INFLUENCE OF BARLEY INCLUSION METHOD AND PROTEASE SUPPLEMENTATION ON GROWTH PERFORMANCE, ENERGY AND NUTRIENT UTILISATION AND GASTROINTESTINAL TRACT DEVELOPMENT IN BROILER STARTERS

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

Master of Science

in

Animal Science

at Massey University, Manawatu

New Zealand

Lindon McEnroe Tari

2020

Abstract

The influence of barley inclusion method and protease supplementation on the performance and coefficient of apparent ileal digestibility (CAID) of nutrients and energy and gastrointestinal tract development in broiler starters fed wheat-based diets from 1 to 21days of age were examined in this study. A normal starch hulled barley was ground in a hammer mill to pass through the screen size of 2.5 mm and 8.0 mm. Three basal diets containing normal starch hulled barley, either finely ground (2.5 mm), coarsely ground (8.0 mm) or whole barley, were used to develop six dietary treatments either with or without protease enzyme. A total of 288 one-day-old male broilers (Ross 308) were individually weighed and allocated into 36 cages. Each of the six treatments was randomly assigned into six cages each housing eight birds. The pellet durability index (PDI) was higher ($P < 0.05$) in pellets made from diets based on finely ground barley than those made from coarsely ground or whole barley. The main effect of barley inclusion method was significant ($P < 0.01$) for weight gain and feed conversion ratio (FCR) from day 1 to 21. Birds fed diets containing coarse particles and whole barley showed higher ($P < 0.05$) weight gain compared to those fed diets containing fine particles. Feed conversion ratio was lower ($P < 0.05$) in birds fed whole barley compared to birds fed fine particles. Diets made from coarsely ground and whole barley had higher ($P < 0.05$) CAID of dry matter, nitrogen, calcium, and gross energy compared to the diets made from finely ground barley. Fine and coarse grinding increased ($P < 0.05$) CAID of phosphorus compared to whole barley diets. Diets made from fine and coarse barley had higher ($P < 0.05$) gizzard pH compared to those made from whole barley. Diets made from fine and coarse grinding increased ($P < 0.05$) the relative weight of proventriculus and decreased ($P < 0.05$) the relative weight of gizzard compared to whole barley diets. An interaction ($P < 0.05$) was observed for the relative weight of caeca. The relative weight of caeca was reduced numerically with protease supplementation in fine and coarse ground diets, whereas, in whole barley diets, protease supplementation numerically increased relative weights of caeca compared to diets without enzyme. The main effect of protease supplementation and interaction of barley inclusion method x protease supplementation were not significant ($P > 0.05$) for growth performance, nutrient digestibility, energy and nitrogen-corrected apparent metabolizable energy parameters. Overall, the present data showed that coarse grinding and whole inclusion of barley, through enhanced nutrient digestibility, are beneficial to the growth performance in broiler starters.

Acknowledgements

First, I would like to express my sincere gratitude to my supervisor, Dr. Reza Abdollahi, for his guidance, advice, encouragement, and expertise in poultry nutrition and commitment in conducting and writing this thesis.

I would also like to thank my co-supervisor, Dr. Fifi Zaefarian for her valuable comments, encouragement, advice, and commitments in writing this thesis.

I would also like to thank Dr. Velmurugu Ravindran for his encouragement and guidance. I appreciate his directions right at the start of commencing my research.

Special appreciation to my colleague PhD students who have provided me the support I needed during my experiment. I appreciate Nipuna Perera for her support in helping me enrich my experience.

I would also like to thank the staff of the Massey University Poultry Research Unit, Edward James, Colin Naftel, Shaun de Malmanche, and Kalwyn Pereka. I am thankful for the friendship we had and the support during my experiment. And to those I forgot to mention.

A special appreciation to the Government of New Zealand through the Ministry of Foreign Affairs and Trade (MFAT) for the scholarship funding and the opportunity to study at Massey University. My gratitude extends to the International Student Support team, Jamie Hooper, Saba Azeem, Dianne Reilly, Tian Yang, and Dandan Wang.

I would like to thank my family, my mum and my siblings for their support and encouragement. My heartfelt gratitude is expressed to my wife, Noilanie and my two children, Kianna and Kelisha, for their never-ending love, understanding, patience, support, encouragement and prayers and to whom this thesis is dedicated.

Above all, I thank God for this achievement and to Him be the glory.

Table of Contents

List of Tables

Chapter 2

Chapter 3

List of Figures

List of Abbreviations

AME	Apparent metabolisable energy
AMEn	Nitrogen-corrected apparent metabolisable energy
ANOVA	Analysis of variance
Ca	Calcium
CAID	Coefficient of apparent ileal digestibility
cm	Centimetre
CP	Crude protein
DF	Dietary fibre
DM	Dry matter
FCF	Free choice feeding
FCR	Feed conversion ratio
g	Gram
GE	Gross energy
GIT	Gastrointestinal tract
GLM	General linear model
GMD	Geometric mean diametre
GSD	Geometric standard deviation
IDE	Ileal digestible energy
kg	Kilogram
MF	Mixed feeding

mg	Milligram
MJ	Mega joule
mm	Millimetre
MMT	Million metric ton
N	Nitrogen
NSP	Non-starch polysaccharides
P	Phosphorus
PDI	Pellet durability index
PP	Post-pelleting
PRP	Pre-pelleting
SF	Sequential feeding
TiO2	Titanium dioxide
µm	Micrometre

CHAPTER 1

General Introduction

The demand for meat products such as poultry meat, has increased over the decades and consequently, the demand for feed and raw materials has also increased and will continue to increase in the future. There is a growing gap between supply and demand which results in higher prices for conventional feed ingredients used in animal production. This provides a convincing cause for exploring alternative feed ingredients and their potential in feed formulations and also practices that can be applied to reduce the cost of feed. Barley is one of the alternative feed ingredients that remains under-utilised in poultry feed due to numerous problems in practical implementation. Barley has been less preferred as a poultry feed ingredient due to its relatively low protein and energy and high fibre content (Singh et al., 2014a). Barley has high content of non-starch polysaccharides (NSP; Jacob and Pescatore, 2012). The prominent NSP present in barley is β-glucan, which limits its nutritive value by encapsulating the nutrients within the endosperm cells (Åman and Graham, 1987) and results in low digestibility of nutrients. This physicochemical properties of NSPs increase the intestinal viscosity which impair digestion and reduce the efficiency of absorption of nutrients (Classen, 1996). However, the supplementation of NSP-degrading enzymes such as β-glucanase and xylanase have been reported to be effective by eliminating the intestinal viscosity and eliminating the cage effect of NSP, thus improving the nutritive value of barley for poultry (Classen, 1996; Choct, 2006).

Firstly, studies evaluating the effect of particle size in different cereal grains used in poultry are limited. In recent years, the attention toward particle size of feed ingredients has increased, as the industry continues to search for ways of optimising feed utilisation and improving production efficiency. Renewed attention in the area of particle size is also driven partly by increasing interest in feeding of whole grains. Recommendation regarding optimum particle size, have been contradictory, due to the number of factors including, grain type, endosperm hardness, grinding method, form of the diet, pellet quality and particle size distribution.

