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**INFLUENCE OF BIRD TYPE, DIETARY FIBRE AND
PARTICLE SIZE ON APPARENT ILEAL DIGESTIBILITY OF
NUTRIENTS AND ENERGY UTILISATION**

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

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List of abbreviations

AA	Amino acid
ADF	Acid detergent fibre
AID	Apparent ileal digestibility
ALA	Alanine
AME	Apparent metabolisable energy
AMEn	Nitrogen-corrected apparent metabolisable energy
ANOVA	Analysis of variance
ARG	Arginine
ASP	Aspartic acid
°C	Celsius
Ca	Calcium
CAID	Coefficient of apparent ileal digestibility
Cu	Copper
CYS	Cysteine
d	days
DDGS	Distiller's dried grains with solubles
DF	Dietary fibre
DM	Dry matter
FI	Feed intake
g	Gram

GE	Gross energy
GIT	Gastrointestinal tract
GLM	General linear model
GLU	Glutamic acid
GLY	Glycine
GMD	Geometric mean diameter
GSD	Geometric standard deviation
h	hours
HCl	Hydrochloric acid
HIS	Histidine
ICP-MS	Inductively Coupled Plasma-Mass Spectrometry
ICP-OES	Inductively Coupled Plasma-Optical Emission Spectroscopy
ISO	Isoleucine
K	Potassium
Kcal	Kilo calories
kg	Kilogram
kJ	Kilo joule
LEU	Leucine
LSD	Least significant difference test
LYS	Lysine
MET	Methionine

Mg	Magnesium
MJ	Mega joule
mm	Millimetre
Mn	Manganese
N	Nitrogen
Na	Sodium
NDF	Neutral detergent fibre
NE	net energy
NRC	National research council
P	Phosphorus
<i>p</i>	Probability
pH	Power of hydrogen
PHE	Phenylalanine
PRO	Proline
SAS	Statistical analysis system
SEM	Standard error of mean
SER	Serine
SID	Standardized ileal digestibility
THR	Threonine
Ti	Titanium
TID	True ileal digestibility

VAL	Valine
Zn	Zinc
μm	microns

CHAPTER 1

General introduction

Poultry require dietary nutrients including amino acids, minerals and vitamins for body maintenance, growth and egg production. Feed represents the major cost item in poultry production accounting for 60 to 70% of the total production cost. The ever-increasing cost of raw materials has placed more emphasis on the use of alternative feed ingredients in order to reduce the costs of production. As a result, there has been an increased interest in exploring new approaches to improve nutrient digestibility in poultry. A major growing area on poultry nutrition research has been the use of digestible amino acids (Huang et al., 2006b) and other nutrients instead of total nutrient contents in feed formulations. The use of digestible nutrient contents of feed ingredients would enhance the economics of production by meeting the bird's nutrient requirements more closely. Besides other factors including sex (Doeschate et al., 1993; Batal and Parsons, 2002; 2003), age (Huang et al., 2005; Garcia et al., 2007), genotype (Ravindran et al., 1999b; Rideau et al., 2014) and dietary nutrient composition (Parsons et al., 1997), type of birds, level of dietary fibre (DF) and particle size of the diet are possible factors that can affect the nutrient digestibility in poultry.

There are several published data on the amino acid digestibility of feed ingredients for broilers, but studies comparing the nutrient digestibility in different bird types are scant. However, there is some available information concerning the effect of bird type on the digestibility of amino acids in feed ingredients (Huang et al., 2006b; 2007). Huang et al. (2007) showed higher apparent ileal digestibility of most amino acids in maize for broilers compared to those for layers. Huang et al. (2006b) found higher apparent ileal digestibility of amino acids in soybean meal for layers compared with those for broilers. Adedokun et al. (2009) showed higher apparent ileal amino acid digestibility of maize (0.891) and lower digestibility of meat and bone meal (0.647) in broilers than those in layers (0.714 and 0.750, respectively). Huang et al. (2000a)

reported lower protein digestibility in sorghum (0.764) and higher digestibility of soybean meal (0.889) in layers than broilers (0.815 and 0.852, respectively). Huang et al. (2000b) showed greater protein digestibility for canola meal in broilers (0.780) than those in layers (0.740). The observed influence of bird type on the nutrient digestibility in different feed ingredients suggests that the use of nutrient digestibility values generated with one type of bird may not be appropriate for other types of birds.

Inclusion of insoluble fibre sources in poultry diets has received renewed attention in the poultry research community and their inclusion is currently recommended in the commercial poultry industry (Mateos et al., 2012). It is noteworthy that factors such as type and inclusion rate of fibre sources might contribute to the variation of nutrient digestibility in poultry (Hetland et al., 2004; Mateos et al., 2012). Studies have shown that the extent to which DF improves nutrient digestibility is variable, depending on the bird type (Walugembe et al., 2014; Kimiaetalab et al., 2018). Layers fed diets with increased fibre content reported to have better utilisation of nutrients compared to broilers, suggesting that laying hens have an increased nutrient digestibility capability to the increased DF content compared to broilers (Walugembe et al., 2014). However, despite its importance, there is no clear information regarding optimal amount of fibre sources to be included in the diets of different bird types.

The effects of feed particle size on performance and nutrient digestibility in poultry have been extensively examined in the last two decades. Particle size reduction which is a common practice in commercial poultry feed production, is believed to increase both the number of particles and the surface area per unit volume, allowing greater access to digestive enzymes in the digestive tract (Amerah et al., 2007a). However, recommendations regarding optimum feed particle sizes in poultry diets are contradictory, due to number of confounding factors including feed physical form, grain type, endosperm hardness, grinding method and particle size distribution (Amerah et al., 2007b). Whilst most of research has focused on broiler chickens (Parsons et al., 2006; Amerah et al., 2007a, b; Amerah and Ravindran 2009), the impact of feed particle size on the nutrient digestibility in laying hens has not received much attention.

Despite the practical importance and possible differences in digestibility response of different bird types, studies comparing the effect of DF (Walugembe et al., 2014; Kimiaetalab et al., 2016) and particles size on the digestibility of nutrients in different bird types are limited. Studies have shown that the dietary inclusion of insoluble fibre sources (Hetland et al., 2003; Hetland et al., 2004; González-Alvarado et al., 2010; Mateos et al., 2012) and, medium and coarse grinding (Nir and Ptichi, 2001; Engberg et al., 2002; Dahlke et al., 2003; Parsons et al., 2006; Rougière et al., 2009) of cereals stimulate the development and functionality of the digestive tract and can have a beneficial effect on the dietary nutrient digestibility and utilisation (Pacheco et al., 2013; Ruhnke et al., 2015). However, the extent of beneficial effects of insoluble fibre sources and coarse particles has not been compared in different bird types. Further studies are warranted to investigate the interaction between bird type and DF content or particle size on nutrient digestibility. These studies would help decision making regarding optimum DF level or feed particle size to improve nutrient utilisation in different bird types.

Whilst limited published data are available on the effect of bird type on the nutrients digestibility and the interaction between DF content and bird type on nutrient digestibility, no study has investigated the interaction between dietary particle size and bird type on nutrient digestibility. In the present thesis work, it was hypothesised that the interaction exists in nutrient digestibility responses of different bird types to increased dietary fibre content and particle size, with layers showing better digestion efficiency in high fibre diets and to increased dietary particle size.

The objectives of the experimental research presented in this thesis are,

1. To investigate the influence of bird type (broilers, pullets and layers) and DF content (low fibre and high fibre) on apparent ileal nutrient digestibility and energy utilisation
2. To investigate the influence of bird type (broilers and layers) and maize particle size (fine, medium and coarse) on apparent ileal digestibility of nutrients and energy utilisation

This thesis consists of five chapters. Chapter 1 covers a general introduction to the thesis. Chapter 2 reviews the available data on the effects of insoluble fibre and particle size on nutrient digestibility and utilisation in different bird types. Chapters 3 and 4 present the experimental work conducted. Each chapter includes an abstract, introduction, materials and methods, results, discussion and conclusions.

Chapter 5 covers the general discussion of the summarised experimental research findings and, presents conclusions and suggestions for future research.

CHAPTER 2

Literature review

2.1 Introduction

Cereals and plant protein sources have been used as major feed ingredients in poultry diets for decades. One of the major challenges in poultry industry is the ever-increasing price of grains and protein sources. This leads to the use of least cost high fibre feed ingredients to reduce the cost of production. Together with the use of least cost fibre ingredients, the appropriate inclusion levels must be added into the diet to improve dietary nutrient digestibility and utilisation. Poultry feed formulation based on digestible nutrient have been recently practiced to more closely meet the nutrient requirements of different bird types and to reduce wastage of nutrients to the environment (Ravindran et al., 1999c; 2005; Kluth and Rodehutschord, 2006). The digestible values provide greater control of diet quality (Green and Kiener, 1989) and enhances the production economy (Wang and Parsons, 1998). It has been reported that, the digestibility of nutrient particularly amino acid (AA), and energy utilisation may vary depending on the physiological status of the bird (Huang et al., 2007). Noteworthy, the available data on the nutrient digestibility of feed ingredients are variable due probably to different bird type, site of sample collection (excreta or ileal) and correction for endogenous AA losses (true or apparent) (Green et al., 1987; Ravindran et al., 1999b; 2005; Kadim et al., 2002).

The strategies to improve dietary nutrient digestibility turned into the use of dietary fibre sources and reduction of the feed particle sizes in poultry diets (Rogel et al., 1987a; Jorgensen et al., 1996). Dietary fibre (DF) is believed to influence feed intake, gastrointestinal tract development (GIT) and nutrient digestibility in poultry (Jorgensen et al., 1996; Sklan et al., 2003; De Vries et al., 2012) but the effect depends on the type and amount of fibre added into the diet (Mateos et al., 2012). The research conducted with broilers (González-Alvarado et al., 2008; Amerah et al., 2009b; Jiménez-

Moreno et al., 2009a; 2009c; 2013a), pullets (Guzmán et al., 2015a; 2015b), layers (Hetland and Svihus, 2001; Hetland et al., 2005) and turkeys (Sklan et al., 2003) suggested that, addition of moderate amount of insoluble fibre sources in the diets improved the development and functionality of the GIT organs, especially the gizzard. A well-developed gizzard increases GIT motility and cholecystokinin release (Svihus et al., 2004) in turn stimulates the secretion of digestive enzymes (Duke, 1992). It also increases the reflux of the digesta content from duodenum to the gizzard, facilitating the contact between nutrient and digestive enzymes (Hetland and Svihus, 2001) hence improving nutrient digestibility. High fibre feed ingredients are retained for a longer time in the proventriculus and gizzard increasing hydrochloric acid (HCl) secretion and reducing digesta pH (González-Alvarado et al., 2008), favoring secretion and activation of digestive enzymes (Duke, 1992; González-Alvarado et al., 2007; Amerah et al., 2009b; Svihus, 2011) and nutrient digestibility.

Over the last two decades there has been an increased attention on the importance of feed particle size on nutrient digestibility, handling and mixing of the poultry feeds (Parsons et al., 2006). The coarse feed particle size reported to have beneficial effect on gizzard functions and gut development (Hetland et al., 2002; Svihus et al., 2004; Choct, 2009). Similar to fibre inclusion, coarse feed particles improved nutrient digestibility due to high retention time of digesta in the upper gut along with greater gizzard development (Nir et al., 1994a; Hetland and Svihus, 2001; Engberg et al., 2002).

Feeding different bird type the diets with high amount of medium and coarse particles increased gizzard weight compared to those fed on fine dietary particle size (Dahlke et al., 2003; Charbeneau and Roberson 2004; Péron et al., 2005; Parsons et al., 2006; Chewning et al., 2012; Jacobs and Parsons 2013b; Xu et al., 2015a,b,c; Naderinejad et al., 2016). Moreover, birds fed on medium and coarse particles reduced the pH of gizzard (Nir et al., 1994a; Engberg et al., 2002; Jiménez-Moreno et al., 2013b) and proventriculus (Nir et al., 1995) hence improving enzymes activation which in turn improves nutrient digestibility (Gabriel et al., 2003; 2008). In order to understand the

influence of bird type on digestibility of nutrient and energy in diet with different fibre content and particle sizes, the objectives of this literature review were to;

1. Review on the nutrient digestibility in poultry feed by considering digestible nutrient and methods of measuring digestibility
2. Review on the influence of bird type on nutrient digestibility and energy utilisation
3. Review of DF digestibility in different bird types; the effect of digesta retention time, passage rate and GIT development on DF digestibility
4. Review on the influence of particle size on nutrient digestibility, GIT development, digesta retention time and passage rate

2.2 Digestibility of nutrient and energy utilisation

The essential nutrient including AA, vitamins and minerals are required by the poultry for body maintenance, growth and production purposes. Digestibility is the process of breaking down the ingested feed and absorption of the nutrient through the GIT (Lemme et al., 2004).

2.2.1 Parts of digestive system of chicken and its functions

The GIT of the chicken consist of the mouth (beak), oesophagus, crop, proventriculus (glandular stomach), gizzard (muscular stomach), small intestine (duodenum, jejunum and ileum) and large intestine (ceca and colon) and cloaca (Figure 2.1). The functions of digestive organs of chicken includes grinding, acidifying, hydrolysing, emulsifying, absorption and transportation (Klasing, 1999; Mahmud et al., 2015).

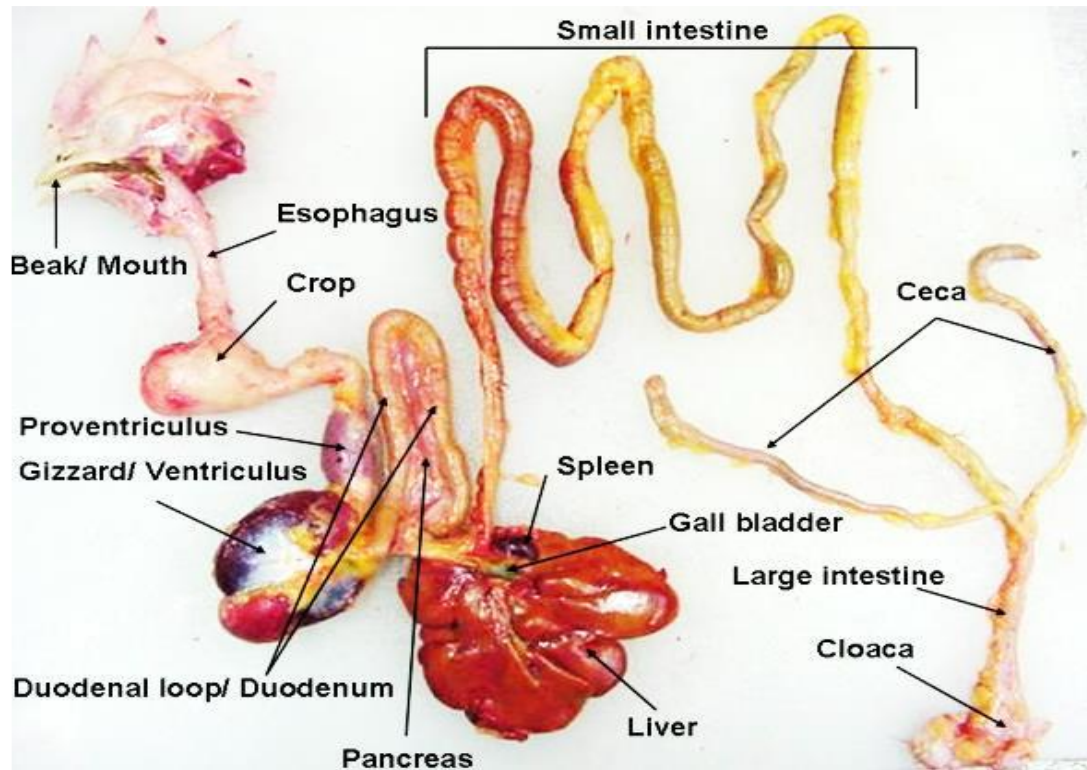


Figure 2.1. The anatomy of the broiler gastrointestinal tract (Jacob and Pescatore, 2013a)

The chicken beak and tongue functions are: grasping, testing, lubricating and propelling the food to the oesophagus. The oesophagus carries food from the mouth to the crop. Swallowed feed and water are temporarily stored in the crop. From the crop, food enters into the proventriculus. In the proventriculus, HCl and digestive enzymes are added to the feed for enzymatic digestion. In the gizzard, the food is ground and mixed through contraction of the gizzard walls. After the gizzard, the food enters the small intestine which is made up of the duodenum, jejunum and ileum. Part of the digestion occurs in the duodenum but most nutrients are absorbed mainly in jejunum and ileum (Sauer and Ozimek 1986). The hind gut is made of ceca and large intestine where microbial fermentation takes place. Due to the effect of hindgut microbial fermentation, the ileal nutrient digestibility is a mostly preferred method for digestibility measurement compared to total tract (excreta collection) method.

2.2.2 Methods of measuring digestibility in poultry

Two commonly methods namely total tract and ileal digestibility, used for the measurement of digestibility of nutrients in poultry.

2.2.2.1 Total tract method

Total tract method involves the collection of excreta from intact or caectomised birds where the digestibility values are obtained by subtracting the unabsorbed nutrient in the excreta from the feed ingested. Total tract method has been criticised due to the effects of hind gut microflora on dietary nutrient fermentation and contribution of microbial protein in the excreta (Payne et al., 1968; Parsons et al., 1982; Raharjo and Farrell, 1984). The microbial fermentation in the ceca (McNab, 1973) and the microbial protein contributes about 25% of the total excreta protein (Parsons et al., 1982), thus ileal method considered to be more accurate than excreta method (Ravindran et al., 1999c).

2.2.2.2 Ileal digestibility method

The criticisms of the accuracy of excreta method were corrected by the use of ileal method. It has shown that AA digestibility in broiler chickens was influenced by the site of measurement, with the values being higher in ileal digestibility method than the corresponding excreta AA digestibility values (Ravindran and Bryden, 1999). In the ileal method, digesta are collected from the distal part of the ileum. The possible errors due to microbial contamination from the hind gut of the birds are eliminated (Parsons et al., 1982; Ravindran et al., 1999c; Kadim et al., 2002). Several researchers have been reported variability of digestibility values measured using excreta and ileal methods on the same diets (Tanksley et al., 1981; Ravindran and Bryden, 1999; Karakas et al., 2001; Perttilä et al., 2001).

Ileal digesta samples can be collected either from the terminal ileum following euthanasia or alternatively from a cannula inserted into the distal ileum of the bird. The use of ileal cannulation in poultry is difficult, time consuming and there is frequent

rejection of the cannula by the birds (Tanksley et al., 1981; Ravindran et al., 1999c), thus the use of euthanasia is mostly preferred. The birds can be euthanased by using sodium pentobarbitone, carbon dioxide or cervical dislocation but the sodium pentobarbitone is preferred to minimise peristalsis, movement of the digesta and shading of the mucosal membrane (Ravindran et al., 2005). The small intestine is exposed and immediately the digesta is collected from the lower half of the ileum (Ravindran et al., 1999c; Adedokun et al., 2007; 2008). Ileum is the portion of the small intestine extending from Meckel's diverticulum to approximately 40mm proximal to the ileo-caecal junction (Ravindran et al., 2005). Digesta are gently flushed from the intestine with distilled water and stored into the airtight containers and immediately frozen to avoid fermentation process. The collected samples are lyophilised, ground and analysed for the determination of different nutrients.

In the ileal method, birds are fed experimental diets with an indigestible marker over an experimental period of time prior collection of the digesta (Adedokun et al., 2008). The dietary indigestible marker is used to relate the nutrient contents in the diet to those in the digesta (Lemme et al., 2004). Chromic oxide, titanium dioxide and acid-insoluble ash are the common inert markers being used. However, the digestibility results may differ slightly depending on the marker used (Scott and Boldaji, 1997). The ileal digestibility values are expressed as apparent (Lemme et al., 2004; Ravindran et al., 2005), standardised (Adedokun et al., 2007; Bandegan et al., 2009) or true (Mutucumarana and Ravindran 2016) depending on how endogenous losses are considered in the digestibility measurement.

