nitrogen (N) and amino acids of soybean meal at different ages of broilers[2]

	Age (d)							Orthogonal polynomial contrasts	
Parameter	7	14	21	28	35	42	Pooled SEM	Linear	Quadratic
N	0.838	0.848	0.851	0.841	0.859	0.857	0.0045	0.004	0.966
Indispensable amino acids									
Arg	0.916	0.908	0.907	0.913	0.931	0.916	0.0035	0.016	0.244
His	0.876	0.878	0.876	0.878	0.895	0.884	0.0040	0.011	0.755
Ile	0.859	0.866	0.865	0.862	0.883	0.870	0.0046	0.021	0.823
Leu	0.858	0.868	0.866	0.863	0.879	0.865	0.0044	0.088	0.309
Lys	0.879	0.891	0.894	0.892	0.903	0.892	0.0034	0.001	0.012
Met	0.869	0.895	0.887	0.886	0.895	0.890	0.0046	0.010	0.053
Thr	0.793	0.796	0.801	0.794	0.821	0.810	0.0055	0.002	0.746
Trp	0.843	0.843	0.846	0.845	0.872	0.866	0.0049	0.001	0.176
Val	0.844	0.856	0.856	0.849	0.872	0.857	0.0048	0.019	0.388
IAA	0.859	0.867	0.866	0.865	0.884	0.872	0.0043	0.002	0.426
Dispensable amino acids									
Ala	0.838	0.857	0.856	0.853	0.865	0.855	0.0048	0.017	0.033
Asp	0.855	0.850	0.849	0.846	0.864	0.852	0.0047	0.636	0.378
Cys[2]	0.732	0.726	0.723	0.725	0.776	0.769	0.0077	0.001	0.005
Glu	0.899	0.896	0.893	0.898	0.909	0.898	0.0039	0.211	0.661
Gly[2]	0.819	0.817	0.822	0.823	0.847	0.836	0.0050	0.001	0.432
Pro	0.847	0.844	0.843	0.845	0.869	0.856	0.0050	0.009	0.275
Ser	0.839	0.839	0.850	0.847	0.860	0.853	0.0052	0.005	0.541
DAA	0.833	0.833	0.834	0.834	0.856	0.845	0.0049	0.004	0.475
TAA	0.848	0.852	0.852	0.851	0.871	0.861	0.0045	0.004	0.881

[1]Each value represents the mean of six replicates (14, 12 and 10 birds per replicate for 7, 14 and 21-d old birds, respectively; eight birds per replicate for 28 and 35-d old birds; and six birds per replicate for 42-d old birds).

[2]Semi-indispensable amino acids for poultry.

DAA = Average digestibility of dispensable amino acids; IAA = Average digestibility of indispensable amino acids; TAA = Average digestibility of all amino acids.

The SIDC of N in SBM showed a tendency to be affected (quadratic; P = 0.075) by age (Table 8.6). No linear or quadratic response was observed for the age effect on the average SIDC of IAA (P > 0.05) and TAA (P > 0.05). Among the IAA, the SIDC of Arg (P < 0.05), Thr (P < 0.01) and Trp (P < 0.05) was influenced in a quadratic manner by age. The SIDC of Arg and Thr was higher at d 7, declined at d 14, and plateaued until d 28; an increase in the SIDC was observed at d 35, followed by a decrease at d 42. The SIDC of Trp increased at d 35 with no further changes to d 42.

In the case of DAA, the average SIDC of DAA, and SIDC of Cys and Pro were influenced by bird age (quadratic; P < 0.05), with values being higher at d 7, decreased at d 14 with no change to 28, and followed by an increase at d 35. While the SIDC of Cys was unaffected to d 42, the SIDC of Pro decreased further at d 42. A tendency for a quadratic age effect was observed for the SIDC of Asp (quadratic; P = 0.081) and Gly (quadratic; P = 0.051).

Table 8.6. Standardised ileal digestibility coefficients[1] of nitrogen (N) and amino acids of soybean meal at different ages of broilers[2]

	Age (d)							Orthogonal polynomial contrasts	
Parameter	7	14	21	28	35	42	Pooled SEM	Linear	Quadratic
N	0.882	0.871	0.873	0.864	0.882	0.873	0.0045	0.529	0.075
Indispensable amino acids									
Arg	0.934	0.917	0.916	0.923	0.940	0.922	0.0035	0.666	0.042
His	0.898	0.890	0.887	0.889	0.906	0.891	0.0040	0.633	0.237
Ile	0.887	0.880	0.879	0.876	0.895	0.878	0.0046	0.992	0.530
Leu	0.884	0.880	0.879	0.877	0.892	0.874	0.0044	0.617	0.851
Lys	0.899	0.900	0.903	0.902	0.912	0.898	0.0034	0.381	0.137
Met	0.908	0.913	0.905	0.906	0.913	0.900	0.0046	0.282	0.510
Thr	0.863	0.835	0.838	0.829	0.858	0.838	0.0055	0.159	0.008
Trp	0.874	0.860	0.864	0.865	0.890	0.879	0.0049	0.008	0.037
Val	0.878	0.874	0.873	0.867	0.889	0.869	0.0048	0.897	0.841
IAA	0.892	0.883	0.882	0.882	0.899	0.883	0.0043	0.908	0.396
Dispensable amino acids									
Ala	0.872	0.874	0.873	0.871	0.882	0.866	0.0047	0.788	0.310
Asp	0.880	0.863	0.862	0.859	0.876	0.860	0.0047	0.124	0.081
Cys[3]	0.807	0.768	0.763	0.764	0.818	0.802	0.0077	0.072	0.001
Glu	0.918	0.905	0.902	0.908	0.919	0.904	0.0039	0.496	0.198
Gly[3]	0.857	0.836	0.841	0.843	0.867	0.850	0.0050	0.172	0.051
Pro	0.885	0.864	0.864	0.864	0.888	0.870	0.0050	0.945	0.019
Ser	0.883	0.862	0.873	0.869	0.884	0.870	0.0052	0.909	0.343
DAA	0.872	0.853	0.854	0.854	0.876	0.860	0.0049	0.798	0.039
TAA	0.883	0.870	0.870	0.869	0.889	0.873	0.0045	0.854	0.147

[1]Apparent digestibility values were standardised using the following basal ileal endogenous flow values (g/kg DM intake), determined by the feeding N-free diet at different ages (Chapter 5):
D7: N, 3.59; Arg, 0.68; His, 0.29; Ile, 0.63; Leu, 0.97; Lys, 0.64; Met, 0.27; Thr, 1.35; Trp, 0.21; Val, 0.81; Ala, 0.75; Asp, 1.41; Cys, 0.47; Glu, 1.71; Gly, 0.78; Pro, 0.91; and Ser, 1.06.
D14: N, 1.87; Arg, 0.30; His, 0.16; Ile, 0.33; Leu, 0.49; Lys, 0.30; Met, 0.12; Thr, 0.73; Trp, 0.12; Val, 0.43; Ala, 0.37; Asp, 0.73; Cys, 0.27; Glu, 0.80; Gly, 0.39; Pro, 0.48; and Ser, 0.55.
D21: N, 1.79; Arg, 0.31; His, 0.15; Ile, 0.32; Leu, 0.49; Lys, 0.28; Met, 0.12; Thr, 0.71; Trp, 0.12; Val, 0.43; Ala, 0.36; Asp, 0.72; Cys, 0.26; Glu, 0.80; Gly, 0.40; Pro, 0.48; and Ser, 0.56.
D28: N, 1.82; Arg, 0.36; His, 0.16; Ile, 0.34; Leu, 0.55; Lys, 0.32; Met, 0.14; Thr, 0.67; Trp, 0.13; Val, 0.45; Ala, 0.41; Asp, 0.74; Cys, 0.25; Glu, 0.89; Gly, 0.43; Pro, 0.48; and Ser, 0.54.
D35: N, 1.81; Arg, 0.32; His, 0.15; Ile, 0.30; Leu, 0.49; Lys, 0.28; Met, 0.12; Thr, 0.71; Trp, 0.12; Val, 0.42; Ala, 0.37; Asp, 0.72; Cys, 0.26; Glu, 0.81; Gly, 0.40; Pro, 0.47; and Ser, 0.56.
D42: N, 1.29; Arg, 0.20; His, 0.10; Ile, 0.20; Leu, 0.31; Lys, 0.18; Met, 0.07; Thr, 0.52; Trp, 0.09; Val, 0.29; Ala, 0.25; Asp, 0.49; Cys, 0.21; Glu, 0.53; Gly, 0.28; Pro, 0.34; and Ser, 0.40.

[2]Each value represents the mean of six replicates (14, 12 and 10 birds per replicate for 7, 14 and 21-d old birds, respectively; eight birds per replicate for 28 and 35-d old birds; and six birds per replicate for 42-d old birds).

[3]Semi-indispensable amino acids for poultry.

DAA = Average digestibility of dispensable amino acids; IAA = Average digestibility of indispensable amino acids; TAA = Average digestibility of all amino acids.

Broiler age tended (quadratic; P = 0.075) to affect the SID protein content of SBM (Table 8.7). No age effect (P > 0.05) was observed for the SID contents of total IAA, DAA and total AA. The SID contents of Arg, Thr, Trp, Cys and Pro were influenced (quadratic; P < 0.05) by

age. The broiler age tended to quadratically influence the SID contents of Asp (P = 0.081) and Gly (P = 0.051).

Table 8.7. Influence of age on standardised digestible protein (CP) and amino acid contents[1] (g/kg) of soybean meal, as-received basis

	Age (d)							Orthogonal polynomial contrasts	
Parameter	7	14	21	28	35	42	Pooled SEM	Linear	Quadratic
CP	394	389	390	385	394	390	2.0	0.529	0.075
Indispensable amino acids									
Arg	30.1	29.6	29.5	29.8	30.3	29.7	0.11	0.666	0.042
His	10.5	10.4	10.3	10.4	10.6	10.4	0.05	0.633	0.237
Ile	18.4	18.2	18.2	18.1	18.5	18.2	0.09	0.992	0.530
Leu	29.7	29.6	29.5	29.5	30.0	29.4	0.15	0.617	0.851
Lys	24.5	24.5	24.7	24.6	24.9	24.5	0.09	0.380	0.137
Met	5.41	5.43	5.39	5.39	5.43	5.36	0.027	0.282	0.509
Thr	14.7	14.2	14.3	14.1	14.6	14.2	0.09	0.159	0.008
Trp	5.23	5.15	5.18	5.18	5.33	5.27	0.029	0.008	0.037
Val	18.9	18.8	18.8	18.7	19.2	18.8	0.10	0.897	0.841
IAA	158	156	156	156	159	156	0.73	0.983	0.482
Dispensable amino acids									
Ala	16.8	16.9	16.8	16.8	17.0	16.7	0.09	0.788	0.310
Asp	43.9	43.1	43.0	42.9	43.7	43.0	0.23	0.124	0.081
Cys[2]	4.51	4.29	4.26	4.26	4.56	4.47	0.043	0.072	0.001
Glu	72.7	71.8	71.5	72.1	72.8	71.7	0.31	0.496	0.199
Gly[2]	16.0	15.6	15.7	15.8	16.2	15.9	0.09	0.172	0.051
Pro	18.8	18.3	18.3	18.3	18.9	18.5	0.11	0.945	0.020
Ser	18.9	18.4	18.7	18.6	18.9	18.6	0.11	0.909	0.343
DAA	192	190	188	189	192	189	0.94	0.330	0.161
Total AA	349	345	344	345	351	345	1.7	0.797	0.235

Standardised digestible amino acid content (g/kg) = [ingredient amino acid content (g/kg) × standardised ileal digestibility (%)]/100.