Secondly, in recent years, whole-grain feeding has also received renewed attention in the commercial poultry industry and is being increasingly used in many parts of the world. The primary aim for whole-grain feeding is to reduce feed costs by eliminating the grinding step. Furthermore, this also meets consumer demands for a natural feeding system and improved animal welfare (Gabriel et al., 2008). Published data on the effects of whole-grain feeding on performance of broilers have been contradictory, with some reports showing beneficial effects, while others failing to show any advantages. The discrepancy among published reports is due to several factors, including differences in experimental methodology, type and quality of grain, age of birds, feeding regime and inclusion level of whole grain. Moreover, most published data are based on whole wheat and data on other grains such as barley are scarce and little attempt has been made to use whole barley in poultry diets.

In addition, studies on the digestibility of proteins and amino acids have shown that valuable amounts of protein in broiler diets are secreted and end up in the excreta (Wang and Parsons, 1998; Angel et al., 2011). This means that there is a need to improve protein utilisation and to reduce nitrogen emissions to the environment from intensive broiler production. This could be achieved by the use of supplemental exogenous proteases in broiler diets (Angel et al., 2011; Freitas et al., 2011). Using protease enzyme in the feed can help to increase digestibility of protein in broilers and thus represents a means to address this need.

Therefore, the objective of this study is to investigate the possible interactions between the three basal diets containing normal starch hulled barley, either finely ground (2.5 mm), coarsely ground (8.0 mm) or whole barley, and protease supplementation on the performance, nutrient digestibility, energy utilisation, and GIT development in broiler starters fed wheat-based diets. There are three chapters covered in this thesis. Chapter 1 provides a general introduction to the thesis. Chapter 2 provides a review on barley as a poultry feed ingredient, including the anti-nutritive effects associated. It also highlights the effects of particle size and whole grain inclusion on performance, nutrient digestibility, energy utilisation and the gastrointestinal tract (GIT) development. Effects of supplemental exogenous enzymes in broiler diets are also discussed in this chapter. Chapter three presents the experimental work of this thesis, which consist of an abstract, introduction, materials and methods, results, discussion, and conclusion.

CHAPTER 2

Literature Review

2.1 Introduction

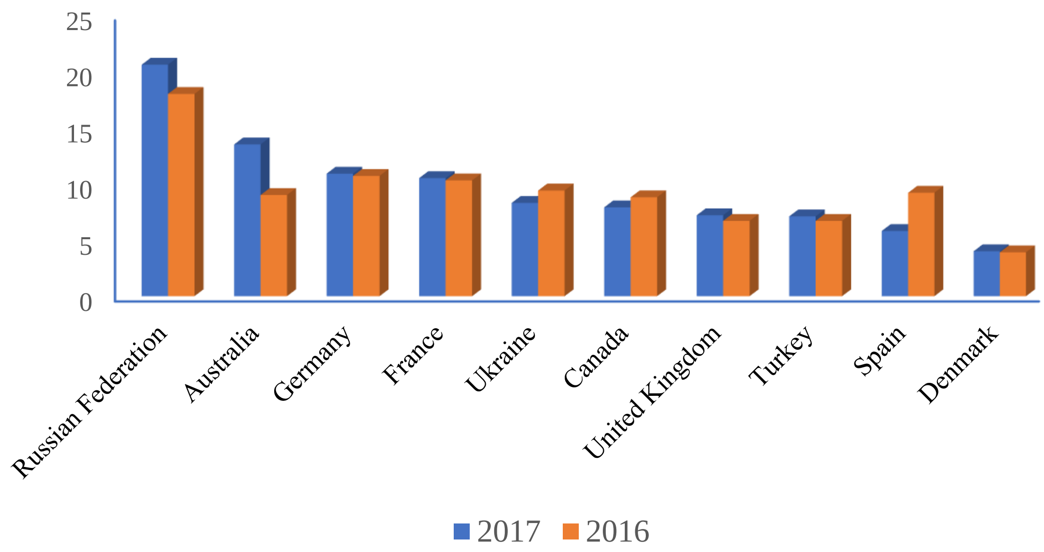
The global feed ingredient supply has changed over the years due to several factors including the population growth, climatic changes, limited land availability for crop production, the increase in world fuel prices, and diversion of feed ingredients to biofuel manufacture. This is a major concern for sustainable poultry production, especially in developing countries, as they move towards animal protein regimen. The future requirements for the traditional feed ingredients such as maize, wheat, and soybean meal cannot be met, even according to optimistic forecasts (Abdollahi and Ravindran, 2019). As a result, it has become the major challenge for the poultry industry to source good quality and more sustainable feed ingredients at acceptable prices. Therefore, it is important to search for potential alternative raw materials as a strategy to overcome this global problem. This review will focus on barley (*Hordeum vulgare L.)* as an alternative feed ingredient and its nutrient composition in poultry diets. It will also cover the effect of particle size of barley, whole barley inclusion, and supplementation of protease on broiler performance, nutrient digestibility and energy utilisation, and gastrointestinal tract (GIT) development in broilers fed wheat-based diet.

2.2 Background of barley

Barley *(Hordeum vulgare L.)* was one of the first crops that was domesticated from its wild relative *Hordeum spontaneum* (Badr et al., 2000). It ranks fourth globally in cereal production behind maize, rice and wheat (Rasmusson, 1985, Ullrich, 2010). It is a short-seasoned, early maturing crop that has been adapted to a wide variety of climate; however, it does not tolerate the high humidity and warm climate of the tropics. Much of the world's barley is produced in regions with climates not favourable to produce other major cereals. Barley has the characteristics to tolerate conditions of drought and high salinity (Mano et al., 1996, 1998; Fayez and Bazaid, 2014); thus, it has adapted to these climatic conditions over other crops.

The Russian Federation was the largest barley producer in 2016 and 2017 (Figure 2.1). Australia increased its barley production in 2017. Figure 2.2 shows that Europe is the largest producer of barley, followed by Asia.

(a)



(b)