2.2.2.3 Apparent, standardised and true digestibility

Apparent digestibility is the estimates of both dietary nutrient or AA and endogenous losses. The apparent ileal digestibility (AID) is influenced by the level of dietary nutrient or AA and does not include a correction for endogenous AA losses. At low dietary protein intakes, greater amount of AA from endogenous origins will be found in the digesta proportional to the AA of dietary origin (Stein et al., 2005). Noteworthy, the AID increases with the increase in dietary AA concentration (Fan et al., 1994; Lemme et al., 2004). The AA found in the excreta or digesta not only originate

from the diet components (Angkanaporn et al., 1994) but also from endogenous origin. The ileal endogenous losses are divided into basal and specific losses. The basal (nonspecific) losses are influenced by dry matter intake and not diet composition whilst specific losses are related to the diet composition (Lemme et al., 2004).

The digestibility estimate based on apparent digestibility might be a better practical basis for diet formulations as there is no reliable method for measuring endogenous losses under different dietary conditions. However, there are some limitations with using apparent digestibility data in diet formulations. Firstly, the additivity of apparent digestibility values of individual feed ingredients during diet formulations. Secondly, underestimation of AID in the feed ingredients with low protein relative to feed ingredients with high protein content due to relatively increased proportion of endogenous AA losses. The limitations of using AID values could be corrected by converting AID values to standardised digestibility values (SID) or true ileal digestibility (TID) by correcting the endogenous AA losses. Both SID and TID involve corrections for endogenous AA losses but these two concepts are different due to how the endogenous losses are corrected.

The TID values are obtained through correction for basal AA and diet induced ileal endogenous AA outflow. In TID, the total endogenous AA losses are subtracted from the ileal outflow of AA from the ingested feed. The TID values depend on the composition of the dietary AA of the feed ingredients (Stein et al., 2007b). The SID values involve corrections of only basal AA endogenous losses. In SID the basal ileal endogenous AA losses are subtracted from the total ileal digesta AA losses (Stein et al., 2007b). The SID is considered to be independent of AA intake (Angkanaporn et al., 1996; Stein et al., 2005). SID has been suggested to be a preferred means of determining the endogenous AA losses in calculating digestibility coefficients that are more practical for poultry feed formulation (Ravindran and Bryden 1999; Stein et al., 2001; Lemme et al., 2004) and also additive for different feed ingredients (Stein et al., 2005; 2007a) and have a minimal variability with the dietary condition (Ravindran and Bryden, 1999; Stein et al., 2001). The SID values of AA in several feed ingredients for chickens of

different ages have been evaluated and recommended (Bandegan et al., 2009; Szczurek, 2009; Woyengo et al., 2010).

2.3 Energy utilisation in birds

Energy is contained within the organic matter of feeds including carbohydrates, fat and protein. These constituents are digested and the end products are oxidized within cells to provide energy (NRC, 1994). The gross energy (GE) is the total energy contained within feeds. The GE of the consumed feed minus the GE of the faeces is the apparent digestible energy (NRC, 1994). The GE of the feed minus the GE of the excreta gives the metabolisable energy (ME) which is referred to as the apparent metabolisable energy (AME). The ME minus the lost energy as heat increment (NRC, 1994) is the NE (Figure 2.2). The nitrogen-corrected AME (AME_n) adjusts the AME values for the N content of the food and excreta (Farrell 1999) by correcting for zero N retention by assuming 36.54 kJ per gram N retained in the body (Hill and Anderson, 1958).

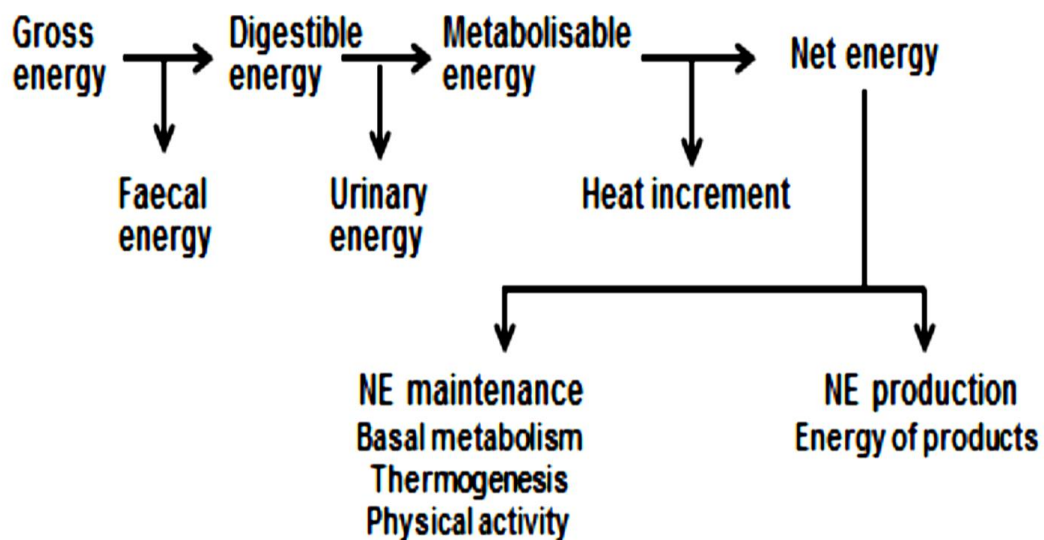


Figure 2.2. Energy partitioning in poultry (Source: Larbier, 1994).

2.4 Influence of bird type on nutrient utilisation

The digestibility of dietary nutrient is not only a function of feed ingredients used in the diet formulation but also the bird type that consumed the tested diet (Ravindran et al., 2005; Huang et al., 2006b; 2007; Adedokun et al., 2009). The differences in the digestibility of dietary nutrient between bird types seemed to be associated with different morphology and functionality of their GIT organs (Slinger et al., 1964; Masic et al., 1974; Nir et al., 1993; Shires et al., 1987; Hetland et al., 2003; Rideau et al., 2014). There is limited information on the effect of different bird types on nutrient digestibility (Huang et al., 2007) and energy utilisation (Mollah et al., 1983). Broilers are mostly selected for their fast growth rates and high feed efficiency (Sacranie et al., 2012), whereas pullets are selected to improve feed intake for maximising future egg production (Kimiaeitalab et al., 2016). Nir et al. (1993) reported that fast growing birds such as broilers have an improved nutrient digestibility to support their growth than layers. However, the information on the ileal digestibility of dietary nutrients for layers are limited (Huang et al., 2007).

Assuming that there is no difference between nutrient digestibility of feed ingredients in different bird types, the digestibility values generated with adult roosters are currently used for the diet formulation of broilers and layers and, the digestibility values for broilers are also applied in the layers feed formulation (Huang et al., 2000a; Ravindran and Hendriks, 2004; Huang et al., 2006b). This practice may create inaccuracies in feed formulation because birds might differ in their GIT structure, digestive enzyme secretion and absorptive capacity which will affect the availability of nutrients to the bird (Ravindran et al., 1999b; Huang et al., 2007). It is also recognised that, several factors such as rate of passage, physiological status related to growth rate, feed intake and dietary nutrient composition may also be responsible for digestibility variability between birds types (Huang et al., 2007).

Nutrient digestibility and energy utilisation in poultry has been reported to vary depending on the age (Lodhi et al., 1969; Huang et al., 2005; Garcia et al., 2007), genotype (Rideau et al., 2014) and gender (Lodhi et al., 1969; Mollah et al., 1983) of a birds. The study by Huang et al. (2005) showed that the digestibility coefficients of AA

for the birds consumed the diet with canola meal were significantly higher at 28 and 42 d of age compared to those at 14 d (Table 2.1). Energy utilisation was also observed to be lower in chicks (4 weeks) compared to adult hens (60 weeks) (Table 2.2).

Wakita et al. (1970), Zuprizal et al. (1992) and Doeschate et al. (1993) reported that, gender of birds could influence nutrient digestibility however, Wallis and Balnave (1984) found no difference in the digestibility of N or AA of male and female birds. In the studies by Wakita et al. (1970) and Zuprizal et al. (1992) male chickens showed higher AA digestibility values than female chickens, suggesting that the rate of AA supply to the fast growing tissues of male broilers may be proportional to the rate of growth. In contrast, the general N and AA digestibility values for the female chicken were 3% higher than the male chickens (Doeschate et al., 1993). These researchers suggested that the digestibility studies need to use birds of the same gender to avoid digestibility discrepancies and ensure proper supply of nutrients to meet birds dietary nutrient requirements.

It is documented that, there is variation among genotypes in nutrients digestibility (Ravindran et al., 1999b). The AME values for broiler strain selected for feed conversion was greater compared to those selected for body weight (Doeschate et al., 1993). Péron et al. (2006) showed that D+ (high digestive efficiency) birds had higher total tract digestibility of starch (0.936), protein (0.808), fat (0.774) and AMEn (12.9 MJ/kg) than D- (low digestive efficiency) with 0.908, 0.763, 0.621 and 11.8 MJ/kg, respectively. The strains differences in nutrient digestibility might be due to inherent traits in the efficiency of nutrient digestibility and utilisation among genotypes. These differences could include the GIT structure, digestive secretions, absorptive capacity and digesta retention time. Nevertheless the differences in apparent ileal digestibility of nutrients speculated to partly caused by endogenous AA losses due to DF sources (for example mucin) (Ravindran and Hendriks 2004).

Table 2.1. The apparent ileal digestibility of crude protein and amino acids in canola meal for broiler birds at the age of 14, 28 and 42 days¹ (Huang et al., 2005)

Parameters	Age (d)			Probability P _≤
	14	28	42	
Crude protein	0.75 ^a	0.77 ^{ab}	0.78 ^b	0.05
Indigestible amino acids				
Threonine	0.69 ^b	0.71 ^a	0.72 ^a	0.05
Valine	0.77 ^b	0.79 ^a	0.78 ^a	0.001
Methionine	0.91	0.92	0.91	NS
Isoleucine	0.78 ^b	0.80 ^a	0.79 ^a	0.01
Leucine	0.82 ^b	0.83 ^a	0.83 ^a	0.01
Phenylalanine	0.81	0.82	0.82	Ns
Histidine	0.8	0.81	0.8	Ns
Lysine	0.79	0.79	0.8	Ns
Arginine	0.85 ^a	0.86 ^a	0.88 ^a	0.01
Dispensable amino acids				
Aspartic acid	0.74 ^b	0.75 ^b	0.78 ^a	0.001
Serine	0.68 ^b	0.71 ^a	0.72 ^a	0.01
Glutamic acid	0.93 ^a	0.94 ^a	0.89 ^b	0.001
Glycine	0.75 ^b	0.78 ^a	0.78 ^a	0.05
Alanine	0.84 ^b	0.85 ^a	0.81 ^c	0.001
Tyrosine	0.78	0.79	0.79	NS
Average ²	0.80 ^b	0.81 ^a	0.81 ^a	0.05

^{a-c}Means with different letters are significantly different <0.05

¹Means of 5 replicates pens (12 birds per pen at 14d of age, 6 birds per pen at 28d and 42 d of age.

NS not significant (P>0.05)

²Means of 15 amino acids

Table 2.2. Comparison of metabolisable energy values for male and female chicks and adult laying hens^{1,2} (Peterson et al., 1976)

Feed ingredients	Metabolisable energy (MJ/kg)		
	Chicks		Hens
	Male	Female	
Maize	13.01a ±.15	13.22a ±.16	14.60b ±.22
Wheat	12.80a ±.11	12.97a ±.12	13.14a ±.21
Barley	9.83a ±.20	10.08a ±.21	12.76b ±.25
Wheat bran	6.03a ±.28	5.82a ±.29	4.94a ±.51
Fish meal	15.52a ±.16	15.69a ±.12	15.77a ±.20
Meat meal	10.38a ±.32	10.50a ±.32	12.47b ±.16
Soybean meal	6.90a ±.24	7.11a ±.29	11.05b ±.24
Sunflower meal	5.23a ±.41	4.44a ±.42	8.91b ±.37
Peas	8.95a ±.16	9.21a ±.17	11.92b ±.15
Dehulled alfalfa meal (25%)	2.55a ±.45	2.89a ±.41	5.02b ±.41
Dehulled alfalfa meal (40%)	3.89a ±.53	3.85a ±.55	4.44b ±.32
Barley hulls (25%)	-1.21a ±.18	-0.92a ±.13	2.01b ±.38
Barley hulls (40%)	2.22a ±.25	2.38a ±.27	5.10b ±.30

¹All values are means ± standard deviation

²The means with different letters are significantly different <0.05

In a study by Huang et al. (2000a), ileal digestibility of protein in soybean meal was greater in broilers and cockerels than layers, and wheat protein was poorly digested by layers and broilers compared to cockerels (Table 2.3). The ileal digestibility of protein in maize, sorghum, soybean meal and meat meal was similar between layers and broilers whereas, with wheat, cottonseed meal and canola meal the digestibility in layers was lower than that of broilers (Huang et al., 2000b). According to Walugembe et al. (2014), digestibility response to the higher DF was greater for layer chicks compared to broiler chicks suggesting that the layer digestive system has a greater capability for digestion of a high fibre diet compared to broilers. Conclusively, due to the different digestibility responses among bird types, using digestibility values generated with one type of bird, for example broilers, may not be appropriate for the feed formulation for other bird types such as roosters, pullets and layers (Huang et al., 2006b; 2007).

Table 2.3. Apparent ileal digestibility of protein in different feed ingredients for cockerels, layers and broilers (Huang et al., 2000a)

Feedstuff	Cockerels	Layers	Broilers	Pooled SEM
Maize	0.793	0.778	0.797	0.016
Sorghum	0.782 ^{ab}	0.764 ^a	0.815 ^b	0.0109
Wheat	0.713 ^a	0.779 ^b	0.791 ^b	0.015
Soybean meal	0.839 ^a	0.889 ^b	0.852 ^a	0.008
Canola meal	0.736	0.761	0.748	0.104
Cottonseed meal	0.716	0.72	0.719	0.109
Meat and bone meal	0.728	0.741	0.742	0.015

^{a,b} Means with different superscripts are significantly different (P<0.05)

2.5 Influence of dietary fibre on nutrient utilisation

Whilst use of alternative feed ingredients can reduce feed costs and enhance the sustainable use of feed sources for poultry production, some of these ingredients contain high levels of DF (Angkanaporn et al., 1994). Dietary fibre encompasses plant cell walls comprising cellulose, non-starch polysaccharides and lignin (Knudsen, 2001; Abdallah et al., 2015).

Inclusion of insoluble DF to poultry diets can improve nutrient digestibility and growth performance by maintaining the gut health, development of the upper parts of GIT, improving motility of the digestive tract (Mateos et al., 2002) and animal welfare especially in layers (Hartini et al., 2002). The influence of fibre concentration in poultry diets on feed intake and nutrient digestibility is a subject to debate. Dietary fibre is considered as a diluent in chicken diets (Mateos et al., 2002) and can reduce nutrient digestibility (Hamberg et al., 1989; Abdallah et al., 2015). However, it has been proposed that the effect of DF on digestibility of nutrients depend on the fibre concentration in the diet (Mateos et al., 2012). The inclusion of moderate amount of fibre in the diets for chickens has been shown to increase gizzard activity and development, and improve mixing of the digesta with digestive enzymes and motility of the digestive tract (Mateos et al., 2002; 2012), reduce the passage rate and increase the retention time of digesta in GIT (González-Alvarado et al., 2008) thus increasing the nutrient digestibility (Duke, 1992).

Although inclusion of DF in poultry diets might benefit nutrient digestibility, its effect may depend on the type of bird and physiochemical characteristics of fibre used (Lentle and Janssen 2008). Additionally, bird factors (age and gender) should be considered on estimation of DF requirements. The information regarding the optimal fibre level in the diet for different bird types is limited (Svihus, 2011). The fibre requirements in different bird types appears to depend not only on dietary fibre concentration but also on the type of fibre added (Mateos et al., 2012).

2.5.1 Types of dietary fibre

Dietary fibre constitute a major part of plant feed stuffs such as cereals and legumes which is found in plant cell walls and includes polysaccharides, oligosaccharides and lignin. The type of DF varies according to its chemical composition and physical features (Knudsen, 2001). Fibre, based on the physiochemical properties, is divided into soluble and insoluble fibre (Dikeman and Fahey, 2006). Some of the plant feed ingredients contain high amounts of fibre with the majority being insoluble (Knudsen, 1997; Knudsen, 2001). The insoluble fibre sources have little effects on intestinal viscosity (Knudsen, 2001), increase HCl, digestive enzymes and bile acids secretion (Rogel et al., 1987a; Hetland et al., 2003) and promote GIT development, thus enhancing nutrient utilisation (Hartini et al., 2002; Hetland et al., 2004; 2005; Mateos et al., 2012). Soluble fibre increases water holding capacity and digesta viscosity in the small intestine (Choct, 2009) which reduces gut mixing and hinders the interaction of the substrate with digestive enzymes, all resulting in impaired nutrient digestibility (Van der Klis et al., 1993; McDougall et al., 1996). High digesta viscosity can also reduce feed intake (Serena et al., 2007).

Dietary fibre is also classified into neutral detergent fibre (NDF), acid detergent fibre (ADF) and crude fibre. The crude fibre method can be used to measure the fibre content of the diet, however, this has been out-dated by the detergent method of Van Soest (1963; 1967) which measures NDF and ADF in the feed.

2.5.2 The digestibility of dietary fibre in different bird types

Dietary fibre digestibility may be influenced by bird factors (species, age, sex), type of fibre (soluble or insoluble) and fibre source inclusion level (Montagne et al., 2003). Some reported data showed that, bird types fed on different source of DF and fibre inclusion level had different digestibility response (Table 2.4). Bird types respond differently to DF inclusion due to the different physiological status of their GIT organs. For example, the diet with 15 g/kg NDF reduced the feed intake for broiler chicks by 9.3% without any effect in layers chicks (Walugembe et al., 2014). Kimiaetalab et al. (2016) found no effect on the feed intake of broilers and pullets fed a diet with the NDF of 10.3 g/kg.

Table 2.4. The improvement in nutrient utilisation in broilers and layers fed diets with different source of fibre

	Sources of fibre	References
Increased AME		
8%	40 g/kg oat hulls	Hetland and Svihus (2001)
3%	30 g/kg oat hulls	González-Alvarado et al. (2007)
5%	Wood shavings <i>ad lib</i>	Hetland and Svihus (2007)
8%	60 g/kg wood shavings	Amerah et al. (2009b)
4%	30 g/kg oat hulls	Jiménez-Moreno et al. (2009a)
Increase in digestibility		
14% excreta starch	100 g/kg oat hulls	Rogel et al. (1987b)
2% ileal starch	100 g/kg oat hulls	Hetland and Svihus (2001)
2% ileal starch	100 g/kg oat hulls	Hetland et al. (2003)
3% ileal starch	Wood shavings <i>ad lib</i>	Hetland et al. (2003)
3% excreta starch	30 g/kg oat hulls	González-Alvarado et al. (2007)
6% excreta fat	30 g/kg oat hulls	Jiménez-Moreno et al. (2009a)
11% ileal starch	60 g/kg wood shaving	Amerah et al. (2009b)

Studies conducted on broilers (Hetland and Svihus, 2001; González-Alvarado et al., 2008; Amerah et al., 2009b; González-Alvarado et al., 2010; Mateos et al., 2012; Jiménez-Moreno et al., 2013a), pullets (Guzmán et al., 2015a; Kimiaetalab et al., 2016) and layers (Hetland et al., 2005) showed different digestibility response to the DF. The study by Walugembe et al. (2014) showed increased ileal digestibility of NDF and AMEn for broilers and layer chicks fed high dietary fibre. Noteworthy, layer chicks showed greater response to the increased DF than broiler chicks suggesting that layers digestive organs are more sensitive to DF compared to broilers. Layer chicks fed on high fibre diet showed greater total tract NDF digestion than broiler chicks (Table 2.5). Some studies suggested that broilers require a minimum amount of DF to maintain gizzard activity and improve nutrient digestibility (Hetland et al., 2003; González-Sánchez et al., 2010). Broilers fed on high fibre diet, decreased ileal digestibility of protein and AA than that of layers, indicating that broilers have a reduced nutrient digestibility response to diets with increased fibre concentration (Table 2.6).