[1]Each value represents the mean of six replicates (14, 12 and 10 birds per replicate for 7, 14 and 21-d old birds, respectively; eight birds per replicate for 28 and 35-d old birds; and six birds per replicate for 42-d old birds).

[2]Semi-indispensable amino acids for poultry.

DAA = Total digestible dispensable amino acid contents; IAA = Total digestible indispensable amino acid contents; Total AA = Total digestible content of all amino acids.

8.4.4. Ileal digestibility coefficients of N and AA in canola meal

The influence of broiler age on AIDC, SIDC and SID content of N and AA in CM is presented in Tables 8.8, 8.9 and 8.10, respectively. The AIDC of N, average AIDC of DAA and TAA was influenced in a quadratic (P < 0.05; Table 8.8) manner. A linear pattern (P < 0.001) was observed for the average AIDC of IAA. The AIDC of N, average of IAA, DAA and TAA increased only at d 35 and then plateaued until d

42. The AIDC of all individual AA (except Leu, Met and Ala) remained constant from d 7 to 28, increased at d 35 with no further changes to d 42, as indicated by linear or quadratic (P < 0.05 to 0.001) patterns with advancing broiler age. The lowest AIDC of Leu (linear; P < 0.001), Met (quadratic, P < 0.05) and Ala (linear, P < 0.001) was recorded at d 7, which increased between d 14 and 28, followed by a further increase at d 35 with no change afterwards.

Table 8.8. Apparent ileal digestibility coefficients[1] of nitrogen (N) and amino acids of canola meal at different ages of broilers[2]

	Age (d)							Orthogonal polynomial contrasts	
Parameter	7	14	21	28	35	42	Pooled SEM	Linear	Quadratic
N	0.725	0.733	0.722	0.733	0.767	0.768	0.0067	0.001	0.023
Indispensable amino acids									
Arg	0.845	0.839	0.839	0.848	0.878	0.880	0.0033	0.001	0.001
His	0.803	0.804	0.794	0.800	0.832	0.830	0.0043	0.001	0.001
Ile	0.754	0.769	0.768	0.770	0.804	0.797	0.0057	0.001	0.571
Leu	0.784	0.803	0.800	0.800	0.830	0.822	0.0050	0.001	0.946
Lys	0.741	0.749	0.742	0.752	0.779	0.778	0.0057	0.001	0.089
Met	0.839	0.863	0.862	0.861	0.875	0.873	0.0037	0.001	0.046
Thr	0.678	0.688	0.680	0.688	0.728	0.722	0.0073	0.001	0.096
Trp	0.762	0.761	0.753	0.762	0.809	0.804	0.0059	0.001	0.001
Val	0.750	0.762	0.758	0.761	0.794	0.789	0.0057	0.001	0.252
IAA	0.773	0.782	0.777	0.782	0.814	0.811	0.0048	0.001	0.073
Dispensable amino acids									
Ala	0.768	0.791	0.786	0.789	0.818	0.812	0.0054	0.001	0.884
Asp	0.721	0.716	0.715	0.714	0.755	0.752	0.0066	0.001	0.006
Cys[2]	0.691	0.690	0.701	0.699	0.725	0.736	0.0062	0.001	0.038
Glu	0.843	0.847	0.839	0.847	0.871	0.870	0.0038	0.001	0.008
Gly[2]	0.735	0.730	0.718	0.731	0.772	0.777	0.0061	0.001	0.001
Pro	0.706	0.704	0.692	0.704	0.746	0.748	0.0066	0.001	0.001
Ser	0.699	0.713	0.718	0.717	0.754	0.744	0.0070	0.001	0.898
DAA	0.738	0.742	0.739	0.743	0.778	0.777	0.0054	0.001	0.016
TAA	0.758	0.764	0.760	0.765	0.798	0.796	0.0050	0.001	0.035

[1]Each value represents the mean of six replicates (14, 12 and 10 birds per replicate for 7, 14 and 21-d old birds, respectively; eight birds per replicate for 28 and 35-d old birds; and six birds per replicate for 42-d old birds).

[2]Semi-indispensable amino acids for poultry.

DAA = Average digestibility of dispensable amino acids; IAA = Average digestibility of indispensable amino acids; TAA = Average digestibility of all amino acids.

A quadratic increase (P < 0.001; Table 8.9) was observed for the SIDC of N, average SIDC of IAA, DAA and TAA in CM with age. The SIDC of N and average SIDC of DAA and TAA were similar from d 7 to 14, decreased at d 21, followed by an increase beyond d 28. The

average SIDC of IAA remained consistently lower from d 7 to 28, followed by an increase and plateauing between d 35 and 42.

Among the IAA, the SIDC of Arg, His, Lys, Thr, Trp and Val was influenced in a quadratic manner (P < 0.05 to 0.001). Nevertheless, the SIDC of Ile, Leu and Met increased linearly (P < 0.001) with broiler age. With the exception of Ala (linear; P < 0.001) and Ser (linear; P < 0.05), the SIDC of individual DAA increased in a quadratic manner (P < 0.001) with advancing broiler age.

Table 8.9. Standardised ileal digestibility coefficients[1] of nitrogen (N) and amino acids of canola meal at different ages of broilers[2]

	Age (d)							Orthogonal polynomial contrasts	
Parameter	7	14	21	28	35	42	Pooled SEM	Linear	Quadratic
N	0.778	0.760	0.748	0.760	0.794	0.787	0.0067	0.008	0.001
Indispensable amino acids									
Arg	0.873	0.851	0.852	0.862	0.891	0.889	0.0033	0.001	0.001
His	0.830	0.819	0.807	0.815	0.846	0.840	0.0043	0.001	0.001
Ile	0.791	0.788	0.787	0.790	0.822	0.809	0.0057	0.001	0.136
Leu	0.818	0.820	0.817	0.819	0.847	0.833	0.0051	0.001	0.368
Lys	0.771	0.762	0.755	0.766	0.792	0.786	0.0057	0.001	0.011
Met	0.870	0.878	0.870	0.878	0.889	0.881	0.0037	0.007	0.446
Thr	0.755	0.730	0.720	0.726	0.768	0.751	0.0073	0.105	0.001
Trp	0.798	0.781	0.775	0.784	0.830	0.819	0.0059	0.001	0.001
Val	0.788	0.783	0.778	0.782	0.813	0.802	0.0057	0.001	0.036
IAA	0.810	0.801	0.796	0.802	0.833	0.823	0.0050	0.001	0.001
Dispensable amino acids									
Ala	0.809	0.811	0.806	0.811	0.839	0.826	0.0054	0.001	0.272
Asp	0.769	0.741	0.740	0.739	0.780	0.769	0.0065	0.046	0.001
Cys[3]	0.744	0.743	0.731	0.726	0.754	0.759	0.0056	0.035	0.001
Glu	0.866	0.858	0.850	0.859	0.882	0.877	0.0038	0.001	0.001
Gly[3]	0.771	0.748	0.737	0.751	0.791	0.790	0.0061	0.001	0.001
Pro	0.743	0.723	0.712	0.724	0.766	0.762	0.0066	0.001	0.001
Ser	0.762	0.746	0.751	0.749	0.787	0.767	0.0070	0.015	0.086
DAA	0.778	0.769	0.762	0.766	0.800	0.793	0.0058	0.001	0.004
TAA	0.796	0.787	0.781	0.786	0.819	0.810	0.0052	0.001	0.001

[1]Apparent digestibility values were standardised using the following basal ileal endogenous flow values (g/kg DM intake), determined by the feeding N-free diet at different ages (Chapter 5); see Table 8.6.

[2]Each value represents the mean of six replicates (14, 12 and 10 birds per replicate for 7, 14 and 21-d old birds, respectively; eight birds per replicate for 28 and 35-d old birds; and six birds per replicate for 42-d old birds).

[3]Semi-indispensable amino acids for poultry.

DAA = Average digestibility of dispensable amino acids; IAA = Average digestibility of indispensable amino acids; TAA = Average digestibility of all amino acids.

Age had a quadratic (P < 0.001) influence on the SID protein content of CM with a range of 290 - 295 g/kg between d 7 to 28, followed by an increase to 42 (Table 8.10). The SID contents of IAA, DAA and total AA plateaued from d 7 to 28, and then increased (linear; P < 0.001) beyond d 35. Except Cys, the SID contents of all individual AA in CM increased (linear or quadratic; P < 0.05 to 0.001) as the birds grew older. The SID content of Cys was influenced in a quadratic (P < 0.001) manner with the value being higher at d 7, then decreasing at d 28, followed by an increase beyond d 35.