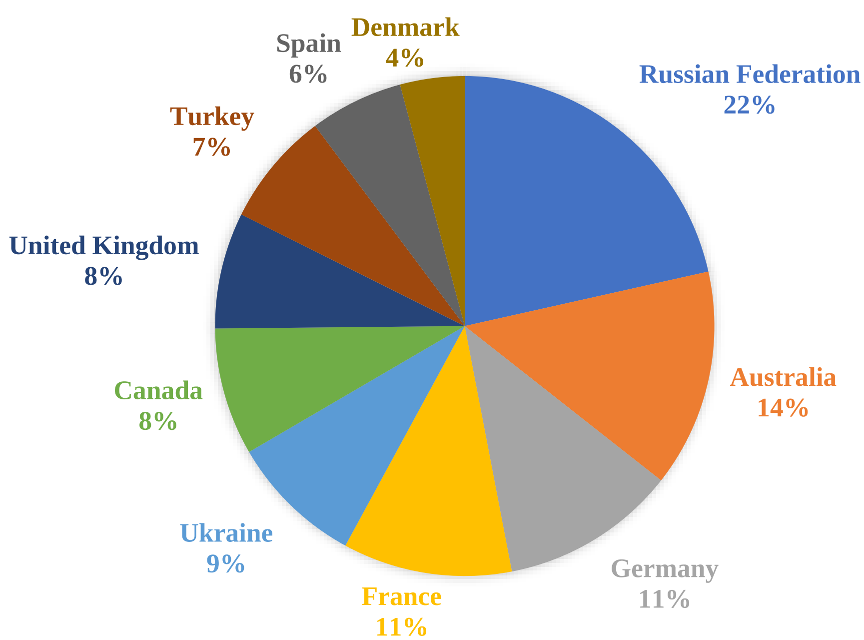
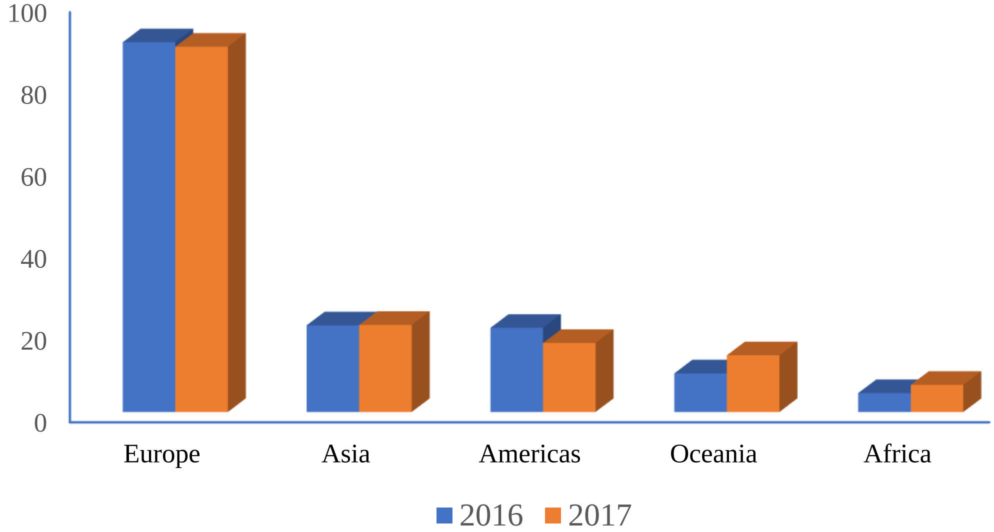


Figure 2.1. (a) Major barley producing countries in 2017 and 2016; (b) Production (%) in major producing countries in 2017 (Source: Food and Agriculture Organisation, 2017).

(a)



(b)

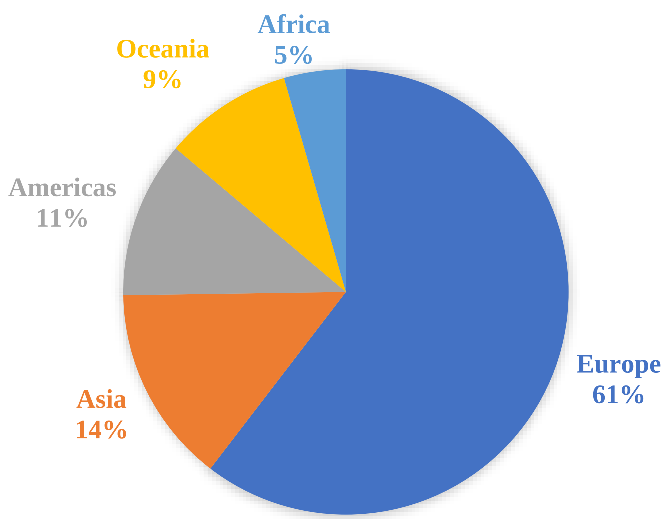


Figure 2.2. (a) Regional barley production (MMT); (b) Production (%) in different regions in 2017 (Source: Food and Agriculture Organisation, 2017).

Barley is the essential ingredient for the production of beer and malt whiskey and is an important component of farm animal diets in many European countries, particularly in the north (McNab and Smithard, 1992; Jacob and Pescatore, 2014). The global malting industry accounts for an annual malt production, of which 90% is made from barley (Oliveira et al., 2012). In livestock feeding rations, approximately 40% of barley was fed to cattle, 34% to dairy cows, 20% to pigs, 6% to grazing ruminants and less than 1% to poultry (Black et al., 2005).

Barley has been classified in several ways based on the number of seeds on the stalk, growth habits, presence or absence of awn, presence or absence of a hull, starch type, aleurone colour, and growth height (Jacob and Pescatore, 2012). The number of seeds on stalk includes the two-row and the six-row barley cultivars. The two-row cultivars are primarily the wild barley, and the six-row cultivars were developed in the early domestication of barley and were the result of a recently identified gene mutation (Komatsuda et al., 2007). The two-row varieties are grown in Europe because they are most adapted to dry climates, while the six-row varieties are mostly grown in North America.

Firstly, the cultivars of the two-row and six-row both have winter and spring types. Vernalisation is a process when the seedlings of winter barley type are exposed to cold which enables them to produce heads and grains normally; thus, the best sowing season is during autumn (Anderson et al., 1995). Spring barley does not require this condition for cold temperatures, so it can be sown in spring or summer. The short growing season of spring barley makes it possible to grow the crop at high altitudes (Jeroch and Dänicke, 1995).

Secondly, the absence or presence of an awn can be used to classify barley cultivars, such as awns being smooth or rough. Awns are useful in photosynthesis, transpiration and grain yield (Grundbacher, 1963; Khan, 1988); thus, the awn-less varieties have low yields compared to those that have awns (Tsvetkov and Tsvetkov, 2007).

Thirdly, the proportion of hull to kernel can differ widely between the barley cultivars. The hull can represent 10-13% of the dry weight of barley kernel and consists mainly of cellulose, hemicellulose, lignin, and a small quantity of protein (Palmer and Bathgate, 1976; Bhatty, 1986). Thus, the hull is a major contributor to the crude fibre content of barley, and if the hull is removed, the crude fibre content will be similar to that of maize and wheat. Hull-less or naked barley cultivars lose the hulls as they mature and are completely removed during harvesting (Bhatty, 1986).

Fourthly, the main component of cereal is starch and the content of barley starch ranges between 52 and 60%. Barley contains two major classes of starch, namely amylose and amylopectin. Normal starch barley contains about 27% amylose and 73% amylopectin whereas waxy starch barley contains about 2-10% amylose and 90-98% amylopectin. (Anker-Nilssen et al., 2006).

Furthermore, barley cultivars can also be classified according to the colour of the aleurone layer; which can be colourless, white, yellow, or blue (Jacob and Pescatore, 2012). The aleurone is part of the endosperm, acting as the storage tissue of the seed providing nourishment to the growing seedling at germination.

In addition, the growth heights of the barley cultivars include the tall, semi-dwarf and short cultivars. Tall barley cultivars are used in rain-fed production regions, whereas the short dwarf and semi-dwarf cultivars are used in less favourable growing conditions (Jacob and Pescatore, 2012). Most Europe barley cultivars are semi-dwarf cultivars, while the dwarf cultivars are common in Japan (Dahleen et al., 2005).

2.2.1 Composition

The composition and properties of barley have gained interest in nutritional studies in determining the availability of its nutrients in animal feeds, as well as human food. The different cultivars of barley present a large variation in structural and chemical compositions contributing to the properties of the barley grain. The variation of the physicochemical properties is due to genetic and climatic factors, stage of maturity, use of nitrogen fertilisers, and the storage time (Jeroch and Dänicke, 1995). Extensive researches on barley as animal feed revealed variations in nutrient composition of different cultivars (Åman et al., 1985); thus, better understanding the nutrient composition is useful in the formulation of least-cost rations that meet nutrient requirements of animals.

