Harper Adams University

UNDERSTANDING AND IMPROVING THE FEEDING VALUE OF WHEAT FOR BROILERS

MUHAMMAD RIZWAN AZHAR

BSc (Hons) Animal Husbandry (University of Agriculture Faisalabad, Pakistan)

MSc Animal Nutrition (University of Plymouth, UK)

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ABSTRACT

Wheat is the main cereal used in broiler feed formulations in North West Europe, the Americas and Australasia. The nutritional value of wheat and variation in feeding quality have significant commercial importance, as it influences efficiency and rate of broiler chicken growth. This thesis deals with the investigation of variation in nutrient composition of currently available wheat samples for commercial broiler diets in the UK, the relationship between wheat characteristics, AME and growth performance of broilers, and the use of exogenous xylanase on the bioavailability of nutrients and AME of wheat.

The first study revealed variation in CP, ash and soluble NSP contents of wheat samples. There were differences ($P < 0.05$) in AME and N-corrected AME (AMEn) of wheat. The AME of wheat had a maximum range of 1.13 MJ/kg DM between wheat samples. The main energy yielding nutrients (starch and protein) of wheat did not relate with the large variation in AME, moreover, there was no relationship between specific weight and AME. There were differences (13 – 14%; $P < 0.05$) in daily feed intake and weight gain of broilers fed different wheat samples, however, differences were not associated with chemical composition and physical characteristics of wheat. The study also indicated no relationship between AME of wheat and growth performance of broilers. The second study indicated that growing site of wheat crop affected ($P < 0.05$) the nutrient composition, AME and growth performance, whereas, wheat variety had no influence. The variations in AME were due to differences in polysaccharides composition of wheat between growing sites. The xylanase response to improve AME was related to NSP content (the level of arabinoxylans) of wheat. The findings suggested that the supplementation of xylanase to wheat-based diets should be carefully considered if wheat samples consist of varieties sourced from different growing locations. The third study revealed that the *in vivo* rate of starch digestion differed ($P < 0.05$) by 25% between two wheat samples with similar proximate nutrients and starch content. Previously, both wheat samples resulted in large differences (11 – 12%; $P < 0.05$) in growth performance of broilers. The study proposed that differences in feed intake and growth rate of broilers may be influenced by the *in vivo* rate of starch digestion and should be investigated using a large set of wheat samples.

This project proposed that the future selections of wheat samples for broiler feeds are based on the actual analysis of wheat instead of predicted values. Conventional analytical tests of determining the nutrient composition are unable to predict the AME of wheat. Future research is warranted on factors affecting the growing conditions of wheat crop and the possible influence of growing condition on the nutritive value of wheat for broiler chickens.

DECLARATION

I declare that this thesis has been entirely composed by the author and is a record of work undertaken by the author on an original line of research. All help given by others is acknowledged and no part of this thesis or whole thesis has been presented in any previous application for a degree. It contains work that has been published by the author in peer reviewed journals and conferences.

Rizwan Azhar

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PUBLICATIONS

Chapter 2 and 3

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AZHAR, M. R., S. P. ROSE, M. R. BEDFORD, V. R. PIRGOZLIEV, and A. M. Mackenzie. 2018. "Metabolisable energy of wheat for broilers." In *Proceedings of International Poultry Scientific Forum, Atlanta, USA, 29-20 January 2018*. p. 15. Abstract no. M46.

AZHAR, M. R., S. C. MANSBRIDGE, S. P. ROSE, M. R. BEDFORD, and V. R. PIRGOZLIEV. 2018. "Wheat cultivar choice modulates broiler chicken jejunal gene expression and wheat feeding value." In *Proceedings of International Poultry Scientific Forum, Atlanta, USA, 29-20 January 2018*. p. 97. Abstract no. p334.

AZHAR, M. R., S. P. ROSE, M. R. BEDFORD, V. R. PIRGOZLIEV, and A. M. MACKENZIE. 2017. "Variability of metabolisable energy in different wheat cultivars for broilers." *British Poultry Science Abstracts* 13:1. p.15. doi:10.1080/17466202.2017.1394641.

AZHAR, M. R., S. P. ROSE, M. BEDFORD, A. M. MACKENZIE, and V. PIRGOZLIEV. 2017. "Does the wheat cultivar or growing site affect broiler growth performance?" In *Proceedings of 21st European Symposium on Poultry Nutrition, Salou, Spain, 8-11 May 2017*. p.162. doi.org/10.3920/978-90-8686-851-3.

Chapter 4

AZHAR, R., S. P. ROSE, S. C. MANSBRIDGE, M. R. BEDFORD, and V. R. PIRGOZLIEV. 2018. "Growing site influences wheat apparent metabolisable energy value for broiler chickens". *British Poultry Abstracts* 14:1, 1-35, p. 20. doi:10.1080/17466202.2018.1553427.

Chapter 5

AZHAR, R., S. P. ROSE, S. C. MANSBRIDGE, M. BEDFORD, and V. R. PIRGOZLIEV. 2019. "The rate of starch digestion in broilers fed wheat-based diets." In *Proceedings of World's Poultry Science Association (UK Branch), Edinburgh, UK, 10-11 April 2019*. p. 11.

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NEALE, H. 2018. "The Feeding Value of Three Hardness Characterised Wheat Cultivars, with or without Exogenous Xylanase Enzyme Treatment for Broiler Chickens.": being an *honours research project submitted in partial fulfilment of the requirements for the BSc (Honours) Degree in Agriculture with Animal Science*. Newport: Harper Adams University.

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LIST OF ABBREVIATIONS

AA	Amino acid
AAD	Amino acid digestibility
AHDB	Agriculture and Horticulture Development Board
AID	Apparent ileal digestibility coefficients
AME	Apparent metabolisable energy
AMEn	Nitrogen-corrected apparent metabolisable energy
ANOVA	Analysis of variance
BW	Body weight
Ca	Calcium
CP	Crude protein
cP	Centipoise
CV	Coefficient of variation
Cu	Copper
DAA	Dispensable amino acid
DM	Dry matter
DMD	Coefficient of dry matter digestibility
DMR	Coefficient of dry matter retention
DE	Digestible energy
df	Degree of freedom
DJ	Distal jejunum
DV	Dynamic water extract viscosity
Dig. vis	Ileal digesta viscosity
DI	Distal Ileum
EE	Ether extract

EH	Endosperm hardness
FBW	Final body weight
FD	Coefficient of total tract fat digestibility
FI	Daily feed intake
FCE	Feed conversion efficiency
FCR	Feed conversion ratio
GE	Gross energy
HFN	Hagberg falling number
IAA	Indispensable amino acid
Insol NSP	Insoluble non-starch polysaccharides
Kg	Kilograms
KV	Kinematic water extract viscosity
ME	Metabolisable energy
MRT	Mean retention time
N	Nitrogen
ND	Coefficient of ileal nitrogen digestibility
NE	Net energy
NR	Coefficient of total tract nitrogen retention
NSP	Non-starch polysaccharides
P	Phosphorus
PDI	Pellet durability index
PJ	Proximal jejunum
PI	Proximal ileum
SBM	Soybean meal
SD	Standard deviation

SE	Standard error
SEM	Standard error of means
SED	Standard error of difference
Sol NSP	Soluble non-starch polysaccharides
SW	Specific weight
TAA	Total amino acid
TGW	Thousand grain weight
TIO_2	Titanium dioxide
TME	True metabolisable energy
WG	Daily weight gain
WPI	Wheat protein isolate
Zn	Zinc

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CHAPTER 1: LITERATURE REVIEW

GENERAL INTRODUCTION

Wheat is the second largest crop in the world after rice. It is grown on around 218 million hectares with an annual production of about 759.7 Mt (million tonnes) in 2017/18 (FAO 2018). The largest producers of wheat in the world are the European Union (EU), China, India, Russia, the United States of America, Canada, Pakistan Ukraine, Argentina and Turkey, and accounted for 83.2% of total wheat produced in the world (USDA 2019) (Figure 1.1). In the EU, France, Germany, the United Kingdom (UK) and Poland are the leading producers of wheat and accounted for 60% of total wheat production in the EU states (Eurostat 2018a). In the UK, wheat is the largest arable crop by area, and it is widely grown in the country but centred towards eastern part of England with East Anglia, south-east and East Midland regions. The annual wheat production in 2017/18 was recorded at 14.84 Mt whereas, consumption of wheat in animal feed was estimated at 7.51 Mt (AHDB 2019). In the UK, wheat is the main cereal used in animal feeds followed by barley, maize and oats (AHDB 2019). In the UK, since 2014, the consumption of wheat in animal feeds is increasing, and the major consumers for wheat in the animal industry are poultry, followed by pig, cattle and sheep.

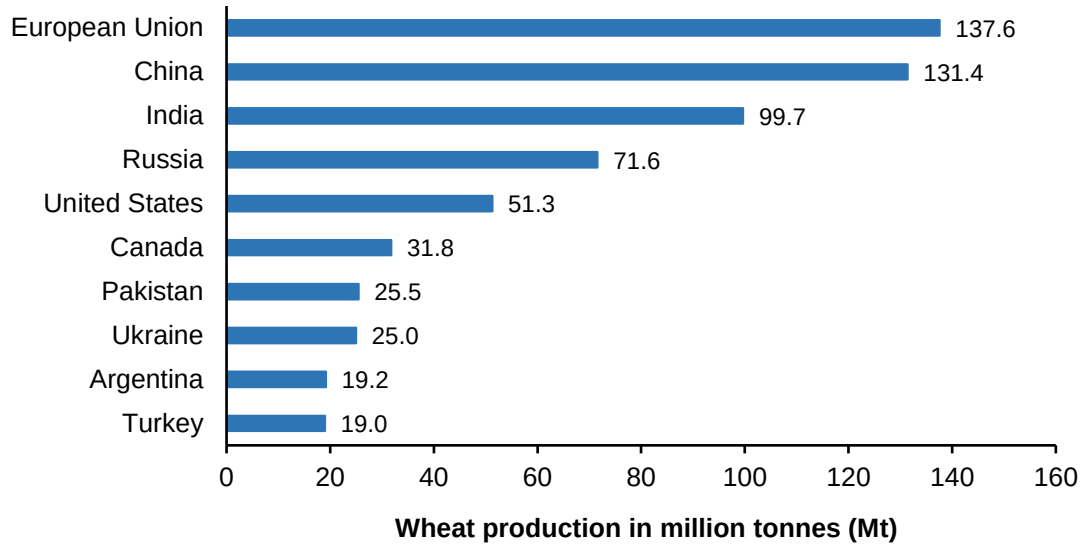


Figure 1. 1. The leading wheat producers worldwide in 2018/19.
(Source: USDA 2019)

The main goal of efficient broiler production is to achieve good growth performance with high feed efficiency. Wheat, specifically grown for feed and also that in excess of milling processes (bread, biscuits and cakes), is the main cereal used in commercial broiler diet

formulations in the UK, northern Europe, Canada and Australia (McNab 1996; Scott et al. 1998a; Steenfeldt 2001; Wiseman 2000, Kim et al. 2003). High grain yield and low cost available energy (price/MJ of metabolisable energy) make wheat one of the most economically competitive cereals in poultry feeds, accounting for up to 70% of the metabolisable energy (ME) and 35% of the protein requirements of commercial broilers (Gutierrez del Alamo et al. 2008b). There is, however, a considerable variation in ME content of wheat, with ranges between 8.50 – 15.90 MJ/kg DM (Mollah et al. 1983; Wiseman 2000; McCracken et al. 2002; Pirgozliev et al. 2003). This large variation in ME content of wheat makes it challenging for nutritionists to predict the feeding value of wheat for broilers.

The majority of the research on UK wheat samples and their effect on apparent metabolisable energy (AME) and broiler growth performance was conducted 15 – 20 years ago (Waldron 1997; McCracken and Quintin 2000; Wiseman 2000; Rose et al. 2001; Pirgozliev et al. 2003). These past studies have not demonstrated conclusively on how the chemical composition and physical characteristics of wheat are related to AME and growth performance of broilers; however, these studies indicated that there were differences in AME of wheat (Waldron 1997; Scott et al. 1998a; Steenfeldt 2001). Starch is the main energy yielding component of wheat, accounting over 50% of energy intake (Svihus 2011), but inconsistent relationships between starch and AME value of wheat have been reported (McCracken et al. 2002; Svihus and Gullord 2002). In contrary, the digestibility of starch was closely associated with AME of wheat rather than total starch content (Wiseman et al. 2000; Carré et al. 2005).

Broiler growth response to wheat-based diets in many previous studies have also been due to the differences in protein content of wheat samples (Steenfeldt 2001; Pirgozliev et al. 2003; Hetland et al. 2004). The protein content of the wheat is variable and ranging between 8 – 17% depending on variety and the growing condition (Scott et al. 1998a; Wiseman 2000; McCracken et al. 2002). Therefore, there is a need to consider the factors other than protein content such as starch, non-starch polysaccharides (NSP) and investigate their relationship with broiler growth performance and AME. Current wheat cultivars have undergone numerous changes in chemical composition, quality and yield due to plant breeding. Feed wheat varieties are specifically grown for animal feeds. High yield wheat varieties with better resistance to diseases have been produced (AHDB 2015). Wheat genotype, soil composition, seasonal changes, crop husbandry and agronomic factors have changed UK wheat significantly in the last two decades (AHDB 2015). Wheat varieties with low arabinoxylans content are now available, which have the benefit of conferring a low ileal digesta viscosity for broilers resulting in improvement in growth performance of broilers (Choct and Annison 1992a, b; Pirgozliev et al. 2015). The changes in the current UK wheat

cultivars stresses updates on the nutrient availability of wheat for broilers and how these changes can affect broiler response.

The differences in nutritional value of wheat may affect the growth performance of broilers but there is a need to understand that differences in wheat cultivars that are not limited by the number of wheat samples used in a study. Currently, available information on wheat-based studies for broilers are limited to sample size, variables studied, and the geographic distribution of wheat. Wheat-based studies in broilers were either restricted to a small number of wheat samples, or samples were collected over years and certain geographically distribution does not represent a conclusive data. Gutierrez del Alamo et al. (2008a) found that variations in wheat cultivars affected the broiler performance (e.g., feed intake, body weight) based on a study using four different wheat samples in broilers diets. Wiseman (2000) reported that variation in physical characteristics of wheat, e.g., specific weight (SW) and thousand grain weight (TGW) were not related to variation in AME using ten wheat varieties grown at specific sites. Rose et al. (2001) found that physical characteristics (endosperm hardness (EH) and water extract viscosity) of six wheat cultivars influenced the feed conversion ratio (FCR) of broilers. Pirgozliev et al. (2003) reported that differences in broiler performance were related to the variation in EH and ash content of wheat samples (23) grown over three years. McCracken and Quintin (2000) studied the effect of six wheat cultivars with different SW on growth performance of broilers and revealed that difference in growth performance was due to variation in chemical composition of wheat (level of NSP). Differences during the growth of wheat crop can affect the nutritive value of the crop. Various growing condition (e.g., growing site, climate, husbandry techniques) could result in differences in the harvested crop which can significantly affect the AME and broiler growth performance (Waldron 1997; Choct and Hughes 1999). The sample size, limited variable studied, and selection of sample over certain region could not provide conclusive evidence on nutritional composition of wheat and their effect of growth performance of broilers. There is a need to understand the causes of variation in nutritive value of wheat by using a large data set of wheat samples and investigate the effect of both chemical and physical characteristics of wheat on AME and growth performance.

The commercial broiler industry relies heavily on wheat being the main cereal grain in broiler feed formulations in the UK and many countries, therefore, it is pertinent to characterise the currently available wheat samples for broiler feeds. The broiler feed industry requires a study to define a range of representative current UK wheat samples and investigate how the differences in chemical composition and physical characteristics of wheat samples are related to AME and growth performance of broilers. The information on these wheat samples can be incorporated into the commercial feed manufacturing industry standards to

re-evaluate the feeding value of wheat and therefore, requires adjusting broiler diet formulations.

The specific objectives of this project were:

- To evaluate the chemical composition and physical characteristics of current grown wheat in the UK.
- To investigate the differences in AME value of wheat and growth performance of broilers.
- To examine if differences were related to chemical composition, physical characteristics and nutrient utilisation of wheat.
- To explore the use of exogenous xylanase on nutrient availability of wheat and AME.
- To improve the feeding value of the UK wheat through enzyme technologies.

1.1. WHEAT CHEMICAL COMPOSITION

The nutritive value of a feedstuff is influenced by its chemical composition, and depends on how birds are able to digest, adsorb and utilise these nutrients of specific feedstuff (Wiseman and Inborr 1990). Nutritional value of wheat samples varies according to their nutrient contents and digestibility of these nutrients (Ravindran and Amerah 2009). Variations in nutrient contents may be linked with cultivars, growing season and genetic origin of the cultivars (Gutierrez-del-Alamo et al. 2008b). The chemical composition of wheat varies depending upon growing location, use of fertilisers, moisture content and other agronomic factors (Ravindran and Amerah 2009). According to NRC (1994), on as-fed basis, wheat grain contains 870 g/kg DM, 140 g/kg CP, 25 g/kg crude oil (as ether extract) and 30 g/kg crude fibre.

1.1.1. Wheat grain structure

The wheat grain (known as wheat kernel) can be divided into three major parts, bran layer, endosperm and germ (or embryo). A mature wheat seed consists of about 13% bran layer (5% pericarp, 8% aleurone layer), 85% endosperm, and 2% embryo (also called germ) (Pomeranz 1988). Figures 1.2 and 1.3 present the structure of a wheat grain and its cross section layers, respectively.

1.1.1.1. Bran

The bran consists of pericarp, testa (seed coat), nucellar cells and aleurone layers (Figure 1.2). The pericarp and testa are the outside layers. Botanically there is clear distinction between pericarp and testa, but they are physically united with the innermost tissues of pericarp and firmly attached with the cuticle on the outer surface of testa (Evers and Bechtel 1988; Bechtel et al. 1990). The bran is formed from the fusion of pericarp and testa. The bran is composed of cellulose, lignin and non-starch polysaccharides (NSP), vitamin B complex and minerals. The pericarp mainly consists of cellulose and other polysaccharides (e.g., hemicelluloses and pentosans) (Lasztity 1999). The most distinctive property of the seed coat is its permeability to water and gases. The seed coat creates a barrier between the embryo and its immediate environment. Germination cannot occur as long as water entry is blocked in embryo (Pomeranz 1988).

The inside layer of the bran is called the aleurone layer. During seed maturation, the peripheral cells of endosperm multiply to form small rectangular cells in one or two layers. The walls of these cells become thickened and produce protein bodies and the layers of cell form the aleurone layer. The aleurone cells in wheat grain are generally a one cell thick

layer and form the outermost layer of the endosperm tissues and surround the starchy endosperm and part of the embryo except that which is attached with scutellum (Evers and Bechtel 1988). Although, aleurone is anatomically part of the endosperm, millers consider it as innermost layer of the bran. The aleurone layer surrounding the endosperm are typically block shaped when viewed in cross or longitudinal section (Figure 1.3).

Aleurone layers contains oil droplets as storage reserve. The aleurone cell wall is mainly composed of arabinoxylans (AX), β-glucans and proteins. The majority of the mineral content of grain is located in aleurone and it contains almost one third of the thiamine content of grain (Grundas 2003). The aleurone layer contains less dietary fibre than the pericarp or the testa. The cells of aleurone layer are rich in protein, oil, vitamins and minerals (Kent and Evers 1994). In comparison to other parts of the wheat grain, aleurone is high in protein, ash, fat, phytate phosphorus and vitamins (especially niacin). The thiamine and riboflavin contents are higher in aleurone layer than in other bran layers (Somers et al. 1945; Pollock and Geddes 1951). The niacin represents about 80% of total amount in wheat grain. The protein bodies are associated with a chemical called phytate (also called phytin). Phytate, a potassium, magnesium and calcium salt of *myo-inositol* hexaphosphoric acid, is the storage form of phosphorus. It acts as a major store of phosphate and macronutrient mineral elements within the cereal grain. The aleurone layer plays an important role in the germination of grain seeds because it secretes the α-amylase, which breaks down the endosperm starch into glucose to supply energy to the developing embryo (Stevens et al. 1988).

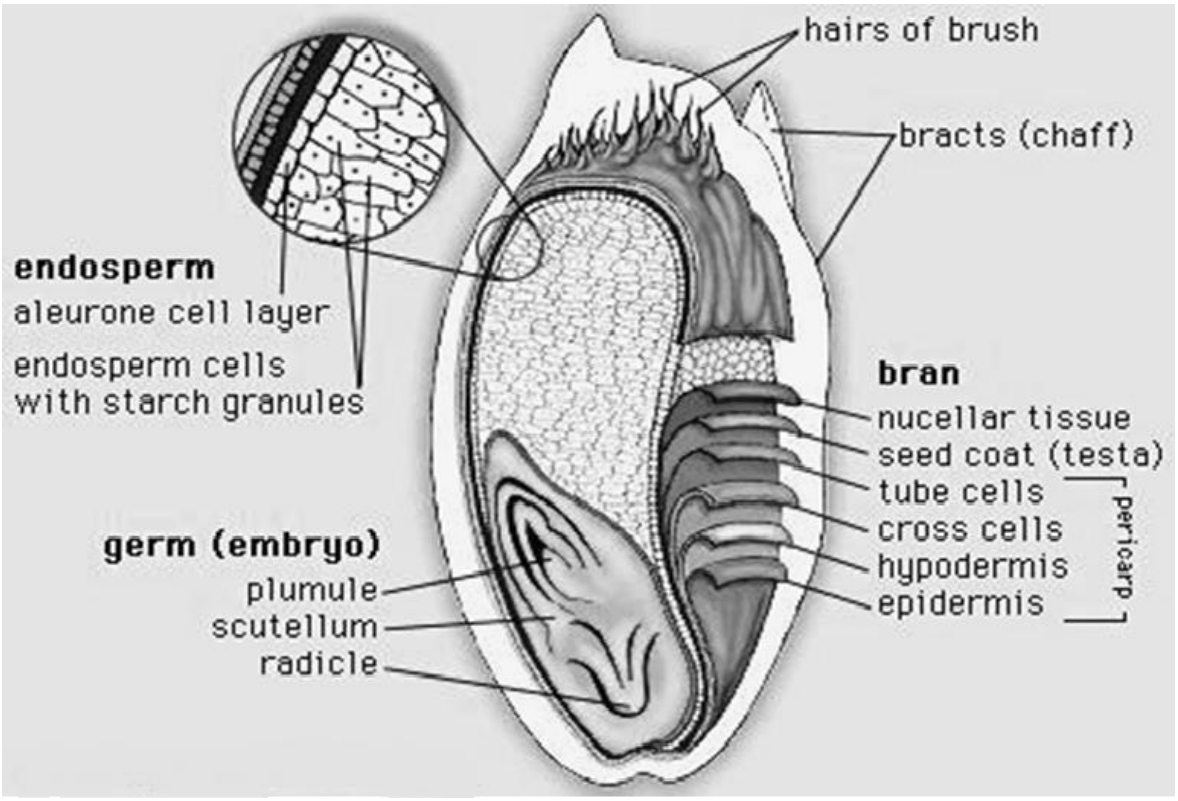


Figure 1. 2 The outer layer and internal structures of a wheat kernel.

(Source: adapted from Encyclopedia Britannica 2010)

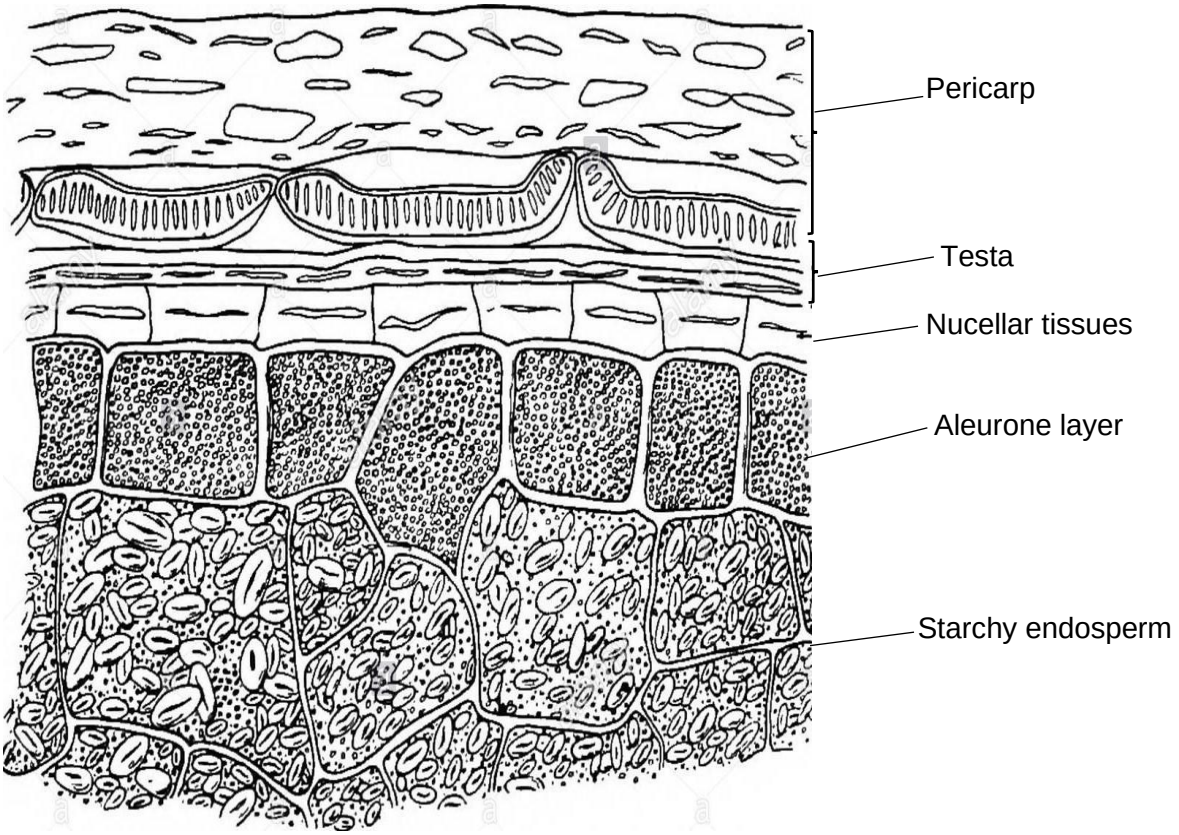


Figure 1. 3. Cross section layer of a wheat kernel.

(Source: adapted from Delcour and Hoseney 2010)

1.1.1.2. Endosperm

Endosperm is a starchy tissue that forms during grain development. The endosperm varies in its composition from its outer portion, just beneath the aleurone layer to its centre. The ratio of protein and ash decrease from the outer portion to the centre of endosperm. Endosperm comprises about 80 – 85% of the mature wheat grain (Pomeranz 1988). It provides energy for the developing embryo and for the seedling after the germination until it can establish. It contains a variety of storage materials such as starch, lipids, protein and hemicelluloses, however, the principal components of endosperm cells are starch and protein and these two components make up the bulk of the endosperm. The total lipid content of milled endosperm was reported as 0.75 – 2.16%, averaging 1.08% (MacMasters et al. 1971). The amount of lipid in endosperm largely depends on the method of extraction. The endosperm cell walls are made of pentosans and β- glucans.

The starchy endosperm is the material from which white flour is made. The endosperm cells are packed with starch granules embedded in a protein matrix. The high protein fractions (33 – 54%) is present near the subaleurone endosperm cells. The fractions with the highest protein contents consisted of subaleurone cells with the little starch and confined to the periphery of the cells. Starch granules present in the endosperm are produced by amyloplasts which are present in the cells of the developing seed (Cornell and Hoveling 1998).

1.1.1.3. Germ (Embryo)

Wheat germ (embryo) consists of two parts, the embryonic axis and scutellum (cotyledon) and is rich in proteins, lipids, vitamins and minerals. The embryo is commonly called germ by the millers. The embryo is developed from the fusion of egg nucleus and the second sperm nucleus. Wheat germ is high in vitamin E (α-tocopherol), B vitamins, lipids, protein and contains some enzymes. It is high in protein (25%), sugar (18%), oil (16% of embryonic axis and 32% of scutellum are oil) and ash (5%) (Grundas 2003). According to a review by Barnes (1982), wheat germ contains 27.6% protein, 10.6% fat, 4.3% ash, 17.0% sugar, 21.3% starch, and 3.2% crude fibre (CF). The embryo is structurally completed before the start of the grain filling stage; however, storage reserves are not deposited. Storage reserves in the embryo start to deposit towards the end of endospermic cell division phase (Pomeranz 1988). The primary reserves of the embryo are protein bodies and lipid droplets. Lipid droplets are present in low numbers initially but increase in quantity during grain filling stage. The embryo is an immature new plant (sporophyte) that is arrested in a dormant state in the seed (Heyne 1987).

The scutellum is a storage organ and known as cotyledon. The scutellum is adjacent to the endosperm on one side and bordered by the embryonic axis on other side. The mature scutellum is a major storage for protein, phytin and lipid droplets (Swift and O'Brian 1972). It also contains higher proportion of ash, manganese, thiamine and lower in protein. Scutellum contains two third of the grain thiamine content. The scutellum is the highest part of the embryo and the main function of scutellum is the mobilisation of food reserves during germination.

1.1.2. Polysaccharides composition

There are two main categories of polysaccharides in plant material, the storage polysaccharides (the starch) and the structural (the cell wall polysaccharides) which are known as non-starch polysaccharides (NSP) (Englyst and Cumming 1988). Polysaccharides constitute up to 80% of dry matter (DM) of wheat grain and variation in their composition (starch and non-starch polysaccharides) influenced the nutritional value of wheat for broilers (Gutierrez del Alamo et al. 2008b). Starch is stored in seeds in two forms: amylose and amylopectin. Non-starch polysaccharides include cellulose and non-cellulose polysaccharides. Cellulose is the main component of plant cell walls and is insoluble in water. Non-cellulose polysaccharides are mainly water soluble and include arabinoxylans, β-glucans, and pectin.

1.1.2.1 Starch

Starch is the most abundant carbohydrate found in plants and in wheat, comprising 60 to 75% of grain. Starch content in wheat has been reported by several authors (Svihus and Gullord 2002; Kim et al. 2003; Pirgozliev et al. 2003; Ball et al. 2013a, b) and the amounts quoted in the literature depends on the technique used for starch determination as different techniques result in variation in starch content. The starch content of wheat used for broiler diets in different broilers studies is presented in Table 1.1.

Starch is the main energy yielding component of wheat and its concentration is inversely related to protein content of wheat (Svihus and Gullord 2002; Pirgozliev et al. 2003; Ravindran and Amerah 2009). The variation in starch content depend on variety and growing condition. Growing conditions can affect the starch quality and starch synthesis (Metayer et al. 1993; Scott et al. 1998a; Kim et al. 2003). Sometimes grains are smaller or shrunken due to frost in winter season and this type of wheat is rejected for the milling industry, instead used in the feed industry (Leeson and Summers 2005). Starch is found as discrete granules within the cells of the endosperm. Modern milling practices can remove the bran and germ, producing a flour that essentially contains endosperm which is rich in starch.

Table 1. 1. Starch content (g/kg DM) of wheat samples used in different broiler studies.

Mean	Range	*n*	Country	References
677	658 - 728	16	Denmark	Steenfeldt (2001)
648[a]	629 - 662	6	UK	Rose et al. (2001)
627	612 - 656	12	UK	McCracken et al. (2002)
665	614 - 712	16	Norway	Svihus and Gullord (2002)
691	594 - 732	23	UK	Pirgozliev et al. (2003)
653	585 - 737	18	Australia	Kim et al. (2003)
706	664 - 732	15	France	Carré et al. (2005)
644	567 - 719	10	UK	Ball et al. (2013a)

n = number of samples in each study.
[a] Starch content in g/kg.

1.1.2.1.1. Amylose and amylopectin

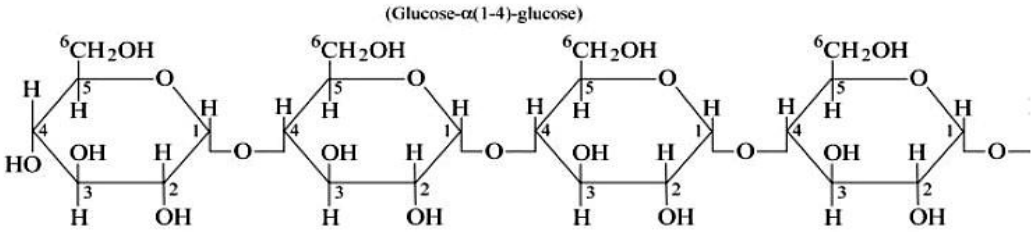
Starch contains two major carbohydrate components, amylose and amylopectin. Both are high molecular weight polymers. Amylose is a linear polymer composed of glucose units linked through α- D- (1→4) glycosidic linkage (Figure 1.4A) and some branching at α- (1→6) linkage. Amylose has a molecular weight of around 1×10^5 – 1×10^6. Amylose is 99% α- (1→4) linked polymers and differ in size and structure depending on botanical origin (Tester 1997; Buleon et al. 1998; Tester et al. 2004).

Amylopectin is a branched chain polymer with frequent branches at α-(1→6) bonds occurring every 24 to 30 glucose units (Figure 1.4B). Amylopectin has a much higher molecular weight 1×10^7 – 1×10^9 and is heavily branched with 95% α- (1→4) and 5% α- (1→6) linkage (Buleon et al. 1998). Hizukuri et al. (1997) described the branching points of amylose and amylopectin in starch granules and suggested that the half of the amylose is branched, and the amount of branch points is less than 20 per molecule. Amylopectin contains one branch point per 20 glucose units (Figure 1.5).

The ratio of amylose and amylopectin varies according to botanical origin of the plant. Starch generally contains 20 to 25% amylose and 75 to 80% amylopectin by weight (Tester et al. 2004). Starch is divided into three types depending on amylose to amylopectin ratio. The waxy starches contain less than 15% amylose, normal starch contains amylose 20 – 35% and high amylose starch (Amylo) contains greater than 40 % amylose (Tester et al. 2004). Hybridisation in wheat cultivars resulted in wheat with no amylose content (waxy) or very little amylose (partial waxy) content (Graybosch 1998). Starch digestibility is influenced by starch structure, amylose to amylopectin ratio, interaction with other components

(protein) of endosperm and processing condition (Svihus et al. 2005). Starch digestibility is reduced with higher amylose content as amylose is a more stable molecule compared to amylopectin due to the presence of large number of hydrogen bonds that interlink polymers of glucose (Ahuja et al. 2013). It might be also due to the interaction between amylose and fatty acids, which results in the formation of a less digestible complex on the surface of starch granules (Crowe et al. 2000).

Amylose (A)



Amylopectin (B)

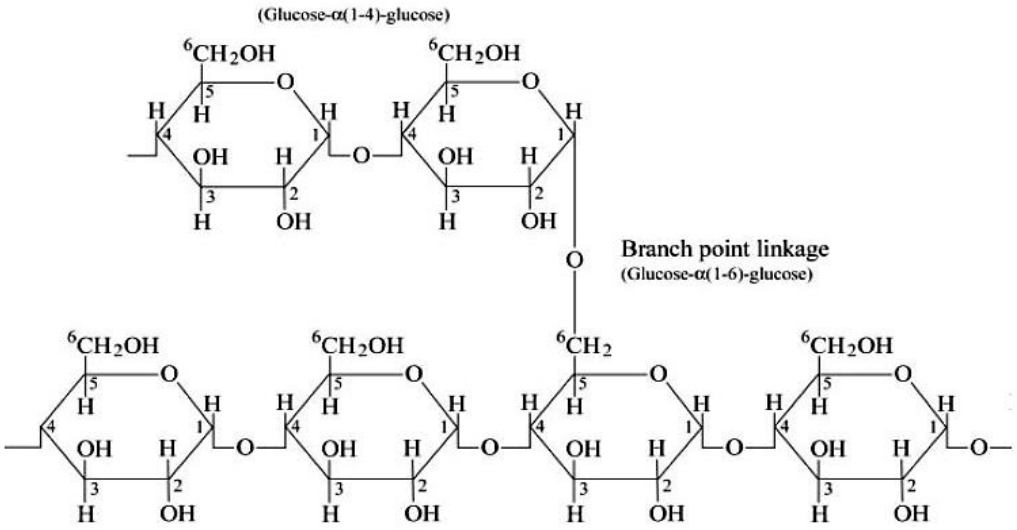
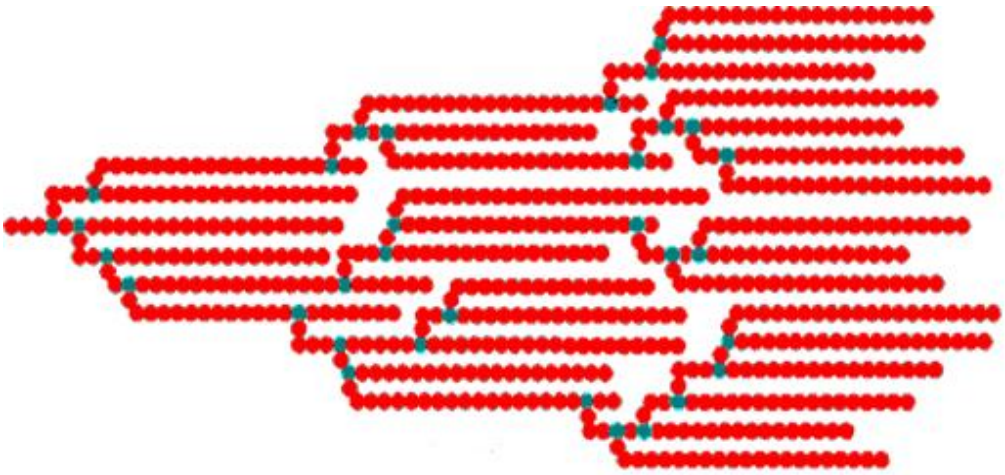


Figure 1. 4. Structure of Amylose (A) and Amylopectin (B).
(Source: Tester et al. 2004)



● α-1,4 linked D-glucose, ● α-1,4,6 D-glucose branch points (each dot representing glucose molecules).

Figure 1. 5. Amylopectin branched structure.

(Source: adapted from Hizukuri 1986)

1.1.2.1.2. Starch granules

Starch is present as granules of varying sizes in cereal endosperm. Starch granules are synthesized by amyloplasts. Starch granule organisation has been reviewed in detail by Morell et al. 1995 and Buleon et al. 1998. Starch granule size ranges from 1 – 50 µm. Starch granules in wheat have been categorised into three types. Type A (lenticular shape) are the large size granules ranges from 15 to 50 µm, type B (spherical shape) medium granule size ranges from 1 to 10 µm and type C granules size ranges from 1 – 5 µm (Bechtel et al. 1990; Buleon et al. 1998; Salman et al. 2009). In a mature grain, type A granules constitute 51.6%, type B 45% and type C 3.4% of the total mass of starch in endosperm (Bechtel et al. 1990).

The shape of starch granules is dependent upon the proportion of amylose and amylopectin (Svihus 2014; Tester et al. 2004; Zaefarian et al. 2015). Granules containing proportionally large amount of amylose compared to amylopectin in the starch leads to more round shapes whereas those containing more amylopectin are more flattened (Morell et al. 1995). Peterson and Fulcher (2001) suggested that starch granule size distribution varied within cultivars. The starch granule size is also affected by environmental factors like temperature. It was suggested that high temperature during the grain filling stage of cereal resulted in less starch per endosperm with smaller granule size and higher amylose contents (Tester et al. 1991). Starch granules are located in the protein matrix, which complicates the digestion process. Starch granules are insoluble in water, but in the presence of heat can swell in aqueous medium. Swelling is reversible, but when a certain temperature threshold is reached, swelling become irreversible (Hoover 1995; Svihus et al. 2005). The structure of starch changes significantly during pelleting of feed, and this process is called

gelatinisation. The temperature at which gelatinisation occurs is called gelatinisation temperature. Gelatinisation opens the starch structure, making it more susceptible for amylolytic degradation because of the loss of crystalline structure of starch (Rooney and Pflugfelder 1986). Wheat starch normally gelatinises at temperatures ranges between 52 – 65°C (Lund and Lorenz 1984). Water is a prerequisite for starch gelatinisation, however the processes like grinding, milling and crushing also cause gelatinisation to a certain extent (Abdollahi et al. 2013).

1.1.2.1.3. Endosperm hardness

Endosperm hardness of the wheat is an important criterion of determining the end use of wheat, whether to select it for bread making, biscuits or cakes. It also determines the particle size of wheat flour. The hardness or softness of a grain is the relative resistance of grain to deformation or crushing when an external force is applied (Turnbull and Rahman 2002). Hardness of endosperm affect the milling characteristics of wheat. During milling, hard wheat shatters, with regular particle sizes and large surface area. Wheat with soft endosperm results in flour with irregular particle sizes, smaller size, fine powder flour, little starch granule damage and intact starch granules (Pomeranz et al. 1984; Rose et al. 2001). During milling, hard endosperm wheat varieties produce large particles with cleaved starch granules. The solubility of cleaved starch granules is higher when they are intact, subsequently resulting in increases in growth performance of broilers (Rose et al. 2001). The most important physical difference between endosperm of hard and soft wheat is due to adhesive strength of starch granules with the protein matrix which surrounds it (Simmonds et al. 1973). Endosperm hardness is an important characteristic in the quality of wheat.

MacRitchie (1980) described that physical hardness of starch and protein, the strength of their interaction within endosperm cells, and the interaction of individual cells to produce the overall grain structures are the major factors which affect endosperm hardness. Barlow et al. (1973) suggested that grain hardness may be caused by the degree of starch and protein interaction in the endosperm. Hard wheat possesses a strong starch protein matrix compared to soft wheats. Hardness also depends upon the physical structure of the protein matrix. The quantity of protein increases towards the surface of starch granule and a large amounts of protein present on the surface of starch granules are low molecular weight protein ranges 5 – 60 kDa (kilodaltons), whereas, proteins associated with the internal of starch granule ranges between 60 –150 kDa (Baldwin 2001). In wheat, a starch surface protein, friabilin is thought to be associated with hardness of endosperm (Greenwell 1986, Schofield 1994, Baldwin 2001). Greenwell (1986) found abundant amounts of friabilin on the surface of starch granule in soft wheat, while its concentration was lower in hard wheat.

The authors suggested that friabilin is concentrated at the starch-protein interface which may impede the bonding between starch and protein matrix in soft wheat and resulted in soft endosperm. Environmental conditions, protein content, the quantity and quality of pentosans and moisture content (Cornell and Hoveling 1998; Turnbull and Rahman 2002), are other factors considered to be influencing the endosperm hardness of wheat starch.

Grain hardness is important for the flour industry because it has a significant impact on milling, baking and qualities of wheat. The texture of endosperm influences certain physical properties like tempering requirements, flour particle size, flour density, starch damage, water absorption and milling yield (Pasha et al. 2010) and indicates the suitability of specific flour for different products. The endosperm hardness is vital to wheat growers because hard wheat received higher prices due to protein content differences as compared to soft wheats (Pasha et al. 2010).

1.1.2.2. Non-starch polysaccharides

Non starch polysaccharides (NSP) are the main constituents of the cell wall, closely associated with other polysaccharides or non-carbohydrate material such as protein and lignin. They are known as the portion of plant tissue that is not digested by endogenous secretions in the digestive tract (Choct 2015). The NSP covers a range of polysaccharides excluding α-glucans (starch), and are classified into three main groups, cellulose, non-cellulosic polysaccharides and pectic polymers (Bailey 1973) (Figure 1.6). The non-cellulosic polysaccharides include several polysaccharides and are generally termed as hemicellulose. The non-cellulose polysaccharides include arabinoxylans (also known as pentosans), mixed-linked β-glucans, mannans, galactans, xyloglucans and fructans. Pectic polymers consist mainly of polygalacturonic acids substituted with arabinan, galactan and arabinogalactan.

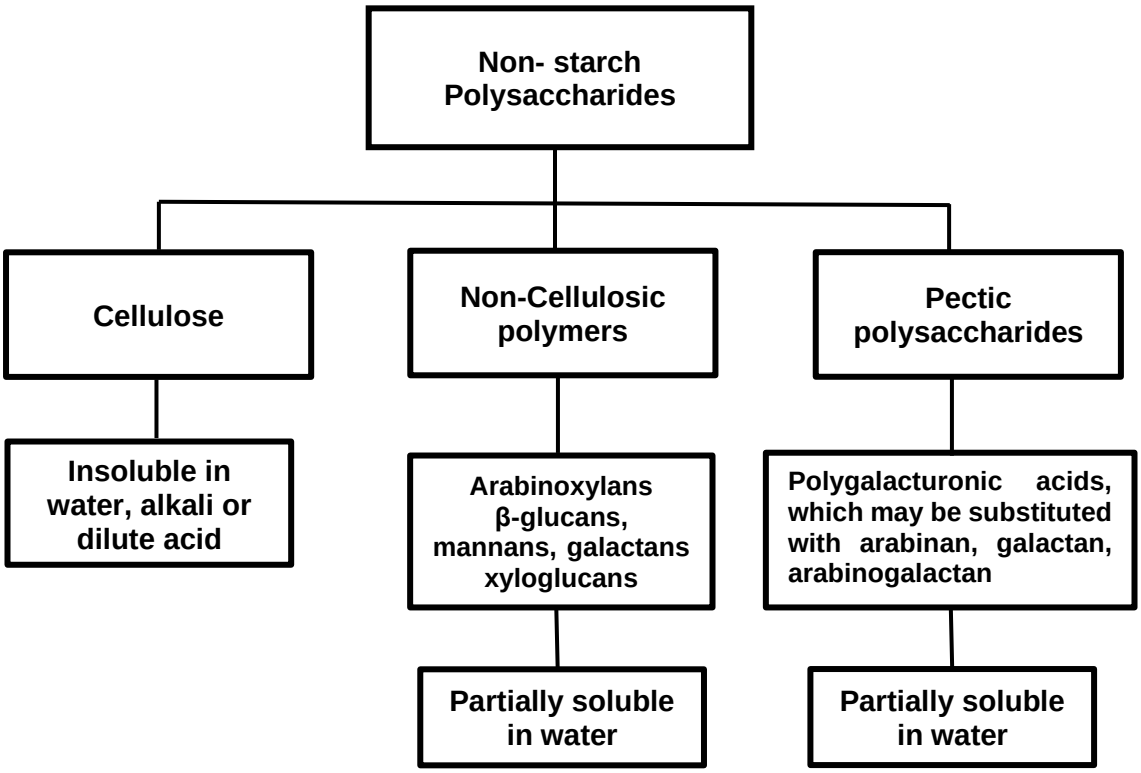


Figure 1. 6. Main constituents of non-starch polysaccharides (NSP).
(Source: adapted from Bailey 1973)

The cell wall polysaccharides of cereals (rye, wheat, barley) are mainly arabinoxylans (AX) and β-D-glucans. Cereal β-D-glucans are linear glucose polymers of glucose with (1→3), (1→4) glucosidic links. The relative amounts of AX and β-D-glucans vary considerably among cereals. Commonly used cereals in poultry diets consist of 10 – 20% NSP (Choct 1997). In cereals, the bulk of the NSP are composed of predominantly AX, cellulose and β-glucans, whereas only trace amount of pectic polymers are found in stem and leaves of cereals (Choct 1997). Wheat contains less total and soluble β-glucans in comparison to barley, rye or oats and less AX as compared to rye and oats (Table 1.2). Maize contains very low level of β-glucans and AX.

Table 1. 2. Comparison of NSP content (g/kg DM) of commonly used cereals for poultry diets.

Non-starch polysaccharides	Wheat	Barley	Rye	Oat	Maize
Soluble NSP	24	45	46	48	9
Insoluble NSP	90	122	86	30	47
Total NSP	114	167	132	78	56
Total arabinoxylans	61	33	89	21	42[2]
Soluble arabinoxylans	10	7	25	2	-
β-D-glucan	5	76	12	34[3]	1
Soluble β-D-glucan	5	29	7	21[3]	-
Cellulose	20	39	15	7	20

Source: Englyst et al. (1989) ; Annison (1991)[2] ; Henry (1985)[3]

The NSP in cereal grains can be further divided into two groups (viscous and non-viscous cereals) based on the amount of soluble NSP in the grain. Rye, wheat, barley, triticale and oats are grouped as viscous cereals, whereas, maize, sorghum, rice and millet are classified as non-viscous cereals (Choct 1997, 2015). The AX and β-glucans present in wheat, rye, barley, triticale and oats are partially soluble and can form highly viscous solutions and therefore, these grains are classified as viscous cereals. Maize, sorghum and rice contain a low NSP content and of which a very small amount of NSP is soluble, however, it does not form viscous solutions and therefore are classified as non-viscous cereals (Table 1.3).

Wheat endosperm is high in AX and low in β-glucans. In wheat grain, β -glucans are present in small quantity and even their proportion does not exceed from 1% in some varieties (Pomeranz 1988). Wheat grain contains 10 – 12% NSP, of which 8% is AX, 2% cellulose and 1% β-glucans (Ravindran and Amerah 2009). Arabinoxylans and β-glucans are the two most important water-extractable dietary fibre polysaccharides in cereal food products. The structure and molecular weight distributions of these polymers determine their physical properties like viscosity, extractability, solubility and gelling behaviour, as well as nutritional properties. The level of NSP and composition of their fractions is variable in wheat (Knudsen 1997; Choct and Hughes 1999; Svihus and Gullord 2002; Smeets et al. 2014). A summary of varied level of NSP content of wheat in different studies is presented in Table 1.4.

Table 1. 3. The NSP fractions of commonly used cereals and their byproducts (g/kg DM).

Cereals		Arabinoxylans	B-glucans	Cellulose	Mannose	Galactose	Total
Wheat	Soluble	18	4	-	t	2	24
	Insoluble	63	4	20	t	1	90
Barley	Soluble	8	36	-	t	1	45
	Insoluble	71	7	39	2	1	122
Rye	Soluble	34	9	-	1	1	46
	Insoluble	55	11	15	1	2	86
Triticale	Soluble	13	2	-	0.2	1	17
	Insoluble	95	15	25	6	4	146
Oat	Soluble	2	28	-	-	1	48
	Insoluble	19	-	7	2	1	30
Sorghum	Soluble	1	1	-	t	t	2
	Insoluble	20	1	22	1	1.5	46
Maize	Soluble	1	t	-	-	t	1
	Insoluble	51	-	20	2	6	80
Rice	Soluble	t	1	-	t	1	3
	Insoluble	2	-	3	t	t	5
Wheat bran	Soluble	26	2	-	t	1	32
	Insoluble	260	-	108	1	6	384

Source: Choct (1997; 2015). t: trace amount

Table 1. 4. A summary of NSP content (g/kg DM) of different wheat samples used in broiler diets.

NSP		Steenfeldt (2001) (*n*= 16) Denmark	Pirgozliev et al. (2003) (*n* = 23) UK	McCracken et al. (2002) (*n* = 12) UK	Svihus and Gullord (2002) (*n* =16) Norway	Choct and Hughes (1999) (*n* = 81) Australia
Soluble NSP	Mean	23	26.8	22	20	12.2
	Range	10 – 39	15 – 49	16 – 26	14 – 28	9.0 – 18
Insoluble NSP	Mean	83	73	98	110	92.6
	Range	73 – 94	66 – 85	86 –118	104 –112	72 – 139
Total NSP	Mean	106	100	120	130	105
	Range	98 – 117	85 – 128	106 –144	118 –140	81 – 157

n = number of samples in each study.

The NSP have anti-nutritive properties in chickens because they lack the endogenous enzymes to digest nutrients in cereal grains. Nutrients like starch and protein can be trapped with fibrous cell wall due to high fibre contents and becomes unavailable to birds, hence resulting in reduced digestibility of nutrient. The soluble fibres form viscous gels in the chicken gut that trap nutrients and slow down the rate of digestion and passage of feed in gut, reducing feed intake and subsequent growth.

Wheat NSP have anti-nutritive properties even at low levels (10%) and their high viscosity property contribute to their anti-nutritional effects (Annison 1990, 1991; Choct and Annison 1990; Bedford and Schulze 1998; Steenfeldt 2001). Soluble and insoluble NSP in wheat have different properties and different effects on poultry digestion. The maximum amount of the arabinoxylans (6 – 7%) in wheat are insoluble because they are attached to the cell walls. High level of NSP increase digesta viscosity, reduce nutrient digestibility resulting in a reduction of bird performance (Choct and Annison 1992a; Choct et al. 1995a, b). High intestinal viscosity resulted in, decrease in the rate of passage of feed, reduced feed intake, increased water consumption, changes in gastrointestinal tract (GIT) environment and poor litter quality (Choct and Annison 1990; Choct et al. 1996; Silversides and Bedford 1999). Ball et al. (2013a) reported a positive relationship between *in vitro* viscosity and soluble and total NSP content and a negative correlation between in vitro viscosity and ileal starch and protein digestibility. The use of NSP degrading enzymes is a common practice to treat wheat-based diets to reduce digesta viscosity and improve nutrients availability and growth performance of broilers (Bedford 1996; 2000; Olukosi et al. 2007; Amerah et al. 2009a, b).

1.1.3. Protein

Protein is the second most abundant constituent of cereal grains, and in wheat the crude protein content (CP) varies from 80 – 160 g/kg, depending on variety and growing conditions (Lasztity 1999). The average CP content of wheat is higher than other cereals including maize, a most widely used cereal in poultry diets. Protein is distributed in all parts of the wheat grain but the majority of it is concentrated in the endosperm and aleurone layer. In wheat grain, endosperm contains 72%, aleurone layer 15%, testa 4%, scutellum 4.5% and embryo contains 3.5% protein of the total protein content (Pomeranz 1988; Pirgozliev 2000). The CP content of wheat used in different broiler studies was found to be highly variable (Table 1.5).

Table 1. 5. Variability in crude protein (CP) content (g/kg DM) of different wheat samples used in broiler diets.

Mean	Range	*n*	Country	References
131	89 – 183	81	Australia	Choct et al. 1999
120	112 - 127	16	Denmark	Steenfeldt (2001)
126	116 - 147	12	UK	McCracken et al. (2002)
130	109 - 154	16	Norway	Svihus and Gullord (2002)
114	85 - 151	23	UK	Pirgozliev et al. (2003)
134	97 - 191	18	Australia	Kim et al. (2003)
109	90 - 137	4	Spain	Gutiérrez del Alamo et al. (2008a)
118	78 - 150	94	UK	Ball et al. (2013a)

n = number of samples in each study.

Proteins in wheat grains can be divided into four fractions depending on their solubility as proposed by Osborne (1907): albumins, globulins, gliadins, and glutenins. Albumins are soluble in water and neutral buffer, whereas, globulins are soluble in dilute salt solution but insoluble in water. Gliadins (also known as prolamins) are soluble in aqueous alcohol (70% ethanol) and glutenins are soluble in dilute acetic acid but insoluble in neutral aqueous solutions, saline solutions or alcohol (Wrigley and Bietz 1988; Cornell and Hoveling 1998). Albumins and globulins are known as cytoplasmic and metabolically active proteins, whereas, gliadin and glutenins are mostly storage proteins (Pomeranz 1988), commonly referred as the gluten. Gluten proteins are generally characterised by having a high content of proline and glutamic acid. The water-soluble proteins are important for baking quality of flour. The omission of soluble proteins in baking generally results in a decrease in the loaf volume (Pomeranz 1988). Glutenins are responsible for the elasticity of gluten complex. Gliadins and glutenins are the main protein fractions of wheat and consists of 80% of total protein, whereas, albumins and globulins each contributes 5 –10% (Lasztity 1999). The protein in hard wheat is useful in bread making, while the soft varieties are used in cookies, cakes and biscuits. Although, wheat breeders routinely select for protein content in their breeding programme e.g., high protein for bread making and low protein for feed or other uses, however the current range of variation in protein content is limited in commercial cultivars. Snape et al. (1993) indicated that wheats used for bread making in the UK typically contains higher protein content by 2% as compared to feed wheats.

Protein is a high cost feed ingredient, and meat and egg production in poultry is dependent on conversion of feed protein to animal protein, therefore, protein is always of special significance to nutritionist. Body tissues are mostly made up of proteins, with structural proteins also being present in bones, skin and muscles. Protein is made up of amino acids but the combination and sequential arrangements of amino acids in protein differ considerably. The job of a nutritionist is to ensure the provision of adequate levels of available nitrogen and essential amino acids necessary for optimum protein synthesis in the birds, at each stage of production. This involves determining the exact combination of the various protein to be obtained from available raw materials and meeting the bird's requirements from those components. The amount of protein in most of the cereals is not adequate to meet the bird requirements for growth, therefore additional protein is required to support the growth using additional source of protein e.g., soybean meal, sunflower meal, rape seed meal and fish meal etc.

1.1.3.1. Amino acids

Amino acids (AA) are classified as essential and non-essential amino acids. Essential amino acids (also referred as indispensable) cannot be synthesised by the animal in the body and therefore, must be supplied by the diet, whereas non-essential amino acids can be synthesised in the body from other amino acids. Of the non-essential amino acids (known as dispensable), a few cannot be synthesised at a faster rate for maximum growth and therefore, should be included in the diet. The essential amino acids are further subdivided into three categories depending on ability of animal to achieve limited or no synthesis:

(a) Lysine and threonine have no intermediary precursor, therefore 100% must be supplied in the diet.

(b) Leucine, isoleucine and valine can be synthesised from precursor intermediary metabolites; however, the production is very limited and may only supply 2 – 5% of the requirements.

(c) Arginine and histidine can also be synthesised from intermediates and can yield 5 – 8% of animal requirements under normal circumstances.

In most biological systems, there are 20 common AA identified as protein components. Among 10 essential AA, eight are critical AA, whereas, others are usually present in adequate amount in most diets. The critical AA in poultry are lysine, methionine, threonine, cystine, isoleucine, arginine, tryptophan and valine. Of the non-essential AA, the major AA found in animal tissues and feedstuffs is glycine, which can be synthesised from serine and choline and plays an integral part in uric acid synthesis. Synthetic forms of critical AA are

available and added in significant amounts in poultry diets to meet up the AA requirements of poultry. Protein consists of long chains of AA joined in a definite and characteristic manner. The linkage within these chains are called peptide bonds. The polypeptide chains are formed when there are more than 20 linked AA and protein are formed when there are 50 or more linked amino acids (Berg et al. 2002). Protein quality of a feed is determined by its amino acid profile and not by the total amount of CP in the diet. If CP of a grain has a high content of all essential AA, it is described as having a high biological value. However, if any single essential AA is missing, then the biological value of protein is reduced.

The AA composition of cereal grains is influenced by the type of protein (metabolically active protein or storage protein). Storage proteins contain a high amount of glutamic acid and proline and only a small amount of arginine, lysine, threonine and tryptophan. Metabolically active proteins (albumins and globulins) contain less glutamic acid and proline but a high amount of lysine and arginine which gives these proteins high nutritional value. Wheat flour contains less lysine, arginine, and methionine. The concentration of essential AA in different cereals in presented in Table 1.6.

Table 1. 6. The content of essential amino acid (AA) in different cereal grains.

	Wheat	**Maize**	**Rice**	**Barley**	**Rye**
Phenylalanine	4.6	3.8	5.2	5.2	5.0
Histidine	2.0	2.3	2.5	2.1	2.4
Isoleucine	3.0	2.9	4.1	3.6	3.7
Leucine	6.3	10.0	8.6	6.6	6.4
Lysine	2.3	2.6	4.1	3.5	3.5
Methionine	1.2	1.8	2.4	2.2	1.6
Threonine	2.4	2.9	4.0	3.2	3.1
Tryptophan	2.4	0.6	1.4	1.5	0.8
Valine	3.6	4.0	5.8	5.0	4.9

Source: adapted from McKevith (2004) and NRC (1994)

The AA concentration in wheat increases linearly with the increase in protein content of wheat (Ravindran et al. 1998; 2005). Maize is the most widely used cereal grain in poultry diets in the world, however, maize contains lower CP content (80 – 85 g/kg) and AA levels as compared to wheat (Table 1.7). Wheat being high in protein content is considered as a better source of AA as compared to maize and in practical broiler feed formulation, diets based on wheat require less protein and AA supplementation than maize-based diets.

Table 1. 7. Amin acid content (g/kg) in wheat containing different level of protein and comparison to maize.

	Protein level in wheat			Maize
	90 g/kg	120 g/kg	160 g/kg	80 g/kg
Indispensable amino acids				
Arginine	4.7	5.7	7.5	3.9
Histidine	2.6	3.2	4.2	2.4
Isoleucine	3.7	4.6	6.4	3.3
Leucine	6.8	8.4	11.3	10.9
Lysine	3.1	3.6	4.7	2.5
Methionine	1.3	1.6	2	1.4
Phenylalanine	4.5	6	8	4.3
Threonine	3.2	3.8	5.2	3.7
Valine	4.5	5.5	7.2	4.4
Dispensable amino acids				
Alanine	3.8	4.6	5.6	6.7
Aspartic acid	5.3	6.1	8.5	5.7
Glycine	4.3	5.2	7.1	3.5
Glutamic acid	28.4	40.1	54.5	16.2
Serine	5.5	6.8	8.3	4.6
Tyrosine	2.4	3.3	4.8	4.4

Source: Ravindran and Amerah (2009)

1.1.4. Lipids

Lipids are minor constituents of wheat, but play a major role in production, storage, processing and nutritional quality. Lipids are present on the surface of starch granules in most cereals (Baldwin et al. 1997). Most of the lipids in wheat are fatty acids esters of glycerol, and the remainder include free (unesterified) fatty acids (FFA) and several types of sterol-based lipids and glycosphingolipids (Morrison 1988, 1994). The principal fatty acids are palmitic acid (16:0), stearic acid (18:0), oleic acid (18:1), linoleic acid (18:2) and linolenic acid (18:3) (Morrison 1988). The lipids (known as oil) in wheat are stored in oil droplets or spherosomes present in the scutellum and aleurone, but also in the subaleurone starch endosperm (Morrison 1994). In wheat, the contents of lipids are unevenly distributed in different parts of the grain. Germs contains about 25 – 30%, aleurone layer 22 – 33%, starchy endosperm 40 – 50% and pericarp contain 4% lipids (Morrison 1988; Pirgozliev et al. 2000). Endosperm contains 20 – 31% non-starch lipids and 16 – 22% starch lipids. Wheat contains low level of lipids, averaging 25 g/kg (NRC 1994). The lipid content of wheat is variable and ranges from 10 – 30 g/kg DM (Table 1.8).