Table 8.10. Influence of age on standardised digestible protein (CP) and amino acid contents[1] (g/kg) of canola meal, as-received basis

	Age (d)							Orthogonal polynomial contrasts	
Parameter	7	14	21	28	35	42	Pooled SEM	Linear	Quadratic
CP	292	291	290	295	304	302	1.9	0.001	0.084
Indispensable amino acids									
Arg	19.3	19.1	19.1	19.4	20.0	19.9	0.08	0.001	0.001
His	7.87	7.87	7.82	7.91	8.13	8.07	0.035	0.001	0.024
Ile	11.9	12.0	12.2	12.2	12.6	12.4	0.08	0.001	0.222
Leu	20.9	21.3	21.4	21.5	22.0	21.7	0.12	0.001	0.079
Lys	14.9	15.0	15.1	15.3	15.6	15.5	0.09	0.001	0.865
Met	6.58	6.70	6.72	6.71	6.79	6.73	0.029	0.001	0.024
Thr	11.6	11.5	11.6	11.7	12.1	11.9	0.09	0.001	0.239
Trp	4.03	4.01	4.02	4.08	4.27	4.21	0.026	0.001	0.037
Val	15.1	15.3	15.3	15.4	15.8	15.6	0.10	0.001	0.554
IAA	112	113	113	114	117	116	0.62	0.001	0.869
Dispensable amino acids									
Ala	13.0	13.2	13.3	13.4	13.7	13.5	0.08	0.001	0.103
Asp	19.9	19.7	19.8	19.9	20.7	20.4	0.16	0.001	0.111
Cys[2]	5.92	5.91	5.81	5.78	6.00	6.04	0.044	0.035	0.001
Glu	57.1	57.2	57.0	57.7	58.9	58.5	0.23	0.001	0.152
Gly[2]	14.4	14.3	14.2	14.5	15.1	15.0	0.09	0.001	0.001
Pro	15.9	16.3	16.0	16.3	16.9	16.8	0.09	0.001	0.035
Ser	11.3	11.4	11.6	11.6	12.1	11.8	0.09	0.001	0.318
DAA	138	138	138	139	143	142	0.71	0.001	0.144
Total AA	250	251	254	253	261	258	1.30	0.001	0.379

Standardised digestible amino acid content (g/kg) = [ingredient amino acid content (g/kg) × standardised ileal digestibility (%)]/100.

[1]Each value represents the mean of six replicates (14, 12 and 10 birds per replicate for 7, 14 and 21-d old birds, respectively; eight birds per replicate for 28 and 35-d old birds; and six birds per replicate for 42-d old birds).
[2]Semi-indispensable amino acids for poultry.

DAA = Total digestible dispensable amino acid contents; IAA = Total digestible indispensable amino acid contents; Total AA = Total digestible content of all amino acids.

8.4.5. Uplift in digestibility coefficients due to correction for age-appropriate endogenous amino acid losses

The percentage of increase in the digestibility coefficients of N and AA after correction of AIDC for basal endogenous N and AA losses at different broiler ages is shown in Table 8.11.

Regardless of age, standardisation of apparent data for age-appropriate endogenous N and AA losses resulted an increase in the SIDC estimates. The extent of increase declined with advancing broiler age. The correction of AIDC increased the average TAA digestibility coefficients in SBM by 4.13% (d 7), 2.24% (d 14), 2.11% (d 21), 2.12% (d 28), 2.07% (d 35), and 1.39% (d 42). The corresponding increases in the CM were 5.01% (d 7), 3.01% (d 14), 2.76% (d 21), 2.75% (d 28), 2.63% (d 35), and 1.76% (d 42), respectively.

Table 8.11. Percentage increase in digestibility coefficients of nitrogen (N) and amino acids in soybean meal and canola meal after correction of apparent ileal digestibility coefficients for age-appropriate endogenous amino acid losses of broilers

	SBM						CM					
	Age (d)						Age (d)					
Parameter	7	14	21	28	35	42	7	14	21	28	35	42
N	5.25	2.71	2.59	2.73	2.68	1.87	7.31	3.68	3.60	3.68	3.52	2.47
Indispensable amino acids												
Arg	1.97	0.99	0.99	1.10	0.97	0.66	3.31	1.43	1.55	1.65	1.48	1.02
His	2.51	1.37	1.26	1.25	1.23	0.79	3.36	1.87	1.64	1.87	1.68	1.20
Ile	3.26	1.62	1.62	1.62	1.36	0.92	4.91	2.47	2.47	2.60	2.24	1.51
Leu	3.03	1.38	1.50	1.62	1.48	1.04	4.34	2.12	2.12	2.37	2.05	1.34
Lys	2.28	1.01	1.01	1.12	1.00	0.67	4.05	1.74	1.75	1.86	1.67	1.03
Met	4.49	2.01	2.03	2.26	2.01	1.12	3.69	1.74	0.93	1.97	1.60	0.92
Thr	8.83	4.90	4.62	4.41	4.51	3.46	11.4	6.10	5.88	5.52	5.49	4.02
Trp	3.68	2.02	2.13	2.37	2.06	1.50	4.72	2.63	2.92	2.89	2.60	1.87
Val	4.03	2.10	1.99	2.12	1.95	1.40	5.07	2.76	2.64	2.76	2.39	1.65
IAA	3.84	1.85	1.85	1.97	1.70	1.26	4.79	2.43	2.45	2.56	2.33	1.48
Dispensable amino acids												
Ala	4.06	1.98	1.99	2.11	1.97	1.29	5.34	2.53	2.54	2.79	2.57	1.72
Asp	2.92	1.55	1.53	1.54	1.39	0.94	6.66	3.49	3.50	3.50	3.31	2.26
Cys[1]	10.3	6.13	5.53	5.38	5.41	4.29	7.67	7.68	4.28	3.86	4.00	3.13
Glu	2.11	1.00	1.01	1.11	1.10	0.67	2.73	1.30	1.31	1.42	1.26	0.80
Gly[1]	4.64	2.33	2.31	2.43	2.36	1.67	4.90	2.47	2.65	2.74	2.46	1.67
Pro	4.49	2.37	2.49	2.25	2.19	1.64	5.24	2.70	2.89	2.84	2.68	1.87
Ser	5.24	2.74	2.71	2.60	2.79	1.99	9.01	4.63	4.60	4.46	4.38	3.09
DAA	4.68	2.42	2.40	2.40	2.34	1.78	5.42	3.64	3.11	3.10	2.83	2.06
TAA	4.13	2.24	2.11	2.12	2.07	1.39	5.01	3.01	2.76	2.75	2.63	1.76

[1]Semi-indispensable amino acids for poultry.

DAA = Average digestibility of dispensable amino acids; IAA = Average digestibility of indispensable amino acids; TAA = Average digestibility of all amino acids.

CM = canola meal; SBM = soybean meal.

8.5. Discussion

The complexity of AA digestibility measurements is that a myriad of interrelated factors is involved. These include dietary [(ingredient type, Bryden *et al.*, 2009a; AA content, Lemme *et al.* (2004); antinutritional factors (Choct and Annison, 1992a); feed form (mash vs. pellet; Chapters 3, 4)], bird [(age, Huang *et al.*, 2005; sex, ten Doeschate *et al.*, 1993; class of bird, Huang *et al.* (2006)]; and environmental factors (ambient temperature; Zuprizal *et al.*, 1993). In practice, nutrient digestibility is affected by the age of bird but only limited reports (Fonolla *et al.*, 1981; Wallis and Balnave, 1984; Zelenka and Liska,

1986; Huang *et al.*, 2005) exist documenting the age impact on protein or AA digestibility in broilers, with contradictory results.

Several compilations (Lemme *et al.*, 2004; Bryden *et al.*, 2009a; Blok and Dekker, 2017) are available on the AA digestibility of ingredients, but all have been determined in older broilers (mostly 21-35 d). Some studies have determined the SID AA in different PS, including the SBM and CM (Adedokun *et al.*, 2009a; Woyengo *et al.*, 2010; Kim *et al.*, 2012b; Ravindran *et al.*, 2014b; Adewole *et al.*, 2017). Several studies (Garcia *et al.*, 2007; Adedokun *et al.*, 2007a, 2008; Toghyani *et al.*, 2015; Szczurek *et al.*, 2020) compared the SID AA in different feed ingredients at two or three broiler ages. Among these reports, the SID AA in five plant ingredients, including CM and SBM (Adedokun *et al.*, 2008), have been reported at d 5 and 21. Toghyani *et al.* (2015) reported the SID AA in expeller-extracted CM for 10 and 24-d-old broilers.

The specific objective of the present experiment was to assess the SIDC AA in two commonly used PS, namely the SBM and CM, at six broiler ages (d 7, 14, 21, 28, 35 and 42). The distinctive feature of the current work was that values for age-appropriate basal EAA flows were used for the correction of apparent AA digestibility at all six ages. Previously only few researchers (Adedokun *et al.*, 2007a, 2008; Toghyani *et al.*, 2015; Szczurek *et al.*, 2020) have standardised the AIDC using age-appropriate basal EAA flows, but corrections were limited to two or three ages.

Nutrient composition

The proximate nutrient, including AA, composition of SBM and CM falls within the range of previously published data (Chapter 4; Bryden *et al.*, 2009a; Khajali and Slominski, 2012; Ravindran *et al.*, 2014b). Soybean meal contained a higher CP content and lower NDF content compared to CM. The CP content of SBM (484 g/kg) was comparable to the value (485 g/kg) reported by NRC (1994) and close to the range (464-482 g/kg) of Ravindran *et al.* (2014b). In the case of CM, the CP content (356 g/kg) was within the range (266 to 394 g/kg) of Bryden *et al.* (2009a) and comparable to those in previous reports (365-380 g/kg; NRC, 1994; Khajali and Slominski, 2012). The NDF content in CM (250 g/kg) in present study was concordant with the range (218-265 g/kg) reported by Toghyani *et al.* (2015) studying CM samples of Australian origin.

The TAA content in SBM (416 g/kg) was higher than CM (290 g/kg). The IAA and DAA contents in SBM and CM were similar or close to published literature (Chapter 4; Woyengo *et al.*, 2010; Newkirk, 2011; Ravindran *et al.*, 2005, 2014b). The Lys content of SBM (28.9 g/kg) was notably higher compared to CM (19.2 g/kg), while the Met (6.36 vs. 6.83 g/kg) and Cys (6.94 vs. 7.86 g/kg) contents in SBM were lower than CM. Similar observations have been made by Newkirk (2011). Depending on the processing condition, the AA composition in CM can be variable with Met content ranging from 6.40 to 7.20 g/kg (Adewole *et al.*, 2016) that match with the Met content (6.83 g/kg) recorded in current study. The Lys (19.2 g/kg) and Thr (14.6 g/kg) contents for CM

in present study were close to the ranges (20 to 23 g/kg and 15.1 to 16.2 g/kg, respectively) as reported by Adewole *et al.* (2016). As stated by Evers *et al.* (1999), the variation in AA composition in ingredients of plant origin could be attributed to differences in season, cultivars and harvesting practices. The climate, dehulling and processing also influence the AA content in SBM (Ravindran *et al.*, 2014b). In the case of CM, differences in processing conditions, fibre content, and the soil conditions (Bell and Keith, 1991) might influence the nutrient composition.