Table 2.5. The effect of increasing dietary fibre in broilers and layers chicks from 1 to 21 days on nitrogen corrected apparent metabolisable energy (AMEn), ileal and total tract digestibility of neutral detergent fibre (NDF) (Walugembe et al., 2014)

Line	Dietary fibre	AMEn	Ileal aNDF	Excreta aNDF
Broiler		13.42	0.21	0.19 ^b
Layer		13.41	0.22	0.23 ^a
SEM ¹		0.119	0.007	0.010
	High fibre	13.42	0.25 ^a	0.23 ^a
	Low fibre	13.41	0.18 ^b	0.20 ^b
SEM ¹		0.119	0.007	0.010
P value				
Line		0.96	0.39	0.01
Dietary fibre		0.99	0.01	0.02
Line x dietary fibre		0.41	0.66	0.75

^{a,b}Values in the same column with different superscript differ significantly at $P \leq 0.05$

¹Pooled standard error of mean.

Table 2.6. Apparent ileal digestibility of crude protein and amino acids in wheat middlings for broilers and layers^a (Huang et al., 2007)

Parameters	Broilers	Layers	SEM	P-value
Crude protein (N X 6.25)	0.73b	0.76a	0.006	
Indigestible amino acids				
Threonine	0.61b	0.70a	0.009	0.001
valine	0.72b	0.78a	0.006	0.001
Methionine	0.79	0.82	0.012	0.057
Isoleucine	0.74b	0.84a	0.005	0.001
Leucine	0.76b	0.81a	0.006	0.001
Phenylalanine	0.76b	0.81a	0.006	0.001
Histidine	0.74b	0.79a	0.007	0.001
Lysine	0.79	0.79	0.006	0.979
Arginine	0.79b	0.81a	0.004	0.003
Dispensable amino acids				
Aspartic acid	0.71b	0.78a	0.006	0.001
Serine	0.66b	0.78a	0.007	0.001
Glutamic acid	0.86	0.86	0.005	0.797
Glycine	0.67b	0.75a	0.008	0.001
Alanine	0.72b	0.78a	0.007	0.001
Tyrosine	0.69b	0.76a	0.008	0.001
Average ^b	0.74b	0.79a	0.006	0.001

a The values are means of 5 replicate pens (5 birds/replicate)

b Mean of 15 amino acids

Means in a row with different letters (a, b) are significantly different (P < 0.05)

The inclusion of moderate amount of insoluble fibre in the poultry diets increased nutrient digestibility in different bird types Table 2.7. A study by Rogel et al. (1987a) showed that the digestibility of starch in broilers fed a diet without oat hulls and with oat hulls (100 g/kg) increased from 0.976 to 0.995 but further inclusion to 150 g/kg reduced digestibility to 0.781. Jorgensen et al. (1996) found reduced ileal dry matter digestibility from 0.860, 0.800 to 0.670 in broilers fed the diet with 0, 187 and 375 g/kg oat bran inclusion, respectively. The digestibility of dry matter in broiler was 0.731 for the diet without barley inclusion whilst the diet with 75 g/kg of barley hulls inclusion improved digestibility to 0.753 but more addition of barley hulls to 150g/kg failed to increase digestibility 0.754 (Adibmoradi et al., 2016).

Table 2.7. Effect of dietary pea hulls on the coefficients of total tract apparent nutrient retention, nitrogen - corrected metabolisable energy (AMEn, MJ/kg) of the diet and apparent ileal digestibility (CAID) of nutrient in broilers (Source: Jiménez-Moreno et al., 2011)

Item	Pea hulls g/kg				P-value
	0	25	50	75	
Dry matter	0.772 ^y	0.791 ^x	0.780 ^y	0.755 ^z	0.001
Organic matter	0.817 ^y	0.834 ^x	0.821 ^y	0.796 ^z	0.001
Nitrogen	0.627 ^y	0.670 ^x	0.677 ^x	0.647 ^y	0.047
Soluble ash	0.407 ^z	0.483 ^{xy}	0.491 ^x	0.465 ^y	0.001
Ether extract	0.907 ^y	0.921 ^x	0.918 ^x	0.913 ^{xy}	0.317
AMEn (MJ/kg)	13.41 ^x	13.46 ^x	13.33 ^x	13.10 ^y	0.001

^aAverage of data at 6, 12 and 18 d of age

^{x-z}Means within a same row not sharing a common superscript are different ($P \leq 0.05$).

The study by Hetland and Svihus (2001) showed increased ileal starch digestibility from 0.974 in the diet without oat hulls to 0.986 and 0.992 at 40 and 100 g/kg oat hulls inclusion, respectively. The DF inclusion in broiler diet (25 to 75 g/kg pea hulls) reduced digestibility of protein from 82.1 to 76.02 and AMEn values 13.48 to 13.08 MJ/kg respectively but with 50 g/kg pea hulls inclusion had higher starch digestibility 0.939 compared to 0.902 for the diet without pea hulls (Mateos et al., 2012). Kimiaetalab et al. (2016) found higher nutrient retention (DM, N and GE) for a diet with 30 g/kg sunflower hulls inclusion compare to diet without sunflower inclusion with a greater response in broilers compared to pullets (Table 2.8).

Table 2.8. Comparison of nutrient retention and nitrogen corrected metabolisable energy (AMEn) in broilers and pullets at 21 d of age¹ (Source: Kimiaetalab et al., 2016)

Item	Broilers	Pullets	P-value ²
			Line
Dry matter (%)	68.9	67.3	0.001
Nitrogen (%)	64.1	55	0.001
Gross energy (%)	75.3	74.7	0.072
AMEn (MJ/kg)	12.89	12.77	0.097

¹The experimental unit was a cage with 6 birds at 21 d of age.

²The interactions between chicken line and SFH inclusion were not significant ($P > 0.05$).

2.5.3 Effect of dietary fibre on digestive tract development, digesta retention time and passage rate

2.5.3.1 Effect of dietary fibre on gastrointestinal tract development

The insoluble fibre ingredients are required to be added in a poultry diets for proper development and functioning of the GIT organs (Mateos et al., 2012). Birds respond so fast to the DF concentration by modifying the weight and length of the GIT organs. The magnitude of the DF effects on the development of the poultry digestive organs depends on the level and type of fibre added (Jiménez-Moreno et al., 2011; Jiménez-Moreno et al., 2013b) and also the particle size of the fibre ingredients (Jiménez-Moreno et al., 2010).

The importance effects of DF on nutrient digestibility may be related on gizzard development, increased HCl secretion and digestive enzymes secretion. Feeding chickens with higher DF concentration increases the accumulation of digesta content in the upper parts of GIT. The accumulation of the digesta content in the gizzard to be ground prior leaving the gizzard, stimulates the muscular contraction of the gizzard walls thus results into an increased distension of the gizzard walls and increasing the gizzard size (Jorgensen et al., 1996; Jiménez-Moreno et al., 2013a,b). A well-developed gizzard improves GIT motility, increase GIT refluxes and cholecystokinin release and stimulates the secretion of pancreatic enzymes (Duke, 1992; Svihus et al., 2004). Besides increasing gizzard development and functionality, the DF inclusion has been shown to reduce the pH of the gizzard digesta (Figure 2.3). Longer retention time and better functioning of the gizzard and secretion of HCl in the gizzard may reduce gizzard digesta pH. The digesta pH reduction promotes enzyme activity and increases minerals solubility and nutrient digestibility (Guinotte et al., 1995).

Figure 2.3. Effects of the inclusion of dietary fibre sources on a) relative weight of the empty gizzard and b) digesta pH of the gizzard (Source: Mateos et al., 2012).

Some of the studies showed the influence of the level of DF on development and functionality of the poultry GIT organs (Jorgensen et al., 1996; Hetland and Svihus 2001; Hetland et al., 2004; 2005; Serena et al., 2007; Jiménez-Moreno et al., 2009c; Mateos et al., 2012). Whilst feeding a moderate amount of DF can improve development and functionality of the gizzard (González-Alvarado et al., 2007; Amerah et al., 2009b; Svihus, 2011; Mateos et al., 2012), the high inclusion of insoluble fibre in diet of young birds may result in reduced feed intake and nutrient digestibility due to the under development of their GIT organs (Sklan et al., 2003). González-Alvarado et al. (2008) suggested that an inclusion of 30 g/kg oat hulls in broiler diets is able to stimulate the development of the upper parts of the GIT and gizzard function. The broilers fed the diet with 40 g/kg of DF increased their gizzard weight by 8.7% (González-Alvarado et al., 2008). Abdallah et al. (2015) reported that broilers consumed the diet with increased fibre content from 36.5 to 46.5 and 59.2 g/kg had increased gizzard weights of 14, 19, and 21 g respectively. Kimiaeitlab et al. (2016) reported higher absolute weight of gizzard (24.2 versus 10.0 g) and proventriculus (4.50 versus 1.87 g) for broilers than pullets when fed a diet with 30 g/kg sunflower hulls.

2.5.3.2 Effect of dietary fibre on passage rate and digesta retention time

The feed passage rate and retention time influenced nutrient digestibility by determining the time available for digestive enzymes to act on nutrients together with the interactions between nutrient and the intestinal absorptive surfaces. Gizzard regulates the feed passage rate to the small intestine (Svihus et al., 2002). The insoluble fibre contents are difficult to grind, therefore, the ingested insoluble fibre sources, retained in gizzard for a long time compared to non-dietary fibre contents (Mateos et al., 2012).

The effects of DF on the feed passage rate and retention time depends on the fibre inclusion level, type of fibre added in the diet (Jiménez-Moreno et al., 2009b; 2010; 2011) and particle size of the DF ingredients (Saki et al., 2011; Mateos et al., 2012; Lindberg, 2014). Hetland et al. (2003) found the increased volume of gizzard contents for the birds fed on the diet with high concentration of DF compared to those in low DF concentration. High DF contents are selectively retained in the gizzard to be ground before passing through other parts of the GIT compared to non DF ingredients (Hetland et al., 2005). The increased accumulation of digesta content in the gizzard lowers the feed passage rate. A slow passage rate and increased retention time of DF in the gizzard, increased gizzard contraction, reduces digesta pH, increases digestive enzymes secretions (Jones and Taylor, 2001) and accessibility of the digesta to digestive enzymes in turns improves nutrient digestibility (Svihus et al., 2002; Jiménez-Moreno et al., 2011).

2.6 Particle size

2.6.1 Particle size reduction

Cereal grains are commonly processed before being mixed into the poultry diets. The particle size reduction of the cereal grains is obtained by initially disrupting the outer grain hulls and exposure of the seed endosperm through either a hammer mill or a roller mill. Particle size reduction increases the number of particles, and improves handling and mixing of the feed ingredients (Koch, 1996; Laurinen et al., 2000; Lentle et al.,

2006; Amerah et al., 2007b; Addo et al., 2012). The average particle size of the feed is given by the geometric mean diameter (GMD) which is normally expressed in millimeters (mm) or microns (μm). The range of variation of the feed particle sizes is called geometric standard deviation (GSD) and is an indicator of the feed particle size uniformity (Nir et al., 1994a). From the nutritional point of view, particle size reduction increases surface area per unit volume of the feed thus facilitates contact between nutrient and digestive enzymes allowing increased access to dietary nutrient (Amerah et al., 2007b; Addo et al., 2012).

2.6.2 Particle size determination

Dry and wet sieving are two commonly methods used for particle size determination (Baker and Herrman 2002). In dry sieving method, the feed particle size is determined by using representative sample of 100 g. The ground samples are passed through series of sieves of different sizes on a shaker for 5 minutes and the amount of the sample retained on each sieve determined as initial weight of the sample. The GMD and GSD of the retained sample are calculated using standard formula or by using computer software. Particle size determination by wet sieving method is mainly used for pelleted diets. In wet sieving method, feed samples are suspended in 50ml of water and left to stand for 30 minutes prior sieving to ensure adequate hydration. Then the sample is washed through a set of sieves and the material retained on each sieve is filtered and dried for 24 hours at 80°C. The weight of the retained particles from each sieve is considered as percent of total dry matter recovered (Lentle et al., 2006).

2.6.3 Effect of particle size on nutrient digestibility in different bird types

Cereal grains particle size reduction for poultry diets is recently considered as a way of optimizing nutrient digestibility and utilisation. The effect of particle size on nutrients digestibility has been studied with wheat (Péron et al., 2005; Amerah et al., 2008a,b), maize (Zang et al., 2009; Jacobs et al., 2010; Pacheco et al., 2013) and soybean meal (Pacheco et al., 2013). Particle size has been reported as a major factor determining FI in

broilers (Amerah et al., 2009b) and layers (Safaa et al., 2009). Feeding finely-ground particles in layer diets have been reported to reduce FI, increase feed selection and feed wastage (Safaa et al., 2009; Herrera et al., 2016). Birds differentiate feed particle sizes by mechanoreceptors located in their beak (Nir et al., 1990; Oryschak and Zijlstra, 2002; Gabriel et al., 2003; Jiménez-Moreno et al. 2016). Birds have greater preference for coarse feed particles compared to the fine particles (Schiffman, 1968; Portella et al., 1988; Nir et al., 1994a). However, there are practical limits to the level of particle size reduction because chickens of different ages may experience difficulties in consuming very coarse or very fine feed particles.

Although the particle size reduction has been suggested to improve digestibility of nutrients, the studies comparing the effect of dietary particle size on nutrient digestibility in different bird type is scant. The effect of feed particle size on nutrient digestibility has been mostly conducted with broilers but limited data for layers (Table 2.9). Moreover, the effect of feed particles size in layers and pullets has mainly focused on bird performance, digestive tract development and egg quality rather than digestibility (Hamilton and Proudfoot, 1995; MacIsaac and Anderson, 2007; Frikha et al., 2009; Safaa et al., 2009; Röhe et al., 2014; Herrera et al., 2016). It has been suggested that in broiler diets at least 20% of the particle size of cereal grains should be greater than 1.5 to 2.0 mm in size to stimulate gizzard activity and development (Svihus, 2011). The study by Goodband et al. (2000) suggested that layers diets should be formulated using medium and coarse particles and no any advantage of reducing feed particle size below 0.800 mm. Broilers and layers had different physiological status of their digestive organs. The digestive system of layers is more mature compared to broilers; therefore, transferring data obtained from broilers studies to layers diet formulations is tenuous.

The extent of reducing cereal grains particle size for poultry diets can improve the digestive tract development and functioning in turns increase nutrient digestibility. The fine feed particles reported to reduce digesta retention time in the gizzard, reduce gizzard activity thus impair nutrient digestibility (Mateos et al., 2012). The use of medium and coarse feed particles in poultry diets are preferred by the poultry industry

on improving nutrient digestibility compared to fine grinding (Amerah et al., 2007a). However, the documented effects of medium and coarse grindings to improve dietary nutrient digestibility in poultry are contradictory with some studies showing improved digestibility (Reece et al., 1985; Nir et al., 1994a,b; Röhe et al., 2014; Ruhnke et al., 2015) whilst others failing to show any benefits on nutrient utilisation (MacIsaac and Anderson, 2007; Safaa et al., 2009). However, the contradictory data on the effect of particle size on nutrient digestibility might be due to some factors including feed form, type of grain, endosperm hardness, grinding method (Amerah et al., 2007b) and dietary particle size distribution.

The study by Ganzer et al. (2017) showed 6.3% reduction in AA digestibility in broiler diets formulated with finely-ground maize compared with coarsely ground maize (0.62 vs. 0.96 mm, respectively). Parsons et al. (2006) showed higher nitrogen and lysine retention in coarse maize particle size compared to fine particles. The increased maize particle size from 781 to 2,242 μm improved nitrogen retention in broilers (Parsons et al., 2006). The contradictory results showed that, feeding broiler birds with fine grindings increased digestibility of starch (Péron et al., 2005), AME values (Kilburn and Edwards, 2001) and protein (Carré et al., 1998) compared to coarse grinding. Svihus et al. (2002); Amerah et al. (2007b) found no AMEn differences between diets formulated with medium or coarse particle size (3 and 7 mm, respectively). Similarly, Singh et al. (2014) found no particle size effect on ileal digestibility of DM, N, starch. Amerah and Ravindran (2009) found that the AMEn, Ca, N retention and ileal N digestibility in broiler birds were not influenced by feed particle size of 5 and 7 mm.

The feed particle size reported to have effect on minerals digestibility and retention. In minerals digestibility, the coarse particle size are more preferred than fine grindings. The coarse grindings increase retention time in the gizzard, allowing more time for HCl secretion, reducing digesta pH in turns improve mineral solubility and absorption (Guinotte et al., 1995). Kasim and Edwards (2000) reported that broilers fed on ground particles of maize grains from 484 to 573 and 894 μm significantly increased digestibility of calcium, total phosphorus and phytate phosphorus. The diets with the larger particle size (GMD, 1239 μm), improved feed efficiency and phosphorus retention

than fine particles (GMD, 891 μm) (Kilburn and Edwards, 2004). Kilburn and Edwards (2001) also showed increased Ca and P digestibility in a coarse diet with GMD of 3474 μm than a fine diet with GMD of 1051 μm .

Table 2.9. The influence of particle size on the nutrient digestibility and energy utilisation in poultry

Nutrient	Bird type	Grain	Particle size			References
			Fine	Mediu m	Coarse	
Protein	Broilers	Maize	0.848	-	0.862	Pacheco et al. (2013)
Protein	Broilers	Maize	0.820	-	0.790	Bhuiyan et al. (2013)
Gross energy	Broilers	Maize	0.780	-	0.680	
Crude protein	Broilers	Maize	0.760	-	0.790	Siegert et al. (2017)
Crude protein	Broilers	Soybean	0.790	-	0.740	
Starch	Layers	Maize	0.957	-	0.969	Ruhnke et al. (2015)
AME MJ/kg	Broilers	Maize	13.64	-	14.27	Kilburn and Edwards (2001)
Starch	Broilers	Wheat	0.854	-	0.925	Péron et al. (2005)
AME MJ/kg	Broilers	Wheat	5.17	-	5.45	
AMEn MJ/kg	Broilers	Maize	10.37	-	10.72	Jacobs and Parsons (2013b)
Starch	Broilers	Pea	0.964	-	0.888	Carré et al. (1998)
AMEn MJ/kg	Broilers	Pea	13.60	-	12.50	
DM	Turkey	Maize	0.730	0.719	0.750	Favero et al. (2012)
Nitrogen	Broilers	Maize	0.740	-	0.766	Xu et al. (2015a)
AME MJ/kg	Broilers	Maize	61.88	-	66.07	

2.6.4 Effect of particle size on digestive tract development, digesta retention time and feed passage rate

2.6.4.1 The effect of particle size on digestive tract development

In the poultry feed industry, there is a growing interest on studying the influence of dietary particles size on the development of the GIT of chickens. The reported data showed that, feed particle size has a great influence on the development of the poultry GIT organs especially gizzard (Nir and Ptichi 2001; Engberg et al., 2002; Dahlke et al., 2003; Choct et al., 2004; González-Alvarado et al., 2007). The influence of feed particle size on development of GIT organs is well documented (Table 2.10). The gizzard activity and gizzard development depends on either the genetic origin of the birds (Péron et al., 2006; Rougrière and Carré, 2010) or the texture of the consumed diets (Rougrière and Carré, 2010).