Table 1. 8. Comparison of oil content* (g/kg DM) of wheat samples used in selected broiler studies.

Mean	Range	*n*	Country	References
24.5	21 - 27	16	Denmark	Steenfeldt (2001)
13.4	11-15	6	UK	Rose et al. (2001)
16	12-22	12	UK	McCracken et al. (2002)
26	22-34	16	Norway	Svihus and Gullord (2002)
17	15-21	23	UK	Pirgozliev et al. (2003)
18	17-18	4	Spain	Gutiérrez del Alamo et al. (2008a)
10	8-14	10	UK	Pirgozliev et al. (2015)

n = number of samples used in study.
*Oil as ether extract.

Changes in lipids occur slowly in suitable storage condition whereas deterioration in baking quality is much faster in damp stored flour. However, changes in lipids occur rapidly in milled products due to the dispersal of lipids and improved access of enzymes to substrates. Hydrolysis is predominantly quick in wholemeal flour, especially in finely ground flour and is attributed to lipase (s) located in the outer bran layers. During the milling of wheat grain, the aleurone and germ lipids redistributes and if milled wheat is stored for longer period, can become more susceptible to lipolysis. Cereal oils are unsaturated, they contain mainly linoleic acid and oleic acid, can become rancid quickly. Lipids can be extracted from milled grain by cold or hot extraction process using organic solvents. The amount and type of lipid extracted varies with the type of solvent used, the variety of wheat and conditions for solvent extraction. Soxhlet extraction using petroleum spirit or diethyl ether removes up to 1.5% of the lipids (free lipid) but leaves a significant amount of lipid to the flour.

1.1.5. Endogenous enzymes

Wheat grain contains enzymes that are responsible for the hydrolysis of endospermic starch, releasing energy for the growth and development of the embryo when sprouting begins (Stevens et al. 1988). There are four enzymes which are involved in complete hydrolysis of starch in wheat: α-amylase, β-amylase, debranching enzymes and α-glucosidases.

The enzyme α-amylase is considered to be more important while β-amylase had synergetic effect. It is believed that increased α-amylase activity results in starch damage and its activity is associated with the baking quality of wheat. This enzyme is only present in small amounts in mature grain and its level is maintained during dry storage condition. The

aleurone layer is the main site of α-amylase presence. As the process of germination increases, the enzyme moves towards the endosperm for the mobilisation of starch. In damped storage condition, level of α-amylase increases, and this results in degradation of starch. The α-amylase enzyme hydrolyses α-(1-4)-D glucosidic linkage in starch. The baking industry indirectly measures the activity of α-amylase by a method devised by Sven Hagberg (Hagberg 1960) and known as the Hagberg Falling Number (HFN) method. Higher activity of α-amylase in wheat flour means increased degradation of starch and reduced viscosity in heated wheat flour, which results in decreased falling number (FN). The method is based on the measurement of viscosity characteristics after heating a slurry of milled wheat flour and water. The viscosity is measured as the time (in seconds) it takes for a plunger to fall a certain distance through the gelatinised paste after a standard mixing time. The analysis is used as a measure of pre-harvest sprouting in wheat. The HFN values above 300s are considered free of sprouting, while below 160s are generally considered α-amylase activity, mostly unsuitable for bread making. The degree of sprouting is equated to the amount of enzyme α-amylase in the slurry. Wheat with a low HFN is mostly rejected for food and is used as animal feed in most countries.

Wheat grain also contains xylanases and phytase. Xylanases in wheat grain degrades the walls of aleurone and endosperms cells during the germination stage, which results in starch and protein being readily available to amylases and proteases (Mares and Stone, 1973a, b; Bonnin et al. 1998; Cleemput et al. 1995). In addition, wheat also contains endogenous phytase which increase the availability of phytate-bound phosphorus. The releasing activity of phytase results in higher availability of phosphorus in wheat as compared to other cereals (Selle and Ravindran 2007). During the pelleting process of poultry diets, heat generated during friction can reduce the availability of these endogenous enzyme (Slominski et al. 2007; Amerah 2015). In poultry, it is a common practice to add exogenous xylanase in wheat-based diet to eliminate the adverse effect caused by high level of NSP.

1.1.6. Vitamins and minerals

Vitamins are essential dietary factors required by the birds in small quantities. There are two groups of vitamins: fat soluble and water soluble. All of them require some body fat for their metabolism. Fat soluble vitamins include vitamin A (retinol), D, E (α-tocopherol) and K. The fat-soluble vitamins can be store in the fatty tissue of the bird to some extent. The important water-soluble vitamins are thiamine (B1), riboflavin (B2), niacin, pyridoxine, pantothenic acid, biotin, folic acid, B12, choline and vitamin C. Birds are unable to store any of water-soluble vitamins except B12. Animals require all water-soluble vitamins in the diet except vitamin C. When a feed contains excess level of water-soluble vitamins, they are

excreted through urine, therefore, it is important that birds receive the exact dose of vitamins on a daily basis.

Wheat grain is good source of vitamin E, thiamine, niacin and low in riboflavin and pyridoxine (Table 1.9). Wheat contains high level of biotin compared to maize. Wheat germ is rich in vitamin E. Wheat germ and bran contains high proportion of vitamins, especially the bran is a good source of B complex vitamins (Šramkova et al. 2009). Wheat is a good source of choline (1090 mg/kg) (NRC 1994). Wheat grain contains more vitamins than wheat flour, because of separation of bran and germ during milling process of grains. Milling and degree of flour extraction affects vitamins and mineral analysis of flour and other milled products. The natural vitamins present in a feedstuff can make an important contribution to meet bird's need and useful for nutritionist to be aware of their contents in particular feedstuff. However, modern poultry diets require additional vitamins and minerals supplements as premixes, because the available content of both vitamins and minerals in cereal-based diets are not sufficient to meet up the bird's requirement.

Minerals must be present in the diet of bird at a sufficient level for proper enzyme activity, immune function and skeletal development. The classification of minerals into major minerals (macro) and trace mineral (micro) depends on their concentration in the animal. Among macro minerals Ca and P are the two single most important minerals and often discussed together due to their interacting effect on each other. Poultry diets contains a high proportion of cereals grains which are deficient in calcium and high in phosphorus. Wheat grain contains high level of sodium, potassium, magnesium, copper, iron, manganese, selenium and zinc as compared to maize (NRC 1994) (Table 1.9). Although, wheat is a good source of macro and micro minerals, however, low in calcium, sodium, chlorine, selenium, manganese, iron concentrations as compared to the requirements of poultry. Phosphorus is present in the form of phytate which decreases its availability. Minerals and vitamins are present in high concentrations in the aleurone layer, but a significant amount is also present in germ. Wheat endosperm contains small quantities of minerals (Pomeranz 1988).

Table 1. 9. Comparison of mineral and vitamins composition of wheat and maize.

		Wheat	**Maize**
Minerals	*units*		
Calcium	g/kg	0.5	0.2
Total phosphorus	g/kg	3.7	2.8
Non phytate P	g/kg	1.3	0.8
Sodium	g/kg	0.4	0.2
Chlorine	g/kg	0.5	0.4
Potassium	g/kg	4.5	3
Magnesium	mg/kg	1700	1200
Copper	mg/kg	6	3
Iron	mg/kg	60	45
Manganese	mg/kg	32	7
Selenium	mg/kg	0.2	0.03
Zinc	mg/kg	34	18
Vitamins			
E	mg/kg	13	22
Thiamine (B1)	mg/kg	4.5	3.5
Riboflavin (B2)	mg/kg	1.4	1
Niacin	mg/kg	48	24
Pantothenic acid	mg/kg	9.9	4
Pyridoxine (B6)	mg/kg	3.4	7
Biotin	mg/kg	0.11	0.06
Folic acid	mg/kg	0.4	0.4
B12	mg/kg	-	-
Choline	mg/kg	1090	620

(Source: NRC 1994)

1.2. ENERGY AND NUTRIENT AVAILABILITY OF WHEAT

The nutritional quality of wheat for broilers relates to its nutrient content, availability and digestibility of nutrients, however, the availability and digestibility of nutrients of wheat is variable. The factors affecting the availability of these nutrients could be intrinsic or extrinsic. The intrinsic factors include variety and chemical composition while extrinsic factors are growing condition and storage time (Gutierrez et al. 2008b). The precise and accurate values of energy and nutrient availability is important when formulating balanced diets for broilers.

1.2.1. Availability of energy

A feedstuff must be digested, absorbed and metabolised to be used as a source of energy. Birds eat food to satisfy their energy requirements and nutrient requirements of bird depends on their bioavailable energy intakes. In poultry, feed intake is controlled by the energy content of feed, provided birds are offered *ad libitum* nutritionally adequate diets (Sibbald 1982). To determine the total amount of energy in a feedstuff, a term called gross (GE) is used extensively, which is measured by estimating the amount of heat liberated when a feedstuff is burnt in the presence of oxygen (Maclean et al. 2003). Digestible energy is determined by measuring the gross energy (GE) of the feed consumed and subtracting the GE of the faeces. However, poultry void faeces and urine through a common cloaca, therefore, this system is not generally applied for poultry. Instead metabolisable energy (ME) is the most widely used system for describing available energy content of common feedstuffs used in poultry feeds. The ME is determined by measuring the GE of feed consumed and subtracting the GE in faeces, urine and gaseous production during digestion (ME is the difference between digestible energy and the excreta GE). A correction for nitrogen retained in the body is made to determine nitrogen-corrected ME (MEn) (NRC 1994). Hill and Anderson (1958) proposed a correction value of 34.4 MJ/kg DM, which correspond to the amount of energy obtained by the complete combustion of one (1) g of urinary N in the form of uric acid. In previous years, ME has been used interchangeably with apparent metabolisable energy (AME). The prefix “apparent” is used to recognise that energy in faecal matter is not solely attributed to energy derived from feed. Endogenous losses, bile secretion, mucosal cells and unabsorbed intestinal secretions are also present in the faeces (Sibbald 1982). Determination of accurate estimates of available energy of poultry feedstuffs is essential for uniform broiler growth, efficient broiler production and to minimise the production cost.

1.2.1.1. Apparent metabolisable energy value of wheat

Apparent metabolisable energy is the most important measurement used in characterising the nutritional value of a feed for poultry. The determination of AME not only indicates the nutrient composition of a feed but also how the bird will respond to that feed. Therefore, the AME value of raw ingredients and compound feed is determined as a first step for nutritional studies of cereal grains for poultry. Wheat is known as a variable cereal grain in its chemical composition and physical characteristics as compared to other available cereal grains for poultry, therefore the determination of AME of wheat is vital for better utilisation of wheat in practical feed formulations. There is an enormous amount of data available on AME of wheat and wheat-based diets, with values ranging from 8.32 to 16.60 MJ/kg DM (Mollah et al. 1983; Wiseman 2000; McCracken et al. 2002). Studies from several parts of the world have shown that AME value of wheat for broilers varies considerably (Table 1.10). The AME value of Australian wheats was found to be varied considerably (9.16 to 15.89 MJ/kg DM) and low AME values were attributed to a high level of NSP (arabinoxylans) of wheat grains (Mollah et al. 1983; Rogel et al. 1987a; Annison 1991; Choct et al. 1999).

Table 1. 10. The variability in apparent metabolisable energy (AME) value of wheat for broilers.

No of samples	Country	AME MJ/kg DM	Reference
25	Canada	12.30 −16.57	Sibbald and Slinger (1962)
13	Australia	11 - 15.89	Mollah et al. (1983)
38	Australia	10.35 − 14.81	Rogel et al. (1987a)
13	Australia	11.25 − 13.60	Annison (1991)
-	North America	13.22	NRC (1994)
108	Canada	13.70 − 15.30	Scott et al. (1998a)
81	Australia	9.16 − 14.97	Choct et al. (1999)
12	UK	8.32 − 13.72	Austin et al. (1999)
50	UK	8.49 − 12.45	Wiseman (2000)
80	New Zealand	10.20 − 15.94	Ravindran et al. (2001)
23	UK	14.02 − 15.38	Pirgozliev et al. (2003)
20	Norway	12.25 − 14.10	Hetland et al. (2007)
94	UK & Northern Ireland	12.75 − 14.70	Ball et al. (2013a)

The high variability in AME of wheat could influence growth performance of broilers. However, the relationship between AME and growth performance is inconsistent (Scott et al. 1998a; Steenfeldt 2001; Svihus and Gullord 2002; Pirgozliev et al. 2003). Steenfeldt (2001) investigated the effect of 16 wheat cultivars on AME and growth performance of broilers and found a poor correlation between growth performance and AME. Svihus and Gullord (2002) found a significant negative correlation ($r = -0.70$, $P < 0.05$) between AME and feed intake of broilers. Pirgozliev et al. (2003) found a negative correlation between AME of wheat and feed intake, however, there was no relationship of AME with weight gain of broilers. Based on inconsistent or lack of relationships between AME of wheat and growth performance of broilers, various authors suggested that care should be taken using AME alone to predict the nutritive value of wheat and wheat-based diets.

The large variability in the AME of wheat is challenging for nutritionist to predict the accurate feeding value of wheat. An understanding of the factors contributing to the variation in the availability of energy of wheat is very important for better utilisation of wheat in poultry diets and for efficient broiler production. The AME of the wheat depends both on content and digestibility of starch, protein and lipids (McCracken and Quintin 2000; Svihus and Gullord 2002; Wiseman et al. 2000; Carré et al. 2007). Wheat being variable in its gross content (e.g., starch and protein) and anti-nutritive content (NSP) would result in variation in its AME. Previously, the reasons of low AME in some Australian wheats were caused by the presence of total and soluble NSP contents (especially arabinoxylans) (Mollah et al. 1983; Rogel et al. 1987a; Choct et al. 1995, 1996).

There are inconsistent reports of relationship between wheat chemical composition (especially major energy yielding component starch, protein) and AME (McCracken and Quintin 2000; Pirgozliev et al. 2003; Gutierrez et al. 2008a). McCracken and Quintin (2000) reported significant positive correlation between starch content of wheat and AME. In contrary, McCracken et al. (2002) found no relationship between starch content of wheat and AME. A study by Pirgozliev et al. (2003) found that the amount of starch, protein and fat content of wheat samples in combination were significantly correlated with the AME of diets. Svihus and Gullord (2002) found positive correlation between AME and starch and fat contents of wheat, and a negative correlation between AME and protein content. Starch is the main energy yielding component of wheat grain but according to various broiler studies based on wheat-based diets, the relationship of starch with AME was not consistent. Instead of starch content, digestibility of starch in wheat-based diets was more closely associated with AME (Wiseman et al. 2000; Carré et al. 2005). Rogel et al. (1987a) found significant differences in starch digestibility and AME of 38 Australian wheats and there was a positive relationship between starch digestibility and AME ($R^2 = 0.85$, $P < 0.05$). A study by Choct et al. (1999) found significant differences in AME of Australian wheats and

revealed that there were significant negative correlations between AME and total, soluble and insoluble NSP content of wheat samples. The authors found no correlation between AME and starch content of wheat samples. Ball et al. (2013a) found a negative correlation between AME and total and soluble NSP contents of wheat samples collected from different locations across the UK.

There are also reports of inconsistent relationships between physical characteristics of wheat and AME (McCracken et al. 2002; Wiseman 2000; Hetland et al. 2007). Wiseman (2000) found variation in the AME of ten different wheat samples grown at five different location from 8.49 to 12.45 MJ/kg DM, however, study was unable to establish any relationship between specific weight (SW), thousand grain weight (TGW) and AME of wheat for broilers. Svihus and Gullord (2002) also found no relationship between TGW, SW of wheat samples and AME content.

In conclusion, AME values of wheat are variable and variability could be due to various factors including chemical composition especially polysaccharides content of wheat and their digestibility. Moreover, the relationship between AME of wheat and starch is not consistent, instead, AME is strongly related with starch digestibility in broilers. Therefore, there is a need to understand the factors influencing the AME values of currently available wheats for broiler diets and how the variation in AME values relates to chemical composition and physical characteristics of wheat.

1.2.2. Starch availability

Wheat starch is highly digestible by adult birds (up to 95% of available starch). Most of the studies with UK wheat samples have found starch digestibility coefficients ranged between 0.90 – 0.95 or even more (Longstaff and McNab 1986; Weurding et al. 2001; Hetland et al. 2002, 2003). Studies with the European wheat samples have found starch digestibility coefficients within 0.9 – 1.0 in young and adult birds (Carré et al. 2005; Steenfeldt et al. 1998b). Low starch digestibility in some Australian wheat-based studies were reported previously, which was speculated due to anti-nutritional properties of soluble NSP (Mollah et al. 1983; Rogel et al. 1987a; Choct et al. 1995). Studies by Mollah et al. (1983) and Rogel at al. (1987a) found starch digestibility in broilers ranges between 80 to 99% and 82 to 100%, respectively. In both studies, starch digestibility in wheat-based diets was highly correlated with AME, suggesting that variation in starch digestibility was responsible for low AME. Similarly, Carré et al. (2005) also found up to 10% variation in starch digestibility in wheat-based diets.

The starch digestibility in wheat-based diets also depend on the type of diets fed to broilers (mash, pellet, cold pelleted diet). Starch digestibility was found to be low when broilers were fed cold pelleted diets (without using any steam). Svihus (2001) reported low starch digestibility coefficients (0.76 – 0.83) in broilers fed cold pelleted wheat-based diets. However, Svihus (2001) also reported high ileal starch digestibility up to 95% when broilers were fed wheat-based mash diets. Steenfeldt et al. (1998b) reported a high ileal starch digestibility (97 – 99%) in broilers fed mash diets containing different wheats. A study by Abdollahi et al. (2011) found a significant reduction (14%) in the ileal starch digestibility coefficients of a wheat-based diets when broiler chickens were switched from mash diet (0.96) to a pellet diet (0.84). A review of ileal starch digestibility of wheat-based diets in broilers is presented in Table 1.11.

Table 1. 11. Ileal starch digestibility in broilers fed wheat-based diets at different age of growth.

Age of bird	Ileal starch digestibility	Reference
29	0.95	Choct et al. (1999)
21	0.79	Svihus (2001)
21	0.79	Svihus and Hetland (2001)
29	0.94	Weurding et al. (2001)
24	0.97	Hetland et al. (2002) *
38	0.94	Hetland et al. (2002) *
33	0.97	Hetland et al. (2003)
25	0.95 - 0.98	Svihus et al. (2004)
21	0.81 - 0.98	Abdollahi et al. (2011)
21	0.92 - 0.98	Abdollahi et al. (2013)

*Ileal starch digestibility at different age in same broiler study.

Starch digestibility varies due to the structure of starch (shape and size of granules), the proportion of amylose to amylopectin, interaction of starch with protein and processing conditions during feed manufacturing (Svihus et al. 2005; Singh et al. 2007; Svihus 2014). Wheat containing more regular endosperm organisation and a greater number of type “A” starch granules can be digested quickly and readily gelatinised in gut (Zaefarian et al. 2015). Hard, regular endosperm structure tend to result in uniform availability of these starch granules for enzymatic breakdown. The larger “A” type granules are more easily ruptured during feed processing and digestion in birds because of their surface to volume ratio,

therefore, starch contained in those granules will be more readily available for hydrolysis by enzyme. The smaller B and C type granules are more difficult to rupture and often escape digestion due to their size and small surface to volume ratio (Svihus et al. 2005; Zaefarian et al. 2015).

The digestibility of cereal starch is dependent upon its amylose and amylopectin content (Rogel et al. 1987a, b; Pirgozliev et al. 2002; Svihus 2014). Amylose is considered to be more digestible than amylopectin because of its simple linear molecule structure. The branched structure of amylopectin makes it poorly digestible by α-amylase which results in larger digestion products which must be hydrolysed before they can be absorbed from intestinal lumen. Supplementation of exogenous enzymes (xylanases and β-glucanases) in wheat and barley-based diet, respectively are known to increase starch digestibility (Svihus and Gullord 2002; Gutierrez del Alamo et al. 2008a).

Starch hydrolysis is carried out mostly by pancreatic α-amylase in the duodenum and jejunum. It hydrolyses most of the α-(1—4) glycosidic linkages in amylose and amylopectin. Amylose is hydrolysed into maltose and maltotriose, while amylopectin is degraded to maltose, maltotriose and α-dextrins (Moran 1982). These molecules are water soluble but cannot pass through the intestinal wall. They are further hydrolysed by maltase and isomaltase located at the brush border membrane of enterocytes. Glucose is absorbed from the small intestine and transported across the intestinal wall by a specific glucose carrier which depends on the presence of Na+ in the lumen. The driving force behind this transport is the Na-K pump which pumps the Na+ back again into the lumen (Gray 1992). Some of the glucose absorbed is oxidised and serves as energy while the remainder is transported by the portal vein and supply energy to other tissues or stored as glycogen or fat for further energy requirements.

1.2.3. Non-starch polysaccharides availability

Non-starch polysaccharides are carbohydrate fractions excluding starch and free sugars. These are polymeric carbohydrates differing in structure and composition from amylose and amylopectin.

Dietary fibre is categorised into insoluble dietary fibres (IDF) and soluble dietary fibre (SDF), based on their solubility in water. Most of the NSP present in feed ingredients are insoluble in water because they are attached to the cell walls by alkali-labile ester like cross links (Choct 1997). Therefore, insoluble NSP act as a physical barrier to the digestive enzymes in poultry. Poultry lack endogenous enzymes to hydrolyse the cell wall; and because starch and protein may be surrounded by cell walls in the lumen, it may escape digestion or slow down decrease digestion. The insoluble NSP make up the bulk of total fibre in the diet, but

they have no or little effect on nutrient utilisation in monogastric animals (Annison 1991; Angkanaporn et al. 1994). It is evident from earlier work that insoluble NSP enhances GIT development in poultry (Choct 2006, Hetland et al. 2007). Previous studies have demonstrated that performance of broilers and nutrient digestibility were not affected when diets were supplemented with moderate level of insoluble fibre (Hetland and Svihus 2001; Hetland et al. 2002, 2003). It has been suggested that appropriate ratio between soluble and insoluble NSP is important to reduce the negative effect of soluble NSP (Choct 1997). The IDF in monogastric diets is generally considered as a diluent of nutrients (Edwards 1995) and since it is not digested in the bird's digestive tract, it tends to reduce the AME of the diet. The IDF has been reported to improve gut health, gut microbial profile and digestive organ development which consequently may increase the digestibility of non-fibre components (Rogel et al. 1987a; Hetland et al. 2003, 2004; Amerah et al. 2009a; González-Alvarado et al. 2010; Svihus 2011). Therefore, the AME of the whole diet depends on the balance between negative and positive effects of IDF on AME. If the balance is positive, feed intake is reduced in order to adjust to the ME intake requirement.

Soluble NSP usually refers to the portion of NSP which is soluble in water and is an important physicochemical feature of the anti-nutritional properties of NSP for monogastric animals, especially poultry. The antinutritive effects of soluble NSP in poultry diets are well recognised (Choct and Annison 1990, 1992; Choct 2006). Soluble fibres with high molecular weight are found in cereal grains and increase intestinal viscosity, altering the digesta passage rate, microbiota, metabolites and efficacy of digestion. Viscosity is not related to linkage type or the sugar composition of polysaccharides, rather, it is determined by the physical properties of the polysaccharides, such as molecular weight, distribution and structure. Soluble DF including arabinoxylans (wheat, maize, sorghum, rye millet, and triticale) and β-glucans (barley and oat) reduced the digestibility of nutrients especially starch (Choct and Annison 1992a, b; Choct 1997). The soluble NSP can cause an increase in digesta viscosity in the small intestine and inhibit digestion and absorption of nutrients. High gut viscosity caused by soluble NSP result in slower passage rate of digesta (increased residence time of digesta in small intestine) and changes in gut microflora balance which ultimately result in a stable environment for microbial proliferation in the gut (Van der Klis et al. 1993). Diets with high level of soluble NSP result in general inhibition of nutrients in the diet. Choct and Annison (1992a) revealed that the addition of soluble NSP isolated from wheat to a broiler diet reduced the digestibility of starch, protein and lipid by 14.6, 18.7 and 25.8%, respectively. The detailed mechanism of soluble NSP and its anti-nutritive effects are discussed in Section 1.3.1.

1.2.4. Protein and amino acid availability

The nutritive value of protein in feed ingredients is determined by the total content and availability of AA. In modern feed manufacturing, feed formulations are based on AA content rather the total protein content, as birds have requirements for AA not for protein *per se*. The accurate knowledge regarding the digestibility of cereals AA is critical for precise feed formulation for poultry and also for sustainable use of feed ingredients. The purpose of describing digestible AA is superior rather than on a total AA content of feed ingredients because it represents the amounts that is actually available to the birds for maintenance and production (Lemme et al. 2004).

The analytical methods used to determine AA digestibility are also critical for the evaluation of digestibility values in broilers. The studies on AA digestibility in broilers needs to be carefully evaluated as different methodology were in practice in studies. Ravindran et al. (1999) compared ileal digestibility with excreta digestibility and found that ileal digestibility in broilers is higher than the excreta digestibility. Ileal digestibility is a more accurate method of determining the nutrient digestibility because of inaccuracies in the excreta method and occurrence of contamination in excreta collection (Ravindran et al. 1999). Excreta digestibility method is simpler and does not require birds to be slaughtered. However, digestibility measurements on excreta are criticised because of possible effects of avian hindgut microorganism on protein utilisation and contamination of microbial protein to amino acid secretion in faeces (Parsons et al. 1982; Ravindran et al. 1999). Excreta analysis does not measure AA digestibility as it defines, instead it determines AA metabolisability because faeces and urine are voided together in birds. Ileal digestibility of AA is a widely used method to determine AA digestibility in poultry. In this method, digesta are recovered from the distal part of ileum and analysed for AA content. This method eliminates error originating from urine AA and the modifying effect of hindgut microbiota. Ileal digestibility of AA is calculated in reference to indigestible markers. In poultry, chromium dioxide (Cr_2O_3), celite as a source of acid insoluble ash (AIA) and titanium dioxide ($TiO_{2)}$ are routinely used as indigestible markers.

Lysine and threonine are the least digestible AA among indispensable AA especially when broilers are fed wheat-based diets. Previous works have shown that ileal digestibility of AA in broilers fed wheat-based diets varies (Table 1.12). The AA digestibility of different cereals varies in broiler chickens. Lysine and threonine have low apparent digestibility than other AA in wheat protein. Low lysine and threonine digestibility in wheat, maize and sorghum was reported by McNab (1991) and Ravindran et al. (1999). The AA digestibility in broilers varies depending upon the age of the birds and feeding pattern of assay diets. Haung et al. (2005) found that age of broilers influenced the apparent AA digestibility of different cereals

grains in broilers. The authors reported that AA digestibility coefficient of wheat in broilers at the age of 14 days were higher than in those at 28 and 42 days, whereas, those fed maize, the AA digestibility was higher at 28 and 42 days of the age. The AA digestibility of sorghum was higher at 42 days than those at 24 days of age. The authors concluded that the AA digestibility coefficient determined at 42 days of age in broilers fed wheat and maize can be applied for the feed formulation at 28 days broilers but not those at 14 days of age.

Ravindran et al. (1999) compared ileal digestibility of wheat, maize and sorghum with excreta digestibility. The authors found that the ileal digestibility values of wheat, maize and sorghum were higher as compared to excreta digestibility. However, they found low lysine (0.77) and threonine digestibility (0.69) coefficient values in wheat-based diets fed to broilers. The authors also found low digestibility values of lysine and threonine in maize (0.74, 0.62) and sorghum-based diets (0.75, 0.66) in broilers. The level of protein also affects the AA digestibility in broilers fed wheat. Ravindran et al. (1998; 2005) reported amino acids in high protein wheat cultivars are more digestible than those in low protein cultivars.

Table 1. 12. Apparent ileal digestibility of amino acids of wheat for broilers.

	Ileal digestibility			
Amino acids	Ravindran et al.1999[a]	Hew et al. 1998[a]	Haung et al. 2005[a]	Kadim et al. 2002[b]
Indispensable				
Threonine	0.69	0.65	0.59	0.49
Valine	0.81	0.77	0.74	0.69
Methionine	0.85	0.86	0.75	0.85
Isoleucine	0.84	0.80	0.78	0.75
Leucine	0.86	0.84	0.81	0.72
Phenylalanine	0.87	0.80	0.82	0.75
Histidine	0.83	0.81	0.70	0.70
Lysine	0.77	0.75	0.64	0.81
Arginine	0.81	0.81	0.74	0.70
Dispensable				
Aspartic acids	0.75	0.72	0.66	0.56
Serine	0.81	0.78	0.72	0.72
Glutamic acids	0.94	0.93	0.89	0.90
Alanine	0.79	0.77	0.69	0.55
Tyrosine	0.73	0.73	0.61	0.65

[a] Digestibility at 42 days of age.
[b] Digestibility at 34 days of age.

1.2.5. Lipids availability

Changes in the lipids occur during the storage of wheat however, under suitable storage conditions, changes are very slow. During humid condition, changes in lipids are faster and it can result in the deterioration of feeding and baking quality of wheat. The fat digestibility (FD) of wheat ranges from 0.70 – 0.80 depending upon diet form, inclusion level of wheat, level of NSP and increase in viscosity (Steenfeldt 2001; Carré et al. 2002; 2007). Steenfeldt (2001) found that diets containing high levels of wheat (815 g/kg) resulted in a low FD coefficient (mean value of 0.67), whilst diets containing less wheat inclusion (650 g/kg) level was found to have high FD (mean value of 0.73). The author also found high variability (21%) in FD of diets containing higher level of wheat and concluded that the reason for low FD was due to increased level of arabinoxylans in diets with higher wheat inclusion.

Researchers ascribed that variation in soluble NSP content of wheat affect the lipid digestibility in broilers. Variation in lipid digestibility have been attributed to increase *in vitro* water extract viscosity caused by soluble AX in wheat. Carré et al. (2002) found 15% variation in lipid digestibility in broiler diets containing 50% wheat. The authors also found a negative correlation between wheat soluble AX, *in vitro* water extract viscosity and lipids digestibility. In a study by Choct and Annison (1992b), the addition of wheat pentosans (AX) to cereal based broiler diets resulted in a decrease in AME and FD and decrease in FD was due to increased digesta viscosity. In conclusion, there are variations in FD of wheat in broilers and these variations are mainly caused by the level of AX in wheat and increased viscosity caused by soluble AX.

1.2.6. Phytate-Phosphorus availability

Phosphorus (P) is stored in the form of phytate in plant seeds and is poorly available for pigs and poultry. Phytate is a polyanionic molecule with the potential to chelate positively charged minerals especially calcium, iron and zinc, which is almost certainly fundamental to the anti-nutritive properties of phytate. The phytate protein interaction in the upper digestive tract at low pH has been considered as compromising the utilisation of protein/amino acids, energy, calcium, sodium and trace minerals (Selle and Ravindran 2007; Adeola and Cowieson 2011; Humer et al. 2014). The un-degraded phytate-P is excreted in the environment and contributes to pollution, especially in the areas where poultry production is intense (Smith et al. 1999; Tayyab and McLean 2015).

Three different terms are used in the literature to describe phytate which are phytate, phytic acid and phytin. The most commonly used term, phytate, refers to the mixed salt of phytic acid (*myo*-inositol hexaphosphate; IP_6). The term ‘phytin’ is the insoluble mixed phosphorus, potassium, magnesium, and calcium salt of *myo*-inositol hexaphosphoric acid and it occurs in plant materials, whereas phytin is free from IP_6 (Selle and Ravindran 2007).

Phosphorus is crucial for skeletal integrity and growth performance, therefore, formulating an optimum level is essential for broiler growth and for a sustainable environment. The requirements of non-phytate-P in broilers diets are different at various stages of growth and range from 4.5 g/kg (0 – 3 weeks), 3.5 g/kg (3 – 6 weeks) to 3.0 g/kg (6 – 8 weeks) (NRC 1994). In order to efficiently utilise P in plant material by poultry, phytate must be hydrolysed into inorganic phosphate. Poultry can utilise phytate-P provided there is active phytase available in the diet. Phytate-P is largely unavailable for utilisation by poultry due to a lack of an effective endogenous phytase.

Phosphorus in wheat is mostly present as phytate and there is wide variation in phytate level depending upon wheat cultivars and growing condition. Selle et al. (2003) found variation in phytate-P level in Australian wheats ranging from 1.35 to 3.20 g/kg. The content of phytate in commonly used different cereals and feedstuffs for poultry varies (Table 1.13).

Table 1. 13. Phytate-P content and availability for broilers in common feed ingredients.

Ingredient	**Phytate-P** g/kg	**Phytate-P as** % of total P
Wheat	2.4 (1.9 - 2.9)	68 (61 - 78)
Maize	2.0 (1.6 - 2.6)	73 (61 - 85)
Sorghum	2.2 (1.9 - 2.9)	68 (61 - 76)
Oats	2.8 (1.6 - 3.5)	69 (48 - 78)
Barley	2.1 (1.9 - 2.4)	58 (55 - 62)
Wheat bran	8.8 (6.0 - 12.7)	76 (68 - 93)
Soybean meal	3.7 (2.8 - 4.0)	57 (46 - 61)
Canola meal	6.5 (4.6 - 7.8)	58 (36 - 70)

(Source: Ravindran 1995)

1.3. ANTI-NUTRITIONAL PROPERTIES

1.3.1. Non-starch polysaccharides

The negative effects of NSP (mainly AX) on nutrients utilisation and bird performance are well established and widely discussed (Bedford 2006; Choct 1997, 2006). Bedford and Schulze (1998) proposed two key mechanisms to explain the anti-nutritional activities of NSP. The first mechanism is associated with the insoluble NSP acting as a physical barrier. Starch and protein are encapsulated by the cell wall in wheat endosperm cells. Cell wall is composed mainly of cellulose, hemicellulose (mostly AX), pectin and lignin. Most of the AX in wheat are insoluble and are not available to bird for digestion because they are attached to the cell walls by alkali-labile ester like cross links (Choct 1997), hence can act as a physical barrier to digestive enzymes. The endogenous enzymes in the birds are unable to hydrolyse the cell wall, so the starch and the protein surrounded by cell wall may thus escape digestion.

The second mechanism is associated with the increment in viscous nature of digesta due to soluble NSP and their interaction with the gut microflora. The degree of viscosity depends on the solubility of NPS and their molecular weight. The solubility of NSP depends on the chemical structure of NSP (linear or branched) and their association with the rest of cell wall components. High intestinal digesta viscosity can cause various negative effects (Van der Klis et al. 1993; Smits and Annison 1996; Choct 1997; Bedford and Schulz 1998):

- Reduction in passage rate of digesta in the intestine (Edwards et al. 1988; Bedford and Classen 1992).
- Reduced mixing of digestive enzymes with the substrates (Choct 1999).
- Increased secretion of endogenous enzymes and modification of the endogenous secretion of water, electrolytes, proteins (amino acids) and lipids (Johnson and Gee 1981; Angkanaporn et al. 1994). Changes in the gut function also result in enhanced digestive secretions and decreased nutrient absorption.
- Higher soluble NSP content increase the resident time of digesta in the gut (due to slower rate of digesta in the gut), which may decease oxygen tension in the small intestine and result in the proliferation of anaerobic microflora (Van der Klis et al. 1993; Smits and Annison 1996). The slower passage rate increases the time available for digesta associated bacteria to multiply in the small intestine, coupled with reduce rate of digestion by the host.

- Increased relative weights of digestive organs due to higher digesta viscosity, which can increase metabolic cost of maintaining the gut (Choct 1997).

The high viscous intestinal environment, slower rate of feed passage and presence of significant amounts of undigested materials are also likely to lead to the proliferation of microflora in the small intestine. Microflora in the gut ferment and utilise the carbohydrates and protein, thus competing effectively with the host for nutrients (Bedford 1995; Choct et al. 1996). The anti-nutritional effects of NSP has supported the viscosity mechanism in the literature extensively caused by soluble fraction, but both mechanisms (insoluble and soluble) are likely to be involved (Bedford 2006; Cowieson et al. 2006). The proposed effects of NSP on gut and metabolic parameters of birds fed wheat-based diets are summarised in Figure 1.7.

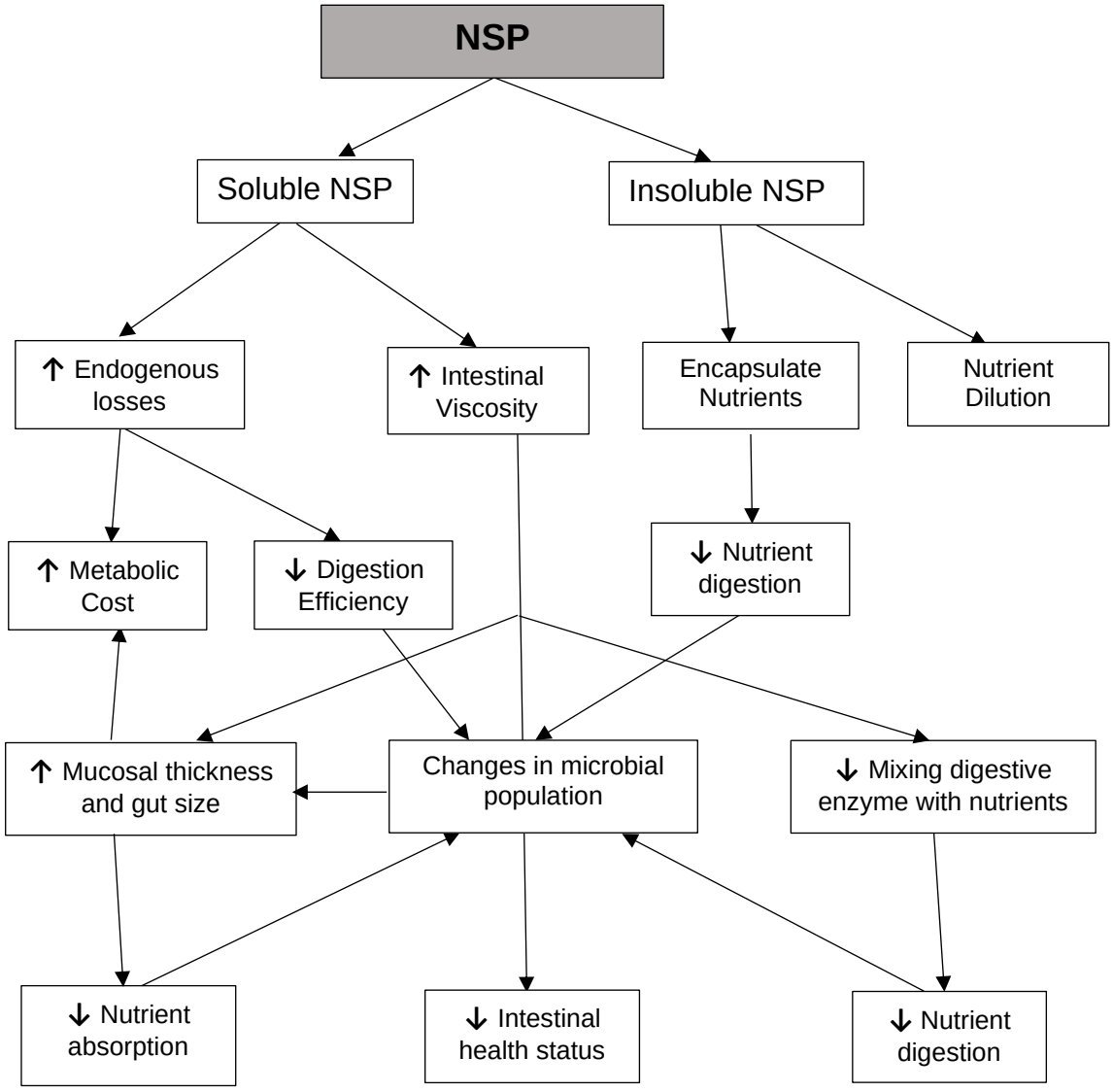


Figure 1. 7. Mode of action of non-starch polysaccharides (NSP) of wheat in poultry. (Source: adapted from Ravindran and Amerah 2009)

In poultry, high soluble NSP are well known for their anti-nutritive activities and reduce nutrient digestibility, AME and bird's performance (Choct and Annison 1990, 1992a, b; Choct et al. 1995; Choct 2006). The anti-nutritive activities of soluble NSP are manifested through the inhibition of starch, protein and lipid in small intestine (Choct and Annison 1992). Choct and Annison (1992a) revealed that the addition of soluble NSP (isolated from wheat) to broiler diets reduced the digestibility of starch, protein and lipid by 14.6, 18.7 and 25.8%, respectively. Rogel et al. (1987a) found a large variation in intestinal starch digestibility (0.818 – 0.999) of broilers fed 38 Australian wheat samples and attributed variation to soluble NSP content of wheat. In contrary, Carré et al. (2002) reported that viscosity cannot be proposed for reduction in total tract starch digestibility of wheat-based diets in broilers. It can be anticipated that the extent of reduction in starch digestibility due to viscosity variations is low. In practical feed formulations, the anti-nutritional activities caused by NSP of wheat have been largely overcome by the use of appropriate exogenous enzymes.

1.3.2. Phytate

Phytate molecule in cereal grains is considered as anti-nutritive and its presence in the ileum results in the significant loss of endogenous nutrients (amino acid) (Cowieson et al. 2004) and energy in the form of mucin, intestinal cells and pancreatic enzymes (Selle and Ravindran 2007). The anti-nutritional effect of phytate in wheat is evident by reducing nutrient utilisation, resulting in increases in the production cost and also impacting the environment (Selle and Ravindran 2007). The phytate P utilisation in poultry ranges from 0 – 50% depending upon age of the birds, ingredients, dietary level of Ca, P and vitamin D (Ravindran et al. 1995). The hydrolysis of phytate in birds improves the productive performance, the availability of P and the sparing effect of these dietary components which ultimately results in better utilisation of net energy component of the diet (Selle et al. 2003; Selle and Ravindran 2007).

1.4. ENZYME SUPPLEMENTATION TO IMPROVE THE FEEDING VALUE OF WHEAT

Enzymes have been used in the animal feed industry over the past 40 years and their use is increasing in commercial poultry production to improve productive performance and reduce the variability within feedstuff used in poultry diets. The addition of exogenous enzymes to poultry diets results in improvements in utilisation of nutrients, energy contents of the diet, intestinal health and gut function and litter quality (Bedford 2000; Choct 2006; Cowieson et al. 2006). The primary aim of using exogenous enzymes is to improve bird performance and productivity and this is achieved through enhanced digestion of dietary components e.g., protein, amino acids, starch, lipids and consequently energy in feed ingredients. Enzyme are also added in poultry diets because of their additional benefits including:

- The enzyme addition increases flexibility in the selection of feedstuffs in least-cost feed formulations, e.g., the addition of xylanase, glucanase to wheat and barley based diets, respectively.

- Enzymes help to reduce variability in nutritive value between different batches of feed ingredients e.g., wheat, barley. Enzyme can improve the nutritive value of poor quality ingredient, thus reducing the variability between a poor and a good sample of particular feed ingredient (Classen et al. 1995; Scott et al. 1998a; Bedford 2000). As a result, variation in subsequent bird performance is reduced and improve flock uniformity and also more uniform production from flock to flock.

- Decrease moisture contents of excreta and reduce wet litter problem in birds fed high level of NSP in the diet. Wet litter can increase incidence of foot lesions, hock burn and carcass downgrading (Collett 2012).

- The positive effect of enzyme addition results in improved digestion and absorption of nutrients (starch and protein) in the gut and fewer amount of undigested material reaching in the lower part of the gut and large intestine (Cowieson and Bedford 2009).

- Improvement in digestion and absorption of nutrients result in favourable microflora in the gut which cause an improvement in gut health, intestinal morphology and overall positive effect on bird health (Yegani and Korver 2008; Bedford and Cowieson 2012).

- Better utilisation of nutrients results in lower excretion of phosphorus and nitrogen in the litter, ultimately a positive impact on environment and improved production efficiency (Choct 2006).

The mode of action of enzymes are highly substrate specific. Each enzyme breaks down specific substrates in the feed ingredients at a specific reaction site. Therefore, to achieve maximum benefit from enzyme supplementation, enzymes should be selected based on the substrates in the feed ingredients. The effectiveness of enzymes depends on various factors such as specific reaction site, moisture content, pH, temperature, enzyme concentration and substrate level in the feed ingredient (Bedford 2000).

There are different types of enzymes currently used in poultry diets and their usage is dependent on the feed ingredients and their substrates, falling into two broad categories. First the use of phytase to improve the availability of P and also to alleviate the negative effect caused by P excretion in the environment (Choct 2006; Adeola and Cowieson 2011). The second group aids or enhances the digestion of the various feed components of the diet that supply protein and energy to the birds. The second group is also the group containing fibre degrading enzymes to improve the availability of nutrients and mitigate the adverse effects of anti-nutritional factors caused mainly by NSP. This group includes xylanase, β-glucanase, proteases, amylase, mannanase and lipase. The distinction is based on the site of activity within the gastrointestinal tract (GIT) of the chicken. A list of commercially available enzymes mainly used in poultry diets to improve nutrient availability and digestion of feedstuffs is presented in Table 1.14.

Table 1. 14. Types of commercial feed enzymes and target substrate in different feed ingredients.

Enzyme	Target Substrate	Target Feedstuffs
Phytases	Phytic acid	All plant-derived ingredients
Xylanases	Arabinoxylans	Wheat, rye, barley, triticale, maize, fibrous plant materials
β-Glucanases	β-Glucan	Barley, oats, rye
Amylase	Starch	Cereal grains, grain legumes
Lipase	Lipids	lipids in feed ingredients
Proteases	Proteins	All plant protein sources
α-Galactosidases	Oligosaccharides	Soybean meal, grain legumes
Mannanases, cellulases, hemicellulase, pectinases	Cell wall matrix (fibre components)	Plant-derived ingredients, fibrous plant materials

(Source: Ravindran 2013)

Commercially available enzymes may contain single enzyme or a blend/cocktail of several enzymes that contain guaranteed level of certain enzymes. Phytase is widely used in wheat or maize-based diets. Commercially available phytases act largely at low pH environments (pH 4 – 5) which means they are active in in upper digestive tract, mainly the crop, gizzard and proventiculus (with the crop being the first site of phytase activity). Carbohydrases and proteases act in the neutral environment of lower digestive tract at pH 6.5 – 7.5. This means that if a mixture of enzymes is supplemented in the diet, phytase will be the first enzyme to be active before other enzymes.

1.4.1. Phytase

Phytase has been used in poultry diets since 1990s as a feed enzyme to release the phytate bound P and to reduce the release of phosphorus levels in effluent from intensive poultry units (Ravindran et al. 1995). The addition of phytase to inadequate P diets have shown improvement in growth performance and reduction in P excretion in litter. The response to phytase supplementation is variable depending on nutrient specification, phytate content in feed ingredient and inclusion level of phytase in the diet (Selle and Ravindran 2007). In broilers, the response to phytase supplementation also varies depending on feed ingredient used in diet. Ravindran et al. (1999) found that phytase addition increased ileal digestibility of essential AA in wheat (9.2%) as compared to maize-based diet (3.3%). Similarly, Rutherford et al. (2002) found an improvement of 13.4% in essential AA digestibility in wheat whereas, there was less improvement in maize (3.9%). The addition of phytase (600

FTU/kg) to wheat-based diets in broilers improved AME of wheat by an average of 0.5 MJ/kg DM as compared to non-supplemented control diets (Ravindran et al. 1999; Selle et al. 2003, 2005). However, the data on phytase response needs to be carefully evaluated as variable responses in broilers have been documented depending on the inclusion level of phytase and the content of non-phytate-P. In a study by Simons et al. (1990), phytase addition (1500 FTU/kg) to diets containing 4.5 g/kg total P improved weight gain from 338 to 733 g and feed conversion ratio from 1.85 to 1.50 of broilers between 0 to 24 days of age. Cabahug et al. (1999) reported that a phytase addition of 400 FTU/kg to a wheat and sorghum-based broiler diet showed maximum improvements in feed intake (9.0%), weight gain (18.8%), and feed efficiency (7.9%) and further inclusion of phytase (800 FTU/kg) showed little improvement in growth performance.

1.4.2. Xylanase

Xylanases have the ability to degrade either soluble or insoluble arabinoxylans. Commercially available xylanases have broad spectrum activity on both soluble and insoluble arabinoxylans in the feed (Ravindran and Amerah 2009). The xylanase addition improves energy and nutrient utilisation of wheat-based diets by the degrading NSP in the cell wall matrix, release of encapsulated starch and protein, reduction in digesta viscosity, increase accessibility of nutrients to endogenous enzymes, improvement in feed passage rate and stimulation of intestinal motility (Bedford and Schulze, 1998; Cowieson et al. 2006; Choct 2006). The variation in broiler performance caused by the differences in chemical and physical structure of wheat can be reduced by the supplementation of xylanase (Bedford 1996, 2000; Choct 2006).

Xylanase supplementation to broiler diets is a common practice to alleviate the adverse effects of NSP and to minimise the variation in AME and performance of poultry fed wheat-based diets (Choct et al. 1995a; Bedford 1996; Adeola and Cowieson 2011; Kiarie et al. 2014). However, the response of broiler to enzyme supplementation in wheat-based diets is variable. Gutierrez del Alamo et al. (2008a) reported no influence of xylanase supplementation on growth performance of broilers fed different wheat cultivars. The addition of xylanase could not eliminate the differences in growth performance of broilers however, improved starch digestibility and AMEn. Factors that may cause variation in the response of broilers to xylanase addition include: the type of xylanase, quality of wheat and breed and age of birds (Bedford 1997). The response of xylanase in broilers may also depend on the hardness of endosperm (hard, soft) of wheat. Amerah et al. (2009b) reported improvements in feed per gain (FCR) and AMEn in broilers fed diets containing wheats based on hard endosperm, however there was no improvement in those fed diets containing soft wheat.

A study by Steenfeldt (1998a) showed improvements in weight gain and FCR of broilers fed wheat-based diets supplemented with xylanase and also reduction in jejunal and ileal digesta viscosity. The response of xylanase addition in chickens fed wheat-based diets also depends on form of wheat inclusion (whole wheat or ground). A study by Wu and Ravindran (2004) reported that xylanase addition improved weight gain of broilers fed whole wheat and ground wheat, however improvement in ground wheats were significantly higher than those fed whole wheats. The researchers also observed a significant wheat form × xylanase interaction for feed intake and xylanase supplementation increased feed intake of those fed ground wheat diets, whereas, reduced in those fed whole wheat diets. The authors suggested that the improvement in weight gain of birds feeding whole wheat inclusion can be enhanced by the use of xylanase and this was associated with larger gizzard weight, increased grinding activity of gizzard and enhanced mixing of substrate with supplemented xylanase. Cowieson and Masey O' Neill (2013) found an improvement in FCR and weight gain of broilers fed diets supplemented with xylanase. There was also an improvement of 0.35 MJ/kg in ileal digestible energy. Gao et al. (2008) reported improvement in weight gain and FCR of broilers between 7 – 49 days of age fed wheat-based diets supplemented with xylanase and a significant reduction in viscosity of proventiculus and the jejunum. Overall, xylanase supplementation has been successfully used in wheat-based diets to reduce variation in AME and improvement in nutrient availability and growth performance of broilers.

1.5. THE UK POULTRY INDUSTRY

The EU is one of the largest poultry meat producers. In 2018, the EU had a record poultry meat production of 15.2 million tonnes (Mt) with an increase of 3.2 Mt since 2010. Six member states in the EU produced about 70% of total poultry meat in 2018 (Eurostat 2018b, c). Poland is the largest producer of poultry meat (16.8%). The UK is the second largest producer of poultry meat (12.9%), followed by France (11.4 %), Spain (10.7 %), Germany (10.4 %) and Italy (8.5 %) (Figure 1.8). The demand for poultry meat is growing year after year and it is expected that in 2019, poultry meat production will increase by 2.5%. Increased consumer preference assisted by the poultry being a more affordable meat protein and high levels of efficiency with the lower production costs have contributed to continued growth in the EU member states.

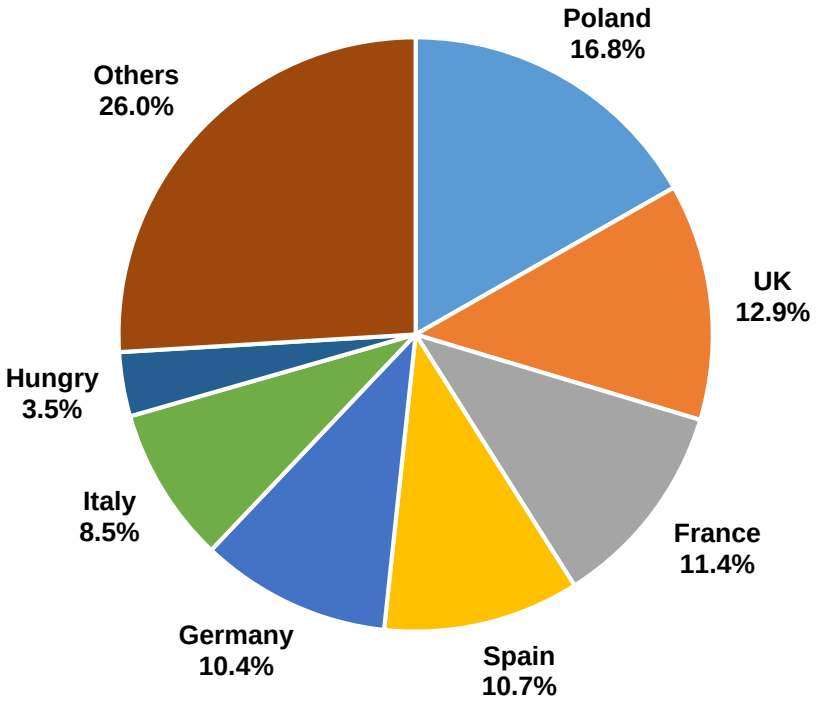


Figure 1. 8. Poultry meat production: % share of EU-28 total, based on carcass weight.

Source: (Agriculture production-livestock and meat, Eurostat 2018b; Poultry meat production, Eurostat 2018c)

In the UK, production of poultry meat has increased significantly during last 20 years, and this shows consumer preference and confidence in buying poultry meat over other meat types. Broiler meat production in the past few years had benefitted from the continued move towards leaner meat by the UK consumers. In 2018, total poultry meat production was 1937 thousand tonnes (Tt) with an increase of around 5.4% against 1837 Tt in 2017 (DEFRA 2019a, Eurostat 2019). Broilers are the largest contributor (86.4%) to poultry meat in the UK, whereas, there is also some contribution by turkeys (8.1%), ducks (1.6%) and boiling fowl (3.9%), respectively (DEFRA 2019a). In the UK, due to high demand of chicken meat, broiler production is on the rise during the last two decades. In 2018, broiler meat production was at the highest, with a record production of 1673 Tt, an increase of 7.7% as compared to 2017 (Figure 1.9). The average live weight of broilers is 2.2 kg/bird at the time of slaughter (DEFRA 2019a).

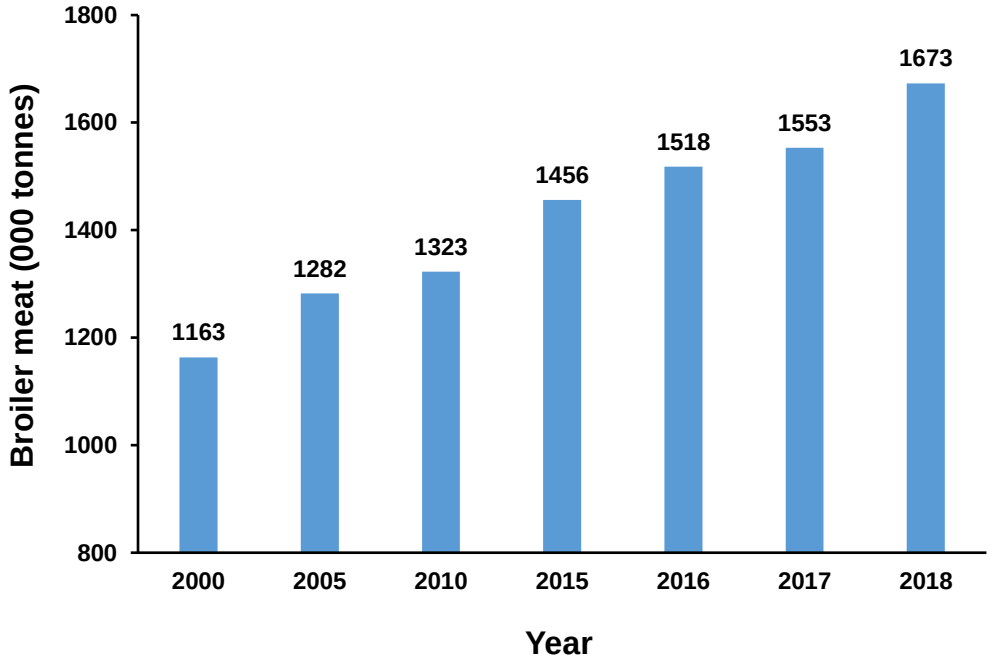


Figure 1. 9. Broiler meat production (thousand tonnes) in the UK since 2000.
Source: (AHDB poultry pocketbook-2018; DEFRA 2019a; Eurostat 2019)

In the UK, the numbers of poultry slaughtered, and chick placing has increased during the last two decades. In 2018, 1083 million broilers were slaughtered with an increase of 4.5% as compared to 2017 (Table 1.15). The broiler chicks placing increased from 1047 to 1089 million, whereas, commercial layers number also improved from 38 to 40 million (AHDB poultry pocketbook-2018, DEFRA 2019b). The turkey numbers remained in a range of 15 – 16 million in the past few years. The turkey production is largely seasonal, and demand increases around Christmas period. The UK poultry industry is characterised by steady growth and this trend is anticipated to continue despite some challenges.

Table 1. 15. The numbers of poultry slaughtered, and chick placing in the UK.

Million head	2000	2005	2010	2015	2016*	2017	2018
Poultry Slaughtered							
Broilers	760	827	863	953	993	1037	1083
Turkeys	27	19	16	17	16	14	15
Boiling fowls**	38	38	41	45	57	53	54
Chick placings							
Broilers	809	857	903	972	1013	1047	1089
Commercial layers	32	30	35	36	38	38	40
Turkeys	27	19	16	16	16	15	16

Source: (AHDB poultry pocketbook-2018, DEFRA 2019a, b)
*53 weeks statistical year.
**Broiling fowl includes spent hens and spent breeders.

Strong and continued growth in poultry production make the UK, 75% self-sufficient in poultry meat. There are also imports of around 977 Tt (AHDB poultry pocketbook-2018), mainly salted meat and process meat products. The structure of the UK broiler industry places it in a strong position, since almost all the fresh market, and significant amount of prepared chicken sector, already farmed and produced in the UK. This reduces the reliance on import of finished meat products and drives a degree of self-sufficiency. Consumers continue to choose chicken meat for its health benefits and advantage price over other proteins and preference is expected to grow further in coming year. The UK per capita consumption of poultry meat is 36.3 kg/person/year, well above than pig, beef and veal meat. The UK egg industry is also in strong position, with annual egg production of 899 million dozen in 2016. The UK egg market is 85% self-sufficient in egg production. The remaining 15% is imported mainly from the EU countries as liquid egg and egg products.

Although, the UK poultry industry in general and broiler meat production specifically hold a strong position in the agriculture sector, however the industry is under immense pressure of rising production cost. The price of broiler meat (price/kg of meat) in the UK is comparatively higher as compared to the EU. Feed is the single largest cost to poultry, up to 70% and rising prices of raw materials could further increase the feed cost. The cost of feed in the UK is already higher as compared to other large poultry meat producing countries. Expensive labour cost also adds in production cost. In order to keep poultry production economical and broiler chicken meat a price competitive and valuable protein source, it is essential that the cost of production is kept at minimum. This is achievable through the use of high-quality price competitive cereals. The availability of raw materials and home-grown cereals is pre-requisite for efficient broiler and poultry meat production.

The UK broiler feed industry relies heavily on wheat being the main raw material in feed formulations. Wheat is grown intensively in the UK as milling and feed wheats and largely available to be used in poultry diets. However, it is imperative that high-quality wheat is available for broiler diets at affordable price for efficient broiler production. Furthermore, the differences between wheat varieties are fully investigated and accounted for in practical feed formulations to reduce the variation in broiler growth performance.

1.6. CONCLUSIONS AND KNOWLEDGE GAP

Wheat is known as the most variable cereal in terms of its nutritional composition as compared to other routinely used cereals in broiler diets. The review of literature has clearly indicated that chemical composition of wheat is highly variable and availability of nutrients to broilers also varies. The variability in availability of nutrients especially starch and protein could be problematic for using wheat in broiler diets due to the compromising effect of high NSP levels of wheat. In addition, variability in AME of wheat is most challenging for nutritionist to predict the accurate energy value of wheat. The use of exogenous xylanase and phytase have been effective in mitigating the adverse effect of NSP, utilisation of phytate P, reducing the variation in AME and overall result in improvements in growth performance of chickens. Wheat is the most commonly used raw material in the UK and numerous European countries and their inclusion level could exceed up to 70% in commercial broilers diets. However, information on the chemical composition and physical characteristics of currently available UK wheat samples for broiler diets is scarce. Clearly, there is also a lack of knowledge on the nutrient availability of these wheat cultivars for broilers. In addition, the information on the effect of these currently available wheat cultivars on AME of wheat and growth performance is limited. The hypothesis of this thesis was that there are differences in chemical composition and physical characteristics of currently available wheat samples for broiler diets and variation between wheat samples may result in differences in AME and growth performance of broilers. Therefore, this project aims to fill the knowledge gap by characterising the currently available UK wheat samples for broiler diets and by investigating the variability in their chemical composition and physical characteristics. Bioavailability and digestibility of nutrients of these current wheat cultivars will be explored and relationship between nutrients availability and AME and growth performance will be examined. In addition, the effect of exogenous xylanase will also be investigated to improve the feeding quality of currently available wheats for broilers.