Performance, gizzard pH and jejunal digesta viscosity

Regardless of the PS, the DFI and DWG increased as the birds grew older. The decrease in gizzard pH at d 14, compared to d 7, in both the SBM (3.17 vs. 2.92) and CM (3.16 vs. 2.70) was in agreement with the findings of David *et al.* (2020). The cause of reduction in gastric pH is likely to be due to increased gastric secretions in the proventriculus from d 2 to 15 (Rynsburger, 2009). Though concurrent secretions of pepsin and HCl from proventriculus increase with age (Nitsan *et al.*, 1991), the pH increases in gizzard digesta after d 14 in both PS because of greater feed intake as the birds grow (Ravindran, 2013). With advancing age, the increased load of feed with neutral pH in the gastrointestinal tract (GIT) dilutes the HCl elevating the gizzard pH. The dietary proteins are exposed to HCl in the proventriculus and the acidic conditions initiate protein digestion by denaturing the protein and exposing the peptide bonds to enzyme hydrolysis. The first step in protein digestion is the conversion of pepsinogen to pepsin that requires

an acidic environment (Rynsburger, 2009). Therefore, the expectation was that the elevation of gizzard pH with advancing broiler age will negatively influence the protein digestion. In the current study, no correlation ($r = 0.158$; $P > 0.05$) was observed between the gizzard pH and the average SIDC of TAA in SBM. On the other hand, in the case of CM, a positive correlation ($r = 0.553$; $P < 0.001$) was exhibited, a finding that is difficult to explain.

A tendency (quadratic; $P = 0.060$) for age effect was observed for the jejunal digesta viscosity in birds fed the SBM diet. In the CM, the viscosity increased from d 7 to 21 and then decreased from d 28 to 35, followed by an increase at d 42. The CM contains 14.3 g/kg soluble non-starch polysaccharides (NSP; Meng and Slominski, 2005). According to Choct and Annison (1992b), the soluble NSP is positively related to digesta viscosity. Increased feed intake by age and, consequently, increased intake of dietary soluble NSP may have contributed to the gradual rise of intestinal viscosity until d 21. The decreased viscosity after d 21 may be due to the increased resilience of older birds to soluble NSP because of the increased activity and stability of gut microbiota (Choct and Annison, 1992a). The cause of higher viscosity at d 42 in CM is hard to explain. It is noteworthy that the jejunal viscosity in both SBM ($r = -0.202$; $P > 0.05$) and CM ($r = -0.038$; $P > 0.05$) were not correlated with the average SIDC of TAA. Besides the gizzard pH and viscosity, numerous other factors, discussed below, may potentially affect the AA digestibility at different broiler ages.

Ileal digestibility coefficients of nitrogen and amino acids

Limited reports (Huang *et al.*, 2005; Adedokun *et al.*, 2007a, 2008) indicate that the AIDC AA differs depending on broiler age. In the present study, the AIDC of N, and the average AIDC of IAA, DAA and TAA in SBM increased as the birds grew older. With the exceptions of Leu, Asp and Glu, an increase was also recorded in the AIDC of all individual AA. The average AIDC of TAA from d 7 to 28 was 2.3% lower compared to d 35 and 1.2% than d 42. The lower AIDC AA in SBM at earlier age may be partly explained by the presence of α-galactosides (raffinose, verbascose, stachyose), which is not digested by young broilers (Carre´*et al.*, 1995).

The trend of increased AIDC of N and AA with age in SBM is in agreement with previous findings (Noy and Sklan, 1995; Huang *et al.*, 2005; Adedokun *et al.*, 2008). Noy and Sklan (1995) reported an increase in the ileal N digestion from d 4 (78%) to 21 (92%) following the feeding of maize-SBM-based diets to broilers. Uni *et al.* (1995), in a study with maize-SBM-based diets, reported an increase in N digestion in heavy and light strain chicks increased from 70% on d 4 to 90% on d 14. Adedokun *et al.* (2008) reported the AIDC AA in SBM and CM at two ages of broilers (d 5 vs. 21). The AIDC of TAA in SBM were reported to be 0.810 (d 5) and 0.860 (d 21), respectively. The AIDC AA in CM also increased by age. Huang *et al.* (2005), investigating the age effect (d 14, 28 and 42) on the AIDC AA in a wide range of ingredients (wheat, sorghum, maize, SBM, CM, meat and bone meal; MBM, cotton seed meal and mill run), concluded that the AIDC

AA increased in older birds though the age effect varied depending on the ingredient and AA. In line with present findings, and compared to d 14, the AIDC AA was found to be higher at d 28 and 42 in SBM, CM and MBM. The average AIDC of TAA in SBM at d 28 and 42 was similar and 2.35% higher than those at d 14.

In the current study, the AIDC of N, average AIDC of IAA, DAA and TAA in CM increased with advancing broiler age. The digestibility estimates were lower from d 7 to 28 and increased thereafter. Compared to the average AIDC of TAA from d 7 to d 28, the AIDC of TAA at d 35 and d 42 were 4.72 and 4.46% higher, respectively. The AIDC of individual AA increased at different magnitudes. Toghyani *et al.* (2015) reported a decrease in the AIDC of CP and most AA at d 10 compared to d 24 of broiler in expeller extracted CM processed at different temperatures (90, 95 and 100 ºC). A study by Huang *et al.* (2005) found that the average AIDC of TAA in CM at d 14 was 1.23% lower compared to either d 28 or 42 with no differences in the digestibility estimates between these two ages. Adedokun *et al.* (2008) observed 3.59% lower AIDC of TAA in CM at d 5 compared to d 21. Canola meal contains relatively high contents of fibre and phytate, both can lower AA utilisation (Cowieson *et al.*, 2009). The protein-phytate complex in CM is poorly hydrolysed decreasing the availability of some AA (Bell and Keith, 1991). The dietary phytate also increase the EAA flows at the ileal level of birds by increasing secretion and/or with the reduction of digestion and reabsorption of AA (Cowieson and Ravindran, 2007; Cowieson *et al.*, 2009). Edwards *et al.* (1989)

reported that older birds can utilise phytate-P more than the young chicks. The presence of greater endogenous phytase activity in the GIT of older birds may increase the utilisation of phytate (Ravindran *et al.*, 1995), thus removing the negative effects on AA.

Three explanations may be provided for the increased AIDC AA in SBM and CM with age. First, the GIT and digestive physiology are immature at hatch and development in these systems with age (Ravindran and Abdollahi, 2021) is the key factor contributing to the increased AA digestibility. Nitsan *et al.* (1991) found that the pancreatic weight of newly hatched chicks increased at d 8 and 23 by 10- and 30-folds, respectively. The corresponding increases in small intestinal weight were recorded to be 10-folds (d 8) and 20-folds (d 23), respectively. The insufficient secretion and lower activities of proteases (trypsin, chymotrypsin) in the pancreas and small intestine at early ages is a significant factor contributing to the compromised AA digestibility (Nitsan *et al.*, 1991). Second, digesta passage rate is slower through the small intestine (Hurwitz and Bar, 1966; Sklan *et al.*, 1975) and GIT (Hillerman *et al.*, 1953; Shires *et al.*, 1987) in older birds than young birds. The digesta passage rate directly influences nutrient digestion by regulating the contact time among the nutrients, digestive enzymes and intestinal surface (Mateos *et al.*, 1982; Shires *et al.*, 1987). Third, trends in the AIDC AA in SBM and CM are clearly affected by the contribution of AA of endogenous origin in the ileal digesta. As observed in our previous study (Chapter 5), the EAA flow varied depending on broiler age. The EAA flow was higher at d 7, declined on

d 14 with no further change until d 35, and decreased again on d 42. The EAA flow is measured as a balance between the secretion and reabsorption of AA and reductions of EAA flow with advancing age have been attributed to decreased mucin production, longer digesta retention, and increased digestion and re-absorption of EAA (Adedokun *et al.*, 2007b; Chapter 5).

Although the AIDC of N and AA increased by age in SBM, the SIDC of N, average SIDC of IAA and TAA remained unchanged across broiler ages. The average SIDC of DAA and the SIDC of some individual AA (Arg, Thr, Trp, Cys and Pro) were influenced in a quadratic manner by age. The results confirm that the age effect on the AIDC AA in SBM was masked by the EAA flows, especially in young broilers. The influence of age on SIDC estimates in CM were also inconsistent. The SIDC of N, average SIDC of DAA and TAA were higher at d 7 to 14, declined at d 21, followed by an increase from d 28 to 42. The average SIDC of IAA in CM from d 7 to 28 was lower in comparison with d 35 (3.72%) and 42 (2.55%), respectively. In line with the present study, Toghyani *et al.* (2015) recorded higher SIDC for most AA at d 24 compared to d 10 in the CM.

The underlying physiological mechanisms for the variation observed among the digestibility of individual AA are intricate. The AA differ in their chemical structure and have different linkages. Each protease (trypsin, chymotrypsin, elastase, carboxypeptidases A and B, and aminopeptidases) exhibits cleavage specificity and differences in conformation and the AA sequence of polypeptide chains are related in

the breakdown and release of individual AA (Riviere and Tempst, 2001; Chen, 2017). Then the transport across the brush border and basolateral membranes of GIT is facilitated by passive or active (Na+ or H+ co-transporters) pathways (Frenhani and Burini, 1999). Dietary factors, identified above, further influence the absorption rates from the lumen, metabolism in the gut wall and the release of AA into the portal circulation. The amount and quality of protein, and the presence of glucose are also major factors influencing the final outcome (Chen, 2017). According to Webb (1990), among AA, Lys exhibited the slowest absorption rate and Met the highest.

Striking differences observed in age effect trends between the AIDC and SIDC AA estimates for both PS were in agreement with previous studies (Chapters 6 and 7; Adedokun *et al.*, 2008; Szczurek *et al.*, 2020). In the study of Adedokun *et al.* (2008), AIDC AA in SBM and CM increased with broiler age (d 5 vs. 21), but the SIDC AA in SBM and CM was unaffected. Two recent studies in our laboratory (Chapters 6 and 7) determined the AIDC of N and AA at six broiler ages (d 7, 14, 21, 28, 35 and 42) in four grains (wheat, sorghum, maize and barley). With advancing age, the average AIDC of TAA in wheat, maize and barley increased and no age effect was observed in sorghum. The average SIDC of TAA was unaffected by age in wheat and decreased in sorghum. While the trend was an inconsistent pattern in maize, the average SIDC of TAA in barley increased with age.