Table 2.10. Influence of feed particle size on the relative weight (g/kg body weight) of the organs of gastrointestinal tract (GIT)^a in broilers and layers

GIT segments	Bird type	Grain type	Feed particles			References
			Fine	Medium	Coarse	
Gizzard	Layers	Maize	15.4	-	16.6	Röhe et al. (2014)
Gizzard	Broilers	Wheat	13.0	-	15.0	Péron et al. (2005)
Gizzard	Layers	Maize	27.1	28.5	29.5	Herrera et al. (2016)
Proventriculus	Broilers	Maize	3.9	4.1	3.9	Naderinejad et al. (2016)
Gizzard	Broilers	Maize	12.9	14.9	15.0	
Small	Broilers	Maize	22.8	23.1	22.1	
Proventriculus	Broilers	Maize	-	5.8	5.1	Amerah et al. (2007b)
Gizzard	Broilers	Wheat	-	22.0	20.1	
Small	Broilers	Wheat	-	28.5	25.7	
Proventriculus	Broilers	Wheat	-	4.5	4.6	Amerah and Ravindran (2009)
Gizzard	Broilers	Maize	-	19.8	20.7	
Proventriculus	Broilers	Wheat	3.9		4.0	Amerah et al. (2008a)
Gizzard	Broilers	Wheat	9.0	-	10.0	
Gizzard	Broilers	Wheat	16.8	-	18.0	Engberg et al. (2002)
Gizzard	Broilers	Maize	18.2	-	19.9	Pacheco et al. (2013)
Gizzard	Broilers	Maize	9.1	-	10.4	Huang et al. (2006a)
Gizzard	Broilers	Wheat	-	17.9	19.2	Amerah et al. (2008b)
Ileum	Broilers	Wheat	-	7.8	7.6	
Proventriculus	Broilers	Maize	2.4	-	2.3	Xu et al. (2015a)
Gizzard	Broilers	Maize	8.1	-	9.2	
Ileum	Broilers	Maize	10.9	-	10.4	
Proventriculus	Broilers	Maize	4.4	-	4.6	Rougière et al. (2009)
Gizzard	Broilers	Maize	14.7	-	18.9	
Ileum	Broilers	Maize	9.9	-	8.8	
Proventriculus	Broilers	Maize	2.3	-	2.1	Xu et al. (2015b)
Gizzard	Broilers	Maize	9.5	-	11.0	
Gizzard	Broilers	Maize	1.9	-	2.5	Jacobs and Parsons (2013b)
Proventriculus	Broilers	Maize	4.3	5.3	5.5	Xu et al. (2015d)
Gizzard	Broilers	Maize	21.0	25.7	28.3	
Gizzard	Broilers	Maize	15.1	-	18.1	Parsons et al. (2006)
Gizzard	Layer	Wheat	10.9	-	15.7	Hetland et al. (2003)
Gizzard	Broilers	Maize	4.0	4.5	4.9	Nir et al. (1994b)

^aGIT segments

Gizzard is known as a muscular organ that reduces feed particle size through mechanical grinding. The finely grinding of feed particles by the feed mills, has negative effect on gizzard development and functioning (Taylor and Jones, 2004). The birds consumed finely ground feed particle size results into underdeveloped gizzard and the proventriculus become enlarged (Figure 2.4). At this point, gizzard functions as a transit rather than grinding organ (Amerah et al., 2007b). This shows that, there is positive relationship between poultry feed particle size and the relative gizzard weight and functioning (Nir and Ptichi 2001).

It has been hypothesized that feeding larger particle sizes of feed ingredients improved bird health as well as GIT development and functions (Nir et al., 1995). Coarse feed particles stimulate the activity of the gizzard (Nir et al., 1995) and improve the muscular strength and development of the gizzard (Preston et al., 2000; Amerah et al., 2007b; 2008b; Svihus, 2014; Xu et al., 2015a) and increasing gizzard contractions (Williams et al., 2008; Rougière et al., 2009). A well-developed gizzard increased grinding activity resulting into an increased reduced particle size of digesta contents entering the small intestine thus increasing the accessibility to digestive enzymes (Gabriel et al., 2003).

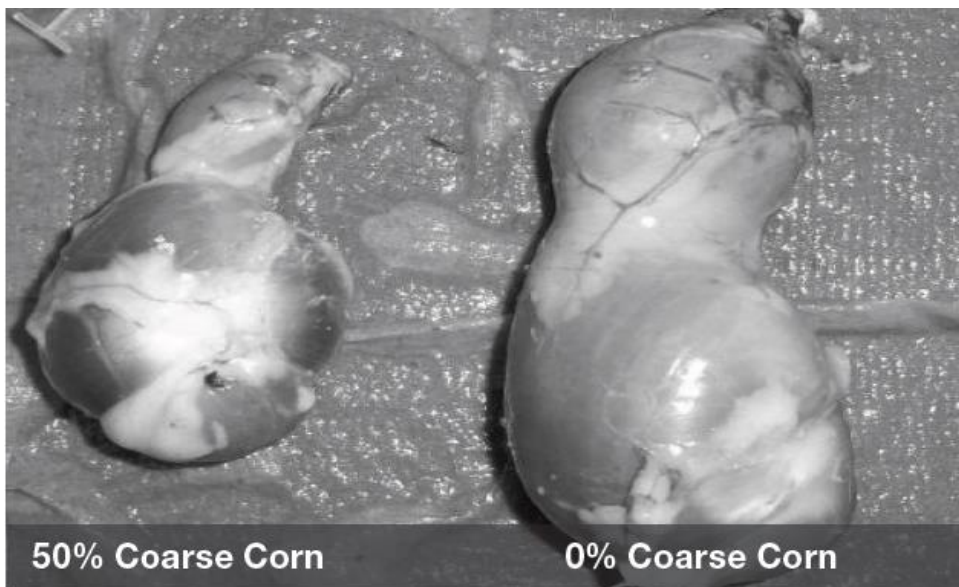


Figure 2.4. The effect of coarse maize addition on the gizzard and proventriculus development (Source: Wilmer et al., 2015).

Feeding birds with medium (3 mm) and coarse (8 mm) maize particles increased gizzard weight by 26 and 41%, respectively than those fed fine particles (2 mm) (Nir et al., 1994b). The increased GMD values of the dietary particle size showed an increased gizzard weight (Table 2.11). In order to stimulate gizzard development and functionality, it has been suggested that poultry diets should contain at least 20 to 30% cereal particles larger than 1 mm (Svihus 2011). A well-developed gizzard mixes the ingested food with digestive enzyme, (Amerah et al., 2007a,b) and improved GIT motility (Ferket, 2000; González-Alvarado et al., 2008) in turns improve nutrient digestibility and utilisation. A well-developed gizzard allows more accumulation of digesta in the gizzard which increases HCl secretion and reduces gizzard pH (González-Alvarado et al., 2008). The lower pH of the digesta content in the gizzard and increase enzyme activity (Gabriel et al., 2003) therefore improve nutrient digestibility.

Birds fed on coarse feeds have a lower gizzard and proventriculus pH (Nir et al., 1994a,b; 1995; Engberg et al., 2002) in comparison to those fed on fine feed particle. Dahlke et al. (2003) showed a reduction of gizzard pH from 3.82 to 3.65, 3.20 and 2.87 as a result of an increase in feed particle sizes from 0.336 to 0.585, 0.856 and 1.12 mm, respectively. However, some studies have found that the increased particle sizes in mash diets had no effect on gizzard and proventriculus pH (Charbeneau and Roberson, 2004; Singh et al., 2014; Naderinejad et al., 2016). The undeveloped gizzard has an elevated pH due to shorter retention time of feed particle in the GIT, reducing enzymes activity and nutrient digestibility (Ravindran, 2013).

However, feed particle size has been reported to influence the development of other segments of the poultry digestive tract. Nir et al. (1994b) reported the reduced length of the small intestine when the birds fed fine mash diets. Naderinejad et al. (2016) found an increased broilers proventriculus and a reduced length of the small intestine by increasing particle size from fine (2 mm) to coarse (8 mm). Feeding coarse particles to broilers reported to reduce the relative weight of duodenum compared to those fed finely ground diets (Nir et al., 1995).

The differences on development responses of digestive organs due to feed particle size might be associated with anatomical and physiological differences in

digestive system among bird genotypes. The study by Péron et al. (2006) and Rougrière and Carré, (2010) showed greater gizzard weight in D+ (high digestive efficiency) compared with D- (low digestive efficiency) broilers fed on coarse particles. The reason for the different responses to the increased proventriculus and gizzard weights between these chicken lines was related with the lower gizzard digesta retention time in bird D- (Rougrière and Carré, 2010) and reduced gizzard activity (Carré, 2004).

Table 2.11. Effect of maize particle size on broiler gizzard weight (% of body weight) (Adapted from Jacobs et al., 2010)

Experiment	Geometric mean diameter of maize particles, (µm)				Pooled SEM
	557	858	1,210	1,387	
Experiment 1	2.26 ^c	2.50 ^b	2.62 ^{ab}	2.69 ^a	0.05
Experiment 2	1.50 ^c	1.94 ^b	2.14 ^a	2.20 ^a	0.04
Experiment 3	1.54 ^c	1.92 ^b	2.00 ^{ab}	2.14 ^a	0.07

^{a-c}Means with different letters differ significantly (P<0.05)

2.6.4.2 The effect of particle size on digesta retention time and passage rate on

The feed passage rate and retention time of digesta content along the GIT organs, depends on the dietary particle sizes (Van der Klis et al., 1993; Amerah and Ravindran 2009). The high passage rate of digesta contents along the GIT lowers time available for nutrients digestion and absorption. In the small intestine the feed passage rate is determined by the frequencies of peristaltic and anti-peristaltic movements. Several factors reported to affect the digesta passage rate including age of birds (Shires et al., 1987), bird strain (Denbow, 2000) diet factors (particle size).

The fine feed particles do not need to be ground to such an extent as coarse particles in the gizzard this reduced retention time and increased passage rate through the GIT (Hetland et al., 2002; Svihus et al., 2002) compared to the coarse particles. Thus fast passage rate of fine particles in the GIT reduces time for enzyme activity as a result lowers nutrient digestibility. The coarse particles are retained longer in the gizzard to be ground before passing to lower parts of the GIT (Moore, 1999). The high retention time

of coarse particles in the gizzard stimulates gizzard activity (Jiménez-Moreno et al., 2010) also enhances GIT motility and the intestinal reflex (Rogel et al., 1987a; Jiménez-Moreno et al., 2010; Svihus, 2011). Coarse oat hulls have been shown to accumulate in the gizzard and reduced the digesta passage rate than those in ground oat hulls (Figure 2.5). A number of studies documented on the reduced digesta passage rate in the GIT for the bird fed on coarse particles (Nir et al., 1994a; Nir and Ptichi 2001; Benedetti et al., 2011), increase exposure time of nutrients to digestive enzymes, thus improve nutrient digestibility and absorption (Preston et al., 2000; Kilburn and Edwards, 2004).

Figure 2.5. Passage rate of ground and coarse oat hulls through the gizzard (Source: Hetland et al., 2005)

2.7 Conclusion

Optimum supply of the required nutrient should be considered during poultry feed formulation to improve bird health and production. The use of digestible nutrients is the best criterion to meet the dietary nutrient requirements of different bird types and to reduce wastage of nutrients to the environment. Dietary fibre together with feed particle size has a significant influence on digestive tract development and nutrient digestibility in poultry. Identifying the optimum concentration of insoluble fibre and particle size of the feed ingredients to be included in the poultry feed is therefore important to optimise the digestibility of nutrients. Most of the digestibility studies conducted using broilers and the available data has been used for diet formulation for other type of birds. There is limited information on digestibility response of layers and pullets to different diet types. Moreover, there is a lack of information on the possible interaction between dietary fibre content or feed particle size and bird type on ileal nutrient digestibility. A different digestibility response might be expected due to the fact that different physiological status of bird digestive organs can impact nutrient digestibility.

CHAPTER 3

Influence of bird type and dietary fibre content on the apparent ileal digestibility of nutrients and energy utilisation

3.1 Abstract

The interaction between bird type and dietary fibre content on the apparent ileal digestibility of nutrients and energy utilisation was investigated in the current study. A 2 x 3 factorial arrangement of treatment was used with two fibre contents (10.3 and 19.3 g/kg neutral detergent fibre) and three bird types (broilers, pullets and layers). The low fibre diet was based on maize and soybean meal whilst the high fibre diet was developed by the inclusion of palm kernel meal, canola meal and oat hulls. Titanium dioxide was used as an inert marker to calculate the digestibility. The digesta were collected from terminal ileum after feeding the experimental diets for 7 days.

Significant interactions ($P < 0.05$) between dietary fibre content and bird type were observed for all nutrients. Overall, nutrient digestibility was higher in broilers, intermediated response in pullets and lowest in layers in both diet types. The digestibility of nutrients in the three bird types was higher ($P < 0.05$) in the high fibre diet than in the low fibre diet, but the magnitude of increases differed. Layers showed markedly higher digestibility responses compared to broilers and pullets.

There were interactions ($P < 0.05$ to 0.001) between dietary fibre content and bird type for all AA, except aspartic acid and lysine. The digestibility of aspartic acid and lysine was highest ($P < 0.05$) in broilers, intermediate in pullets and lowest in layers. The average AA digestibility improved with increased fibre content with markedly greater responses in layers compared to broilers and pullets.

Overall, layers showed greater digestibility of nutrients in response to the increased fibre content. These findings suggest that layers require high dietary fibre contents to efficiently utilise nutrients compared to broilers and pullets.

3.2 Introduction

Poultry nutrition aims to supply optimum levels of nutrients in poultry diets to ensure cost effective and efficient feed utilisation by maximising nutrient digestibility and minimising wastage of nutrients into the environment. An adequate supply of nutrients can be achieved by formulating diets on the basis of digestible nutrients. In recent years, there had been an increasing interest in exploring ways to improve nutrient digestibility by the inclusion of dietary fibre and through consideration of particle size.

A large volume of published reports on the amino acid (AA) digestibility of feed ingredients for broilers is available, but studies comparing the nutrient digestibility in different bird types are scant. However, there is some data available concerning the effect of bird type on the digestibility of AA in feed ingredients (Huang et al., 2006b; 2007). Nutrient digestibility estimates can vary depending on assay methodology (ileal or excreta), bird factors (bird type, age and gender) and dietary factors [type and level of fibre, and anti-nutritional factors] (Green and Kiener, 1989; Parsons et al., 1997; Kadim et al., 2002; Huang et al., 2005; Ravindran et al., 2005). Ravindran et al. (1999a) showed a significant strain effect on AA digestibility and energy utilisation in broilers and suggested that this might be due to genetic variation in nutrient utilisation among genotypes. However, contradictory results on nutrient digestibility have been reported due to the influence of the age, bird type and sex of the birds (Batal and Parsons 2002; Huang et al., 2005) and the fibre inclusion level in the diet (Svihus and Hetland 2001).

Dietary fibre has been reported to reduce feed intake and nutrient digestibility in poultry, but the extent of reduction depends on the type and level of fibre inclusion (Mateos et al., 2002; Rougière and Carré 2010). Currently there is no data to fully understand the optimum dietary fibre inclusion level for different type of birds. Studies in broilers (Hetland and Svihus 2001; González-Alvarado et al., 2010; Mateos et al., 2012), pullets (Guzmán et al., 2015a,b; Kimiaetalab et al., 2016) and layers (Hetland et al., 2005) have reported increased nutrient digestibility in birds fed diets with moderate inclusion levels of dietary fibre. Moderate amounts of fibrous ingredients, such as oat hulls, pea hulls or wood shavings, improve the development and function of the gizzard (Hetland and Svihus 2001; Hetland et al., 2003; Hetland et al., 2005; Jiménez-Moreno et

al., 2011; Mateos et al., 2012). Guzmán et al. (2015b) showed increased relative weights of gizzard (38.2 versus 40.2g) and proventriculus (5.41 versus 5.44g) and reduced length of small intestine in 10-week old pullets fed a diet with 20-40g/kg dietary fibre inclusion. In addition, dietary fibre enhances peristaltic movement of the gut, (Jamroz et al., 2001), reduces gizzard digesta pH, improves enzyme activities (Hetland et al., 2003; Jiménez-Moreno et al., 2010), and solubility and absorption of minerals (Van der Ar et al., 1983; Guinotte et al., 1995). Fibrous sources are known to increase retention time in the upper digestive tract, thereby improving the nutrient digestibility by allowing more time for enzymatic action (González-Alvarado et al., 2007).

The utilisation of dietary fibre increases with advancing age of birds. González-Alvarado et al. (2010) reported an increased gizzard development and nutrient digestibility in broilers with the consumption of increased dietary fibre at the age of 32 days compared to 25 days of age. However, bird types seem to influence the digestibility of nutrients and dietary fibre. Layers fed diets containing 50-200 g/kg maize distillers dried grains with solubles (DDGS) showed better productivity due to improved nutrient digestibility (Roberson et al., 2005). The inclusion of DDGS and wheat bran (60-80 g/kg) was observed to improve nutrient digestibility in layer chicks compared to broiler chicks (Walugembe et al., 2014). The improved digestibility in layer chicks was suggested as being associated with improved capability of layer digestive tract to utilise fibrous ingredients more efficiently compared to broilers.

The effects of dietary fibre on growth (Jiménez-Moreno et al., 2010; Jiménez-Moreno et al., 2011; Mateos et al., 2012) and production performance (Incharoen and Yamauchi, 2009; Incharoen and Maneechote, 2013) have been investigated in different bird types; however, limited study has examined the interaction between dietary fibre content and bird type on nutrient digestibility. It was hypothesised that an interaction will exist for digestibility responses of layers, pullets and broilers, and diets with different dietary fibre contents. Due to the well-known tolerance of layers to fibrous feed ingredients, we hypothesised that layers will have a relatively better digestion of dietary nutrients when fed high fibre diets. The objective of the present study was to

evaluate the influence of diet type (low and high fibre) and bird type (broilers, pullets and layers) on the ileal nutrient digestibility and energy utilisation.

3.3 Materials and methods

Two diets (low and high fibre diets) and three bird types (broilers, pullets and layers) were used. This provided six experimental treatments as shown in Table 3.1.

Table 3.1. Summary of the experimental treatments

Treatment	Diet type	Bird type
A	Low fibre	Broilers
B	Low fibre	Pullets
C	Low fibre	Layers
D	High fibre	Broilers
E	High fibre	Pullets
F	High fibre	Layers

3.3.1 Dietary treatments

The diets were formulated and mixed using the same batch of ingredients (Table 3.2). The low fibre diet was based on maize and soybean meal. In the high fibre diet, palm kernel meal, canola meal and oat hulls were included to increase the fibre concentration. Titanium dioxide (5.0 g/kg) was included in the diets as an indigestible marker for digestibility calculations.

Table 3.2. Ingredients composition and calculated analysis of the experimental diets (g/kg, as fed basis)

Ingredient	Low fibre	High fibre
Maize	711.8	546.1
Soybean meal	248.6	217.1
Palm kernel meal (crude fibre, 19.7%)	0	80.0
Canola meal (crude fibre, 10.5%)	0	40.0
Oat hulls (crude fibre, 28.3%)	0	40.0
Soybean oil	0.2	38.8
Dicalcium phosphate	16.5	16.2
Limestone	10.7	9.6
Sodium chloride	2.3	2.1
Sodium bicarbonate	2.6	2.8
Vitamin premix ¹	0.8	0.8
Trace mineral premix ¹	1.5	1.5
Titanium dioxide ²	5.0	5.0
Total	1000	1000
Calculated values		
Apparent metabolisable energy, (MJ/kg)	12.55	12.13
Crude protein	180	180
Crude fibre	23.8	50.6
Crude fat	29.8	69.8
Methionine	3.0	3.0
Methionine and cysteine	6.0	6.0
Lysine	9.2	9.0
Threonine	6.7	6.7
Tryptophan	2.3	2.2
Calcium	8.5	8.5
Total phosphorus	6.6	6.8
Non-phytate phosphorus	4.2	4.2
Sodium	1.8	1.8
Chloride	1.8	1.8
Analysed values		
Starch	410	356
Fat	20	51
Crude protein (nitrogen x 6.25)	183	161
Gross energy (MJ/kg)	15.70	16.84
Neural detergent fibre	10.32	19.33
Calcium	10.5	10.6
Total phosphorus	6.0	6.5

¹Supplied 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.

²Inert marker for ileal digestibility measurements

3.3.2 Birds and housing

Experimental procedures complied with the Massey University Animal Ethics Committee guidelines (Approval number, 17/13).