CHAPTER 2: CHEMICAL COMPOSITION AND PHYSICAL CHARACTERISTICS OF SEVENTEEN CURRENT UK WHEAT SAMPLES

2.1. INTRODUCTION

The commercial broiler industry relies heavily on wheat being the main raw material in broiler feed formulation in the UK and many countries in the Europe, therefore, it is very important to characterise the current wheat cultivars samples. Currently in the UK poultry feed industry, wheat available for broiler feed could be milling wheat (bread, biscuits and cakes) and feed wheat. Excessive milling wheats after human consumption, bread making, biscuits and other bakery products is also used for poultry feed. Wheat being the highly variable cereal in its nutrient composition is always challenging to be used in broiler feed formulations. Nutritional evaluation of wheat cultivars can lead to different information, especially if the wheat cultivars are grown at different growing sites, because variation in the soil, micro-climate, and husbandry techniques may confound the nutrient composition (Gutierrez-Alamo et al. 2008b). There is a need to examine a range of representative UK wheat samples currently available for broiler feed formulations and determine the variability in their nutrient composition. The main objectives of this study were to define the variability in chemical composition, energy content and physical characteristics of seventeen (17) current UK grown wheat samples.

2.2. MATERIALS AND METHODS

2.2.1. Wheat samples

Seventeen current UK wheat samples harvested in the year 2015, sourced from Klien Wanzlebener Saatzucht (KWS UK Ltd), grown at four different sites in the UK (Yorkshire, Nottinghamshire, Lincolnshire and Cambridgeshire) were used in this study (Table 2.1). The samples were among currently listed UK wheat varieties (AHDB 2015, 2016) and comprised of feed wheats (Leeds and Santiago) and milling wheats (Lili, Trinity, Barrel and Basset). Among 17 wheat samples, sample ten (10) and twelve (12) were the second crop, while rest of the samples were first crop. First wheat crop was cultivated after oil seed crop, while second crop was cultivated after the previous crop of wheat. Wheat samples used in this study were comprised of group 1, group 2, group 3, soft group 3 and hard group 4 (classification by National Association of British and Irish Millers) and were currently listed on HGCA recommended list for 2015/2016 (AHDB 2015). Wheat varieties are grouped on the basis of their milling quality for the milling market.

Group 1 wheat varieties are used for bread making and produce consistent milling and baking performance. Millers offer premium above base price, if they achieve specified quality requirements. Group 2 varieties are also used for bread making, but because of their inherent consistency or specific characteristics, are not suited to all grist. These varieties mostly attract varying market price. Group 3 contains soft varieties for biscuit, cake and other flour where main requirement is soft milling characteristics, low protein, good extraction rates and an extensible but not elastic gluten. Group 4 varieties are mainly known as feed wheat and subdivided into hard endosperm and soft endosperm types. In 2013, 56% of total wheat produced in the UK was group 4 wheat, used as feed wheat, whereas, in 2014, 58% of total wheat end use was as feed wheat (AHDB 2015).

Leeds is a high yielding soft group 4 feed wheat variety and is good for distilling and blending for export. Santiago is a hard group 4 feed wheat variety. It performs well in both first and second crop. KWS Lili is a group 2 hard milling wheat suitable for bread making market. It has highest yield and good grain quality (AHDB recommended list 2015/2016). Wheat growers find it acceptable to wider range market because of good grain quality and high yield. KWS Lili has robust agronomic characteristics and includes great standing ability and excellent disease resistance. KWS Trinity is a hard group 1 milling wheat suitable for bread making. It is a high yielding and relatively early maturity variety with exceptional disease resistance. It is one of the leading breads making variety in the market. It suits on all regions of UK on light and heavy land and is a high yielding group in the east region trials. KWS Barrel is a high yielding soft group 3 variety with good grain quality and suitable for distilling. KWS Basset is a soft group 3 variety with good grain quality and disease resistance. It is a good choice as a second wheat. Both Barrel and Basset are suitable for cakes and biscuit making market. Among 17 wheat samples used in this study, four (4) samples consist of Leeds and four (4) samples were Santiago. Two (2) samples consist of Lili, four (4) samples were Trinity, two (2) samples were Barrel, and one (1) samples was from Basset variety.

Wheat samples were received in 25 kg tote bags and stored in a dry place at ambient temperature until analyses and bird trial. Each sample was mixed homogenously for 10 minutes (min), and random samples were collected for analyses. Around 300 g of each wheat sample was freshly milled to pass through a 0.75 mm screen using rotor mill Retsch ZM 200 (Retsch GmbH, Haan, Germany). All chemical analyses were performed in duplicate and reported on dry matter (DM) basis. Physical analyses of wheat grains were determined in triplicate.

Table 2. 1. List of experimental wheat samples.

Sample ID	Variety	Growing site	Type*	Usage
1	Leeds	Nottinghamshire	Feed (Soft)	Feed wheat
2	Leeds	Yorkshire	Feed (Soft)	Feed wheat
3	Leeds	Lincolnshire	Feed (Soft)	Feed wheat
4	Leeds	Cambridgeshire	Feed (Soft)	Feed wheat
5	KWS Santiago	Yorkshire	Feed (Hard)	Feed wheat
6	KWS Santiago	Nottinghamshire	Feed (Hard)	Feed wheat
7	KWS Santiago	Lincolnshire	Feed (Hard)	Feed wheat
8	KWS Santiago	Cambridgeshire	Feed (Hard)	Feed wheat
9	KWS Lili	Yorkshire	Milling (Hard)	Bread
10	KWS Lili	Yorkshire	Milling (Hard)	Bread
11	KWS Trinity	Yorkshire	Milling (Hard)	Bread
12	KWS Trinity	Yorkshire	Milling (Hard)	Bread
13	KWS Trinity	Lincolnshire	Milling (Hard)	Bread
14	KWS Trinity	Cambridge	Milling (Hard)	Bread
15	KWS Barrel	Lincolnshire	Milling (Soft)	Cakes, biscuits
16	KWS Barrel	Cambridgeshire	Milling (Soft)	Cakes, biscuits
17	KWS Basset	Cambridgeshire	Milling (Soft)	Cakes, biscuits

*Varieties are listed on AHDB (Agriculture and Horticulture Development Board) recommended list 2015/16 and 2016/17.

2.2.2. Chemical composition of wheat samples

2.2.2.1. Proximate analysis of samples and gross energy

Dry matter (DM**)** was determined by drying samples in a forced draft oven at 105 °C to a constant weight (AOAC 2012; 934.01). Crude protein (CP) (N × 6.25) was determined by dry combustion method (AOAC 2012; 990.03) using a LECO FP-528 N (Leco Corp., St. Joseph, MI, USA) with EDTA as a standard. Crude oil (as ether extract) was extracted with 40 – 60 ^{0}C petroleum ether by ether extraction method (AOAC 2012; 920.39) using a Soxtec system (Foss UK Ltd, Warrington, UK). Gross energy (GE) was determined in a bomb calorimeter (model 6200; Parr Instrument Co., Moline, IL, USA). Benzoic acid was used as a standard. Crude ash of samples was determined by combustion in a muffle furnace (Gallenkamp Muffle Furnace, Size 2, GAFSE 620, Gallenkamp, Loughborough, UK) at 550 °C for 4 h (AOAC 2012; 942.05).

2.2.2.2. Carbohydrate analysis

Starch and non-starch polysaccharide (NSP) analysis was carried out by Englyst Carbohydrates Ltd (Southampton Science Park, Southampton, UK).

2.2.2.2.1. Total starch

Total starch (TS) was analysed following the method of Englyst et al. (2000). Milled wheat samples were treated with protease enzymes to disrupt any starch – protein interaction. Then samples were incubated with amylolytic enzymes at 37 ^{0}C, pH 5.2 for 2 h which hydrolysed the digestible starch portion. The samples were then treated with 2M potassium hydroxide to disperse resistant starch and after buffering to pH 4.5 was incubated with glucoamylase to complete the starch hydrolysis. Released glucose was determined by ion chromatography on a Thermo ICS-5000 system.

2.2.2.2.2. Non-Starch polysaccharides

Wheat NSP fractions were determined by the method of Englyst et al. (1994). Starch was first dispersed by dimethyl sulphoxide (DMSO) and then hydrolysed by amylolytic enzymes. The total NSP fraction was isolated by precipitation in 80% ethanol and the insoluble NSP fraction was isolated by pH7 aqueous washing, with the soluble fraction calculated as the difference between total and insoluble NSP. The dried residues were hydrolysed by successive treatments with 12M and 2M sulphuric acid at 35 ^{0}C and 100 ^{0}C respectively. The released sugars were measured by gas chromatography as their alditol acetate derivatives on a Shimadzu 2010 GC system with flame ionisation detection.

2.2.2.3. Amino acids

Amino acid (AA) contents of wheat samples were determined using the easy-fast amino acid kit supplied by Phenomenex according to official Journal of the European Union Commission directives 152/2009 Annex III method F except tryptophan. Samples were hydrolysed for 24 hours in a nitrogen atmosphere with 6N hydrochloric acid. The solution was filtered, and then run with the Phenomenex EZ: FAAST system of sample clean up and reaction with propyl chloroformate. The solution was run on a Waters Alliance 2695 HPLC system with Waters Quattro Micro triple quad mass spectrometry detection against known standard materials. The AA contents of wheat samples were analysed by DM Scientific Ltd (main site, Dalton, Thirsk, North Yorkshire).

2.2.3. Physical characteristics of wheat samples

2.2.3.1. Endosperm hardness (EH)

Endosperm Hardness (EH) was measured by Single Kernel Characterisation System (SKCS 4100, Perton Instrument, Hagersten, Sweden). Three hundred (300) kernels of cleaned wheat were assessed for each sample and their mean was taken as average hardness index. The SKCS 4100 isolates individual kernels (grain), weighs and crushes them between a toothed rotor and a progressively narrowing crescent gap. As a kernel is crushed, the force between the rotor and crescent and the conductivity between the rotor and the electrically isolated crescent are measured. The SKCS completes a test in around 3 min and gives mean values and standard deviation of individual kernel data of 300 kernels. Hardness classification is constructed from the average hardness index of wheat kernels and the distribution of individual kernel hardness measurements within soft, hard and mixed as defined by the Grain Inspectors, Packers and Stockyard Association, United States Department of Agriculture (GIPSA-USDA). Average hardness index was expressed in relative units (0 – 120, soft – hard). The mean hardness index > 46 is classified as hard and index is ≤ 46 is considered as soft (Maghirang and Dowell 2003). The SKCS also reports diameter, size, moisture and weight of kernels, however in this study only used for EH.

2.2.3.2. Hagberg falling number (HFN)

Hagberg falling number (HFN) was determined by HFN apparatus model 1400 (Falling Number AB, Stockholm, Sweden) (AOAC 976.13). The falling number analysis is based on the measurement of viscosity characteristics after heating a slurry of whole-meal flour and water, and viscosity is measured as the time (in seconds) it takes for a plunger to fall a certain distance through the gelatinised paste after a standard mixing time. The analysis is

used as a measure of pre-harvest sprouting, giving wheat flours of high α-amylase activity and of reduced viscosity upon gelatinisation.

Seven (7) grams of each wheat sample in duplicate was mixed with 25 ml of distilled water in a viscometer tube and shake vigorously to obtain a homogenous suspension. After mixing, the viscometer tube with a stirrer was inserted into boiling water in HFN apparatus and waited for stirrer to start. The stirrer released automatically after 60 seconds (5+55) and allowed to fall under the force of gravity through the mixture, and time (in seconds) it takes for a stirrer to fall a certain distance was recorded. The value of time in seconds is the HFN of wheat flour. The principle of HFN is based that when the tubes are placed in the boiling water bath, the starch begins to gelatinise, and the slurry becomes more viscous. The mixing makes sure the gelatinisation is homogeneous in the slurry. At high temperature, α -amylase enzyme starts to break down the starch and the viscosity of wheat starts to decrease. The amount of starch break-down is dependent on the α-amylase activity and higher activity of the α-amylase will result in lower viscosity of gelatinised flour.

2.2.3.3. Specific weight (SW)

Specific weight (SW) was determined by Chondrometer (Farm Tec, Yorkshire, UK). Wheat grains were filled in a cylinder with a slide and plunger and then weighed. At the start of the measurement, the cut-off slide was inserted in the cylinder. Then plunger weight was dropped into the cylinder, ensuring that it is resting flat on the cut-off slide. Wheat grains were dropped from a height of approximately 25 mm and slowly filled the cylinder up to the top. Then the slide was removed and grains with plunger inside were allowed to fall under gravity. The slide was re-inserted through the column of grains to isolate the sample. The surplus grains were tipped out above the slide. Chondrometer with sample grains, plunger and slide was weighed and reading was recorded in grams. Each reading was checked against chart provided with Chondrometer to convert into kilogram/hectolitre (kg/hl).

2.2.3.4. Weight of thousand kernels (TWG)

Thousand grain weights (TGW) of wheat samples was determined by weighing one thousand (1000) randomly selected grains. Each wheat sample was mixed homogenously, and representative samples were taken for TGW. The grains used for analysis were randomly selected without separating small or medium size grains. Additionally, the grains were intact, undamaged and whole kernels.

2.2.3.5. Dynamic water extract viscosity (DV)

Dynamic water extract viscosity (DV) was determined by a rotating cone and cup viscometer (model DV-II + LV, Brookfield Engineering Laboratories, Stoughton, MA, USA) as described by Pirgozliev et al. (2003). The milled wheat sample (2 g) was weighed in a 10 ml plastic tube and 4 ml of distilled water was added to the tube. The contents of tube were thoroughly mixed by a vortex mixer. Then tube was incubated in a water bath at 40°C for 30 min. After incubation, tube was centrifuged at 10,000 × *g* for 2 min and left at room temperature for 15 min. A 0.5 ml aliquot was taken from the liquid portion of each tube in triplicates and viscosity of this supernatant was measured by viscometer.

2.2.3.6. Kinematic water extract viscosity (KV)

Kinematic water extract viscosity (KV) of wheat samples was measured using an automated viscometer (AVS 370 SCHOTT Instruments, SI Analytics, Germany) fitted with an Ostwald capillary tube (Saulnier et al. 1995). One gram of each wheat sample was weighed in triplicates into 15 ml Falcon tubes and 4 ml water was added. The contents of tube were thoroughly mixed using a vortex mixer to ensure the sample was completely suspended. The samples tubes were agitated for 15 min on spiramix. Then tubes were centrifuged for 10 min (5000 *g*) at 25 °C in Eppendorf benchtop centrifuge. The supernatant was immediately transferred to a clean Falcon tube and placed in a boiling water bath for 10 min. Later, the supernatant was transferred to 15 ml Cortex tubes and centrifuge at 10 000 × *g* for 10 min to sediment flocculated proteins. Clear supernatant was filtered in a clean tube through 0.45um Millex PVDF disposable filters and aliquots were subjected to viscosity measurement on AVS 370.

2.2.4. Statistical analysis

Microsoft Excel 2013 was used for calculation and descriptive statistical analysis. The coefficients of variation (CV%) of different wheat variables were determined to express the variations between the wheat samples.

Pearson's correlation coefficients were generated to test linear relationship between proximate nutrients, chemical composition and physical characteristics of wheat samples. Relationships were reported at significance level ($P < 0.05$, r = 0.482; $P < 0.01$, r = 0.606; $P < 0.001$, r = 0.725 and $P < 0.1$, r = 0.412).

2.3. RESULTS

2.3.1. Proximate analysis and gross energy

There were variations in CP, ash and oil contents of wheat samples (Table 2.2). The mean CP, ash, oil and GE contents of wheat samples were 121, 16, 14.6 g/kg DM and 17.99 MJ/kg DM, respectively. The CP content of wheat samples ranged from 97 to 143 g/kg DM (CV = 8.3%). The GE content of wheat samples ranged from 17.81 to 18.24 MJ/kg DM and less variable (CV = 0.6%) between samples. There was a difference of 0.43 MJ/kg DM in GE between the lowest and highest sample. The oil content (as ether extract) in wheat samples ranged from 10.9 to 17.4 g/kg DM (CV = 13.0%). The ash content between samples ranged from 12.8 to 19.6 g/kg DM (CV= 9.9%). The mean DM content of wheat samples was 894 g/kg and ranged from 873 to 910 g/kg (CV = 1.2%).

Table 2. 2. Proximate composition and gross energy content of seventeen wheat samples (g/kg DM).

Wheat Samples	DM[1]	Protein[2]	GE[3]	Ash	Oil
1	873	143	18.11	19.6	10.9
2	905	117	17.94	16.0	16.4
3	882	127	17.88	15.3	12.9
4	887	116	17.81	12.8	12.4
5	904	117	17.89	15.9	16.1
6	877	138	18.13	17.9	14.9
7	898	116	17.98	16.2	17.4
8	890	123	17.94	18.0	13.9
9	898	122	18.03	16.3	15.0
10	904	117	18.09	15.1	14.4
11	910	126	18.24	16.9	12.7
12	904	119	18.06	16.4	15.7
13	891	127	18.00	14.2	15.4
14	893	116	17.97	14.8	15.9
15	886	122	18.02	14.9	17.1
16	902	114	17.98	15.3	11.6
17	891	97	17.81	17.2	14.8
Mean	894	121	17.99	16.0	14.6
Mini	873	97	17.81	12.8	10.9
Max	910	143	18.24	19.6	17.4
CV%	1.2	8.3	0.63	9.88	13.03

[1]Dry matter (g/kg), [2]Crude protein (N × 6.25), [3]Gross energy (MJ/kg DM) mega joules/kilogram on dry matter.
Each analysis was performed in duplicate.

2.3.2. Polysaccharides composition

The polysaccharides composition of 17 wheat samples is presented in Table 2.3. The mean starch content of wheat samples was 706 g/kg DM and ranged from 671 to 728 g/kg DM (CV = 2.2%). The mean total, insoluble and soluble NSP contents were 88.0, 71.3 and 16.7 g/kg DM, respectively. The total NSP contents of wheat samples ranged from 80.1 to 98.2 g/kg DM (CV = 4.9%). The total soluble and insoluble NSP contents of wheat samples ranged from 11.2 to 23.2 (CV = 23.1%) and 63.7 to 80.2 g/kg DM (CV = 5.3%), respectively. Among NSP fractions, total arabinose in wheat samples ranged from 19.8 to 25.6 (CV = 6.2%) (Table 2.4). The total xylose content of samples ranged from of 33.1 to 39.9 g/kg DM (CV = 5.3%). There was variation in total glucose content of wheat samples and ranged from 20.7 to 29.0 g/kg DM (CV = 9.6%). There was large variation in soluble arabinose and xylose contents of wheat samples (CV = 28.2 and 29.5%, respectively). The predominant constituent sugars of total NSP in descending order were, xylose, glucose, arabinose, galactose and mannose. Large CV were observed for total galactose (9.4%). The other constituents of NSPs (rhamnose, fucose, glucuronic acid, galacturonic acid) were not found in studied wheat samples.

Table 2. 3. Polysaccharides composition of seventeen wheat samples (g/kg DM).

Wheat samples	Starch	total NSP*	soluble NSP	insoluble NSP
1	678	98.2	17.9	80.2
2	704	92.8	21.3	71.5
3	704	90.5	16.2	74.2
4	696	87.7	13.8	73.9
5	697	86.6	15.1	71.4
6	722	90.6	18.4	72.2
7	671	80.1	12.3	67.8
8	728	87.7	11.2	76.4
9	699	86.7	18.8	67.9
10	704	90.7	22.6	68.2
11	707	82.2	11.3	71.0
12	727	86.9	23.2	63.7
13	708	83.5	15.9	67.6
14	715	84.7	13.6	71.1
15	704	92.9	21.7	71.2
16	709	87.5	17.3	70.3
17	726	87.4	13.9	73.5
Mean	706	88.0	16.7	71.3
Mini	671	80.1	11.2	63.7
Max	728	98.2	23.2	80.2
CV%	2.2	4.9	23.1	5.3

*NSP= Non-starch polysaccharides.
Each analysis was performed in duplicate.

Table 2. 4. Polysaccharides fractions composition of seventeen wheat samples (g/kg DM).

Fraction		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	CV%
Arabinose	Soluble	4.8	5.0	4.7	4.8	4.8	6.1	5.7	1.0	4.9	4.8	2.6	5.5	3.9	3.6	4.3	3.8	3.9	28.2
	Insoluble	20.8	17.8	17.4	17.6	16.4	17.8	16.4	19.7	16.2	16.9	17.2	16.4	17.1	17.2	17.0	17.4	17.9	6.7
	Total	25.6	22.8	22.1	22.4	21.3	23.9	22.1	20.7	21.1	21.7	19.8	21.9	21.0	20.8	21.3	21.2	21.7	6.2
Xylose	Soluble	8.4	7.7	7.8	8.1	8.8	10.3	5.2	1.8	10.2	10.3	4.8	11.1	9.5	7.6	8.7	6.7	7.8	29.5
	Insoluble	31.3	29.7	27.5	26.1	28.4	29.6	27.9	34.8	28.0	28.6	29.4	25.4	26.0	28.1	28.9	28.8	29.5	7.5
	Total	39.7	37.4	35.3	34.3	37.2	39.9	33.1	36.6	38.2	39.0	34.2	36.5	35.4	35.7	37.6	35.5	37.2	5.3
Mannose	Soluble	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.2	2.8
	Insoluble	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	Total	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.2	2.8
Galactose	Soluble	1.0	1.7	0.9	0.1	0.2	0.8	0.5	0.4	0.6	1.6	1.6	1.9	1.5	0.7	1.0	1.1	0.7	58.3
	Insoluble	3.1	2.9	3.3	3.7	3.4	3.1	2.7	3.6	3.0	2.7	2.8	2.5	2.4	3.2	3.0	2.8	2.8	12.3
	Total	4.0	4.7	4.2	3.8	3.6	4.0	3.1	4.0	3.6	4.3	4.3	4.4	3.9	3.9	4.0	3.9	3.5	9.4
Glucose	Soluble	2.6	5.7	1.6	0.0	0.2	0.0	0.0	1.0	2.0	4.8	1.2	3.5	0.0	0.5	6.6	4.6	0.3	108.5
	Insoluble	25.3	21.1	26.2	26.1	23.3	21.8	20.7	24.2	20.8	20.0	21.8	19.6	22.3	22.8	22.4	21.4	23.6	8.9
	Total	27.9	26.9	27.8	26.1	23.5	21.8	20.7	25.2	22.9	24.8	23.0	23.1	22.3	23.4	29.0	26.0	23.9	9.6
Total NSP	Soluble	17.9	21.3	16.2	14.1	15.1	18.4	12.3	11.2	18.8	22.6	11.3	23.2	15.9	13.6	21.7	17.3	13.9	23.0
	Insoluble	80.2	71.5	74.2	73.6	71.4	72.2	67.8	76.4	67.9	68.2	71.0	63.7	67.6	71.1	71.2	70.3	73.5	5.3
	Total	98.2	92.8	90.5	87.7	86.6	90.6	80.1	87.7	86.7	90.7	82.2	86.9	83.5	84.7	92.9	87.5	87.4	4.9

Each analysis was performed in duplicate.

2.3.3. Amino acids

The indispensable amino acids (IAA) composition of wheat samples is summarised in Table 2.5. The mean lysine, methionine, and threonine contents of wheat samples were 3.1, 1.8 and 3.4 g/kg DM, respectively. The lysine and methionine contents of wheat samples ranged from 2.6 to 3.6 g/kg DM (CV = 7.9%) and 1.3 to 2.1 g/kg DM (CV = 12.3%), respectively. The threonine content between samples ranged from 2.8 to 3.8 g/kg DM (CV = 8.5%). The mean methionine content was the lowest as compared to other IAA, whereas, leucine had the highest concentration (7.6 g/kg DM), followed by arginine (5.5 g/kg DM).

Among dispensable amino acids (DAA), glutamic acid had the highest mean concentration (35.5 g/kg DM) followed by proline (10.7 g/kg DM), aspartic acid (5.9 g/kg DM), serine (5.5 g/kg DM), glycine (4.5 g/kg DM), alanine (3.8 g/kg DM), cysteine (2.8 g/kg DM) and tyrosine (1.7 g/kg DM) (Table 2.6). The concentration of glutamic acid, glycine, cysteine, aspartic acid, serine and alanine ranged from 27.4 to 42.0 g/kg DM (CV = 9.8%), 3.6 to 5.0 g/kg DM (CV = 8.2%), 2.4 to 3.3 g/kg DM (CV = 8.9%), 4.7 to 6.8 g/kg DM (CV = 8.2%), 4.6 to 6.3 g/kg DM (CV = 7.6%) and 3.0 to 4.3 g/kg DM (CV = 7.6%), respectively. Among the DAA, tyrosine had the highest variation (CV = 16.6%), whereas, alanine and serine had the lowest CV values (7.6%).

Table 2. 5. Indispensable amino acid (IAA) composition of seventeen wheat samples (g/kg DM).

Wheat samples	Lysine	Methionine	Threonine	Phenyl-alanine	Leucine	Iso-Leucine	Histidine	Arginine	Valine
1	3.5	2.0	3.8	6.2	8.9	4.5	3.1	6.2	5.4
2	3.1	1.5	3.1	5.0	7.3	3.8	2.3	5.7	4.8
3	3.1	1.8	3.4	5.3	7.7	4.1	2.8	5.8	5.1
4	2.9	1.6	3.2	4.7	7.0	3.6	2.6	5.3	4.8
5	3.1	1.8	3.3	5.2	7.4	3.8	2.4	5.3	4.8
6	3.6	2.1	3.8	6.4	8.9	4.6	3.0	6.4	5.7
7	3.1	1.8	3.2	5.3	7.3	3.9	2.3	5.0	4.9
8	3.3	2.0	3.4	5.5	7.9	4.0	2.6	5.4	5.3
9	2.9	1.6	3.2	5.5	7.5	3.9	2.3	5.3	4.9
10	2.9	1.5	3.1	5.4	7.4	3.9	2.3	5.1	4.8
11	3.2	1.8	3.6	5.1	7.8	4.1	2.6	5.5	5.3
12	3.0	1.7	3.3	4.8	7.4	3.8	2.5	5.0	5.0
13	3.1	1.9	3.7	5.2	8.0	4.2	2.6	5.4	5.3
14	3.4	2.1	3.6	5.3	8.3	4.3	2.7	6.0	5.6
15	3.2	1.9	3.5	5.3	7.6	4.0	2.6	5.5	5.2
16	3.0	1.7	3.0	5.1	7.2	3.8	2.3	5.2	5.0
17	2.6	1.3	2.8	3.8	6.3	3.1	2.4	4.5	4.0
Mean	3.1	1.8	3.4	5.2	7.6	3.9	2.6	5.5	5.0
Mini	2.6	1.3	2.8	3.8	6.3	3.1	2.3	4.5	4.0
Max	3.6	2.1	3.8	6.4	8.9	4.6	3.1	6.4	5.7
CV	7.9	12.3	8.5	10.7	8.4	8.4	9.4	8.7	7.7

Each analysis was performed in duplicate.

Table 2. 6. Dispensable amino acid (DAA) composition of seventeen wheat samples (g/kg DM).

Wheat samples	Alanine	Aspartic acid	Cysteine	Glutamic acid	Glycine	Proline	Serine	Tyrosine
1	4.2	6.7	3.0	41.7	4.9	12.1	6.2	2.1
2	3.8	5.9	2.4	33.3	4.4	10.3	5.2	1.5
3	4.1	6.0	2.5	37.1	4.7	11.1	5.8	1.6
4	3.7	5.6	2.5	32.4	4.2	10.5	5.2	1.5
5	3.7	5.9	2.7	34.1	4.1	10.1	5.3	1.9
6	4.2	6.8	3.3	42.0	4.8	12.8	6.3	2.2
7	3.7	5.9	2.8	33.5	4.1	9.6	5.2	1.7
8	3.9	6.2	2.9	35.9	4.3	11.0	5.5	1.7
9	3.8	5.7	2.8	36.0	4.5	11.3	5.3	1.7
10	3.7	5.6	2.5	34.7	4.5	11.1	5.3	1.8
11	4.0	6.0	3.0	37.6	4.7	11.1	5.8	1.6
12	3.6	5.6	2.7	34.9	4.4	10.8	5.3	1.7
13	4.0	6.0	2.9	38.1	4.9	10.8	5.6	1.6
14	4.3	6.0	3.0	37.2	5.0	11.9	5.7	1.5
15	4.0	5.9	2.8	35.2	4.5	10.3	5.5	1.8
16	3.8	5.3	2.7	32.3	4.3	9.6	5.0	1.3
17	3.0	4.7	2.5	27.4	3.6	7.6	4.6	1.0
Mean	3.8	5.9	2.8	35.5	4.5	10.7	5.5	1.7
Mini	3.0	4.7	2.4	27.4	3.6	7.6	4.6	1.0
Max	4.3	6.8	3.3	42.0	5.0	12.8	6.3	2.2
CV	7.6	8.2	8.9	9.8	8.2	10.8	7.6	16.6

Each analysis was performed in duplicate.

2.3.4. Physical characteristics of wheat samples

The EH of wheat samples ranged from 21 to 87 relative units (soft to hard) (Table 2.7). The values of EH were variable (CV = 43.7%) between samples because of different wheat types (feed wheat, soft and hard milling wheat). The values of HFN between wheat samples ranged from 130 to 384 (CV = 28.7%). The SW of wheat samples ranged from 75.4 to 82.4 kg/hl (CV = 2.8%). The TGW ranged between 45.7 to 59.9 g (CV = 7.8%). The KV ranged between 1.17 to 1.56 cSt (centistokes) (CV = 7.71%), whereas, the DV ranged between 2.4 to 6 cP (centipoise) (CV = 27.5%).

2.3.5. Relationship between chemical composition and physical characteristics of wheat samples

The correlation matrix between chemical and physical measurements of wheat samples is presented in Table 2.8. There was no relationship ($P > 0.05$) between SW and starch or CP contents of wheat samples. The CP content was positively correlated with GE of wheat samples (r = 0.606; $P < 0.01$). There was a positive correlation between GE content and EH and HFN (r = 0.496, r = 0.629; $P < 0.05$, $P < 0.01$, respectively) of wheat samples. The oil content was negatively correlated (r = −0.553; $P < 0.05$) with insoluble NSP content of wheat samples. The ash content of wheat sample was positively correlated (r = 0.503, $P < 0.05$) with xylose content. There was no relationship between starch and NSP content of wheat samples, however, Starch content was negatively correlated (r = −0.567; $P < 0.05$) with ratio of arabinose to xylose. The CP was negatively correlated (r = −0.762, $P < 0.01$) with TGW of wheat samples.

Table 2. 7. Physical characteristics of seventeen wheat samples.

Wheat samples	EH	HFN	SW (kg/hl)	TGW	DV (cP)	KV (cSt)
1	35	240	77.8	45.7	3.4	1.56
2	32	233	82.3	52.3	6.0	1.44
3	26	130	76.6	48.6	3.9	1.32
4	21	210	78.6	52.6	4.5	1.32
5	87	181	80.0	52.4	3.4	1.29
6	82	291	75.4	46.3	4.6	1.42
7	71	197	78.1	52.6	2.6	1.17
8	75	241	81.3	52.9	2.7	1.20
9	85	301	81.8	50.9	3.6	1.40
10	74	308	80.2	50.2	3.8	1.27
11	79	380	82.4	53.2	4.4	1.42
12	68	374	79.0	55.1	4.3	1.49
13	64	368	77.2	54.7	2.8	1.42
14	56	384	80.8	59.9	2.4	1.27
15	30	237	77.1	54.4	2.8	1.30
16	27	252	82.3	59.6	2.7	1.27
17	31	206	78.7	59.4	2.5	1.30
Mean	55	266	79.4	53.0	3.5	1.34
Mini	21	130	75.4	45.7	2.4	1.17
Max	87	384	82.4	59.9	6.0	1.56
CV	43.7	28.7	2.77	7.80	27.50	7.713

EH= endosperm hardness (relative units 0 –120), HFN= Hagberg falling number,
SW= specific weight, TGW= thousand grain weight (g), DV= dynamic water extract viscosity,
KV= kinematic water extract viscosity.

Table 2. 8. Correlation coefficients between proximate, chemical composition and physical characteristics of wheat samples.

	CP	GE	Oil	Ash	Starch	tNSP	sNSP	iNSP	Arab	Xylose	Arab/Xyl	EH	HFN	SW	TGW	DV
GE	**0.606**															
Oil	-0.314	-0.107														
Ash	0.414	0.415	-0.207													
Starch	-0.227	-0.065	0.048	0.045												
tNSP	0.394	0.046	-0.313	0.327	-0.055											
sNSP	0.128	0.242	0.194	-0.052	0.069	**0.574**										
iNSP	0.319	-0.193	**-0.553**	0.425	-0.133	**0.557**	-0.360									
Arab	0.485	0.060	-0.260	0.431	-0.356	**0.695**	0.325	0.462								
Xylose	0.350	0.276	-0.034	**0.503**	0.175	**0.745**	**0.599**	0.241	**0.524**							
Arab/Xyl	0.172	-0.211	-0.221	-0.032	**-0.567**	0.030	-0.210	0.247	**0.581**	-0.387						
EH	0.209	**0.496**	0.332	0.276	0.096	-0.450	-0.104	-0.408	-0.299	0.133	-0.438					
HFN	0.152	**0.629**	0.081	-0.032	0.353	-0.308	0.134	-0.487	-0.325	0.029	-0.397	0.440				
SW	-0.357	0.088	-0.106	-0.008	0.093	-0.233	-0.123	-0.141	-0.499	-0.171	-0.387	0.164	0.261			
TGW	**-0.762**	-0.358	0.219	-0.398	0.383	-0.486	-0.213	-0.337	-0.628	-0.456	-0.253	-0.215	0.253	0.396		
DV	0.257	0.200	-0.064	-0.003	0.016	0.302	0.389	-0.051	0.311	0.184	0.159	-0.019	0.019	0.110	-0.474	
KV	0.544	0.445	-0.282	0.336	0.010	0.441	0.429	0.067	0.493	0.428	0.094	-0.045	0.350	-0.124	-0.394	0.549

df = 15; Correlation coefficients > 0.482, 0.606, 0.725 and 0.412 are statistically significant at $P < 0.05$, $P < 0.01$, $P < 0.001$ and $P < 0.1$ respectively.
Significant correlations are highlighted in bold.

CP, GE, tNSP, sNSP, iNSP: crude protein, gross energy, total, soluble and insoluble non-starch polysaccharides contents of wheat samples.
Arab, Arab / xylose: arabinose, ratio of arabinose to xylose of wheat samples.
EH, HFN, SW, TGW, DV, KV: endosperm hardness, Hagberg falling number, specific weight, weight of 1000 kernels of wheat, dynamic water extract viscosity, kinematic water extract viscosity of wheat samples.

2.4. DISCUSSION

The main aim of this study was to investigate variability in the chemical composition and physical characteristics of a range of representative current UK grown wheat samples available for broiler feed formulations. All wheat samples were among the currently listed wheat varieties and grown at specific locations in the UK. The nutrient composition of wheat varies, depending upon variety and growing condition. Growing condition refers to wheat grown at certain geographical location, soil type, use of fertilisers, growing season, amount of rain fall (Gutierrez del Alamo et al. 2008b).

2.4.1. Chemical composition

Each wheat sample used in this study was carefully mixed homogenously and representative subsamples were collected for analyses. Standard laboratory techniques of using at least two or more replicates of each sample was used for analyses. Using more replicates of each sample may able to reflect the true variation between the samples and represents the more accurate differences between wheat samples. However, due to the cost and time involved to complete analyses of more replicates and complexities of some laboratory procedures, more replicates may not always be a viable option. In routine analytical tests, duplicate of each sample are considered to be suitable for laboratory analyses and similar approach is followed in commercial feed industry. Variation among the studied wheat samples were expressed by coefficient of variation (CV%) values.

The proximate nutrient composition and GE content of wheat samples were in a similar range to results published by several studies (Preston et al. 2000; Rose et al. 2001; Steenfeldt 2001; McCracken et al. 2002; Kim et al. 2003; Pirgozliev et al. 2003; 2015a). Chemical composition of wheat varies due to numerous factors including growing season, soil type, location, crop husbandry and genetic origin of the wheat (Gutierrez del Alamo et al. 2008b, Ravindran and Amerah 2009; Ball et al. 2013a, b).

There was less variation in moisture content of wheat samples (CV = 1.2%). Sample one and six had the highest moisture contents (12.7 and 12.3%, respectively). Both wheat samples were grown at a similar location (Nottinghamshire). The CP content between wheat samples was variable (CV = 8.3%) and variation between the samples were in agreement with the reported results (McCracken et al. 2002; Hetland et al. 2007; Gutierrez del Alamo et al. 2008a. The CP content of sample one and six was the highest (143 and 138 g/kg DM, respectively) among all wheat samples. Sample 15, 16 and 17 had relatively low protein contents as compared with rest of the wheat samples. These wheat samples were comprised of soft milling wheats. Soft varieties contain relatively low protein and high starch

contents and their use is mainly for biscuits, cake making and some other flours (AHDB 2015). Sample 17 with low CP content of 97 g/kg DM was retested, but still gave similar result, therefore CP value of 97 g/kg DM was accepted. Ravindran and Amerah (2009) reported a variation of 8 – 16% in protein content of wheat samples and attributed that variation depends on type of wheat, climate and soil fertility of wheat crop. The variability in this study had a similar range between 9.7 – 14.3%. A study by Kim et al. (2003) reported variation up to 13.4% in Australian wheat samples and attributed that variation could be due to high rain fall in some areas. Interestingly, the two wheat samples (samples 1 and 6) grown at Nottinghamshire site with higher CP content also had greater moisture level as compared to other wheat samples. The causes of high protein content in these samples are unclear and could also be due to soil type, use of N application. The GE content of wheat samples were less variable between wheat samples and values were in agreement with earlier results (Pirgozliev et al. 2003, 2015a). The ash content of wheat samples was variable (CV = 9.9%), however in agreement with those reported by McCracken et al. (2002) and Pirgozliev et al. (2003, 2015a). McCracken et al. (2002) reported similar size of variation studying wheat varieties grown at different location in the UK. In this study, wheat samples grown at Nottinghamshire site had higher ash content as compared to samples grown at rest of three growing sites. The variations in ash content of wheat samples may be due to various factors including soil contamination, use of harvester, type of soil (sandy, clay, mixed soil), however no such information was available regarding growing locations of these wheat samples. The studied samples were collected from different growing sites and in the UK, soil type is different and also depends on factors like water holding capacity and mineral content etc. Nottinghamshire growing site is a sandy light soil that can create significant amount of crop stress in summer due to drought. Relatively large variability (13%) was observed in crude oil content of wheat samples, however, the values were in agreement to reported results (McCracken et al. 2002; Pirgozliev et al. 2003; 2015a). Steenfeldt (2001) reported slightly higher oil content ranging from 21 – 27 g/kg DM in Danish wheat and found slightly low variation (8%) between wheat samples containing feed wheat and milling wheat. Wheat generally contains low fat content as compared to other cereals, on average 0.9 – 3% (Amerah 2015) and contribution is low towards available energy from wheat. The wheat-based diets require additional feedstuffs (e.g., soya oil) to meet up the energy requirements of birds. Overall, in this study, the proximate nutrient composition and GE values were in the range of reported values in the literature.

The starch content of 17 wheat samples was less variable (CV = 2.2%) and variation in starch content between wheat samples were in agreement with previous findings (Rose et al. 2001; Steenfeldt 2001; Svihus and Gullord 2002; McCracken et al. 2002; Carré et al. 2005). In this study, the amount of starch in wheat samples was in the range of 67 – 73%

and similar range of values were reported previously (Steenfeldt 2001; Svihus and Gullord 2002; Pirgozliev et al. 2003). Wheat contains high starch content (60 – 70%) and starch is the main energy yielding component of wheat (Ravindran and Amerah 2009). A study by Kim et al. (2003) reported slightly higher variation in starch content (CV = 5.4%) of Australian wheat samples. The authors also found higher variation in CP content (CV = 19.4%) of similar wheat samples. The authors compared nutrient composition of wheat varieties grown in three different rainfall zones between two growing seasons. The possible cause of large variation in starch content in their study could be due to the difference in rainfall during the growing season. Pirgozliev et al. (2003) found variation (5%) in starch content of wheat samples and speculated that variation could be due to samples grown in different seasons. Grain carbohydrate level is influenced by rainfall during different stages of plan growth (vegetative, growing, and ripening) (Gutierrez del Alamo 2008b). Variability in starch and protein concentration may result in differences in available energy of wheat and growth performance of broilers (Metayer et al. 1993). The amount of total NSP was within the range of previous work on wheat by several authors (Steenfeldt 2001; Choct et al. 2006; Pirgozliev et al. 2015a). The variability in total NSP contents was relatively low (4.9%). There was a range of variability in insoluble NSP content (CV= 5.5%). Insoluble NSP are attached to cell walls and inaccessible to birds during digestion process. Large variability in insoluble NSP content may result in decrease in nutrients availability and hence growth performance is affected (Choct et al. 1995). Large CV was observed for soluble NSP but in line with similar range of variation reported by Steenfeldt (2001), studying Danish wheat samples containing milling wheat and feed wheat. Pirgozliev et al. (2003) reported large CV (31%) in soluble NSP content of wheat samples collected from three different years. It is noteworthy that soluble NSP content are determined through subtraction of insoluble NSP from total NSP, therefore, the concentration of soluble NSP is linked to amount to insoluble NSP and its ratio with the total NSP content. The soluble constituents of sugar (arabinose, xylose) were highly variable between wheat samples. Among total NSP fraction, xylose had the least variability between wheat samples (5.3%), whereas arabinose had slightly higher variability (6.2%). Steenfeldt (2001) reported similar CV for arabinose studying 16 wheat samples. In conclusion, there was variation in CP, ash, oil and soluble NSP contents of a range of currently available UK wheat samples.

2.4.2. Amino acid composition

The IAA and DAA contents of wheat samples were in a similar range to published values (Bedford et al. 1998; Ravindran et al. 1999; McCracken and Quintin 2000; Steenfeldt 2001; Ravindran and Amerah 2009). Generally, moderate variation was observed in IAA content of wheat samples except methionine and phenylalanine. The methionine content was highly variable (CV = 12.3%) between wheat samples, whereas, phenylalanine was the second

highest varied IAA (CV = 10.7%) after methionine. In this study, the reason of relatively high variability in AA content between wheat samples could be due to large variation in CP content. A study by Steenfeldt (2001) found less variation in lysine, methionine and phenylalanine contents and related to less difference in CP content of wheat samples. The low lysine, methionine and threonine content of wheat samples were in correspondence to CP content of respective wheat samples, e.g., sample 17 with the lowest CP content had the lowest lysine, methionine, and threonine contents. The routine quality tests are unable to predict the CP and AA content of wheat and variation in AA content. The variability in IAA content of wheat samples suggested that routinely used wheat samples should be analysed for CP and AA content on arrival at the mill gate using some robust technique to adjust the AA level of wheat in feed formulation matrix rather using predicted values. In wheat, methionine content is relatively lower as compared to other IAA and results of this study confirmed the low level of methionine in all wheat samples. Lysine had moderate variation among wheat samples as compared to methionine and phenylalanine. Among DAA, moderate CV values were observed for majority of DAA except proline and tyrosine. The low levels of DAA of wheat sample 17 were in line with the low CP content. Overall, wheat samples with high CP value had higher AA content, e.g., sample one (1) had the highest CP content (143 g/kg DM), so had the highest threonine, leucine, histidine, glutamic acid and relatively high lysine, methionine. Similarly, wheat sample six (6) with CP content of 138 g/kg DM had highest lysine, threonine, phenylalanine, leucine, isoleucine, histidine and arginine content. In wheat, the AA content increases linearly with the increase in CP content (Ravindran et al. 1995; 2005) and the results of this study confirmed that wheat samples with highest CP content also had high levels of AA. In comparison to maize, wheat is considered as a good source of protein and AA contents and in commercial feed formulations, diet based on wheat require less AA supplementation as compared to maize. Overall, the variability in AA content of wheat samples were in line with the variation in CP content of correspondence samples.

2.4.3. Physical characteristics

The relative units of EH were in agreement of previous findings (Rose et al. 2001; Pirgozliev et al. 2003; Amerah et al. 2009b). In the current study, the use of wheats with different wheat types (soft and hard) can explain the reason of large variation in EH units. Due to the fact wheat samples consists of mixed pool of hard and soft wheats and feed wheats, therefore variation in EH was expected. The relative units of EH were higher in this study because of different equipment (SKCS) used for EH measurements, however it was comparable to previous data. The published data on smaller units of EH is based on near infrared analysis (NIR) while, SKCS method measures the force to break each wheat kernel and expressed EH index as a mean of 300 kernels. Wheat endosperm hardness is an

important characteristic in the quality of wheat for bread making, cakes and biscuits. Hardness of wheat affects the milling performance of the wheat. Hard wheat shatters during milling process and the flour is fine, with regular particle size and large surface area, whereas, soft wheat results in irregular particle size and smaller surface area (Rose et al. 2001; Ball et al. 2013a). The EH measurement provides an opportunity to flour millers to accept the wheat samples arriving at the mill and decision on their suitability for bread making or biscuits. Interestingly, the study also found slight variation in EH values of similar wheat variety grown at different locations, e.g., samples 4 (variety Leeds) grown at Cambridgeshire site had lower EH as compared to similar variety grown at Nottinghamshire. Rose et al. (2001) proposed that EH is affected by the crop growth and harvesting condition. The variation in EH of similar wheat variety suggested that the direct measurement of EH of wheat samples arriving at the feed mills is more accurate than discriminating them based on their expected values.

The values of HFN in wheat samples were similar to those reported previously (Rose et al. 2001; Hetland et al. 2007), although their reported HFN values were higher, which could be the due to the difference in growing condition, effect of weather, storage condition. The values of HFN varied between 17 wheat samples (CV = 28.7%), and soft and hard milling wheats had relatively high HFN as compared to feed wheat samples. The high variability in HFN values were due to different wheat types (soft – hard) of samples. The HFN is an alternative way of measuring α-amylase activity in wheat grains and high HFN means less or no α-amylase activity. High α-amylase activity was observed in feed wheats (Leeds and Santiago) with a lower HFN. Both Leeds and Santiago are feed wheat varieties and generally had low HFN (AHDB 2016).

The SW was less variable (CV = 2.8%) between wheat samples and values of SW were in accord with previous findings (Wiseman 2000; Gutierrez del Alamo et al. 2008a, Ball et al. 2013a, b). Specific weight (generally known as bushel weight) is used as a measure of quality of wheat by the feed millers to accept wheat samples based on their yield (kg/hl). In the UK, feed compounders accept wheat on the basis of minimum SW of 72 kg/hl and in this study, all wheat samples had SW above 72 kg/hl. The values of SW were in range of currently available UK wheat samples (AHDB 2015/2016). The TGW of wheat samples were in the expected range and values were in agreement with published values (Pirgozliev et al. 2003; Ball et al. 2013a). The TGW was variable between feed wheat and milling wheat. The values of DV were in the range as expected for wheat samples and had variation between samples (CV= 27.5%). The large variation in DV was due to high DV value of sample two. The sample was re-analysed but found similar value, therefore it was accepted. In this study, findings indicated that variation in some of physical characteristics (EH, HFN) were associated with the type of wheat and depend on hardness of wheat samples.

The positive correlation between some NSP content and fractions of NSP (soluble, insoluble arabinose, xylose) was expected and in agreement with earlier findings (Rose et al. 2001, Pirgozliev et al. 2003; Ball e al. 2013a). The positive relationship between GE and EH and HFN may be due to the large variation in values of EH and HFN because of different wheat types. However, there was no association of both EH and HFN with any of other chemical measurements of wheat samples, therefore the relationship may not be used as conclusive evidence to predict the nutritive value of wheat. In current study, the lack of association of SW with starch content of wheat samples was in accord with the previous findings (Pirgozliev et al. 2003; Ball et al. 2013a). The possible reason of absence of relationship may be due to less variation in SW and starch content of wheat samples. There was no association of SW with CP of wheat samples which may be due to less variation in SW of wheat samples. A study by Rose et al. (2001) found no relationship between CP and SW of wheat samples and the values of SW were less variable between different wheats. The findings from this study suggested that SW cannot be used as a predictor of nutritive value of wheat for broiler feeds because SW did not relate with routine chemical analysis of wheat samples.

2.5. CONCLUSIONS

The findings indicated that there are significant variations in the nutrient composition of currently available the UK wheat samples for poultry diets. Wheat is known for variable CP content and this study confirms that there is variation in CP content of currently available UK wheat samples. The present study also revealed variability in lysine, methionine and threonine contents of current wheat samples and suggest that feed specification matrix should be adjusted accordingly based on updated analysis of AA content of wheat on regular basis. Due to the fact, the routine quality tests used for wheat are unable to predict the AA level and variation between wheat samples, the updated AA values of wheat would help nutritionists to accurately estimate the required level of AA in feed formulations. The observed variability in the ash content of wheat samples was mainly due to two samples grown at a specific location. Overall, wheat samples grown at Nottinghamshire were higher in CP and ash contents. Starch content was less variable between wheat samples. Soluble NSP contents were greatly variable between samples but similar range of variability have been reported in the literature. The SW is widely used in commercial poultry feed industry to predict the feeding quality of wheat, however, this study indicated that SW cannot be used to predict the nutritive quality of wheat for broiler feeds because of absence of relationship between SW and starch and CP content of wheat samples. In conclusion, there was no relationship between physical characteristics and chemical analysis of wheat samples.

CHAPTER 3: EFFECT OF SEVENTEEN WHEAT SAMPLES ON APPARENT METABOLISABLE ENERGY, NUTRIENT UTILISATION AND GROWTH PERFORMANCE OF BROILERS

3.1. INTRODUCTION

Wheat is known for its variable nutrient content (Steenfeldt 2001; Gutierrez del Alamo et al. 2008a; Ball et al. 2013a, b). The findings from Chapter 2 confirmed that currently available UK wheat samples varied in their chemical composition and physical characteristics. Variation in nutrient composition of wheat can significantly affect AME and growth performance of broilers (Svihus and Gullord 2002; Pirgozliev et al. 2003). Therefore, it is critically important to determine wheat characteristics which may influence AME and growth performance of broiler chickens. To test this, 17 wheat samples studied in Chapter 2 were incorporated to formulate broiler grower diets and their effect on AME and growth performance of broilers was investigated.

Variation in protein content of wheat can significantly affect AME and growth performance of broilers. Broiler growth response to wheat-based diets in some of previous studies has been due to differences in protein content of the wheat sample (Steenfeldt 2001; Hetland et al. 2004; Pirgozliev et al. 2003). The findings of Chapter 2 confirmed large variation in CP content of currently available UK wheat samples. To eliminate the possible influence of variation in CP content of wheat samples on AME and growth performance of broilers, all diets were balanced for protein in this study. This enables to investigate the effects of other chemical characteristics of wheat such as starch, NSP and their relationship with AME and growth performance.

Majority of published data on wheat studies is based on mash feeding to broilers due to the simplicity of mash diet manufacturing and unavailability of resources to pellet diets. Broiler chickens responded differently to wheat-based mash diets as compared with pellet diets (Amerah et al. 2007a, b; Abdollahi et al. 2011; Pirgozliev et al. 2016). It is commercially relevant that diets are fed in pellet form to investigate the effect of wheat-based diets on AME and growth performance. Due to the fact that small volume of diets is required for research trials, whereas, commercial facilities are not designed to produce small quantities. Moreover, commercially pelleted diets for research trials may have chances of error e.g., handling of small batches of diets for research studies, mixing errors between diets, cross contamination of raw ingredients between batches etc. To avoid these possible errors during diet manufacturing, all experimental diets were pelleted at NIPH (The National Institute of Poultry Husbandry) Harper Adams University to ensure uniformity in the diet,

minimise variations during mixing, avoidance of contamination with other diets and control of temperature for all diets during pelleting.

The objectives of this study were:

(a) To investigate the differences in the AME of currently available wheat samples in the UK.

(b) To determine the differences in growth performance of broilers when these wheat samples were fed as part of nutritionally balanced diet.

(c) To determine if differences were related to chemical composition and physical characteristics of wheat.

(d) To determine the relationship between the AME of wheat and growth performance of broilers.

3.2. MATERIALS AND METHODS

3.2.1. Wheat samples

The wheat samples analysed previously in Chapter 2 were incorporated into broiler grower diets for this study.

3.2.2. Diet preparation

Each wheat sample was milled to pass through a 3 mm screen. Hammer mill was cleaned after milling each wheat samples to avoid any cross contamination of different wheat samples. Each milled wheat sample was mixed with a balancer feed separately. A balancer feed (Target Feeds Ltd, Whitchurch, UK) was formulated including major ingredients of 521.3 g/kg soybean meal (SBM), 299.2 g/kg of full-fat soya meal, 60.5 g/kg soya oil, and contained 374.5 g/kg CP and 12.45 MJ/kg AME (Table 3.1). Seventeen diets were prepared by mixing 670 g/kg of each of the 17 experimental wheat samples with 330 g/kg of a balancer.

Table 3. 1. Ingredients and chemical composition (g/kg as-fed) of balancer and wheat-based grower diet for broiler chickens.

Ingredients	**Basal**	**Diet**
Wheat	–	670.0
Soybean meal (48)	521.3	172.0
Full-fat soybean meal	299.2	99.0
Soya oil	60.5	20.0
Monocalcium phosphate	35.4	12.0
Limestone	40.9	13.0
NaCl	9.1	3.0
L- Lysine HCL	9.1	3.0
DL Methionine	12.4	4.0
Vitamin mineral premix[1]	12.1	4.0
Titanium Dioxide*	15	5
Total	1015	1005
Calculated analysis (as fed basis)		
CP (g/kg)	374.5	204
ME (MJ/kg)	12.45	12.96
Crude fat (g/kg)	119.3	48.8
Ca (g/kg)	23.3	8.3
Available P (g/kg)	9.7	4.1
Lysine (g/kg)	31.3	13.0
Methionine (g/kg)	15.1	6.1
Threonine (g/kg)	13.4	6.6
Methionine + Cysteine (g/kg)	20.4	9.3
Tryptophan (g/kg)	4.6	2.2
Analysed values (as fed)		
DM (g/kg)	915	890 -916
CP (N × 6.25) (g/kg)	369	202- 203
Crude fat (g/kg)	127	38.9 - 49.6

Each experimental diet met or exceeded the diet specification for Ross 308 broilers (Aviagen 2014a).
*Titanium dioxide (TiO_2) was added on top in balancer diet @ 15 g/kg.
[1]The vitamin and mineral premix contained vitamins and trace elements to meet the breeder's recommendations (Aviagen Ltd, Edinburgh, UK). The premix provided (units/kg diet): retinol, 12000 IU; cholecalciferol, 5000 IU; α-tocopherol, 34 mg; menadione, 3 mg; thiamine, 2 mg; riboflavin,7 mg; pyridoxine,5 mg; cobalamin, 15 µg; nicotinic acid, 50 mg; pantothenic acid,15 mg; folic acid,1 mg; biotin,200 µg; 80 mg Fe as iron sulphate (30%); 10 µg Cu as copper sulphate (25%); 100 mg Mn as manganous oxide (62%); 80 mg Zn as zinc oxide (72%); 1 mg I as calcium iodate (52%); 0.2 mg Se as sodium selenite (4.5%) and 0.5 mg Mo as sodium molybdate (40%).

The AME was not determined on balancer diet because it would have contained an inappropriate supply of nutrients for broilers. Three additional diets (18, 19 and 20) were formulated by mixing 470, 570 and 770 g/kg of one of the wheats (sample 8) with 530, 430 and 230 g/kg of balancer feed, respectively, for AME determination of the balancer feed by slope ratio method (Finney 1978). Sample 8 was chosen at random to formulate additional three diets. The nutritional profile of each additional diet was closed to the current nutrient specification for Ross 308 broilers. The AME of balancer diet was predicted by regression analysis of diet 8, 18, 19 and 20.

In diets 1 –17, each of 17 wheat samples was included at 670 g/kg of the final diet. Diets 18, 19 and 20 were those containing wheat sample 8, at inclusion levels of 470, 570 and 770 g/kg, respectively of final diet. Each diet was supplemented with titanium dioxide TiO_2 as an external marker @ inclusion level of 5 g/kg of final diet for determination of ileal digestibility for starch and AA. Diets were free of coccidiostats, antimicrobial growth promoters or any other additives. Each experimental diet met or exceeded the current diet specification for Ross 308 broiler chicken (Aviagen 2014a).

3.2.2.1. Balance of energy and protein

Diets were made iso-nitrogenous by adding wheat protein isolate (WPI) (LifeSource Foods, USA) to each wheat sample by substituting wheat with WPI. The additional quantity of wheat protein isolate (WPI) to be added was estimated on analysed protein value of each wheat sample on *as fed basis*. The additional amount of WPI was added to each wheat sample and to balance the final quantity of each wheat sample inclusion (670 g/kg) in the diet, maize starch was added e.g., a mixture of wheat protein isolate (WPI) and maize starch totalling 25.5 g/kg was added to each wheat sample. The highest amount of wheat protein isolate was added to sample 17 at 25.5 g / 670 g wheat. All 17 diet had the same amount of protein. A relatively small contribution of energy provided by additional WPI and maize starch was taken into consideration during AME determination of each diet. The determined AME of the diet in this study was the AME of the mixture (wheat plus WPI).

3.2.2.2. Pelleting of diet

The diets were pelleted at NIPH (The National Institute of Poultry Husbandry) Harper Adams University using a pelleter (KAHL, Amandus Kahl GmbH & Co. KG, Reinbek, Germany). The pelleter was connected with a feeder and outlet jacket (Figure 2.1). Diets were pelleting without using steam. The frequency of pelleter was maintained at 50Hz and temperature of the jacket ranged between 44.5°C – 46.5°C during pelleting. Pellets were produced at temperature ranged between 59°C – 62.5°C. The whole pelleting process was in a controlled environment, strictly monitoring speed of the feeder, frequency of pelleter

and temperature of pellet produced. The diameter and length of pellet was 3 mm and 4 – 7 mm, respectively. Pellets were dispensed in plastic trays using a metal chute to avoid physical damage and were spread evenly in plastic trays for air to circulate. Pellets were cool down to ambient temperature by ventilated cool air for 30 mins and later stored in bags at temperature below 18°C. Extra care was taken during bagging up pellets to avoid any breakage or mechanical damage.

Figure 3. 1. Pelleting of broiler diet using Kahl Amandus Pelleter.
(Source: Author's own)

3.2.2.3. Pellet durability index (PDI)

Pellet durability index (PDI) was determined in duplicate using a Holmen Pellet Tester (Holmen NHP100 Portable Pellet Durability Tester, TekPro Ltd, Willow Park, Norfolk, UK) by simulating the pellet transportation environment from mill to trough. A 100 g of clean pellet samples with no fines were used for testing. Pellets were rapidly circulated in an air stream around a perforated test chamber for 30 seconds. Fines were removed continuously through the perforations (2 mm in diameter) during the test cycle for accurate readings. After the test cycle, pellets were removed manually and weighed. The PDI was calculated as the ratio of the pellets not passing through the perforations after test to whole pellets at

the start of test. The values of PDI ranged from 73.4 to 83.8% (CV = 3.73%) (Table 3.2). The dry matter (DM) of all experimental diets was also determined as described in chapter 2 (Section 2.2.2.1) and ranged from 890 to 916 g/kg DM (Table 3.2).

Table 3. 2. Dry matter (DM) and pellet durability index (PDI %) of seventeen grower diets[1].

Diet	DM g/kg	PDI %
1	894	77.4
2	909	79.2
3	897	80.2
4	901	80.6
5	902	79.0
6	890	83.0
7	904	80.2
8	899	78.6
9	902	74.8
10	906	73.4
11	916	78.4
12	905	83.8
13	896	76.2
14	901	74.0
15	894	76.9
16	900	81.5
17	894	78.5
Mean	901	78.6
Min	890	73.4
Max	916	83.8
CV %	0.7	3.73

[1]Each value represents mean of 2 replicates.

3.2.3. Birds husbandry and experimental design

All procedures were approved by Harper Adams University Research Ethics Committee. Eight hundred (800), one day old male Ross 308 broilers were obtained from a commercial hatchery (Cyril Bason ltd, Craven Arms, Shropshire, UK), weighed individually and allocated randomly to 160 raised floor pens with 0.60 m^2 solid floors area, five birds in each pen. Each diet was randomly assigned to eight pens within blocks and fed from 0 – 21 days. Blocks were positioned within the broiler house. Birds were fed experimental wheat-based mash diets from 0 – 7 days and pellet diets from 7 to 21 d of age. Feed and water were offered *ad libitum* to birds throughout the experiment. Each pen was equipped with a feeding trough outside the pen and automatic drinkers inside the pen. Temperature was maintained at 32°C for the first day of the study and was reduced gradually to 21°C at the end of study (d 21). A standard light programme was followed, decreasing the light: dark ratio from

23h : 1h from day old to 18h : 6h at day 7, which was maintained till day 21 (Aviagen Ross broiler management handbook, Aviagen Ltd. Edinburgh, UK). Body weights were recorded at the beginning (day 0) and at the end of experiment (day 21). The birds were inspected daily, and dead birds were weighed at the time of removal. Feed intake per pen (FI) was recorded and feed conversion ratio (FCR) was calculated on a pen weight basis. The body weights of dead birds were also included when calculating FCR.

3.2.4. Nutrient utilisation measurements

3.2.4.1. Determination of wheat apparent metabolisable energy

At day 18, the solid floor of each pen was replaced with a wire mesh and plastic trays were placed underneath for excreta collection. The AME, N-corrected AME (AMEn) and total tract nutrients retention was determined using total collection method. Although TiO_2 was added in the diet to determine nutrient digestibility, however not used in AME determination due to possible chance of less digesta available for starch and AA digestibility. Excreta were collected every 12 h per pen for three consecutive days (19 – 21 days) and immediately dried at 60°C in a forced draft oven, weighed and milled by Retsch ZM 200 (Retsch GmbH, Haan, Germany) using a 0.75 mm screen. Feathers and scales were removed carefully to avoid any contamination. The feed intake was recorded for the same period. The dry matter (DM), GE, nitrogen (N) and fat (as ether extract) contents of excreta and the experimental diets were determined as described for the wheat samples (Section 2.2.2.1). All analyses were performed in duplicate.

The AME values of the diets on a DM basis were determined by using the equation 3.1.

Equation 3.1. AME calculation

$$AME_{diet}\,(MJ/kg\ DM) = \frac{(Feed\ intake \times GE_{diet}) - (Excreta\ output \times GE_{excreta})}{Feed\ intake}$$

N-corrected AME (AMEn) was determined by correction for zero N retention by simple multiplication with 34.39 MJ/kg N retained as described by Hill and Anderson (1958) (Equation 3.2).