2.2.1.1 Structural composition

The endosperm of barley is the storage tissue of the seed that provides nourishment to the growing seedling at germination. It contains three layers of different cell types: the starchy endosperm, the basal transfer layer and the aleurone (Olsen, 2001, 2004; Jacob and Pescatore, 2012). The starchy endosperm is the major storage site for starch and proteins while the transfer layer functions in uptake of nutrients from the plant during seed development. The aleurone layer regulates the germination process by secreting amylase enzymes to break down the stored starch, providing the growing seedlings with sugars for energy and growth. The cell walls of the aleurone contain β-glucans, arabinoxylans and phenolic acids, and the ratio of β-glucan and arabinoxylan is 25:75, which is the reverse for the endosperm cell walls (Zhang and Li, 2010). The sub-aleurone layer is located between the aleurone and endosperm and it contains proteins embedded in starch the granules (Macewicz et al., 2006). The embryo is the fertile part of the barley seed and contains starch, proteins and lipids (Zhang and Li, 2010). These are used when the seeds commence germination. The barley husk amounts to approximately 13% of grain weight but can range between 7-25% depending on the cultivar, growing environment and grain size (Evers et al., 1999). Variation occurs between row types; six-row have a larger proportion of husk compared to two-row type. The hull plays an important role before and after harvest. It prevents pre-harvest sprouting (Benech-Arnold et al., 1999), and protect germ during harvesting (Olkku et al., 2005).

2.2.1.2 Chemical composition

There is a large variation in barley chemical composition, nutritive value, and bioavailable energy content, due to genetic and environmental factors and interaction between the two (Zhang et al., 1994; Valaja et al., 1997; Andersson et al., 1999). A study by Svihus and Gullord (2002) on five barley varieties, grown on two locations on two different production years, showed that the nutrient content did not vary considerably among batches of barley; however, starch content was affected by barley variety, fat content was affected by year of growing, and protein content was affected by the interaction between location and year. Hughes and Choct (1999) also reported effect of variation in protein content due to location and the year of production.

i) Starch

Starch, comprising up to 70-80% of most cereal grains, is the primary source of energy in poultry diets (Zaefarian et al., 2015). Starch accumulates in granules in the endosperm and consists of two different glucose polymers, namely amylose and amylopectin (Classen, 1996; Zaefarian et al., 2015). Amylose is a linear (1→4)-α-glucan, and amylopectin is a branched polymer with (1→6)-α-linkages at the branch points (Henry, 1988; Alberle et al., 1994). Andersson et al. (1999) and Holtekjølen et al. (2006) reported that the content of starch in barley generally ranged from 513-644 g/kg. The types of barley are evaluated based on the amylose:amylopectin ratio and the four main types of barley based on this ratio are: i) normal, ii) high amylose, iii) waxy, and iv) zero amylose waxy. Normal barley starch consists of 15-25% amylose and 75-85% amylopectin; high amylose consists of 40% amylose and 60% amylopectin; while the waxy barley starches consist of 97-100% amylopectin (Ullrich et al., 1986; MacGregor and Fincher, 1993). The waxy gene was originally found in maize and later incorporated into barley (Jacob and Pescatore, 2012). The waxy cultivars do not produce one of the enzymes needed for starch synthesis; therefore, they produce starch composed primarily of amylopectin. Waxy starch is more digestible than normal starch because amylopectin is more susceptible to α-amylase than amylose (Ankrah et al., 1999). This could explain why high amylose:amylopectin ratio resulted in slower degradation of starch (Anker-Nilssen et al., 2006). Furthermore, there is a wide range of amylose to amylopectin that has been reported in previous studies (Table 2.1). Relatively small variations in the total dietary starch content and amylose to amylopectin ratio can affect the growth performance of poultry (Pirgozliev et al., 2010). In addition, to the ratio of amylose:amylopectin, starch granule size, shape, surface area and interactions with other nutrients can affect the accessibility of enzymes to starch granules and thus affect the rate of digestion.

Mature barley endosperms consist of two distinct starch granules, the large, disc-shaped A-granules and small, spherical B-granules (Ao and Jane, 2006; Figure 2.3). Starch granules may occur individually or clustered as compound granules and occur in bimodal size distribution (Song and Jane, 2000; Ao and Jane, 2006; Copeland et al., 2009). The granule size distribution and shape are important for the functional properties of starch (Svihus et al., 2005). This suggests that the size of granule may also affect digestibility, as the relationship between surface area and volume, and thus contact between substrate and enzyme, decreases as the granule sizes increase. Moreover, cereals with small starch granules have a higher surface area to volume, therefore, have a greater starch digestibility than those with large starch granules. On the other hand, compound granule reduces the capacity for amylase to bind to the granule surface and therefore restricts the hydrolysis of starch (Tester and Karkalas, 2006).