Day-old male broilers (Ross 308) were obtained from a local hatchery, raised in floor pens and fed commercial broiler starter (d 1 to 21) and finisher (d 22 to 35) diets. The birds were 35 days old (average body weight, 2.60 kg) at the start of the test period. The pullets (Hy-Line Brown, 10-weeks old, average body weight, 1.05 kg) and layers (Hy-Line Brown, 59-weeks old, average body weight, 1.95 kg) were obtained from a local layer farm. For each bird type, 96 birds with uniform body weight were selected and assigned to 24 cages, so that the average initial weight per cage was similar. For each of the bird type treatment, two adjacent cages were treated as a replicate (four birds per cage, eight birds per replicate) and each diet was randomly assigned to 6 replicates. Birds were fasted for 12 hours before the introduction of treatment diets. The diets, in mash form, were offered *ad libitum* for 7 days prior to the collection of ileal digesta. Water was freely available throughout the trial period via nipple drinkers.

3.3.3 Measurements

3.3.3.1 Apparent metabolisable energy (AME) determination

Feed intake and excreta output were recorded over the last 4 days of experimental period for the determination of AME. Daily excreta collections were pooled within a replicate mixed in a blender and sub-sampled. The sub-sample was lyophilised (Model 0610, Cuddon Engineering, Blenheim, New Zealand), ground to pass through a 0.5 mm sieve and stored in airtight plastic containers at 4 °C until laboratory analysis. Excreta samples were analysed for dry matter (DM), gross energy (GE) and nitrogen (N).

3.3.3.2 Ileal digesta collection

After 7 days on treatment diets, all birds were euthanised by intravenous injection (1 ml/2 kg live weight) of sodium pentobarbitone (Provet NZ Pty Ltd., Auckland, New Zealand) and ileal digesta was collected from the lower half of the ileum (Ravindran et

al., 2005). The ileum was defined as the portion of the small intestine extending from Meckel's diverticulum to ~40 mm proximal to ileo-caecal junction. The ileum was then divided into two halves and the digesta were collected from the lower half towards the ileo-caecal junction by gentle flushing with distilled water. Digesta from birds of the same replicate were pooled together giving six samples per treatment. The digesta were immediately frozen, lyophilised (Model 0610, Cuddon Engineering, Blenheim, New Zealand), ground to pass through a 0.5-mm sieve and stored at 4 °C until laboratory analysis for DM, gross energy (GE), N, amino acids (AA), titanium (Ti), starch, fat and neutral detergent fibre (NDF).

3.3.3.3 Chemical analysis

All analyses, except nitrogen and amino acids, were conducted in ISO17025 accredited laboratory (Nutrition Laboratory, Massey University). Amino acids and N were determined at the Evonik animal nutrition analytical laboratory (Evonik SEA Pte. Ltd., Singapore).

Dry matter was determined using standard procedures (method 930.15 and 925.10; AOAC, 2005). The GE was determined by adiabatic bomb calorimetry (Gallenkamp Autobomb, London, UK) standardised with benzoic acid. The NDF was determined using standard procedures (method AOAC 2002.04/973.18). Titanium was determined on a UV spectrophotometer following the method of Short et al. (1996). Nitrogen was determined by combustion (method 968.06; AOAC, 2005) using the Leco CNS 2000 auto-analyser (LECO Corporation, St. Joseph, MI). Crude protein content was calculated as N X 6.25. Starch was determined using the assay procedure (Megazyme Total Starch Assay Procedure; Megazyme International Ireland Ltd., Wicklow, Ireland) based on thermostable α -amylase and amyloglucosidase. Fat was determined using the Soxhlet extraction procedure (method 991.36; AOAC, 2005).

Amino acids were determined using (method 994.12 AOAC, 2005). Briefly, the samples were hydrolysed with hydrochloric acid-phenol for 24h in a heating oven at 110 \pm 2 °C. Amino acids were detected using an AA analyser, and the chromatograms were integrated using dedicated software (Biochrom, Version 20 plus, Biochrom Ltd.,

Cambridge, UK) with the amino grams simultaneously detected at 570 and 440 nm. Cysteine and methionine were analysed as cystic acid and methionine sulphone, respectively, by oxidation with performic acid-phenol for 16h at 0 °C prior to hydrolysis.

For mineral analysis, the samples were wet acid digested with nitric and perchloric acid mixture, and concentrations of Ca, P, K, Mg and Na were determined by Inductively Coupled Plasma-Optical Emission Spectroscopy (ICP-OES) using a Thermo Jarrell Ash IRIS instrument (Thermo Jarrell Ash Corporation, Franklin, MA). The concentrations of Cu, Mn and Zn were determined by Inductively Coupled Plasma-Mass Spectrometry (ICP-MS) using a Perkin Elmer Elan 6000 instrument (Melbourne, Victoria, Australia).

3.3.3.4 Calculations

The AME values were calculated using the following formula with appropriate corrections made for differences in DM content.

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

Nitrogen-corrected AME (AMEn) was determined by correction for zero N retention by simple multiplication by assuming 36.54 kJ per gram N retained in the body as described by (Hill and Anderson 1958).

The coefficient of apparent ileal digestibility (CAID) of nutrients was calculated using the following formula:

$$\text{CAID of diet component} = \frac{[(\text{Component/Ti})_{\text{diet}} - (\text{Component/Ti})_{\text{ileal}}]}{(\text{Component/Ti})_{\text{diet}}}$$

Where;

(Component/Ti) diet = ratio of component to titanium in the diet, and

(Component/Ti) ileal = ratio of component to titanium in the ileal digesta.

3.3.3.5 Statistical analysis

Replicates were considered as the experimental unit. The data were analysed by two-way ANOVA to determine the main effects (diet type and bird type) and their interaction using the General Linear Models procedure (GLM) of the SAS Institute (SAS, 2004) using the model below.

$$Y_{ijk} = \mu + A_i + \beta_j + A_i \times B_j + e_{ijk}$$

Where:

Y_{ijk} = k^{th} observation in the i^{th} treatment group A (diet type) and j^{th} treatment group B (bird type)

μ = a general mean

A_i = Fixed effect of i^{th} treatment group (diet type)

B_j = Random effect of the j^{th} treatment group (bird type)

$A_i \times B_j$ = Fixed interaction (diet type x bird type)

e_{ijk} = random residual error

Differences were considered to be significant at $P < 0.05$ and significant differences between means were separated by the Least Significant Difference test (Kaps and Lamberson, 2009).

3.4 Results

3.4.1 Apparent ileal digestibility of nutrients

The ileal digestibility of dry matter (DM), starch, fat, NDF and gross energy (GE) of the diets for the three bird types is shown in Table 3.3. There were significant ($P < 0.05$ to 0.001) interactions between the bird type and dietary fibre content for ileal digestibility of all nutrients. Digestibility responses to increased fibre content differed between bird types. Digestibility of all nutrients, in general, increased in the high fibre diet, but the responses were markedly greater in layers compared to those in the broilers and pullets (Table 3.4). The digestibility improvements in layers ranged from 16.5 (for nitrogen) to

62.0% (for fat). The improvements in broilers and pullets ranged from 0.3 (for GE) to 35.0% (for fat) and 1.3 (for starch) to 27% (for fat), respectively.

In the low fibre diet, the digestibility of DM in broilers was higher ($P < 0.05$) than those in pullets and layers, while in the high fibre diet the digestibility was similar ($P > 0.05$) among all three bird types. The ileal digestibility of starch was higher ($P < 0.05$) in broilers and pullets than in layers fed the low fibre diet, but all bird types showed similar ($P > 0.05$) starch digestibility in the high fibre diet. With the low fibre diet, the highest ($P < 0.05$) fat digestibility was determined in pullets and the lowest ($P < 0.05$) in layers. Fat digestibility in broilers was lower ($P < 0.05$) than that in pullets, but higher ($P < 0.05$) than that in layers. In the high fibre diet, fat digestibility was similar ($P > 0.05$) among all bird types. In the low fibre diet, NDF was digested only in broilers and the digestibility estimates were negative in pullets and layers. However, on the high fibre diet, NDF was digested in all bird types and the digestibility estimates were similar ($P > 0.05$). In the low fibre diet, ileal GE digestibility in broilers was higher ($P < 0.05$) than those in pullets and layers, and the energy digestibility in layers was lower ($P < 0.05$) than that in pullets. In the high fibre diet, layers showed lower ($P < 0.05$) GE digestibility than broilers, but similar digestibility ($P > 0.05$) to that of pullets.

The interaction between bird type and fibre content was significant ($P < 0.05$) for the AMEn (Table 3.3). At both fibre contents, the AMEn in pullets was higher than those in the broilers and layers, and that in layers was lower ($P < 0.05$) than that of broilers. But the differences among bird types were lower in the high fibre diet.

Table 3.3. Influence of bird type and diet type on the apparent ileal digestibility of dry matter (DM), starch, fat, neutral detergent fibre (NDF) gross energy (GE) and nitrogen corrected apparent metabolisable energy (AMEn)

Item		CAID ^a					
Diet type	Bird type	DM	Starch	Fat	NDF	GE	AMEn ^b
Low fibre	Broilers	0.685a	0.950b	0.696c	0.275a	0.736a	12.93bc
	Pullets	0.604c	0.981ab	0.749b	-0.058b	0.661c	13.35a
	Layers	0.495d	0.771c	0.569d	-0.051b	0.550d	11.72e
High fibre	Broilers	0.666ab	0.978ab	0.943a	0.340a	0.738a	12.66c
	Pullets	0.628bc	0.994a	0.952a	0.234a	0.712ab	13.04ab
	Layers	0.627bc	0.963ab	0.924a	0.270a	0.688bc	12.16d
SEM ^c		0.019	0.014	0.014	0.044	0.017	0.119
Main effects							
Diet type							
Low fibre		0.595	0.901	0.671	0.055	0.649	12.67
High fibre		0.640	0.978	0.940	0.281	0.712	12.62
Bird type	Broilers	0.675	0.964	0.820	0.307	0.737	12.79
	Pullets	0.616	0.988	0.850	0.088	0.686	13.20
	Layers	0.561	0.867	0.746	0.110	0.619	11.94
Probabilities, P ≤							
Diet type		0.007	0.001	0.001	0.001	0.001	0.652
Bird type		0.001	0.001	0.001	0.001	0.001	0.001
Diet type x bird type		0.002	0.001	0.001	0.013	0.001	0.005

Means in a column not sharing a common letter (a, b, c, d) are significantly different (P < 0.05)

^a Each value represents the mean of six replicates (eight birds per replicate) measured after 7 days on treatment diets

^b Each value represents the mean of six replicates (eight birds per replicate) measured over the last 4 days on treatment diets

^c Pooled standard error of the mean

Table 3.4. Percentage improvements in the digestibility of dry matter (DM), starch, fat, nitrogen (N), average amino acids (AA), gross energy (GE) and AMEn in the three bird types due to increased dietary fibre content^a

Bird type	DM	Starch	Fat	N	Ave. AA	GE	AMEn
Broilers	3.0	3.0	35.0	4.6	4.9	0.3	2.1
Pullets	4.0	1.3	27.0	6.5	6.4	7.7	2.4
Layers	27.0	25.0	62.0	16.5	17.5	25.0	3.8

^aEach value represents percentage increase in nutrient digestibility in the high fibre diet over the low fibre diet.

3.4.2 Apparent ileal digestibility of nitrogen and amino acids

The influence of bird type and dietary fibre content on the ileal digestibility of N and AA is summarised in Tables 3.5A and B. The ileal N digestibility increased with increase in dietary fibre content in all bird types, but the magnitude of responses differed resulting in a bird type x diet type interaction ($P < 0.05$). The digestibility improvements for N were distinctly greater in the layers followed by pullets and least in broilers, 16.5, 6.5 and 4.6% respectively.

Both the bird type and dietary fibre content had significant ($P < 0.001$) effects on the ileal digestibility of AA. However, there were interactions ($P < 0.05$ to 0.001) between diet type and bird type for all AA, except aspartic acid and lysine. The digestibility of AA in the three bird types was higher in the high fibre diet ($P < 0.05$) compared to the low fibre diet, but the magnitude of increases differed. The digestibility of AA was higher in broilers ($P < 0.05$), compared to pullets and layers, in both fibre diet types. The response to increased fibre was, however, greater in layers. The improvements due to high fibre content in average AA digestibility of broilers, pullets and layers were 4.9, 6.4 and 17.5%, respectively (Table 3.4).

The main effects of bird type and diet type were significant ($P < 0.001$) for the digestibility of aspartic acid and lysine and there were no interactions ($P > 0.05$). The digestibility of these AA increased with increasing fibre content. Broilers had the

highest and layer the lowest digestibility of aspartic acid and lysine, with pullets being intermediate.

Table 3.5A. Influence of bird type and diet type on the apparent ileal digestibility of nitrogen and amino acids

Item		CAID ^a								
Diet type	Bird type	N	ASP	SER	GLU	PRO	GLY	ALA	CYS	ISO
Low fibre	Broilers	0.798b	0.806	0.806b	0.865bc	0.808b	0.771b	0.808b	0.752ab	0.822b
	Pullets	0.754c	0.757	0.769c	0.852c	0.769c	0.696c	0.802b	0.645c	0.808b
	Layers	0.673d	0.712	0.647d	0.780d	0.628d	0.613d	0.693c	0.381d	0.720c
High fibre	Broilers	0.835a	0.839	0.846a	0.892a	0.847a	0.813a	0.864a	0.797a	0.866a
	Pullets	0.803b	0.805	0.812b	0.878ab	0.814ab	0.770b	0.857a	0.724b	0.858a
	Layers	0.784b	0.791	0.780bc	0.856c	0.782bc	0.741b	0.818b	0.631c	0.826b
SEM ^b		0.010	0.010	0.012	0.007	0.011	0.012	0.009	0.022	0.009
Main effects										
Diet type										
Low fibre		0.742	0.758b	0.741	0.833	0.735	0.693	0.768	0.593	0.784
High fibre		0.807	0.812a	0.813	0.875	0.814	0.775	0.846	0.717	0.850
Bird type	Broilers	0.817	0.823a	0.826	0.878	0.828	0.792	0.836	0.775	0.844
	Pullets	0.779	0.781b	0.791	0.865	0.792	0.733	0.829	0.684	0.833
	Layers	0.728	0.752c	0.714	0.818	0.705	0.677	0.755	0.506	0.773
Probabilities, P ≤										
Diet type		0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001
Bird type		0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001
Diet type x bird type		0.002	0.071	0.001	0.001	0.001	0.006	0.001	0.001	0.003

Means in a column sharing a common letter (a, b, c, d) are the same ($P > 0.05$).

^a Each value represents the mean of six replicates (eight birds per replicate); measured after 7 days on treatment diets.

^b Pooled standard error of mean

Table 3.5B. Influence of bird type and diet type on the apparent ileal digestibility of amino acids and average digestibility of amino acids

Item		CAID ^a								
Diet type	Bird type	LEU	PHE	HIS	LYS	ARG	VAL	MET	THR	Average AA
Low fibre	Broilers	0.837b	0.854b	0.834bc	0.834	0.889b	0.803bc	0.850b	0.743b	0.818bc
	Pullets	0.837b	0.848b	0.801d	0.808	0.859c	0.764d	0.846b	0.662c	0.783d
	Layers	0.722c	0.765c	0.693e	0.773	0.826d	0.660e	0.729c	0.526d	0.679e
High fibre	Broilers	0.882a	0.895a	0.871a	0.867	0.907a	0.849a	0.896a	0.797a	0.858a
	Pullets	0.880a	0.890a	0.847ab	0.850	0.886b	0.826ab	0.886a	0.746b	0.833ab
	Layers	0.845b	0.864b	0.812cd	0.827	0.875bc	0.787cd	0.846b	0.690c	0.798cd
SEM ^b		0.009	0.008	0.01	0.008	0.006	0.011	0.009	0.016	0.010
Main effects										
Diet type										
Low fibre		0.799	0.822	0.776	0.805b	0.858	0.743	0.808	0.644	0.760
High fibre		0.869	0.883	0.843	0.848a	0.889	0.820	0.876	0.744	0.830
Bird type	Broilers	0.860	0.875	0.852	0.851a	0.898	0.826	0.873	0.77	0.838
	Pullets	0.858	0.869	0.824	0.829b	0.872	0.795	0.866	0.704	0.808
	Layers	0.783	0.815	0.753	0.800c	0.851	0.723	0.787	0.608	0.739
Probabilities, P ≤										
Diet type		0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001
Bird type		0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001
Diet type x bird type		0.001	0.001	0.001	0.435	0.042	0.001	0.001	0.004	0.001

Means in a column sharing a common letter (a, b, c, d) are the same ($P > 0.05$).

^a Each value represents the mean of six replicates (eight birds per replicate); measured after 7 days on treatment diets.

^b Pooled standard error of mean.

3.4.3 Apparent ileal digestibility of minerals

The influence of diet type and bird type on the ileal digestibility of minerals is shown in Table 3.6.

The diet type had no effect ($P > 0.05$) on the digestibility of Ca and there was no interaction ($P > 0.05$) between the bird type and diet type. The main effect of bird type was significant, with layers showing considerably higher Ca digestibility compared to pullets and broilers. Calcium digestibility in pullets and broilers was similar ($P > 0.05$). The average Ca digestibility coefficients in broilers, pullets and layers were 0.248, 0.192 and 0.769, respectively. Phosphorus P digestibility differed among bird types, but the responses to increased fibre content varied causing a significant ($P < 0.05$) interaction. Similar to Ca digestibility, P digestibility was markedly higher in the layers and was not affected by increased fibre content. In broilers and pullets, P digestibility was increased in high fibre diets. The average P digestibility coefficients in broilers, pullets and layers were 0.372, 0.321 and 0.688, respectively.

The main effects of bird type and diet type were significant ($P > 0.05$) for Mg digestibility. The digestibility of Mg was higher for birds fed the high fibre diet than those fed the low fibre diet. Layers had higher ($P < 0.05$) ileal digestibility of Mg than pullets and broilers, and the digestibility was higher ($P < 0.05$) in broilers compared to pullets. The main effect of bird type and an interaction ($P < 0.05$) was observed for K digestibility, with digestibility increasing in the high fibre diet for pullets, but decreasing for broilers and layers.

The ileal digestibility of Na and Zn were influenced ($P < 0.05$) by diet type, but the values were negative. The digestibility of Cu was negative in the low fibre diet. However, Cu was digested in the high fibre diet, with layers having a higher ($P < 0.05$) digestibility than broilers and pullets. The main effect of bird type was noted for Mn digestibility, with negative values for broilers and positive values for the other two bird types.

Table 3.6. Influence of bird type and diet type on the apparent ileal digestibility of minerals

Item		CAID ^a							
Diet type	Bird type	Ca	P	Mg	K	Na	Cu	Mn	Zn
Low fibre	Broilers	0.235	0.322cd	0.123	0.851bc	-0.347c	-0.460	-0.199	-0.488
	Pullets	0.226	0.262d	-0.076	0.828c	-0.967b	-0.530	0.031	-0.450
	Layers	0.780	0.696a	0.234	0.890a	-1.740a	-0.240	0.035	-0.547
High fibre	Broilers	0.261	0.422b	0.176	0.825c	-0.312c	0.404	-0.054	-0.263
	Pullets	0.159	0.381bc	0.108	0.853bc	-0.823b	0.360	0.017	-0.191
	Layers	0.758	0.679a	0.240	0.863ab	-0.815b	0.451	0.020	-0.446
SEM ^b		0.033	0.029	0.041	0.012	0.137	0.056	0.037	0.080
Main effects									
Diet type									
Low fibre		0.414	0.427	0.094b	0.856	-1.018	-0.410b	-0.044	-0.495b
High fibre		0.393	0.494	0.175a	0.847	-0.650	0.405a	-0.006	-0.300a
Bird type	Broilers	0.248b	0.372	0.150b	0.838	-0.330	-0.028b	-0.126b	-0.376a
	Pullets	0.192b	0.321	0.016c	0.841	-0.895	-0.085b	0.024a	-0.321a
	Layers	0.769a	0.688	0.237a	0.877	-1.277	0.106a	0.028a	-0.497b
Probabilities, P ≤									
Diet type		0.439	0.007	0.021	0.335	0.003	0.001	0.211	0.006
Bird type		0.001	0.001	0.001	0.004	0.001	0.006	0.001	0.097
Diet type x bird type		0.380	0.050	0.092	0.048	0.005	0.169	0.060	0.588

Means in a column sharing a common letter (a, b, c, d) are the same ($P > 0.05$).