Equation 3.2. AMEn calculation

$$AMEn_{diet}\,(MJ/kg\ DM) = \frac{((Feed\ intake \times GE_{diet}) - (Excreta\ output \times GE_{excreta})) - (N_{retained} * 34.39)}{Feed\ intake}$$

The AME of wheat was determined by substitution method using equation 3.3. Linear regression analysis was used to test the linear response of bioavailable energy and total tract nutrient retention at four inclusion levels of wheat (470, 570, 670 and 770 g/kg) in the diets. There was a linear response ($P < 0.05$) to inclusion levels of wheat for AME, AMEn and nutrient retention with no evidence of non-linearity ($P > 0.05$) (Table 3.3). The AME of balancer diet was determined by linear regression analysis of diets 8, 18, 19 and 20 and values were used in equation 3.3 to calculate the AME of wheat.

Equation 3.3. AME, AMEn of wheat

$$\text{AME}_{\text{Wheat}}\ (\text{MJ/kg DM}) = \frac{\text{AME}_{\text{diet}} - (\text{AME}_{\text{balancer diet}} \times 0.33)}{0.67}$$

$$\text{AMEn}_{\text{Wheat}}\ (\text{MJ/kg DM}) = \frac{\text{AMEn}_{\text{diet}} - (\text{AMEn}_{\text{balancer diet}} \times 0.33)}{0.67}$$

Where 0.33 is the inclusion level of balancer diet and 0.67 is the inclusion level of wheat in whole diet.

3.2.4.2. Coefficients of total tract nitrogen retention, fat digestibility and dry matter retention

The coefficients of total tract nitrogen retention (NR), fat digestibility (FD) and dry matter retention (DMR) of diets were determined as the differences between the intake and the excretion of nutrients divided by their respective nutrient intake (Equation 3.4).

Equation 3.4. NR, FD, DMR calculations:

$$\text{NR coefficient}_{\text{diet}} = \frac{(\text{Feed intake} \times \text{N}_{\text{diet}}) - (\text{Excreta output} \times \text{N}_{\text{excreta}})}{\text{Feed intake} \times \text{N}_{\text{diet}}}$$

$$\text{FD coefficient}_{\text{diet}} = \frac{(\text{Feed intake} \times \text{fat}_{\text{diet}}) - (\text{Excreta output} \times \text{fat}_{\text{excreta}})}{\text{Feed intake} \times \text{fat}_{\text{diet}}}$$

$$\text{DMR coefficient}_{\text{diet}} = \frac{(\text{Feed intake} \times \text{DM}_{\text{diet}}) - (\text{Excreta output} \times \text{DM}_{\text{excreta}})}{\text{Feed intake} \times \text{DM}_{\text{diet}}}$$

The total nutrient retention of wheat was determined as illustrated for the wheat using equation 3.5. The nutrient retention constant of balancer diet was determined by linear regression analysis of diets 8, 18, 19 and 20 and values were used in equation 3.5 to calculate total tract nutrient retention of wheat.

Equation 3.5. NR, FD, DMR of wheat:

$$\text{Nutrient retention }_{\text{Wheat}} = \frac{(\text{Nutrient }_{\text{diet}}) - (\text{Nutrient }_{\text{balancer diet}} \text{ x } 0.33)}{0.67}$$

3.2.4.3. Digesta viscosity

At the end of trial, at 21 days of age, birds in each pen were weighed and killed by cervical dislocation. The contents of ileal digesta from Meckel's diverticulum to ileal-caecal junction were gently pressed from each bird into a plastic container and pooled by pen and homogenised. A sample of this homogenised digesta was centrifuged (10 000 × *g* for 2 mins) to determine ileal digesta viscosity. The viscosity of the supernatant was measured using a rotating cone and cup viscometer (model DV – II + LV, Brookfield Stoughton, MA, USA) as described by Bedford and Classen (1992).

3.2.4.4. Ileal digestibility of starch, protein and amino acids

The digesta from each pen were immediately transferred to freezer at −20°C and subsequently freeze-dried. The dried ileal digesta were then ground to pass through 0.75 mm screen Retsch ZM 200 (Retsch GmbH, Haan, Germany) and stored in airtight containers in a freezer at −20°C for chemical analyses of GE, N, starch and TiO_2.

The Ileal CP (N x 6.25) content was determined using Leco FP 528 as described for wheat samples (Section 2.2.2.1). The starch contents in ileal digesta and balancer diet was determined as described in chapter 2 (Section 2.2.2.2). The concentration of amino acids (AA) in the digesta and balancer diet was determined as described in chapter 2 (Section 2.2.2.3). The concentration of AA and starch in the diet was determined by using analysed value of AAs and starch in wheat samples and balancer feed and through multiplication with their respective inclusion levels (wheat 0.67, balancer 0.33). Titanium Dioxide (TiO_2) in digesta and balancer diet was analysed by DM Scientific Ltd (main site, Dalton, Thirsk, North Yorkshire). The ashed samples were boiled in sodium sulphate and concentrated sulphuric acid, later the samples were dissolved in hydrogen peroxide. The addition of hydrogen peroxide resulted in orange colour, the intensity of colour dependent upon the concentration of titanium. The concentration of TiO_2 in the sample aliquots was read on spectrophotometry against standard titanium solution.

Equation 3.6. Apparent ileal digestibility of starch, protein and amino acids (AA)

The ileal digestibility of starch was determined by the following equation:

$$\text{Apparent starch digestibility}_{\text{diet}} = \frac{((\text{Starch}/\ TiO_2)_{\text{diet}} - (\text{Starch}/\ TiO_2)_{\text{digesta}}))}{(\text{Starch}/\ TiO_2)_{\text{diet}}}$$

Apparent Ileal digestibility of protein (N × 6.25) was determined by the following equation:

$$\text{Apparent protein digestibility}_{\text{diet}} = \frac{((\text{Protein}/\ TiO_2)_{\text{diet}} - (\text{Protein}/\ TiO_2)_{\text{digesta}}))}{(\text{Protein}/\ TiO_2)_{\text{diet}}}$$

The apparent ileal digestibility of AA in diets was determined by the following equation as described by Ravindran et al. (1999).

$$\text{Apparent amino acid digestibility}_{\text{diet}} = \frac{((AA/\ TiO_2)_{\text{diet}} - (AA/\ TiO_2)_{\text{digesta}}))}{(AA/\ TiO_2)_{\text{diet}}}$$

Where $(AA/TiO_2)_{\text{diet}}$ is the ratio of amino acids to Titanium dioxide (TiO_2) in diet and $(AA/TiO_2)_{\text{digesta}}$ is the ratio of amino acid to Titanium dioxide (TiO_2) in ileal digesta.

The ileal digestibility of starch, protein and AA in wheat samples were calculated as described in equation 3.3 after determining the constant for basal diet as illustrated in section 3.2.4.1.

3.2.5. Statistical analysis

All broiler data were collated, and calculations were performed using Microsoft Excel 2013. Statistical analyses were performed in GenStat statistical software (GenStat 17th edition supplied by VSN international Ltd, UK). Broiler growth performance, AME, AMEn values of wheat samples and nutrient retention coefficients were subjected to analysis of variance (ANOVA) in a randomised block design, with a single pen representing experimental unit (replicate). Treatments and block were fixed effects. The variables that described growth performance were feed intake gram per bird per day (FI), weight gain gram per bird per day (WG), final body weight kilogram per bird over entire study period (FBW), feed conversion ratio corrected for mortality (gram of feed intake per gram of weight gain) (FCR). Differences were reported as significant at $P \leq 0.05$ and trends were reported at $P < 0.1$. Means were separated using Duncan's multiple range test and differences were reported significant at $P < 0.05$. Least significant difference (LSD) test was used for illustration purpose to report the significant differences between means of variables.

Pearson's correlation coefficients were generated to test linear relationship between chemical composition plus physical characteristics of wheat samples with growth performance and AME and nutrient utilisation of wheat. Relationships were reported at significance level ($P < 0.05$; r = 0.482, $P < 0.01$; r = 0.606, $P < 0.001$; r = 0.725 and $P < 0.1$; r = 0.412).

Simple and stepwise multiple linear regression analysis was used to assess the relationship between broiler growth performance, determined AME of wheat and characteristics of wheat samples (chemical composition and grain quality). A stepwise regression technique was used to evaluate the effects of the independent variables into a linear model. Chemical composition and physical characteristics of wheat samples were used as independent variables in stepwise regression. The variables FI, WG, FCR and determined AME of wheat samples were used separately as dependent variables into regression model. The variables were added one at a time in the model starting with highest correlation with dependant variables. Contribution of each variable was analysed before entering next variable. If a non-significant variance was found, it was removed from the model. Variables were added to independent variables until there was no further improvement in variance and addition of variables were statistical significance ($P < 0.05$) in the equation. The effect of wheat variety and growing site on biological data was analysed by unbalanced design using GenStat regression.

3.3. RESULTS

3.3.1. Linearity between different inclusions levels of wheat

There was a linear response to the inclusion levels of wheat samples (47, 57, 67 and 77 %) for AME, AMEn, NR, FD and DMR (Table 3.3).

Table 3. 3. Linearity between wheat inclusion levels and apparent metabolisable energy and nutrient availability.

Variables	Inclusion level of wheat				SEM[1]	*P value*		
	47%	57%	67%	77%		Treatment	Linear	Quadratic
AME DM	14.67	14.5	13.97	13.07	0.424	0.052	0.008	0.507
AMEn DM	12.56	12.78	12.65	13.37	0.139	0.001	<0.001	0.107
NR	0.621	0.624	0.602	0.588	0.0125	0.166	0.041	0.590
FD	0.852	0.826	0.756	0.774	0.0197	0.006	0.003	0.173
DMR	0.665	0.685	0.695	0.736	0.0071	<0.001	<0.001	0.260

[1]Stanadrad error of means.

3.3.2. Apparent metabolisable energy of wheat

The determined AME of 17 individual wheat samples ranged from 13.68 to 14.81 MJ/kg DM (CV $^{m\ (of\ 17\ individual\ samples)}$ = 4.2%) (Table 3.4). The determined AMEn of wheat samples ranged from 13.32 to 14.36 MJ/kg DM (CV ^{m} = 4.1%), respectively. There were differences (P < 0.05) in AME and AMEn between individual wheat samples. There were differences (P < 0.05) in GE metabolisability of wheat samples and AME: GE ratio ranged from 0.762 to 0.822 (CV ^{m} = 4.2%), whereas, the AMEn: GE ratio ranged from 0.742 to 0.797 (CV ^{m} = 4.1%). The determined AME, AMEn and nutrient utilisation of 17 diets is presented in Appendices (Appendix A).

Table 3. 4. The effect of wheat samples on [1]apparent metabolisable energy (AME MJ/kg DM), N-corrected AME (AMEn MJ/kg DM), gross energy metabolisability (GE J/J).

Wheat samples	AME	AMEn	AME: GE	AMEn: GE
1	14.00^{abcd}	13.65^{abcd}	0.773^{abc}	0.754^{ab}
2	14.25^{abcde}	13.92^{abcde}	0.794^{abcd}	0.776^{abc}
3	13.73^{ab}	13.40^{ab}	0.768^{ab}	0.750^{ab}
4	14.44^{bcde}	14.03^{bcde}	0.811^{cd}	0.788^{bc}
5	14.38^{abcde}	14.05^{bcde}	0.804^{bcd}	0.785^{bc}
6	13.82^{abc}	13.44^{abc}	0.762^{a}	0.742^{a}
7	14.20^{abcde}	13.86^{abcde}	0.790^{abcd}	0.771^{abc}
8	13.68^{a}	13.32^{a}	0.762^{a}	0.743^{a}
9	14.03^{abcd}	13.64^{abcd}	0.778^{abc}	0.756^{ab}
10	14.55^{de}	14.17^{de}	0.804^{bcd}	0.783^{bc}
11	14.05^{abcd}	13.71^{abcde}	0.770^{ab}	0.752^{ab}
12	14.50^{cde}	14.11^{cde}	0.803^{bcd}	0.782^{bc}
13	14.36^{abcde}	13.96^{abcde}	0.798^{abcd}	0.776^{abc}
14	14.05^{abcd}	13.65^{abcd}	0.782^{abc}	0.759^{abc}
15	14.81^{e}	14.36^{e}	0.822^{d}	0.797^{c}
16	14.40^{bcde}	13.96^{abcde}	0.801^{abcd}	0.777^{abc}
17	14.36^{abcde}	13.99^{abcde}	0.807^{bcd}	0.785^{bc}
Mean	14.21	13.84	0.790	0.769
Min	13.68	13.32	0.762	0.742
Max	14.81	14.36	0.822	0.797
CV%	4.2	4.1	4.2	4.1
SEM	0.212	0.203	0.0118	0.0113
P value	0.012	0.017	0.004	0.005

[1]Each value represents mean of 8 experimental unit (pen) of 5 birds each. Values are based on total collection from 19 to 21 days of age.
[2]Standard error of means (df = 109).
Means within a column with no common superscripts differ significantly (P < 0.05).

3.3.3. Nutrient utilisation of wheat

There was no difference ($P > 0.05$) in the coefficient of NR between wheat samples. Differences were observed for coefficients of FD and DMR ($P < 0.05$, $P < 0.001$, respectively) between wheat samples (Table 3.5). The coefficients of NR ranged from 0.545 to 0.607(CV ^{m} = 8.2%), FD ranged from 0.605 to 0.742 (CV ^{m} = 11.6%) and DMR ranged from 0.763 to 0.811, (CV ^{m} = 3.6%).

Table 3. 5. The effect of wheat samples on coefficients[1] of nitrogen retention (NR), fat digestibility (FD) and dry matter retention (DMR) in broilers.

Wheat samples	NR	FD	DMR
1	0.545	0.637^{ab}	0.765^{a}
2	0.557	0.658^{abc}	0.795^{abc}
3	0.588	0.658^{abc}	0.799^{bc}
4	0.607	0.678^{abc}	0.807^{c}
5	0.599	0.685^{abc}	0.802^{c}
6	0.565	0.614^{a}	0.763^{a}
7	0.560	0.742^{c}	0.768^{ab}
8	0.563	0.696^{abc}	0.764^{a}
9	0.575	0.605^{a}	0.781^{abc}
10	0.572	0.668^{abc}	0.806^{c}
11	0.559	0.672^{abc}	0.767^{ab}
12	0.591	0.710^{bc}	0.804^{c}
13	0.606	0.728^{bc}	0.807^{c}
14	0.570	0.677^{abc}	0.780^{abc}
15	0.597	0.742^{c}	0.811^{c}
16	0.605	0.737^{c}	0.790^{abc}
17	0.572	0.717^{bc}	0.789^{abc}
Mean	0.578	0.684	0.788
Min	0.545	0.605	0.763
max	0.607	0.742	0.811
CV%	8.2	11.6	3.6
SEM[2]	0.0168	0.0281	0.0101
P value	0.179	0.007	<0.001

[1]Each value represents mean of 8 experimental unit (pen) of 5 birds each. Values are based on total collection from 19 to 21 days of age.
[2]Standard error of means (df = NR: 109, FD: 108, DMR: 110).
Means with a column with no common superscripts differ significantly ($P < 0.05$).

3.3.4. Starch and protein digestibility of wheat

There was a difference ($P < 0.05$) in ileal digestibility of starch between wheat samples (Table 3.6). Starch digestibility ranged from 0.749 to 0.869 ($CV^m = 9.9\%$) and there was a difference of 16% in starch digestibility between lowest and highest wheat sample. Ileal digestibility of protein was different ($P < 0.05$) between wheat samples. Protein digestibility of wheat samples ranged from 0.782 to 0.873 ($CV^m = 5.1\%$) and there was a difference of 11.6% in protein digestibility between lowest and highest wheat sample.

3.3.5. Amino acid digestibility of wheat

There were differences ($P < 0.05$) in ileal digestibility of methionine and leucine (Table 3.7). There was also a difference ($P = 0.05$) in phenylalanine digestibility between wheat samples. There was no difference ($P > 0.05$) in lysine and threonine digestibility between wheat samples. Among dispensable amino acids (DAA), differences ($P < 0.05$) were observed in proline and tyrosine digestibility between wheat samples (Table 3.8).

Table 3. 6. Apparent ileal digestibility coefficients[1] of starch and protein (N x 6.25) of wheat samples for broilers.

Wheat samples	Starch	Protein
1	0.754^{a}	0.828^{abcd}
2	0.819^{ab}	0.782^{a}
3	0.784^{ab}	0.824^{abcd}
4	0.864^{b}	0.788^{ab}
5	0.810^{ab}	0.843^{cd}
6	0.786^{ab}	0.830^{abcd}
7	0.749^{a}	0.832^{abcd}
8	0.751^{a}	0.834^{bcd}
9	0.804^{ab}	0.819^{abc}
10	0.867^{b}	0.814^{abc}
11	0.780^{ab}	0.802^{abc}
12	0.869^{b}	0.836^{bcd}
13	0.837^{ab}	0.873^{d}
14	0.804^{ab}	0.793^{abc}
15	0.818^{ab}	0.823^{abc}
16	0.833^{ab}	0.839^{cd}
17	0.860^{b}	0.799^{abc}
Mean	0.811	0.821
Min	0.749	0.782
Max	0.869	0.873
CV%	9.9	5.1
SEM[2]	0.0284	0.0148
P value	0.017	0.004

[1]Each value represents mean of 8 experimental unit (pen) of 5 birds each.
[2]Standard error of means (df = starch: 112, protein: 111).
Means within a column with no common superscripts differ significantly ($P < 0.05$).

Table 3. 7. Apparent ileal digestibility coefficient[1] of indispensable amino acids (IAA) of wheat samples for broilers.

Wheat samples	Lysine	Methionine	Threonine	Phenyl-alanine	Leucine	Iso-leucine	Histidine	Arginine	Valine
1	0.845	0.893^{abcd}	0.852	0.881	0.879^{bc}	0.850	0.853	0.896	0.788
2	0.793	0.899^{abcd}	0.764	0.854	0.832^{ab}	0.810	0.814	0.910	0.767
3	0.832	0.911^{abcd}	0.685	0.866	0.864^{abc}	0.859	0.840	0.898	0.782
4	0.812	0.871^{ab}	0.796	0.851	0.872^{bc}	0.860	0.833	0.904	0.729
5	0.841	0.881^{abc}	0.798	0.883	0.880^{bc}	0.841	0.851	0.910	0.800
6	0.870	0.893^{abcd}	0.867	0.901	0.907^{bc}	0.907	0.891	0.944	0.869
7	0.861	0.936^{bcd}	0.822	0.907	0.897^{bc}	0.876	0.872	0.938	0.824
8	0.870	0.950^{cd}	0.760	0.911	0.899^{bc}	0.893	0.877	0.917	0.847
9	0.844	0.930^{bcd}	0.819	0.890	0.860^{abc}	0.898	0.852	0.917	0.821
10	0.848	0.926^{abcd}	0.782	0.906	0.898^{bc}	0.867	0.894	0.923	0.819
11	0.865	0.953^{cd}	0.769	0.888	0.882^{bc}	0.856	0.848	0.900	0.795
12	0.886	0.964^{d}	0.800	0.911	0.908^{bc}	0.888	0.890	0.937	0.820
13	0.839	0.917^{abcd}	0.788	0.887	0.880^{bc}	0.828	0.838	0.923	0.794
14	0.808	0.854^{a}	0.731	0.843	0.838^{ab}	0.847	0.808	0.911	0.824
15	0.823	0.924^{abcd}	0.732	0.907	0.868^{bc}	0.847	0.863	0.948	0.815
16	0.916	0.921^{abcd}	0.863	0.912	0.934^{c}	0.909	0.886	0.933	0.834
17	0.790	0.898^{abcd}	0.751	0.824	0.794^{a}	0.815	0.825	0.935	0.786
Mean	0.844	0.913	0.787	0.884	0.876	0.862	0.855	0.920	0.807
Min	0.790	0.854	0.685	0.824	0.794	0.810	0.808	0.896	0.729
Max	0.916	0.964	0.867	0.912	0.934	0.909	0.894	0.948	0.869
CV%	9.6	6.9	14.6	6.6	7.4	9.5	8.5	6.2	11.3
SEM[2]	0.0287	0.0222	0.0407	0.0205	0.0229	0.0289	0.0258	0.0203	0.0321
P value	0.200	0.036	0.133	0.048	0.015	0.368	0.358	0.810	0.414

[1]Each value represents mean of 8 experimental unit (pen) of 5 birds each. Ileal digesta was collected from broilers at 21 days of age.
[2]Standard error of means.
Means within a column with no common superscripts differ significantly ($P < 0.05$).

Table 3. 8. Apparent ileal digestibility coefficient[1] of dispensable amino acids (DAA) of wheat samples for broilers.

Wheat samples	Alanine	Aspartic acid	Glutamic acid	Cystine	Glycine	Proline	Serine	Tyrosine
1	0.833	0.785	0.925	0.942	0.847	0.919^{bc}	0.940	0.838^{abc}
2	0.735	0.759	0.923	0.869	0.841	0.864^{ab}	0.718	0.809^{abc}
3	0.699	0.769	0.905	0.828	0.812	0.882^{bc}	0.869	0.805^{abc}
4	0.761	0.801	0.942	0.932	0.893	0.893^{bc}	0.946	0.803^{abc}
5	0.747	0.809	0.926	0.937	0.890	0.886^{bc}	0.797	0.840^{abc}
6	0.808	0.868	0.969	0.925	0.805	0.938^{c}	0.978	0.876^{c}
7	0.858	0.818	0.918	0.981	0.905	0.919^{bc}	0.735	0.865^{c}
8	0.842	0.823	0.942	0.955	0.852	0.920^{bc}	0.943	0.866^{c}
9	0.802	0.761	0.911	0.917	0.789	0.891^{bc}	0.943	0.814^{abc}
10	0.824	0.818	0.933	0.964	0.899	0.920^{bc}	0.940	0.859^{bc}
11	0.754	0.766	0.928	0.882	0.838	0.896^{bc}	0.901	0.818^{abc}
12	0.903	0.795	0.962	0.965	0.834	0.915^{bc}	0.764	0.861^{bc}
13	0.864	0.761	0.939	0.933	0.777	0.897^{bc}	0.846	0.820^{abc}
14	0.715	0.671	0.921	0.896	0.810	0.871^{abc}	0.928	0.778^{ab}
15	0.829	0.739	0.921	0.914	0.856	0.895^{bc}	0.846	0.826^{abc}
16	0.809	0.835	0.984	0.953	0.848	0.920^{bc}	0.920	0.879^{c}
17	0.682	0.712	0.917	0.882	0.649	0.819^{a}	0.566	0.767^{a}
Mean	0.792	0.782	0.933	0.922	0.832	0.897	0.858	0.831
Min	0.682	0.671	0.905	0.828	0.649	0.819	0.566	0.767
Max	0.903	0.868	0.984	0.981	0.905	0.938	0.978	0.879
CV%	19.9	15.9	7.7	9.8	16.6	6.3	29.5	8.5
SEM	0.0556	0.0439	0.0253	0.0321	0.0487	0.0199	0.0893	0.0249
P value	0.222	0.287	0.776	0.096	0.099	0.018	0.110	0.039

[1]Each value represents mean of 8 experimental unit (pen) of 5 birds each. Ileal digesta was collected from broilers at 21 days of age.
[2]Standard error of means.
Means within a column with no common superscripts differ significantly ($P < 0.05$).

3.3.6. Broiler growth performance

There were differences in FI (*P* = 0.013, CV ^{m} = 8.5%), WG (*P* = 0.023, CV ^{m} = 7.9%) and FBW (*P* = 0.022, CV ^{m} = 7.5%) of broilers fed 17 wheat-based diets (Table 3.9). The daily FI and WG ranged from 37.5 to 43.1 g/b/d DM and 30.4 to 34.9 g/b/d, respectively. The FBW of broilers at day 21 ranged from 0.679 to 0.773 kg. Although, there were differences in FI and WG of broilers but no differences (*P* > 0.05) in FCR were observed between wheat samples and values of FCR ranged from 1.197 to 1.243 (CV ^{m} = 3.2%). There were no differences (*P* = 0.062, CV ^{m} = 26.3%) in ileal digesta viscosity of broilers determined at day 21. The values of FI and WG were lower than Aviagen standards for Ross 308 (Aviagen 2014b), however, FCR was within Aviagen standards (1.270) for Ross 308 male broilers (Aviagen 2014b). Birds remained healthy throughout the experiment and mortality was low 0.13% during the entire study.

3.3.7. Effect of wheat variety and growing site on growth performance of broilers and apparent metabolisable energy of wheat

Significant differences were found in FI (*P* = 0.013) and WG (*P* = 0.023) of broilers fed 17 wheat samples when compared statistically by ANOVA. The information was available about variety and growing site of these 17 wheat samples; therefore, data was also analysed for the effect of wheat variety and growing site on growth performance and AME. Further analysis of biological data by unbalanced design using regression indicated that there was no consistent (*P* > 0.05) effect of wheat variety on FI, WG, FCR and AMEn (Table 3.10). Similarly, no differences (*P* > 0.05) were observed in FI, WG, FCR and AMEn between wheat growing sites (Table 3.11).

Table 3. 9. The [1]daily feed intake, weight gain, feed conversion ratio, final body weight and ileal digesta viscosity of broilers fed experimental wheat-based diets.

Diets	**FI (g/b/d DM)**	**WG (g/b/d)**	**FCR (g: g)**	**FBW (Kg)**	**Digesta Viscosity(cP)***
1	42.5^{bc}	34.9^{c}	1.213	0.773^{c}	2.63
2	37.7^{a}	30.4^{a}	1.235	0.679^{a}	2.16
3	39.7^{abc}	33.2^{abc}	1.211	0.738^{abc}	2.09
4	39.4^{abc}	32.1^{abc}	1.225	0.714^{abc}	1.65
5	42.3^{bc}	34.4^{c}	1.224	0.763^{c}	2.01
6	38.5^{ab}	32.0^{abc}	1.201	0.711^{abc}	2.14
7	37.5^{a}	30.8^{ab}	1.213	0.687^{ab}	1.97
8	43.1^{c}	34.6^{c}	1.232	0.766^{c}	1.61
9	42.1^{bc}	33.7^{bc}	1.243	0.748^{bc}	2.44
10	40.4^{abc}	33.2^{abc}	1.211	0.737^{abc}	2.93
11	41.8^{bc}	33.5^{bc}	1.238	0.745^{bc}	2.39
12	41.5^{abc}	34.0^{c}	1.220	0.754^{c}	2.38
13	40.1^{abc}	32.2^{abc}	1.237	0.716^{abc}	2.66
14	39.9^{abc}	32.9^{abc}	1.209	0.730^{abc}	1.75
15	41.1^{abc}	33.5^{abc}	1.197	0.743^{abc}	1.73
16	38.8^{ab}	32.1^{abc}	1.210	0.713^{abc}	1.96
17	42.2^{bc}	34.3^{c}	1.233	0.760^{c}	2.04
Mean	40.5	33.1	1.221	0.734	2.15
Min	37.5	30.4	1.197	0.679	1.61
Max	43.1	34.6	1.243	0.773	2.93
CV%	8.5	7.9	3.2	7.5	26.3
SEM[2]	1.21	0.92	0.0138	0.0194	0.282
P	0.013	0.023	0.478	0.022	0.062

[1]Each value represents mean of 8 experimental unit (pen) of 5 birds each. Values are based on feeding period from day old to 21 days of age.
[2]Standard error of means, (df = 112).
Means within a column with no common superscript differ significantly ($P < 0.05$).
FI= average daily feed intake (gram/bird/day on dry mater), WG= average daily body weight gain (gram/bird/day), FCR= mortality corrected feed conversion ratio (g/g DM), FBW= average final body weight at d 21, *Ileal broiler digest viscosity at d 21.

Table 3. 10. Influence of wheat variety on mean feed intake, weight gain, feed conversion ratio and N-corrected apparent metabolisable energy.

Variety	FI DM g/b/d[1]	WG g/b/d[2]	FCR DM[3]	AMEn DM MJ/kg[4]
Leeds	39.82	32.6	1.224	13.80
	(±0.669)	(±0.508)	(±0.0072)	(±0.110)
Santiago	40.34	32.87	1.219	13.71
	(±0.669)	(±0.508)	(±0.0072)	(±0.108)
Lili	41.24	33.44	1.228	13.90
	(±0.921)	(±0.700)	(±0.0101)	(±0.149)
Trinity	40.75	33.14	1.225	13.83
	(±0.657)	(±0.500)	(±0.0070)	(±0.108)
Barrel	39.97	32.81	1.203	14.13
	(±0.922)	(±0.701)	(±0.0101)	(±0.154)
Basset	42.21	34.31	1.233	13.99
	(±1.302)	(±0.990)	(±0.0138)	(±0.210)
Min SED[a]	0.921	0.700	0.0099	0.150
Max SED	1.596	1.213	0.0171	0.261
P value	0.538	0.736	0.443	0.173

[a]Standard error of difference. Values in parenthesis are standard errors of each value.
[1]FI DM g/b/d: Feed intake gram/bird/day on dry matter.
[2]WG g/b/d: Weight gain gram/bird/day.
[3]FCR DM: Feed conversion ratio on dry matter.
[4]AMEn DM MJ/kg: N-corrected apparent metabolisable energy on dry matter mega joules/kilogram.

Table 3. 11. Influence of wheat growing site on mean feed intake, weight gain, feed conversion ratio and N-corrected apparent metabolisable energy.

Site	FI DM g/b/d[1]	WG g/b/d[2]	FCR DM[3]	AMEn DM MJ/kg[4]
Cambridgeshire	40.43	32.99	1.220	13.71
	(±0.623)	(±0.474)	(±0.0067)	(±0.101)
Lincolnshire	39.58	32.43	1.217	13.84
	(±0.661)	(±0.503)	(±0.0072)	(±0.112)
Nottinghamshire	40.5	33.44	1.207	13.55
	(±0.921)	(±0.700)	(±0.0098)	(±0.149)
Yorkshire	40.77	33.08	1.229	13.93
	(±0.586)	(±0.446)	(±0.0062)	(±0.095)
Min SED[a]	0.856	0.650	0.0092	0.138
Max SED	1.134	0.862	0.0122	0.186
P value	0.646	0.611	0.394	0.138

[a]Standard error of difference. Values in parenthesis are standard errors of each value.
[1]FI DM g/b/d: Feed intake gram/bird/day on dry matter.
[2]WG g/b/d: Weight gain gram/bird/day.
[3]FCR DM: Feed conversion ratio on dry matter (g: g).
[4]AMEn DM MJ/kg: N-corrected apparent metabolisable energy on dry matter mega joules / kilogram.

3.3.8. Relationship between chemical composition of wheat, apparent metabolisable energy of wheat and growth performance of broilers

A correlation matrix was initially used to assess the relationships between wheat samples laboratory analyses and AME and broiler growth performance (Table 3.12). Ash was negatively correlated with AME and AMEn (r = −0.513, −0.489; $P < 0.05$, respectively). There was a tendency of a negative relationship between insoluble NSP and AME and AMEn (r = −0.466, −0.464; $P < 0.1$). There was a tendency of negative correlation between CP of wheat and with AME and AMEn (r = −0.472, 0.481; $P < 0.05$). Although, there were differences in FI and WG of broilers fed different wheat samples, however, there was no relationship ($P > 0.05$) between growth performance and wheat chemical composition.

3.3.9. Relationship between physical characteristics of wheat, apparent metabolisable energy of wheat and growth performance of broilers

Specific weight (SW) was the only physical characteristics of wheat samples which was positively correlated (r = 0.515, $P < 0.05$) with FCR only (Table 3.13). No other determined physical characteristics of wheat relate to broiler growth performance and AME. The FI of broilers was correlated (r = 0.953, $P < 0.001$) with WG of broilers. Broiler growth performance did not correlate ($P > 0.05$) with AME and AMEn of the wheat samples. Simple linear regression with groups (growing site was used as group) indicated that there was an interaction of growing site of wheat with endosperm hardness for FI of broilers ($R^2 = 0.50$, $P < 0.05$).

Table 3. 12. Correlation coefficients between broiler growth performance, metabolisable energy, nutrients utilisation and chemical composition of wheat samples.

	CP	Fat	Ash	Starch	NSP	NSPsol	NSPins	GE	Dig.vis	NR	DMR	FD	FI	WG	FCR	AME	AMEn
CP	1																
Fat	-0.314	1															
Ash	0.414	-0.207	1														
Starch	-0.227	0.048	0.045	1													
NSP	0.394	-0.313	0.327	-0.055	1												
NSPsol	0.128	0.194	-0.052	0.069	0.574	1											
NSPins	0.319	-0.553	0.425	-0.133	0.557	-0.360	1										
GE	0.606	-0.107	0.415	-0.065	0.046	0.242	-0.193	1									
Dig.Vis	0.316	-0.155	0.206	-0.172	0.151	0.444	-0.279	0.559	1								
NR	-0.292	0.031	-0.737	0.146	-0.195	0.126	-0.350	-0.432	-0.210	1							
DMR	-0.386	0.222	-0.744	0.080	0.094	0.484	-0.383	-0.421	0.061	0.773	1						
FD	-0.511	0.282	-0.345	0.038	-0.389	-0.152	-0.289	-0.295	-0.314	0.450	0.348	1					
FI	0.049	-0.215	0.410	0.240	0.133	-0.115	0.267	0.083	0.121	-0.062	-0.074	-0.102	1				
WG	0.101	-0.293	0.438	0.237	0.241	-0.054	0.329	0.070	0.106	-0.043	-0.070	-0.121	**0.953**	1			
FCR	-0.225	0.043	0.038	0.122	-0.351	-0.301	-0.095	-0.125	0.194	-0.079	0.042	-0.142	0.333	0.087	1		
AME	-0.472	0.319	**-0.513**	-0.099	0.009	0.471	-0.466	-0.109	0.090	**0.534**	**0.730**	**0.560**	-0.069	-0.101	0.153	1	
AMEn	-0.481	0.341	**-0.489**	-0.132	0.002	0.460	-0.464	-0.113	0.116	**0.496**	**0.726**	**0.552**	-0.068	-0.104	0.122	0.995	1

df = 15; Correlation coefficients > 0.482, 0.606, 0.725, 0.412 are statistically significant at $P < 0.05$, $P < 0.01$, $P < 0.001$ and $P < 0.1$, respectively.
Significant correlations are highlighted in bold.

CP, NSPins, NSPsol, NSP, GE: crude protein, insoluble and soluble non-starch polysaccharides, gross energy of wheat samples.
NR, FD, DMR: coefficients of nitrogen retention, fat digestibility and dry matter retention of wheat samples.
FI, WG, FCR, Dig.vis: feed intake, weight gain, feed conversion ratio and ileal digesta viscosity of birds.
AME, AMEn: apparent metabolisable energy, N corrected apparent metabolisable energy of wheat samples.

Table 3. 13. Correlation coefficients between broiler growth performance, metabolisable energy and physical characteristics of wheat samples.

	EH	HFN	SW	TGW	FI	WG	FCR	AME	AMEn
EH	1								
HFN	0.440	1							
SW	0.164	0.261	1						
TGW	-0.215	0.253	0.396	1					
FI	0.252	0.119	0.155	-0.005	1				
WG	0.169	0.034	0.004	-0.057	**0.953**	1			
FCR	0.238	0.175	**0.515**	0.129	0.333	0.087	1		
AME	-0.239	0.090	0.002	0.408	-0.069	-0.101	0.153	1	
AMEn	-0.214	0.059	0.013	0.378	-0.068	-0.104	0.122	0.995	1

df= 15; Correlation coefficients > 0.482, 0.606, 0.725, 0.412 are statistically significant at $P < 0.05$, $P < 0.01$, $P < 0.001$ and $P < 0.1$, respectively.
Significant correlations are highlighted in bold.

EH, HFN, SW, TGW: endosperm hardness, Hagberg falling number, specific weight, weight of 1000 kernels of wheat.
FI, WG, FCR: feed intake, weight gain, feed conversion ratio corrected for mortality.
AME, AMEn= apparent metabolisable energy, N-corrected apparent metabolisable energy of wheat samples.

3.3.10. Relationship between nutrient availability, ileal starch digestibility and apparent metabolisable energy of wheat

Simple linear regression analysis indicated positive relationships between the coefficients of NR, FD and AMEn (R^2 = 0.25, 0.31 $P < 0.05$, respectively) (Figure 3.2, 3.3). The DMR was positively correlated with AMEn (R^2 = 0.53, $P < 0.001$) (Figure 3.4). The ileal starch digestibility was positively related with AMEn (R^2 = 0.49, $P < 0.01$) (Figure 3.5). The CP content of wheat samples was negatively correlated (R^2 = 0.31, $P < 0.05$) with ileal starch digestibility of wheat samples (Figure 3.6). There was no relationship ($P > 0.05$) between ileal CP digestibility and AMEn and starch digestibility.

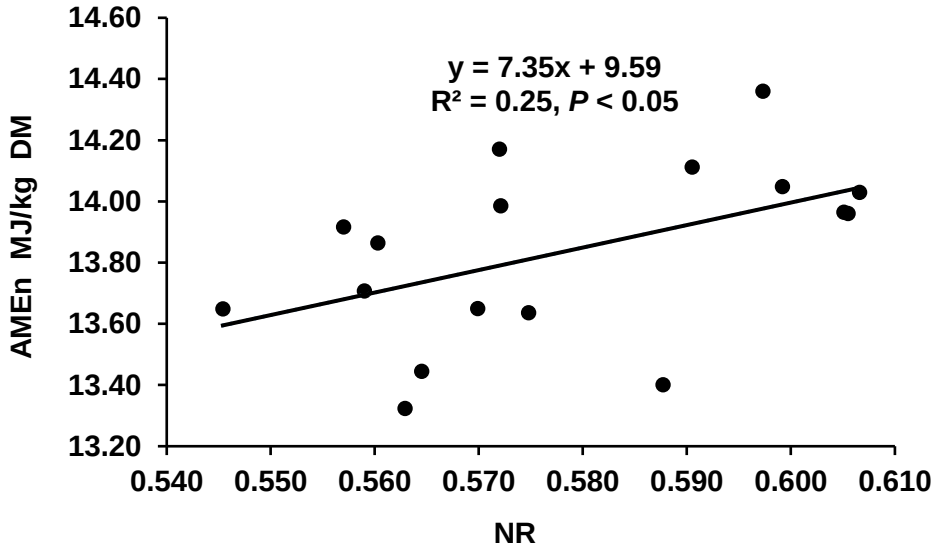


Figure 3. 2. Relationship between wheat AMEn and coefficients of nitrogen retention (NR) measured in excreta collected from broilers during last three days of the study.

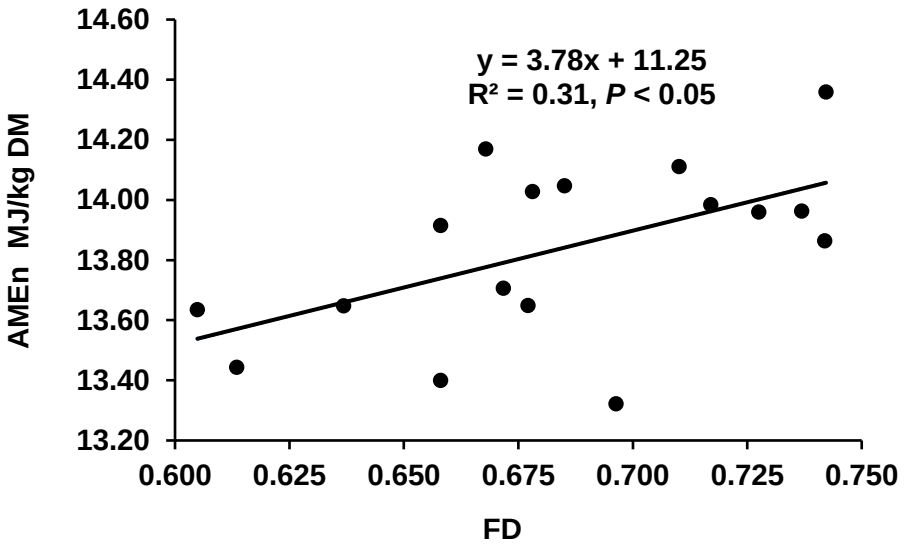


Figure 3. 3. Relationship between wheat AMEn (MJ/kg DM) and coefficients of fat digestibility (FD) of wheat determined in excreta collected from broilers during last three days of the study.

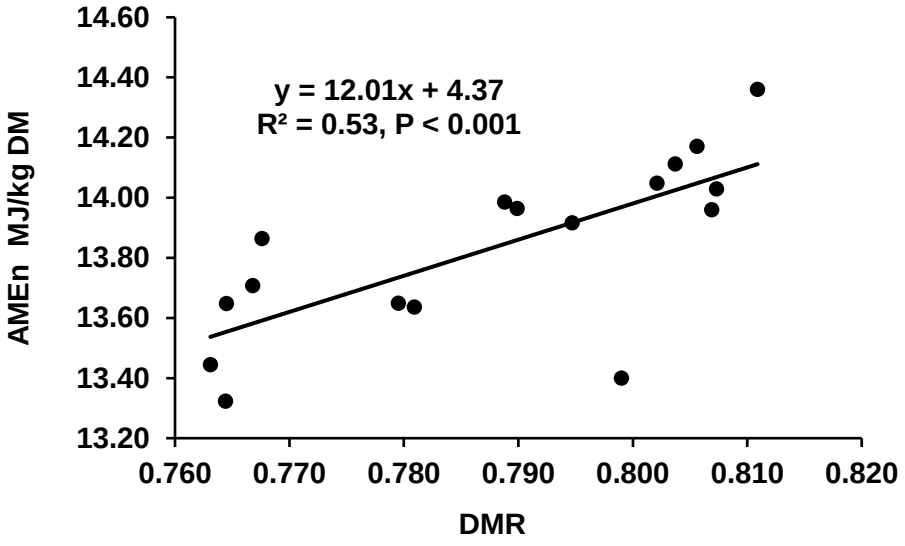


Figure 3. 4. Relationship between Wheat AMEn (MJ/kg DM) and coefficients of dry matter retention (DMR) of wheat determined in excreta collected from broilers during last three days of the study.

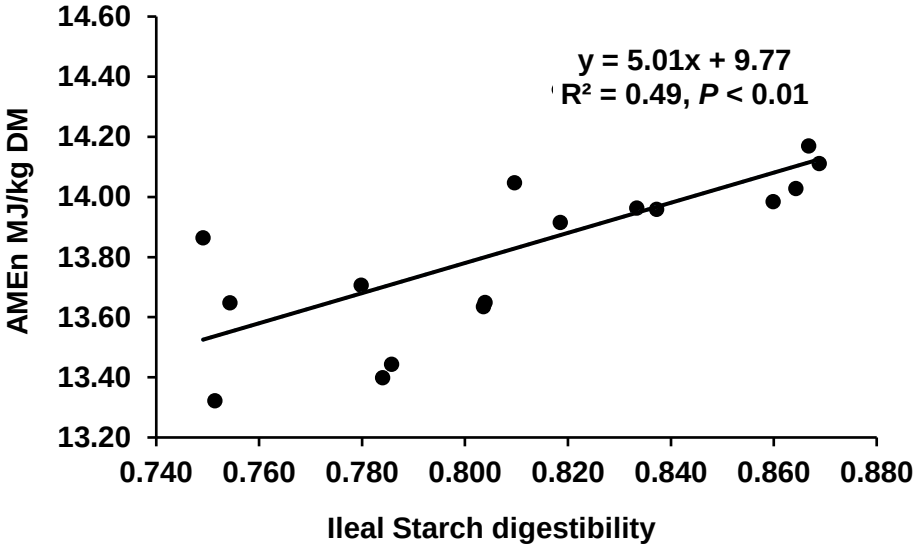


Figure 3. 5. Relationship between wheat AMEn (MJ/kg DM) and coefficients of ileal starch digestibility (SD) of wheat measured in ileal digesta collected from broilers at day 21.

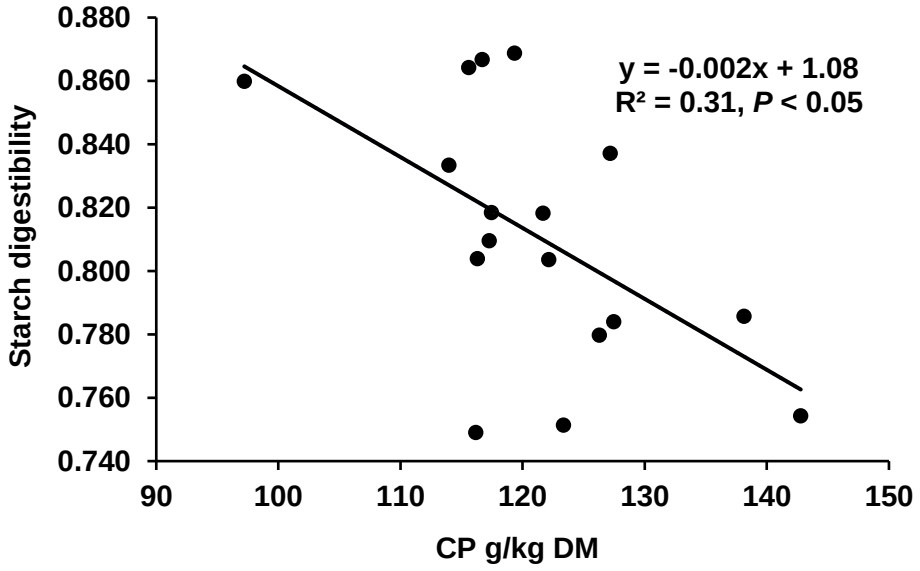


Figure 3. 6. Relationship between CP content (g/kg DM) of wheat samples and coefficients of ileal starch digestibility (SD) of wheat measured in ileal digesta collected from broilers at day 21.

3.3.11. Relationship between combination of wheat chemical composition and apparent metabolisable energy of wheat

The stepwise multiple regression analysis predicted the wheat chemical composition variables that minimised the residual mean squares for AME. The CP, ash and soluble NSP contents of wheat samples gave the best explanation (R^2 = 0.59; $P < 0.05$) of variation in AME but only accounted for 59% variability in AME (Table 3.14). The addition of any further explanatory wheat variables did not ($P > 0.05$) reduce the residual mean squares in the determined AME. The determined AME and wheat characteristics variables were also tested for non-linear regression, however, there was no evidence of non-linear ($P > 0.05$) response between AME and wheat variables. Moreover, there was no relationship ($P > 0.05$) between the combination of wheat chemical composition, physical characteristics and growth performance of chickens.

Table 3. 14. The relationship between apparent metabolisable energy of wheat samples and their analysed chemical composition.

Response variate		**Explanatory variates**				R^2	SEO[1]	*P* value
		Constant	CP	Ash	sol NSP*			
AME		16.03	− 0.012	− 0.062	0.040	0.59	0.219	0.007
	SE[2]	± 0.753	± 0.0061	± 0.0381	± 0.0143			
AMEn		15.53	−0.012	− 0.052	0.037	0.57	0.209	0.009
	SE	± 0.720	± 0.0058	± 0.0365	± 0.0137			

*Soluble non–starch polysaccharides.
[1]Standard error of observation.
[2]Standard error.

3.4. DISCUSSION

The study evaluated the effect of wheat samples that represented the range and quality of wheat currently available to the UK poultry feed industry, with different chemical composition and physical characteristics on AME, nutrient utilisation of wheat and growth performance of broilers. The findings of this study are important for feed industry because a large set of currently available UK wheat samples for poultry feeds were studied and the effects of variability between different samples on AME and growth performance of broiler chickens were investigated.

3.4.1. Energy availability and nutrient utilisation

The size of differences in AME values of experimental wheat samples were in the expected range and in agreement with previous reports (McCracken and Quintin 2000; Steenfeldt 2001; Pirgozliev et al. 2003; Smeets et al. 2015). The values of AME between individual wheat samples were significantly different (*P* = 0.012; LSD = 0.60 MJ/kg DM), similarly, AMEn values of individual wheat samples were also significantly different (P = 0.017; LSD = 0.57 MJ/kg DM). Some of the previous studies reported no difference in AME between wheat samples (Wiseman 2000; Amerah et al. 2009b), but only physical characteristics were measured in the former study, while only two samples were analysed in the latter. In this study, maximum range of differences in AME and AMEn between samples were 1.13 and 1.04 MJ/kg DM, respectively. The AME and AMEn value of sample 15 was 8.3 and 7.8% higher than the lowest AME value in sample 8. The difference of 1 MJ/kg is commercially important for wheat-based diets in broiler feed formulation and indicates that there is important variation between AME of different currently available UK wheat samples.

The coefficients of NR, FD and DMR were in expected range and in accord with previous reports (Steenfeldt 2001; Pirgozliev et al. 2015a, b; Smeets et al. 2015). The values of DMR of sample 15 were 6.15% higher than in sample 8 which corresponded to difference in AME between these two samples. There was a difference of 21.5% in FD between average lowest and highest values of wheat, and the size of variation between wheat samples was in line with variability reported by Steenfeldt (2001).

3.4.2. Starch and amino acids digestibility

In the current study, ileal starch digestibility was relatively low (0.749 – 0.869) but the values were within the range of reported results (Wiseman et al. 2000, Svihus 2001, Svihus and Hetland 2001; Carré et al. 2005). Svihus (2001) reported similar range of starch digestibility values when cold pellet diets were fed to broilers. In broilers, high starch digestibility values were based on feeding wheat-based mash diets (Steenfeldt et al. 1998b; Gutierrez del

Alamo et al. 2008a, 2009a), while in the present study, diets were pelleted without using steam. Gutierrez del Alamo et al. (2009a, b) revealed a starch digestibility up to 96 – 97% in distal ileum in broilers fed mash diets, whereas, Svihus (2001) and Svihus and Hetland (2001) found lower values of starch digestibility (83, 79%; respectively) when birds were fed pelleted diet without using steam. Svihus and Hetland (2001) indicated that low digestibility of wheat-based cold pellet diet increased when broilers were switched to mash diet. Steam conditioning during pelleting help in gelatinisation and appropriate temperature is essential for starch gelatinisation. Starch granules diffuse into water at gelatinisation temperature during steam conditioning and opens the starch structure and enzymes can enter the starch structure, making it susceptible for amylolytic degradation (Svihus et al. 2005). In this study, relatively low values of starch digestibility may be due to manufacturing pellet diets without using steam where incomplete gelatinisation may have resulted in lower starch digestibility. Wheat is consistently reported to result in low starch digestibility when used at high level in broilers diets and also when diets are pelleted without using steam (Svihus and Gullord 2002; Gutierrez del Alamo et al. 2008b). The current study showed variation up to 16% in ileal starch digestibility between individual wheat samples (sample 7 and 12). Previously, Mollah et al. (1983) and Rogel et al. (1987a) also reported variation up to 20 – 22% in ileal starch digestibility of broilers fed different wheat samples. Although, there were variations between individual wheat samples, however, only three wheat samples (1,7 and 8) resulted in significantly lower starch digestibility values.

The values of ileal digestibility coefficients of IAA of wheat samples were in accord with previous findings (Ravindran et al. 2005; Huang et al. 2005; Ravindran and Amerah 2009). The digestibility values of DAA were in a similar range of earlier reported results (Ravindran et al. 1999; Ravindran and Amerah, 2009). Among IAA, arginine was highly digestible AA (0.920), followed by methionine (0.913). A difference of 12.9% and 17.6% was observed in digestibility of methionine and leucine, respectively between the lowest and highest wheats. The difference in digestibility of methionine was largely due to one sample (14) which resulted in significantly lower digestibility values. Lysine and threonine are known as least digestible essential amino acids in wheat. In the current study, threonine was the least digestible AA, followed by lysine and results were in line with Ravindran et al. (1999). In the present study, the values of digestibility coefficient of lysine and threonine were comparable with published values of their digestibility in wheat-based diets, however in contrast with results of Hew et al. (1998) and Huang et al. (2007). In their studies, wheat was included at 91% in broilers diet, whereas, in current study, all broiler diets contained 67% wheat. This may indicate that higher inclusions of wheat may confer with amino acids digestibility especially lysine and threonine. The digestibility values of methionine, phenylalanine and leucine were similar to results of Ravindran et al. (2005) and Huang et al. (2006).

3.4.3. Relationship between chemical composition, physical characteristics, nutrient utilisation and apparent metabolisable energy of wheat samples

There was no significant association of major energy yielding components of wheat including starch and protein with AME. The only significant ($P < 0.05$) relationship was, a negative association of ash with AME ($R^2 = 0.21$, $P < 0.05$). Simple linear regression analysis indicated that only 21% of the variation in AME was explained by the ash content of wheat samples. The analysis of stepwise multiple linear regression indicated that only 59% variability in AME of studied wheat samples was explained ($P < 0.05$) when ash, soluble NSP and CP contents were used in combination. The addition of any other chemical composition variables of wheat did not further explain variability in AME. Researchers have found negative correlations between CP and AME (Svihus and Gullord 2002; Ball et al. 2013a) but this study indicated only a tendency for a negative correlation between CP content and AME and AMEn of wheat samples. The tendency of a negative association between insoluble NSP and AME was in accord with previous published data (Annison 1991, 1993; Smeets et al. 2015). Studies have indicated that variation in NSP content of wheat could affect ME and broilers growth performance (Choct et al. 1995; Hetland et al. 2004). The NSP can be encapsulated within the cell wall, making it difficult for endogenous enzyme to release them. Most of the arabinoxylans in wheat are insoluble and inaccessible to birds as nutrients (Bedford and Morgan 1996; Choct 2006). In the present experiment, there were variations (CV = 23.1%) in soluble NSP content of wheat samples; however, there was no association of soluble NSP with AME, which was in agreement of previous findings (Steenfeldt 2001; Choct et al. 2006; Pirgozliev et al. 2015a). Soluble NSP have been reported to increase ileal digesta viscosity, decrease passage rate, resulting in reduced nutrient utilisation and affect bird's performance (Choct, 1992, 1995). The tendency of association between soluble NSP and ileal digesta viscosity ($r = 0.444$, $P < 0.1$) suggests that soluble NSP are not always associated with a reduction in ME by increasing ileal digesta viscosity.

The lack of relationship between starch and AME was not surprising and has been reported previously (McCracken et al. 2002; Gutierrez del Alamo et al. 2008a). In wheat endosperm, starch is encapsulated within cells with protein. Steam conditioning during pelleting can damage cell walls to release starch, resulting in improved AME. In the present study, diets were pelleted without using steam, and the absence of steam conditioning may have not released starch completely. Furthermore, the absence of relationship of starch with AME could also be due to less variability (CV = 2.2%) in starch content of wheat samples.

The absence of correlation between SW and AME was in accord with previous reports (Wiseman 2000; Svihus and Gullord 2002) and could be due to less variation in SW (CV= 2.8%) between wheat samples. The SW is most commonly used by feed millers to accept wheat samples based on their yield (kg/hl). High yield varieties do not always correspond to high AME. In this study, the physical characteristics of wheat samples did not relate to AME, which suggested that physical characteristics cannot be relied upon to determine the feeding value of wheat. Researchers so far have been unable to establish a consistent relationship between wheat physical characteristics and AME (McCracken et al. 2002; Hetland et al. 2007; Ball et al. 2013a).

A significant positive correlation between FD and AMEn was in agreement of previous results of Steenfeldt (2001) and could be due to high variability (CV = 11.6%) in FD coefficients. In this study, the positive relationship between FD and AMEn showed that although starch is the main energy-yielding component in wheat, high FD may also contribute towards higher AMEn of wheat to some extent, though this contribution is not significant enough to be accounted for major variation in AMEn. The positive correlation between nutrient retention and AMEn indicates that AMEn is only improved if diets have highly digestible nutrients and alongside starch, other nutrients may also contribute towards available energy from wheat.

In the present study, a positive correlation between ileal starch digestibility and AME was observed and findings were in accord of Mollah et al. (1983); Rogel at al. (1987a) and Wiseman et al. (2000). However, past studies indicated that relationship between starch digestibility and AME was not always consistent. A recent study by Karunaratne et al. (2018a) found no relationship between *in vivo* starch digestibility and AME of Canadian wheats. The authors found less variation in starch digestibility at distal ileum. Although, there was a significant positive relationship between ileal starch digestibility and AME, however the R^2 value was weak (R^2 = 0.49) and significance may be attributed to large variation (CV = 10%) in starch digestibility of wheat. Even though, there was a correlation between starch digestibility and AME, but starch digestibility did not relate with growth performance of broilers.

3.4.4. Relationship between chemical composition, physical characteristics of wheat samples and growth performance of broilers

The current study revealed that there were large differences (13 – 14 %) in FI and growth rate of broilers, which cannot be fully explained by the wheat chemical composition. The tendency of relationship between ash content of wheat samples and WG was in agreement with the previous work of Pirgozliev et al. (2003). The variations in ash content of wheat samples could be due to soil contamination, use of harvester and type of soil (sandy, clay,

mixed soil). The studied samples were sourced from different growing sites. In the UK, soil type is distinct and depends on various factors e.g., water holding capacity and mineral content etc. Nottinghamshire growing site had a sandy light soil that can create significant amount of crop stress in summer due to drought. The lack of association of FI with all other measurements of wheat sample indicated that there were some unexplained factors which may influence the FI of broilers. Factors such as growing condition, agronomy and crop husbandry may influence the wheat chemical composition that may affect the feeding value of wheat for broilers. Growing conditions refer to wheat grown at different geographical location, season, soil type and rain fall.

The study found no relationship between EH of wheat and growth performance of broilers. Although, there was a range (21 – 87) in relative units of EH between wheat sample, however, the study was unable to detect any effect of EH on growth performance variables (FI, WG, FCR). The published literature on the effect of EH on growth performance is inconsistent. Rose et al. (2001) reported a positive correlation between EH of wheat samples and FI and WG of broilers. Amerah et al. (2009b) found increases in FI of broilers fed soft wheat-based diets supplemented with enzyme, but no improvement in FI of those fed hard wheat. Salah Uddin et al. (1996) reported no effect of EH on broiler performance in pellet diet. Pirgozliev et al. (2016) compared pellet versus mash diets containing wheat with soft and hard endosperm and only found differences in FI and WG of broilers fed on pellet diets. Soft wheat tends to produce flour with smaller surface area and relatively little starch damage due to intact starch granules, whereas, in hard wheat, particles are large with irregular shapes and starch granules are cleaved (Rose et al. 2001). Cleaved starch granules solubilise more quickly than when they are intact. Endosperm hardness may influence the quality of pellet. Hard endosperm produces good quality pellets because large particle size absorbs more water during pelleting process which helps in gelatinisation (Abdollahi et al. 2011, 2013). However, in the current study, there was less variation (3.7%) in pellet durability index (PDI) of wheat-based diets and there was no association of EH with PDI of diets. The lack of influence of EH on pellet quality may be due to pelleting diets without using steam. Moreover, there was no relationship between PDI and growth performance of broilers.

The lack of association between HFN of wheat samples and growth performance was in accord with the findings of Hetland et al. (2007). The reported effect of HFN on growth performance are inconsistent (Rose et al. 2001, Svihus and Gullord 2002) and require further investigation. The HFN is used in the milling industry to access the wheat suitability for bread making, and a high HFN is considered to produce a good quality loaf for bread making. The HFN is a measure of α- amylase activity to determine pre-harvest sprouting. High α- amylase activity means less viscous wheat flour upon gelatinisation. The positive

correlation between SW of wheat and FCR was interesting but SW did not correlate to any of the other broiler performance attributes, making it difficult to explain this relationship. Although the relationship was significant, but the correlation coefficient was not strong enough to explain the significance. Overall, the lack of relationship between SW and growth performance variables was in agreement with previous findings (Rose et al., 2001; Pirgozliev et al. 2003; Ball et al. 2013a). The absence of relationship of SW with AME and growth performance of broilers suggested that SW cannot be relied upon to determine the nutritive value of what for poultry.

Subsequent analysis of wheat variety and growing sites further confirmed that differences in broiler growth were not influenced by variety or growing location of wheat. The studied 17 samples were from six different varieties grown at four different sites, however, they were not balanced, as it was not possible to source more samples from certain sites. An interaction between EH and growing site of wheat samples on FI was observed but this interaction was negative for Lincolnshire and Nottinghamshire while positive for Yorkshire and Cambridgeshire. Different response of EH with growing site for FI requires more samples from these growing sites to investigate this interaction in detail. The factors such as growing location, soil type, irrigation, use of fertiliser, cultivation practices can affect the grain hardness (Pomeranz and Williams 1990). The growing season (e.g., precipitation and temperature during maturation) could also affect the EH. Glen et al. (1991) reported that strength of EH is related to moisture content whilst studying the influence of water on endosperm mechanical strength and attributed that rainfall during harvesting can affect hardness of grain. The purpose of this study was not to examine the effect of growing site on endosperm hardness, but findings were interesting and warrant further investigations.

In the current study, although, there were significant differences in AME of wheat and growth performance of broilers but there was no association of AME of wheat with growth performance of broilers. The absence of a relationship between growth performance and AME in this study agreed with previous findings (Steenfeldt 2001; Ball et al. 2013a; Pirgozliev et al. 2015a). Moreover, the large differences observed in AME of wheat samples were not evidently related to variations in wheat chemical composition or physical characteristics. Wheat provides high proportion of ME (up to 70%) in practical broilers diets and any differences in AME between different wheat samples are important. However, this study has confirmed that care should be taken in using wheat chemical composition and physical characteristics information as predictor of AME of individual wheat samples.