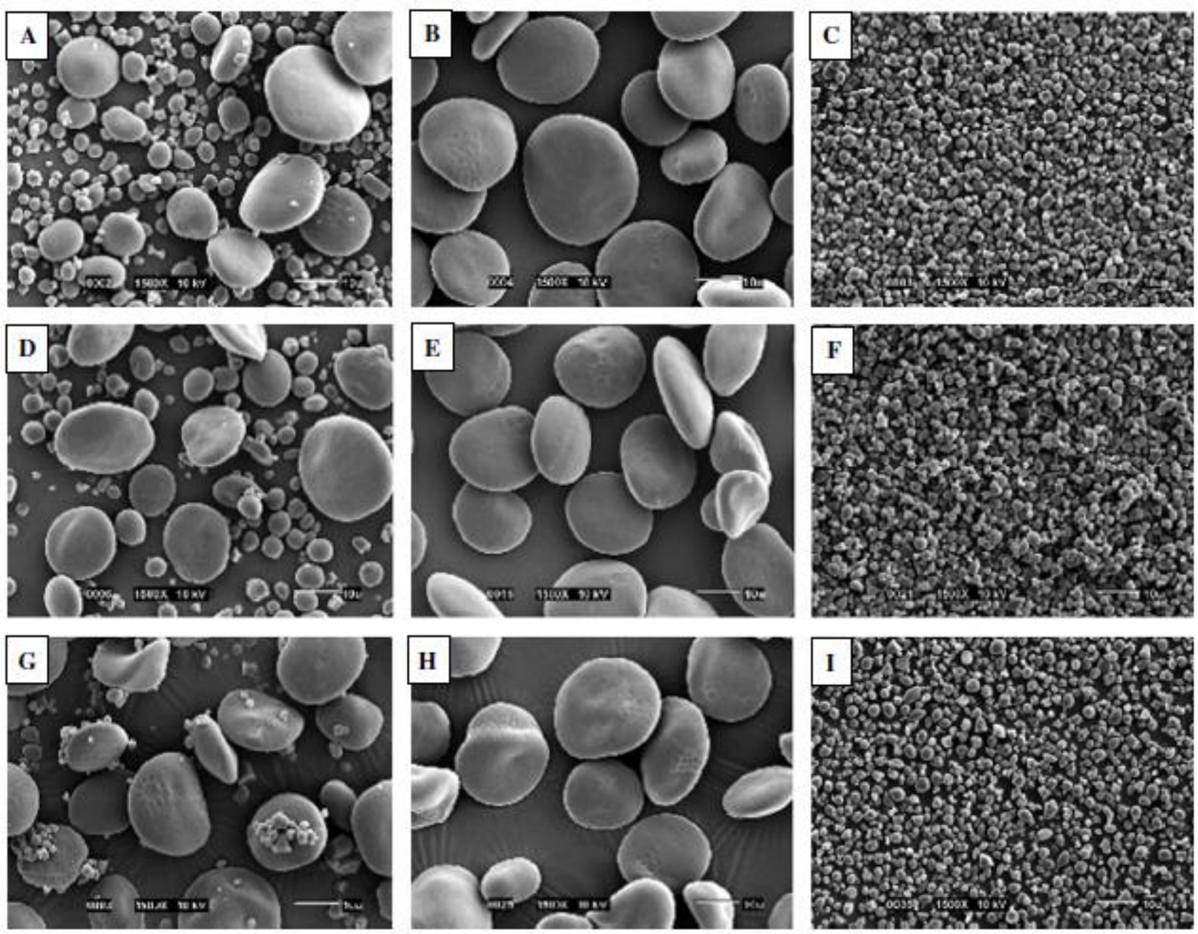


Figure 2.3. Scanning electron micrographs of native wheat (A), triticale (D), and barley (G) starch granules, and their fractional large, A-granules (B, E, and H, respectively) and small, B-granules (C, F, and I, respectively), Source: Ao and Jane (2006).

The amylose:amylopectin ratio and amylopectin branch chain length have a strong correlation with granule size and size distribution of hull-less barley (waxy, normal amylose, and high amylose; Li et al., 2001). A typical bimodal distribution in starch granule size has been reported in wheat, rye and barley with smaller granule diameter < 10 µm and large granule diameter > 10 µm. It has been reported that in normal barley the crystallinity of B-granules (23.0%) was greater than the A-granules (20.3%) compared to waxy barley, which the crystallinity of A-granules is greater (36.6%) than the B-granules (33.0%; Tang et al., 2002). The large A-granules contain more amylose than small B-granules (Ao and Jane, 2007). The total number and the weight of A- and B-granules differ among different genotypes (Li et al., 2001). The A-granules constitute

Table 2.1. Comparison of starch, amylose and amylopectin contents (g/kg) in different cereal grains

	Nutrient composition of cereal grains (g/kg)						
References	Cereal grain	Hulled/ hull-less	Starch type	N	Starch	Amylose	Amylopectin
Oscarsson et al., 1996	Barley	Hulled	High amylopectin, waxy	3	571		
			High protein, normal	2	571		
			Low β-glucan in grain, normal	1	600		
			Low β-glucan in wort, high enzyme activity, normal	2	627		
			Normal starch	1	560		
			High amylose	1	494		
		Hull-less	High amylopectin, waxy	1	602		
			High protein and lysine, from hiproly, normal	1	494		
			Normal (gene from Chinese material)	3	654		
			High amylose	1	584		
Knudsen, 1997	Barley	Hulled	-	10	587		
		Hull-less	-	6	645		
	Maize			3	690		
	Wheat			5	651		
	Barley	dehulled	waxy	-	630		
	Barley	dehulled	normal	-	673		
	Barley	dehulled	high amylose	-	607		
Li et al., 2001	Barley	Hull-less	Zero amylose	1	585	0	585
			Waxy	2	622	34	588
			Waxy (compound granule)	1	582	27	555
			Normal amylose (compound granule)	2	604	177	427
			Normal amylose	2	642	173	469
			High amylose	2	563	251	312
Ravindran et al., 2007	Barley	Hulled	normal		598	281	719
		Hull-less	normal		655	249	751
		hull-less	waxy		642	83	917
		hull-less	waxy		586	29	971
Biel and Jacyno, 2013	Barley	Hulled	normal	4	591-616		
Perera et al., 2019	Barley	Hulled	Normal	1	610	267	343
		Hull-less	waxy	1	554	77.2	477
	Wheat			1	537	229	308

[1]*n* = Number of samples analysed.

only about 10-15% in mature grains but can constitute up to 90% of the total weight of starch (Bleidere and Gaile, 2012).

Environmental factors, such as the growth temperatures during grain filling, have been shown to affect both granule size and starch properties. Starch accumulation is reduced when endosperms develop at high temperatures (Macleod and Duffus, 1988). It has been suggested that high temperatures have adverse effects on the sucrose cleavage enzyme UDP-sucrose synthase activity, which is necessary for the development of grain (Macleod and Duffus, 1988). Tester et al. (1991) examined effects of ambient temperatures (10, 15 and 20 ^{o}C) during grain filling on four different barley genotypes (one waxy, two normal, and one high-amylose barley) and the results showed that grain that was grown at high temperatures had lower starch accumulation, smaller A- and B-granules, and fewer B-granules. Furthermore, Savin and Nicolas (1996) reported that drought, combined with elevated temperatures, resulted in reduced grain weight of barley.

ii) Protein and amino acid

Cereal grains contain a relatively small amount of protein averaging about 10-12% of DM compared to legume seeds (Shewry and Halford, 2002). The protein content in the grains depends on the cultivar being grown, practices involved in the cultivation, such as nitrogen (N) fertilisation, and environment. The six-row cultivars of barley have a higher protein content compared to the two-row cultivars (Jeroch and Dänicke, 1995). Oscarsson et al. (1998) reported that increasing levels of N fertilisation rates in different barley cultivars at 45, 90 and 135 kg/ha, increased yields in all the barley cultivars, irrespective of the hull and starch type. Oscarsson et al. (1997, 1998) reported that the protein content of barley varied from 7.1-14.8%. The relative levels of essential amino acids decrease as the protein content of the grain increases with nitrogen fertilisation (Jeroch and Dänicke, 1995; Jacob and Pescatore, 2012).

Furthermore, the absence of the hull seems to affect the content of protein in barley. Andersson et al. (1999) reported that the protein content in naked barley samples was lower compared to normal barley and they suggested that the absence of a hull to be the cause. In contrast, Holtekjølen et al. (2006) reported that due to the absence of husk, the hull-less barley varieties were higher in protein content than hulled barley while the waxy and high-amylose varieties had higher protein, β-glucan and soluble fibre content. This highlights a strong positive correlation between β-glucan and protein contents. Ravindran et al. (2007) reported that the crude protein (CP) content is independent of hulls, highlighting that the differences in amino acid composition might not be influenced by the starch type, but rather by the CP content (Table 2.2). Moreover, Biel and

Jacyno (2013) reported that the barley varieties with a higher CP content had a lower quality of proteins, with lysine being the most limiting amino acid examined in all barley varieties.