^a Each value represents the mean of six replicates (eight birds per replicate); measured after 7 days on treatment diets

^b Pooled standard error of mean

3.5 Discussion

3.5.1 Digestibility of dry matter, starch, fat, neutral detergent fibre gross energy, and apparent metabolisable energy

The ileal digestibility of DM, starch, fat, NDF and GE were influenced by dietary fibre content and bird type, but the digestibility responses to the increased dietary fibre content varied among bird types. The increases were pronounced in layers compared with broilers and pullets. The improvements with the increased fibre content in the ileal digestibility of DM, starch, fat and GE in layers was 27, 25, 62 and 25%, respectively. The corresponding improvements in broilers and pullets were 3.0, 3.0, 35 and 0.3%, and 4.0, 1.3, 27 and 7.7%, respectively. The marked digestibility responses in layers may suggest that their digestive tract is more sensitive to feed structure than the broilers and pullets (Moran and Evans, 1977). Studies have shown that birds fed diets with dietary fibre retained the digesta longer in the gizzard, stimulating gizzard activity and increasing gizzard weight. Layers fed diets with 10g/kg wood shavings, 40g/kg oat hulls and 30g/kg wood shavings increased gizzard weight by 50% (Hetland et al., 2003), 60% (Hetland et al., 2005) and 70% (Hetland et al., 2007), respectively, than those fed the control diets.

Variable responses by different bird types on nutrient digestibility have been reported in the literature at different fibre levels. Broilers fed diets containing 0, 10, 20 and 30g/kg high fibre sunflower cake showed reduced DM digestibility with increasing inclusions from 0.77, 0.70, 0.66 and 0.61, respectively (Kalmendal et al., 2011). Inclusion of oat bran (187 and 375g/kg) in broiler diets showed reduced starch digestibility from 0.97 to 0.94 (Jorgensen et al., 1996). Layers fed a whole wheat diet with 10g/kg wood shavings had increased starch digestibility from 0.95 to 0.98 (Hetland et al., 2003). The differences in nutrient digestibility among bird types in the current study may also be partly reflective of age effects. Layers are mature birds, whereas pullets and broilers are growing birds. The older birds fed higher dietary fibre were reported to have longer retention time in the gizzard compared to young birds (Shires et al., 1987; González-Alvarado et al., 2010). Longer retention time (Hetland et al., 2005; Mateos et al., 2012), reduced gizzard pH (González-Alvarado et al., 2010) and increased digesta exposure to digestive

enzymes (Hetland and Svihus 2001) lead to improvements in nutrient digestibility (González-Alvarado et al., 2007; Mateos et al., 2012).

In the low fibre diet, the ileal digestibility of fat was highest in pullets, followed by broilers and lowest in layers; however, these differences were evened out in the high fibre diet. In all three bird types, fat digestion was increased with increased dietary fibre content, which may be associated with the better development of the digestive tract (González-Alvarado et al., 2008). It should be also noted that most of the dietary fat in the low fibre diet originated from intact fat within maize grains, whilst a portion of the fat in the high fibre diets was in the form of supplemental soybean oil. Supplemental soybean oil has been shown to be more digestible than the encapsulated lipids within the cell wall matrix (González-Alvarado et al., 2007). The encapsulated lipids within the cell walls become a physical barrier to digestive enzymes (Hetland et al., 2004) and this may explain the lower fat digestibility for all bird types in the low fibre diet. Moreover, increased dietary fibre content possibly increased gastrointestinal refluxes causing greater solubilisation and digestibility of the dietary fat.

Interestingly, NDF digestibility estimates in pullets and layers fed the low fibre diet were negative suggesting that the fibre was not digested. In contrast, some NDF digestion occurred in the broilers fed the low fibre diet. These findings were unexpected and difficult to explain. Increasing the dietary fibre content had no effect on the NDF digestibility in broilers, but increased those in the pullets and layers. In the high fibre diets, NDF digestion was found to be similar in all bird types. Walugembe et al. (2014) reported a higher ileal NDF digestibility in a high dietary fibre (0.25; 80g/kg DDGS and wheat bran) than a low dietary fibre (0.18; 60g/kg DDGS and wheat bran). In their study, broilers and layers showed similar ileal NDF digestibility in both diet types.

Ileal energy digestibility was highest in broilers compared to other two bird types and was not influenced by the dietary fibre content. On the other hand, pullets and layers responded to the increased fibre content with increases in GE digestibility of 7.7 and 25.0%, respectively. In the present study, the AMEn was not influenced by dietary fibre content, but the AMEn differed among bird types at both dietary fibre contents. Walugembe et al. (2014) reported similar AMEn between layer (13.41

MJ/kg) and broilers chicks (13.42 MJ/kg) fed diets containing 60 or 80g/kg DDGS and wheat bran. Jiménez-Moreno et al. (2013a) reported AMEn values 13.53, 13.81 and 13.37 MJ/kg with the inclusion of 0, 50 and 75g/kg oat hulls, respectively, in broiler diets. Inclusion of 30g/kg sunflower hulls versus no sunflower inclusion resulted in higher AMEn values 12.90 versus 12.72 MJ/kg (Kimiaeitalab et al., 2016) and 12.18 MJ/kg versus 12.02 MJ/kg (Kimiaeitalab et al., 2018). In this study the responses of broilers and pullets to the increased fibre contents were similar.

Overall, the current findings indicate that, the laying hens ostensibly require higher dietary fibre contents to efficiently digest and utilise the nutrients and energy.

3.5.2 Apparent ileal digestibility of nitrogen and amino acids

The ileal N and AA digestibility were increased with the increased dietary fibre content in all bird types, but the responses were greater in layers than in broilers and pullets. Feeding of high fibre diets increased the digestibility of N and average AA in layers by 16.5 and 17.5%, respectively, compared to broilers (4.6 and 4.9%, respectively) and pullets (6.5 and 6.4%, respectively). These findings further demonstrate that layers need higher fibre contents in their diets for efficient protein digestion. It has been reported that layers can tolerate relatively high inclusions of fibrous feeds compared to broilers (Lumpkins and Batal, 2005; Masa'deh et al., 2012; Matterson et al., 1966; Roberson et al., 2005; Walugembe et al., 2014). However, increased N and AA digestibility responses to increased dietary fibre in all bird types may be due to the development and functioning of the upper digestive tract (Batal and Parsons, 2002), increased enzyme activity (Gabriel et al., 2008) and increased absorptive cells in the intestine (Washburn, 1991).

Regardless of the dietary fibre content, ileal digestibility of N and AA were highest in broilers, intermediate in pullets and the lowest in layers. Several factors may be responsible for the observed differences among the bird types, including age, gender and genotype. The influence of age on nutrient digestibility is well recognised (Ravindran et al., 2005; Garcia et al., 2007; Huang et al., 2007; Adedokun et al., 2008; 2009). Nutrient digestibility of poultry is compromised at the time of hatch, but increases with advancing age (Uni et al., 1995). The digestion and uptake of AA in growing broilers reported to be higher compared to older birds

(Batal and Parsons 2002; Huang et al., 2006b). Batal and Parsons (2002) reported that the total tract digestibility of lysine, arginine, threonine and cysteine in broilers increased from 0.78, 0.88, 0.69 and 0.62 at the age of 2 days to 0.89, 0.94, 0.85 and 0.81 at the age of 21 days respectively. The increased intestinal absorption of nutrients in young chickens may closely associated to their rapid growth rate (Uni et al., 1995). The rate of growth is proportional to the rate of nutrient supply to the growing tissues (Wakita et al., 1970). Therefore, the increased digestibility of AA would be associated with rapid growth rate of broiler chickens. Huang et al. (2005) reported that the digestibility of most AA in canola meal, soybean meal, and meat and bone meal in broilers were higher at both 28 and 42 days (0.81, 0.87 and 0.79, respectively) than at 14 days of age (0.80, 0.85 and 0.77, respectively). Ileal protein digestibility of canola meal, maize and mill run in broilers increased with age from 0.75, 0.77 and 0.67 at 14 days to 0.78, 0.82 and 0.73 at 42 days, respectively (Huang et al., 2005).

The influence of gender on AA digestibility has been contradictory. Wakita et al. (1970) found higher intestinal absorption of glutamic acid, methionine in male (0.338, 0.654 and 0.700) than in female chickens (0.241, 0.621 and 0.692). A study by Zuprizal et al. (1992) reported higher AA digestibility of rapeseed meal in male (0.77) than female (0.70) broilers. In contrast, Doeschate et al. (1993) observed 3% higher protein and AA digestibility in female than in male broilers.

Different genetic strains may have different digestion capacities (Kluth and Rodehutschord 2006). A study by Ravindran et al. (1999b) found the average AA digestibility in three broiler strains to be 0.858 (Strain A), 0.791 (Strain B) and 0.828 (Strain C). Al-Marzooqi et al. (2010) found overall mean AA digestibility coefficients across barley varieties were: 0.77, 0.66, 0.73 and 0.71 for the modern broiler whereas for local broilers were 0.67, 0.50, 0.66 and 0.56 respectively. Al-Marzooqi et al. (2011) reported greater AA digestibility in soybean meal for modern broilers 0.85 compared to the local broilers 0.74.

Huang et al. (2006b) reported the higher average digestibility of AA in broiler chickens by 4.8 and 6.1% than layers and roosters, respectively. These researchers also found higher AA digestibility of maize, wheat and sorghum in broilers than in layers and roosters, and the digestibility of maize and sorghum was

greater in roosters than layers. Huang et al. (2007) reported higher digestibility of most AA in maize in broilers than layers whilst, in wheat middlings, the digestibility was higher in layers than in broilers. Similar to present findings, Kimiaetalab et al. (2016) reported higher N retention in birds fed a diet containing 30 g/kg sunflower hulls- high dietary fibre than that containing no sunflower hulls- low dietary fibre (60.5 versus 58.7%). However, the N retention was higher in broilers (64.1%) compared to pullets (55.0%). The authors suggested that the pullets require less N for growth compared to broilers and therefore the lower retention of N in pullets was to be expected. It is also possible that part of the differences in apparent AA digestibility among bird types may be attributed to differences in endogenous AA and N losses (Huang et al., 2007; Ravindran and Hendriks 2004). In particular, given that bird types differ in their tolerance to handle fibre, these effects may be exacerbated in the high fibre diet.

3.5.3 Apparent ileal digestibility of minerals

With the exception of Ca and P, published data on the ileal digestibility of minerals for poultry are scant. For all minerals, there are no reports examining the influence of bird type or fibre on ileal digestibility. In the current study, the effect of bird type and fibre differed for the different minerals.

Ileal digestibility of Ca was unaffected by increasing dietary fibre content. However, there were differences among bird types, with layers showing markedly higher digestibility compared to pullets and broilers. The average Ca digestibility coefficients in broilers, pullets and layers were 0.248, 0.192 and 0.769, respectively. Interestingly, ileal P digestibility was also considerably higher in the layers. The average P digestibility coefficients in broilers, pullets and layers were 0.372, 0.321 and 0.688, respectively. The digestibility coefficients for Ca and P determined for broilers are comparable to those reported in the literature for non-phytase supplemented diets for 21-day broilers: Ca (0.321), P (0.478) (Ravindran et al., 2006), Ca (0.241), P (0.367) (Ravindran et al., 2008), P (0.384) (Truong et al., 2015) and P (0.435) (Abdollahi et al., 2016). The data also showed that the digestibility of these two minerals were similar for pullets and broilers.

The better digestion of Ca and P by layers was an expected finding because of the high metabolic needs for eggshell formation (Leeson et al., 1993; Narváez-Solarte et al., 2006; Pelicia et al., 2009; Ahmed et al., 2013). Nevertheless, the magnitude of differences observed from other bird types was unexpected. It is possible that the markedly high Ca digestibility in layers may be partly due to the low Ca content of the experimental diets (8.5g/kg) compared to the recommended requirements for layers. The published values for Ca requirements in layers include 32.5 (NRC, 1994), 40.0 (Hy-Line 2006), 36 (Jadhao and Sinha 1998), 34 (Leeson et al., 1993), 38 (Keshavarz, 2003), 34 (Rao et al., 2003; Vieira et al., 2011) and 41 g/kg (An et al., 2016). Laying hens showed increased Ca retention when dietary Ca level was reduced to 18.2 g/kg (0.746) from 39.3 g/kg (0.513) (Hurwitz and Bar, 1969). Sebastian et al. (1996) reported reduced Ca retention of 0.659, 0.490 and 0.435 with increasing dietary Ca concentrations of 6.0, 10.0 and 12.5 g/kg respectively. Plumstead et al. (2008) found decreased apparent ileal digestibility of Ca from 0.468 to 0.386 in broilers with the dietary Ca concentrations of 4.7 and 11.6 g/kg respectively. Other studies reporting improved Ca digestibility at low dietary Ca levels are; Taylor and Kirkley (1967), Hurwitz and Bar (1970), Hurwitz et al. (1970), Hamilton and Ciperá (1981) and Sugiyama et al. (2007). However, recommended Ca requirement for broiler finishers and pullets is 8.0 g/kg (NRC, 1994), which is similar to that supplied by the experimental diets in the current study. Additionally, low dietary Ca levels may increase Ca-binding proteins in the intestinal epithelial cells resulting in increased Ca digestibility (Pelicia et al., 2009) and absorption (Bar and Wasserman 1973). However, Ca digestion and absorption may vary from breed to breed as well as between strains within the same breed. Shafey et al. (1990) found different Ca retention between two strains (0.240 and 0.174) of broilers.

Phosphorus digestibility and absorption is also known to be influenced by dietary Ca and P concentration (Hurwitz and Bar 1965; Van der Klis et al., 1993). The P requirements for laying hens are estimated to be 3.2 g/kg diet (NRC, 1994). The high P digestibility in layers in the current study may therefore explained by the low Ca level in the diet. Phosphorus digestibility in layers was not influenced by increased fibre content. Phytate is a major determinant of P digestibility (Selle et al., 2000). In the current study, however, phytate P concentration of the two diets were

similar. In broilers and pullets, P digestibility was increased in the high fibre diet. Similar improvements in digestibility at the high fibre content were noted for Mg and Cu. It is tempting to speculate that the high dietary fibre through its effects on the gizzard development increased the secretion of hydrochloric acid (González-Alvarado et al., 2010), thus reducing digesta pH and increasing the solubility and absorption of minerals (Shafey et al., 1991). However, it should be noted that the gizzard weights were not recorded in the present study due to the short assay period.

The digestion patterns of the two electrolyte minerals are distinctly different, with K being well digested and Na having a negative digestibility. The negative ileal digestibility of Na has been previously reported (Ravindran et al., 2006; Ravindran et al., 2008; Naderinejad et al., 2016). This reflects secretion of large amounts of endogenous Na into the small intestine, mainly in the form of sodium bicarbonate. Another noteworthy observation in the current study was the lack of absorption of the trace minerals Cu, Mn and Zn. A study by Ravindran et al. (2006) showed the adverse influence of dietary phytate concentration on the ileal digestibility of Cu and Mn. Naderinejad et al. (2016) reported lack of influence of feed form and particle size on the coefficient of apparent ileal digestibility of minerals and also showed negative digestibility for Na, Cu, Mn and Zn. Given the biological significance of trace minerals in growth, metabolism and health, the present data emphasise the need to improve their utilisation in poultry.

The present study was designed to test the hypothesis that layers are more tolerant of fibrous diets and will digest nutrients in high fibre diets more efficiently than broilers and pullets. While acknowledging the significant interaction between fibre content and bird type for all nutrients and AME, the current findings do not support the null hypothesis and the general belief that the efficiency of nutrient digestion is greater in layers than broilers. In general, broilers showed higher nutrient digestibility and energy utilisation than layers, with pronounced differences in the low fibre diet. However, the greatest responses to increased dietary fibre content were observed in layers. It is worth noting that measured parameters, except AMEn, were lower in the low fibre diet for all bird types, possibly due to poor upper digestive tract development. Broilers exhibited no responses (DM, starch, NDF and GE) or minimal responses (fat, N and AA) compared to pullets and layers. Some studies have suggested that broilers require a minimal amount of fibre in their diet to

optimise digestive tract functionality and nutrient digestibility (Hetland et al., 2003; Amerah et al., 2009b). The inclusion of oat hulls in broiler diets up to 50g/kg increased AMEn values, but further increase to 75g/kg showed the reduced value of AMEn (Jiménez-Moreno et al., 2013a).

3.6 Conclusions

In general, the current results suggest that, bird type influenced the digestibility of nutrients in both diet types, with higher digestibility in broilers compared to pullets and layers. Bird types showed different magnitude of digestibility response to the increased dietary fibre content. Nutrient digestibility and AMEn were lower in layers fed the low fibre diet, but most of these reductions were restored in the high fibre diet. Nutrient digestibility responses to dietary fibre content were greater in layers than broilers and pullets, suggesting that the digestive tract development and function in layers is more sensitive to feed structure. The interactions observed between diet type and bird type suggest that, the information available on the dietary inclusion level of fibre sources and nutrient digestibility for one type of bird may not be appropriate for use in diet formulation for other types. Further investigations are warranted on optimum fibre contents in diets for different bird types to improve nutrient digestibility.

CHAPTER 4

Influence of bird type and maize particle size on apparent ileal nutrient digestibility and energy utilisation

4.1 Abstract

The interaction between bird type and maize particle size on the apparent ileal digestibility of nutrient and digestive organ development was examined in this study. Two bird types (broilers and layers) and three particle sizes (fine, medium and coarse) were evaluated in a 2 x 3 factorial arrangement of treatments. Titanium dioxide was used as an inert marker for the measurement of ileal nutrient digestibility. The digesta were collected from terminal ileum after feeding the experimental diets for 7 days.

Broilers consumed more ($P < 0.05$) feed than layers. Increasing maize particle size from fine to coarse resulted in higher ($P < 0.05$) feed intake in both bird types. Significant interactions ($P < 0.05$) between bird type and particle size were observed on ileal digestibility of dry matter (DM), starch, and gross energy (GE). Overall, nutrient digestibility was higher in broilers than in layers except for calcium and phosphorus. The digestibility of DM, starch and GE was higher ($P < 0.05$) in medium and coarse particles compared to fine particles, but the magnitude of increases differed between the bird types.

Neither the main effects nor their interactions were significant ($P > 0.05$) for the apparent metabolisable energy (AME), nitrogen corrected apparent metabolisable energy (AMEn) and ash. Broilers had higher ($P < 0.05$) N retention than layers. N retention was higher on medium and coarse particles than fine particles. Layers had higher ($P < 0.05$) relative gizzard weight and lower gizzard pH than broilers. The gizzard weight in medium and coarse particles was higher ($P > 0.05$) than in fine particles. The relative proventriculus weights were higher in layers.

Medium and coarse particles increased gizzard and proventriculus weights in both bird types, but the digestibility of DM, starch and GE increased only in the layers. These findings suggest that the proximal digestive organs in layers are more sensitive to particle size or feed texture compared to broilers.

4.2 Introduction

Particle size reduction of cereal grains is a common practice in poultry feed formulation. Particle size (average diameter of the individual particles) reduction involves the disruption of seed hulls and fracture of the endosperm (Parsons et al., 2006; Amerah et al., 2007a). Particle size reduction improves the handling and mixing of ingredients during the feed manufacturing process (Koch, 1996) and enhances the accessibility of digestive enzymes to nutrients.

Feed particle size has important effects on health, development and functionality of bird's gastrointestinal tract (GIT) (Nir et al., 1995). Feeding poultry with reduced particle size can regulate feed intake (FI) and increase nutrient utilisation. Feed particle size has been reported as a major factor determining FI in broilers (Amerah et al., 2009a,b) and layers (Safaa et al., 2009; Ruhnke et al., 2015). Larger feed particles are preferred by broilers and layers thus increase FI (Amerah et al., 2009b; Ruhnke et al., 2015). Feeding finely-ground particles have been reported to depress FI (Yasar, 2003), through increasing feed selection (Herrera et al., 2016) and feed wastage in layers (Safaa et al., 2009).