The current study revealed significant differences in FI and growth rate of broilers when fed diets comprising different individual wheat samples. Broilers fed diet containing wheat sample 8 had 14.3 and 13.8% higher FI and WG, respectively as compared to those fed

sample 2. In this study, differences were unlikely due to differences in protein content of wheat samples because all diets were made isonitrogenous and had same amino acid balance. This magnitude of difference in broiler growth performance would be commercially important for the broiler feed industry. The size of difference in FI and WG was identical to findings of Waldron (1997), Steenfeldt (2001) and Gutierrez del Alamo et al. (2008a), where almost similar inclusion (65%) of different wheat cultivars was incorporated in feed formulation. The values of FI and WG of broilers were lower than recommended Aviagen standards for Ross 308 male broilers (Aviagen 2014b). Aviagen recommended FI and WG are based on large scale broiler production, whereas, small experimental studies have some limitations e.g., rearing in pen, bird's density, sample collection. Conversely, in this study, there were no differences between fifteen (15) out of seventeen (17) wheat samples. Wheat samples two (2) and seven (7) were the samples which had significant low growth rate in broilers as compared to other fifteen samples. Further examination of data indicated that these two samples did not have any obvious difference in their chemical composition and physical characteristics. A study by Steenfeldt (2001) also reported 14% reduction in growth rate of broilers when fed diets containing different wheat cultivars at similar inclusion (65%) of wheat. This study also demonstrated that broilers with low growth rate also had lower voluntary feed intakes, but the differences were not related to chemical and physical characteristics of wheat samples. The findings of this study suggest that perhaps nutritionists can identify wheat samples that give poor growth rate using some robust analytical techniques and exclude them from diet formulations.

3.5. CONCLUSIONS

In conclusion, the current UK wheat samples examined in this study varied in their chemical composition and physical characteristics. The results indicated that AME content of currently available UK wheat samples is variable. Ideally, AME of individual batches of wheat samples would be considered at diet formulation stage at commercial feed mills, so it would require a robust prediction method. This study has not been able to specify that wheat chemical composition and physical characteristics can be used for the determination of AME because there was no association of wheat characteristics with AME. Although, the present study illustrated no clear association of starch and protein content of wheat samples with AME but would be interesting to investigate the relationship between digestibility of macronutrients and AME.

The present study has demonstrated that there are substantial differences in growth rate of broilers fed different wheat samples, even though, no difference in feed efficiency was identified. High growth rate in broilers is important because birds can achieve live weight gain at a faster rate resulting in a shorter production cycle. In this study, difference in FI and WG were not related to AME or any single or combination of chemical composition and physical characteristics of wheat. Differences in feed intake of broilers fed different wheat samples warrant further investigation. Moreover, the difference in nutrient composition of wheat grown at specific location warrant further investigation of growing site of wheat.

CHAPTER 4: EFFECT OF WHEAT VARIETY, GROWING SITE AND XYLANASE SUPPLEMENTATION ON APPARENT METABOLISABLE ENERGY, NUTRIENT UTILISATION OF WHEAT AND GROWTH PERFORMANCE OF BROILERS

4.1. INTRODUCTION

The purpose of first study (Chapter 2, 3) was to examine a range of currently available UK wheat samples and their effect on AME of wheat and growth performance of broilers. Results of Chapter 3 indicated that AME content of currently available UK wheat samples varied. Differences in feed intake and growth rate of broilers were also identified when these wheat samples were fed to broilers. As we had information on wheat variety and growing site, therefore samples were also analysed to investigate the effect of wheat variety and growing site on AME and growth performance of broilers. As discussed in Chapter 3, there was an interaction of growing site on FI of broilers. However, due to unbalanced number of samples from each growing site, this interaction required further investigation. Therefore, a balanced experiment was designed to investigate the effect of variety and growing site of wheat on AME and growth performance in the current study.

Over the past four decades, exogenous enzymes (e.g., xylanases, glucanases, proteases, phytase) have been used in the poultry diets to improve productive performance of birds by minimising the variation in metabolisable energy (AME) and improving the nutrient digestibility of diets (Bedford 2000; Cowieson et al 2006; Slominski 2011). The application of xylanase on different wheat cultivars for broilers is reported enormously in the literature (Rafuse et al. 2005; Choct 2006; Bedford 2012, Cowieson and Masey O'Neill 2013), however, the literature on the effect of xylanase on wheat variety and growing site for broilers is scarce. Information on the use of xylanase on wheat variety and growing site would be helpful for poultry nutritionists for the selection of wheat for broiler diets.

The objectives of this study were:

(i) To investigate the effect of wheat variety and growing site on AME content, nutrient utilisation of wheat and their effect on growth performance of broilers.

(ii) To investigate the influence of xylanase on AME, nutrient utilisation of wheat and growth performance of broilers.

4.2. MATERIALS AND METHODS

4.2.1. Wheat samples

Three wheat varieties KWS Lili, KWS Barrel and KWS Kerrin from three different growing sites Lincolnshire, Cambridgeshire and Yorkshire were tested in this study (Table 4.1). The samples were sourced from KWS UK Ltd. All varieties were among listed AHDB 2016/17 varieties (AHDB 2017), and grouping was based on their chemical and physical characteristics. KWS Lili is a group 2 hard wheat and is recommended by AHDB as a hard milling wheat with high demand for bread making. KWS Barrel is a group 3 soft wheat with soft endosperm and used for soft milling purpose for cakes and biscuits. KWS Kerrin is a hard group 4, newly listed variety, added to AHDB recommended list 2017/18 as a feed wheat. It is a high yielding feed wheat variety (AHDB 2017). Three growing sites were the same sites used in study 1 (Chapter 2 and 3). In the UK poultry feed industry, wheat available for broiler diets could be feed wheat varieties or excess milling wheats left after human consumption (bread, cake and biscuits) or milling wheats which could not meet the milling specifications for bread or biscuits.

Wheat samples were stored in a dry place at ambient temperature until study commenced. Samples were received in 25 kg bags. Each sample was mixed homogenously for 10 min, and random samples were collected for chemical and physical analyses. Each wheat sample (300 g) was milled to pass through a 0.75 mm screen using a rotor mill Retsch ZM 200 (Retsch GmbH, Haan, Germany). All chemical analyses were performed in duplicate and were reported on dry matter basis. Physical analyses of wheat grains were determined in triplicate.

Table 4. 1. List of experimental wheat cultivars samples.

Sample ID	Variety	Site	Type*	Usage
LL	KWS Lili	Lincolnshire	Group2 Milling	Bread
LC	KWS Lili	Cambridgeshire	Group2 Milling	Bread
LY	KWS Lili	Yorkshire	Group2 Milling	Bread
BL	KWS Barrel	Lincolnshire	Group3 Soft	Biscuits, cakes
BC	KWS Barrel	Cambridgeshire	Group3 Soft	Biscuits, cakes
BY	KWS Barrel	Yorkshire	Group3 Soft	Biscuits, cakes
KL	KWS Kerrin	Lincolnshire	Group4 Hard	Hard feed wheat
KC	KWS Kerrin	Cambridgeshire	Group4 Hard	Hard feed wheat
KY	KWS Kerrin	Yorkshire	Group4 Hard	Hard feed wheat

*Group 2, 3, 4 listed varieties on AHDB recommended list (AHDB 2016/2017) sourced from KWS UK Ltd.

4.2.2. Chemical composition and physical characteristics

Proximate composition of wheat samples was determined as described in Chapter 2 (Section 2.2.2.1). Polysaccharides composition were determined as described in Section 2.2.2.2. Physical characteristics of wheat samples were determined as described earlier in Section 2.2.3.

4.2.3. Diet formulation, protein balance

Each wheat sample was milled separately to pass through a 3 mm screen in a hammer mill and mixed with balancer feeds (grower and finisher) separately. A grower balancer feed (Target feeds Ltd, Whitchurch, UK) was formulated using major ingredients of 507.7 g/kg soybean meal (SBM), 300 g/kg full-fat soya meal and 51.5 g/kg soya oil (Table 4.2). Nine (9) broiler grower diets were formulated by mixing 670 g/kg of each wheat sample and 330 g/kg of a grower balancer. Broiler grower diets were formulated to provide 12.77 ME and 199 g/kg CP content. A 15 g/kg of TiO_2 was added in the balancer, which was later diluted to 5 g/kg of the final diet when mixed with wheat. So, each broiler grower diet contained 5 g/kg titanium dioxide (TiO_2) as an external marker for AME determination, however, TiO_2 was not used in calculations instead AME was determined by total collection method. An extra diet was also formulated by mixing 470 g/kg of wheat and 530 g/kg of grower balancer for AME determination of balancer as described in Chapter 3 (Section 3.2.2). Sample BL was chosen at random to formulate extra diet.

A finisher balancer diet was formulated using major ingredients of 569.7 g/kg soybean meal (SBM), 181.8 g/kg full fat soya and 136.4 g/kg soya oil (Table 4.3). Nine (9) broiler finisher diets were formulated by mixing 670 g/kg of each wheat sample and 330 g/kg of a finisher balancer. Broiler finisher diets were formulated to provide 13.32 MJ/kg ME and 190 g/kg CP content. Each experimental diet (grower and finisher) met or exceeded the current diet specification for Ross 308 broiler chicken (Aviagen 2014a). Diets were free of coccidiostats, antimicrobial growth promoters or any other additives.

Diets were made isonitrogenous by adding wheat protein isolate (WPI) (Life Source Foods, USA). The additional quantity of WPI to be added was calculated on analysed protein value of each wheat sample on *as fed basis*. To balance the total quantity of each diet, maize starch was added to have the same amount (kg) of each diet. For example, a mixture of wheat protein isolate (WPI) and maize starch combining weight of 8 g/kg was added to each diet. A relatively small amount of energy contribution (0.17 MJ/kg) by the mixture (WPI and maize starch) was also accounted during AME determination of the diet. The determined AME of the diet was the AME of the wheat plus mixture.

Table 4. 2. Ingredients and chemical composition (g/kg as-fed) of grower balancer and wheat-based grower diet for broiler chickens.

Ingredients	**Balancer**	**Diet**
Wheat	–	670.0
Soybean meal (48)	507.7	167.5
Full-fat soybean meal	300.0	99.0
Soya oil	51.5	17.0
Monocalcium phosphate	39.4	13.0
Limestone	42.4	14.0
NaCl	9.1	3.0
L Lysine HCL	6.1	2.0
L Threonine	6.1	2.0
DL Methionine	10.6	3.5
Vitamin mineral premix[1]	12.1	4.0
Titanium Dioxide	15.0	5.0
Total	1000	1000
Calculated analysis (*as fed basis*)		
CP (g/kg)	368.0	198.9
ME (MJ/kg)	11.73	12.77
Crude fat (g/kg)	113.1	49.5
Ca (g/kg)	28.2	9.7
Available P (g/kg)	10.4	4.2
Lysine (g/kg)	27.7	11.5
Methionine	15.4	6.2
Threonine	20.1	8.7
Methionine + Cysteine (g/kg)	20.7	9.3
Tryptophan (g/kg)	4.5	2.3
Analysed values (*as fed basis*)		
DM (g/kg)	910	885-900
CP (Nx6.25) (g/kg)	359	191-207
Crude Fat (g/kg)	110	41.9-44.3

Each experimental diet met or exceeded the current diet specification for Ross 308 broiler (Aviagen 2014a).

[1]The vitamin and mineral premix contained vitamins and trace elements to meet the breeder's recommendations (Aviagen Ltd. Edinburgh, UK). The premix provided (units/kg diet): retinol 13500 IU; cholecalciferol 5000 IU; α-tocopherol 60 IU; menadione 3 mg; thiamine 2 mg; riboflavin 10 mg; pyridoxine 5 mg; cobalamin 15 µg; nicotinic acid 60 mg; pantothenic acid 15 mg; folic acid 1.5 mg; biotin 200 µg; 80 mg Fe as iron sulphate (30%); 10 mg Cu as copper sulphate (25%); 100 mg Mn as manganous oxide (62%); 80 mg Zn as zinc oxide (72%); 1 mg I as calcium iodate (52%); 0.20 mg Se as sodium selenite (4.5%) and 0.5 mg Mo as sodium molybdate (40%).

Table 4. 3. Ingredients and chemical composition (g/kg as-fed) of balancer and wheat-based finisher diet for broiler chickens.

Ingredients	**Balancer**	**Diet**
Wheat	–	670
Soybean meal (48)	569.7	188.0
Full-fat soybean meal	181.8	60.0
Soya oil	136.4	45.0
Monocalcium phosphate	30.3	10.0
Limestone	45.5	15.0
NaCl	9.1	3.0
L Lysine HCL	3.0	1.0
L Threonine	3.0	1.0
DL Methionine	9.1	3.0
Vitamin mineral premix[1]	12.1	4.0
Total	1000	1000
Calculated analysis (*as fed basis*)		
CP (g/kg)	349.3	190.3
ME (MJ/kg)	13.55	13.32
Crude fat (g/kg)	177.6	70.7
Ca (g/kg)	27.7	9.5
Available P (g/kg)	8.3	3.5
Lysine (g/kg)	22.6	9.4
Methionine	13.8	5.6
Threonine	16.7	7.5
Methionine + Cysteine (g/kg)	18.9	8.6
Tryptophan (g/kg)	4.4	2.2
Analysed values (*as fed basis*)		
DM (g/kg)	912	891-906
CP (Nx6.25) (g/kg)	348	193-199
Crude Fat (g/kg)	180	63.4-66.3

Each experimental diet met or exceeded the current diet specification for Ross 308 broiler (Aviagen 2014a).

[1]The vitamin and mineral premix contained vitamins and trace elements to meet the breeder's recommendations (Aviagen Ltd. Edinburgh, UK). The premix provided (units/kg diet): retinol 10000 IU; cholecalciferol 5000 IU; α-tocopherol 60 IU; menadione 3 mg; thiamine 2 mg; riboflavin 7 mg; pyridoxine 5 mg; cobalamin 15 µg; nicotinic acid 60 mg; pantothenic acid 15 mg; folic acid 1.5 mg; biotin 200 µg; 80 mg Fe as iron sulphate (30%); 10 mg Cu as copper sulphate (25%); 100 mg Mn as manganous oxide (62%); 80 mg Zn as zinc oxide (72%); 1 mg I as calcium iodate (52%); 0.25 mg Se as sodium selenite (4.5%) and 0.5 mg Mo as sodium molybdate (40%).

4.2.3.1. Enzyme addition

Diets were split into two parts and half of the diets were then supplemented with xylanase (Econase XT 25, AB Vista, Marlborough, UK), while other half diets were without xylanase supplementation. Xylanase was mixed in the basal diet at 0.3 g/kg for 30 min and then diluted to 0.1 g/kg of the diet by mixing with 67% of wheat for 30 min. The xylanase preparation (Econase XT 25) contained 160,000 units of endo 1, 4-β xylanase activity per gram. One unit of xylanase (XU) is defined as the amount of enzyme that liberates 1 nmol reducing sugars from birch wood xylan, measured as xylose equivalents, under the conditions of the assay (AB Enzymes, Darmstadt, Germany). In total, twenty (20) broiler grower diets and eighteen (18) broiler finisher diets were formulated. Nine (9) grower diets contained 67% of wheat without xylanase, nine (9) finisher diets contained 67% of wheat with xylanase and two extra grower diets contained 47% of wheat with and without xylanase, respectively (Table 4.4).

Table 4. 4. Description of grower and finisher diets containing 67% wheat, 33% balancer with and without xylanase.

Grower		**Finisher**	
Diets	Enzyme	Diets	Enzyme
LL	No enzyme	LL	No enzyme
LC	No enzyme	LC	No enzyme
LY	No enzyme	LY	No enzyme
BL	No enzyme	BL	No enzyme
BC	No enzyme	BC	No enzyme
BY	No enzyme	BY	No enzyme
KL	No enzyme	KL	No enzyme
KC	No enzyme	KC	No enzyme
KY	No enzyme	KY	No enzyme
LL+E	Xylanase	LL+E	Xylanase
LC+E	Xylanase	LC+E	Xylanase
LY+E	Xylanase	LY+E	Xylanase
BL+E	Xylanase	BL+E	Xylanase
BC+E	Xylanase	BC+E	Xylanase
BY+E	Xylanase	BY+E	Xylanase
KL+E	Xylanase	KL+E	Xylanase
KC+E	Xylanase	KC+E	Xylanase
KY+E	Xylanase	KY+E	Xylanase
BL 47%	No enzyme		
BL 47%+E	Xylanase		

4.2.3.2. Pelleting of grower and finisher diets

Each diet was pelleted separately at NIPH (Harper Adams University) using a pelleter (KAHL, Amandus Kahl GmbH & Co. KG, Reinbek, Germany) as described in Section 3.2.2.2. Diets were pelleted without using steam. Both broiler grower and finisher diets were pelleted at the same time and stored in bags below 18°C. Grower pellet diameter and length were 3 mm and 4 – 7 mm, respectively. The diameter and length of the finisher pellet was 3 mm and 5 – 8 mm, respectively. The pellet durability index (PDI) of grower and finisher diets were determined as described in Section 3.2.2.2. Dry matter content of grower and finisher diets was also determined and ranged from 885 to 904 and 885 to 906 g/kg, respectively. The PDI and DM of diets are presented in Table 4.5.

Table 4. 5. Dry matter (g/kg) and pellet durability index (PDI %) of broilers grower and finisher diets.

	Grower		**Finisher**	
Diet ID	DM	PDI	DM	PDI
LL	900	89.4	900	76.2
LC	885	89.8	898	89
LY	891	96	901	94.4
BL	898	89	906	72
BC	891	92.4	891	77
BY	900	92.4	896	71.6
KL	897	94	896	72
KC	898	95.5	901	62
KY	899	92.3	905	92.8
LL+E	894	91.6	900	69
LC+E	893	93	899	77.2
LY+E	901	86.3	903	92.2
BL+E	904	84.4	898	74.5
BC+E	886	85.2	892	78.2
BY+E	889	83.6	898	80.2
KL+E	897	50.4	885	50.2
KC+E	894	52	899	75.4
KY+E	899	52.8	900	87.3
Mean	895	83.9	898	77.3
Min	885	50.4	885	50.2
Max	904	96	906	94.4
CV%	0.59	18.16	0.57	14.52

Each value represents mean of 2 replicates per diet.

4.2.4. Bird husbandry and sample collection.

All animal procedures conducted in this study were approved by Harper Adams University Research Ethics Committee (Project no. 0278-201706-PGMPHD). Approximately, a thousand (1000) day-old male Ross 308 broiler chicks were obtained from a commercial hatchery (Cyril Bason, Cravens Arms, Shropshire, UK) and reared on a single floor pen. Chicks were fed *ab libitum* a proprietary broiler starter diet (Target feed Ltd, Whitchurch, UK) up to seven days of age (Table 4.6). Temperature was maintained at 32°C on day one and then reduced gradually by 0.5 °C every day. A standard light programme was followed, decreasing light to dark ratio 23:1 h from day old to 18:6 h at 7 days of age, which was maintained till the end of the study (Aviagen Ross 308 broiler management handbook).

Table 4. 6. Composition of commercial broiler starter diet (g/kg as-fed).

Ingredients	**Diet**
Barley	105
Wheat	500
Soybean meal	260.0
Full-fat soybean meal	50.0
Soya oil	40.0
Monocalcium phosphate	15.0
Limestone	12.5
NaCl	2.5
L Lysine HCL	4.0
L Threonine	1.5
DL Methionine	4.0
Vitamin mineral premix[1]	4.0
Sodium bicarbonate	1.5
Total	1000
Calculated analysis (*as fed basis)*	
CP (g/kg)	214
ME (MJ/kg)	12.78
Crude fat (g/kg)	63.4
Ca (g/kg)	9.8
Available P (g/kg)	4.8
Lysine (g/kg)	13.4
Methionine + Cysteine (g/kg)	10.2
Tryptophan (g/kg)	2.5

[1]The vitamin and mineral premix contained vitamins and trace elements to meet the breeder's recommendations (Aviagen 2014a). The premix provided (units/kg diet): retinol 13500 IU; cholecalciferol 5000 IU; α-tocopherol 100 mg; menadione 3 mg; thiamine 3 mg; riboflavin 10 mg; pyridoxine 5 mg; cobalamin 20 µg; nicotinic acid 60 mg; pantothenic acid 15 mg; folic acid 2 mg; biotin 200 µg; 40 mg Fe as iron sulphate (30%); 16 mg Cu as copper sulphate (25%); 100 mg Mn as manganous oxide (62%); 80 mg Zn as zinc oxide (72%); 2 mg I as calcium iodate (52%); 0.30 mg Se as sodium selenite (4.5%) and 1 mg Mo as sodium molybdate (40%).

4.2.4.1. Dietary phase

4.2.4.1.1. Grower phase

At day seven, the first day of experiment, chicks were weighed individually and selected weight near or above seven days age (189 ± 7 g) according to recommended Ross 308 male broilers body weight (Aviagen 2014b) leaving nine hundred and sixty birds (960). Eight birds were randomly allocated to one of 120 raised floor pens with 0.60 × 0.60 m solid floor area. Each pen was equipped with a trough feeder outside and automatic drinker inside the pen. The floor of each pen was covered with wood shavings. Each of twenty (20) experimental diets were allocated to six pens within blocks following randomisation. Feed and water were provided *ab libitum* throughout the experiment. Birds were fed experimental wheat-based grower diets from 7 – 21 d age. Standard lighting and temperature programme for Ross 308 broiler chickens were followed (Aviagen Ross broiler management handbook). A light programme of 18 hours (h) light and 6 h dark period was followed and maintained till the end of experiment. Temperature was gradually reduced daily until reached 21°C on day 21 and maintained afterwards until the end of experiment. Body weights were recorded at the beginning (d 7) and at the end of grower phase (d 21). The birds were inspected daily, any dead birds were weighed at the time of removal. Feed intake per pen was recorded and FCR was calculated on a pen weight basis. The weights of dead bird were included to calculate FCR.

4.2.4.1.2. Excreta collection

At day 18, the solid floor of each pen was replaced with a wire mesh and plastic trays were placed underneath wire mash for excreta collection for the determination of AME, total tract nutrients retention and N-corrected AMEn content using total collection method. Excreta were collected every 12 h per pen for three consecutive days (19 – 21) and immediately dried at 60 °C in a forced air oven, weighed and milled to pass through a 0.75 mm screen using Retsch ZM 200 (Retsch GmbH, Haan, Germany). Feathers and scales were removed carefully from excreta to avoid any contamination. The feed intake was also recorded for this period. Although, TiO_2 was added in the diets for nutrient digestibility and retention determination, however, was not used in the calculation. Instead, total collection method was used for nutrient retention determination. The DM, GE, N and crude fat (as ether extract) contents of each dried excreta sample and experimental diets were determined in duplicate as described in section 2.2.2.1. The AME, nitrogen corrected AMEn content and total tract nutrient retention (NR, FD and DMR) of diets and wheat were determined as described in Chapter 3 (Section 3.2.4.1 and 3.2.4.2). In Chapter 3 (Section 3.3.1), a linear response ($P < 0.05$) was already fitted to different inclusion levels of wheat for AME, AMEn and nutrient retention with no evidence of non-linearity ($P > 0.05$). Therefore, there was no

need to prove linearity again between two inclusion levels of wheat (47 and 67%) used in this study. The AME and nutrient retention constant of the balancer diets were determined by linear regression analysis of diets BL, BL 47%, BL + E and BL + E 47% and used in a substitution equation (Equation 3.3 and 3.5, respectively) to predict AME and total tract nutrient retention of wheat as explained previously (Section 3.2.4.1 and 3.2.4.2).

4.2.4.1.3. Ileal digesta viscosity and gizzard measurements

At d 21, all birds per pen were weighed and three birds with a body weight closest to the mean weight of the pen were chosen for ileal digesta collection. The live weights of birds were recorded prior to cervical dislocation. The birds were killed by cervical dislocation and ileal digesta were collected from each of the three birds. The contents of ileal digesta from Meckel's diverticulum to ilealo-caecal junction were gently pressed from each bird into a plastic container and pooled by pen and homogenised. A sample of this digesta was centrifuged at 10 000 × g for 2 min. The viscosity of the supernatant was measured using a rotating cone and cup viscometer (model DV – II + LV, Brookfield Stoughton, MA, USA) as described by Bedford and Classen (1992).

Of the three birds chosen previously, one bird was selected at random for gizzard sample and weighed. The gizzard was carefully excised at the junction of proventiculus and duodenum. First, fresh weight of gizzard with food contents was recorded, then gizzard was cut, emptied of its contents and subsequently weighed. The relative weight (g/kg BW) of gizzard was calculated as ratio to live weight of bird per pen.

4.2.4.1.4. Finisher phase

The remaining five birds were kept in same raised floor pens and fed experimental finisher diets. Each finisher diet was replicated six times and allocated to the same pen as previously allocated the same grower diet. Birds were fed experimental finisher diet from d 21 to d 35. Birds were reared on a solid floor with wood shavings on top of the floor. Standard temperature and lighting programme were followed (Ross 308 broiler management handbook). On d 35, the body weights of birds per pen and feed intake per pen were recorded and FCR was calculated on a pen weight basis. The body weights of dead birds were adjusted for when calculating FCR.

4.2.5. Statistical analysis

Statistical analyses were performed in GenStat (GenStat 17th edition supplied by VSN international Ltd, UK). Proximate nutrient composition, polysaccharides composition and physical characteristics of wheat variety and their growing site were compared statistically by analysis of variance (ANOVA). Broiler growth performance, AME, AMEn, nutrient

utilisation of wheat samples, ileal viscosity and digestive tract measurements were compared statistically by ANOVA in a randomised complete block design using a 3 × 3 × 2 factorial arrangement of treatments, with a single pen representing an experimental unit (replicate) for all response measurements. Treatment and blocks were fixed effects. The main effects were the wheat variety, the growing site and the xylanase (with and without). The variables that described growth performance were feed intake g/bird/day (FI), weight gain g/bird/day (WG), final body weight kg/bird at d 21 and d 35 (FBW), feed conversion ratio corrected for mortality (g feed intake/g weight gain) (FCR). Body weights at day seven were used as co-variate for performance variables (FI, WG, FBW, FCR) during 7 – 21 d and 7 – 35 d. Differences were reported as significant at *P* value less than 0.05 and *P* value between 0.05 and 0.1 was considered as a trend. If a significant difference ($P < 0.05$) for the main effects or the interactions were found, means were separated using Duncan's multiple range test and differences were reported significant at $P < 0.05$.

4.3. RESULTS

Recovery of xylanase in grower balancer and finisher balancer diet was tested by ESC (Enzyme Services and Consultancy (ESC), Ystrad, Mynach, UK) and presented in Table 4.7. Xylanase was added to the diets to achieve an in-feed activity of 16000 BXU/kg in the final diet. Xylanase was mixed in the balancer diets and then diluted with 67% of the wheat, therefore the calculated activity of xylanase in balancer diets was expected to be 48000 BXU/kg (3 times × 16000 BXU/kg). The analysis of both grower and finisher balancer diets confirmed the activity of xylanase greater than the required activity but at acceptable level. The calculated activity of xylanase in appropriate grower and finisher diets was in the required level. The analysis of balancer diets without xylanase also confirmed absence of any xylanase activity.

Table 4. 7. The calculated and analysed activity of xylanase in balancer diets containing Econase XT 25.

Diet	**Activity in balancer diet BXU/kg (analysed)**	**Activity in final diet BXU/kg (calculated)**
Grower Balancer	<2000	
Grower Balancer + Econase	58900	19633
Finisher Balancer	<2000	
Finisher Balancer + Econase	79900	26633

Xylanase activity was analysed by ELISA method by ESC.

4.3.1. Proximate nutrient composition of wheat

Proximate nutrient composition and GE of wheat samples are presented in Table 4.8. There was no difference ($P > 0.05$) in DM content between wheat varieties and growing sites. There was no difference ($P > 0.05$) in CP content (N × 6.25) between wheat varieties. Wheat from Lincolnshire had higher ($P < 0.05$) concentration of CP content (134 g/kg DM) than Cambridgeshire and Yorkshire (average = 127 g/kg DM). There was no difference ($P > 0.05$) in GE content between wheat varieties. The GE content of Yorkshire grown wheat (17.63 MJ/kg DM) was lower ($P < 0.05$) than Lincolnshire and Cambridgeshire (average = 18.36 MJ/kg DM). Wheat from Yorkshire tended ($P = 0.089$) to have lower ash content (15.6 g/kg DM) as compared to wheats from Lincolnshire (17.5 g/kg DM). The crude oil (as ether extract) content of wheat variety Barrel (12.9 g/kg DM) was higher ($P < 0.05$) than Lili and Kerrin (average = 10.6 g/kg DM).

Table 4. 8. Proximate composition and GE content of wheat varieties grown at different locations (g/kg DM).

Sample ID	Variety	Site	DM[1]	Protein (N×6.25)	GE[2]	Ash	Oil[3]
LL	Lili	Lincolnshire	872	136	18.26	17.6	10.7
LC	Lili	Cambridgeshire	863	129	18.46	15.5	10.2
LY	Lili	Yorkshire	874	135	17.46	15.9	9.0
BL	Barrel	Lincolnshire	867	134	18.36	18.3	13.2
BC	Barrel	Cambridgeshire	861	124	18.53	16.3	13.2
BY	Barrel	Yorkshire	875	127	17.81	15.9	12.3
KL	Kerrin	Lincolnshire	868	133	18.20	16.5	11.2
KC	Kerrin	Cambridgeshire	873	124	18.36	16.8	10.8
KY	Kerrin	Yorkshire	878	124	17.61	14.9	11.6
Overall mean			870	129	18.12	16.4	11.4
Treatment factor means							
Variety							
Lili			870	133	18.06	16.3	10.0[a]
Barrel			868	128	18.23	16.8	12.9[b]
Kerrin			873	127	18.06	16.1	11.2[a]
P value			0.338	0.082	0.112	0.51	0.012
Site							
Lincolnshire			869	134[b]	18.27[b]	17.5[b]	11.8
Cambridgeshire			866	126[a]	18.45[b]	16.2[ab]	11.4
Yorkshire			876	129[a]	17.63[a]	15.6[a]	11.0
SEM[4] (variety and site) (df = 4)			2.3	1.4	0.050	0.45	0.37
P value			0.088	0.028	<0.001	0.089	0.419

[1]Dry matter g/kg, [2]Gross energy MJ/kg DM, [3]Oil as ether extract.
[4]Standard error of means.
Each analysis was performed in duplicate.

4.3.2. Polysaccharides composition of wheat

In Table 4.9, only the concentration of major constituents of polysaccharide are presented, whereas, in Table 4.10, constituents of NSP are summarised. There were no differences ($P > 0.05$) in starch content between three wheat varieties. The starch content of wheat sourced from Yorkshire site (700 g/kg DM) was lower ($P < 0.05$) than the Cambridgeshire and the Lincolnshire (average = 725 g/kg DM). There were no differences ($P > 0.05$) in total NSP content between wheat variety and their growing sites. There were no differences ($P > 0.05$) in soluble NSP content between wheat varieties and growing sites. The insoluble NSP content of wheat variety Kerrin (65.2 g/kg DM) were lower ($P < 0.05$) than Lili and Barrel (average 73.3 g/kg DM). Wheat grown at Yorkshire site had lower ($P = 0.05$) insoluble NSP concentration (67.4 g/kg DM) as compared to the Cambridgeshire (73 g/kg DM). Among soluble NSP fractions, there were no differences ($P > 0.05$) in soluble arabinose, xylose, mannose, galactose and glucose between wheat varieties and their growing sites (Table 4.10). The order of distribution of constituent sugars in wheat samples were xylose, glucose and arabinose. There were differences ($P < 0.05$) in some insoluble fractions of NSP between variety and growing sites. Kerrin had the lowest ($P < 0.05$) arabinose (14.9 g/kg DM), xylose (28.0 g/kg DM) and glucose concentration (20.5 g/kg DM). Wheat from growing site Yorkshire had the lowest ($P < 0.05$) concentration of arabinose (15.2 g/kg DM). Wheat variety Kerrin had relatively lower total xylose, arabinose and arabinoxylans (AX) (arabinose plus xylose) (38, 21.3 and 59.3 g/kg DM) contents as compared to Lili (41.4, 21.8 and 63.2 g/kg DM) and Barrel (42.3, 22.1 and 64.4 g/kg DM), respectively.

Table 4. 9. Polysaccharides composition of wheat varieties grown at different locations (g/kg DM).

Sample ID	Variety	Site	Starch	tNSP	sNSP	iNSP
LL	Lili	Lincolnshire	709	90.9	16.6	74.2
LC	Lili	Cambridgeshire	728	97.2	21.3	75.8
LY	Lili	Yorkshire	686	89.2	21.8	67.4
BL	Barrel	Lincolnshire	724	99.7	25.8	73.9
BC	Barrel	Cambridgeshire	714	99.7	22.2	77.5
BY	Barrel	Yorkshire	702	87.2	16.4	70.8
KL	Kerrin	Lincolnshire	736	87.4	21.2	66.2
KC	Kerrin	Cambridgeshire	739	88.1	22.5	65.6
KY	Kerrin	Yorkshire	713	90.8	26.9	63.9
Overall mean			717	92.2	21.6	70.6
Treatment factor mean						
Variety						
Lili			708	92.4	19.9	72.5[b]
Barrel			713	95.5	21.5	74.0[b]
Kerrin			729	88.8	23.5	65.2[a]
P value			0.077	0.330	0.638	0.010
Site						
Lincolnshire			723[b]	92.7	21.2	71.5[ab]
Cambridgeshire			727[b]	95.0	22.0	73.0[b]
Yorkshire			700[a]	89.0	21.7	67.4[a]
SEM[1] (variety and site) (df = 4)			4.9	2.77	2.55	1.12
P value			0.034	0.399	0.974	0.053

tNSP = Total NSP, sNSP = soluble NSP, iNSP = Insoluble NSP.
[1]Standard error of means.
Each analysis was performed in duplicate.

Table 4. 10. Non-starch polysaccharides (NSP) composition of wheat varieties grown at different locations (g/kg DM).

	Variety			Site			*P value*		SEM[1] (df = 4)
	Lili	Barrel	Kerrin	Lincs	Cambs	Yorks	Variety	Site	(for variety and site means)
sNSP									
Arabinose	5.4	5.2	6.4	5.9	5.4	5.7	0.325	0.764	0.51
Xylose	8.7	10.0	10.0	9.5	9.6	9.6	0.708	0.997	1.22
Mannose	2.1	1.8	2.2	2.0	2.0	2.1	0.552	0.910	0.22
Galactose	2.2	2.1	2.3	2.1	2.1	2.3	0.551	0.595	0.16
Glucose	1.6	2.3	2.6	1.7	2.9	2.0	0.731	0.653	0.90
Total sNSP	19.9	21.5	23.5	21.2	22.0	21.7	0.638	0.974	2.55
iNSP									
Arabinose	16.5[b]	16.9[b]	14.9[a]	16.1[b]	17.0[c]	15.2[a]	0.006	0.010	0.21
Xylose	32.7[b]	32.2[b]	28.0[a]	31.3	31.6	30.0	0.009	0.221	0.58
Mannose	0.7	0.7	0.9	0.9	0.9	0.5	0.399	0.184	0.13
Galactose	0.7	0.7	0.9	0.9	0.8	0.6	0.271	0.219	0.08
Glucose	22.0[ab]	23.6[b]	20.5[a]	22.3	22.7	21.0	0.031	0.167	0.51
Total iNSP	72.5	74.0	65.2	71.5	73.0	67.4	0.010	0.053	1.12
tNSP									
Arabinose	21.8	22.1	21.3	22.0	22.4	20.9	0.487	0.158	0.44
Xylose	41.4	42.2	38.0	40.8	41.2	39.6	0.108	0.576	1.10
Mannose	2.8	2.5	3.1	2.8	2.9	2.6	0.033	0.193	0.11
Galactose	2.9	2.8	3.2	3.0	2.9	3.0	0.107	0.759	0.11
Glucose	23.6	25.9	23.1	24.0	25.6	23.0	0.375	0.469	1.32
Total NSP	92.4	95.5	88.8	92.7	95.0	89.0	0.330	0.399	2.77

sNSP = soluble NSP, iNSP = insoluble NSP, tNSP = total NSP.
Lincs = Lincolnshire, Cambs = Cambridgeshire, Yorks = Yorkshire.
[1]Standard error of means.

4.3.3. Physical characteristics of wheat

There were differences ($P < 0.001$) in EH values between wheat varieties (Table 4.11). The HFN values of wheat variety Kerrin (195) were lower ($P < 0.05$) than Lili (358), whereas, Barrel had an intermediate value of HFN (277). There was no difference ($P > 0.05$) in SW between wheat varieties. The SW of wheat from growing site Cambridgeshire (81.6 kg/hl) was higher ($P < 0.05$) than Lincolnshire and Yorkshire (average = 73.9 kg/hl). The TGW of Lili (51.3 g) was lower ($P < 0.05$) as compared to Barrel and Kerrin (average = 58.9 g). The TGW of wheat from growing site Yorkshire (53.5 g) was lower ($P < 0.05$) than Cambridgeshire (59.4 g). There were no differences ($P > 0.05$) in DV between wheat variety and growing site.

Table 4. 11. Physical characteristics of wheat varieties grown at different locations.

Sample ID	Variety	EH	HFN	SW	TGW	DV
LL	Lili	66	402	73.9	52.8	2.8
LC	Lili	74	352	83.0	54.8	2.8
LY	Lili	72	322	70.2	46.3	3.1
BL	Barrel	27	310	75.6	57.6	6.2
BC	Barrel	31	196	82.0	62.7	4.4
BY	Barrel	38	326	74.2	56.0	3.5
KL	Kerrin	54	263	74.9	58.3	2.6
KC	Kerrin	58	178	79.9	60.7	2.5
KY	Kerrin	73	145	74.5	58.2	2.3
Overall mean		55	277	76.5	56.4	3.4
Treatment factor mean						
Variety						
Lili		70^{b}	358^{b}	75.7	51.3^{a}	2.9
Barrel		32^{a}	277^{ab}	77.2	58.8^{b}	4.7
Kerrin		61^{b}	195^{a}	76.5	59.1^{b}	2.5
P value		0.001	0.038	0.632	0.014	0.059
Site						
Lincolnshire		49	325	74.8^{a}	56.2^{ab}	3.9
Cambridgeshire		54	242	81.6^{b}	59.4^{b}	3.2
Yorkshire		61	264	73.0^{a}	53.5^{a}	3.0
SEM[1] (variety and site) (df = 4)		2.5	28.3	1.10	1.15	0.47
P value		0.068	0.216	0.011	0.055	0.476

EH = endosperm hardness (relative units 0 –120), HFN = Hagberg falling number, SW = specific weight (kg/hl), TGW = thousand grain weight (g), DV = dynamic water extract viscosity (centipoise).
[1]Standard error of means.

4.3.4. Influence of wheat variety, growing site and xylanase addition on apparent metabolisable energy and nutrient utilisation of wheat

The growing site influenced AME and AMEn of wheat for broilers (Table 4.12). Wheat grown at Cambridgeshire site had the highest (P < 0.001) AME content (14.77 MJ /kg DM), followed by Lincolnshire (14.40 MJ/ kg DM) and Yorkshire (14.00 MJ/kg DM). Similarly, wheat from Cambridgeshire site had the highest (P < 0.001) AMEn content (14.34 MJ/kg DM), followed by Lincolnshire (13.95 MJ/kg DM) and Yorkshire (13.55 MJ/kg DM). There was no difference (P > 0.05) between wheat varieties in their AME and AMEn content. Overall, xylanase supplementation improved (P < 0.001) AME and AMEn content of wheat by 0.50 and 0.59 MJ/kg DM, respectively. There was an interaction (P = 0.005) between wheat variety and xylanase for AME and AMEn content of wheat. Xylanase supplementation improved (P < 0.05) AME content of Barrel by 0.73 MJ/kg DM (14.09 to 14.82 MJ/kg DM) and Lili by 0.63 MJ/kg DM (13.97 to 14.60 MJ/kg DM) but no improvement in Kerrin (P > 0.05) (Table 4.12, variety × xylanase interaction). Similarly, the addition of xylanase improved (P < 0.05) AMEn content of Barrel by 0.81 MJ/kg DM (13.61 to 14.42 MJ/kg DM) and Lili by 0.74 MJ/kg DM (13.46 to 14.20 MJ/kg DM) and showed no improvement in Kerrin (P > 0.05).

The total tract of nutrient retention values of wheat is presented in Table 4.13. The fat digestibility (FD) of wheat from Cambridgeshire (0.743) was greater (P < 0.05) than Lincolnshire and Yorkshire (average = 0.711). The dry matter retention (DMR) of wheat from Yorkshire (0.839) was greater (P = 0.05) than Lincolnshire and Cambridgeshire (average = 0.827). The coefficient of nitrogen retention (NR) tended to be different (P = 0.07) between growing sites. There was no difference (P > 0.05) in nutrient utilisation between wheat varieties. Overall, the addition of xylanase increased NR (P < 0.001), FD (P = 0.003) and DMR (P < 0.001) of wheat by 5.1, 4.8 and 2.8%, respectively. There was an interaction (P < 0.05) between variety and xylanase for FD and DMR content of wheat. Xylanase supplementation improved (P < 0.05) FD of Lili and Barrel but showed no improvement (P > 0.05) in Kerrin. The addition of xylanase also improved (P < 0.05) DMR of Lili and Barrel but no improvement (P > 0.05) was seen in Kerrin. There was an interaction (P < 0.05) between growing site and xylanase for NR only, where the addition of xylanase improved (P < 0.05) NR of wheat from Lincolnshire (0.575 to 0.619) and Cambridgeshire (0.582 to 0.619) but did not improve (P > 0.05) NR of wheat from Yorkshire.

Table 4. 12**.** Effect of wheat variety, growing site and xylanase addition on AME and AMEn content[1] of wheat for broilers.

	Xylanase	AME MJ/kg DM	AMEn MJ/kg DM
Variety			
Lili		14.28	13.83
Barrel		14.46	14.01
Kerrin		14.43	13.99
SEM[2]		0.070	0.064
Site			
Lincolnshire		14.40[b]	13.95[b]
Cambridgeshire		14.77[c]	14.34[c]
Yorkshire		14.00[a]	13.55[a]
SEM[2]		0.070	0.064
Xylanase			
	−	14.14	13.65
	+	14.64	14.24
SEM[2]		0.057	0.053
Variety × Xylanase			
Lili	−	13.97[a]	13.46[a]
	+	14.60[bc]	14.20[cd]
Barrel	−	14.09[a]	13.61[a]
	+	14.82[c]	14.42[d]
Kerrin	−	14.38[b]	13.89[b]
	+	14.49[bc]	14.10[bc]
SEM[2]		0.099	0.091
Site × Xylanase			
Lincolnshire	−	14.15	13.67
	+	14.65	14.24
Cambridgeshire	−	14.43	13.95
	+	15.12	14.72
Yorkshire	−	13.86	13.34
	+	14.15	13.75
SEM[2]		0.099	0.091
Significance			
Variety		0.180	0.088
Site		<0.001	<0.001
Xylanase		<0.001	<0.001
Variety × Site		0.200	0.264
Variety × Xylanase		0.005	0.003
Site × Xylanase		0.132	0.156
Variety × Site × Xylanase		0.144	0.158

[1]Each value represents mean of 6 experimental units of eight birds each. Values are based on total collection from 19 to 21 days of age.
[2]Standard error of means (df = 81).
Means within a column with no common superscripts differ significantly ($P < 0.05$).

Table 4. 13. Effect of wheat variety, growing site and xylanase addition on coefficients[1] of nitrogen retention (NR), fat digestibility (FD) and dry matter retention (DMR) in broilers.

	Xylanase	NR	FD	DMR
Variety				
Lili		0.600	0.730	0.830
Barrel		0.584	0.716	0.827
Kerrin		0.597	0.718	0.836
SEM[2]		0.0055	0.0094	0.0039
Site				
Lincolnshire		0.597	0.716[a]	0.828[a]
Cambridgeshire		0.600	0.743[b]	0.827[a]
Yorkshire		0.583	0.706[a]	0.839[b]
SEM[2]		0.0055	0.0094	0.0039
Xylanase				
−		0.579	0.704	0.820
+		0.608	0.738	0.842
SEM[2]		0.0045	0.0077	0.0032
Variety × Xylanase				
Lili	−	0.585	0.700[ab]	0.814[a]
	+	0.615	0.761[c]	0.846[b]
Barrel	−	0.562	0.694[a]	0.812[a]
	+	0.606	0.737[bc]	0.842[b]
Kerrin	−	0.589	0.719[ab]	0.833[b]
	+	0.605	0.716[ab]	0.839[b]
SEM[2]		0.0078	0.0134	0.0055
Site × Xylanase				
Lincolnshire	−	0.575[a]	0.686	0.814
	+	0.619[b]	0.745	0.841
Cambridgeshire	−	0.582[a]	0.741	0.811
	+	0.619[b]	0.745	0.843
Yorkshire	−	0.580[a]	0.687	0.834
	+	0.587[a]	0.725	0.844
SEM[2]		0.0078	0.0134	0.0055
Significance				
Variety		0.101	0.495	0.238
Site		0.070	0.019	0.053
Xylanase		<0.001	0.003	<0.001
Variety × Site		0.873	0.655	0.488
Variety × Xylanase		0.205	0.050	0.033
Site × Xylanase		0.040	0.129	0.107
Variety × Site × Xylanase		0.200	0.084	0.545

[1]Each value represents mean of 6 experimental units of eight birds each. Values are based on total collection from 19 to 21 days of age.
[2]Standrad error of means (df = 83).
Means within a column with no common superscripts differ significantly ($P < 0.05$).

4.3.5. Effect of wheat variety, growing site and xylanase addition on growth performance of broilers

4.3.5.1. Grower phase 7 – 21 d

The effect of wheat variety, growing site and xylanase addition on FI, WG, FBW and FCR in the grower phase (7 – 21 d) are summarised in Table 4.14. The growing site of wheat influenced daily FI, WG and FBW of broilers. The daily FI of broilers fed wheat from Lincolnshire and Cambridgeshire was greater ($P < 0.05$; 2.9%) as compared to those fed wheat from Yorkshire (average 70.0 vs 68.3 g/b/d DM). The daily WG of broilers fed wheat from Lincolnshire and Cambridgeshire (average = 53.2 g/b/d) was greater ($P < 0.05$, 2.9%) in comparison to wheat from Yorkshire (51.7 g/b/d). At d 21, the FBW of birds fed wheat from Lincolnshire and Cambridgeshire was greater ($P < 0.05$, 2.6%) than those receiving wheat from Yorkshire (average 936 vs 912 g). Although, there were differences in FI and WG between growing sites but no difference ($P > 0.05$) in FCR between different growing sites was observed. The FI of birds fed wheat Barrel was greater ($P < 0.05$, 3.1%) than those fed wheat Lili (70.6 vs 68.5 g/b/d DM), whereas those receiving wheat Kerrin had intermediate FI. There were no differences ($P > 0.05$) in WG, FBW and FCR between wheat varieties.

Xylanase supplementation improved ($P < 0.001$) FCR by 0.033 (from 1.338 to 1.305). In the grower phase, FI was greater ($P < 0.001$; 70.7 g/b/d DM) in birds fed diets without xylanase as compared to those fed xylanase supplemented diets (68.2 g/b/d). The WG tended to be greater ($P = 0.067$; 53.1 g/b/d) in birds fed diets without xylanase than those fed diets containing xylanase enzyme (52.2 g/b/d). There was an interaction ($P = 0.047$) between wheat variety and growing site for WG only (Table 4.15). The WG of chickens fed wheat variety Barrel from Lincolnshire and Cambridgeshire was greater ($P < 0.05$) than those fed wheat Lili and Barrel from Yorkshire and Kerrin from Cambridgeshire sites. Mortality was low (0.3%) in the grower phase.

4.3.5.2. Finisher phase 21 – 35 d

The effect of wheat variety, growing site and xylanase addition on growth performance of broilers in finisher phase (21 – 35 d) are presented in Table 4.16. The FI of broilers fed wheat sourced from Lincolnshire and Cambridgeshire was greater ($P < 0.05$) than those fed wheat from Yorkshire (average 124.8 vs 116.9 g/b/d DM). The WG of broilers fed wheat sourced from Lincolnshire was greater ($P < 0.05$; 82.3 g/b/d) than those fed wheat from Cambridgeshire and Yorkshire (average 75.2 g/b/d). Although, there were differences in FI and WG between growing sites in the finisher phase, no difference ($P > 0.05$) in FCR between growing sites was found. In the finisher phase, FI was greater ($P < 0.05$) in broilers

fed the diets without xylanase (125.4 g/b/d DM) than those fed the diets supplemented with xylanase (119 g/b/d DM). Similarly, the WG was greater ($P < 0.05$) in broiler fed the diets without xylanase addition (79.7 g/b/d) than those fed the diets supplemented with xylanase (75.4 g/b/d) in this phase.

The FI of broilers fed wheat variety Barrel was greater ($P < 0.05$; 125.7 g/b/d DM) than those fed Lili (118.3 g/b/d DM), whereas birds receiving Kerrin had intermediate FI. There was no difference ($P > 0.05$) between wheat varieties in WG and FCR. During the finisher phase, an interaction ($P = 0.001$) between growing site and xylanase was found for FI only, where xylanase supplementation reduced FI of broilers fed wheat sourced from Cambridgeshire ($P < 0.05$; 14.7%) but did not change the response of those fed wheat other two growing sites. Mortality was low (2.4%) in the finisher phase and was not affected by wheat variety or growing site ($P > 0.05$).

4.3.5.3. Overall performance 7 – 35 d

The effect of wheat variety, growing site and xylanase supplementation on growth performance of broilers in overall period (7 – 35 d) are summarised in Table 4.17. The FI of broilers receiving wheat from growing sites Lincolnshire and Cambridgeshire (average 91 g/b/d DM) was greater ($P < 0.05$, 5%) than those fed wheat from Yorkshire (86.7 g/b/d DM). The WG of broilers fed wheat from Lincolnshire (64.1 g/b/d) was greater ($P < 0.05$; 5.6%) than those fed wheat from Cambridgeshire and Yorkshire (average 60.7 g/b/d). Overall, there was no difference in FCR between growing sites of wheat. At day 35, FBW was greater ($P < 0.01$; 6.6%) in birds fed wheat from growing site Lincolnshire (2.096 kg/b) as compared to Yorkshire (1.966 kg/d) and intermediate for those receiving wheat from Cambridgeshire (2.032 kg/b).

The FI of broilers fed diets containing wheat variety Barrel was greater ($P < 0.05$; 5%) than Lili (91.8 vs 87.4 g/b/d DM), whereas intermediate for those receiving wheat Kerrin (89.5 g/b/d DM). There was no difference ($P > 0.05$) in WG, FBW and FCR between wheat varieties.

Overall, the FI was greater ($P < 0.001$) in chickens fed the diets without xylanase (91.5 g/b/d DM) than those fed the diets supplemented with xylanase (87.6 g/b/d DM). Similarly, the WG was greater ($P < 0.05$) in birds fed the diets without xylanase (62.9 g/b/d) than those fed the diets with xylanase (60.7 g/b/d). The addition of xylanase improved ($P < 0.05$) FCR by 0.015 (from 1.443 to 1.428) during 7 – 35 d. There was an interaction ($P = 0.006$) between the growing site of wheat and xylanase for FI only during 7 – 35 d, where xylanase supplementation reduced FI of broilers fed wheat sourced from Cambridgeshire ($P < 0.05$; 10%) but did not reduce FI of those receiving wheat from Lincolnshire and Yorkshire.

Table 4. 14. Effect of wheat variety, growing site and xylanase addition on growth performance[1] of broilers (7 – 21 days).

	Xylanase	FI DM g/b/d	WG g/b/d	FCR (g: g)	FBW 21d
Variety					
Lili		68.5[a]	52.3	1.319	0.920
Barrel		70.6[b]	53.4	1.325	0.938
Kerrin		69.2[ab]	52.2	1.321	0.926
SEM[2]		0.53	0.43	0.0076	0.0061
Site					
Lincolnshire		70.0[b]	53.4[b]	1.313	0.938[b]
Cambridgeshire		70.0[b]	52.9[b]	1.321	0.934[b]
Yorkshire		68.3[a]	51.7[a]	1.330	0.912[a]
SEM[2]		0.52	0.42	0.0074	0.0060
Xylanase					
−		70.7	53.1	1.338	0.932
+		68.2	52.2	1.305	0.923
SEM[2]		0.42	0.35	0.0061	0.0049
Variety ×Xylanase					
Lili	−	69.7	52.8	1.336	0.925
	+	67.4	51.7	1.302	0.915
Barrel	−	71.8	53.6	1.343	0.940
	+	69.4	53.2	1.306	0.936
Kerrin	−	70.6	52.9	1.335	0.932
	+	67.9	51.6	1.306	0.919
SEM[2]		0.738	0.606	0.0106	0.0085
Site × Xylanase					
Lincolnshire	−	71.1	53.3	1.336	0.937
	+	68.9	53.4	1.290	0.939
Cambridgeshire	−	71.7	53.6	1.339	0.941
	+	68.4	52.1	1.303	0.926
Yorkshire	−	69.3	52.4	1.339	0.919
	+	67.3	50.9	1.321	0.904
SEM[2]		0.73	0.60	0.0105	0.0084
Significance					
Variety		0.050	0.190	0.847	0.186
Site		0.029	0.020	0.264	0.005
Xylanase		<0.001	0.067	<0.001	0.206
Variety × Site		0.347	0.047	0.383	0.071
Variety × Xylanase		0.955	0.703	0.942	0.892
Site × Xylanase		0.673	0.299	0.409	0.514
Variety × Site × Xylanase		0.238	0.299	0.476	0.770

[1]Each value represents mean of 6 experimental units of eight birds each. The body weight of birds at day 7 was used as a covariate.
[2]Standard error of means (df = FI: 84, WG: 83, FCR: 82, FBW: 84).
Means within a column with no common superscripts differ significantly ($P < 0.05$).
FI: feed intake (g/b/d DM), WG: weight gain (g/b/d), FCR: feed conversion ratio (g/g DM), FBW: final body weight (kg/bird) at 21 day.

Table 4. 15. Effect of wheat variety and growing site on weight gain[1] (WG g/b/d) of broilers (7 – 21 days).

	Site				**SEM[2]**			***P Value***		
Variety	Lincs	Cambs	Yorks	Variety (mean)	Variety	Site	Interaction	Variety	Site	Interaction
Lili	52.8^{ab}	53.2^{ab}	50.9^{a}	52.3	0.43	0.42	0.74	0.19	0.02	0.047
Barrel	54.7^{b}	54.0^{b}	51.4^{a}	53.4						
Kerrin	52.7^{ab}	51.4^{a}	52.7^{ab}	52.2						
Site (mean)	53.4	52.9	51.7							

[1]Each value represents mean of 6 experimental units of eight birds each.
Lincs = Lincolnshire, Cambs = Cambridgeshire, Yorks = Yorkshire.
[2]Standard error of means (df = 83).
Means within treatments (interaction effect) within rows not sharing a common superscript differ significantly ($P < 0.05$).

Table 4. 16. Effect of wheat variety, growing site and xylanase supplementation on growth performance[1] of broilers (21 – 35 days).

	Xylanase	FI DM g/b/d	WG g/b/d	FCR (g: g)
Variety				
Lili		118.3[a]	75.3	1.550
Barrel		125.7[b]	79.5	1.573
Kerrin		122.6[ab]	77.8	1.556
SEM[2]		1.78	1.62	0.0157
Site				
Lincolnshire		126.5[b]	82.3[b]	1.547
Cambridgeshire		123.2[b]	75.8[a]	1.587
Yorkshire		116.9[a]	74.5[a]	1.546
SEM[2]		1.78	1.62	0.0157
Xylanase				
−		125.4	79.7	1.549
+		119.0	75.4	1.571
SEM[2]		1.46	1.32	0.0128
Variety × Xylanase				
Lili	−	121.0	76.2	1.543
	+	115.5	74.3	1.557
Barrel	−	129.6	83.8	1.544
	+	121.8	75.3	1.602
Kerrin	−	125.5	79.0	1.559
	+	119.8	76.6	1.554
SEM[2]		2.52	2.286	0.0222
Site × Xylanase				
Lincolnshire	−	128.7[c]	83.1	1.534
	+	124.4[bc]	81.5	1.559
Cambridgeshire	−	131.7[c]	80.5	1.579
	+	114.8[a]	71.0	1.595
Yorkshire	−	115.8[a]	75.3	1.533
	+	118.0[ab]	73.6	1.559
SEM[2]		2.52	2.29	0.0222
Significance				
Variety		0.015	0.178	0.563
Site		<0.001	0.002	0.113
Xylanase		0.003	0.025	0.219
Variety × Site		0.187	0.258	0.860
Variety × Xylanase		0.887	0.282	0.351
Site × Xylanase		0.001	0.145	0.970
Variety × Site × Xylanase		0.545	0.689	0.311

[1]Each value represents mean of 6 experimental units of five birds each.
[2]Standard error of means (df = FI: 85, WG: 82, FCR: 83).
Means within a column with no common superscripts differ significantly ($P < 0.05$).
FI: feed intake (g/b/d DM), WG: weight gain (g/b/d), FCR: feed conversion ratio (g/g DM).

Table 4. 17. Effect of wheat variety, growing site and xylanase addition on growth performance[1] of broilers (7 – 35 days).

	Xylanase	FI DM g/b/d	WG g/b/d	FCR (g: g)	FBW 35d
Variety					
Lili		87.4[a]	60.7	1.432	1.989
Barrel		91.8[b]	63.2	1.444	2.061
Kerrin		89.5[ab]	61.6	1.431	2.045
SEM[2]		0.93	0.84	0.0072	0.0268
Site					
Lincolnshire		91.7[b]	64.1[b]	1.425	2.096[b]
Cambridgeshire		90.3[b]	61.6[a]	1.443	2.032[ab]
Yorkshire		86.7[a]	59.8[a]	1.438	1.966[a]
SEM[2]		0.91	0.83	0.0071	0.0263
Xylanase					
−		91.5	62.9	1.443	2.075
+		87.6	60.7	1.428	1.988
SEM[2]		0.74	0.67	0.0057	0.0215
Variety × Xylanase					
Lili	−	89.1	61.2	1.443	2.015
	+	85.6	60.2	1.420	1.963
Barrel	−	94.2	65.5	1.448	2.123
	+	89.5	60.9	1.440	1.998
Kerrin	−	91.3	62.0	1.439	2.088
	+	87.7	61.2	1.423	2.001
SEM[2]		1.30	1.18	0.0101	0.0376
Site × Xylanase					
Lincolnshire	−	93.2[cd]	64.8	1.432	2.119
	+	90.2[bc]	63.4	1.418	2.074
Cambridgeshire	−	94.6[d]	64.0	1.454	2.121
	+	86.0[a]	59.3	1.433	1.943
Yorkshire	−	86.8[ab]	59.9	1.444	1.986
	+	86.6[ab]	59.6	1.432	1.946
SEM[2]		1.29	1.17	0.0100	0.0372
Significance					
Variety		0.015	0.245	0.341	0.213
Site		<0.001	0.002	0.170	0.004
Xylanase		<0.001	0.031	0.053	0.005
Variety × Site		0.234	0.238	0.604	0.778
Variety × Xylanase		0.889	0.209	0.725	0.627
Site × Xylanase		0.006	0.158	0.871	0.117
Variety × Site × Xylanase		0.413	0.828	0.122	0.799

[1]Each value represents mean of 6 replicates. The body weight of birds at day 7 was used as a covariate.
[2]Standard error of means (df = FI: 84, WG: 83, FCR: 82, FBW: 84).
Means within a column with no common superscripts differ significantly ($P < 0.05$).
FI: feed intake (g/b/d DM), WG: weight gain (g/b/d), FCR: feed conversion ratio (g/g DM),
FBW: final body weight (kg/b) at 35 days.

4.3.6. Influence of xylanase addition on ileal digesta viscosity and gizzard development

There was a difference ($P < 0.05$) in ileal digest viscosity of broilers between wheat variety and growing site (Table 4.18). The addition of xylanase had an interaction ($P < 0.05$) with wheat variety and their growing site for ileal digesta viscosity determined (Figure 4.1, 4.2). The supplementation of xylanase reduced ($P < 0.05$) ileal digesta viscosity in birds fed wheats Lili and Barrel from all three growing sites. However, in regard to wheat Kerrin, xylanase only reduced ($P < 0.05$) ileal viscosity in birds fed wheat sourced from Cambridgeshire but did not decrease ileal viscosity of those fed wheat from Lincolnshire and Yorkshire ($P > 0.05$) (Table 4.18. variety × site × xylanase interaction). There was no effect ($P > 0.05$) of wheat variety, growing site and xylanase addition on relative gizzard weight and gizzard contents of broilers (Table 4.19).

Table 4. 18. Influence of wheat variety, growing site and xylanase addition on ileal digesta viscosity[1] (cP) of broilers (digesta collected at day 21 of age).

	Viscosity (cP)		Xylanase	Viscosity (cP)
Variety		**Variety × Site × Xylanase**		
Lili	6.15[b]	Lili × Lincolnshire	−	5.54[c]
Barrel	6.03[b]		+	4.12[ab]
Kerrin	4.34[a]	Lili × Cambridgeshire	−	9.84[f]
SEM[2]	0.156		+	3.85[ab]
Site		Lili × Yorkshire	−	7.78[d]
Lincolnshire	4.79[a]		+	5.78[c]
Cambridgeshire	6.15[c]	Barrel × Lincolnshire	−	8.25[de]
Yorkshire	5.58[b]		+	3.42[ab]
SEM[2]	0.156	Barrel × Cambridgeshire	−	9.08[ef]
Xylanase			+	3.28[ab]
−	7.12	Barrel × Yorkshire	−	7.71[d]
+	3.89		+	4.43[b]
SEM[2]	0.127	Kerrin × Lincolnshire	−	4.28[ab]
			+	3.12[a]
		Kerrin × Cambridgeshire	−	7.60[d]
			+	3.28[a]
		Kerrin × Yorkshire	−	4.01[ab]
			+	3.74[ab]
		SEM[2]		0.382
Significance				
Variety		<0.001		
Site		<0.001		
Xylanase		<0.001		
Variety × Site		<0.001		
Variety × Xylanase		<0.001		
Site × Xylanase		<0.001		
Variety × Site × Xylanase		0.027		

[1]Each value represents mean of 6 replicates units (digesta collected from three bird from each pen at day 21 and pooled per pen).
[2]Standrad error of means.
Means within a column with no common superscripts differ significantly ($P < 0.05$).