In contrast to the present findings, some studies indicated reduction of AA digestibility in older birds. The apparent excreta protein

digestibility was reduced at d 52 compared to d 21 in a study by Fonolla *et al.* (1981). According to Zuprizal *et al.* (1992), the true excreta digestibility of protein and AA in SBM and rapeseed meal in broilers was higher at week 3 than week 6. In SBM, the corresponding true protein digestibility estimates were reported to be 0.872 (week 3) vs. 0.831 (week 6), respectively. ten Doeschate *et al.* (1993) observed that the true excreta protein digestibility in broilers at d 15 was higher than d 29 and d 43 by 2.3 and 1.1%, respectively. The above contradictions may be attributed to the site of measurement (ileal vs. excreta; Ravindran and Bryden, 1999) and, reflect modifications in AA by hindgut microbial fermentation and urinary contamination. Adedokun *et al.* (2007a), using ileal measurements, found that the apparent digestibility of TAA in beef-originated meat and bone meal at 5 d (0.705) was higher than that of 21 d old broiler (0.595). Correction for endogenous AA failed to make any difference and the SIDC of TAA at d 5 was 20.3% higher than d 21. Conversely, the AIDC of TAA in pork-originated meat and bone meal was 32.1% higher at d 21. The SIDC of TAA also increased with age and the estimated values were 0.588 and 0.752 at d 5 and d 21, respectively.

The calculation of SIDC AA involves standardisation of apparent digestibility coefficients for the basal EAA flow (Lemme *et al.*, 2004) that arises from mucoproteins, various digestive secretions (bile, pancreatic, gastric and intestinal secretions), and desquamated epithelial cells from the GIT (Ravindran, 2021). Studies conducted with combinations of different ingredients demonstrate that the SIDC

AA is more additive than AIDC when used in mixed diets. The use of SIDC AA minimises the chance of underestimating the value of nutritionally critical AA (Cowieson *et al.*, 2019; An *et al.*, 2020) and diets formulated based on SIDC AA matches the birds' requirements more closely.

Despite its limitations, the AIDC AA estimates are presented herein for two reasons: (i) most published AA digestibility data are based on AIDC, and (ii) to better understand the extent of age influence on the SIDC AA after standardisation of AIDC data using age-appropriate EAA flows. Figures 8.1 and 8.2 clearly illustrate the differences between the average AIDC and SIDC of TAA in SBM and CM, respectively, with the use of age-appropriate EAA flows.

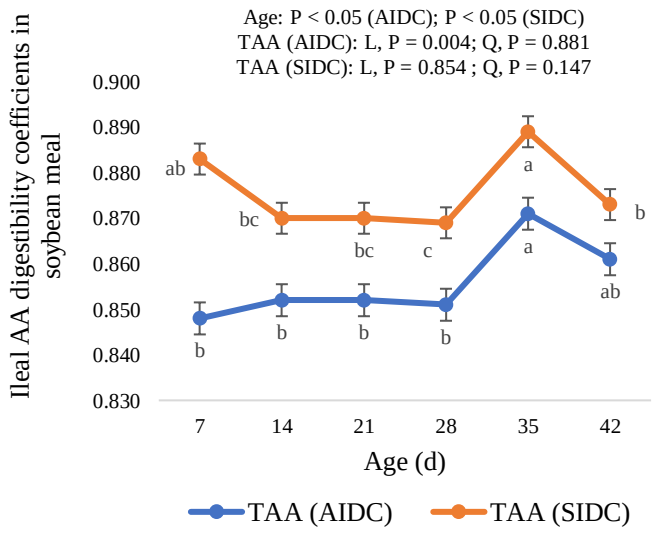

Figure 8.1. Apparent and standardised ileal digestibility coefficients of total amino acids (TAA) in soybean meal (bars represent means ± SE)

as influenced by broiler age. [a,b,c]Values with different superscripts differ significantly (P < 0.05). L, Linear; Q, Quadratic.

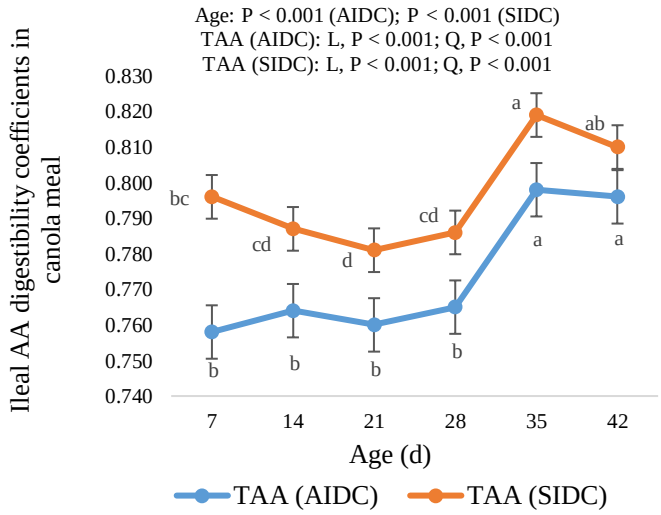

Figure 8.2. Apparent and standardised ileal digestibility coefficients of total amino acids (TAA) in canola meal (bars represent means ± SE) as influenced by broiler age. [a,b,c,d]Values with different superscripts differ significantly (P < 0.05). L, Linear; Q, Quadratic.

Marked differences were observed in the percentage of increase in SIDC AA estimates at different ages over the AIDC data. The percentage uplift in the average SIDC of TAA in SBM at d 7 (4.13%) was almost two times higher than the increase from d 14 to 35 (2.07-2.24%), and three times than those of d 42 (1.39%). In the CM, the percentage of increase in the SIDC of average TAA at d 7 was 5.01% and higher than the increase from d 14 to 35 (2.63-3.01%) and d 42 (1.76%), respectively. These data show that the extent of increase in SIDC AA over AIDC AA decreases with age. The implication is that the use of single EAA flow for standardisation of AIDC data at

different ages underestimates the SIDC AA at early ages and overestimates at later ages.

8.6. Conclusions

The present results provide, for the first time, age-related trends in the AIDC AA and SIDC AA in SBM and CM over the whole broiler growth cycle. The AIDC AA in both PS were increased with advancing age, but the trends in SIDC AA differed between the PS. In the SBM, broiler age had no influence on the average SIDC of IAA and TAA. An inconsistent pattern of age effect was observed for the average SIDC of DAA and the SIDC of some AA (Arg, Thr, Trp, Cys, Pro, Asp). In the CM, an increase in the average SIDC of DAA and TAA was observed from d 7 to 14 that declined at d 21, followed by an increase after d 28. The average SIDC of IAA, however, was lower until d 28 and increased beyond d 35. The results suggest that the age effects on the SIDC AA are dependent on the PS and AA. The use of age-appropriate EAA flows for the standardisation of AIDC might be considered in future AA digestibility assays.

CHAPTER 9 GENERAL DISCUSSION

9.1. Introduction

It is now unquestionably accepted that the digestible amino acid (AA) is superior to total AA (TAA) as it represents the actual AA that becomes available to birds for maintenance and production purposes (Rostagno *et al.*, 1995). Currently, the AA digestibility measured from the terminal ileum of birds is preferred because the excreta digestibility method suffers from several limitations including chance of hindgut microflora action or voiding of faecal and urinary AA that modifies the AA digestibility estimates. These issues are avoided in ileal AA digestibility assays (Lemme *et al.*, 2004). Historically, both the apparent and standardised AA digestibility values have been used in the poultry industry (Lemme *et al.*, 2004; Bryden *et al.*, 2009a). In standardised ileal digestibility (SID) estimates, the apparent ileal digestibility (AID) values are corrected for endogenous AA (EAA) flows from various digestive secretions, pancreatic or enzymatic secretions. According to current reports (Cowieson *et al.*, 2019; An *et al.*, 2020), the AID coefficients (AIDC) of AA undervalue several nutritionally critical AA digestibility and, is less additive in feed formulations than SID coefficients (SIDC). Despite the debate on the procedure of correction of EAA flows, nowadays the SIDC AA is become the norm (Lemme *et al.*, 2004) to determine the magnitude of protein digestion in poultry (Bryden *et al.*, 2009a). Though a range of data are available on the AA digestibility in various ingredients for

broilers (Lemme *et al.*, 2004; Bryden *et al.*, 2009a; Blok and Dekker, 2017), several aspects of practical relevance remain unexplored. This project aimed to address some of the current research gaps and possible solutions.

Firstly, to the author's knowledge, all previous experiments on AA digestibility in broilers have been conducted using mash diets because of simplicity and the fact that most research facilities do not have pelleting equipment. However, pelleted diets are the most prevalent feed form (FF) used for broiler feeding worldwide. Therefore, the applicability of the AA digestibility data generated with mash diets in pelleted diets could be questioned. The first two studies (Chapters 3 and 4) were aimed to determine the effect of FF (mash vs. pellet) on the SIDC of nitrogen (N) and AA in common grains (maize, barley, wheat and sorghum) and protein sources (PS; soybean meal, SBM; canola meal, CM; meat and bone meal) for broilers. The notable findings of these experiments were that diverse factors associated with pelleting might negatively affect the AA digestibility. Secondly, though basal EAA flows in broilers have been measured (Ravindran *et al.*, 2004; Ravindran and Hendriks, 2004; Golian *et al.*, 2008), there is no data on the basal EAA flows from hatching to the end of broiler growth cycle. With the exception of some studies (Adedokun *et al.*, 2007b,c; Adedokun *et al.*, 2008; Szczurek *et al.*, 2020; Toghyani *et al.*, 2015), all previous EAA flow estimates were obtained at the late stage of broiler life (older than 21 d) and have been used to standardise the AIDC AA across all ages. Chapter 5 investigated the basal EAA flows

at six broiler ages (d 7, 14, 21, 28, 35 and 42) following the feeding of a N-free diet (NFD). It was found that the EAA flows were influenced by broiler age and higher in younger birds.

Finally, several previous studies indicated that age has a major impact on the AA digestibility in broilers (Fonolla *et al.*, 1981; Jin *et al.*, 1998). To the best of our knowledge, only a few age-related studies exist on the SIDC AA (Garcia *et al.*, 2007; Adedokun *et al.*, 2007a, 2008; Toghyani *et al.*, 2015; Szczurek *et al.*, 2020), but these are limited to only two or three ages. Currently, the digestibility estimates are generated from older birds (mostly 22 to 35 d) and are used in feed formulations regardless of broiler age. Using a single digestibility estimate for all ages will lead to incorrect evaluation of AA requirement of broilers, and ultimately influence the precision of feed formulations. Experiments in Chapters 6, 7 and 8 determined the SIDC AA at different ages of broilers (d 7, 14, 21, 28, 35 and 42) in wheat and sorghum (Chapter 6), maize and barley (Chapter 7), and SBM and CM (Chapter 8). These studies confirmed that age has a significant effect on AA digestibility and that the age effect is variable depending on the ingredient type and specific AA.