The importance of particle size on nutrient digestibility is increasingly recognised by the poultry industry where medium and coarse feed particles are preferred compared to fine grinding (Amerah et al., 2007a). Published data on the effects of medium and coarse particle size on dietary nutrient digestibility in poultry are contradictory, with some studies showing improved digestibility (Reece et al., 1985; Nir et al., 1994a, b; Röhe et al., 2014; Ruhnke et al., 2015) whilst others failing to show any benefits on nutrient utilisation (MacIsaac and Anderson, 2007; Safaa et al., 2009). Broilers showed greater starch digestibility (0.925) in fine particles compared to (0.854) in coarse particles (Péron et al., 2005). Broilers fed coarse particles improved the ileal digestibility of Ca 0.526 and P 0.509 than in fine particles 0.484 and 0.488 respectively (Kilburn and Edwards 2001).

The improved nutrient digestibility associated with the use of large feed particles can be attributed, to enhanced enzymatic activity and hydrochloric acid (HCl) secretion in the proventriculus, better gizzard development and function, and lower gizzard pH. Large particles are retained longer in the gizzard compared to fine particles (Nir et al., 1994b; Hetland et al., 2004), thus increasing the efficiency of

grinding and mixing of digesta with digestive enzymes (Amerah et al., 2007a; Svihus, 2011).

In contrast to broilers, only minimal attempts have been made to elucidate the impact of maize particle size on the nutrient digestibility in layers. The particle size recommendation for broilers is to have at least 20% of cereal particles greater than 1.5 to 2.0 mm (Svihus, 2011). It is not clear if layers would respond to maize particle size in similar direction and magnitude. Therefore, the present experiment was designed to investigate the interaction between bird type (broilers and layers) and particle size (fine, medium and coarse) on the apparent ileal digestibility of nutrient, apparent metabolisable energy (AME) and the development and pH of gizzard and proventriculus.

4.3 Materials and methods

The two bird types (broilers and layers) and three particle sizes (fine, medium and coarse) were tested, giving a total of six experimental treatments as shown in Table 4.1.

Table 4.1. Summary of the experimental treatments

Treatment	Bird type	Particle size	No of cage	Birds per cage	No. of replicates	Birds per replicate	Birds per treatment
A	Broilers	Fine	12	3	6	6	36
B	Broilers	Medium	12	3	6	6	36
C	Broilers	Coarse	12	3	6	6	36
D	Layers	Fine	12	3	6	6	36
E	Layers	Medium	12	3	6	6	36
F	Layers	Coarse	12	3	6	6	36

4.3.1 Dietary treatments

The diets, based on maize and soybean meal, were formulated and mixed at the same time using the same batch of ingredients for both broilers and layers (Table 4.2). Whole maize was obtained from a commercial supplier (Denver Stock Feeds, Palmerston North, New Zealand), and ground in a hammer mill (Bisley's Farm

Machinery, Auckland, New Zealand) to pass through screen sizes of 2.0, 5.0 and 8.0 mm for fine, medium and coarse grades, respectively. Diets were mixed in a single-screw paddle mixer (Bonser Engineering Co. Pty. Ltd., Merrylands, Australia). Titanium dioxide (5.0 g/kg) was included in the diet as an indigestible marker for digestibility measurements.

Table 4.2. Ingredients composition, calculated analysis and analysed values (g/kg as fed)

Item	Composition
Maize	711.8
Soybean meal	248.6
Soybean oil	0.2
Dicalcium phosphate	16.5
Limestone	10.7
Sodium chloride	2.3
Sodium bicarbonate	2.6
Vitamin premix ¹	0.8
Trace mineral premix ¹	1.5
Titanium oxide ²	5.0
Total	1,000
Calculated values	
Apparent metabolisable energy, (MJ/kg)	12.6
Crude protein	180.0
Crude fibre	23.8
Crude fat	29.8
Methionine	3.0
Methionine and cysteine	6.0
Lysine	9.2
Threonine	6.7
Tryptophan	2.3
Calcium	8.5
Total phosphorus	6.6
Non-phytate phosphorus	4.2
Sodium	1.8
Chloride	1.8
Analysed values	
Starch	459
Fat	22.9
Crude protein (nitrogen x 6.25)	169
Gross energy (MJ/kg)	16.0
Calcium	10.6
Total phosphorus	6.9

¹Supplied 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, 4mg; 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.

²Inert marker for the ileal digestibility measurements

4.3.2 Birds and housing

Experimental procedures complied with the Massey University Animal Ethics Committee guidelines (Approval number, 17/13).

Day-old male broilers (Ross 308) were obtained from a local hatchery, raised in floor pens and fed commercial broiler starter (days (d) 1 to 21) and finisher (d 22 to 35) diets. Layers (Hy-Line Brown) were obtained from a local farm. At the start of the experiment, the broilers were 35 d old (average body weight, 2.60 kg) and the layers 59-weeks old (average body weight, 1.95 kg). For each bird type, 108 birds with uniform body weights were selected and assigned to 36 cages, so the average initial weight per cage was similar. For each bird type, two adjacent cages were treated as a replicate (three birds per cage, six birds per replicate) and each diet was randomly assigned to six replicates. Birds were fasted for 12 hours before the introduction of treatment diets. The diets, with three particles sizes of maize, were offered *ad libitum* for 7 days prior to the collection of ileal digesta. Water was freely available throughout the trial period.

4.3.3 Measurements

4.3.3.1 Determination of particle size distribution

The particle size distribution of the ground maize and the diets was determined using the dry sieving method of Baker and Herrman, (2002). In brief, weighed sample (100 g) of each ground maize and diet was sieved through a set of six steel sieves (Endecotts Ltd., London, UK) with 2000, 1000, 500, 212, 125 and 63 μm sizes for 5 minutes. The dry weight of particles retained by each sieve was expressed as the proportion of initial sample weight. The geometric mean diameter (GMD) and geometric standard deviation (GSD) of the maize and diets were determined as described by Baker and Herrman, (2002).

4.3.3.2 Performance data

Body weights were recorded on the first and last days of the experimental period. Feed intake was recorded on a cage basis for the trial period.

4.3.3.3 Apparent metabolisable energy (AME) determination

Feed intake and excreta output were recorded over the last 4 days of experimental period for the determination of AME. Daily excreta collections were pooled within a replicate mixed in a blender and sub-sampled. The sub-sample was lyophilised (Model 0610, Cuddon Engineering, Blenheim, New Zealand), ground to pass through a 0.5 mm sieve and stored in airtight plastic containers at 4 °C until laboratory analysis. Excreta samples were analysed for dry matter (DM), nitrogen (N) and gross energy (GE).

4.3.3.4 Ileal digesta collection

After 7 days on the treatment diets, all birds were euthanised by intravenous injection (1 ml/2 kg live weight) of sodium pentobarbitone (Provet NZ Pty Ltd., Auckland, New Zealand) and ileal digesta was collected from the lower half of the ileum (Ravindran et al., 2005). The ileum was defined as the portion of the small intestine extending from Meckel's diverticulum to ~40 mm proximal to ileo-caecal junction. The ileum was then divided into two halves and the digesta was collected from the lower half towards the ileo-caecal junction by gentle flushing with distilled water. Digesta from birds of the same replicate were pooled together giving six samples per treatment. The digesta was immediately frozen, lyophilised (Model 0610, Cuddon Engineering, Blenheim, New Zealand), ground to pass through a 0.5-mm sieve and stored at 4 °C until laboratory analysis. The diets and digesta were analysed for DM, N, fat, starch, GE, ash, and titanium (Ti).

4.3.3.5 Gizzard and proventriculus weight and pH measurement

The proventriculus and gizzard of two birds from each replicate (with body weights closest to the mean weight of the cage) that were euthanised for ileal collection were collected as described by Singh et al. (2014). The pH of the digesta was measured with a calibrated digital pH meter. The pH was recorded by inserting the calibrated digital pH meter directly into three different parts (proximal, middle and distal) of the gizzard and proventriculus contents from each bird. Readings were recorded after stabilization of value and average of the three readings was considered as gizzard and proventriculus pH values. The gizzard and proventriculus were emptied of any digesta and the weights of both organs were expressed relative to the body weight (g/kg).

4.3.3.6 Chemical analysis

All analyses were conducted in an ISO17025 accredited laboratory (Nutrition Laboratory, Massey University).

Dry matter was determined using standard procedures (method 930.15 and 925.10; AOAC, 2005). Ash was determined by standard procedures (method 942.05; AOAC, 2005) using muffle a furnace at 550 °C for 16h. Gross energy (GE) was determined by adiabatic bomb calorimetry (Gallenkamp Autobomb, London, UK) standardised with benzoic acid. Titanium was determined on a UV spectrophotometer following the method of Short et al. (1996). Nitrogen was determined by combustion (Method 968.06 (Dumas method); AOAC, 2005) using a carbon nanosphere-200 carbon, Nitrogen and sulphur auto analyser (LECO Corporation, St. Joseph, MI). Crude protein content was calculated as N X 6.25. Starch was determined using the assay procedure (Megazyme Total Starch Assay Procedure; Megazyme International Ireland Ltd., Wicklow, Ireland) based on thermostable α -amylase and amyloglucosidase. Fat was determined using the Soxhlet extraction procedure (method 991.36; AOAC, 2003.06).

For mineral analysis, Calcium (Ca) was determined by colorimetric assay (Flexor E, Vital Scientific NV, Spankeren/Dieren, the Netherlands) following digestion with 6 M HCl to release Ca (method 968.08D; AOAC, 2005). Phosphorus (P) was determined by colorimetric analysis (UV mini 1240 Shimadzu Corp., Kyoto, Japan) at 680 nm (method 968.08D; AOAC, 2005).

4.3.3.7 Calculations

The AME values were calculated using the following formula with appropriate corrections made for differences in DM content.

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

Nitrogen-corrected AME (AMEn) was determined by adjusting to a zero N retention by simple multiplication by assuming 36.54 kJ per gram N retained in the body as described by Hill and Anderson (1958). N retention was calculated by taking the difference between N intake and N in the excreta divide by DM feed intake.

The coefficient of apparent ileal digestibility (CAID) of nutrient was calculated using the following formula:

$$\text{CAID of diet component} = \frac{[(\text{component/Ti})_{\text{diet}} - (\text{component/Ti})_{\text{ileal}}]}{(\text{component/Ti})_{\text{diet}}}$$

Where;

(component/Ti) diet = ratio of diet component to titanium in the diet, and

(component/Ti) ileal = ratio of diet component to titanium in the ileal digesta.

4.3.3.8 Statistical analysis

Replicates were considered as the experimental unit. The data was analysed by two-way ANOVA to determine the main effects (bird type and particle size) and their interaction using the General Linear Models procedure of SAS (2004) using the model below

$$Y_{ijk} = \mu + A_i + \beta_j + A_i \times B_j + e_{ijk}$$

Where:

Y_{ijk} = k^{th} observation in the i^{th} treatment group A (Bird type) and j^{th} treatment group B (particle size)

μ = a general mean

A_i = Fixed effect of i^{th} treatment group (Bird type)

B_j = Random effect of the j^{th} treatment group (Particle size)

$A_i \times B_j$ = Fixed interaction (Bird type x Particle size)

e_{ij} = random residual error

Differences between means were separated by the Least Significant Difference test (LSD) (Kaps and Lamberson, 2009) and differences were considered to be significant at $P < 0.05$.

4.4 Results

4.4.1 Particle size distribution

The GMD values of maize ground through 2.0, 5.0 and 8.0 screen sizes were determined to be 492, 700 and 912 μm with corresponding GSD values of 2.2, 2.4 and 2.4 (Table 4.3). The GMD values of the diets were 477, 642 and 818 μm with corresponding GSD values of 2.5, 2.6 and 2.4 for fine, medium and coarse grades, respectively.

Table 4.3. Particle size distribution (proportion of the retained particles on sieves)^a of ground maize and experimental diets by dry sieving

Particle size	Sieve openings (μm)							GMD ¹	GSD ²
	> 2000	1000-2000	500-1000	212-500	125-212	63-125	< 63		
Ground maize									
Fine	0.05	20.29	35.63	24.36	15.02	4.51	0.15	492	2.2
Medium	10.32	32.60	26.68	16.27	10.81	3.17	0.15	700	2.4
Coarse	25.8	33.62	18.92	9.59	8.99	2.90	0.18	912	2.4
Experimental diets									
Fine	0.03	23.08	35.65	18.35	11.92	8.01	2.95	477	2.5
Medium	9.79	31.10	28.65	14.01	7.10	6.96	2.41	642	2.6
Coarse	20.23	29.14	26.33	13.08	8.19	2.57	0.46	818	2.4

Fine, medium and coarse particle size were achieved using screen sizes of 2.0, 5.0 and 8.0 mm, respectively

^a Each value represents the mean of three replicates.

¹GMD = Geometric mean diameter

²GSD = Geometric standard deviation

4.4.2 Apparent ileal digestibility of nutrient

The effects of particle size and bird type on the apparent ileal digestibility of nutrients are shown in Table 4.4

Feed intake was influenced by bird type ($P < 0.001$) and particle size ($P < 0.05$) with the intake of the medium and coarse particles being greater ($P < 0.05$) compared to fine particles. Broilers showed greater feed intake ($P < 0.05$) than layers in all particle sizes. Feeding fine and coarse particles resulted in the lowest and highest feed consumption, with medium particles being intermediate. No bird type x particle size interaction ($P > 0.05$) was observed for feed intake.

A bird type by diet particle size interaction was observed for the digestibility of DM ($P < 0.01$), starch, GE and digesta starch content ($P < 0.001$). In broilers, digestibility of DM, starch and GE was not influenced ($P > 0.05$) by particle size, whilst in layers, increasing the particle size to medium and coarse resulted in higher digestibility of DM, starch and GE compared to fine particles. Digesta starch content was not influenced by particle size in broiler diets, but significantly decreased in layers as a result of particle size increase to medium and coarse.

The digestibility of N and fat was greater in broilers than layers ($P < 0.05$) and the Ca and P digestibility was greater in layers compared to broilers ($P < 0.05$). Diets with fine and medium particles had a similar digestibility for Ca but the Ca digestibility was lower in the diet with coarse particles ($P < 0.05$). There was no effect of bird type or particle size on the ileal digestibility of ash, AME and AMEn. Nitrogen retention was higher ($P < 0.05$) in broilers than layers and, medium and coarse grinding of the diet resulted in higher ($P < 0.05$) N retention than fine grinding.

Table 4.4. Influence of bird type and particle size on the feed intake (g/bird) and coefficient of apparent ileal digestibility (CAID) of dry matter (DM), nitrogen (N), fat, starch, gross energy (GE), calcium (Ca), phosphorus (P), ash, digesta starch content (% DM basis), nitrogen retention (% intake), apparent metabolisable energy (AME, MJ/kg DM) and nitrogen-corrected AME (AMEn; MJ/kg DM)

Bird type	Particle size	Feed intake	CAID ^a								Digesta starch ^a	N-retention ^b	AME ^b	AMEn ^b
			DM	Fat	Starch	GE	N	Ca	P	Ash				
Broilers	Fine	1092	0.728a	0.743	0.969ab	0.751a	0.824	0.417	0.505	0.444	6.1cd	71.2	15.21	14.43
	Medium	1125	0.748a	0.767	0.976a	0.767a	0.821	0.506	0.517	0.505	5.0d	72.8	15.26	14.46
	Coarse	1189	0.731a	0.719	0.975a	0.748a	0.837	0.499	0.528	0.492	4.9d	74.5	15.34	14.52
Layers	Fine	694	0.497d	0.605	0.769d	0.498d	0.559	0.805	0.696	0.515	24.2a	63.4	15.01	14.31
	Medium	701	0.578c	0.474	0.904c	0.582c	0.547	0.819	0.607	0.469	11.7b	65.0	15.23	14.52
	Coarse	751	0.651b	0.507	0.932bc	0.645b	0.622	0.903	0.670	0.561	9.9bc	65.2	15.20	14.49
SEM ^c		28.3	0.0160	0.0424	0.0136	0.0163	0.0254	0.0281	0.0320	0.0267	1.36	0.93	0.088	0.080
Main effects														
Bird type														
Broilers		1135a	0.736	0.743a	0.973	0.755	0.827a	0.474b	0.517b	0.481	5.3	72.8a	15.27	14.47
Layers		715b	0.576	0.529b	0.868	0.575	0.576b	0.842a	0.657a	0.515	15.3	64.5b	15.15	14.44
Particle size														
	Fine	892b	0.612	0.674	0.869	0.624	0.692	0.611b	0.600	0.480	15.1	67.3b	15.11	14.37
	Medium	912ab	0.663	0.620	0.940	0.674	0.684	0.662ab	0.562	0.487	8.4	68.9a	15.24	14.49
	Coarse	970a	0.691	0.613	0.954	0.697	0.729	0.701a	0.599	0.527	7.4	69.8a	15.27	14.50
Probabilities, P ≤														
Bird type		0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.122	0.001	0.001	0.096	0.636
Particle size		0.028	0.001	0.300	0.001	0.001	0.185	0.012	0.408	0.185	0.001	0.035	0.171	0.212
Bird type × Particle		0.772	0.002	0.205	0.001	0.001	0.464	0.245	0.306	0.092	0.001	0.630	0.589	0.543

Fine, medium and coarse particle sizes were achieved using sieve sizes of 2.0, 5.0 and 8.0 mm, respectively.

Means in a column not sharing a common letter (a, b, c, d) are significantly different (P < 0.05).

^a Each value represents the mean of six replicates (six birds per replicate) measured after 7 days on treatment diets.

^b Each value represents the mean of six replicates (six birds per replicate) measured over the last 4 days on treatment diets.

^c Pooled standard error of the mean

4.4.3 Gizzard weight and pH

Bird type had no effect ($P > 0.05$) on the absolute gizzard weight. Layers had greater ($P < 0.05$) gizzard weight relative to their body weight and a lower ($P < 0.05$) gizzard pH compared to broilers (Table 4.5). Medium and coarse particles resulted in similar ($P > 0.05$) gizzard weights but greater than that of birds on a diet with fine particles (Table 4.5). Particle size did not affect the gizzard pH ($P > 0.05$).

Table 4.5. Influence of bird type and particle size on absolute weight (g), relative weight (g/kg body weight) and pH of the gizzard^a

Bird type	Particle size	Absolute weight	Relative weight	pH
Broilers	Fine	27.5	8.8	3.49
	Medium	30.8	9.7	3.19
	Coarse	33.3	9.9	3.30
Layers	Fine	28.7	15.0	3.06
	Medium	32.2	17.4	2.50
	Coarse	30.8	16.4	2.48
SEM ^b		1.01	0.45	0.22
Main effects				
Bird type				
Broilers		30.5	9.4b	3.33a
Layers		30.6	16.3a	2.68b
Particle size:				
	Fine	28.1b	11.9b	3.27
	Medium	31.5a	13.5a	2.85
	Coarse	32.1a	13.2a	2.89
Probabilities, $P \leq$				
Bird type		0.974	0.001	0.001
Particle size		0.001	0.003	0.133
Bird type × Particle size		0.122	0.241	0.671

Fine, medium and coarse particle sizes were achieved using sieve sizes of 2.0, 5.0 and 8.0 mm, respectively

Means in a column not sharing a common letters (a, b) are significantly different ($P < 0.05$).

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

^b Pooled standard error of mean.

4.4.4 Proventriculus weight and pH

There was no interaction ($P > 0.05$) between bird type x particle size for proventriculus weights and pH. Broilers had a greater proventriculus weight compared to layers ($P < 0.05$) but the relative weight of the proventriculus was greater in layers than broilers ($P < 0.05$; Table 4.6). The diet containing coarse particles tended to increase ($P = 0.062$) the proventriculus weight compared to diets with fine and medium particles (Table 4.6). Particle size had no effect on proventriculus pH.