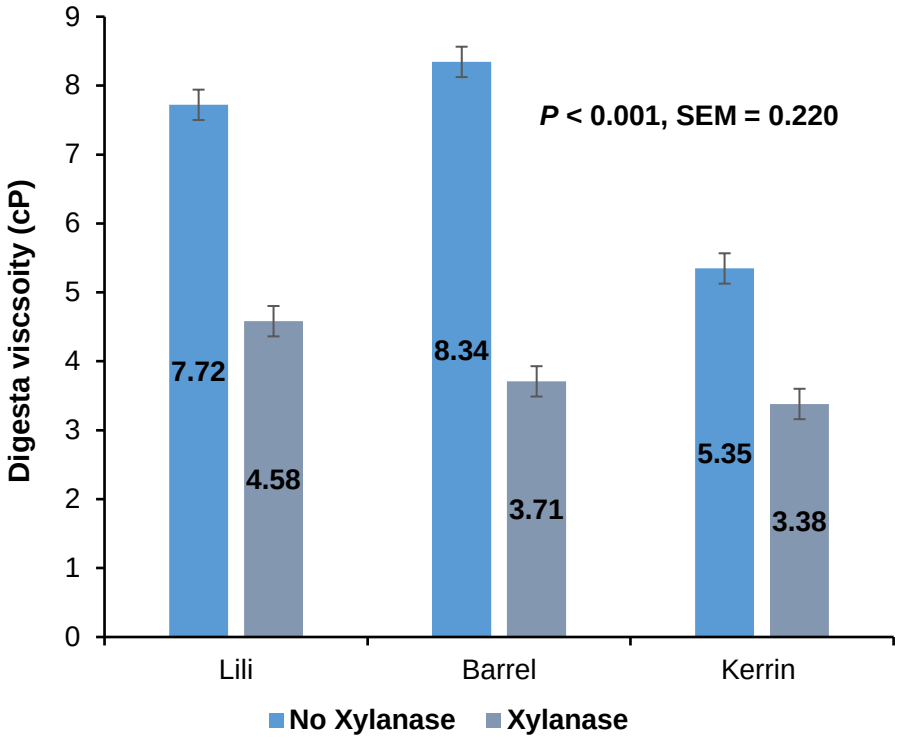


Figure 4. 1. Wheat variety × xylanase interaction on ileal digesta viscosity of broilers.

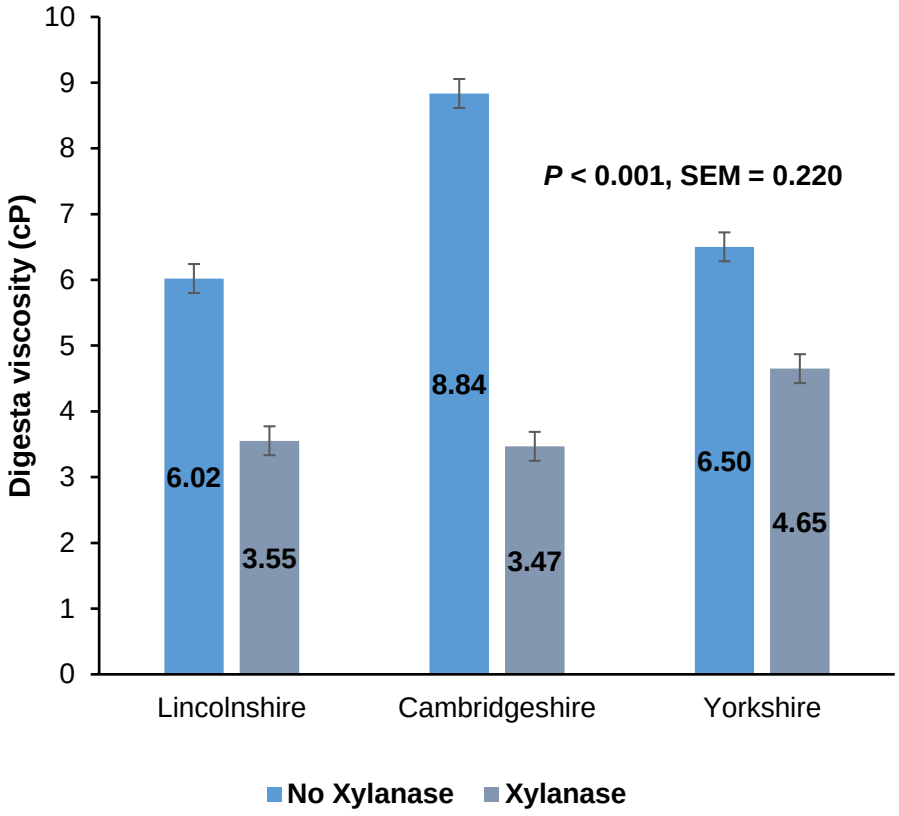


Figure 4. 2. Wheat growing site × xylanase interaction on ileal digesta viscosity of broilers.

Table 4. 19. Effect of wheat variety, growing site and xylanase addition on relative empty gizzard weight[1] and relative gizzard contents at 21 days post-hatch.

	Relative gizzard weight (g/kg BW)	Relative gizzard contents weight (g/kg BW)
Variety		
Lili	11.1	1.4
Barrel	10.6	1.4
Kerrin	10.8	1.2
SEM[2]	0.26	0.20
Site		
Lincoln	10.7	1.2
Cambridge	11.0	1.6
Yorkshire	10.9	1.3
SEM[2]	0.25	0.19
Xylanase		
−	10.8	1.3
+	10.9	1.4
SEM[2]	0.21	0.16
Significance		
Variety	0.550	0.664
Site	0.675	0.297
Xylanase	0.779	0.849
Variety × Site	0.902	0.162
Variety × Xylanase	0.942	0.833
Site × Xylanase	0.556	0.518
Variety × Site × Xylanase	0.334	0.643

[1]Values are mean of 6 experimental units containing 1 bird each, selected randomly and weighed prior to gizzard sample collection.
[2]Standard error of means (df = 81).

4.4. DISCUSSION

The novel aspect of this study was that, all three wheat varieties were grown on same locations, harvested in the same year and this provided an opportunity to investigate the effect of both variety and growing site and their interaction on nutritional value of wheat for broilers.

The AME and growth performance of broilers were influenced by the wheat variety (Wiseman and McNab 1995; Rose et al. 2001; Steenfeldt 2001; Svihus and Gullord 2002; Pirgozliev et al. 2003; Gutierrez del Alamo et al. 2008a), however, the entitled comparison of wheat varieties on growth performance and AME of broilers may have confounded by the growing location of these wheat varieties. Perhaps, the differences in nutrient composition of wheat varieties may be due to the effect of different growing location where those wheat varieties were grown. In literature, the effect of different wheat varieties grown at same location is limited and majority of the previous studies were centred around the effect of wheat varieties (grown on different locations) on growth performance and AME for broilers. Only few studies have tried to investigate the effect of wheat varieties grown on same location and how the similar varieties respond in different growing conditions (Metayer et al. 1993; Scott et al. 1998a; Wiseman 2000; McCracken et al. 2002). Therefore, the findings of this study will only be compared with the results of previous work where wheat varieties were grown at same locations.

4.4.1. Nutrient composition

Proximate composition and GE content of wheat samples were similar to a range reported in previous studies (McCracken et al. 2002; Svihus and Gullord 2002; Pirgozliev et al. 2003, 2015; Gutierrez del Alamo et al. 2008a). The absence of difference in GE content between wheat varieties was in accordance with results of McCracken and Quintin (2000). The results of this study indicated that growing site of wheat affected the nutrient composition of wheat (especially CP, GE) and tended to influence the ash content. The nutrient composition of wheat is influenced by various factors including variety and growing condition (Gutierrez del Alamo et al. 2008b). The term growing condition is referred to wheat grown at different geographical location, growing season, soil types and rainfall. Chemical composition of wheat changes from one variety to other, however, varieties do not respond in a uniform way and even within a variety, composition of nutrients can vary depending on harvest year, harvesting time, growing location (Rose et al. 2001; McCracken et al. 2002; Kim et al. 2003; Pirgozliev et al. 2003). A broiler study by Metayer et al. (1993) found that wheat samples grown in the north of France had higher CP content as compared to those in the south of France. Choct et al (1999) also demonstrated that CP content of Australian

wheat samples varied significantly between different geographical locations. In the current study, the varietal effect was only observed on crude fat content of wheat varieties and there was no effect of wheat variety on any other nutrient composition variables.

4.4.2. Polysaccharides composition

The findings from this study indicated that starch content of same wheat varieties varied between growing locations and the results were in concur with previous findings (Longstaff and McNab 1986; Metayer et al. 1993; Kim et al. 2003). The size of variability in starch content was also similar to reported variation (2.2%) in Chapter 2 studying 17 wheat samples. In the current study, all three wheat varieties grown at Yorkshire site had lower starch (3.6%) and insoluble NSP (7.2%) content as compared to the average of Lincolnshire and Cambridgeshire. The starch content of wheat varieties used in broiler diets varied considerably between growing regions and crop year. Metayer et al. (1993) found significant regional and year effect on starch content of wheat samples grown at 10 geographical regions in France. Longstaff and McNab (1986) found a significant difference of 5% in starch content of three wheat varieties grown at two locations in the UK; with those grown in the south-east part had higher starch content than those grown in the northern regions. The authors also found differences in xylose content of same wheat varieties grown at two different locations and speculated that varietal and environment changes during growing and maturation stages of wheat crop could result in, differences in monosaccharides especially AX of wheat. Kim et al. (2003) found that growing season (wheat varieties grown in two different years) influenced the starch and soluble NSP content of wheat. The authors also found a positive correlation between annual rainfall and starch content of wheat. In current study, Kerrin had significantly lower insoluble NSP content than Lili and Barrel. Insoluble NSP are attached to cell walls and inaccessible to birds during digestion process (Choct 2006). Large variability in insoluble NSP content may result in decrease in nutrient availability due to inaccessibility by enzymes and resulted in variation in growth performance (Choct et al. 1995; Bedford and Schulze 1998). Different growing conditions may affect the level of polysaccharides in wheat grain. Grain composition especially the carbohydrate content is influenced by the rainfall during different stages of growth (vegetative, growing or ripening) (Choct et al. 1999). The level of precipitation during plant growth influenced the carbohydrate composition (Kim et al. 2003). In the current study, a large CV (20.4%) was observed for soluble NSP between wheat samples but a similar range of variation (CV = 29.7%) was reported by Steenfeldt (2001), studying Danish wheat samples containing milling wheat and feed wheat. Pirgozliev et al. (2003) also reported large CV (31%) in soluble NSP content of wheat samples collected from three different years. Overall, this study demonstrated that, the polysaccharides composition of wheat crop is influenced by the growing location of wheat.

4.4.3. Physical characteristics

The determined values of SW were in range of values expected for currently available UK wheat samples (AHDB 2017) and also in accordance with reported values of SW in different studies (Wiseman 2000; McCracken et al. 2002). The effect of growing site on SW of wheat was in accordance with the findings that growing condition influenced the SW of wheat samples (Waldron 1997; McCracken et al. 2002; Pirgozliev et al. 2003; Ball et al. 2013a). A study by Metayer et al. (1993) found that growing location had an effect on SW, TGW of wheat samples grown in ten different regions in France. In their study, wheat grown in the north of France had the highest values of SW and TGW as compare to those grown in the southern part of France. The authors attributed that low values of SW and TGW could be due to rainy condition during the ripening stage of wheat crop. Wiseman (2000) also found a significant effect of growing location (five specific locations in the UK) on SW and TWG of ten wheat cultivars. In the current study, the low values of SW and TGW of wheat varieties grown at Yorkshire site may be due to difference in growing condition especially the level of rainfall during growing and ripening stage.

In the present study, the units of EH of wheats were in line to results of Carré et al. (2002) and Pirgozliev et al. (2003). Due to the fact that the use of wheats in this experiment consist of mixed pool of hard and soft milling wheats and feed wheat, therefore the differences were expected in EH values. The size of variability in HFN was due to different wheat types, however the values of HFN were in a similar range to previous reported results (Rose et al. 2001; Hetland et al. 2007). High α-amylase activity was seen in Kerrin wheat with lower HFN. The values of DV of wheat samples were in agreement with the published results (Carré et al. 2005). The main cause of variability was due to sample Barrel which gave high DV value of 4.7 cP. The high value of DV of Barrel wheat was in correspondence to its total NSP content. The absence of difference in DV between growing sites of wheat was in correspondence to no difference in soluble NSP content between growing sites.

All three wheats were winter varieties, sown in September/October and were harvested in August. Some data was collected regarding soil type of the growing locations of three varieties (Soilscapes data 2018). Lincolnshire is a slowly permeable seasonally wet soil, which impede draining and subsoil can become waterlogged. Cambridgeshire growing site is a freely draining lime rich loamy soil. In these soils, water drain through chalk or limestone. Yorkshire is freely draining lime-rich loamy soils. Lime rich soils contain chalk and limestone in large amounts. The soil description of these three growing locations clearly indicates that soil types differed substantially especially the water draining ability of these soils. The annual rainfall in 2016 in Cambridgeshire and Lincolnshire region was 687.9 mm, while in Yorkshire region it was 873.5 mm (Met office 2019). The rainfall during crop season may

have resulted in changes in nutrient composition of similar wheat varieties (Metayer et al. 1993; Choct et al. 1999). The average rainfall in 2016 in Yorkshire region was significantly higher than Cambridgeshire and Lincolnshire (72.8 vs 57.3 mm). It could be assumed that high rainfall in Yorkshire region may decrease the total starch content of all wheat varieties grown at this specific location. There may be some other unknown factors (within growing conditions) which influenced the growing location of a crop, hence, resulted in changes in nutritional composition of wheat for broilers.

Overall, the analyses of nutrient composition and physical characteristics of wheat samples in this study clearly indicated that growing site influenced the chemical composition and SW of wheat. If growing condition affects chemical composition of wheat, its nutritional value in term of animal performance and available energy is also influenced. Wiseman (1997) and Kim et al. (2004) reported a significant difference of over 1 MJ/kg in DE content of wheat fed to pigs, and the difference was due to different wheat varieties and growing condition especially precipitation level during growing season of wheat. The changes in nutrient composition of wheat caused by the growing site of similar wheat varieties may affect the nutritive value of wheat for broilers (Choct et al. 1999; McCracken et al. 2002; Kim et al. 2003).

4.4.4. Apparent metabolisable energy and nutrient utilisation of wheat

The results of this study indicated that wheat growing site but not wheat variety affected the AME of wheat for broilers. Studies on the effect of wheat cultivars and growing sites in combination on AME are scarce. Most of the findings in broilers are based on the effect of wheat cultivars on AME (Rose et al. 2001; Steenfeldt 2001; Pirgozliev et al. 2003; Gutierrez del Alamo et al. 2008a; Olukosi and Bedford 2019) as it is not always possible to obtain information on growing site of the cereals, however, this study investigated the effect of both variety and growing site of wheat on AME for broilers.

The lack of difference in AME between wheat varieties was due to the absence of difference in their nutrient composition. Metabolisable energy of a cereal grain is dependent on the energy contained, the availability of the energy to the bird, and the presence or absence of anti-nutritive factors such as soluble NSP (Scott et al. 1998a). Starch is the main source of energy in wheat-based diets, and in the present study, there was no difference in starch content between three wheat varieties; furthermore, no difference was found in CP content of varieties. All three wheats were genetically diverse varieties and it was anticipated that wheat varieties will have different CP content because of their inherent milling characteristics based on protein level and significantly different endosperm hardness. However, chemical analysis showed that all three varieties had relatively same CP content, and interestingly, their CP content was influenced by the location of wheat crop. The lack

of difference in main energy yielding nutrient (starch and protein) between three wheat varieties could be the possible reason of absence of difference in AME between wheat varieties. Longstaff and McNab (1986) reported no difference in total metabolisable energy value (TMEn) of six UK wheat varieties for broilers and attributed that the lack of differences in TMEn were due to the absence of difference in their starch content and starch digestibility. A study by Gutierrez del Alamo et al. (2008a) found significant differences in AME values of four wheat varieties, however, the differences in AME may be confounded by the effect of growing site on nutrient composition of wheat varieties as samples were sourced from various growing sites. McCracken et al. (2002) found a significant wheat variety effect on AME of wheat and concluded that highest AME values were associated with lowest soluble NSP content and lowest *in vitro* viscosity of wheat varieties. The authors suggested that although there was no difference in starch content between wheat varieties but the presence of soluble NSP content influenced the AME of wheat varieties, whereas in the current study, the reason of absence of difference in AME between wheat varieties may also be due to lack of difference in soluble NSP content between varieties.

In the present study, the significant differences in AME values between growing sites of wheat varieties were in association with the differences in starch and protein content between growing sites of wheat varieties. The low AME of wheat from Yorkshire site was associated with significantly lower starch content in wheat from Yorkshire as compared to wheats from Lincolnshire and Cambridgeshire. Wheat is known for its high starch content (60 – 70%) and starch constitutes more than half of the energy intake (Svihus 2011), therefore, variation in starch content and its availability will have a direct effect on available energy from wheat. However, the findings of the present study suggested that starch content of wheat varieties could possibly be influenced by the growing condition of the location. A study by Scott et al. (1998a) reported that growing location within a year (environment) had a significant effect on AME of wheat and barley and influenced broiler's growth performance. The authors considered that high AME wheat with low digesta viscosity could be due to low level of NSP in wheat cultivars.

Choct and Annison (1990, 1992b) found variation in AME of different wheats and proposed that variation was due to the differences in NSP content between wheat samples. The authors suggested that high NSP content reduced the availability of starch from wheat. In the present study, the high NSP content of Lili and Barrel may result in reduced availability of starch from these two wheat varieties, whereas, wheat Kerrin had relatively lower NSP and significantly lower insoluble NSP contents which may result in increased starch availability, resulting in higher AME value of Kerrin wheat.

In the present study, the absence of varietal effect on total tract nutrient retention of wheat was partially in line with Smeets et al. (2015) and Olukosi and Bedford (2019). The latter found differences in N retention and DMR but reported no difference in FD, while former

found no difference in DMR and FD in excreta of birds. A study by Gutierrez del Alamo et al. (2008a) reported differences in NR of different wheat cultivars, however difference in NR was due to only one cultivar. In the present study, FD was greatly influenced by the growing site of wheat varieties and the FD of wheat from Cambridgeshire was 4.5% greater than the average of Lincolnshire and Yorkshire. It may be assumed that wheats with higher AME values had better nutrient availability as compared to those with low AME content.

In the present experiment, the addition of xylanase increased NR 5.1%, FD 4.8% and DMR 2.8%. The increments in nutrient retention were in agreement with published results (Gutierrez del Alamo et al. 2008a; Pirgozliev et al. 2015; Olukosi and Bedford 2019). A study by Pirgozliev et al. (2015) published an increase of 2.4% and 2% in DMR and FD by xylanase addition. The interaction between xylanase and wheat variety for FD was only detected on Lili and Barrel and had no influence on Kerrin. The addition of xylanase improved FD of Lili and Barrel by 6.2% and 8.7%, respectively, which may also contribute in increment in AME content of both varieties, albeit the contribution of fat content of wheat is relatively very low in determination of AME.

The interaction between xylanase and growing site was only seen on NR of wheat, and xylanase addition only improved NR of wheats from Lincolnshire and Cambridgeshire growing sites. Interestingly, xylanase addition eliminated the difference in FD and DMR between growing sites and resulted in no interaction between growing site and xylanase, whereas, xylanase enhanced the differences in NR between growing sites. The improvement in NR of wheats from Cambridgeshire and Lincolnshire may relate to nitrogen (N) application at these growing sites. It may be anticipated that beneficial effect of xylanase on wheat may be subjected to soil types and should be carefully evaluated especially if wheat samples are grown at different locations.

In current study, overall, xylanase addition significantly improved AME of wheat variety by 0.5 MJ/kg DM. A positive effect of xylanase addition on wheat-based diets has been reported by several studies (Scott et al. 1998a, b; Choct et al. 1999; Rafuse et al. 2005; Gutierrez del Alamo et al. 2008a) and attributed to many factors including enzymes reducing ileal viscosity and enhancing accessibility of substrates by digestive enzymes. Nevertheless, in this study, the response of xylanase on three wheat varieties was not uniform and xylanase addition was unable to improve AME of the all three varieties. Instead, xylanase supplementation only improved AME of Lili and Barrel by 4.5 and 5.2%. The possible reason of this different response of xylanase to wheat could be associated to the varied level of AX in three wheat varieties. Wheat Kerrin had relatively lower amount of AX than Lili and Barrel. The amount of insoluble xylose and arabinose in Kerrin was significantly lower as compared with Lili and Barrel (Table 4.10). The AX has been found to increase the

gut viscosity in broilers when fed wheat-based diets (Choct and Annison 1990, 1992a, b). The increase in gut viscosity of broilers results in a reduction in passage rate, reduced mixing of digestive enzymes with substrate and transportation of nutrients across gut wall (Choct 1997, 2006; Bedford and Schulze 1998). The supplementation of dietary xylanase to broilers diets has been found to hydrolyse the high molecular fractions of AX into lower molecular weights and reduce the viscous nature of digesta in the broiler's gut, thus improving energy, nutrient availability and consequently growth performance (Bedford and Classen 1992; Choct et al. 1995; Bedford 2000; Adeola and Bedford 2004). In the current study, improvement in the AME of wheats Lili and Barrel was supported by significant reduction in intestinal viscosity of broilers fed Lili and Barrel wheats and their associated samples from respective growing sites, whereas, the response of xylanase to intestinal viscosity was not significant on wheat Kerrin and two of its associated growing sites (Lincolnshire and Yorkshire) (variety × site × xylanase interaction (Table 4.18). In fact, xylanase addition only reduced the ileal digesta viscosity of broilers fed wheat Kerrin grown at Cambridgeshire site. The partial response of xylanase on Kerrin and its associated growing sites could be one of the factors in reduced response of Kerrin to xylanase for increase in AME.

This study also demonstrated that xylanase response was significantly noticeable on wheats with low AME (Lili and Barrel; 13.97 and 14.09 MJ/kg DM, respectively) and there was no significant improvement in high AME wheat Kerrin (14.38 MJ/kg DM). Both Lili and Barrel varieties had relatively higher total NSP contents as compared to Kerrin. The higher insoluble AX of Lili and Barrel may also decrease the accessibility to starch. It could be assumed that in the absence of effective xylanase, higher NSP and insoluble AX contents of wheat varieties Lili and Barrel were not available to the birds and were also encapsulating the starch within endosperm. The high NSP content of these wheats resulted in low AME and when xylanase was supplemented to these wheats with higher NSP content, the response to xylanase was greater as compared to wheat Kerrin. The AX, being the main constituents of NSP contribute to the variation in AME of wheat in poultry. Starch and protein are encapsulated by the cell wall which is mainly composed of cellulose, hemicellulose, pectin and lignin (Bedford and Morgan 1996; Choct 2006). Most of the AX in wheat are insoluble in water, so not readily available to birds (Choct 2006). In the present study, the improvement in AME of Lili and Barrel by the xylanase addition may also be due to the greater reduction in ileal digesta viscosity of broilers fed these wheats in comparison to wheat Kerrin (Figure 4.1). Wheats Lili and Barrel had high digesta viscosity values; hence xylanase addition was more effective, resulting in improvement in their AME. The improvements in AME by the xylanase addition was in agreement with previous findings (Scott et al. 1998a; Gutierrez del Alamo et al. 2008a). Scott et al. (1998a) also found that

wheats with high AME values had lower digesta viscosity and their digesta viscosity was due to lower NSP content, whereas those with low AME values resulted in higher digesta viscosity caused by higher NSP content of wheat. The authors also found higher enzyme response in wheats with low AME. Gutierrez del Alamo et al. (2008a) also published that enzyme addition only improved AME of wheat cultivars which contained high NSP content and reduction in digesta viscosity was greater as compared to other those with low digesta viscosity. The response of xylanase was reported to be more effective in high intestinal viscosity, while have little or no effect on medium to low viscosity. Choct et al. (2004) reported that supplementation of enzymes reduced ileal viscosity and increased AME of wheat with lower AME. McCracken and Quintin (2000) also reported that inclusion of exogenous enzyme had no effect on AME of wheats which already contained high AME values. Another possible explanation behind the lack of response of Kerrin to xylanase may be due to low levels of substrate (NSP) for enzyme activity. Kerrin had lower insoluble NSP content, which illustrated that xylanase had less substrate to work, which may also explain the reduced response of wheat Kerrin to xylanase.

The present study was unable to reveal any effects of variety, growing site of wheat and xylanase addition on gizzard weight and its content. Gizzard size and its weight in broilers is affected by particle size, feed form (pellet vs mash) and whole wheat feeding (Ahmed et al. 2007a, b; Ahmed and Ravindran 2008, Ahmed et al. 2009). In broilers, mash diets resulted in a well-developed and heavier gizzard which was reported due to large particle size in mash diets (2007a). In contrary, Nir et al. (1994, 1995) and Ahmed et al. (2007b) revealed that pellet diets resulted in lower relative gizzard weights and digestive tract organs in broilers and they attributed that lower weights of digestive tract were due the fact that pelleting may neutralise the particle size effect. It was suggested by Ahmed et al. (2007b) that pelleting reduces the grinding requirements of the gizzard, which consequently makes it as a transit organ. In broilers, pellet diets are often supplemented with whole wheat to stimulate gizzard development and its function (Wu and Ravindran 2004; Ravindran et al. 2006). Whole wheat is added at the mill after pelting or at the farm. In this study, the possible explanation of the lack of variety and growing site effect may be due to feeding pelleted diets to broilers. The lack of xylanase response on gizzard weight and its content in broilers may associate with absence of any significant effect of variety or growing site on gizzard weight and its content. The absence of xylanase response was in agreement to earlier work by several authors (Brenes et al. 1993; Wu and Ravindran 2004; Gao et al. 2008; Masey O'Neill et al. 2014; Olukosi et al. 2019).

4.4.5. Broiler growth performance

The main findings of this study showed that wheat growing site has a prominent effect on most of the broiler growth performance variables including FI, WG and FBW in both grower and finisher phase. The growth rate of broilers is affected by voluntary feed intake, nutrient composition of the diet and anti-nutritional factors in the diet. The effect of wheat variety was only noticed on FI of birds in both grower and finisher phases as well as in overall performance period. There was no effect of variety on WG of chickens and FCR in any of the phases. The effect of wheat variety on FI was in agreement with the findings of Olukosi and Bedford (2019), where authors found significant differences in FI between four different UK varieties between 0 – 21 days of age. In the present study, the lack of differences in WG and FCR during growing phase between wheat varieties were contradictory to work of Gutierrez del Alamo et al. (2008a). The authors reported differences in WG and FCR of broilers fed different wheat cultivars in the growing phase, but this study only indicated difference in FI of broilers. Differences in FI of chickens between wheat varieties were unable to pass on to growth rate as there was no association of FI with WG and FCR. The size of differences in FI between wheat varieties were small (3%), considering published high values of differences in FI in previous broilers studies (McCracken et al. 2001; Rose et al. 2001; Steenfeldt 2001). Perhaps, the small differences in FI between varieties were the reasons of not converting into differences in WG. Another possible reason may be due to the lack of differences in starch and protein content between wheat varieties. Wheat contains high proportion of starch and protein; so, the variability in these main components of wheat samples resulted in variation in AME and growth performance (McCracken and Quintin, 2000, Pirgozliev et al. 2003). The lack of difference in growth rate of birds between wheat varieties was also supported by the absence of differences in AME and AMEn between wheat varieties. In the finisher phase, similar pattern of wheat variety effect was observed. The absence of varietal effect on WG and FCR in the finisher phase was in line with results of Gutierrez del Alamo et al. (2008a). The authors reported no effect of wheat cultivars on daily WG and FCR of broilers fed in the finisher phase. In the present study, it seemed that once the absence of wheat variety was established in the earlier broiler growth phases, it carried on to the later growth stages. The results also showed that birds fed diet containing soft wheat had high FI as compared to hard wheat. Previously, the response of broilers to different wheat types is not consistent (Rose et al. 2001; Amerah et al. 2009b). The purpose of this study was not to investigate the effect of wheat hardness on growth performance, instead it was designed to examine the effect of wheat variety and growing sites, however the findings were related to the available literature of wheat with different EH and its effect on growth performance.

Interestingly, the effect of wheat growing site was prominent in all phases of broiler's growth and influenced FI and WG significantly. Growing site is affected by various growing conditions, including growing seasons (temperature, rainfall, sunshine), soil types, N application and husbandry practices (Kim et al. 2003; Gutierrez-Alamo et al. 2008b). In the present study, wheat samples from Yorkshire had higher annual rainfall compared to other two growing site and their lower starch content may be due to high rainfall throughout the crop growth and particularly higher rainfall in the harvest month of August (83.5 vs 47.3mm). Rose et al. (2001) reported a significant difference in feed intake and weight gain when broilers were fed the same wheat cultivars grown in three different years. The authors reported a decrease in FI and WG of chickens fed on wheat grown in a year with a high rainfall during harvesting. In their study, starch content of wheat samples exposed to high rainfall during harvesting were lower as compared to wheat harvested with low rainfall. A study by Ball et al. (2013b) attributed that environmental factor (level of N fertiliser) during wheat growth can result in improvement in bird performance. The results from this study suggested that growing condition of wheat crop may be influenced by rainfall at the particular site and agronomic factors also influenced the soil. The growing condition varies from site to site and influence the nutrient composition of wheat for broilers.

In the present study, the overall effect of xylanase was significantly noticeable on growth performance variables in all phases of growth. The inclusion of xylanase reduced FI and WG in the grower and finisher phases but improved FCR in grower phase and overall period. The improvement in FCR may suggest that the bird's feed intake requirements were compensated by xylanase addition. Overall, xylanase supplementation reduced FI and WG of chickens but absence of interaction between xylanase and variety or growing site in grower phase indicated that addition of xylanase could not eliminate the differences between wheat variety and growing site. In this study, studied wheat samples were consists of milling wheats both hard (Lili) and soft (Barrel) and feed wheat (Kerrin). Broiler response to xylanase supplementation in previous studies were not conclusive, especially if wheat samples used in broilers diet were consists of hard and soft wheat (Hetland et al. 2007; Amerah et al. 2009b). The absence of interaction between wheat variety and xylanase in all phases was supported by previous studies (McCracken and Quintin 2000; Gutierrez del Alamo et al. 2008a; Olukosi and Bedford 2019). Xylanase supplementation to wheat-based diets often demonstrate improvements in AME, however, do not always improve growth rate of broilers as revealed in this study. The findings suggest that the addition of xylanase should not be assumed to reduce variability in growth rates of broilers fed different wheats.

In this study, xylanase addition to wheat from the growing site Cambridgeshire responded with a 14.7% and 10% reduction in FI during finisher phase and overall period, respectively. The possible reason of greater response of wheat from Cambridgeshire may be due to higher NSP content of wheat from Cambridgeshire site as compared to other two growing sites. The significant response of wheat from Cambridgeshire to xylanase was also supported by the greatest reduction in ileal digesta viscosity of all three varieties grown at Cambridgeshire site (Figure 4.2). The greatest reduction in ileal viscosity of broilers fed wheat from Cambridgeshire explained an increased response of xylanase due to the presence of higher level of NSP in the Cambridgeshire wheat. The response of xylanase is in reciprocal to concentration of NSP in wheat samples (Choct et al. 1999). In the present study, different responses of xylanase to growing site of wheat revealed that efficiency of xylanase was influenced by the NSP content of wheat and growing condition of cereal crop affects the chemical composition. It is noteworthy that the interaction between wheat growing site and xylanase was only observed for FI in the finisher phase and overall period. There was no interaction of xylanase with growing site for WG and FCR. The xylanase response to wheat from Cambridgeshire for FI was associated with its higher NSP content and unable to response to those wheats with lower NSP content. Although, reduction in FI was significant but could not convert into any significant reduction in WG for Cambridgeshire wheat. These finding clearly suggest that xylanase response on FI of broilers fed wheat from different growing sites is not uniform and care should be taken adding xylanase to wheat grown at different locations.

In the current study, the addition of xylanase improved FCR by 3 points in grower phase, however, in finisher phase, xylanase supplementation did not improve FCR, which may be due to higher maintenance requirements by the birds in later stages of bird's growth. Overall, birds fed diet supplemented with xylanase had lower FI (4.4%) and WG (3.6%) as compared to those fed diets without xylanase addition. Although, xylanase addition did not improve FI and WG of broilers but improved FCR by two points. The improvements in FCR could be due to better nutrient availability and indicated that birds were able to utilise dietary nutrient efficiently in the presence of supplementary xylanase. The study also demonstrated that the improved FCR in the grower phase was carried to the later stages of broiler growth. Another possible reason for improvement in FCR by xylanase addition could be due to less decrease in WG of broilers in comparison to relatively greater reduction in FI. The improvement of two to three points in FCR is commercially significant for economical broiler production and evidenced the beneficial effect of xylanase addition in wheat-based diet.

4.5. CONCLUSIONS

This study suggested that growing site of wheat had a significant effect on AME of wheat for broilers and variations in AME were due to differences in polysaccharides composition between growing sites. The study shows that differences in crop growth has a significant effect on nutrient composition of wheat for broilers. The three wheat varieties used in this study were genetically diverse varieties and consist of milling (bread), soft (biscuit) and feed wheats. Although, wheat varieties were genetically different in their chemical and physical characteristics but did not demonstrate any differences in their important nutrients (starch and protein) and had no differences in their nutritional value for broilers. Contrary, this study concluded that growing location of wheat appears to be the biggest source of variability in nutritional value of wheat for broilers. The growing location is influenced by various factors during the growth of a crop e.g., rainfall, temperature, soil types, solar radiation, use of N fertiliser, lodging, disease incidence and crop husbandry. Future research may consider investigating these growing conditions and which factors in the growing condition affect the nutritional and chemical composition of wheat for broilers.

This study also concluded that growth rate of broilers was influenced by the growing site of wheat but not the wheat variety. The differences in growth rate were due to differences in the nutrient supply of same wheat varieties sourced from different growing site. The study also indicated that the supplementation of xylanase should be carefully considered if wheat samples used in the broilers diets consists of varieties collected from different locations. The findings proposed that wheat with high AME values were not responding to xylanase addition as efficiently as low AME wheats. Therefore, xylanase addition to a batch of wheat with mixed wheat samples should be carefully considered as may result in variable responses in broilers. The present study also revealed that xylanase response in wheat-based diets to improve AME is related to polysaccharides composition especially the level of arabinoxylans of wheat.

CHAPTER 5: THE RATE OF STARCH DIGESTION IN BROILERS

5.1. INTRODUCTION

The results of study one (Chapter 3) confirmed significant differences in growth rate of broilers when fed different wheat samples but differences were not related to proximate composition and nutrient utilisation of wheat samples. The findings from second study (Chapter 4) suggested a significant growing site effect on growth rate of broilers but was not explained by the nutrient utilisation. The differences in growth rate of broilers were affected by the differences in voluntary feed intake between wheat samples. It could be assumed that differences in feed intake may be associated with the differences in the rate of nutrient utilisation.

Starch is the main energy- yielding component in wheat and understanding of its digestion characteristics is important for better utilisation of wheat in broiler chickens (Wiseman 2000; Liu and Selle 2015; Zaefarian et al. 2015). Diets with similar amount of digestible nutrients, but differences in digestion kinetics, may result in differences in growth performance. Differences in site of starch digestion may have metabolic consequences which can affect feed utilisation. Previously, starch digestion rates in broilers have been found to be different between wheat cultivars (Gutierrez del Alamo et al. 2009a, b), however, the effect of these differences on growth performance were not fully investigated. Liu et al. (2013c) found significant correlation between rate of starch digestion and weight gain of birds studying three sorghum varieties and reported a tendency between rate of starch digestion and FCR. The limited research on kinetics of starch digestion in broilers fed wheat samples and its effect on bird's performance require further investigation to fully understand this major component of wheat and its rate of utilisation.

In broilers, the determination of *in vivo* rate of starch digestion using a large number of wheat samples are technically difficult because of several complexities of animal trials, e.g., variation between animals, time consuming, techniques to collect digesta from small intestine of chickens, a large number of digesta sample collection from different segments of small intestine and various laboratory analyses afterward. Therefore, the current work was considered a preliminary study, to identify two wheat samples which gave differences in growth performance of broilers fed practical wheat-based diets and examine if any difference exists in the *in vivo* rate of starch digestion between wheat samples.

This study has two parts:

(Part A) To identify two wheat samples with differences in growth performance of broilers.

(Part B) To investigate if there is any difference in the *in vivo* rate of starch digestion between these two wheat samples.

PART (A): GROWTH PERFORMANCE OF BROILERS FED EIGHT WHEAT SAMPLES

The objective of this study was to investigate differences in growth performance of broilers fed eight currently available the UK wheat samples.

5.2. MATERIALS AND METHODS

5.2.1. Wheat samples

Eight current wheat samples were collected from different growing sites across the UK (Table 5.1). Wheat samples were freshly sourced for this study because sufficient quantities of wheats were not available from the previous two studies. The samples consist of current listed varieties on AHDB recommended list 2017/18 (AHDB 2017) and comprise of milling wheats (soft, hard) and feed wheat except sample IW, where no variety information was available. Samples were stored in a dry place at ambient temperature until analyses and commencement of bird's trial. Each sample was mixed homogenously for 10 min, and random samples were collected for analyses. Around 300 g of each wheat sample was milled to pass through a 0.75 mm screen using a rotor mill, Retsch ZM 200 (Retsch GmbH, Haan, Germany) and used for chemical and physical analyses.

Table 5. 1. List of experimental wheat samples.

Sample ID	Wheat description	Source
IW	Unknown	Northern Ireland
LILI	Lili	Lancashire
BC	Barrel	Cambridgeshire
SC	Santiago	Cambridgeshire
DW	Diego	Warrington
SD	Siskin	Ormskirk
HAR	Mixed	Harper Adams
TW	Lili	Shropshire

Samples were listed as current wheat varieties on AHDB recommended list 2017/18.

5.2.2. Chemical composition and physical characteristics

The samples were subjected to proximate analyses and physical characteristics as described in Chapter 2 (Section 2.2.2.1, 2.2.3). Polysaccharides composition wheat samples were determined as described in Chapter 2 (Section 2.2.2.2). All proximate and polysaccharides analyses of wheat samples were carried out in duplicate and results were expressed on a DM basis. Physical characteristics of eight wheat samples were determined in triplicate.

5.2.3. Diet formulation, energy and protein balance, pelleting, pellet durability test

Each wheat sample was milled separately in a hammer mill to pass through a 3 mm screen and then mixed with a grower balancer feed (Target Feeds Ltd, Whitchurch, UK). Eight broiler grower diets were formulated by mixing 670 g/kg of each wheat sample and 330 g/kg of a grower balancer. The main ingredients were wheat 670 g/kg, soybean meal 172 g/kg, full fat soya 99 g/kg and 17 g/kg of soya oil (Table 5.2). Each experimental diet met or exceeded the current diet specification for Ross 308 broiler chicken (Aviagen 2014a). Diets were free of coccidiostats, antimicrobial growth promoters or other similar additives.

Diets were made isonitrogenous by adding wheat protein isolate (WPI) (Life Source Foods, USA), by substituting wheat with WPI. The WPI was sourced from the same supplier as used in study 1 (Chapter 3) and study 2 (Chapter 4). The additional quantity of WPI to be added was calculated on analysed protein value of each wheat sample on *as fed basis*. To balance the total quantity of each diet, maize starch was added to have the same amount (kg) of each diet. For example, a mixture of wheat protein isolate (WPI) and maize starch combined weight of 10.76 g/kg was added to each diet. The additional quantity of WPI was calculated based on 67% inclusion of wheat. Each diet was pelleted as described in Chapter 3 (section 3.2.2.2). Grower pellet diameter and length were 3 mm and 4 – 7 mm, respectively.

Table 5. 2. Ingredient composition (g/kg, as-fed) and determined analysis of balancer and broiler grower diets.

Ingredients	**Balancer**	**Diet**
Wheat	–	670
Soybean meal (48)	522.7	172.5
Full-fat soybean meal	300.0	99.0
Soya oil	51.5	17.0
Monocalcium phosphate	39.4	13.0
Limestone	42.4	14.0
NaCl	9.1	3.0
L-Lysine HCL	6.1	2.0
L Threonine	6.1	2.0
DL Methionine	10.6	3.5
Vitamin mineral premix[1]	12.1	4.0
Titanium Dioxide[2]	15.0	5.0
Total	1015	1005
Calculated analysis (as fed basis)		
CP (g/kg)	368.0	198.9
ME (MJ/kg)	11.73	12.77
Crude fat (g/kg)	113.1	49.5
Ca (g/kg)	28.2	9.7
Available P (g/kg)	10.4	4.2
Lysine (g/kg)	27.7	11.5
Methionine	15.4	6.2
Threonine	20.1	8.7
Methionine + Cysteine (g/kg)	20.7	9.3
Tryptophan (g/kg)	4.5	2.3
Analysed values (as fed)[3]		
DM (g/kg)	910	878-897
CP (N x 6.25) (g/kg)	357	196-203

Each experimental diet met or exceeded the current diet specification for Ross 308 broilers (Aviagen 2014a).

[1]The vitamin and mineral premix contained vitamins and trace elements to meet the breeder's recommendations (Aviagen Ltd. Edinburgh, UK). The premix provided (units/kg diet): retinol 13500 IU; cholecalciferol 5000 IU; α-tocopherol 60 IU; menadione 3 mg; thiamine 2 mg; riboflavin 10 mg; pyridoxine 5 mg; cobalamin 15 µg; nicotinic acid 60 mg; pantothenic acid 15 mg; folic acid 1.5 mg; biotin 200 µg; 80 mg Fe as iron sulphate (30%); 10 mg Cu as copper sulphate (25%); 100 mg Mn as manganous oxide (62%); 80 mg Zn as zinc oxide (72%); 1 mg I as calcium iodate (52%); 0.20 mg Se as sodium selenite (4.5%) and 0.5 mg Mo as sodium molybdate (40%).

[2]Titanium Dioxide was added on top in balancer diet @ 15 g/kg.

[3]Analyses were performed in duplicate.

5.2.4. Bird husbandry and experimental design

All procedures were approved by Harper Adams University Research Ethics Committee (Project no. 0023-201801-PGMPHD). A total of four hundred and fifty (450) day old male Ross 308 chicks were obtained from a commercial hatchery (Cyril Bason Ltd, Craven Arms, UK). Chicks were inspected on arrival and separated any unhealthy birds. Birds were kept on a single floor covered with wood shavings and fed *ad libitum* a commercial starter diet that met the nutrient specifications of broilers up to 7 days of age (Aviagen 2014a). Commercial starter diet had the same formulation as used in study 2 (Section 4.2.4, Table 4.6). At d 7 (the first day of experiment), chicks were weighed individually and four hundred (400) of them with similar body weights near or above 7 days age (189 ± 5 g) were selected for the experiment. Five chicks with similar weights were allocated randomly to each of forty (40) raised floor pens with 0.60 × 0.60m solid floor area. Each pen was equipped with a feeding trough outside and an automatic drinker inside the pen. The floor of each pen was covered with wood shavings. Each of the eight wheat-based experimental diets was assigned to ten (10) pens within blocks following randomisation. Birds were fed experimental wheat-based grower diets in pellet form with a pellet diameter 3 mm (4 – 7 mm length) up to day 21. Feed and water were provided *ab libitum* throughout the experimental period. Standard lighting and temperature programme were followed (Aviagen Ross broiler management handbook). The birds were inspected daily, and any dead birds were removed. Date of the death and body weights of dead birds was recorded at the time of removal. The FI and body weights were recorded from d 7 until the end of experiment at d 21 and FCR was calculated on a pen weight basis. The body weights of dead birds were adjusted for to calculate FCR.

5.2.5. Statistical analysis

Statistical analyses were performed in GenStat (GenStat 18th edition, supplied by VSN international Ltd, UK). Broiler growth performance was compared statistically by one-way General Analysis of Variance (ANOVA) in a randomised complete block design, with a single pen representing experimental unit (replicate). The variables that described growth performance were FI g/b/d, WG g/b/d, FBW kg/b at day 21, FCR corrected for mortality (g of FI / g of WG). Body weights at day seven were used as co-variate for performance data (FI, WG, FBW, and FCR). Differences were reported as significant at a *P* value of equal to or less than 0.05 and *P* value between 0.05 and 0.1 was considered as a trend. If a significant difference ($P < 0.05$) for the main effects were found, means were separated using Duncan's multiple range test and differences were reported significant at $P < 0.05$.

5.3. RESULTS

5.3.1. Chemical composition of eight wheat samples

The GE and proximate analysis of eight wheat samples are shown in Table 5.3. The DM content of wheat samples ranged from 874 to 899 g/kg (CV = 1.0%) with a mean DM 883 g/kg. The CP content (N × 6.25) ranged from 114 to 130 g/kg DM (CV = 4.8%) with a mean value of 123 g/kg DM. The GE content of wheat samples ranged from 18.04 to 18.23 MJ/kg DM (CV = 0.4%) with a mean value of GE 18.15 MJ/kg DM. The ash content of wheat samples ranged from 14.0 to 19.6 g/kg DM (CV= 11.1%) and mean ash content was 17.3 g/kg DM. Crude oil content (as ether extract) of wheat samples ranged from of 9.8 to 14.5 g/kg DM (CV = 15.5%). The mean oil content of wheat samples was 11.8 g/kg DM.

Table 5. 3. Proximate composition and GE content of eight wheat samples (g/kg DM).

Sample ID	Wheat	Site	DM[1]	CP[2]	GE[3]	Ash	Oil
IW	Unknown	Northern Ireland	899	126	18.17	17.4	9.8
LILI	Lili	Lancashire	874	127	18.04	14.0	11.1
BC	Barrel	Cambridgeshire	877	114	18.23	14.8	10.1
SC	Santiago	Cambridgeshire	886	122	18.05	17.4	13.4
DW	Diego	Warrington	879	124	18.16	17.9	11.5
SD	Siskin	Ormskirk	892	114	18.15	18.6	10.3
HAR	Mixed	Harper Adams	879	130	18.19	19.6	14.5
TW	Lili	Shropshire	880	126	18.19	18.5	13.8
Mean			883	123	18.15	17.3	11.8
Mini			874	114	18.04	14.0	9.8
Max			899	130	18.23	19.6	14.5
CV%			1.0	4.8	0.4	11.1	15.5

[1] Dry Matter (g/kg), [2]Crude protein (N × 6.25), [3]Gross energy MJ/kg DM.
Each analysis was performed in duplicate.

The polysaccharides composition of wheat samples is summarised in Table 5.4. The mean starch, total NSP, soluble NSP and insoluble NSP contents were 668, 95.7, 28.9, 66.8 g/kg DM, respectively. The amount of starch in wheat samples ranged from 651 to 687 g/kg DM (CV= 1.9%). The total NSP content varied from 84.2 to 106.8 g/kg DM (CV= 7.2%). The soluble NSP content ranged from 15.1 to 37.9 g/kg DM (CV= 24.0%). The amount of insoluble NSP content ranged from 59.6 to 73.3 g/kg DM (CV= 6.6%).

Table 5. 4. Polysaccharides composition of eight wheat samples (g/kg DM).

Sample ID	Wheat	Site	Starch	NSP total	NSP soluble	NSP insoluble
IW	Unknown	Northern Ireland	674	106.8	33.5	73.3
LILI	Lili	Lancashire	676	93.0	23.2	69.7
BC	Barrel	Cambridgeshire	687	93.9	29.6	64.4
SC	Santiago	Cambridgeshire	677	90.2	30.6	59.6
DW	Diego	Warrington	663	84.2	15.1	69.2
SD	Siskin	Ormskirk	663	98.4	31.2	67.2
HAR	Mixed	Harper Adams	651	100.3	37.9	62.4
TW	Lili	Shropshire	654	98.9	30.0	69.0
Mean			668	95.7	28.9	66.8
Mini			651	84.2	15.1	59.6
Max			687	106.8	37.9	73.3
CV%			1.9	7.2	24.0	6.6

NSP = Non-starch polysaccharides.
*Each analysis was performed in duplicate.

5.3.2. Physical characteristics of eight wheat samples

Physical characteristics of wheat samples are presented in Table 5.5. The relative units of EH of wheat samples ranged from 27 to 71 (CV = 31.5%). The SW of wheat samples ranged from 75.7 to 82.3 kg/hl (CV= 3.0%), and mean SW samples was 79.4 kg/hl. The TGW of wheat samples ranged from 39.5 to 58.5 g (CV = 14.0%).

Table 5. 5. Physical characteristics of eight wheat samples.

Sample ID	Wheat	Site	EH	SW	TGW
IW	Unknown	Northern Ireland	31	79.8	39.8
LILI	Lili	Lancashire	52	81.6	52.0
BC	Barrel	Cambridgeshire	27	82.3	58.5
SC	Santiago	Cambridgeshire	71	81.6	51.2
DW	Diego	Warrington	68	78.3	46.3
SD	Siskin	Ormskirk	53	75.7	45.2
HAR	Mixed	Harper Adams	47	79.0	53.7
TW	Lili	Shropshire	64	76.9	39.5
Mean			52	79.4	48.3
Mini			27	75.7	39.5
Max			71	82.3	58.5
CV%			31.5	3.0	14.0

EH = endosperm hardness (relative units 0 –120), SW = specific weight (kg/hl),
TGW = thousand grain weight (g).
*Each analysis was performed in triplicate.

5.3.3. Broiler growth performance

Although, broilers fed wheat sample IW had the highest FI and WG, however, sample consist of mixed grains and no information was available regarding wheat variety, therefore results obtained by sample IW were excluded from further testing. The daily FI of chicken fed diet based on wheat DW was greater ($P < 0.05$) by 11.2% as compared to those fed wheat sample Lili (Table 5.6). Similarly, the WG of broilers fed wheat sample DW was greater ($P < 0.05$) by 12.4% as compared to those fed wheat sample Lili. The FBW of broilers fed wheat DW was greater ($P < 0.05$) by 9.6% than those fed wheat Lili. There were differences ($P < 0.05$) in FCR between individual wheat samples.

Table 5. 6. Growth performance[1] of broilers fed eight different wheat samples (7 – 21 days of age).

Wheat	FI (g/b/d DM)	WG (g/b/d)	FBW (kg)	FCR (g: g)
IW	69.6^{c}	53.4^{c}	0.934^{c}	1.305^{bc}
LILI	61.4^{a}	46.7^{a}	0.841^{a}	1.316^{c}
BC	62.7^{ab}	49.1^{ab}	0.874^{ab}	1.277^{ab}
SC	67.1^{bc}	51.4^{bc}	0.907^{bc}	1.303^{bc}
DW	68.3^{c}	52.5^{bc}	0.922^{bc}	1.301^{abc}
SD	64.9^{abc}	50.1^{abc}	0.888^{abc}	1.296^{abc}
HAR	66.9^{bc}	52.7^{bc}	0.924^{bc}	1.270^{a}
TW	67.1^{bc}	52.2^{bc}	0.917^{bc}	1.286^{abc}
Mean	66.0	51.0	0.901	1.294
SEM[2]	1.60	1.27	0.0177	0.0102
P value	0.007	0.006	0.006	0.034

[1]Each value represents mean of 10 experimental units per treatment. Each experimental unit was a pen containing 5 birds each.
[2]Standard error of means (df = 61).
Means within a column with no common superscript differ significantly ($P < 0.05$).
FI: feed intake (g/b/d DM), WG: weight gain (g/b/d), FBW: final body weight (kg/b) at d 21, FCR: feed conversion ratio (g/g DM).

5.4. DISCUSSION

5.4.1. Chemical composition and physical characteristics

Proximate nutrients of wheat samples were similar to a range determined in other studies (McCracken et al. 2002; Pirgozliev et al. 2003; Gutierrez del Alamo et al. 2009a; Yegani et al. 2013). The CP content of wheat samples were less variable (CV = 4.8%) between eight samples and within a range similar to reported by Pirgozliev et al. (2003) and Gutierrez del Alamo et al. (2009a). The ash and oil contents were variable (CV = 11.1, 15.5%, respectively) between wheat samples; however, the values of both ash and oil were in a similar range as reported by the earlier studies (Steenfeldt 2001; McCracken et al. 2002; Pirgozliev et al. 2015a). The GE content was less variable between samples and in line with results of Pirgozliev et al. (2015a). The results of previous studies (Chapter 2 and 4) also indicated less variability in GE of wheat samples.

The starch content of wheat samples was in line to the results of Waldron (1997); McCracken and Quintin (2000); Carré et al. (2005). The size of variability in starch content (1.9%) of wheat samples was also similar to determined variation (2.2%) in chapter 4 studying nine wheat samples. The amount of total NSP was within the range and in agreement with previous studies on wheat (Steenfeldt 2001; Choct 2006, Gutierrez del Alamo et. 2008a). The variability in total NSP contents was relatively low (7.2%). The moderate variability (CV = 6.6%) in insoluble NSP content was in line with published data of Steenfeldt (2001). The variability in insoluble NSP content of UK wheats studied in chapter 2 and 4 was 5.3 and 7%, respectively. Large variation (CV = 24%) was observed for soluble NSP but in line with a similar range of variation reported by Steenfeldt (2001), studying Danish wheat samples containing milling wheat and feed wheat. Pirgozliev et al. (2003) also reported large variation (CV = 31%) in soluble NSP of wheat samples collected from three different years. In this study, the size of variability in soluble NSP content was also in agreement with variation observed in current UK wheat samples studied in previous studies (Chapter 2, 4).

The relative units of EH were in line with Pirgozliev et al. (2003); Carré et al. (2005). The wheat samples consist of soft and hard wheats, therefore a range of EH values were expected. The values of SW were less variable and variability between samples was in accord with findings of Wiseman (2000) and Ball et al. (2013a). The size of variability in SW in this experiment was also in line with results of 17 wheat samples studied earlier (Chapter 2). The TGW were similar to reported values by previous studies on wheat by Pirgozliev et al. (2003) and McCracken et al. (2002). Overall, in this study, wheat samples had less variability in starch, CP and physical characteristics and the size of variability was within the range of reported values by several previous findings.

5.4.2. Broiler growth performance

The main objective of growth trial was to determine the difference in growth performance of broilers when fed current UK wheat samples and findings confirmed significant difference in growth performance (FI, WG and FCR). The results indicated that broilers fed sample IW had the highest FI and WG, however, sample IW consisted of mixed grains from different wheats and there was no information about wheat variety. Due to the fact that sample IW contained mixed type grains and quality of wheat grains was inconsistent in comparison to other wheat samples. It was anticipated that broiler growth results could be compromised and not representative of wheat samples used in this study. Therefore, results attained by the sample IW were rejected and sample DW was chosen as with the highest FI and WG. In the current study, a large difference of 11.2 and 12.4% in daily FI and daily WG of broilers was observed between sample DW and Lili, respectively. The size of difference in FI and WG of broiler chickens fed both samples was identical to findings of Waldron (1997), Steenfeldt (2001) and Gutierrez del Alamo et al. (2008a), where almost similar inclusion (65%) of different wheat cultivars was incorporated in grower diet formulation. This size of difference in FI and WG is economically significant for commercial broiler production. Broilers fed wheat samples with higher FI and growth rate, can reach target live weight earlier than those fed on wheat resulted in over 11% less FI and WG. The higher FI and WG in broilers results in shorter production cycle and more flocks per year. It is pertinent to achieve target weight at desired age or earlier for efficient broiler production. This would help to predict target weight of chickens accurately and planning for future crops. The values of daily FI and WG were slightly lower than recommended Aviagen standards for Ross 308 male broilers (75 and 55 g, respectively) (Aviagen 2014b), however it was acceptable. The lower FI and WG could be due to limitations of experimental conditions e.g., rearing in pen, bird's density, chick quality and sample collection during trial, whereas, large scale broiler production has no such conditions. Interestingly, both wheat samples DW and Lili were similar in proximate composition and physical characteristics. The CP content of both Lili and DW was almost similar (127, 124 g/kg DM, respectively), whereas the starch content of both wheat samples was in a close range (676, 663 g/kg DM, respectively), however, these two samples resulted in large significant differences in FI and WG of broilers. There was also less variation in physical characteristics of these wheats (SW and TGW). The aim of this simple growth study was to investigate differences in growth performance of broilers when fed different wheat samples and results indicated large differences (11 – 12%) in FI and WG of broilers between two wheat samples which were similar in their key energy yielding nutrient (starch and protein) and also had similar physical characteristics. Additionally, diets were pelleted under similar condition, were formulated to be iso-nitrogenous, yet found large differences in growth performance of broilers. The differences

in growth performance between wheat samples were not related to their nutrient composition. It could be speculated that differences in the rate of nutrient assimilation in small intestine of chickens may possibly result in such a large difference in growth performance.

5.5. CONCLUSIONS

The study concluded that wheat samples with similar proximate composition and starch content resulted in large differences in FI and growth rate of broilers. Both samples were also similar in their physical characteristics. The diets were formulated to be iso-nitrogenous and pelleted under similar condition yet results in large differences in FI and growth rate of broilers. Previously, differences in growth performance were not related to proximate nutrients, chemical composition of wheat and nutrients availability (Chapter 3). It was anticipated that perhaps the rate of nutrient utilisation in the small intestine could be the possible explanation of such a large difference in FI and WG. To test this, it was suggested to determine the rate of *in vivo* starch digestion between these two samples. The sample with highest FI and WG (DW) and lowest FI and WG (Lili) were selected for part B of this study, where *in vivo* rate of starch digestion between these samples was determined.

PART (B): THE RATE OF STARCH DIGESTION IN BROILERS FED TWO WHEAT SAMPLES

5.1. INTRODUCTION

The results from part A of this study demonstrated that broilers fed diet containing wheat DW had the highest FI and WG (68.3 g/b/d DM, 52.5 g/b/d, respectively), whereas, wheat samples Lili resulted in the lowest FI and WG (61.4 g/b/d DM, 46.7 g/b/d, respectively). Both wheat samples were similar in nutrient composition and physical characteristics but resulted in significant differences in FI and WG of chickens. The objective of this study (Part B) was to determine the rate of starch digestion in broilers fed two wheat samples (with similar nutrient composition) which previously differed in FI and WG of broiler chickens.

5.2. MATERIALS AND METHODS

5.2.1. Diet formulation, enzyme addition, pelleting

Both DW and Lili wheat samples were chosen to formulate broiler grower diets. The composition of each diet was similar to the diets used in part A (Section 5.2.3) and contained 670 g/kg wheat and 330 g/kg of a balancer. Wheat samples were mixed with the same balancer used in the growth experiment (part A). Each diet was supplemented with TiO_2 @ 5 g/kg of the diet on top as an external marker. Titanium dioxide (TiO_2) was added in the balancer diet @ 15 g/kg and then balancer was mixed with 67% of wheat. Both diets were made isonitrogenous as described earlier (Section 5.2.3).

The diets were split into two parts and half of the diets were supplemented with xylanase (Econase XT 25, AB Vista feed ingredients, Marlborough, UK, while the other half diets were without xylanase addition (Table 5.7). Xylanase was initially mixed in the balancer diet at 0.3 g/kg for 30 min and then diluted to 0.1 g/kg of the diet by mixing 67% of wheat as described in Chapter 4 (Section 4.2.3). In total, four (4) broiler grower diets were formulated; two (2) diets contained 67% of wheat with xylanase, and other two (2) diets contained 67% of wheat without xylanase. All four diets were pelleted as described in section 3.2.2.2. The frequency of pelleter was 50Hz, whilst, speed of feeder was maintained at 300 rpm during the whole pelleting process. The temperature of jacket was maintained between 59.5 to 62°C. The diameter and length of pellets were 3 mm and 4 – 7 mm, respectively. The DM and PDI of four diets were also determined (Table 5.8).

Table 5. 7. Details of experimental grower diets containing 67% of wheat, 33% of balancer and with and without xylanase.

Treatment ID	Xylanase activity
DW	None
Lili	None
DW + E	Xylanase
Lili + E	Xylanase

Table 5. 8. The dry matter (g/kg) and pellet durability index (PDI %) of four experimental grower diets.

Diet	DM g/ kg	PDI %
DW	879	95.8
Lili	881	96.4
DW + E	879	94.6
Lili + E	882	93.4

Each analysis was performed in duplicate.

5.2.2. Bird husbandry and experimental design

A total of three hundred (300) day old male Ross 308 broilers were housed on a single floor. Birds were fed a commercial starter diet from day old to day 7 and then switched to a commercial broiler grower diet in pellet form till 21 days of age. Both commercial starter and grower diets met or exceed the current nutrient specification for Ross 308 broiler chicken (Aviagen 2014a)). Feed and water were provided *ad libitum*. Standard lighting and heating programme were followed for Ross 308 broilers (Aviagen Ross broiler management handbook). At the first day of experiment (day 21), birds were weighed individually and two hundred and forty (240) of them with body weights similar or above 21 days age (959 g) (Aviagen 2014b) were selected for the experiment. Six birds were allocated to each of forty (40) raised floor pens following randomisation. Each experimental diet was assigned randomly to 10 pens within block and fed from 21 to 28 days of age. In total, four (4) diets were fed to the birds, including two diets with xylanase and two without xylanase. Feed and water were provided *ad libitum* and feed intake was monitored from d 21 until the end of the experiment d 28. At day 27, feed in the open bag and in the trough was weighed, then birds were fed the same diet for next 24 h. At day 28, leftover feed in the trough and open bag was weighed and FI in last 24 h was used to calculate mean retention time (MRT). The body weights of all six birds per pen were recorded at d 28 and FCR was calculated on a pen weight basis. The birds were inspected daily, and the body weights of dead birds were

recorded at the time of removal and accounted for FCR during experimental period of seven days.

5.2.3. Digesta collection

At day 28, all six birds per pen were killed by cervical dislocation and their abdomen was excised. Immediately afterward, the intestine was removed, the mesentery was cut, and jejunum and ileum were separated at Meckel's diverticulum. The four small intestinal segments were demarcated by the end of the duodenal loop, and the ileo-caecal junction and their mid-points. Both jejunum and ileum were split into two parts of equal length, namely, proximal Jejunum (PJ), distal Jejunum (DJ), proximal ileum (PI), distal ileum (DI) as described by Weurding et al. (2001). Digesta samples were collected from each bird per pen in their entirety from the PJ, DJ, PI and DI. Digesta were rinsed out of each part (without squeezing) with demineralized water at 4°C, collected in a clean, dry plastic tub and pooled per pen per part. Digesta samples were stored at –80°C and freeze-dried afterwards. After freeze-drying, the samples were ground through a 0.5 mm sieve using a rotor mill, Retsch ZM 200 (Retsch GmbH, Haan, Germany) and stored at –20°C in plastic bottles until analysis of starch and TiO_2.