9.2. Influence of feed form on standardised ileal amino acid digestibility in grains and protein sources

Data reported in Chapter 3 indicated that, regardless of the grain type, FF had no effect on the SIDC of N and average SIDC of total AA (TAA). However, the SIDC of Cys, Pro and Glu (a tendency) was

compromised in pelleted diets. The SIDC of Cys and Pro was reduced by 4.9 and 3.7%, respectively, by pelleting. Being the most heat labile AA, the digestibility of Cys is affected by hydrothermal treatment (Goodarzi Boroojeni *et al.*, 2016). In the case of PS, the major findings recorded in Chapter 4 were that FF had no effect on the SIDC of most indispensable AA (IAA), except His that was decreased by pelleting. A tendency of reduction in the SIDC by pelleting was observed for Thr, Trp and Val, and average of IAA. Pelleting, however, compromised the SIDC of all individual dispensable AA (DAA), average DAA by 8.8%, and average TAA by 6.5%. Cysteine was the most affected DAA by pelleting with a 15.4% reduction in the SIDC compared to mash. The corresponding SIDC decreases in pellet-fed broilers were reported to be 11.1% in Gly, 9.1% in Pro, 8.4% in Asp, 6.9% in Ser, and 5.1% in Glu. These two studies (Chapters 3 and 4) revealed that the effect of FF on AA digestibility was more prominent in PS compared to cereal grains.

According to Cheftel (1986), favourable conditions for Maillard reactions include high temperature (> 180 ºC) and low moisture (< 15%) combined with shear (> 100 rpm in an extruder). The conditions of pelleting (75-85 ºC temperature, 3-4% moisture) are, however, too mild to initiate the Maillard reaction (Svihus *et al.*, 2004; Svihus and Zimonja, 2011), and therefore other factors associated with pelleting are responsible for the observed SIDC AA effects in PS. Higher consumption and, consequently, nutrient overload in the gastrointestinal tract (GIT; Abdollahi *et al.*, 2018b), dilution of

digestive enzymes and elevated gizzard pH (Engberg *et al.*, 2002) might negatively influence the digestion of nutrients, including AA, when birds are fed pelleted diets. Shorter digesta retention time in birds fed pelleted diets decreases the contact between the nutrients and intestinal absorptive surface, which might also compromise the AA digestion and absorption (Frikha *et al.*, 2009; Abdollahi *et al.*, 2018b).

9.3. Which feed form should be used in AA digestibility assays? Mash or pellet?

When this project was initiated, the pragmatic challenge was to decide the correct FF (mash vs. pellet) to be used in AA digestibility assays. Ample amount of data exists on the FF effect on the digestibility of nutrients in complete broiler diets (Abdollahi *et al.*, 2011, 2014). However, no studies to date have investigated the effect of FF on the AA digestibility in single ingredients. From the first two studies (Chapters 3 and 4), it is evident that FF has a substantial impact on the SIDC AA in different ingredients for broilers and the effect is more pronounced in high-protein feed ingredients than in cereal grains. These findings indicate that the current trend of using the SIDC AA data derived from feeding mash diets may lead to overestimation of the AA availability in feed ingredients, affecting the precision of broiler feed formulation. It is, therefore, recommended that AA digestibility estimates should be generated using pelleted experimental diets to resemble the prevalent FF in the broiler industry. Based on findings from Chapters 3 and 4, assay diets in subsequent age-related studies

(Chapters 6, 7 and 8) were offered in pellet form, which is one of unique features of this thesis research.

9.4. Age-appropriate endogenous AA flows: how can feed formulation benefit from it?

Data reported in Chapter 5 showed that broiler age influences the basal EAA flows. The EAA flow of TAA at d 7 was almost twice the average from d 14 to 35 and three times higher than those at d 42. The EAA flow is a balance between the secretion and absorption of endogenous proteins. The source of endogenous proteins and the entry point into the GIT determine this balance. If easily digestible endogenous enzymes predominate the endogenous flow, then there will be greater digestion and absorption of AA and EAA will be reduced. On the other hand, when there is increased output of hard-to digest mucoproteins in the GIT, EAA flows at the ileal level will be greater (Ravindran, 2021). The higher EAA flows determined at early ages may result from increased EAA secretion, decreased EAA absorption, or a combination of both. It has been reported that when an NFD is fed to birds, the predominant source of the EAA is mucoproteins (Adedokun *et al.*, 2007b). The AA abundant in mucin glycoproteins are Thr, Asp, Pro, Glu, Ser and Asp (Souffrant, 1991; Lien *et al.*, 1997). Higher EAA flows at d 7 compared to older birds in Chapter 5 may be attributed to higher mucin production. The observed higher endogenous flows of Thr (102%), Pro (103%), Ser (103%) Asp (106%), and Glu (123%) on d 7 than the average from d 14 to 42 lend credence to this speculation.

For precise feed formulation, accurate quantification of EAA flows is necessary to correct AIDC values to SIDC AA estimates. From the foregoing discussion, it is evident that the application of single EAA flow estimate for the standardisation of AIDC data for all ages may underestimate the SIDC in early life and overestimate in older birds. Data presented in Chapter 5 provides, for the first time, age-specific basal EAA flows from 7 to 42 d of age that can be used to generate age-specific SID AA in ingredients. A criticism of this study is that an NFD diet was used to measure the EAA flow. Though feeding an NFD is the widely accepted and simplest method for estimating basal EAA flows, this method suffers from underestimation of values and being non-physiological because of the protein-free diet (Donkoh and Moughan, 1999; Stein *et al.*, 2007). Future age-related studies are warranted using alternative methods (feeding diet containing highly digestible protein, or peptide alimentation method), but it must be noted that these approaches have their own advantages and constraints.

9.5. Antinutritive factors in ingredients - do their influence on amino acid digestibility differ at different broiler ages?

Unlike maize and sorghum, wheat and barley contain high concentrations of soluble non-starch polysaccharides (NSP) that remain unhydrolysed in the upper intestinal tract of monogastric animals (Bach Knudsen, 2014). The water-soluble fraction of NSP, specifically arabinoxylans (pentosans) and mixed linkage β-glucans, are viscous and negatively affect the digestion and absorption of nutrients (Choct and Annison, 1992a). In present study, the AIDC of N

and AA in wheat (Chapter 6) and barley (Chapter 7) increased with advancing age. In wheat, the average AIDC of TAA at d 7 was 8.48% lower than those the average from d 14 to 21 and 12.2% lower than the average of d 28, 35 and 42. The average AIDC of TAA in barley at d 7 was 29.2% lower than that at d 14, 37.3% lower than at d 21 and 36.4% lower than the average from d 28 to 42. Lower digestibility at d 7 in wheat and barley may be attributed to the lower tolerance of younger chicks to viscous NSP (Choct and Annison, 1992a). Due to maturity and size of GIT in older birds, the adverse effects induced by soluble NSP are ameliorated (Salih *et al.*, 1991; Almirall *et al.*, 1994).

Data reported in Chapter 8 demonstrated that the average AIDC of IAA, DAA and TAA in SBM and CM increased with advancing broiler age. The average AIDC of TAA in both PS evened out from d 7 to 28 and increased beyond d 35. Lower AIDC AA at early ages in SBM may be attributed, in part, to the presence α-galactosides (raffinose, verbascose, stachyose). Carre´*et al.* (1995) stated that young broilers are incapable of digesting α-galactosides. Canola meal also contains some antinutritive factors, such as soluble and insoluble NSP and, phytic acid. The higher phytate and fibre content in CM can reduce the AA utilisation (Cowieson *et al.*, 2009) because of poor digestion of protein-phytate complex (Bell and Keith, 1991). In addition, the dietary ingestion of phytate increases EAA flows either by increased secretion and/or reduced digestion and reabsorption of AA (Cowieson and Ravindran, 2007). Older birds are known to utilise phytate phosphorus more than young birds (Edwards *et al.*, 1989). In older birds, the higher

endogenous phytase activity in the GIT enhance phytate utilisation (Ravindran *et al.*, 1995), thereby decreasing the negative effects on AA digestion.

9.6. Influence of age on the standardised ileal amino acid digestibility of grains and protein sources

The SIDC of N and AA of wheat and sorghum, maize and barley at six different broiler ages (d 7, d 14, d 21, d 28, d 35 and d 42) are reported in Chapters 6 and 7, respectively. The AIDC data were standardised using the age-specific EAA flows measured in Chapter 5. The SIDC of N and, average digestibility of IAA and DAA in wheat were unaffected by age. The average SIDC of TAA tended (linear, P = 0.09) to increase with advancing age. An opposite trend was observed in the case of sorghum with higher SIDC of N, average SIDC of IAA, DAA and TAA at d 7, which declined at d 14 and plateaued thereafter (Chapter 6). Data reported in Chapter 7 showed that while there was no age effect on the SIDC of N in maize, inconsistent patterns were observed for the average SIDC of IAA, DAA and TAA. The average SIDC of TAA was higher at d 7 that dropped at d 14, increased and plateaued between d 21 and 35, finally declined again at d 42. In barley, the SIDC of N and average SIDC of IAA, DAA and TAA increased as the birds grew older. The digestibility increased from d 7 to 21 and followed by a plateau to d 42. The SIDC of all individual AA in barley increased with age.

Data reported in Chapter 8 showed the age effect on the SIDC of N and AA in SBM and CM. In SBM, no patterns were observed for the age influence on SIDC of N, average SIDC of IAA and TAA. In the case of IAA, the changes in the SIDC of Arg, Thr and Trp with advancing age were inconstant. In CM, the SIDC of N and the average SIDC of DAA and TAA plateaued from d 7 to 14, that declined at d 21 and increased beyond d 28. The average SIDC of IAA were lower until d 28, that increased and evened out between d 35 and 42.

It is noteworthy that the findings of SIDC AA changes from hatching to broiler market age generated in this thesis are novel and the only available data to date. The exact reasons for the variable SIDC AA responses with age in different ingredients is hard to explain and complicated by plethora of factors including the secretion and activity of proteases (Nitsan *et al.*, 1991), allometric growth of digestive organs (Katanbaf *et al.*, 1988), digesta passage rate (Noy and Sklan, 1995), antinutritive factors (Bell and Keith, 1991), and differing basal EAA flows at different ages (Chapter 5).

9.7. Uplift in standardised digestibility coefficients due to correction of apparent data for age-appropriate endogenous amino acid losses

The striking differences between the AIDC and SIDC AA, due to correction using age-specific EAA flows, were as expected. The extent of differences decreased as the birds grew older (Table 9.1).