Table 4.6. Influence of bird type and particle size on absolute weight (g), relative weight (g/kg body weight) and pH of proventriculus^a

Bird type	Particle size	Absolute weight	Relative weight	pH
Broilers	Fine	7.8	2.5	3.10
	Medium	8.1	2.5	3.02
	Coarse	9.1	2.7	2.87
Layers	Fine	6.2	3.2	3.60
	Medium	5.8	3.1	3.21
	Coarse	6.6	3.5	3.23
SEM ^b		0.30	0.12	0.25
Main effects				
Bird type:				
Broilers		8.4a	2.6b	2.99
Layers		6.2b	3.3a	3.34
Particle size:				
	Fine	7.0b	2.9	3.35
	Medium	7.0b	2.8	3.11
	Coarse	7.8a	3.1	3.05
Probabilities, $P \leq$				
Bird type		0.001	0.001	0.101
Particle size		0.011	0.062	0.459
Bird type × Particle size		0.336	0.606	0.832

Fine, medium and coarse particle sizes were achieved using sieve sizes of 2.0, 5.0 and 8.0 mm, respectively

Means in a column not sharing a common letters (a, b) are significantly different ($P < 0.05$)

^a Each value represents the mean of six replicates (two proventriculus per replicate, three pH

^b Pooled standard error of mean.

4.5 Discussion

4.5.1 Particle size distribution

Maize particle size greater than 1000 μ m added in the diets were present in the coarse diet at almost three times (59.4) of the proportion found in the fine diet (20.3). However, the mixed diet for the coarse particles had two times (49.4) the proportion of the particles greater than 1000 μ m compared to fine diet (23.1). Therefore, the grinding and mixing strategies implemented in this study were able to create differences in diet coarseness as expected in the experimental design. Similarly, Naderinejad et al. (2016) found an increased proportion of particle size >1000 μ m in medium 34.54 and coarse 46.22 particles compared to fine particles 11.47, ground using sieve openings of 2, 5 and 8mm, respectively.

4.5.2 Digestive tract development

4.5.2.1 Gizzard development and pH

In the current study, while absolute gizzard weights were similar for broilers and layers, the relative gizzard weight was higher in layers than in broilers by 73%. Feeding diets with more fine particles lowered the gizzard weight compared to birds fed diets with medium and coarse particles. Several studies have shown an increased gizzard weight with increasing dietary particle size from fine to coarse particles (Nir et al., 1994a; Nir and Ptichi 2001; Dahlke et al., 2003; Charbeneau and Roberson, 2004; Péron et al., 2005; Parsons et al., 2006; Jacobs et al., 2010; Chewning et al., 2012; Jacobs and Parsons, 2013; Röhe et al. 2014; (Xu et al., 2015a,b,c). The increased gizzard weight is related to the accumulation of larger particles in the gizzard, which increase the grinding requirement of the gizzard compared to fine particles. The frequency of gizzard contractions as well as gastrointestinal reflexes improves mechanical stimulation and development of this organ (Gabriel et al., 2003; Hetland et al., 2003; Ferket and Gernat, 2006).

In the present study, layers had lower gizzard pH values than broilers. The elevated gizzard pH in broilers might be associated with higher feed intake compared to layers, thus reduced retention time of digesta in the gizzard and less HCl secretion (Svihus, 2011). Gizzard pH was not influenced by the particle size of the diets.

These results agreed with those of Nir et al. (1995) and Jacobs et al. (2010) who reported no changes in gizzard pH of broilers fed with maize particles ranging from 557 to 1,387 μ m. In contrast to the current findings, Nir et al. (1994a) reported lower gizzard pH in broilers fed a diet with a high proportion of coarse particles (2.7) compared to those fed a diets with a greater proportion of fine particles (3.5).

4.5.2.2 Proventriculus development and pH

Broilers had a heavier proventriculus weight. Interestingly, opposite to gizzard, proventriculus pH was higher in layers than broilers, an observation that is difficult to explain. Proventriculus weight was greater in birds fed coarse particles. In the study of Röhe et al. (2014), particle size of a mash diet had no effect on proventriculus weight for layers. While Benedetti et al. (2011) showed that, broilers fed fine maize particles had lower proventriculus weight compared to those fed coarse particles. The lack of proventriculus pH response to particle size in this study, is in agreement with the findings by Dahlke et al. (2003) who found no change in proventriculus pH of broilers fed a mash diet with different particle sizes.

4.5.3 Feed intake, nutrient digestibility and energy utilisation

In the current study, both the bird type and particle size influenced feed intake. Broilers consumed 59% more feed than layers. Feed intake increased on diets with medium and coarse particles compared to fine particles. In agreement with the current finding, Nir et al. (1990) reported feeding medium (4mm) and coarse (8mm) particles increased feed intake (11.4 g and 11.8 g, respectively) compared to fine 2mm particles (10.2 g) during 4 h of feeding. Safaa et al. (2009) found increased feed intake for layers fed with coarse particles (10mm) 110.6 g/d than those fed the fine particles (6mm) 107.9 g/d. According to the Lohmann management guide (Lohmann, 2014), use of coarse particles is recommended in layer diets than fine particles due to the size of their beak. Increased feed consumption in birds fed medium and coarse particles compared to fine particles herein suggests that feed intake for a certain bird type can be restricted or improved by changing particle size of the diet without changing the nutrient composition of the diet.

In the current study, broilers showed greater overall nutrient digestibility compared to layers. Broilers grow faster than layers; therefore, to meet the nutrient requirements for this fast growth, they need to have higher digestibility and greater absorption capacity (Huang et al., 2006b). Increasing the maize particle size from medium to coarse particles resulted in increased nutrient digestibility for all bird types compared to fine particles. Layers consuming coarse wheat particles (GMD, 0.96mm) had a greater ileal starch digestibility compared to layers on a diet with fine particles (Ruhnke et al., 2015). The higher nutrient digestibility herein for the birds consuming coarse particles may be associated with the development of the gizzard and proventriculus. Feeding diets with more coarse particles to layers resulted in a heavier gizzard that improved nutrient digestibility (Gabriel et al., 2003; Williams et al., 2008). A well-developed gizzard will increase grinding activity (Svihus et al., 2010) resulting in increased accessibility of nutrients to digestive enzymes (Palander et al., 2010) and, also provide more time for the secretion of HCl, increase reverse peristalsis that serve to re-expose the digesta to digestive enzymes, all these in turn improving nutrient digestibility (Nir et al., 1995; González-Alvarado et al., 2008; Jacobs et al., 2010; Svihus, 2011; Röhe et al., 2014).

The response of ileal digestibility of DM, starch and GE to increased dietary particle size differed between the bird types. The increases were more pronounced in the layers compared to broilers. The improvements with increased dietary particle size in the ileal digestibility of DM, starch and GE in layers was 31, 21 and 30%, whilst no increases were observed in broilers. The marked digestibility responses to the increased particle size in layers resulted in the interaction between bird type and particle size for ileal digestibility of these nutrients. The increased relative gizzard weight in layers in the current study might suggest the increased digestibility of nutrient in layers. Layers digestive organ may have greater nutrient digestion capability to the increased particle size compared to broilers. The digesta starch content showed interaction between the bird type and particle size due to different magnitude of digestibility responses between layers and broilers. Feeding the diets with more fine particles had a greater digesta starch response compared to medium and coarse particles.

Broilers showed higher N digestibility than layers that may reflect an age effect, as recognised in some studies (Garcia et al., 2007; Adedokun et al., 2009).

The markedly higher digestibility of Ca and P by layers was an expected result because of the high metabolic needs for these minerals for eggshell formation (Taylor and Kirkley, 1967; Hurwitz et al., 1973; Roland et al., 1996; William et al., 2006; Ahmed et al., 2013). However, it is possible that, the markedly high Ca digestibility in layers may be due to the low Ca content of the experimental diets (8.5 g/kg) compared to the recommended requirements for layers. The study by Hurwitz and Bar (1969) reported greater Ca absorption 0.773 for the layers fed low dietary Ca level 19.0 g/kg compared to 0.683 for the high dietary Ca level 35.6g/kg. The published Ca requirements for layers include 32.5 (NRC, 1994), 40.0 (Hy-Line (2006), 34.0 g/kg (Rao et al., 2003; Vieira et al., 2011) and 41.0 g/kg (An et al., 2016). It is also likely that the higher relative gizzard weight and reduced gizzard pH may also have increased Ca digestibility in layers compared to broilers.

The greater Ca digestibility with feeding medium and coarse particles in the current study might be explained by the increased gizzard and proventriculus weights. Medium and coarse particles increase the digesta retention time in the gizzard, improve digestive tract development and functioning (Amerah et al., 2007a; González-Alvarado et al., 2007), increase HCl secretion and reduce the digesta pH, which increases mineral solubility (Guinotte et al., 1995).

The bird type and particle size influenced the N retention with greater retention in broilers than in layers. The greater N retention might be associated with greater N digestibility in broilers compared to layers or due to higher N requirements in broilers compared to layers (Kimiaetalab et al., 2016). Nitrogen retention was greater when the diet contained medium and coarse particles compared to fine particles. This might be due to the increased retention time of digesta in the GIT that provides more time for digestive enzymes to act on the substrate. The study by Parsons et al. (2006) showed 4.75% higher N retention in broilers fed a diet with a greater proportion of coarse particles compared to fine particles.

Lack of significant effect on feeding different particle sizes for AME and AMEn, corresponds with the findings by Kasim and Edwards (2000), Svihus et al. (2004), Amerah et al. (2007b; 2008a), Jacobs et al. (2010) and Naderinejad et al. (2015; 2016) . Likewise, Favero et al. (2012) showed no effect of particle size on AME and AMEn values for turkey. In contrast, Kilburn and Edwards (2001)

observed greater AME values in fine particles of maize 14.10 MJ/kg compared with coarse particles 13.90 MJ/kg.

4.6 Conclusions

The current study was designed to test the hypothesis that there will be interactions between diet type and particle size on nutrient digestibility and layers will digest nutrients more efficiently with increased dietary particle size than broilers. The significant interaction between bird type and particle size for DM, starch and GE partly supported the null hypothesis. It is noteworthy that although feeding medium and coarse particles in the current study was associated with increased gizzard and proventriculus weights in both layers and broilers, only the layers benefited from this effect in terms of DM, starch and GE digestibility, suggesting that digestive system of layers is more sensitive to particle size or feed texture compared to broilers. Moreover, the relatively high digestibility of DM, starch and GE in broilers fed fine particles might have not left any room for digestibility improvements by feeding medium and coarse particles.

The current study demonstrated that broilers had a greater digestibility of dietary nutrients than layers, except Ca and P. Calcium digestibility benefited from medium and coarse grindings in both broilers and layers. Particle size had no effect on the digestibility of nutrients in broilers, but digestibility of DM, starch and GE in layers improved by medium and coarse grinding. These findings suggest that layers can benefit more from coarsely-ground particles. Medium and coarse grinding in mash diets would be preferable because of the enhanced feed intake and nutrient digestibility.

CHAPTER 5

General discussion

5.1 Introduction

Poultry diets are currently formulated using digestible nutrients of feed ingredients, so that the nutrient requirements of birds are precisely met, improving feed utilisation and reducing wastage of undigested nutrients into the environment (Lemme et al., 2004). It is generally assumed that nutrient digestibilities are similar across all bird types, and therefore, broilers nutrient digestibility data are used in diet formulation for other bird types, including layers. Most AA digestibility studies have been done in broilers and limited findings are available comparing digestibility of nutrients in different bird types. Huang et al. (2006; 2007) suggested that bird type influenced the ileal digestibility of AA in different feed ingredients. Factors such as assay methodology (Ravindran et al., 1999b), environment (Gonzalez-Esquerra and Leeson, 2007), sex of birds, (Huang et al., 2006), age, (Huang et al., 2007), genotype (Rideau et al., 2014), dietary fibre content and particle size of the diet can also have an impact on nutrient digestibility.

Inclusion of insoluble fibre (Mateos et al., 2012; Abdallah et al., 2015) and feed ingredients particle size (Amerah et al., 2008a, b; Jacobs et al., 2010) in poultry diets have been found to affect nutrient digestibility. Increased digestibility due to dietary fibre and larger particle size may be associated with increased gizzard development and functionality (Svihus, 2011) but has been noted to be dependent on the type and level of fibre added in the poultry diet (Hetland et al, 2005; Amerah et al., 2007) and feed particle sizes (Gabriel et al., 2003; Amerah et al., 2008b). Birds fed diets with moderate amount of fibre and medium to coarse feed particles observed to increase gizzard grinding activity, hydrochloric acid secretion (HCl), lower digesta pH, and increase digestive enzymes activity, hence improving the nutrient digestibility (Hetland et al, 2005; Mateos et al., 2012). However, responses in terms of nutrient digestibility and gastrointestinal tract (GIT) development due to the increase of dietary fibre (DF) content (Kimiaetalab et al., 2016; 2018) and particle size (Péron et al., 2005) can vary in different bird types.

Several published data on nutrient digestibility in poultry diets using different fibre sources are available; however, studies comparing the effect of fibre inclusion on nutrient digestibility for different bird types are limited. Therefore, the focus of this thesis was to investigate the influence of bird type and dietary fibre content as well as feed particle size on apparent ileal digestibility of nutrients and energy utilisation. In this study two fibre levels (Chapter 3; 10.3 and 19.3 g/kg neutral detergent fibre [NDF]) and three particle sizes (Chapter 4; 2.0, 5.0 and 8.0 mm) were used to evaluate nutrient digestibility among different bird types. It was hypothesised at the start of this study that an interaction might exist between diets and bird types on the apparent ileal digestibility of nutrients, with layers expected to have greater nutrient digestibility than broilers.

5.2 Effect of dietary fibre concentration on nutrient digestibility

The influence of bird type and dietary fibre content on the apparent ileal digestibility of nutrients and energy utilisation was examined in the first study (Chapter 3). As expected, a significant interaction between bird type and diet type was found for all nutrients. The current study also failed to support the general belief that the efficiency of dietary nutrients digestion is greater in layers than broilers. The overall nutrient digestibility was higher in broilers followed by pullets and lowest in layers. Nutrient digestibility was greater in the three bird types on a high fibre diet than in low fibre diets but layers had greater digestibility in response to increased dietary fibre, compared to broilers and pullets. Therefore, the digestibility of nutrients seems to be more sensitive to dietary fibre in layers and may have a greater capability for digestion of dietary fibre compared to broilers and pullets.

The reason for the different magnitude of digestibility responses in different birds could be associated with different ages of bird used. The longer retention time of dietary fibre in the GIT of older birds compared to young birds is documented (Shires et al., 1987; González-Alvarado et al., 2010). Layers were more mature than broilers and pullets. Therefore, the increased retention time in the GIT of layers could have increased exposure of dietary nutrients to the digestive enzymes thus improve nutrient digestibility. Another reason could be attributed to the development of the digestive organs, especially gizzard.

5.3 Apparent ileal digestibility of amino acids

The chapter 3 findings showed that both bird type and diet type significantly influenced the ileal digestibility of AA. Regardless of the dietary fibre content, ileal digestibility of nitrogen (N) and AA was higher in broilers, intermediate in pullets and the lowest in layers. The digestibility variations may be associated with factors such as age (Batal and Parsons, 2002), gender (Doeschate et al., 1993), genotype (Ravindran et al., 1999a) and endogenous losses (Ravindran and Hendriks, 2004). The ileal N and AA digestibility were increased with increased dietary fibre content in all bird types, but the responses were greater in layers than broilers and pullets, resulting in a significant bird type x diet type interaction, except for aspartic acid and lysine. This finding suggests that layers need high fibre sources in their diets for efficient protein digestibility.

5.4 Apparent ileal digestibility of minerals

To our knowledge, no research to-date has examined the influence of bird type or diets with different fibre inclusion on ileal digestibility of minerals. The results presented in Chapter 3 showed that the effect of bird type and fibre content differed for the different minerals. Whilst the Ca digestibility was unaffected, P digestibility increased because of an increase in dietary fibre content. The Ca and P digestibilities were markedly higher in layers than pullets and broilers. The increased digestibility of Ca and P in layers was expected due to the importance of these minerals on eggshell formation for the laying hens. It can also be explained by the fact that Ca content of the experimental diets was lower compared to the recommended requirements for layers. Calcium digestibility has been shown to be higher in layers diets with low Ca level compared to those diets with high Ca levels (Sugiyama et al., 2007; Plumstead et al., 2008). Phosphorus digestibility depends on the dietary Ca:P ratio, therefore the increased P digestibility in the current study in layers may be explained by the low dietary Ca level. Higher Mg and Cu digestibility in high fibre diets may be due to the reduced digesta pH in high fibre diets promoting minerals solubility and absorption (Van der Klis et al., 1990).

The current findings showed greater nutrient digestibility responses in layers to increased dietary fibre content, which may suggest higher sensitivity of layers

digestive organs to the feed texture. However, the information on the optimal amount of fibre sources to be added in diets of different birds is scant and further research is warranted.

5.5 The effect of maize particle size on digestive tract development

Layers showed greater gizzard weight relative to body weight and lower gizzard pH compared to broilers. These findings could suggest that, layers gizzard development is more sensitive to feed texture or structure. A number of studies have shown that, medium and coarse dietary particle size can increase gizzard weight compared to fine particles (Péron et al., 2005; Amerah and Ravindran, 2009; Röhe et al., 2014).

The findings reported in Chapter 4 similarly showed that, medium and coarse particles increased the absolute and relative gizzard weight in all bird type. These results confirmed previous findings that larger feed particles accumulate longer in the gizzard and increase the grinding activity and size of the gizzard (Hetland et al., 2003). The increased gizzard weight to the increased particles size due to medium and coarse particles could have contributed the increased DM, starch and GE digestibility but the pronounced effect was observed only in layers. These findings may suggest that the layers digestive organs have better nutrient digestion capability due to medium and coarse particles compared to broilers.

All bird types showed an increased proventriculus weight. The proventriculus absolute weight was only greater in the birds consumed coarse particles. However, no particle size effect on proventriculus pH was observed.

5.6 The effect of particle size on apparent ileal nutrient digestibility

The interaction between bird type and feed particle size has not been previously investigated. The findings in this thesis showed inconsistent effects of feed particle size on nutrient digestibility between broilers and layers. In overall, broilers showed greater digestibility of all nutrients except for Ca and P compared to layers. Broilers digested and absorbed N and fat to a greater extent than layers. This might be due to high dietary nutrient requirement for the fast growth rate of broilers to meet their

body requirements. As expected and similar to the results from Chapter 3, layers showed higher Ca and P digestibility compared to broilers. The higher requirements of Ca for eggshell formation that promotes more efficient use of dietary Ca might explain this finding (Pelicia et al., 2009).

Feeding medium and coarse particles increased the digestibility of DM, starch and GE compared to the fine particles, a finding that has been reported in number of studies (Nir et al., 1994a, b; Hetland and Svihus, 2001; Engberg et al., 2002; Ruhnke et al., 2015). The particle size had no effect on P digestibility whilst Ca showed increased digestibility due to medium and coarse particles compared to fine particles. Noteworthy, the increased digestibility may be explained by an increased retention time of digesta contents in the digestive tract allowing more time for nutrients digestibility and absorption induced by feeding medium and coarse particles. These findings demonstrate that, the feed particle size must be considered when formulating the diet for different type of birds.

5.7 Summary and main conclusions

The major finding of this research was that the bird type influenced the digestibility of nutrients regardless of diet type, with higher digestibility in broilers compared to pullets and layers. Bird types showed different magnitude of digestibility responses to increased dietary fibre content. Nutrient digestibility responses to dietary fibre content were greater in layers than in broilers and pullets. Although feeding medium and coarse particles was associated with increased gizzard and proventriculus weights in both layers and broilers, only the layers benefited in terms of increase nutrient digestibility. Overall, these findings demonstrate that the digestive tract development and function in layers is more sensitive to feed structure. The implication is that feeding diets low in fibre and finely ground diets to layers should be avoided to achieve optimum digestion. The interactions observed between diet type or particle size and bird type suggest that the information available on the dietary inclusion level of fibre sources and nutrient digestibility for one type of bird may not be appropriate for use in diet formulation for the other type of birds. However, further research is required to determine the dietary fibre level and particle sizes to be considered in diets for different bird types.

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