The freeze dried digesta samples collected from each segment were weighed to determine the MRT in each segment. Starch and TiO_2 were determined in the experimental diets and freeze-dried digesta. Starch concentrations in the diets and digesta were determined using Megazyme total starch kit (Megazyme, Co. Wicklow, Ireland) (AOAC 996.11). The procedure is based on incubating milled digesta samples in the presence of α-amylase and amyloglucosidase, and later absorbance of each solution and standard was read at 510 nm using a spectrophotometer as described by Mahasukhonthachat et al. (2010). Milled digesta/diet samples were weighed accurately (approximately 100 mg) in a glass test tubes (16 x120 mm) and 0.2 ml 80% ethanol (v/v) was added to glass tubes and vortex mix. The addition of ethanol added dispersion. Then immediately 3 ml of thermostable α-amylase (Megazyme, Co. Wicklow, Ireland) was added to each glass tube, vortex mixed and incubated in a boiling water bath for 6 min. During boiling, tubes were constantly vortex mixed after 2, 4 and 6 min. After incubation, tubes were placed in a water bath at 50°C and 0.1 ml of amyloglucosidase (AMG) was added, vortex mixed and left to incubate at 50°C for 30 min. Then tubes were transferred to 100 ml volumetric flask and made up volume up to 100 ml. An aliquot of this solution was centrifuged at 3000 rpm for 10 min. Duplicate aliquots (0.1 ml) of the diluted solution were transferred to the bottom of a clean glass tube. Then 3 ml of GOPOD reagent (Megazyme, Ireland) was added to each tube including standard and reagent blank and incubated the tubes at 50°C for 20 min. Absorbance of each sample and

standard solution was read at 510 nm against blank using a spectrophotometer (Jenway 6305, Bibby Scientific Ltd. UK).

The concentrations of TiO_2 in the diets and digesta were analysed following the method of Short et al. (1996). Duplicate samples of diet and digesta were ashed in crucibles in a muffle furnace. Once cooled, 10 ml of sulphuric acid (H_2SO_4) 7.4M was added to each crucible and contents were gently transferred to Kjeldahl tubes. All samples were boiled until completely dissolved. After cooling, contents were transferred to volumetric flasks and 20ml of hydrogen peroxide (H_2O_2) 30% was added to each flask. The addition of H_2O_2 resulted in an intense orange colour depending upon the concentration of titanium. The absorbance of titanium in the samples and standard solution prepared at the same time were read on spectrophotometer at 410nm.

5.2.4. Calculations

The apparent digestibility coefficients of starch in each segment of intestine (PJ, DJ, PI and DI) were calculated by the following equation:

$$\text{Apparent digestibility coefficients} = \frac{(\%\ \text{starch}/\ \%\ TiO_2)_{\text{diet}} - (\%\ \text{starch}/\ \%\ TiO_2)_{\text{digesta}}}{(\%\ \text{starch}/\ \%\ TiO_2)_{\text{diet}}}$$

Mean retention time (MRT) (minutes) in each segment of intestine was calculated using the following equation (Weurding et al. 2001).

$$\text{MRT (min)} = \frac{1440 \times TiO_{2\,\text{digesta}} \times W}{FI_{24hr} \times TiO_{2\,\text{diet}}}$$

Where, $TiO_{2\,\text{digesta}}$ is the marker concentration in the digesta (mg/g), W is the weight of dry gut content (g), FI_{24hr} is the feed intake over 24 h period, $TiO_{2\,\text{diet}}$ is the marker concentration in the diet (mg/g) and 1440 equals total minutes per day.

The digestion time (t) was calculated from the sum of different MRT determined in each intestinal segment. Starch absorption was not anticipated to take place prior to small intestine (Weurding et al. 2001). The MRT in the duodenum was estimated to be 5 min as previously determined (Weurding et al. 2001; Gutierrez del Alamo et al. 2009a) and was added to MRT in each segment to calculate the digestion time (t). By relating the digestion coefficient achieved in each segment with the digestion time (t), the pattern of rate of starch digestion was studied for each replicate. The curve of digestion was described by

exponential model developed by Ørskov and McDonald (1979) using SigmaPlot (Systat Software Inc):

$$DS = DST \times (1 - e^{-kds \times t})$$

DS is the proportion of starch that digested at time (t), the fraction DST is the amount of potential digestible starch (asymptote) (g/g starch), kds is the digestion rate constant k (per unit time, h^{-1}) indicate how rapidly starch was digested in each segment. A kds value of 2 would mean 200% starch digestion within an hour. A non-linear regression iterative process was used to fit the modelling curves of starch digestion coefficient by digestion time (t) assuming a potential starch digestibility value of 100% and adding extra digestion time (t) points (280, 320, 360, 400 min) using Sigma Plot (Systat Software Inc).

5.2.5. Statistical analysis

Starch digestibility coefficients, MRT in each segment and rate of starch digestion were analysed by analysis of variance (ANOVA) in a randomised complete block design using a 2 × 2 factorial treatment arrangement. The factors were two wheat samples and two levels of xylanase (with and without). Each pen represented an experimental unit (replicate). Treatments and blocks were fixed effects. Significance were reported at $P \leq 0.05$ and tendencies were reported $P < 0.1$.

5.3. RESULTS

Recovery of xylanase in both grower diets was tested by ESC (Enzyme Services and Consultancy (ESC), Ystrad, Mynach, UK) and presented in Table 5.9. Xylanase was added to each diet to achieve an in-feed activity of 16000 BXU/kg in the final diet. The analysis of both grower diets confirmed presence of xylanase in the required level (16000 BXU/kg) for in feed activity.

Table 5. 9. Activity of xylanase in grower diets containing Econase XT 25 (BXU/kg).

Diet	Xylanase activity BXU/kg
Lili + Econase	16900
DW + Econase	16700

Xylanase activity was analysed by ELISA method by ESC.

5.3.1. Starch digestion coefficient

The starch digestibility coefficients in four segments of small intestine of broilers are summarised in Table 5.10. Birds fed sample DW had higher ($P < 0.05$) starch digestibility in DJ, PI and DI. There was no difference ($P > 0.05$) in starch digestion in PJ. Starch was gradually digested in small intestine and in jejunum 75.6% starch was digested, whereas, PI had 88.5% starch digestibility. The DI had the highest amount of starch digestion 91.8%. The addition of enzyme did not influence ($P > 0.05$) starch digestion in any segment of intestine. There was no wheat × enzyme addition interaction ($P > 0.05$) for starch digestibility.

Table 5. 10. Apparent digestibility coefficients[1] of starch in different segments of small intestine of broiler chickens fed diets containing two wheat samples and effect of xylanase on starch digestion.

	Xylanase	Jejunum		Ileum	
		Proximal	Distal	Proximal	Distal
Wheat					
DW		0.559	0.796	0.913	0.942
Lili		0.516	0.716	0.857	0.901
SEM*		0.0258	0.0163	0.0114	0.0106
Xylanase					
−		0.560	0.767	0.878	0.918
+		0.515	0.745	0.892	0.925
SEM*		0.0258	0.0163	0.0114	0.0106
Wheat × Xylanase					
DW	−	0.599	0.807	0.914	0.939
	+	0.520	0.785	0.912	0.945
Lili	−	0.521	0.727	0.842	0.897
	+	0.511	0.705	0.872	0.905
SEM*		0.0365	0.0231	0.0162	0.0150
Significance					
Wheat		0.246	0.002	0.002	0.012
Xylanase		0.235	0.353	0.393	0.622
Wheat x Xylanase		0.351	0.976	0.345	0.932

[1]Each value represents mean of 10 experimental units per treatment. Each experimental unit was a pen containing 6 birds each.
*Standard error of means (df = PJ: 24, DJ: 25, PI: 27, DI: 24).

5.3.2. Rate of starch digestion

Birds fed wheat DW had a faster (P = 0.008) *in vivo* rate of starch digestion (3.18 kds $^{h-1}$) than those fed wheat Lili (2.54 kds $^{h-1}$). The addition of xylanase did not influence ($P > 0.05$) rate of starch digestion in broilers. There was no wheat × xylanase interaction ($P > 0.05$) for rate of starch digestion. An example of pattern of starch digestion along the small intestine of broilers fed wheat samples DW and Lili, its relationship with the digestion time and how it fits with the mathematical model used is shown in figures 5.1 and 5.2. Starch digestion was not assumed to take place before small intestine, thus zero (0) min represents no digestion at the beginning.

Table 5. 11. The rate of starch digestion[1] in small intestine of broilers fed two wheat samples and influence of xylanase on rate of starch digestion.

	Xylanase	Starch digestion rate (kds $^{h-1}$)
Wheat		
DW		3.18
Lili		2.54
SEM*		0.158
Xylanase		
−		2.85
+		2.87
SEM*		0.158
Wheat × Xylanase		
DW	−	3.35
	+	3.02
Lili	−	2.36
	+	2.72
SEM*		0.224
Significance		
Wheat		0.008
Xylanase		0.934
Wheat x Xylanase		0.138

[1]Each value represents mean of 10 experimental units per treatment. Each experimental unit was a pen containing 6 birds each.
Starch digestion characteristics was calculated using exponential curve equation.
$DS = DST*(1- e^{-kds*t})$.
DS is the proportion of starch digested at time (t).
DST is the amount of potential digestible starch (asymptote) that is digested at fractional rate kds (per unit time, h^{-1}).
*Standard error of means (df = 25).

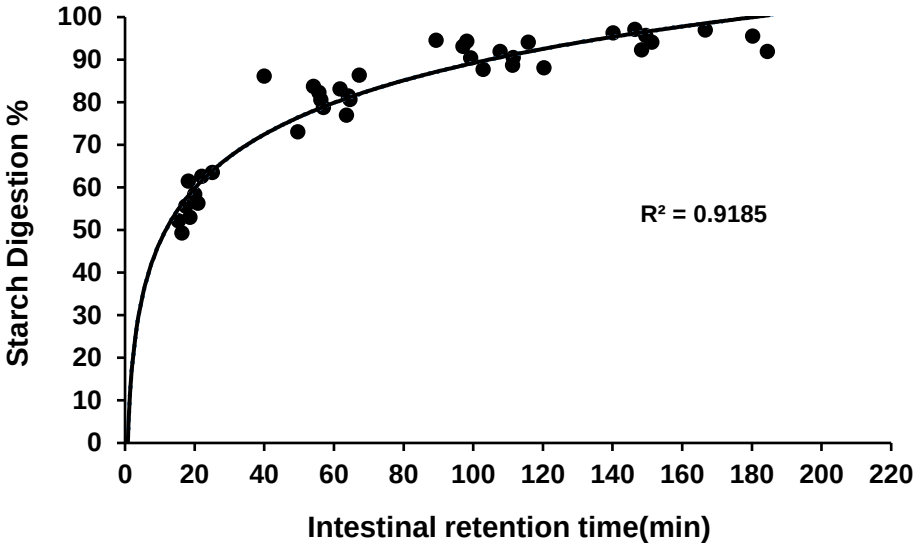


Figure 5. 1. The pattern of starch digestion in small intestine of broilers fed wheat DW.

Each point represents the rate of starch digestion of an experimental unit (six chickens per replicate).

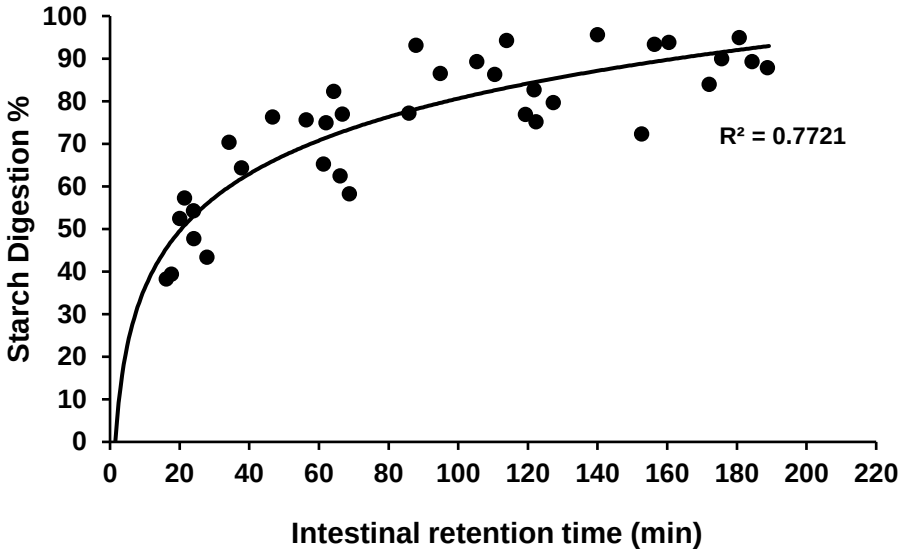


Figure 5. 2. The pattern of starch digestion in small intestine of broilers fed wheat Lili.

Each point represents the rate of starch digestion of an experimental unit (six chickens per replicate).

5.3.3. Mean retention time (MRT)

The MRT in different segment of small intestine and total MRT (jejunum + ileum) are summarised in Table 5.12. Birds fed diet containing wheat DW had lower (P = 0.05) MRT in PI than those fed diet based on wheat Lili. The MRT tend to be lower (P = 0.08) in DJ of broilers fed diet containing wheat DW than those fed diets based on wheat Lili. There was no difference (P > 0.05) in MRT in PJ and DI between DW and Lili. The total MRT (jejunum + Ileum) in broilers fed sample DW was 15.5 min lower (P < 0.05) than those fed wheat Lili (149 vs 164.5 min, respectively). The addition of xylanase did not affect MRT in any segment of intestine or total MRT and no wheat × xylanase interaction (P > 0.05) for MRT was observed.

Table 5. 12. Mean retention time[1] (min) in the small intestine of broiler chickens fed two wheat samples and influence of xylanase on mean retention time.

Xylanase		Jejunum		Ileum		Jejunum + Ileum
		Proximal	Distal	Proximal	Distal	
Wheat						
DW		16.2	34.8	45.8	52.2	149.0
Lili		17.3	38.4	50.7	58.1	164.5
SEM*		1.35	1.41	1.65	2.61	4.45
Xylanase						
−		18.1	38.6	46.4	53.8	156.8
+		15.4	34.6	50.2	56.5	156.7
SEM*		1.35	1.41	1.65	2.61	4.45
Wheat × Xylanase						
DW	−	16.4	38.0	45.9	51.9	152.2
	+	15.9	31.6	45.8	52.6	145.8
Lili	−	19.8	39.1	46.8	55.7	161.4
	+	14.8	37.7	54.6	60.5	167.6
SEM*		1.91	1.99	2.34	3.70	6.29
Significance						
Wheat		0.546	0.084	0.049	0.124	0.021
Xylanase		0.165	0.060	0.115	0.464	0.990
Wheat x Xylanase		0.246	0.223	0.102	0.583	0.330

[1]Each value represents mean of 10 experimental units per treatment. Each experimental unit was a pen containing 6 birds each.
*Standard error of means (df = 24).

There was no difference (P > 0.05) in FI and WG of broilers fed wheat samples DW and Lili during 21 – 28 days feeding period, however, there was a difference (P = 0.048) in FCR (Appendix B). The addition of xylanase did not influence (P > 0.05) the growth performance in similar period. There was no wheat × xylanase interaction (P > 0.05) on any of growth performance variables during this phase.

5.4. DISCUSSION

The key objective of the current study was to investigate the *in vivo* rate of starch digestion in broilers fed two wheat samples which were previously found to be different in FI and growth rate. The results of part A of this study were interesting because two wheat samples which resulted in large significant differences (11 – 12%) in FI and WG were similar in their nutrient composition and physical characteristics. The starch content of both wheat samples was similar, nevertheless, found significant differences in FI and WG of broilers. By using the conventional quality tests, it was hard to discriminate the quality of these two wheat samples and distinguish which sample would result in superior broiler growth rate. It was anticipated that there should be no difference in the *in vivo* rate of starch digestion between two wheat samples because both samples had similar starch and nutrient composition, however, the findings revealed that rate of starch digestion was significantly different between two wheat samples.

5.4.1. Starch digestibility

The coefficients of starch digestibility in different segments of small intestine were in a similar range reported in earlier studies (Weurding et al. 2001; Gutierrez del Alamo et al. 2008a, 2009a, b). Starch was gradually digested in small intestine of broilers and less than 6% starch entered in large intestine. Overall, the present study demonstrated starch digestibility up to 92.2% in the distal ileum and values were in a range similar to those reported by Gutierrez del Alamo et al. (2008a, 2009a, b).

The present experiment clearly demonstrated that wheat sample (DW) which stimulated higher FI and growth rate were found to contain higher starch digestibility as compared with sample Lili which resulted in lower FI and growth rate in the growth experiment (Part A). The higher differences in starch digestion between both samples were observed in middle part of small intestine (DJ and PI), whereas, relatively a small but significant difference in starch digestion occurred in DI. In different sections of small intestine, wheat DW had higher starch digestibility in DJ 11.2%, PI 6.5% and DI 4.5% as compared to wheat Lili. The difference in starch digestibility between two wheat samples suggested that variability exist in starch digestibility between wheat samples, albeit, the samples were similar in proximate composition and their starch content. Additionally, both wheat-based diets were formulated to be iso-nitrogenous yet showed differences in their starch digestibility Conventionally, starch digestibility is largely determined at ileum (Gutierrez del Alamo 2008a; Svihus 2001; Svihus and Hetland 2001; Ball et al. 2013b) because ileum is the point where starch digestion is almost completed. Although, in this study, there were differences in starch digestibility in different sections of intestine, however, difference in distal part of small

intestine was only 4.5% between two wheat samples. The size of difference in starch digestibility at the distal ileum was small to explain the reason for such a large difference (11 – 12%) in FI and growth rate of broilers (Part A). Previously, inconsistent relationships between wheat starch digestibility and AME were reported (Mollah et al. 1983, Rogel et al. 1987a; Wiseman et al. 2000; Gutierrez et al. 2008a; Yegani et al. 2013). In addition, the differences in starch digestibility in broilers does not always seem to be related with the differences in the growth performance (Rogel et al. 1987; Gutierrez et al. 2008a). Higher starch digestibility is not an indication of greater starch digestion rate because rate of digestion is related to retention time of digesta in the small intestine and the digestive capacity of the bird (Weurding et al. 2001; Gutierrez del Alamo et al. 2009b). It is the ability of the bird to digest starch which consequently affects retention time. It may be proposed that the high starch digestibility of wheat DW could be due to the bird's ability to digest starch at a faster rate as compared to those fed wheat sample Lili.

In the current study, although, the starch digestibility values were in a range of published results, but they were slightly lower. The possible reason of slightly lower digestibility values in small intestine may be due to feeding pellets diets using without steam, where incomplete gelatinisation may have resulted in lower starch digestibility. Studies in broilers revealed that higher starch digestibility in wheat-based diet were coincided with feeding mash diets to broilers (Steenfeldt et al. 1998b; Svihus and Hetland 2001; Weurding et al. 2001; Gutierrez del Alamo et al. 2009a, b) whereas, low starch digestibility values were observed when chickens were fed pelleted diets without prior heating (Svihus 2001; Svihus and Hetland 2001; Abdollahi et al. 2011). Gutierrez del Alamo et al. (2009a, b) revealed starch digestibility up to 96 – 97% in distal ileum in broilers fed mash diets, whereas, Svihus (2001) and Svihus and Hetland (2001) found lower values of starch digestibility (83, 79%; respectively) when birds were fed pelleted diet without using steam. Steam conditioning help in gelatinisation of starch and in dispersion of starch molecules from protein, which results in amylolytic action, however, due to the absence of steam during pelleting process, starch gelatinisation is incomplete and results in less starch degradation (Svihus et al. 2005, Abdollahi et al. 2011).

A study by Pirgozliev et al. (2016) found that broilers fed pelleted diets (conditioned with steam) resulted in higher FI and WG but when birds were fed on mash diets, lower FI and WG were observed. In contrary, high intake of cold pellet diets in broilers may result in overload of wheat starch in the gut, which may affect its digestibility. A study by Svihus and Hetland (2001) anticipated that the causes of low starch digestibility in broilers with high feed intake could be due to high amount of fibre contents in the intestinal chyme which acts as an anti-nutritional factor. The use of fibre degrading enzymes for example xylanase can alleviate the negative effect of soluble fibre, thus increasing digestibility of starch. However,

the current study was unable to find any effect of xylanase on starch digestibility in any segment of intestine, therefore it can be assumed that variation in NSP content may not be the only reason of lower starch digestibility in broilers fed wheat Lili. The findings of the current study suggested that cause of lower starch digestibility in wheat Lili was not due to the lower feed intake, but possibly could be the rate at which starch was digested in different sections of small intestine. The chemical analysis of both wheat samples revealed no such difference in their starch content, also proximate nutrients especially CP and oil contents were also similar. The differences in starch digestibility could not be associated with starch or protein content of both wheat samples. Overall, the small difference in starch digestibility between wheat samples were unable to explain the large differences in FI and WG of broilers in growth trial (Part A).

5.4.2. Rate of starch digestion

The current study revealed that the *in vivo* rate of starch digestion differed significantly between two wheat samples. The rate of starch digestion in broilers fed sample DW was 25% higher than those fed wheat Lili. The values of rate of starch digestion (kds) were in a similar range to those published by Gutierrez del Alamo et al. (2009a, b) and the differences between wheat samples were in agreement with reported values by Gutierrez del Alamo et al. (2009b). The authors found values of 2.45 to 3.28 h^{-1} between three wheat samples. It was interesting to observe such a difference in the rate of starch digestion because both samples were similar in proximate composition and starch content.

The present study clearly illustrated that wheat sample which stimulated higher FI and growth rate previously, had faster rate of starch digestion than the sample who found to be involved in lower FI and growth rate of broilers. The findings of this study were unique in a way because differences in FI and WG between two samples were further investigated by studying mechanism of the rate of starch digestion in broilers using the same wheat samples without altering their composition especially the starch content. Previously, a study by Gutierrez del Alamo (2009a) found worst growth response in broilers fed wheat samples with lower rate of starch digestion. The authors investigated the effect of different rate of starch digestion (by manipulating the dietary composition of wheat samples to have different rate of starch digestion) on growth performance and found that birds fed diet containing faster rate of starch digestion (2.2 kds h^{-1}) resulted in the higher weight gain and better FCR, whereas, those fed on diet with lower rate of starch digestion (1.80 kds h^{-1}) resulted in the lowest growth performance. The researchers substituted the composition of wheat starch with peas starch to reduce the rate of starch digestion. Conversely, in the current study, differences in rate of starch digestion were observed between wheat samples with similar inclusion levels of wheat (67%) in both performance (Part A) and rate of starch digestion

trials (Part B). There was no modification in starch content of both wheat samples. In fact, both wheat samples had same amount of starch content. Additionally, the diets were made iso-nitrogenous and pelleted under the similar conditions, similar to growth trial. Further, the analysis of pellet quality indicated no difference in PDI% of both diets (Table 5.8) but still found differences up to 25% in their rate of starch digestion.

The findings of the current study proposed that the rate of starch digestion may have direct influence on voluntary feed intake of broilers fed different wheat samples. Weurding (2002) reported that growth performance of broilers was affected using varying level of starch digestion rate of different feedstuffs. The author reported that broilers fed on diets with slower rate of starch digestion resulted in lower feed intake as compared to those fed on diets with faster rate of starch digestion. Liu et al. (2013c) found a significant correlation between starch digestion rates and weight gain, N retention and AMEn of broilers fed three sorghum varieties. Sorghum is used as energy source in broiler's diet and energy is derived from the starch content of sorghum. The results of current study were partially in line with the findings of Liu et al. (2013c). Waldron (1997) found significant differences (29%) in rate of starch hydrolysis between two wheat samples. It appears that differences in rate of starch digestion may explain the reasons of large differences in growth performance of broilers fed different wheat samples as indicated previously (Waldron 1997; Wiseman et al. 2000; Weurding 2002).

Overall, the total MRT (jejunum plus ileum) in broilers fed wheat sample DW was 15.5 min lower than those fed wheat Lili (149 vs 164.5 min). The wheat DW which stimulated higher FI and growth rate in growth experiment (Part A) had 10% lower total MRT as compared to wheat Lili with lower FI and growth rates. The values of total MRT were in a similar range to those reported by Gutierrez del Alamo et al. (2009b). The authors reported total MRT values in a range of 140.1 to 166.1 min. The values of MRT in different segments of small intestine were also in a similar range to those reported by Weurding et al. (2001) and Gutierrez del Alamo et al. (2009a). Birds fed wheat sample Lili had longer MRT in the middle part of intestine (DJ, PI). In DJ part of intestine, birds fed sample Lili stayed longer (38.4 min) as compared to those fed sample DW (34.8 min) and difference in MRT was significantly reflected in PI where broilers fed sample Lili stayed longer (50.7 min) than those fed sample DW (45.8 min). It could be anticipated that in birds fed wheat Lili, due to slower rate of starch digestion, starch was taking longer time to digest, hence resulted in lower starch digestibility and consequently longer retention time. In contrary, birds offered wheat DW, starch was digested at a faster rate, consequently resulted in higher starch digestibility with shorter retention time. A study by Weurding et al. (2001) reported that MRT is inversely correlated with starch digestibility coefficients in different segments of intestine and confirmed that feedstuffs with longer retention time in each segment had lower starch

digestion coefficients. The findings of the present study also elucidated the similar results; chickens fed wheat Lili had longer total MRT, hence had significantly lower starch digestion coefficients in three segments of intestine (DJ, PI and DI).

The maximum difference in starch digestibility and the retention time between wheat samples occurred in DJ and PI of small intestine. In broiler chickens, the majority of starch digestion occurred in upper part of intestine especially jejunum, hence starch digestion is completed before entering in DI (Weurding et al. 2001; Gutierrez del Alamo 2009a). It may be assumed that starch in wheat (DW) was readily available and digested rapidly as shown in Figure 5.1. The faster rate of starch digested may stimulate the voluntary feed intake in birds. Starch is a predominant nutrient in wheat-based diets and quicker flow of starch through small intestine results in better nutrient availability and increased demand for the food. Weurding et al. (2001) suggested that faster rate of digestion resulted in efficient energy utilisation in broilers. It could be anticipated that in birds fed wheat sample Lili, slower rate of starch digestion may be a possible factor in reduced FI and ultimately decreased WG in the growth trial.

Various factors affect the retention time or rate of passage of feed and depends on composition of diet especially the content of soluble and insoluble NSP (Choct and Annison 1990; Choct et al. 1999), the use of NSP enzymes (Almirall and Esteve-García 1994), feed form (Svihus and Hetland 2001; Hetland et al. 2002) and the size of raw ingredient in the feed (Carré 2002; Svihus et al. 2002). The increased viscosity due to high level of soluble NSP could result in decrease in retention time and reduce digestion and absorption of major nutrients (Choct and Annison 1992a, b; Bedford 2006). High digesta viscosity resulted in decrease in passage rate of digesta (Van der Klis et al. 1993; Choct et al. 1996, 1999). It could be anticipated that high NSP content of wheat Lili may have resulted in higher digesta viscosity which eventually reduced passage rate of digesta and impacted starch digestion and the rate of starch digestion; however, viscosity related mechanism could not be considered fully responsible because there was no effect of xylanase on either starch digestion or retention time. The digesta viscosity was not determined in this study due to low amount of digesta available in different intestinal segments for starch and TiO_2 determination but this can be further investigated by determining the digesta viscosity in different intestinal segments and its relationship with passage rate and rate of starch digestion.

The rate of passage of feed in the broiler's gut is relatively faster as compared to the non-ruminants. A series of work by Liu et al. (2013a, b, c; 2014) found an average retention time of 2.98 h in jejunum and ileum in broiler chickens fed sorghum-based diets and the values of total MRT in the present study were relatively in a similar range. Sorghum is considered

as gradually digestible starch raw material, whereas, wheat is relatively thought to be rapidly digestible starch raw material (Weurding et al. 2001). It can be concluded that the retention time is an important factor in determining the rate of digestion and feed intake of broilers and should be considered in conjunction with the dietary components and factors affecting the rate of digestion of starch and protein. In current study, no difference was observed in FI during last 24 h of digestion trial, moreover, there was no difference in FI during digesta collection phase, which further clarifies that significant variations in rate of starch digestion between wheat samples were independent of amount of voluntary feed intake during the similar period.

It is noteworthy, that current study was a preliminary work and the results indicated that the rate of starch digestion differed between wheat samples with similar starch content, proximate nutrient and physical characteristics. There may be several factors which affect the rate of starch digestion e.g., starch and protein matrix, the size of starch granules, the ratio of amylose to amylopectin (Svihus et al. 2005; Liu and Selle 2015; Zaefarian et al. 2015), however, this was not the aim of this study to investigate the factors affecting starch digestion in broilers. The primary objective of this study was to examine the rate of starch digestion in broilers fed two wheat samples with similar nutrient composition and results demonstrated that there were substantial differences in the rate of starch digestion between wheat samples. Svihus et al. (2005) illustrated that structural feature of starch can affect the rate of starch digestion in broilers. The amylose to amylopectin ratio is negatively correlated with starch digestion. The effect of amylose to amylopectin ratio in starch digestibility is understood through the interaction between starch granule size and structure. The reduced digestibility of starch with high amylose could be due to complex formed between amylose and lipids around starch which would result in reduced rate of enzymatic action on starch (Cui and Oates 1999; Crowe et al. 2000; Tufvesson et al. 2001). The inherent variation in starch and protein contents of cereal grains can affect the rate of digestion of starch and protein in chickens. A study by Weurding et al. (2001) concluded that starch digestion rate in different segments of small intestine differed between feedstuffs with sorghum having the lowest starch digestion rate. However, unique aspect of the current study was that wheat samples were not different in their chemical composition yet showed differences in the rate of starch digestion.

Differences in the amount of starch digested at different segments of small intestine indicate that susceptibility of starch to enzymatic action differs and this implies differences in starch digestion rate. The starch granules of endosperm are embedded in protein matrix. The protein matrix surrounding the starch first needs to be hydrolysed for starch to be exposed to amylase therefore protein can create a physical barrier to starch digestion. McAllister et al. (1993) found that protein matrix was a major factor responsible for differences in starch

digestion in ruminants fed corn and barley diets. Processing of wheat during feed manufacturing and grinding action of gizzard disrupts starch in endosperm and increases the surface area of starch granule for better binding by amylase. Differences in starch digestion could also depend on digestion of protein in small intestine and lower utilisation of CP could also affect starch utilisation. The lower rate of starch digestion of wheat Lili could also be due to the effect of CP digestion and the rate of amino acid absorption. Although, CP digestibility and the rate of CP digestion was not determined in this study because the primary purpose was to investigate the major energy nutrient e.g., starch, however it would be interesting to investigate the rate of CP and amino acid digestion and how they affect the rate of digestion of starch.

The main objective of broiler's production is to achieve rapid and efficient growth by better utilisation of nutrients in the diets. This is only possible by feeding diets which contain readily digestible nutrients. The main energy yielding nutrient, starch needs to be digested to glucose by amylolytic enzymes in small intestine or fermented into volatile fatty acids (VFAs) by hind gut microflora. Starch digestion in poultry yields more energy from starch digestion than fermentation in caeca. It is critically important that broiler diets should contain nutrients which are digested completely, and consequently result in increased feed intake and growth of chickens. So far, majority of the studies with broilers have been unable to find a significant relationship between nutrient composition of wheat and growth performance of chickens (Wiseman 2000; Steenfeldt 2001; McCracken et al. 2002; Pirgozliev et al. 2003) and differences in growth performance of broilers fed wheat samples with similar proximate and chemical composition are still questionable. But the findings from this study indicated that despite similar starch content between wheat samples, it is vital to understand how well starch is digested in small intestine of broilers. In the current study, the indicative findings on differences in the rate of starch digestion in broilers between wheat samples provided some preliminary evidence to understand the reason of differences in feed intake and growth rate of broilers fed wheats similar in nutrient composition.

5.5. CONCLUSIONS

This study concluded that the rate of starch digestion is a relevant characteristic in broiler nutrition and differs between wheat samples. The rate of starch digestion in broilers affects retention time and starch digestion at different segments of small intestine. The determination of the *in vivo* rate of starch digestion in broilers fed a large number of wheat samples are complicated, time consuming and also require a large number of replicates, which was not possible in this project. Furthermore, the techniques to collect digesta from different segments of intestine are complex and require a large number of birds. This study was a preliminary work and only two wheat samples were used to investigate the differences in the rate of starch digestion. The findings indicated that the rate of starch digestion differs significantly between wheat sample with similar proximate composition, starch content and physical characteristics. The current work suggested that the rate of starch digestion is a primary factor to investigate the differences in voluntary feed intake. If food digests and absorbs faster, birds tend to eat more with better nutrient supply, hence resulting in increase in feed intake and growth rate. There could be several factors which could affect the rate of starch digestion. However, the first step would be to establish whether the rate of starch digestion is a primary factor to determine the differences in feed intake of broilers. To test this, a large number of wheat samples would be required to investigate the *in vivo* rate of starch digestion in broilers. Once differences in *in vivo* rate of starch digestion are determined, further work may explore the factors affecting the *in vivo* rate of starch digestion and possibly develop robust *in vitro* techniques to predict the rate of starch digestion in wheat samples. The findings of this study should not be taken as a conclusive evidence for the causes of differences between wheat samples until a series of future experiments are set up to investigate the digestive dynamics in broilers fed different wheat samples.

CHAPTER 6: GENERAL DISCUSSION AND CONCLUSIONS

6.1. INTRODUCTION

Wheat is the main raw material in broiler feed formulation in many parts of the world and variation in its nutrient composition and available energy is challenging for nutritionists. In the UK, wheat is the main cereal in broiler feeds and exact knowledge of currently available UK wheat samples is significantly important for the efficient use of this core ingredient in diet formulations. The UK is the 2nd largest poultry meat producing country with a share of 12.9% of total poultry meat production in the Europe (Eurostat 2018b). The continued and consistent growth of poultry meat production would be possible in future if existing knowledge on nutrients composition of wheat, their bioavailability and the effect on growth performance is being updated continuously and communicated to nutritionists and poultry producers in order to keep the variation in growth rate of broilers at minimum. Differences between wheat samples are strategically important and can provide valuable and economical information for wheat grower and broilers feed manufacturers.

Previous information on wheat samples and their effect on AME and growth performance of broilers is inconsistent (Scot et al. 1998a; Rose et al. 2001; Steenfeldt 2001; Pirgozliev et al. 2003; Ball et al. 2013a, b). Wheat is known for its high available energy for broilers, however, large variability in its AME (8 – 17 MJ/kg DM) is always challenging to predict the growth performance of broilers. In North West Europe, Australasia and Americas, broiler's diets can consist up to 60 – 70% of wheat; and when a raw ingredient is used at such a high level, an understanding of variability in its nutrient composition and how it affects the AME is critically important for efficient diet formulation. The existing knowledge on wheat nutrient composition, bioavailability of nutrients and AME relies on research conducted 15 – 20 years ago (Waldron 1997; Wiseman 2000; McCracken et al 2002; Pirgozliev et al. 2013). These studies were not able to demonstrate the characteristics of wheat which could relate to AME and growth performance of broilers, however reported differences in AME and growth performance. Commercial poultry feed industry uses NIR method generally to predict the nutrient composition and AME of raw ingredients, based on the already built in values of several analyses. However, the values predicted by NIR method could not relate to the results of proximate and chemical analysis of wheat samples due to large variability in wheat. Additionally, the AME values of wheat predicted by NIR method often differ from the actual AME determined upon broiler assay. Furthermore, NIR method is unable to predict the growth performance of broilers. Due to the fact, that large AME value of wheat does not always respond in higher growth rate, the prediction by NIR method may be bit irrelevant to predict the feeding value of wheat for broilers. Therefore, to understand the variability and feeding value of currently available wheat samples, the broiler feed industry

requires a study where a range of representative UK wheat samples are characterised by fully investigating their nutrient composition and how the variability in wheat samples affect the AME and growth performance of broilers. The current project aims to provide information which would be potentially relevant to poultry feed industry and future feed formulations for broilers would also be adjusted considering the variability between different wheat samples.

The specific objectives of this project were to investigate the variability in nutrient composition of a range of currently available UK wheat samples for broiler feeds and their effect on AME and growth performance of broilers. The secondary objective was to investigate the relationship between nutrient composition and AME of the wheat and growth performance of broilers. The possible influence of xylanase to improve the feeding value of currently available wheats for broilers was also explored.

6.2. NUTRIENT COMPOSITION OF CURRENT UK WHEAT SAMPLES AND BIOAVAILABILITY OF ENERGY

It was determined from the results of Chapter 2 that nutrient composition of currently available UK wheat samples for broiler feeds is variable. The CP, oil, ash and soluble NSP content of wheat samples were variable between different wheat samples. The soluble arabinose and xylose contents of currently available wheat samples also varied. It was noticed that variability in CP and ash content of wheat samples were due to samples grown at different locations. The size of variability in nutrient composition of wheat samples were in agreement with the previous findings on different wheat samples for broilers (Steenfeldt 2001, Pirgozliev et al. 2003; Ball et al. 2013a, b).

The first broiler study (Chapter 3) revealed large variation (1.13 MJ/kg DM) in AME of currently available UK wheat samples for broiler feeds. The difference of over 1 MJ/kg DM is commercially important in broiler feed formulation and clearly indicated that there is a substantial variation in AME of currently available UK wheat samples for broiler diets. However, the variation in AME of wheat samples was not related to the nutrient composition and bioavailability of the nutrients. The main energy yielding nutrients e.g., starch and protein were not associated with the variation in the AME. Wheat is known for its high starch and protein content and contribute up to 70% of AME in broilers finisher diet. Starch can contribute more than half of the ME intake (Svihus 2011), however the results revealed that starch and protein content of wheat could not be used to predict the AME of wheat for broilers. The study indicated that CP, soluble NSP and ash contents of wheat samples in combination only accounted for 59% variability in AME of the wheat ($R^2 = 0.59$; $P < 0.05$) and still there was a large proportion of AME which was not explained by determined

chemical and physical characteristics of wheat. Although, the variability in CP content between wheat samples was minimised by formulating the diets to be iso-nitrogenous but still resulted in significant differences in AME of wheat. This study was unable to find any relationship between physical characteristics of wheat (e.g., specific weight) and AME and growth performance. There are reports of inconsistent relationship between physical characteristics of wheat and AME (McCracken et al. 2002; Hetland et al. 2007) and this study further confirmed that physical characteristics of wheat in general and specific weight particularly cannot be relied upon to determine the feeding values of wheat for broilers. Overall, the study suggested that the AME of currently available UK wheat samples could not be predicted by the nutrient composition of wheat and require some robust approach to predict the AME.

The literature publishing large differences in AME of wheat and growth performance could be confounded by the effect of growing sites on the nutrient composition of wheat samples (Rogel et al. 1987a; Rose et al. 2001; Steenfeldt 2001; Pirgozliev et al. 2003). The differences in chemical composition of wheat may be due to different growing conditions during crop growth (Gutierrez del Alamo et al. 2008b; Ball et al. 2013b). Due to the fact, wheat samples in previous studies were collected from several growing locations and different growing condition may have resulted in variability in nutrient composition of wheat. Therefore, to test the possible effect of growing site of wheat on nutrient composition, a study was designed to investigate the effect of wheat variety and growing site on nutrient composition, their bioavailability and AME of the wheat. The study (Chapter 4) clearly demonstrated that growing site of wheat crop affected the nutrient composition of the wheat, not the wheat variety. Previously, only few studies have investigated the effect of wheat variety and growing site on nutrient composition and their bioavailability (Scott et al. 1998a; Wiseman 2000; McCracken et al. 2002) and the findings of the current study were in agreement with these studies. The results showed that growing site of wheat crop influenced starch, protein, insoluble NSP and arabinose contents of wheat varieties. The growing site also affected the specific weight of wheat varieties. Interestingly, all three wheat varieties were genetically different in their nutrient composition and inherent milling characteristics but their nutritional value for broilers was influenced primarily by their growing location. The significant differences in AME content between growing sites of wheat were in association with the differences in starch and protein contents. The low AME of wheat was due to the low starch and protein contents of wheat grown at specific growing sites. The study indicated that variability in nutrient composition of wheat and its effect on AME is influenced by the growing location of wheat crop. The findings from this study suggested that growing condition of a wheat crop is the main reason of variability in the nutrient composition of wheat samples. Various growing conditions affect the growth of a

wheat crop. Future work should focus on an understanding of the factors affecting the growing conditions e.g., soil type, level of rainfall (pre harvesting and during harvesting), N application, temperature, disease prevalence and husbandry techniques to investigate the variability in the nutrient composition of wheat varieties.

In conclusion, there were large significant differences in AME of currently available the UK wheat samples, and the differences were not associated with the nutrient composition of wheat varieties. Instead, the differences in AME were due to the effect of growing site on nutrient composition of wheat varieties. The conventional routine quality tests were unable to predict the AME of wheat for broilers. These studies revealed that care should be taken using wheat chemical composition and physical characteristics as a predictor of AME of individual wheat samples for broiler diets.

The nutritional value of wheat can be improved using exogenous xylanase (Choct 2006; Slominski 2011; Adeola and Cowieson 2011). However, the use of xylanase in wheat-based diets does not always result in improvements in nutritional value of wheat. In study (Chapter 4), the response of xylanase to improve AME was not uniform and only improved AME of certain wheat varieties. The improvements in AME using xylanase were related to the NSP content of wheat and viscosity reducing mechanism. The study suggested that it is vital to know the exact NSP content of wheat samples before adding xylanase to the wheat-based diets. If poultry feed industry can accurately predict the NSP content of wheat samples on arrival at the mill, batches of wheat can be separated into low and high AME wheats based on their NSP content. The application and dose of xylanase can be adjusted depending upon the NSP content and AME of wheat. This may benefit to improve the AME of low AME wheats and further it can save the unnecessary addition of xylanase to wheats with high AME values. If some robust analytical techniques can predict the nutrient composition of wheat on arrival, millers can pay extra price for high-quality wheats due to the fact that wheats with high nutritive values would result in high AME, thus saving extra cost to match the energy requirements of broilers.

6.3. GROWTH PERFORMANCE OF BROILERS

The first broiler study (Chapter 3) revealed there were large differences (13 – 14%) in FI and growth rate of broilers, which were not fully explained by wheat chemical composition and physical characteristics. There were wheat samples which resulted in up to 14.3 and 13.8% decrease in FI and WG, respectively, in comparison to those performed with highest FI and growth rate. The large differences in FI and growth rate of broilers were not related to the nutrient composition and physical characteristics of wheat samples. Moreover, differences in FI and growth rate were not associated with nutrients bioavailability and AME

of wheat samples. The size of difference in growth performance were in accord with several studies using similar inclusion level of wheat in broiler diets (Steenfeldt 2001; Ball et al. 2013a; Pirgozliev et al. 2015). Differences in growth performance were unlikely due to the differences in CP content of wheat samples because all diets were formulated to be isonitrogenous and had same amino acid balance. Such as magnitude of difference in growth performance is commercially important for the broiler feed industry. The study suggested that if nutritionists can identify wheat samples which result in poor growth rate and reject them, diets can be formulated using wheat samples of superior feeding potential for broilers. The use of high feeding value wheat samples in diets would result in less variation in growth rate of broiler chickens, improve uniformity within the flock and less rejection at the processing plants. The results of study 2 (Chapter 4) suggested that growing site of wheat has a prominent effect on daily FI and WG of broilers and the effect of wheat variety was minimal. The variation in feed intake and growth rate between growing sites of wheat were not related to differences in AME and nutrient composition.

Although, the addition of exogenous xylanase improved AME of wheat (Chapter 4) but there was no improvement in FI and growth rate of broilers, whereas, the improvement in FCR was due to better nutrient availability of wheat. The study concluded that feeding quality of currently available UK wheat varieties is affected by the growing location and various factors affect the growing location. Further work is warranted to investigate the factors affecting growing location and care should be taken when sourcing wheat varieties grown at different locations.

The quantity of a nutrient absorbed at different segments of small intestine is a simple function of feed intake, its concentration in the diet and digestibility coefficients (Liu and Selle 2015). It is noteworthy that digestive dynamics of starch and protein should not be considered in isolation but in combination because a balanced provision of glucose and amino acids at sites of protein synthesis is a fundamental prerequisite for efficient growth in broilers. In practical feeding programme, broilers have unrestricted access to the feed and *ad libitum* feeding pattern are thought to accommodate any differences in rate of digestion or absorption of nutrients. Nevertheless, *ad libitum* feeding programme does not equate continuous access to the feeding, and practically, broilers do not spend all time in eating when the lighting is on, instead they only eat in the beginning or at the end of the day or both but not in the middle (Jensen et al. 1962; Savory 1980). Therefore, despite *ad libitum* feeding access to broiler chickens in practical production systems; it seems that consumption patterns still provide scope for asynchronies in digestion and absorption of nutrients which ultimately may have an impact on broiler performance.

Although, large differences (13 – 14%) in FI and growth rate of broilers were found (Chapter 3) but there was no association of wheat chemical and physical characteristics with the differences in growth performance. Moreover, differences in FI and growth rate were not associated with large variability in AME of wheat samples. The lack of relationship between starch and FI and WG proposed that may be the rate of starch assimilation in intestine which influenced the voluntary feed intake in broilers. However, in broilers, the *in vivo* rate of starch digestion is difficult to conduct on large scale due to the complexity of techniques involved to collect digesta in different segments of small intestine. Moreover, the trials are time consuming and require a large number of digesta samples. Therefore, the final study was a preliminary study to investigate the *in vivo* rate of starch digestion in broilers fed two currently available the UK wheat samples with similar proximate composition and starch content. The novel aspect of this study was that wheat samples which resulted in significant differences (11 – 12%) in FI and WG during growth experiment were chosen to determine the *in vivo* rate of starch digestion without changing the dietary starch composition. These samples were similar in proximate composition, starch content and physical characteristics. All diets were made isonitrogenous and pelleted under similar conditions. The initial growth study (Chapter 5, Part A) reconfirmed that large variability exits in growth performance of broilers fed two currently available the UK wheat samples as indicated in previous two studies. The starch digestion study (Chapter 5, Part B) showed that there were large differences (25%) in the *in vivo* rate of starch digestion between two wheat samples with similar nutrient composition. The sample (DW) which resulted in greater FI and growth rate was found to have faster rate of starch digestion as compared to the sample (Lili) which resulted in lower FI and growth rate. The findings of this study provided a preliminary assumption that rate of starch digestion may have influenced the voluntary feed intake in broilers and could be one of the reasons of difference in feed intake and growth rate of broilers. It was interesting to observe such a large difference in the rate of starch digestion, albeit both samples were similar in their proximate nutrient composition. It could be speculated that starch content or its digestibility is not related to voluntary FI, instead FI depends on how fast starch is digested and absorbed in small intestine. The possible explanation of higher FI and growth rate in broilers fed diet with faster rate of starch digestion could be due to the ability of birds to digest starch at a faster rate as compared to those with slower rate of starch digestion. Additionally, the differences in the rate of starch digestion between wheat samples were not influenced by the feed intake during digesta collection period because there was no difference in FI. Starch is a predominant nutrient in wheat and faster rate of starch digestion reflects better nutrient availability and increased demand for the food.

The current work on rate of starch digestion in this thesis was a preliminary study using only two wheat samples and the findings suggested that the *in vivo* rate of starch digestion may be the primary factor to investigate the causes of large differences in FI and growth rate of chickens. However, various factors affect the rate of starch digestion, for example, ratio of amylose to amylopectin, starch and protein matrix, the size of starch granules, transit time of food in intestine, particle size of feed and gut health (Svihus et al. 2005; Liu and Selle 2015; Zaefarian et al. 2015). Further work is warranted to set up a study using a large number of wheat samples and investigate the *in vivo* rate of starch digestion in broilers. If the work establishes that the rate of starch digestion differs between wheat samples of similar nutrient composition, thereafter, future work may focus on factors affecting the rate of starch digestion in broilers. The further research could also develop some robust techniques to determine the *in vitro* rate of starch digestion in wheat which could potentially help poultry industry to select high feeding value wheats for broilers.

6.4. CHALLENGES TO THE UK POULTRY INDUSTRY

Despite strong growth in poultry meat production during the last two decades, the UK poultry sector in general and broiler meat production in particular is currently facing several challenges. The main challenges are listed as follows:

- One of the biggest challenges faced by the UK poultry industry is high production cost (price/kg live weight) (86p/kg). The feed cost is higher as compared to other countries in the EU (average 56p/kg live weight). In addition, high labourer cost makes the poultry industry a bit more difficult to compete with other markets in the EU and rest of the world. Moreover, there is a shortage of skilled workers in the poultry industry and currently 60% of workforce is from the EU states. The potential impact of post Brexit immigration policies would also affect skilled workforce numbers in the UK. Although, consumer choice and preference over other meat make broiler meats still favourable low-cost protein, however the price of chickens should remain within the reach of consumers.

- There is an increased pressure to reduce the use of antibiotics in poultry production. The UK has taken up this challenge and managed to reduce antibiotics by 82% in the last 6 years (BPC antibiotics report 2019) and there is increased awareness to further reduce its use. However, stopping routine medication treatments and changing to targeted therapeutic use of antibiotics has added greater pressure on management and has further emphasised the skill gap and identified existing production practices that need improvement, e.g., terminal hygiene practices.

- There is a high demand to reduce greenhouse gases (GHG) emissions produced by poultry houses and the safe disposal of poultry manure and wastes. Poultry produces less GHG emissions as compared to ruminants and pigs. The majority of GHG emissions are associated with growing of cereals crops and use of low-quality feed ingredients which could end up in litter as undigested feed materials. Poultry production require significant amount of energy to maintain temperature in poultry houses. The use of renewable sources of energy can make a significant impact in reducing GHG emissions, however it does require significant investment on the part of the producers.

- The future use of sustainable cereals and soya would add additional cost to poultry feeds and production. The cost of imported soya incurs substantial cost to poultry feeds; hence the use of sustainable soya and other imported cereals could further increase the feed price. The increased feed price will reduce the margin for feed companies and poultry producers.

- Feed wheat varieties are high yielding varieties and the demand of feed wheats in animals especially poultry feeds are growing. In the UK, crop growers prefer to cultivate feed wheats due to relatively less cost involved in the management of feed wheat (less use of chemicals and fertilisers compared to milling wheats). Moreover, there are less chances of rejections as compared to milling wheats. Generally, if milling wheats do not meet the specifications for milling purpose, there are more chances of rejection, whereas, feed wheats generally trade on moisture content (%) and specific weight. There is a need to keep the supply and demand in balance and also the price of feed wheats should remain viable for feed mills.

All these above challenges faced by the UK poultry industry could further increase the cost of current broiler meat production. In addition to the above issues, the use of low-quality raw materials in broiler feeds could add extra costs to production. However, by selecting wheat samples of high feeding value, nutritionists could minimise the losses caused by variation in growth performance due to low-quality ingredients. The selection of high-quality feed ingredients could also benefit broiler producers by achieving optimum growth rate at the desired age. Due to relatively high production cost and low margin on live weight of broiler chickens in the UK, it is essential that variation in growth rate of broilers is reduced to minimum and growth remains uniform from crop to crop. If wheat available for broiler feeds differ significantly in their nutrient composition and result in a variability of 13 – 14% in feed intake and live weight gain of broilers, this could result in a significant loss in production, e.g., decrease in production cycle per year, lack of uniformity within the crop and more rejects at the processing plants, hence economical loss to broiler producers. However, if variability within the flock is reduced, this would help poultry producers to accurately predict the target live weight of crop, improved uniformity and less rejections at the processing plants.

6.5. GENERAL CONCLUSIONS AND RECOMMENDATIONS

- The nutrient composition (starch, CP and soluble NSP contents) of currently available wheat samples for broiler feeds in the UK varies significantly. This variability in the nutrient composition and specifically starch and protein contents, is mainly due to the growing location of wheat crop. To improve the consistency of the finished feed, the poultry feed industry needs to know this variability in nutrient composition before the diet manufacture.

- Growing site of wheat crop affects the nutrient composition, bioavailability of nutrients and AME of wheat. The possible influence of growing site should be considered when selecting wheat varieties grown at different locations. Further work investigating the factors affecting growing condition would help in determining the nutritive value of wheat for broilers.

- Specific weight is not the good predictor of feeding value of wheat for broilers. Therefore, the feed industry would require some robust methods to determine the nutritive value of wheat before accepting a load of wheat at the feed mill. A rapid qualitative test to determine the nutrient composition would help the feed industry in selection of wheat samples for broiler feeds.

- There are differences in AME content of currently available wheat samples, and these differences are not predicted by the conventional nutrient analyses of wheat. The feed industry needs to develop robust *in vitro* methods to predict the AME of wheat for broiler chickens.

- The addition of xylanase was only beneficial on wheats with low AME and did not improve high AME wheats. The supplementation of xylanase to wheat-based diets and its beneficial effects are associated with the NSP content of wheat samples. Robust techniques to determine the NSP content of wheat samples would help to fully utilise the beneficial effects of exogenous xylanase in wheat-based diets and save cost of adding xylanase to high AME wheats.

- There are large differences in feed intake and growth rate of broilers fed different wheat samples and differences in growth performance are not related to the nutrient composition and the AME of wheat.

- The *in vivo* rate of starch digestion differs significantly between wheat samples with similar proximate composition and starch content. The rate of starch digestion may be the primary factor in determining the causes of large differences in voluntary feed intake and growth rate of broilers fed different wheat samples. Further studies are required to determine the *in vivo* rate of starch digestion in broilers fed large number of wheat samples and investigation of factors affecting the rate of starch digestion.

6.6. FUTURE PROSPECTS OF WHEAT IN POULTRY FEED FORMULATIONS

Feed is the major cost in poultry meat production and contributes up to 65% of cost/kg of live weight of broiler chicken. The volatile prices of raw ingredients used in the broiler feeds could add further cost to poultry producers. However, due to its low-cost available energy and high nutritional value, wheat is the favourable raw ingredient in current broiler diets in the UK and many parts of the world. The accurate knowledge of nutrient composition of currently available wheat samples for the broiler feeds and the determination of factors influencing its nutritional value is vital. In order to use wheat at high inclusion levels in the broiler feed formulations, the poultry feed industry should continue developing strategies to determine the actual feeding value of wheat for broilers. This would require robust analytical techniques to determine the nutrient composition of wheat arriving at the feed mill. Additionally, further research would need to develop *in vitro* techniques to predict the AME of currently available wheat samples.

The influence of growing condition on nutrient composition of wheat, bioavailability of nutrients and AME require further work. Factors affecting growing condition would help to understand the variability in nutritive value of wheat for broilers. Future studies should consider determining certain growing conditions (e.g., rainfall, climate, soil type) which may influence the nutritional composition of wheat. This will require joint work by agronomist and poultry nutritionist to develop strategies to select wheat varieties of high nutritive values for broilers.

The segregation of wheats into low and high AME based on their NSP content could help the feed industry to select wheats of higher feeding value. The selection of high AME wheats in broiler feed formulations would be advantageous in the least cost formulation. However, the separation of wheats into low and high AME require commercial modelling and investment in installation of additional silos at the feed mills. If using high AME wheats could bring cost benefit to feed formulations, then the future commercial planning could be modified considering the added value of additional wheat silos at the mill. Moreover, the application and dose of exogenous xylanase may also be adjusted based on the NSP

content and the AME value of wheat. This would benefit to improve the AME content of low energy wheats and also save cost of adding xylanase to wheats with high AME value.

A simple cost benefit analysis between high and low energy wheats is summarised below and indicates potential value to the wheat grower and the feed manufacturer of using a high energy wheat.

The model is based on using a wheat with AMEn value of 13 MJ/kg, a price for the wheat of £164.50/tonne (t), and the wheat being included in the diet at 69%. The diet is a standard commercial broiler finisher type feed and the final diet cost is £279/t. If the AMEn of wheat is reduced to 12.5 MJ/kg, the cost of the reformulated diet increases to £287/t, an additional cost of £8.00 to produce a diet with a similar specification. However, if high energy wheat with AMEn value of 13.5 MJ/kg is used, this would result in with a saving of £8.00 against the standard. This example indicates that there is a simple saving of £8.00 by using 0.5MJ/kg high energy wheat in the least cost formulation and this equates to a value equivalent to £1.60 per 0.1 MJ/kg of AME. If half of the value is shared with the wheat grower, the farmer could possibly get extra £5.80 premium per tonne of wheat, leaving a similar amount for the feed manufacturer to invest in additional handling facilities at the mill.

Wheat contributes a significant amount of protein to current poultry diets, e.g., If wheat with average CP content of 10% is included at 65%, it contributes 6.5% CP of finished feed. In the current climate of continued evaluation of raw materials, their nutrients and questioning of protein sources being included in poultry diets (e.g. soya), the contribution of protein from wheat in the diet will potentially become more valuable. In recent years, cultivation of high yielding feed wheat varieties has increased, however, as the yield of conventional feed wheat varieties increases, the average protein content reduces. Therefore, it is pertinent that future feed wheat varieties may need to be higher in protein content as well as a good source of dietary energy.

REFERENCES

ABDOLLAHI, M., V. RAVINDRAN, T. WESTER, G. RAVINDRAN, and D. THOMAS. 2011. "Influence of Feed Form and Conditioning Temperature on Performance, Apparent Metabolisable Energy and Ileal Digestibility of Starch and Nitrogen in Broiler Starters Fed Wheat-Based Diet." *Animal Feed Science and Technology* 168 (1-2): 88-99.

ABDOLLAHI, M., V. RAVINDRAN, T. WESTER, G. RAVINDRAN, and D. THOMAS. 2013. "Influence of Pellet Diameter and Length on the Quality of Pellets and Performance, Nutrient Utilisation and Digestive Tract Development of Broilers Fed on Wheat-Based Diets." *British Poultry Science* 54 (3): 337-345.

ADEOLA, O. and M. R. BEDFORD. 2004. "Exogenous Dietary Xylanase Ameliorates Viscosity-Induced Anti-Nutritional Effects in Wheat-Based Diets for White Pekin Ducks (*Anas Platyrinchos Domesticus*)." British Journal of Nutrition 92 (1): 87-94.

ADEOLA, O. and A. COWIESON. 2011. "Board-Invited Review: Opportunities and Challenges in using Exogenous Enzymes to Improve Nonruminant Animal Production." *Journal of Animal Science* 89 (10): 3189-3218.

AHDB (Agriculture and Horticulture Development Board) 2015. AHDB Recommended list 2015/2016 for Cereals and Oilseeds. [Online]. Warwickshire, UK. https://cereals.ahdb.org.uk/media/576554/recl1516-recommended-list-2015-2016-summer-edition.pdf [Accessed: Jan 15, 2016].

AHDB (Agriculture and Horticulture Development Board) 2016. AHDB Recommended List 2016/17 for Cereal and Oilseeds. [Online]. Warwickshire, UK. https://cereals.ahdb.org.uk/media/883980/1-RLDL_2016-17_Winter_Wheat_RL-V6.pdf [Accessed: Jan 15, 2016].

AHDB (Agriculture and Horticulture Development Board) 2017. AHDB Recommended List 2017/18 for Cereal and Oilseeds. [Online]. Warwickshire, UK. https://cereals.ahdb.org.uk/media/800462/lr-ahdb-recommended-list-2017-18.pdf [Accessed: Nov 22, 2017].

AHDB (Agriculture and Horticulture Development Board) 2018. Poultry Pocketbook 2018. [Online].https://pork.ahdb.org.uk/media/275384/poultry-pocketbook-2018.pdf [Accessed: June 25, 2019].

AHDB (Agriculture and Horticulture Development Board) 2019. AHDB Cereal and Oilseeds Early UK Supply and Demands Estimates. [Online]. Warwickshire, UK. https://cereals.ahdb.org.uk/media/1445091/AHDB-Early-Balance-Sheet-18-19.pdf [Accessed: July 18, 2019].

AHUJA, G., S. JAISWAL, and R. N. CHIBBAR. 2013. "Starch Biosynthesis in Relation to Resistant Starch." In *Resistant Starch: Sources, Applications and Health Benefits,* edited by Yong-Cheng. Shi and Clodualdo C. Maningat, 1-22. Chichester, UK: Wiley.

ALMIRALL, M. and E. ESTEVE-GARCIA. 1994. "Rate of Passage of Barley Diets with Chromium Oxide: Influence of Age and Poultry Strain and Effect of β-Glucanase Supplementation." *Poultry Science* 73 (9): 1433-1440.

AMERAH, A., V. RAVINDRAN, R. LENTLE, and D. THOMAS. 2007a. "Feed Particle Size: Implications on the Digestion and Performance of Poultry." World's Poultry Science Journal 63 (03): 439-455.

AMERAH, A., V. RAVINDRAN, R. LENTLE, and D. THOMAS. 2007b. "Influence of Feed Particle Size and Feed Form on the Performance, Energy Utilization, Digestive Tract Development, and Digesta Parameters of Broiler Starters." *Poultry Science* 86 (12): 2615-2623.

AMERAH, A. and V. RAVINDRAN. 2008. "Influence of Method of Whole-Wheat Feeding on the Performance, Digestive Tract Development and Carcass Traits of Broiler Chickens." Animal Feed Science and Technology 147 (4): 326-339.

AMERAH, A., V. RAVINDRAN, and R. LENTLE. 2009a. "Influence of Insoluble Fibre and Whole Wheat Inclusion on the Performance, Digestive Tract Development and Ileal Microbiota Profile of Broiler Chickens." *British Poultry Science* 50 (3): 366-375.

AMERAH, A., V. RAVINDRAN, and R. LENTLE. 2009b. "Influence of Wheat Hardness and Xylanase Supplementation on the Performance, Energy Utilisation, Digestive Tract Development and Digesta Parameters of Broiler Starters." *Animal Production Science* 49 (1): 71-78. doi:10.1071/EA08162

AMERAH, A. M. 2015. "Interactions between Wheat Characteristics and Feed Enzyme Supplementation in Broiler Diets." *Animal Feed Science and Technology* 199: 1-9.

ANGKANAPORN, K., M. CHOCT, W. L. BRYDEN, E. F. ANNISON, and G. ANNISON. 1994. "Effects of Wheat Pentosans on Endogenous Amino Acid Losses in Chickens." *Journal of the Science of Food and Agriculture* 66 (3): 399-404.

ANNISON, G. 1990. "Polysaccharide Composition of Australian Wheats and the Digestibility of their Starches in Broiler Chicken Diets." *Australian Journal of Experimental Agriculture* 30 (2): 183-186.