In barley, the proteins are characterised by an unbalanced amino acid pattern compared to the requirements of poultry (Jeroch and Dänicke, 1995). Barley also has low lysine content but compared to wheat and maize, barley has a more favourable amino acid (Jeroch and Dänicke, 1995). Since a formulated poultry diet consists of a large proportion of cereal grains, there is a potential of using barley in poultry diet formulations; however, when the CP content in barley increases, the protein quality deteriorates due to reduced relative proportions of lysine and other essential amino acids. Since barley has a higher CP content compared to maize, and with a strong positive correlation with β-glucan which impedes starch and protein utilisation, the addition of NSP-degrading enzymes and protease enzyme could improve the ileal digestibility of starch and protein and thus improve the nutritive value of barley.

iii) Non-starch polysaccharide

Polysaccharides are polymers of monosaccharides joined by glycosidic linkages, and the term non-starch polysaccharides (NSP) covers a large variety of polysaccharide molecules, excluding the α-glucan (starch; Choct, 1997). The fibre content of the grains consists primarily of NSP, which in cereals, form part of the cell wall structure (Choct, 1997). The physicochemical properties of NSPs in broiler diets are responsible for the anti-nutritive activities observed in broiler chickens. It contributes to the encapsulation of nutrients within the endosperm cell walls and increases in the intestinal viscosity, both of which impair digestion and reduce the efficiency of absorption of nutrients in broilers fed barley-based diets. The NSPs are categorised according to their solubility in water into two groups, namely, the soluble and insoluble NSP (Choct, 2015). Table 2.3 compares the NSP contents in barley, wheat, and maize in different studies and also the solubility of the NSPs. Soluble NSPs reduce the digestibility of starch, proteins and fat, leading to changes in gut physiology, gut microflora, and gut health, whereas insoluble and non-viscous NSPs have beneficial effects on the GIT development and endogenous enzyme secretion (Smits and Annison,

Table 2.2. Crude protein and amino acid composition (g/kg) in various barley varieties and wheat

	Ravindran et al. (2005)	Ravindran et al. (2007)				Biel and Jacyno (2013)	Perera et al. (2019b)		
	Barley	Barley				Barley	Barley		Wheat
		Hulled normal	Hull-less normal	Hull-less waxy	Hull-less waxy	Hulled normal	Hulled normal	Hull-less waxy	
Crude Protein	97	116	104	105	137	117-136	101	133	141
Alanine	4.3	4.54	4.12	3.69	5.79	3.81	4.28	4.98	4.99
Arginine	5.1	5.55	4.91	4.08	6.39	4.0325	5.28	6.44	6.79
Aspartic acid	6.6	7.73	6.72	6.37	10.86	5.755	6.82	8.09	7.46
Cysteine	2.4	2.33	2.26	2.21	2.41	1.395	2.65	3	3.5
Glycine	4.2	4.62	4.02	3.56	5.54	3.715	4.38	4.99	5.95
Histidine	2.4	3.11	2.58	2.3	3.45	2.4275	2.35	2.82	3.46
Isoleucine	3.5	4.18	3.89	3.54	5.24	3.3275	3.69	4.87	4.94
Leucine	7.1	8.15	7.24	6.47	10.07	6.185	7.02	8.99	9.82
Lysine	3.7	4.06	3.43	3.07	5.23	3.66	3.84	4.55	3.95
Methionine	1.8	1.85	1.69	1.66	1.89	1.5825	2.16	2.23	2.52
Phenylalanine	5.0	6.56	5.13	4.51	8.16	5.02	5.13	7.31	6.99
Serine	4.3	4.53	4.26	3.63	5.25	4.0625	4.5	5.23	7.1
Threonine	3.2	3.77	3.55	3.1	4.68	3.145	3.67	4.18	4.14
Tryptophan	2.9	1.22	1.03	1.05	1.16	1.1825	-	-	-
Valine	5.0	5.95	5.46	4.88	7.08	4.35	5.54	6.82	6.49
Proline	-	14.22	11.35	10.37	18.32	9.5375	10.6	16.1	15.2
Tyrosine	-	3.8	3.38	2.51	4.38	2.5275	3.41	4.36	4.68
Glutamic acid	24.4	31.81	27.53	24.16	37.93	24.7425	23.6	34.4	45.1

1996; Hetland et al., 2004; Choct, 2015). The two major dietary fibre (DF) polysaccharides in barley are β-glucans (3.8-7.9%) and arabinoxylans (4.8-9.7%; Oscarsson et al., 1996). The definition of DF provokes a great deal of controversy because there have been numerous meanings over the years, including those based on the physiological effects and those based on methods of determination. In monogastric animal nutrition, DF is the sum of NSP and lignin (Choct, 2015), thus understanding the differences in the definition in different studies is important.

a) Soluble

The presence of soluble NSPs in poultry diets elicit the anti-nutritive properties and result in a significant increase in gut viscosity that causes a reduced apparent metabolisable energy (AME) of the diet, depressed feed efficiency and growth performance of birds (Choct et al., 1996). The partially mixed-linked (1→3), (1→4) β-D-glucan and arabinoxylans are the main NSP present in both wheat and barley compared to maize. In barley and oats, the predominant soluble NSP is the β-glucan whereas, in wheat, rye and triticale, arabinoxylan is the predominant NSP (Classen, 1996; Smits and Annison, 1996; Iji, 1999). It was observed that there was a moderate to high significant positive correlation that exists between viscosity and soluble fibre contents in barley (Svihus et al., 2000). β-glucans are the least digestible, while the arabinoxylan is intermediate (Iji, 1999). The solubility of β-glucan affects the soluble:insoluble NSP ratio, which is greater in barley (1.50 g/100g) than in wheat (0.47 g/100g; Messia et al., 2016). The effect of intestinal viscosity caused by NSPs has been shown to reduce the diffusion of endogenous enzymes and nutritional substrates in which can cause an increased feed passage time (Fengler and Marquardt, 1988).

The mixed-linked (1→3), (1→4)-β-D-glucans is a soluble NSP, joined by the (1→3) and (1→4) glycosidic bonds (Jacob and Pescatore, 2014). The structure of the chain depends on the relative number of (1→3) and (1→4) glycosidic bonds between the repeating glucose molecule. Compared to the insoluble cellulose with the regularity of the polymer chains with the β-(1→4)-links, the mixed-linked β-glucans cannot form this extensive inter-chain cross-links, resulting in polymers binding with water and readily forming viscous gels (McNab and Smithard, 1992; Jacob and Pescatore, 2012, 2014). The problem elicited by barley is due to the soluble, viscous, β-glucan component in endosperm cell walls (Burnett, 1966; White et al., 1983). The β-glucan content is high in barley, making it unpopular as a constituent of poultry diets due to increases in digesta viscosity and consequently, loss of performance due to interference with digestion and absorption of nutrients (McNab and Smithard, 1992). Furthermore, the viscosity is not related to the linkage type or sugar composition of a polysaccharide, but rather, determined by the physical properties of the polysaccharide, such as molecular weight, distribution and structure (Choct, 2015). For instance, the molecular weights of soluble β-glucans are greater than those of the insoluble glucans (Gajdošová et al., 2007).

In barley, β-glucan constitutes about 70% of the starch endosperm cell walls and the remaining 25% consists of the aleurone cell walls (Åman and Graham, 1987). The total β-glucan content of barley cultivars varies between 26 and 79 g/kg DM with 23-69 g/kg DM as soluble β-glucan (Jeroch and Dänicke, 1995). The AME of barley as a feedstuff for poultry is greatly affected by the content of β-glucan (Kocher et al., 1997). In contrast, the cereal varieties rich in arabinoxylans and β-glucan have a strong potential for the production of health food products that contain high overall dietary fibre content (Messia et al., 2016). Diets containing barley β-glucans have been associated with lowering plasma cholesterol (LDL-cholesterol and triglycerides) in men and women (Behall et al., 2004; Izydorczyk and Dexter, 2008; Talati et al., 2009). It was reported that a relationship exists between the consumption of food rich in soluble fibres such as β-glucan, and the reduced risk of heart disease (De Paula et al., 2017). This highlights the benefits of soluble NSPs on reducing the risk of heart diseases in humans.