Table 9.1. Percentage increase in the SIDC over AIDC of average of total AA after correction of apparent data for age-specific EAA flows

Chapter	Ingredients	Age (d)		
		7	14 - 35	42
6	Wheat	23.8	10.8 - 11.5	7.43
	Sorghum	20.9	10.7 - 11.4	7.25
7	Maize	32.5	14.2 - 17	9.85
	Barley	31.6	10.0 - 11.5	7.17
8	Soybean meal	4.13	2.07 - 2.24	1.39
	Canola meal	5.01	2.63 - 3.01	1.76

For instance, the increase in average SIDC of TAA at d 7 was almost twice the increase in d 14 to 35, and three times higher than d 42 in all ingredients. The trends in magnitude of age effect on SIDC AA were reflective of those on age-specific EAA flows as reported in Chapter 5. These results demonstrate that variations in EAA flows at different broiler ages have significant influence on the SIDC AA estimates. The findings in this thesis are the only available SIDC AA data in six common ingredients where the AIDC AA estimates were corrected using age-specific EAA flows.

9.8. How can the broiler industry benefit from this thesis research?

This project explores a hitherto unexplored, but an important, area in AA nutrition investigating the age-dependent SIDC AA of six common feed ingredients used in broiler feeds worldwide. The results of this work are both of scientific and practical interest.

That the digestibility and utilisation of nutrients are compromised in young broiler chicks and change with age has long been known. 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 SIDC AA in feed ingredients over the whole broiler growth cycle, this is a timely addition to existing AA digestibility database. It creates an opportunity to apply the SIDC AA data for specific ages of broilers that will increase the precision of feed formulations. In addition, the findings provide a clear understanding about some of the factors affecting the SIDC AA in broiler from hatch to the end of commercial broiler life.

As discussed previously, several factors associated with pelleting significantly influence the SIDC AA. The negative effects of pelleting are more prominent in the SIDC AA of ingredients with higher protein contents than grains. But pellets are the commonly used FF worldwide in broiler production. The application of existing data derived from mash diets will therefore result in the overestimation of AA digestibility. Considering the FF (mash vs. pellet) effect on the SIDC AA (Chapters 3 and 4), the assay 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 SIDC AA data generated in this thesis are more applicable in practical feed formulations for broilers.

The age-specific EAA data generated in this thesis provide an opportunity to modify existing AA databases for different feed ingredients. This will eliminate the risk of underestimation of the SIDC in early life and overestimation in older birds, and will improve the

precision of feed formulations and the sustainability of broiler production.

The challenge confronting the nutritionists will be how to capture the determined weekly data over the six-week growth period in feed formulations. Although the number of broiler feeding phases has increased to five in some recent recommendations by breeding companies (Aviagen, 2019; Target live weight of 3.1 to 4.0 kg), the development of six separate broiler diets might neither be practical nor economical. The best option will be to incorporate the weekly values into existing broiler feeding programmes; for example, using the four feeds specified in recommendations by breeding companies (Aviagen, 2019). Averaging the determined estimates into the four phases, namely at 7 d (starter), 14 and 21 d (grower), 28 and 35 d (finisher 1), and 42 d (finisher 2) may be a workable approach. Table 9.2 summarises the SIDC and the SID contents of all amino acids in the six ingredients at four age groups. It is proposed that the age of broiler should be a variable within the ingredient matrix. Modern software could easily capture this stochastic nature of AA digestibility. Finally, the benefits of this approach must be tested and confirmed in well-planned feeding trials.

Table 9.2. The SIDC and SID content (g/kg) of amino acids in feed ingredients at four broiler feeding phases

			Indispensable amino acids									Dispensable amino acids						
Phases	Ingredients	N	Arg	His	Ile	Leu	Lys	Met	Thr	Trp	Val	Ala	Asp	Cys[1]	Glu	Gly[1]	Pro	Ser
Starter (d 7)	Wheat	SIDC	0.953	0.904	0.929	0.934	0.839	0.886	0.965	0.852	0.899	0.881	0.813	0.896	0.981	0.894	0.972	0.948
		SID content	4.46	1.96	2.98	5.89	2.39	1.49	2.88	1.06	3.70	3.12	4.06	1.91	25.3	3.67	8.54	4.09
	Sorghum	SIDC	0.961	0.837	0.915	0.906	0.934	0.874	0.967	0.869	0.891	0.912	0.916	0.810	0.927	0.911	0.873	0.943
		SID content	3.94	1.96	3.82	12.8	2.06	1.49	3.27	1.05	4.59	8.87	6.39	1.47	20.1	2.97	7.54	4.50
	Maize	SIDC	0.914	0.839	0.844	0.902	0.704	0.899	0.912	0.769	0.807	0.896	0.863	0.968	0.912	0.784	0.845	0.902
		SID content	3.14	1.74	2.10	7.34	1.64	1.18	2.36	0.45	2.78	4.60	4.11	1.33	11.3	2.24	4.73	2.97
	Barley	SIDC	0.724	0.652	0.574	0.650	0.382	0.611	0.595	0.564	0.578	0.566	0.509	0.806	0.771	0.513	0.755	0.623
		SID content	3.82	1.55	2.28	4.89	1.42	1.08	2.10	0.76	3.19	2.57	3.45	1.79	18.8	2.28	8.12	2.75
	SBM	SIDC	0.934	0.898	0.887	0.884	0.899	0.908	0.863	0.874	0.878	0.872	0.880	0.807	0.918	0.857	0.885	0.883
		SID content	30.1	10.5	18.4	29.7	24.5	5.41	14.7	5.23	18.9	16.8	43.9	4.51	72.7	16.0	18.8	18.9
	CM	SIDC	0.873	0.830	0.791	0.818	0.771	0.870	0.755	0.798	0.788	0.809	0.769	0.744	0.866	0.771	0.743	0.762
		SID content	19.3	7.87	11.9	20.9	14.9	6.58	11.6	4.03	15.1	13.0	19.9	5.92	57.1	14.4	15.9	11.3
Grower (Avg. d 14 and 21)	Wheat	SIDC	0.912	0.898	0.903	0.915	0.832	0.895	0.901	0.879	0.885	0.861	0.817	0.898	0.966	0.861	0.953	0.916
		SID content	4.25	1.95	2.91	5.78	2.37	1.51	2.60	1.09	3.65	3.05	4.08	1.91	24.9	3.54	8.38	3.95
	Sorghum	SIDC	0.863	0.771	0.856	0.889	0.844	0.849	0.805	0.835	0.825	0.892	0.842	0.727	0.899	0.759	0.820	0.833
		SID content	3.60	1.92	3.63	12.6	1.83	1.47	2.85	1.02	4.35	8.41	5.67	1.45	19.9	2.57	7.56	4.13
	Maize	SIDC	0.886	0.838	0.837	0.910	0.721	0.882	0.775	0.734	0.813	0.888	0.818	0.907	0.901	0.750	0.842	0.824
		SID content	3.04	1.74	2.08	7.41	1.68	1.16	2.01	0.43	2.80	4.56	3.90	1.24	11.2	2.14	4.72	2.71
	Barley	SIDC	0.805	0.771	0.777	0.812	0.711	0.813	0.764	0.754	0.772	0.753	0.730	0.857	0.858	0.696	0.833	0.785
		SID content	4.25	1.83	3.09	6.12	2.64	1.44	2.70	1.02	4.26	3.42	4.95	1.91	20.9	3.09	8.96	3.46
	SBM	SIDC	0.917	0.889	0.880	0.880	0.902	0.909	0.837	0.862	0.874	0.874	0.863	0.766	0.904	0.839	0.864	0.868
		SID content	29.6	10.4	18.2	29.6	24.6	5.41	14.3	5.17	18.8	16.9	43.1	4.28	71.7	15.7	18.3	18.6
	CM	SIDC	0.852	0.813	0.788	0.819	0.759	0.874	0.725	0.778	0.781	0.809	0.741	0.737	0.854	0.743	0.718	0.749
		SID content	19.1	7.85	12.1	21.4	15.1	6.71	11.6	4.02	15.3	13.3	19.8	5.86	57.1	14.3	16.2	11.5

Finisher (Avg. d 28 and 35)	Wheat	SIDC	0.931	0.919	0.934	0.949	0.872	0.941	0.918	0.917	0.916	0.880	0.884	0.934	0.972	0.891	0.966	0.937
		SID content	4.36	1.99	3.01	5.99	2.49	1.58	2.66	1.15	3.78	3.12	4.41	1.99	25.0	3.66	8.49	4.05
	Sorghum	SIDC	0.899	0.776	0.853	0.864	0.843	0.874	0.821	0.852	0.834	0.858	0.863	0.793	0.863	0.781	0.809	0.850
		SID content	3.69	1.82	3.56	12.2	1.86	1.50	2.78	1.03	4.31	8.35	6.01	1.44	18.8	2.54	6.98	4.06
	Maize	SIDC	0.935	0.858	0.887	0.931	0.816	0.938	0.864	0.829	0.883	0.912	0.878	0.895	0.925	0.830	0.873	0.902
		SID content	3.21	1.78	2.21	7.58	1.91	1.23	2.24	0.48	3.04	4.69	4.19	1.23	11.5	2.37	4.89	2.97
	Barley	SIDC	0.843	0.773	0.807	0.837	0.770	0.851	0.822	0.789	0.814	0.786	0.789	0.836	0.858	0.749	0.837	0.834
		SID content	4.45	1.84	3.22	6.31	2.87	1.50	2.91	1.07	4.50	3.57	5.35	1.87	20.9	3.32	9.0	3.68
	SBM	SIDC	0.932	0.898	0.886	0.885	0.907	0.909	0.844	0.878	0.878	0.877	0.868	0.791	0.914	0.855	0.876	0.877
		SID content	29.6	10.5	18.3	29.8	24.8	5.41	14.4	5.26	18.9	16.9	43.3	4.41	72.5	16.0	18.6	18.8
	CM	SIDC	0.877	0.831	0.806	0.833	0.779	0.884	0.747	0.807	0.798	0.825	0.760	0.740	0.871	0.771	0.745	0.768
		SID content	19.7	8.02	12.4	21.8	15.5	6.75	11.9	4.18	15.6	13.6	20.3	5.89	58.3	14.8	16.6	11.9
Finisher (d 42)	Wheat	SIDC	0.914	0.913	0.921	0.937	0.863	0.923	0.900	0.911	0.905	0.863	0.864	0.926	0.967	0.881	0.957	0.928
		SID content	4.28	1.98	2.97	5.91	2.46	1.55	2.60	1.14	3.73	3.05	4.31	1.98	24.9	3.62	8.41	4.01
	Sorghum	SIDC	0.879	0.771	0.843	0.865	0.814	0.859	0.799	0.849	0.826	0.859	0.844	0.781	0.863	0.756	0.799	0.841
		SID content	3.60	1.80	3.53	12.2	1.79	1.47	2.70	1.03	4.26	8.36	5.88	1.42	18.7	2.47	6.89	4.01
	Maize	SIDC	0.909	0.844	0.859	0.915	0.776	0.904	0.821	0.792	0.861	0.887	0.843	0.877	0.903	0.807	0.866	0.876
		SID content	3.12	1.75	2.14	7.45	1.81	1.18	2.13	0.46	2.96	4.56	4.02	1.20	11.2	2.29	4.85	2.88
	Barley	SIDC	0.824	0.756	0.793	0.819	0.758	0.823	0.784	0.769	0.799	0.764	0.763	0.809	0.844	0.729	0.827	0.808
		SID content	4.35	1.79	3.16	6.17	2.82	1.46	2.78	1.04	4.41	3.47	5.17	1.80	20.5	3.24	8.89	3.56
	SBM	SIDC	0.922	0.891	0.878	0.874	0.898	0.900	0.838	0.879	0.869	0.866	0.860	0.802	0.904	0.850	0.870	0.870
		SID content	29.7	10.4	18.2	29.4	24.5	5.36	14.2	5.27	18.8	16.7	43.0	4.47	71.7	15.9	18.5	18.6
	CM	SIDC	0.889	0.840	0.809	0.833	0.786	0.881	0.751	0.819	0.802	0.826	0.769	0.759	0.877	0.790	0.762	0.767
		SID content	19.9	8.07	12.4	21.7	15.5	6.73	11.9	4.21	15.6	13.5	20.4	6.04	58.5	15.0	16.8	11.8