ANNISON, G. 1991. "Relationship between the Levels of Soluble Nonstarch Polysaccharides and the Apparent Metabolizable Energy of Wheats Assayed in Broiler Chickens." *Journal of Agricultural and Food Chemistry* 39 (7): 1252-1256.

ANNISON, G. and M. CHOCT. 1991. "Anti-Nutritive Activities of Cereal Non-Starch Polysaccharides in Broiler Diets and Strategies Minimizing their Effects." *World's Poultry Science Journal* 47 (03): 232-242.

ANNISON, G. 1993. "The Role of Wheat Non-Starch Polysaccharides in Broiler Nutrition." *Australian Journal of Agricultural Research* 44 (3): 405-422.

AOAC. 2012. *Official Methods of Analysis*, 19th edition. Gaithersburg, USA: Association of Official Analytical Chemists.

AUSTIN, S., J. WISEMAN, and A. CHESSON. 1999. "Influence of Non-Starch Polysaccharides Structure on the Metabolisable Energy of UK Wheat Fed to Poultry." *Journal of Cereal Science* 29 (1): 77-88.

AVIAGEN. 2014a. "Ross 308 Broiler: Nutrition Specifications." http://en.aviagen.com/assets/Tech_Center/Ross_Broiler/Ross308BroilerNutritionSpecs2014-EN.pdf [Accessed: December 2015; June 2016; November 2017].

AVIAGEN. 2014b. "Ross 308 Broiler: Performance Objectives." http://en.aviagen.com/tech-center/Ross-308-Broiler-PO2014-EN.pdf [Accessed: June 2016].

BAILEY, R. W. 1973."Chemistry and Biochemistry of Herbage." edited by G.W. Butler. London, UK: Academic Press.

BALDWIN, P. M. 2001. "Starch granule-associated Proteins and Polypeptides: A Review." *Starch/Stärke* 53 (10): 475-503.

BALDWIN, P., C. MELIA, and M. DAVIES. 1997. "The Surface Chemistry of Starch Granules Studied by Time-of-Flight Secondary Ion Mass Spectrometry." *Journal of Cereal Science* 26 (3): 329-346.

BALL, M. E., B. OWENS, and K. J. MCCRACKEN. 2013a. "Chemical and Physical Predictors of the Nutritive Value of Wheat in Broiler Diets." *Asian-Australasian Journal of Animal Sciences* 26 (1): 97-107. doi:10.5713/ajas.2012.12178

BALL, M. E., B. OWENS, and K. J. MCCRACKEN. 2013b. "The Effect of Variety and Growing Conditions on the Chemical Composition and Nutritive Value of Wheat for Broilers." *Asian-Australasian Journal of Animal Sciences* 26 (3): 378-385. doi:10.5713/ajas.2012.12180

BARLOW, K., M. BUTTROSE, D. SIMMONDS, and M. VESK. 1973. "The Nature of the Starch-Protein Interface in Wheat Endosperm." *Cereal Chemistry* 50 (4): 443-454.

BARNES, P. 1982. "Lipid Composition of Wheat Germ and Wheat Germ Oil." *Fette, Seifen, Anstrichmittel* 84 (7): 256-269.

BECHTEL, D. B., I. ZAYAS, L. KALEIKAU, and Y. POMERANZ. 1990. "Size-Distribution of Wheat Starch Granules during Endosperm Development." *Cereal Chemistry* 67 (1): 59-63.

BEDFORD, M. R. 1996. "The Effect of Enzymes on Digestion." *The Journal of Applied Poultry Research* 5 (4): 370-378.

BEDFORD, M. R. 2000. "Exogenous Enzymes in Monogastric nutrition—their Current Value and Future Benefits." *Animal Feed Science and Technology* 86 (1): 1-13.

BEDFORD, M. R. and H. L. CLASSEN. 1992. "Reduction of Intestinal Viscosity through Manipulation of Dietary Rye and Pentosanase Concentration is effected through Changes in the Carbohydrate Composition of the Intestinal Aqueous Phase and Results in Improved Growth Rate and Food Conversion Efficiency of Broiler Chicks." *The Journal of Nutrition* 122 (3): 560-569.

BEDFORD, M. 2006. "Effect of Non-Starch Polysaccharidases on Avian Gastrointestinal Function." *Avian Gut Function in Health and Disease.* Oxon: Wallingford: 159-170.

BEDFORD, M. 1995. "Mechanism of Action and Potential Environmental Benefits from the use of Feed Enzymes." *Animal Feed Science and Technology* 53 (2): 145-155.

BEDFORD, M. and A. COWIESON. 2012. "Exogenous Enzymes and their Effects on Intestinal Microbiology." *Animal Feed Science and Technology* 173 (1-2): 76-85.

BEDFORD, M. and A. MORGAN. 1996. "The use of Enzymes in Poultry Diets." *World's Poultry Science Journal* 52 (1): 61-68. doi:10.1079/WPS19960007

BEDFORD, M. and H. SCHULZE. 1998. "Exogenous Enzymes for Pigs and Poultry." *Nutrition Research Reviews* 11 (01): 91-114.

BEDFORD, M., T. SCOTT, F. SILVERSIDES, H. CLASSEN, M. SWIFT, and M. PACK. 1998. "The Effect of Wheat Cultivar, Growing Environment, and Enzyme Supplementation on Digestibility of Amino Acids by Broilers." *Canadian Journal of Animal Science* 78 (3): 335-342.

BEDFORD M. R., ed. 1997. "Reduced Viscosity of Intestinal Digesta and Enhanced Nutrient Digestibility in Chickens Given Exogenous Enzymes." In *Enzyme in Poultry and Swine Nutrition*, edited by R. R. Marquardt and Z. Han, 161-173. Ottawa, Canada: International Development Research Centre.

BERG, J. M., J. L. TYMOCZKO, and L. STRYER. 2002. "Protein Structure and Function." In *Biochemistry*, 5th edition, 159-173: New York, W H Freeman and Company.

BONNIN, E., A. LE GOFF, L. SAULNIER, M. CHAURAND, and J. THIBAULT. 1998. "Preliminary Characterisation of Endogenous Wheat Arabinoxylan-Degrading Enzymic Extracts." *Journal of Cereal Science* 28 (1): 53-62.

BRENES, A., M. SMITH, W. GUENTER, and R. R. MARQUARDT. 1993. "Effect of Enzyme Supplementation on the Performance and Digestive Tract Size of Broiler Chickens Fed Wheat- and Barley-Based Diets." *Poultry Science* 72 (9): 1731-1739.

BPC (British Poultry Council) Antibiotics Stewardship Report 2019. [Online] https://www.britishpoultry.org.uk/bpc-antibiotics-report-2019/ [Accessed: Dec 15, 2019].

BULÉON, A., P. COLONNA, V. PLANCHOT, and S. BALL. 1998. "Starch Granules: Structure and Biosynthesis." *International Journal of Biological Macromolecules* 23 (2): 85-112.

CABAHUG, S., V. RAVINDRAN, P. SELLE, and W. BRYDEN. 1999. "Response of Broiler Chickens to Microbial Phytase Supplementation as Influenced by Dietary Phytic Acid and Non-Phytate Phosphorus Contents. I. Effects on Bird Performance and Toe Ash." *British Poultry Science* 40 (5): 660-666.

CARRÉ, B. 2004. "Causes for Variation in Digestibility of Starch among Feedstuffs." *World's Poultry Science Journal* 60 (1): 76-89.

CARRÉ, B., N. MULEY, J. GOMEZ, F.-. OURY, E. LAFFITTE, D. GUILLOU, and C. SIGNORET. 2005. "Soft Wheat Instead of Hard Wheat in Pelleted Diets Results in High Starch Digestibility in Broiler Chickens." *British Poultry Science* 46 (1) (02/01): 66-74. doi:10.1080/00071660400023847

CARRÉ, B., A. IDI, S. MAISONNIER, J. MELCION, F. OURY, J. GOMEZ, and P. PLUCHARD. 2002. "Relationships between Digestibilities of Food Components and Characteristics of Wheats (Triticum Aestivum) Introduced as the Only Cereal Source in a Broiler Chicken Diet." *British Poultry Science* 43 (3): 404-415.

CARRÉ, B., S. MIGNON-GRASTEAU, A. PÉRON, H. JUIN, and D. BASTIANELLI. 2007. "Wheat Value: Improvements by Feed Technology, Plant Breeding and Animal Genetics." *World's Poultry Science Journal* 63 (04): 585-596.

CHOCT, M. 2006. "Enzymes for the Feed Industry: Past, Present and Future." *World's Poultry Science Journal* 62 (01): 5-16.

CHOCT, M. 2015. "Feed Non-Starch Polysaccharides for Monogastric Animals: Classification and Function." *Animal Production Science* 55 (12): 1360-1366.

CHOCT, M., M. SINLAE, R. A. M. AL-JASSIM, and D. PETTERSSON. 2006. "Effects of Xylanase Supplementation on between -Bird Variation in Energy Metabolism and the Number of Clostridium Perfringens in Broilers Fed a Wheat Based Diet." *Australian Journal of Agriculture Research* (57): 1017-1021.

CHOCT, M., R. J. HUGHES, J. WANG, M. R. BEDFORD, A. MORGAN, and G. ANNISON. 1996. "Increased Small Intestine Fermentation is Partly Responsible for the Anti-Nutritive Activity of Non-Starch Polysaccharides in Chickens." *Poultry Science* (37): 609-621.

CHOCT, M. and G. ANNISON. 1990. "Anti-nutritive Activity of Wheat Pentosans in Broiler Diets." *British Poultry Science* 31 (4): 811-821.

CHOCT, M. and G. ANNISON. 1992a. "Anti-nutritive Effect of Wheat Pentosans in Broiler Chickens: Roles of Viscosity and Gut Microflora." *British Poultry Science* 33 (4): 821-834.

CHOCT, M. and G. ANNISON. 1992b. "The Inhibition of Nutrient Digestion by Wheat Pentosans." *British Journal of Nutrition* 67 (01): 123-132.

CHOCT, M. and R. HUGHES. 1999. "Chemical and Physical Characteristics of Grains Related to Variability in Energy and Amino Acid Availability in Poultry." *Australian Journal of Agricultural Research* 50 (5): 689-702.

CHOCT, M., R. HUGHES, and G. ANNISON. 1999. "Apparent Metabolisable Energy and Chemical Composition of Australian Wheat in Relation to Environmental Factors." *Australian Journal of Agricultural Research* 50 (4): 447-451.

CHOCT, M. 1997. "Feed Non-Starch Polysaccharides: Chemical Structures and Nutritional Significance." *Feed Milling International* 191 (June issue): 13-26.

CHOCT, M., R. J. HUGHES, R. P. TRIMBLE, K. ANGKANAPORN, and G. ANNISON. 1995. "Non-Starch Polysaccharide-Degrading Enzymes Increase the Performance of Broiler Chickens Fed Wheat of Low Apparent Metabolizable Energy." *The Journal of Nutrition* 125 (3): 485.

CLASSEN, H. L., T. A. SCOTT, G. G. IRISH, P. HUCL, M. SWIFT, and M. R. BEDFORD. 1995. "The Relationship of Chemical and Physical Measurements to the Apparent Metabolisable Energy (AME) of Wheat when Fed to Broiler Chickens with and without a Wheat Enzyme Source." In *Proceedings on the Second European Symposium on Feed Enzymes*, *Oct 1995, 65-69*.

CLEEMPUT, G., W. BLEUKX, M. VAN OORT, M. HESSING, and J. DELCOUR. 1995. "Evidence for the Presence of Arabinoxylan Hydrolysing Enzymes in European Wheat Flours." *Journal of Cereal Science* 22 (2): 139-145.

COLLETT, S. R. 2012. "Nutrition and Wet Litter Problems in Poultry." *Animal Feed Science and Technology* 173 (1-2): 65-75.

CORNELL, H. J., and A. W. HOVELING. 1998. "*Wheat: Chemistry and Utilization.*" Pennsylvania: Technomic Publication.

COWIESON, A., T. ACAMOVIC, and M. BEDFORD. 2004. "The Effects of Phytase and Phytic Acid on the Loss of Endogenous Amino Acids and Minerals from Broiler Chickens." *British Poultry Science* 45 (1): 101-108.

COWIESON, A. and M. BEDFORD. 2009. "The Effect of Phytase and Carbohydrase on Ileal Amino Acid Digestibility in Monogastric Diets: Complimentary Mode of Action?" *World's Poultry Science Journal* 65 (4): 609-624.

COWIESON, A. J. and H. V. M. O'NEILL. 2013. "Effects of Exogenous Xylanase on Performance, Nutrient Digestibility and Caecal Thermal Profiles of Broilers Given Wheat-Based Diets." *British Poultry Science* 54 (3): 346-354.

COWIESON, A. J., M. HRUBY, and E. E. M. PIERSON. 2006. "Evolving Enzyme Technology: Impact on Commercial Poultry Nutrition." *Nutrition Research Reviews* (19): 90-103.

CROWE, T. C., S. A. SELIGMAN, and L. COPELAND. 2000. "Inhibition of Enzymic Digestion of Amylose by Free Fatty Acids *in Vitro* Contributes to Resistant Starch Formation." *The Journal of Nutrition* 130 (8): 2006-2008.

CUI, R. and C. OATES. 1999. "The Effect of amylose–lipid Complex Formation on Enzyme Susceptibility of Sago Starch." *Food Chemistry* 65 (4): 417-425.

DELCOUR, J. and R. HOSENEY. ed. 2010. "Structure of Cereals." In *Principles of Cereal Science and Technology*, 3rd edition, 1-22. St. Paul, Minnesota: AACC International.

DEFRA (Department for Environment, Food and Rural Affairs) 2019a. "UK Poultry Slaughterings, Weights and Poultry Meat Production - monthly dataset. " [Online]. https://www.gov.uk/government/statistics/poultry-and-poultry-meat-statistics [Accessed: Dec 17, 2019].

DEFRA (Department for Environment, Food and Rural Affairs) 2019b. UK Chick and Poultry Placings - monthly dataset. [Online] https://www.gov.uk/government/statistics/poultry-and-poultry-meat-statistics [Accessed: Dec 12, 2019].

EDNEY, M., G. CAMPBELL, and H. CLASSEN. 1989. "The Effect of β-Glucanase Supplementation on Nutrient Digestibility and Growth in Broilers Given Diets Containing Barley, Oat Groats or Wheat." *Animal Feed Science and Technology* 25 (1-2): 193-200.

EDWARDS, C. A., I. T. JOHNSON, and N. W. READ. 1988. "Do Viscous Polysaccharides Slow Absorption by Inhibiting Diffusion Or Convection?" *European Journal of Clinical Nutrition* 42 (4): 307-312.

EDWARDS, V. M.1995. "The Physiological Effect of Dietary Fibre". In *Dietary Fibre in Health and Disease*, edited by D. Kritchewsky and C. Bonfield, 58-71. St. Paul, Minnesota, USA: Eagen Press.

ENCYCLOPAEDIA BRITANNICA 2010. In *Cereal Processing*, edited by D. W Kent-Jones and R.P. Singh. [Online]. Encyclopaedia Britannica, Inc. https://www.britannica.com/technology/cereal-processing [Accessed: June 10, 2016].

ENGLYST, H. N., M. E. QUIGLEY, and G. J. HUDSON. 1994. "Determination of Dietary Fibre as Non-Starch Polysaccharides with gas–liquid Chromatographic, High-Performance Liquid Chromatographic or Spectrophotometric Measurement of Constituent Sugars." *Analyst* 119 (7): 1497-1509.

ENGLYST, H., S. BINGHAM, S. RUNSWICK, E. COLLINSON, and J. CUMMINGS. 1989. "Dietary Fibre (non-starch Polysaccharides) in Cereal Products." *Journal of Human Nutrition and Dietetics* 2 (4): 253-271.

ENGLYST, KLAUS N., GEOFFREY J. HUDSON, and HANS N. ENGLYST. ed. 2000. *Starch Analysis in Food*: Wiley Online Library. doi.org/10.1002/9780470027318.a1029.

ENGLYST, H. N. and J. H. CUMMINGS. 1988. "Improved Method for Measurement of Dietary Fiber as Non-Starch Polysaccharides in Plant Foods." *Journal - Association of Official Analytical Chemists* 71 (4) (Jul-Aug): 808-814.

EVERS, A., and D. BECHTEL. 1988."Microscopic Structure of the Wheat Grain." *In Wheat: Chemistry and Technology,* 3rd edition, edited by Y. POMERANZ, 47-95, St. Paul, MN: Association of American Cereal Chemists.

EUROSTAT 2018a. "Agriculture, Forestry and Fishery Statistics. 2018 edition. " [Online]. https://ec.europa.eu/eurostat/statistics-explained/index.php/Agricultural_production_-_crops#Cereals [Accessed: May 10, 2019].

EUROSTAT 2018b. "Agriculture Production- Livestock and Meat Statistics 2018. " [Online]. https://ec.europa.eu/eurostat/statisticsexplained/index.php?title=Agricultural_production_-_livestock_and_meat#Poultry [Accessed: December 10, 2019].

EUROSTAT 2018c. "Poultry Meat Production 2018 Statistics." [Online]. https://ec.europa.eu/eurostat/web/products-eurostat-news/-/DDN-20190325-1 [Accessed: December 10, 2019].

EUROSTAT 2019. "Production of Meat: Poultry, Eurostat Statistics." [Online]. https://ec.europa.eu/eurostat/databrowser/view/tag00043/default/table?lang=en [Accessed: May 23, 2019].

FAO (FOOD and AGRICULTURE ORGANISATION OF THE UNITED NATIONS) 2003. *Food Energy Methods of Analysis and Conversion Factors*.

FAO (FOOD and AGRICULTURE ORGANISATION OF THE UNITED NATIONS) 2019. "FAO Cereal Supply and Demand Briefs." [Online]. http://www.fao.org/worldfoodsituation/csdb/en/ [Accessed on April 15, 2019].

FINNEY, D. J. ed. 1978. *Statistical Method in Biological Assay*. 3rd edition. London and High Wycombe: Charles Griffin & Co.

GAO, F., Y. JIANG, G. H. ZHOU, and Z. K. HAN. 2008. "The Effects of Xylanase Supplementation on Performance, Characteristics of the Gastrointestinal Tract, Blood Parameters and Gut Microflora in Broilers Fed on Wheat-Based Diets." *Animal Feed Science and Technology* 142 (1–2) (4/15): 173-184.

GLENN, G., F. YOUNCE, and M. PITTS. 1991. "Fundamental Physical Properties Characterizing the Hardness of Wheat Endosperm." *Journal of Cereal Science* 13 (2): 179-194.

GONZÁLEZ-ALVARADO, J., E. JIMÉNEZ-MORENO, D. GONZÁLEZ-SÁNCHEZ, R. LÁZARO, and G. MATEOS. 2010. "Effect of Inclusion of Oat Hulls and Sugar Beet Pulp in the Diet on Productive Performance and Digestive Traits of Broilers from 1 to 42 Days of Age." *Animal Feed Science and Technology* 162 (1-2): 37-46.

GRAY, G. M. 1992. "Starch Digestion and Absorption in Nonruminants." *The Journal of Nutrition* 122 (1): 172-177.

GRAYBOSCH, R. A. 1998. "Waxy Wheats: Origin, Properties, and Prospects." *Trends in Food Science & Technology* 9 (4): 135-142.

GREENWELL, P. 1986. "A Starch Granule Protein Associated with Endosperm Softness in Wheat." *Cereal Chemistry.* 63: 379-380.

GRUNDAS, S. T. 2003. "Grain Structure of Wheat and Wheat-Based Products." In *Encyclopaedia of Food Sciences and Nutrition*, edited by B. Caballero, 2nd edition, 6137-6146. Oxford: Academic Press. doi:https://doi.org/10.1016/B0-12-227055-X/01286-4

GUTIERREZ DEL ALAMO, A., M. VERSTEGEN, L. DEN HARTOG, P. PEREZ DE AYALA, and M. VILLAMIDE. 2009a. "Wheat Starch Digestion Rate Affects Broiler Performance." *Poultry Science* 88 (8): 1666-1675.

GUTIERREZ DEL ALAMO, A., P. PÉREZ DE AYALA, L. DEN HARTOG, M. VERSTEGEN, and M. VILLAMIDE. 2009b. "Wheat Starch Digestion Rate in Broiler Chickens is Affected by Cultivar but Not by Wheat Crop Nitrogen Fertilisation." *British Poultry Science* 50 (3): 341-349.

GUTIERREZ DEL ALAMO, A., M. W. VERSTEGEN, L. A. DEN HARTOG, P. PEREZ DE AYALA, and M. J. VILLAMIDE. 2008a. "Effect of Wheat Cultivar and Enzyme Addition to Broiler Chicken Diets on Nutrient Digestibility, Performance, and Apparent Metabolizable Energy Content." *Poultry Science* 87 (4): 759-767.

GUTIERREZ-ALAMO, A., P. P. DE AYALA, M. VERSTEGEN, L. DEN HARTOG, and M. VILLAMIDE. 2008b. "Variability in Wheat: Factors Affecting its Nutritional Value." *World's Poultry Science Journal* 64 (1): 20-39.

HAGBERG, S. 1960. "A Rapid Method for Determining Alpha-Amylase Activity." *Cereal Chem* 37 (2): 218-222.

HUANG, K., V. RAVINDRAN, X. LI, and W. BRYDEN. 2005. "Influence of Age on the Apparent Ileal Amino Acid Digestibility of Feed Ingredients for Broiler Chickens." British Poultry Science 46 (2): 236-245.

HENRY, R. J. 1985. "A Comparison of the non-starch Carbohydrates in Cereal Grains." *Journal of the Science of Food and Agriculture* 36 (12): 1243-1253.

HETLAND, H., M. CHOCT, and B. SVIHUS. 2004. "Role of Insoluble Non-Starch Polysaccharides in Poultry Nutrition." *World's Poultry Science Journal* 60 (4): 415-422. doi:10.1079/WPS200325

HETLAND, H., B. SVIHUS, and Å. KROGDAHL. 2003. "Effects of Oat Hulls and Wood Shavings on Digestion in Broilers and Layers Fed Diets Based on Whole or Ground Wheat." *British Poultry Science* 44 (2): 275-282.

HETLAND, H., B. SVIHUS, and V. OLAISEN. 2002. "Effect of Feeding Whole Cereals on Performance, Starch Digestibility and Duodenal Particle Size Distribution in Broiler Chickens." *British Poultry Science* 43 (3): 416-423.

HETLAND, H., A. UHLEN, K. VIKEN, T. KREKLING, and B. SVIHUS. 2007. "Hagberg Falling Number and the Nutritional Value of Wheat in Broiler Chicken Diets." *British Poultry Science* 48 (1): 12-20. doi:10.1080/00071660601045763

HETLAND, H. and B. SVIHUS. 2001. "Effect of Oat Hulls on Performance, Gut Capacity and Feed Passage Time in Broiler Chickens." *British Poultry Science* 42 (3): 354-361.

HEW, L., V. RAVINDRAN, Y. MOLLAH, and W. BRYDEN. 1998. "Influence of Exogenous Xylanase Supplementation on Apparent Metabolisable Energy and Amino Acid Digestibility in Wheat for Broiler Chickens." *Animal Feed Science and Technology* 75 (2): 83-92.

HEYNE, E. G., ed. 1987. *Wheat and Wheat Improvement*. 2nd edition. Madison, WI, USA: American Society of Agronomy.

HILL, F. and D. ANDERSON. 1958. "Comparison of Metabolizable Energy and Productive Energy Determinations with Growing Chicks." *Journal of Nutrition* 64: 587-603.

HIZUKURI, S., Y. TAKEDA, J. ABE, I. HANASHIRO, G. MATSUNOBU, and H. KIYOTA. 1997. "Analytical Developments: Molecular and Microstructural Characterization." In *Starch: Structure and functionality*, edited by P. J. FRAZIER, P. RICHMOND and A. M. DONALD, 121–128: London, UK: Royal Society of Chemistry.

HIZUKURI, S. 1986. "Polymodal Distribution of the Chain Lengths of Amylopectins, and its Significance." *Carbohydr. Res.* 147: 342-347.

HOOVER, R. 1995. "Starch Retrogradation." *Food Reviews International* 11 (2): 331-346. doi:10.1080/87559129509541044

HUANG, K., X. LI, V. RAVINDRAN, and W. BRYDEN. 2006. "Comparison of Apparent Ileal Amino Acid Digestibility of Feed Ingredients Measured with Broilers, Layers, and Roosters." *Poultry Science* 85 (4): 625-634.

HUANG, K., V. RAVINDRAN, X. LI, and W. BRYDEN. 2005. "Influence of Age on the Apparent Ileal Amino Acid Digestibility of Feed Ingredients for Broiler Chickens." *British Poultry Science* 46 (2): 236-245.

HUANG, K. H., V. RAVINDRAN, X. LI, G. RAVINDRAN, and W. L. BRYDEN. 2007. "Apparent Ileal Digestibility of Amino Acids in Feed Ingredients Determined with Broilers and Layers." *Journal of the Science of Food and Agriculture* 87 (1): 47-53.

HUMER, E., C. SCHWARZ, and K. SCHEDLE. 2014. "Phytate in Pig and Poultry Nutrition." *Journal of Animal Physiology & Animal Nutrition* 99 (4): 605-625. doi:10.1111/jpn.12258

JENSEN, L. S., L. H. MERRILL, C. REDDY, and J. MCGINNIS. 1962. "Observations on Eating Patterns and Rate of Food Passage of Birds Fed Pelleted and Unpelleted Diets." *Poultry Science* 41 (5): 1414-1419.

JMARES, D. and B. STONE. 1973. "Studies on Wheat Endosperm II. Properties of the Wall Components and Studies on their Organization in the Wall." *Australian Journal of Biological Sciences* 26 (4): 813-830.

JOHNSON, I. T. and J. M. GEE. 1981. "Effect of Gel-Forming Gums on the Intestinal Unstirred Layer and Sugar Transport in Vitro." *Gut* 22 (5): 398-403. doi:10.1136/gut.22.5.398

KADIM, I., P. MOUGHAN, and V. RAVINDRAN. 2002. "Ileal Amino Acid Digestibility Assay for the Growing Meat Chicken--Comparison of Ileal and Excreta Amino Acid Digestibility in the Chicken." *British Poultry Science* 43 (4): 588-597. doi: 10.1080/0007166022000004507

KARUNARATNE, N., D. ABBOTT, P. HUCL, R. CHIBBAR, C. POZNIAK, and H. CLASSEN. 2018. "Starch Digestibility and Apparent Metabolizable Energy of Western Canadian Wheat Market Classes in Broiler Chickens." *Poultry Science* 97 (8): 2818-2828. doi.org/10.3382/ps/pey115

KENT, N. L., and A. D. Evers. ed. 1994. *Technology of Cereals: An Introduction for Students of Food Science and Agriculture.* 4th edition. Oxford: Elsevier Science.

KIARIE, E., L. ROMERO, and V. RAVINDRAN. 2014. "Growth Performance, Nutrient Utilization, and Digesta Characteristics in Broiler Chickens Fed Corn or Wheat Diets without or with Supplemental Xylanase." *Poultry Science* 93 (5): 1186-1196.

KIM, J., B. MULLAN, P. SIMMINS, and J. PLUSKE. 2003. "Variation in the Chemical Composition of Wheats Grown in Western Australia as Influenced by Variety, Growing Region, Season, and Post-Harvest Storage." *Australian Journal of Agricultural Research* 54 (6): 541-550.

KIM, J., B. MULLAN, P. SIMMINS, and J. PLUSKE. 2004. "Effect of Variety, Growing Region and Growing Season on Digestible Energy Content of Wheats Grown in Western Australia for Weaner Pigs." Animal Science 78 (1): 53-60.

KNUDSEN, K. E. B. 1997. "Carbohydrate and Lignin Contents of Plant Materials used in Animal Feeding." *Animal Feed Science and Technology* 67 (4): 319-338.

LASZTITY, R. ed. 1995. *The Chemistry of Cereal Proteins.* 2nd edition. NY: CRC Press.

LEESON, S., and J. D. SUMMERS. ed. 2005. *Commercial Poultry Nutrition*. 3rd edition, Nottingham: Nottingham University Press.

LEMME, A., V. RAVINDRAN, and W. BRYDEN. 2004. "Ileal Digestibility of Amino Acids in Feed Ingredients for Broilers." *World's Poultry Science Journal* 60 (04): 423-438.

LIU, S., P. SELLE, and A. COWIESON. 2013a. "Influence of Conditioning Temperatures on Amino Acid Digestibility Coefficients at Four Small Intestinal Sites and their Dynamics with Starch and Nitrogen Digestion in Sorghum-Based Broiler Diets." *Animal Feed Science and Technology* 185 (1-2): 85-93.

LIU, S., P. SELLE, and A. COWIESON. 2013b. "Influence of White-and Red-Sorghum Varieties and Hydrothermal Component of Steam-Pelleting on Digestibility Coefficients of Amino Acids and Kinetics of Amino Acids, Nitrogen and Starch Digestion in Diets for Broiler Chickens." *Animal Feed Science and Technology* 186 (1-2): 53-63.

LIU, S., P. SELLE, and A. COWIESON. 2013c. "The Kinetics of Starch and Nitrogen Digestion Regulate Growth Performance and Nutrient Utilisation of Broilers Fed Coarsely Ground, Sorghum-Based Diets." *Animal Production Science* 53 (10): 1033-1040.

LIU, S., P. SELLE, A. KHODDAMI, T. ROBERTS, and A. COWIESON. 2014. "Graded Inclusions of Sodium Metabisulphite in Sorghum-Based Diets: II. Modification of Starch Pasting Properties in Vitro and Beneficial Impacts on Starch Digestion Dynamics in Broiler Chickens." *Animal Feed Science and Technology* 190: 68-78.

LONGSTAFF, M. and J. MCNAB. 1986. "Influence of Site and Variety on Starch, Hemicellulose and Cellulose Composition of Wheats and their Digestibilities by Adult Cockerels." *British Poultry Science* 27 (3): 435-449.

LUND, D. and K. J. LORENZ. 1984. "Influence of Time, Temperature, Moisture, Ingredients, and Processing Conditions on Starch Gelatinization." *Critical Reviews in Food Science and Nutrition* 20 (4): 249-273. doi:10.1080/10408398409527391

MACLEAN, W.C., J. M. HARNLY, J. CHEN, S. CHEVASSUS-AGNES, G. GILANI, G. LIVESEY, B. MATHIOUDAKIS, M. MUNOZ DE CHAVEZ, M.T. DEVASCONCELLOS,

and P. WARWICK. 2003. "Food energy - methods of analysis and conversion factors." Food and Agriculture Organization of the United Nations (FAO) Technical Workshop Report, vol. 77. Rome, Italy.

MacMASTERS, M. M., J. J. C., Hinton, and D. Bradbury. 1971. "Microscopic Structure and Composition of the Wheat Kernel." In *Wheat: Chemistry and Technology*, edited by Y. Pomeranz, 2nd edition, 51-113. St. Paul, Minnesota: American Association of Cereal Chemist.

MacRITCHIE, F. 1980. "Physicochemical Aspects of some Problems in Wheat Research." *Advances in Cereal Science and Technology (USA)* 3: 271-326.

MAGHIRANG, E. B. and F. E. DOWELL. 2003. "Hardness Measurement of Bulk Wheat by single-kernel Visible and near-infrared Reflectance Spectroscopy." *Cereal Chemistry* 80 (3): 316-322.

MAHASUKHONTHACHAT, K., P. SOPADE, and M. GIDLEY. 2010. "Kinetics of Starch Digestion and Functional Properties of Twin-Screw Extruded Sorghum." *Journal of Cereal Science* 51 (3): 392-401.

MARES, D.J., and B. STONE. 1973a. "Studies on Wheat Endosperm I. Chemical Composition and Ultrastructure of the Cell Walls." Australian Journal of Biological Sciences 26 (4): 793-812.

MARES, D.J., and B. STONE. 1973b. "Studies on Wheat Endosperm II. Properties of the Wall Components and Studies on their Organization in the Wall." Australian Journal of Biological Sciences 26 (4): 813-830.

MASEY-O'NEILL, H., M. SINGH, and A. COWIESON. 2014. "Effects of Exogenous Xylanase on Performance, Nutrient Digestibility, Volatile Fatty Acid Production and Digestive Tract Thermal Profiles of Broilers Fed on Wheat-Or Maize-Based Diet." British Poultry Science 55 (3): 351-359.

McALLISTER, T., R. PHILLIPPE, L. RODE, and K. CHENG. 1993. "Effect of the Protein Matrix on the Digestion of Cereal Grains by Ruminal Microorganisms." *Journal of Animal Science* 71 (1): 205-212.

McCRACKEN, K., M. BEDFORD, and R. STEWART. 2001. "Effects of Variety, the 1B/1R Translocation and Xylanase Supplementation on Nutritive Value of Wheat for Broilers." *British Poultry Science* 42 (5): 638-642.

McCRACKEN, K., C. PRESTON, and C. BUTLER. 2002. "Effects of Wheat Variety and Specific Weight on Dietary Apparent Metabolisable Energy Concentration and Performance of Broiler Chicks." *British Poultry Science* 43 (2): 253-260. doi:10.1080/00071660120121472

McCRACKEN, K. and G. QUINTIN. 2000. "Metabolisable Energy Content of Diets and Broiler Performance as Affected by Wheat Specific Weight and Enzyme Supplementation." *British Poultry Science* 41 (3): 332-342. doi:10.1080/713654927.

McKEVITH, B. 2004. "Nutritional Aspects of Cereals." *Nutrition Bulletin* 29 (2): 111-142.

McNAB, J. 1991. *Factors Affecting the Nutritive Value of Wheat for Poultry*. HGCA Project report no. 43. London: Home Grown Cereal Authority.

McNAB, J.M. 1996. "Factors Affecting the Energy Value of Wheat for Poultry." *World's Poultry Science Journal* 52 (1): 69-73.

METAYER, J., F. GROSJEAN, and J. CASTAING. 1993. "Study of Variability in French Cereals." *Animal Feed Science and Technology* 43 (1-2): 87-108.

METOFFICE 2019. "Met Office Hadley Centre Observations Datasets." [Online]. https://www.metoffice.gov.uk/hadobs/hadukp/data/download.htm [Accessed: March, 2019].

MOLLAH, Y., W. BRYDEN, I. WALLIS, D. BALNAVE, and E. ANNISON. 1983. "Studies on Low Metabolisable Energy Wheats for Poultry using Conventional and Rapid Assay Procedures and the Effects of Processing." *British Poultry Science* 24 (1): 81-89.

MORAN JR, E. 1982. "Starch Digestion in Fowl." *Poultry Science* 61 (7): 1257-1267.

MORELL, M., S. RAHMAN, S. ABRAHAMS, and R. APPELS. 1995. "The Biochemistry and Molecular Biology of Starch Synthesis in Cereals." *Functional Plant Biology* 22 (4): 647-660.

MORRISON, W. R. 1988. "Lipids in Cereal Starches: A Review." *Journal of Cereal Science* 8 (1): 1-15.

MORRISON, W. 1994."Wheat Lipids: Structure and Functionality." In *Wheat: Production, Properties and Quality,* edited by W. Bushuk and V.F. Rasper, 128-142. London: Springer, Boston, MA.

NIR, I., Y. TWINA, E. GROSSMAN, and Z. NITSAN. 1994. "Quantitative Effects of Pelleting on Performance, Gastrointestinal Tract and Behaviour of meat-type Chickens." British Poultry Science 35 (4): 589-602.

NIR, I., R. HILLEL, I. PTICHI, and G. SHEFET. 1995. "Effect of Particle Size on Performance. 3. Grinding Pelleting Interactions." Poultry Science 74 (5): 771-783.

NRC (National Research Council). 1994. *Nutrient Requirements of Poultry*. 9th edition Washington: National Academies Press.

OLUKOSI, O. and M. BEDFORD. 2019. "Comparative Effects of Wheat Varieties and Xylanase Supplementation on Growth Performance, Nutrient Utilization, Net Energy, and Whole-Body Energy and Nutrient Partitioning in Broilers at Different Ages." *Poultry Science* 98 (5): 2179-2188.

OLUKOSI, O. A., A. J. COWIESON, and O. ADEOLA. 2007. "Age-Related Influence of a Cocktail of Xylanase, Amylase, and Protease or Phytase Individually or in Combination in Broilers." *Poultry Science* 86 (1): 77-86.

ØRSKOV, E. and I. MCDONALD. 1979. "The Estimation of Protein Degradability in the Rumen from Incubation Measurements Weighted According to Rate of Passage." *The Journal of Agricultural Science* 92 (2): 499-503.

OSBORNE, T. B. 1907. *The Proteins of the Wheat Kernel*. Carnegie Institution of Washington.

PARSONS, C., L. POTTER, and R. BROWN JR. 1982. "Effects of Dietary Protein and Intestinal Microflora on Excretion of Amino Acids in Poultry." *Poultry Science* 61 (5): 939-946.

PASHA, I., F. ANJUM, and C. MORRIS. 2010. "Grain Hardness: A Major Determinant of Wheat Quality." *Food Science and Technology International* 16 (6): 511-522. doi: org/10.1177/1082013210379691

PETERSON, D. and R. FULCHER. 2001. "Variation in Minnesota HRS Wheats: Starch Granule Size Distribution." *Food Research International* 34 (4): 357-363.

PIRGOZLIEV, V. R. 2000. "The Effect of Wheat Cultivars on the Growth Performance and Energy Retention of Broiler Chickens." PhD Thesis. UK: Open University.

PIRGOZLIEV, V., S. ROSE, and R. GRAYBOSCH. 2002. "Energy and Amino Acid Availability to Chickens of Waxy Wheat." *Archiv Fuer Gefluegelkunde* 66 (3): 108-113.

PIRGOZLIEV, V. R., C. L. BIRCH, S. P. ROSE, P. S. KETTLEWELL, and M. R. BEDFORD. 2003. "Chemical Composition and the Nutritive Quality of Different Wheat Cultivars for Broiler Chickens." *British Poultry Science* 44 (3) 464-475.

PIRGOZLIEV, V., S. P. ROSE, T. PELLNY, A. M. AMERAH, M. WICKRAMASINGHE, M. ULKER, M. RAKSZEGI, Z. BEDO, P. R. SHEWRY, and A. LOVEGROVE. 2015a. "Energy Utilization and Growth Performance of Chickens Fed Novel Wheat Inbred Lines Selected for Different Pentosan Levels with and without Xylanase Supplementation." *Poultry Science* 94 (2): 232-239. doi:10.3382/ps/peu059.

PIRGOZLIEV, V., D. BRAVO, M. W. MIRZA, and S. P. ROSE. 2015b. "Growth Performance and Endogenous Losses of Broilers Fed Wheat-Based Diets with and without Essential Oils and Xylanase Supplementation." *Poultry Science* 94 (6): 1227-1232.

PIRGOZLIEV, V., M. W. MIRZA, and S. P. ROSE. 2016. "Does the Effect of Pelleting Depend on the Wheat Sample when Fed to Chickens?" *Animal* 10 (4): 571-577. doi:10.1017/S1751731115002311

POLLOCK, J. M. and W. GEDDES. 1951. "The Distribution of Thiamine and Riboflavin in the Wheat Kernel at Different Stages of Maturity." *Cereal Chemistry* 28: 289-299.

POMERANZ, Y., H. BOLLING, and H. ZWINGELBERG. 1984. "Wheat Hardness and Baking Properties of Wheat Flours." *Journal of Cereal Science* 2 (3): 137-143.

POMERANZ, Y. and P. WILLIAMS. 1990. "Wheat Hardness: Its Genetic, Structural, and Biochemical Background, Measurement, and Significance." *Advances in Cereal Science and Technology (USA)* 471-544.

POMERANZ, Y. ed. 1988. *Wheat: Chemistry and Technology.* 3rd edition. Minnesota: American Association of Cereal Chemists.

PRESTON, C., K. MCCRACKEN, and A. MCALLISTER. 2000. "Effect of Diet Form and Enzyme Supplementation on Growth, Efficiency and Energy Utilisation of Wheat-Based Diets for Broilers." *British Poultry Science* 41 (3): 324-331. doi:10.1080/713654933

RAVINDRAN, V. 2001. "Apparent Metabolisable Energy Content of New Zealand Wheats." In *Proceedings of the Australian Poultry Science Symposium,* 2001, *University of Sydney*. p. 240.

RAVINDRAN, V. 2013. "Feed Enzymes: The Science, Practice, and Metabolic Realities." *Journal of Applied Poultry Research* 22 (3): 628-636.

RAVINDRAN, V. 1995. "Phytases in Poultry Nutrition. An Overview." In *Proceedings, Australian Poultry Science Symposium*. Vol 7. 135-139.

RAVINDRAN, V. 1995. "Phytates: Occurrence, Bioavailability and Implications in Poultry Nutrition." Poultry and Avian Biology Reviews 6: 125-143.

RAVINDRAN, V., W.L. BRYDEN, W.L, and L.I. HEW. 1998. "*Digestible Amino Acids in Poultry Feedstuffs*." Rural Industries Research and Development Corporation, Canberra and Poultry Research Foundation: The University of Sydney.

RAVINDRAN, V., L. HEW, G. RAVINDRAN, and W. BRYDEN. 2005. "Apparent Ileal Digestibility of Amino Acids in Dietary Ingredients for Broiler Chickens." *Animal Science* 81 (1): 85-97.

RAVINDRAN, V., L. HEW, G. RAVINDRAN, and W. BRYDEN. 1999. "A Comparison of Ileal Digesta and Excreta Analysis for the Determination of Amino Acid Digestibility in Food Ingredients for Poultry." *British Poultry Science* 40 (2): 266-274.

RAVINDRAN, V., Y. WU, D. THOMAS, and P. MOREL. 2006. "Influence of Whole Wheat Feeding on the Development of Gastrointestinal Tract and Performance of Broiler Chickens." *Australian Journal of Agricultural Research* 57 (1): 21-26.

RAVINDRAN, V., and A. M. AMERAH. 2009."Wheat: Composition and Feeding Value for Poultry." In *Soybean and Wheat Crops: Growth, Fertilization and Yield*, edited by S. Davies and G. Evans, 245-259. New York: Nova Science Publisher, Inc, Hauppauge.

ROGEL, A., E. ANNISON, W. BRYDEN, and D. BALNAVE. 1987a. "The Digestion of Wheat Starch in Broiler Chickens." *Australian Journal of Agricultural Research* 38 (3): 639-649.

ROGEL, A., D. BALNAVE, W. BRYDEN, and E. ANNISON. 1987b. "Improvement of Raw Potato Starch Digestion in Chickens by Feeding Oat Hulls and Other Fibrous Feedstuffs." *Australian Journal of Agricultural Research* 38 (3): 629-637.

ROONEY, L. and R. PFLUGFELDER. 1986. "Factors Affecting Starch Digestibility with Special Emphasis on Sorghum and Corn." *Journal of Animal Science* 63 (5): 1607-1623.

ROSE, S., L. TUCKER, P. KETTLEWELL, and J. COLLIER. 2001. "Rapid Tests of Wheat Nutritive Value for Growing Chickens." *Journal of Cereal Science* 34 (2): 181-190. doi:10.1006/jcrs.2001.0390

RUTHERFURD, S., T. CHUNG, and P. MOUGHAN. 2002. "The Effect of Microbial Phytase on Ileal Phosphorus and Amino Acid Digestibility in the Broiler Chicken." *British Poultry Science* 43 (4): 598-606.

SALAH UDDIN, M. S., S. ROSE, T. HISCOCK, and S. BONNET. 1996. "A Comparison of the Energy Availability for Chickens of Ground and Whole Grain Samples of Two Wheat Varieties." *British Poultry Science* 37 (2): 347-357.

SALMAN, H., J. BLAZEK, A. LOPEZ-RUBIO, E. P. GILBERT, T. HANLEY, and L. COPELAND. 2009. "Structure–function Relationships in A and B Granules from Wheat Starches of Similar Amylose Content." *Carbohydrate Polymers* 75 (3): 420-427.

SAULNIER, L., N. PENEAU, and J. F. THIBAULT. 1995. "Variability in Grain Extract Viscosity and Water-Soluble Arabinoxylan Content in Wheat." *Journal of Cereal Science* 22 (3): 259-264. https://doi.org/10.1006/jcrs.1995.0062

SAVORY, C. 1980. "Diurnal Feeding Patterns in Domestic Fowls: A Review." *Applied Animal Ethology* 6 (1): 71-82.

SCHOFIELD, J.D., 1994. "Wheat Proteins: Structure and Functionality in Milling and Breadmaking." *In Wheat Production, Properties and Quality,* edited by W. BUSHUK, and V. F. RASPER, 73-106. London: Springer, Boston, MA.

SCOTT, T., F. SILVERSIDES, H. CLASSEN, M. SWIFT, and M. BEDFORD. 1998a. "Effect of Cultivar and Environment on the Feeding Value of Western Canadian Wheat and Barley Samples with and without Enzyme Supplementation." *Canadian Journal of Animal Science* 78 (4): 649-656.

SCOTT, T., F. SILVERSIDES, H. CLASSEN, M. SWIFT, M. BEDFORD, and J. HALL. 1998b. "A Broiler Chick Bioassay for Measuring the Feeding Value of Wheat and Barley in Complete Diets." Poultry Science 77 (3): 449-455.

SELLE, P. H. and V. RAVINDRAN. 2007. "Microbial Phytase in Poultry Nutrition." *Animal Feed Science and Technology* 135 (1): 1-41.

SELLE, P.H., V. RAVINDRAN, G. RAVINDRAN, and W.L. BRYDEN. 2005. "Amino Acid Digestibility and Growth Performance Interactions to Phytase and Lysine Supplementation of Lysine-Deficient Broiler Diets." In *Proceedings of the 17th Poultry Science Symposium, Sydney, New South Wales, Australia, 7-9 February*, 234-237. Poultry Research Foundation.

SELLE, P., A. WALKER, and W. L. BRYDEN. 2003. "Total and Phytate-Phosphorus Contents and Phytase Activity of Australian-Sourced Feed Ingredients for Pigs and Poultry." *Animal Production Science* 43 (5): 475-479.

SHEWRY, P. R. 2009. "Wheat." *Journal of Experimental Botany* 60 (6): 1537-1553.

SHORT, F. J., P. GORTON, J. WISEMAN, and K. N. BOORMAN. 1996. "Determination of Titanium Dioxide Added as an Inert Marker in Chicken Digestibility Studies." *Animal Feed Science and Technology* 59 (4): 215-221.

SIBBALD, I. 1982. "Measurement of Bioavailable Energy in Poultry Feedingstuffs: A Review." *Canadian Journal of Animal Science* 62 (4): 983-1048.

SIBBALD, I. 1980. "Metabolizable Energy in Poultry Nutrition." *Bioscience* 30 (11): 736-741.

SIBBALD, I. and S. SLINGER. 1962. "The Metabolizable Energy of Materials Fed to Growing Chicks." *Poultry Science* 41 (5): 1612-1613.

SILVERSIDES, F. G. and M. R. BEDFORD. 1999. "Effect of Pelleting Temperature on the Recovery and Efficacy of a Xylanase Enzyme in Wheat-Based Diets." *Poultry Science* 78 (8) (Aug): 1184-1190.

SIMMONDS, D., K. BARLOW, and C. WRIGLEY. 1973. "Biochemical Basis of Grain Hardness in Wheat." *Cereal Chemistry* (50): 553-562.

SIMONS, P., H. A. VERSTEEGH, A. JONGBLOED, P. KEMME, P. SLUMP, K. BOS, M. WOLTERS, R. BEUDEKER, and G. VERSCHOOR. 1990. "Improvement of Phosphorus Availability by Microbial Phytase in Broilers and Pigs." *British Journal of Nutrition* 64 (2): 525-540.

SINGH, J., L. KAUR, and O. MCCARTHY. 2007. "Factors Influencing the Physico-Chemical, Morphological, Thermal and Rheological Properties of some Chemically Modified Starches for Food applications - A Review." *Food Hydrocolloids* 21 (1): 1-22.

SLOMINSKI, B., T. DAVIE, M. NYACHOTI, and O. JONES. 2007. "Heat Stability of Endogenous and Microbial Phytase during Feed Pelleting." Livestock Science 109 (1-3): 244-246.

SLOMINSKI, B. A. 2011. "Recent Advances in Research on Enzymes for Poultry Diets." *Poultry Science* 90 (9): 2013-2023.

SMEETS, N., F. NUYENS, L. VAN CAMPENHOUT, and T. NIEWOLD. 2014. "Variability in the *in Vitro* Degradation of Non-Starch Polysaccharides from Wheat by Feed Enzymes." *Animal Feed Science and Technology* 187: 110-114.

SMEETS, N., F. NUYENS, L. VAN CAMPENHOUT, E. DELEZIE, J. PANNECOUCQUE, and T. NIEWOLD. 2015. "Relationship between Wheat Characteristics and Nutrient Digestibility in Broilers: Comparison between Total Collection and Marker (Titanium Dioxide) Technique." *Poultry Science* 94 (7): 1584-1591. doi:10.3382/ps/pev116

SMITH, V. H., G. D. TILMAN, and J. C. NEKOLA. 1999. "Eutrophication: Impacts of Excess Nutrient Inputs on Freshwater, Marine, and Terrestrial Ecosystems." *Environmental Pollution* 100 (1-3): 179-196.

SMITS, C. H. and G. ANNISON. 1996. "Non-Starch Plant Polysaccharides in Broiler nutrition–towards a Physiologically Valid Approach to their Determination." *World's Poultry Science Journal* 52 (2): 203-221.

SNAPE, J. W., V. HYNE, and K. AITKEN. 1993. "Targeting Genes in Wheat using Marker Mediated Approaches." In *Proceedings of the Eighth International Wheat Genetics Symposium*, *20 July 1993*. vol. 2, 749-759.

SOMERS, G., M. COOLIDGE, and K. HAMNER. 1945. "The Distribution of Thiamine and Riboflavin in Wheat Grains." *Cereal Chemistry* 22: 333-340.

SOILSCAPES 2018. Cranfield soil and Agrifood Institute. Cranfield University. [Online]. http://www.landis.org.uk/soilscapes/index.cfm [Accessed: October 2018].

ŠRAMKOVÁ, Z., E. GREGOVÁ, and E. ŠTURDÍK. 2009. "Chemical Composition and Nutritional Quality of Wheat Grain." *Acta Chimica Slovaca* 2 (1): 115-138.

STEENFELDT, S. 2001. "The Dietary Effect of Different Wheat Cultivars for Broiler Chickens." *British Poultry Science* 42 (5): 595-609. doi:10.1080/00071660120088416.

STEENFELDT, S., A. MÜLLERTZ, and J. F. JENSEN. 1998a. "Enzyme Supplementation of Wheat-Based Diets for Broilers: 1. Effect on Growth Performance and Intestinal Viscosity." *Animal Feed Science and Technology* 75 (1): 27-43.

STEENFELDT, S., M. HAMMERSHØJ, A. MÜLLERTZ, and J. F. JENSEN. 1998b. "Enzyme Supplementation of Wheat-Based Diets for Broilers: 2. Effect on Apparent Metabolisable Energy Content and Nutrient Digestibility." *Animal Feed Science and Technology* 75 (1): 45-64.

STEPHEN, A. M. and J. H. CUMMINGS. 1979. "Water-Holding by Dietary Fibre in Vitro and its Relationship to Faecal Output in Man." *Gut* 20 (8): 722-729.

STEVENS, B. J., R. R. SELVENDRAN, C. E. BAYLISS, and R. TURNER. 1988. "Degradation of Cell Wall Material of Apple and Wheat Bran by Human Faecal Bacteria in Vitro." *Journal of the Science of Food and Agriculture* 44 (2): 151-166.

SVIHUS, B. and M. GULLORD. 2002. "Effect of Chemical Content and Physical Characteristics on Nutritional Value of Wheat, Barley and Oats for Poultry." *Animal Feed Science and Technology* 102 (1): 71-92.

SVIHUS, B. and H. HETLAND. 2001. "Ileal Starch Digestibility in Growing Broiler Chickens Fed on a Wheat-Based Diet is Improved by Mash Feeding, Dilution with Cellulose Or Whole Wheat Inclusion." *British Poultry Science* 42 (5): 633-637.

SVIHUS, B., E. JUVIK, H. HETLAND, and Å. KROGDAHL. 2004. "Causes for Improvement in Nutritive Value of Broiler Chicken Diets with Whole Wheat Instead of Ground Wheat." *British Poultry Science* 45 (1): 55-60.

SVIHUS, B. 2011. "Limitations to Wheat Starch Digestion in Growing Broiler Chickens: A Brief Review." *Animal Production Science* 51 (7): 583-589.

SVIHUS, B., A. K. UHLEN, and O. M. HARSTAD. 2005. "Effect of Starch Granule Structure, Associated Components and Processing on Nutritive Value of Cereal Starch: A Review." *Animal Feed Science and Technology* 122 (3): 303-320.

SVIHUS, B. 2014. "Starch Digestion Capacity of Poultry." *Poultry Science* 93 (9): 2394-2399.

SWIFT, J. and T. O'BRIEN. 1972. "The Fine Structure of the Wheat Scutellum during Germination." *Australian Journal of Biological Sciences* 25 (3): 469-486.

TAYYAB, U. and F. MCLEAN. 2015. "Phosphorus Losses and on-Farm Mitigation Options for Dairy Farming Systems: A Review." *Journal of Animal & Plant Sciences* 25 (2):318-327.

TESTER, R. 1997. "Starch: The Polysaccharide Fractions." *Starch: Structure and Functionality*. 163-171, Cambridge: Royal Society of Chemistry.

TESTER, R., J. SOUTH, W. MORRISON, and R. ELLIS. 1991. "The Effects of Ambient Temperature during the Grain-Filling Period on the Composition and Properties of Starch from Four Barley Genotypes." *Journal of Cereal Science* 13 (2): 113-127.

TESTER, R. F., J. KARKALAS, and X. QI. 2004. "Starch—composition, Fine Structure and Architecture." *Journal of Cereal Science* 39 (2): 151-165.

TUFVESSON, F., V. SKRABANJA, I. BJÖRCK, H. L. ELMSTÅHL, and A. ELIASSON. 2001. "Digestibility of Starch Systems Containing amylose–glycerol Monopalmitin Complexes." *LWT-Food Science and Technology* 34 (3): 131-139.

TURNBULL, K. and S. RAHMAN. 2002. "Endosperm Texture in Wheat." *Journal of Cereal Science* 36 (3): 327-337.

USDA Foreign Agricultural Service. 2019. Global wheat production from 2011/2012 to 2018/201. Statista Inc. [Online]. https://www.statista.com/statistics/267268/production-of-wheat-worldwide-since-1990/ [Accessed: July 18, 2019].

USDA (United States Department of Agriculture) 2019. Largest Wheat Producing Countries Worldwide in 2018/2019. Statista Inc. [Online]. https://www.statista.com/statistics/237912/global-top-wheat-producing-countries/ [Accessed: July 18, 2019].

VAN DER KLIS, J., A. VAN VOORST, and C. VAN CRUYNINGEN. 1993. "Effect of a Soluble Polysaccharide (Carboxy Methyl Cellulose) on the Physico-Chemical Conditions in the Gastrointestinal Tract of Broilers." *British Poultry Science* 34 (5): 971-983.

WALDRON, L. A. 1997. "The Nutritive Value of Different Wheat Varieties for Broiler Chickens." PhD Thesis. Newport, UK: Harper Adams Agriculture College.

WEURDING, R. E. 2002. "Kinetics of Starch Digestion and Performance of Broiler Chickens." PhD Thesis. Wageningen: Wageningen University, Netherland.

WEURDING, R. E., A. VELDMAN, W. A. VEEN, P. J. VAN DER AAR, and M. W. VERSTEGEN. 2001. "Starch Digestion Rate in the Small Intestine of Broiler Chickens Differs among Feedstuffs." *The Journal of Nutrition* 131 (9): 2329-2335.

WISEMAN, J. 1997. "Assigning Energy Values to Ingredients for Pigs." In *Proceedings of the Feed Ingredient, Asia, Singapore International Convention and Exhibition Centre*. http://www.Sian.Info.ve/porcinos/publicaciones/producerdos/articulo6.Htm.

WISEMAN, J. 2000. "Correlation between Physical Measurements and Dietary Energy Values of Wheat for Poultry and Pigs." *Animal Feed Science and Technology* 84 (1): 1-11. doi:10.1016/S0377-8401(00)00107-3

WISEMAN, J., N. NICOL, and G. NORTON. 2000. "Relationship between Apparent Matabolisable (AME) Values and *in vivo/in vitro* Starch Digestibility of Wheat for Broilers." *World's Poultry Science Journal* 56 (4): 305-318.

WISEMAN, J. 2006. "Variations in Starch Digestibility in Non-Ruminants." *Animal Feed Science and Technology* 130 (1-2): 66-77.

WISEMAN, J., and J. INBORR. 1990."The Nutritive Value of Wheat and its Effect on Broiler Performance." *Recent Advances in Animal Nutrition*. 1990: 79-102.

WRIGLEY, C. W., and J. A. BIETZ. 1988."Proteins and Amino Acids." In *Wheat: Chemistry and Technology,* edited by Y. POMERANZ, 3rd edition, 159-252: American Association of Cereal Chemists.

WU, Y. B. and V. RAVINDRAN. 2004. "Influence of Whole Wheat Inclusion and Xylanase Supplementation on the Performance, Digestive Tract Measurements and Carcass Characteristics of Broiler Chickens." *Animal Feed Science and Technology* 116 (1-2): 129-139.

POMERANZ, Y. 1988. Chemical Composition of Kernel Structures. In *Wheat: Chemistry and Technology*, edited by Y. Pomeranz. 3rd edition, 97-158. Minnesota: American Association of Cereal Chemist.

YEGANI, M. and D. KORVER. 2008. "Factors Affecting Intestinal Health in Poultry." *Poultry Science* 87 (10): 2052-2063.

YEGANI, M., M. SWIFT, R. ZIJLSTRA, and D. KORVER. 2013. "Prediction of Energetic Value of Wheat and Triticale in Broiler Chicks: A Chick Bioassay and an *in Vitro* Digestibility Technique." *Animal Feed Science and Technology* 183 (1): 40-50.

ZAEFARIAN, F., M. R. ABDOLLAHI, and V. RAVINDRAN. 2015. "Starch Digestion in Broiler Chickens Fed Cereal Diets." *Animal Feed Science and Technology* 209: 16-29.

APPENDICES

Appendix A

Chapter 3. The AME, AMEn and nutrient utilisation of seventeen wheat-based diets.

Table. The AME, AMEn and coefficients of nitrogen retention (NR), fat digestibility (FD) and dry matter retention (DMR) of seventeen wheat-based diets.

Diet	AME	AMEn	NR	FD	DMR
1	13.50^{ab}	12.76^{abc}	0.591	0.741^{ab}	0.695^{a}
2	13.78^{abcd}	13.06^{abcde}	0.598	0.755^{abc}	0.715^{abc}
3	13.37^{a}	12.64^{a}	0.619	0.755^{abc}	0.718^{bc}
4	13.83^{abcd}	13.06^{abcde}	0.632	0.768^{abc}	0.723^{c}
5	13.96^{bcd}	13.23^{de}	0.627	0.773^{abc}	0.720^{c}
6	13.49^{ab}	12.73^{ab}	0.603	0.725^{a}	0.694^{a}
7	13.76^{abcd}	13.03^{abcde}	0.601	0.811^{c}	0.697^{ab}
8	13.39^{a}	12.65^{a}	0.602	0.780^{abc}	0.695^{a}
9	13.66^{abc}	12.90^{abcd}	0.610	0.719^{a}	0.706^{abc}
10	14.02^{cd}	13.26^{de}	0.609	0.761^{abc}	0.722^{c}
11	13.60^{abc}	12.87^{abcd}	0.600	0.764^{abc}	0.696^{ab}
12	14.00^{cd}	13.23^{de}	0.621	0.790^{bc}	0.721^{c}
13	13.91^{bcd}	13.13^{bcde}	0.631	0.801^{bc}	0.723^{c}
14	13.64^{abc}	12.87^{abcd}	0.607	0.768^{abc}	0.705^{abc}
15	14.18^{d}	13.37^{e}	0.625	0.811^{c}	0.726^{c}
16	14.00^{cd}	13.20^{cde}	0.631	0.808^{c}	0.712^{abc}
17	13.93^{bcd}	13.17^{bcde}	0.609	0.794b^{c}	0.711^{abc}
Mean	13.77	13.01	0.613	0.772	0.71
Min	13.37	12.64	0.591	0.719	0.694
Max	14.18	13.37	0.632	0.811	0.726
CV%	2.9	2.9	5.2	6.9	2.7
SEM[2]	0.141	0.135	0.01126	0.0188	0.0067
P value	<0.001	<0.001	0.179	0.007	<0.001

[1]Each value represents mean of 8 experimental unit (pen) of 5 birds each. Values are based on total collection from 19 to 21 days of age.
[2]Standard error of means.
Means within a column with no common superscripts differ significantly ($P < 0.05$).

Appendix B

Chapter 5. Part B. Growth performance of broilers fed two wheat samples during a feed period of 21 – 28 days.

Table. Growth performance[1] of broilers fed two wheat samples and the addition of xylanase on growth performance (data based on feed period from 21- 28 days).

	Xylanase	FI g/b/d DM	WG g/b/d	FBW (kg)	FCR (g: g)
Wheat					
DW		140.0	104.3	1.761	1.391
Lili		142.3	103.7	1.757	1.368
SEM		1.44	0.769	0.0054	0.008
Xylanase					
−		142.5	103.5	1.755	1.386
+		139.8	104.6	1.763	1.373
SEM		1.46	0.773	0.0054	0.008
Diet x Xylanase					
DW	−	142.9	103.6	1.756	1.399
	+	137.1	105.0	1.765	1.384
Lili	−	142.1	103.3	1.753	1.374
	+	142.5	104.2	1.760	1.362
SEM		2.06	1.094	0.0077	0.011
Significance					
Wheat		0.277	0.604	0.603	0.048
Xylanase		0.194	0.314	0.314	0.234
Wheat x Xylanase		0.147	0.855	0.854	0.881

[1]Each value represents mean of 10 experimental units per treatment. Each experimental unit was a pen containing 6 birds.
Standard error of the means.
FI g/b/d: feed intake (gram/bird/day on DM), WG: weight gain (gram/bird/day), FBW: final body weight in kg at day 28, FCR: mortality corrected feed conversion on DM (g of feed intake / g of body weight gain).

Harper Adams University

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