The hulled high amylose variety of barley has a higher total and unextractable (insoluble) β-glucan than the waxy types, both of which are higher than normal starch varieties (Oscarsson et al., 1996; Andersson et al., 1999; Gajdošová et al., 2007; Ravindran et al., 2007). A strong positive correlation was observed between the β-glucan and the amount of soluble NSP, and protein contents (Holtekjølen et al., 2006), while an inverse relationship was observed between total β-glucan content and starch content (Izydorczyk et al., 2000). Therefore, it is important to carefully consider the amylose:amylopectin ratio and the β-glucan content in barley-based diets for poultry when determining the feeding value of broiler diets (Perera et al., 2019b).

The structure of cereal arabinoxylans (pentosans) is composed predominantly of two pentoses, namely arabinose and xylose, and their molecular structure consists of a linear (1→4)-β-xylan backbone (Perlin, 1951). Arabinoxylan is a minor component of the barley endosperm cell wall, and a major component in aleurone cell wall; however, it is mainly located in the outer layers of barley kernel and husk (Izydorczyk et al., 1998; Holtekjølen et al., 2006). A strong negative correlation was observed between the amount of arabinoxylans and the degree of branching, as well as a negative correlation between β-glucan and arabinoxylan (Holtekjølen et al., 2006). The molecular characteristics of β-glucans and arabinoxylans are important determinants of their physical properties such as water solubility, viscosity, and gelation properties, as well as their physiological functions in the GIT (Izydorczyk and Dexter, 2008). This highlights the importance of considering viscosity based on both the concentration of NSPs as well as the molecular weight.

b) Insoluble

The insoluble fibre fraction has traditionally been regarded as a nutrient diluent in monogastric animal diets (Hetland et al., 2004; Michard et al., 2011). Insoluble and non-viscous NSP affect the gut functions and modulates nutrient digestibility of protein, starch, and fat (Smits and Annison, 1996; Hetland et al., 2004). The insoluble polysaccharides such as cellulose and xylans can hold water as they behave like sponges, but their viscosity is very low (Smits and Annison, 1996). The main effects of the water-holding capacity of insoluble NSPs are the ability to increase the bulk of the chyme and to enhance the digesta passage rate in the small and large intestine (Smits and Annison, 1996). It has been observed that starch digestibility is higher, and digesta passage rate is faster when moderate levels of insoluble fibre are present in diets (Hetland et al., 2004). Oat hulls improved the digestibility of starch in young chicks fed unpelleted wheat diets, and the fibrous texture of oat hulls have a physiological effect in the gizzard, causing gizzard hypertrophy resulting in heavier gizzards (Rogel et al., 1987; Sacranie et al., 2012). Similarly, Amerah et al. (2009) also reported that wood shavings in the diet increased relative gizzard size and improved feed efficiency, ileal starch digestibility and excreta quality. Moreover, in a wheat-based diet, Svihus and Hetland (2001) reported that the starch digestibility increased significantly from 0.97 to 0.99 when oat hulls were included in the diet. They observed that the feed intake in diets containing oat hulls was high and suggested that the inclusion of oat hulls increased gut volume and passage rate. The effects of insoluble fibre on GIT development and broiler performance differs depending on the source and coarseness of fibre (Amerah et al., 2009). The insoluble fractions accumulate in the gizzard resulting in gizzard hypertrophy which then improves the grinding capacity to reduce particle sizes entering through the pyloric sphincter into the duodenum, and this contributes to the increased nutrient availability and utilisation (Michard, 2011; Sacranie et al., 2012). The general practical recommendation of coarse and durable fibres in broiler diets is between 2-3% (Michard, 2011; Mateos et al., 2012).

In barley grains, the cellulose content is generally highest in the hulled types, and this is due to the presence of the hull (Oscarsson et al., 1996). The insoluble NSP contents varied from 10.6-27.3% in hulled two- and six-rowed Norwegian varieties, while the hull-less waxy barley had lower insoluble NSP contents (Holtekjølen et al., 2006). The cellulose content varied from 8.0-17.7% with the highest amounts in the hulled waxy, followed by hull-less waxy, and high amylose barley. Atypical variety among the hull-less varieties had a higher amount of cellulose, which could imply that starch type may also influence the cellulose content. However, in general, the normal hulled barley varieties contain a high amount of total NSP and have more insoluble than soluble NSP (Holtekjølen et al., 2006).

iv) Other components (fat and mineral)

Several studies on lipid and fatty acid contents of barley have been reported (Bhatty et al., 1974; Price and Parsons, 1975; Fedak and Roche, 1977; Osman et al., 2000; Liu, 2011). It has been reported that the crude fat content of barley has not been improved over the years. The crude fat content of barley grain ranged between 10 and 37 g/kg DM (Bhatty et al., 1974; Fedak and Roche, 1977; Oscarsson et al., 1996; Osman et al., 2000; Svihus and Gullord, 2002, Liu, 2011). The range of fat content is narrow, and the contents are low among the diverse cultivars of barley, making the prospects of improving the fat content unlikely (Fedak and Roche, 1977). Oscarsson et al. (1996) reported that both hull and hull-less barley showed no difference in crude fat contents regardless of hull type. In contrast, naked barley varieties have higher contents of fat than hulled grains to some extent because the presence of hull reduces the fat content in barley grains (Andersson et al., 1999; Liu, 2011).

The fatty acid content in barley is affected by both genotype and the environment (De Man, 1985). The most abundant fatty acids in barley are palmitic (C16:0), oleic (C18:1) and linoleic (C18:2) acids. It has been reported that linoleic acid content is comparatively high in barley grains, representing an average of 10.52% compared to sorghum averaging 1.46% (Osman et al., 2000). Furthermore, Liu (2011) revealed that from the outer surface to the inner core of seeds, oil content

Table 2.3. The non-starch polysaccharide types and contents in different cereal sources (g/kg DM)