Avg. = average; CM = canola meal; SBM = soybean meal

[1]Semi-indispensable amino acids for poultry.

Wheat, sorghum (Chapter 6); maize, barley (Chapter 7); SBM, CM (Chapter 8)

9.9. Recommendations for future amino acid digestibility assays

This project was aimed to determine the standardised ileal AA digestibility in common feed ingredients used in broiler diets. In future digestibility assays, age-specific evaluations must be extended to alternative feed ingredients, especially those with poor protein quality. Antinutritive factors, such as soluble NSP, substantially affect the AA digestibility in viscous grains like wheat (Chapter 6) and barley (Chapter 7), and the effect is more prominent in younger birds, specifically during the first 7 d. No exogenous NSP-degrading enzyme was used in the assay diets in this project, as the objective was to investigate the actual age effects on AA digestibility. Similarly, no exogenous proteases were used and an extension of the project would be to determine the influence of proteases on the SIDC AA at different broiler ages, especially in ingredients with low AA digestibility. The efficacy of protease has been reported to be two times higher for every 10% reduction in ileal AA digestibility (Cowieson and Ross, 2014). Based on meta-analysis of 25 poultry and pig studies, Cowieson and Ross (2014) predicted that the response to protease in wheat/canola-based diet in broiler increases from 3% at d 14 to 4% in d 42 age. In maize/SBM-based diets, the protease effect reduces from 2.2% at d 14 to 1.8% at d 42. These results are suggestive of interactions between bird age × diet type in response to protease supplementation. Future studies are warranted using exogenous enzymes to investigate the extent of AA digestibility responses at different ages and to develop age-dependent enzyme matrix values.

An unsolved question relates to the applicability of AA digestibility measured in broilers to laying hens. Published data on the AA digestibility of ingredients for laying hens are scanty (Huang *et al.*, 2006, 2007). Hens are mature birds and it is reasonable to expect that the nutrient digestion will be much more efficient. Future studies may be extended to investigate digestible AA of ingredients in laying hens.

Another issue of some interest is the effect of sex on the AA digestibility in broiler chickens. Although the available data are inconsistent and inconclusive, evidence suggests that there are some differences between male and female broilers in their ability to digest and utilise nutrients (Wallis and Balnave, 1984; ten Doeschate *et al.*, 1993). This may be a subject worth exploring in the future.

9.10. 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 ileal AA digestibility: FF and age of broilers. Feed form substantially impacted the SIDC AA in ingredients. The negative influence of pelleting on the AA digestibility was more notable in high protein feed ingredients. The SID AA from hatch to market age in six most commonly used feed ingredients in broiler feeds was reported. A key finding was that the age effect on the SID AA was variable depending on the ingredient type and specific AA. The results also showed that the difference in the EAA flows at different broiler ages is the prime contributing factor affecting SIDC AA estimates, especially in grains. Therefore, the use of age-specific EAA data for the

standardisation of AIDC AA is recommended. It is proposed that the age of broiler should be accommodated as a variable within the ingredient matrix in formulation packages. This stochastic nature of digestible AA could be easily handled in modern software. Application of age-appropriate SID AA data will enable the poultry industry to improve the precision of feed formulations, broiler performance, profitability and sustainability of poultry production.

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APPENDICES

Appendix A. Chapter 5

Table 1. Basal endogenous amino acid flow values (g/kg dry matter intake) in broilers (data from Massey University)

						Indispensable amino acids										Dispensable amino acids							
References	Age (d)	Diet type (g/kg)	CP	Total AA	N	Arg	His	Ile	Leu	Lys	Met	Phe	Thr	Trp	Val	Ala	Asp	Cys[1]	Glu	Gly[1]	Pro	Ser	Tyr
										N-free diet method													
Unpublished data, 2014	28	Dex, 869	10.8	7.40	1.72	0.44	0.15	0.37	0.60	0.39	0.15	0.42	0.52	0.13	0.47	0.40	0.77	0.22	0.98	0.42	0.45	0.52	NR
Unpublished data, 2014	34	MS/Dex, 420:420	7.28	4.83	1.16	0.20	0.11	0.22	0.33	0.21	0.07	0.21	0.43	NR	0.33	0.25	0.46	0.18	0.53	0.32	0.40	0.38	0.20
Unpublished data, 2014	22	MS/Dex, 437:437	4.63	3.83	0.74	0.18	0.08	0.16	0.25	0.18	0.05	0.16	0.37	NR	0.24	0.20	0.38	0.15	0.43	0.23	0.28	0.34	0.15
Unpublished data, 2014	28	Dex, 869	5.42	4.12	0.87	0.19	0.09	0.17	0.27	0.17	0.06	0.18	0.43	NR	0.27	0.21	0.40	0.16	0.45	0.24	0.30	0.36	0.17
Unpublished data, 2014	21	Dex, 847	7.91	6.11	1.27	0.34	0.10	0.30	0.48	0.27	0.12	0.28	0.49	NR	0.40	0.33	0.58	0.15	0.84	0.33	0.39	0.43	0.28
Unpublished data, 2014	21	Dex, 847	6.67	5.13	1.07	0.26	0.07	0.24	0.38	0.21	0.10	0.23	0.45	NR	0.33	0.26	0.48	0.17	0.66	0.29	0.37	0.40	0.23
Unpublished data, 2016	24	Dex, 842	12.1	8.16	1.94	0.39	0.16	0.37	0.56	0.31	0.13	0.37	0.77	NR	0.54	0.41	0.77	0.34	0.97	0.46	0.61	0.67	0.33
Unpublished data, 2016	28	Dex, 869	10.7	7.19	1.71	0.44	0.14	0.37	0.60	0.39	0.15	0.42	0.52	NR	0.46	0.40	0.76	0.21	0.97	0.41	0.44	0.51	NR
Unpublished data, 2017	31	Dex, 869	9.93	6.75	1.59	0.35	0.13	0.35	0.55	0.39	0.13	0.27	0.55	0.08	0.46	0.39	0.68	0.18	0.92	0.41	0.45	0.46	NR
Unpublished data, 2018	31	Dex, 869	7.20	4.51	1.15	0.21	0.09	0.21	0.34	0.19	0.07	0.15	0.42	0.06	0.31	0.25	0.48	0.17	0.57	0.29	0.33	0.37	NR
Unpublished data, 2018	28	Dex, 869	10.2	6.72	1.64	0.42	0.15	0.31	0.51	0.40	0.13	0.25	0.53	0.13	0.41	0.36	0.70	0.19	0.98	0.38	0.41	0.46	NR
Ravindran *et al.* (2004)	37	Dex, 819	7.33	5.82	1.17	0.28	0.16	0.29	0.44	0.21	0.10	0.29	0.51	0.09	0.42	0.29	0.61	0.23	0.72	0.51	NR	0.42	0.25
Ravindran *et al.* (2009)	35	Dex, 847	7.69	6.20	1.23	0.24	0.13	0.25	0.36	0.23	0.09	0.19	0.53	NR	0.28	0.29	0.55	0.20	0.69	0.48	1.07	0.41	0.21
Ravindran *et al.* (2014a)	34	Dex, 847	8.16	6.89	1.31	0.37	0.17	0.34	0.51	0.29	0.10	0.26	0.60	NR	0.45	0.37	0.59	0.22	0.87	0.62	0.33	0.52	0.28
Ravindran *et al.* (2017)	35	MS/Dex, 200:640	12.3	8.66	1.97	0.49	0.20	0.42	0.69	0.52	0.16	0.49	0.62	0.16	0.55	0.48	0.84	0.25	1.11	0.50	0.55	0.63	NR
Cowieson *et al.* (2019)	28	WS, 842	10.1	7.89	1.61	0.37	0.19	0.33	0.55	0.35	0.12	0.34	0.70	NR	0.47	0.41	0.96	0.29	0.91	0.44	0.53	0.59	0.34
Cowieson *et al.* (2020)	28	WS, 842	7.54	5.84	1.21	0.32	0.10	0.29	0.46	0.26	0.11	0.26	0.47	NR	0.38	0.31	0.57	0.15	0.79	0.32	0.38	0.41	0.26
Perera *et al.* (2019)	24	Dex, 842	7.03	6.12	1.12	0.31	0.13	0.27	0.44	0.31	0.13	0.27	0.51	NR	0.37	0.30	0.60	0.22	0.77	0.35	0.41	0.48	0.25

[1]Semi-indispensable amino acids for poultry.

d = days; CP = crude protein; Dex = dextrose; MS = maize starch; N = nitrogen; NR = not reported; WS = wheat starch.

Appendix B. 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.

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- Please provide the full reference of the Research Output:

Barua, M., Abdollahi, M. R., Zaefarian, F., Wester, T. J., Girish, C. K. and Ravindran, V. (2021). Influence of feed form on the standardised ileal amino acid digestibility of common grains for broiler chickens. Animal Feed Science and Technology, 272, 114743.

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