Reference	Grain type	Hulled/ Hull-less	Starch type	*n*[1]	Soluble/ Insoluble	NSP[2]										Klason Lignin	Total NSP (%)
						AX	A	X	BG	CEL	MA	GAL	UA	GLU	Total		
Andersson et al. (1999)	Barley	Hulled	Normal	1	Soluble	77	2.4	3.2	22		0.7	0.7	1.5	32	40	15	17
					Insoluble		21	50	25	40	3.6	2.0	2.9	55	200		83
		Hulled	High amylose	1	Soluble	90	4	5.6	26		1.4	0.8	1.7	49	63	15	20
					Insoluble		23	57	43	47	6.7	2.3	3.4	67	249		80
		Hulled	Waxy	1	Soluble	75	3.3	4.6	31		0.9	0.8	2.1	46	58	14	23
					Insoluble		22	45	30	35	3.7	2	3.1	50	191		77
		Hulled	Normal	1	Soluble	83	2.6	3.2	15		0.8	0.7	1.1	21	29	17	13
					Insoluble		23	55	13	42	6.7	2.1	3.5	49	194		87
		Hull-less	Normal	1	Soluble	52	3.5	4.9	24		1	1.1	1.5	32	44	7.4	26
					Insoluble		17	27	22	19	3.9	1.6	1.9	33	125		74
		Hull-less	High amylose	1	Soluble	57	4.5	6.6	26		1.4	0.8	1.7	48	63	11	28
					Insoluble		18	28	48	16	4.7	1.5	1.9	42	160		72
		Hull-less	Waxy	1	Soluble	48	2.8	3.6	30		0.9	0.7	1.9	37	46	6.9	27
					Insoluble		18	24	26	14	4.2	1.8	1.7	33	123		73
		Hull-less	Waxy	1	Soluble	120	7.8	13	12		3.7	1.8	2.4	123	152	10	30
					Insoluble		38	61	137	41	10	2.9	3.3	67	360		70
Holtekjølen et al. (2006)	Barley	Hulled	Normal	28	Soluble	13.7	-	-	34						106		31
					Insoluble	116	-	-		127	-	-	-	-	232		69
		Hull-less	Normal	6	Soluble	22.6	-	-			-	-	-	-	124		49
					Insoluble	66.1	-	-		91.8	-	-	-	-	127		51
		Hull-less	Waxy	3	Soluble	24.1	-	-			-	-	-	-	200		64
					Insoluble	62.9	-	-		126.7	-	-	-	-	114		36
		Hull-less	High amylose	1	Soluble	20.5	-	-			-	-	-	-	222		64
					Insoluble	68	-	-		140	-	-	-	-	125		36
		Hulled	Waxy	1	Soluble	15.5	-	-	65		-	-	-	-	184		45
					Insoluble	109.4	-	-		177	-	-	-	-	223		55
Choct (2015)	Barley			1	Soluble	8	-	-	36		t	1	t		45		27
					Insoluble	71	-	-	7	39	2	1	2		122		73
	Wheat			1	Soluble	18	-	-	4		t	2	t		24		21
					Insoluble	63	-	-	4	20	t	1	2		90		79
	Maize			1	Soluble	1	-	-	t		t	t	t		1.0		1.0
					Insoluble	51	-	-	-	20	2	6	t		80		99

and C18:1 and linolenic acid (C18:3) decreased while C16:0 and stearic acid (C18:0) increased, and C18:2 changed slightly. The results of the study by Liu (2011) provided two major reasons for improved oxidative stability of pearled barley grains: i) reduced oil content and ii) shifting fatty acids towards more saturated and less unsaturated. In barley, the oil is concentrated in the germ and the bran region than the inner endosperm. This explains why the pearling of barley removes the regions where oil concentration is high, therefore lowers the lipid content and consequently reducing the chances of oxidation, thus improves the storage stability of the pearled barley (Liu, 2011).

Table 2.4. Mineral composition of different cereal sources

Minerals	Jang et al. (2003)				Rodehutscord et al. (2016)			Perera et al. (2019b)		
	Barley		Maize		Barley	Wheat	Maize	Barley		
	WT[1]	LP[1]	WT	LP				NSH[1]	WSHL[1]	Wheat
n[2]	1	1	1	1	21	29	27	1	1	1
Calcium	0.6	0.6	0.02	0.03	0.59	0.4	0.04	0.39	0.36	0.35
Total Phosphorus	4.1	3.3	3.2	3.2	4.3	3.67	3.17	3.25	3.86	4.26
Phytate P	2.3	1.1	2.2	0.9	2.81	1.92	2.26	1.32	1.79	2.22
Non-phytate P	1.8	2.2	1.8	2.3	1.49	1.75	0.91	1.93	2.07	2.04
Magnesium	1.3	1.3	1.3	1.2	1.63	1.56	1.45	1.28	1.39	1.45
Potassium	-	-	-	-	5.53	4.33	3.96	4.25	5.62	4.93
Sodium	-	-	-	-	0.050	0.005	0.003	0.2	0.1	0.06
Iron	-	-	-	-	0.044	0.041	0.022	0.06	0.06	0.06
Chloride	-	-	-	-				1.31	1.27	0.71
Manganese	0.017	0.020	0.007	0.007	0.015	0.032	0.005	-	-	-
Zinc	0.030	0.037	0.014	0.014	0.024	0.022	0.021	-	-	-
Copper	0.009	0.011	0.006	0.006	0.005	0.004	0.002	-	-	-

[1]WT = Wild type, LP = Low phytate, NSH = Normal starch hulled, WSHL = Waxy starch hull-less.
[2]*n* = Number of samples analysed.

Regarding minerals present in barley, few studies have compared the composition in some barley varieties and other cereal grains (Table 2.4). A recent study revealed that potassium is the major mineral in barley followed by phosphate (Perera et al., 2019b). The study further stated higher contents of magnesium and potassium in the hull-less waxy than the normal hulled barley, while potassium content was higher in the hull-less waxy barley than in wheat. In addition, in normal hulled barley, the content of sodium is markedly higher than in the hull-less waxy barley. All minerals were generally higher in the hull-less waxy barley except sodium, chloride and iron (Perera et al., 2019b).

2.2.2 Optimum inclusion rate of barley in the diet

The optimum inclusion rate of barley in poultry diets is important to consider due to the adverse effects that impede digestion and absorption of nutrients caused by the high levels of NSPs, particularly β-glucan and arabinoxylan. Table 2.5 shows the optimum inclusion level of barley, the duration of and the basal diets used in different studies. The range of inclusion is between 283 to 350 g/kg of diet. Perera et al. (2019a) reported 283 g/kg of the diet is the optimum inclusion level of barley to improve weight gain, feed intake and FCR. Inclusion over 283 g/kg resulted in reduced weight gain regardless of carbohydrase enzyme supplementation.

Table 2.5. Inclusion rate of barley

Reference	Optimum inclusion rate (g/kg diet)	Age (d)	Basal diet
Friesen et al. (1992)	350	17	Wheat diet
Jerock and Danicke (1995)	200-300	-	-
Hetland et al. (2002)	300	38	Wheat diet
Perera et al. (2019a)	283	21	Wheat diet

2.3 Effects of feed particle size of diet in broilers

The current industry practice of feed processing involves the reduction in particle size by grinding. It has been postulated that finer grinding increases substrate availability for enzymatic digestion; however, there is evidence that coarser grinding to a uniform particle size improves the performance of birds. This counter-intuitive effect may result from the positive effect of feed particle size on gizzard development (Amerah et al., 2007a). A more developed gizzard is associated with an increased grinding activity, increased gut motility, and improved digestibility of nutrients and overall performance (Amerah et al., 2007a).

2.3.1 Measurement of particle size

Feed particle size can be defined as the average diameter of individual particles of feed or the ‘fineness of grind’ of the feed (Amerah et al., 2007a). Early studies used the general terms ‘fine, medium and coarse’ to describe particle size, but these terms prevent meaningful comparisons of data. Therefore, the average particle size is described as the geometric mean diameter (GMD), expressed in millimetre (mm) or micrometre (μm) and the range of variation is described by geometric standard deviation (GSD), such that a larger GSD represents a high presence of fine dust particles, resulting in lower uniformity (Nir et al., 1994a; Amerah et al., 2007a).

The two common methods of particle size measurements are i) dry sieving method which is used to measure particle size of feed samples; and ii) wet sieving method which is used to measure particle size of digesta and excreta samples (Amerah et al., 2007a). In a dry sieving method, ground feed samples are passed through a sieve stack with a set of sieves from coarsest on top and finest at the bottom (2000, 1000, 500, 250, 125, and 63 μm, respectively), and the shaker is allowed to run for 10 minutes (Baker and Herrman, 2002). The amount of sample retained on each sieve can be weighed, and the GMD and GSD of each sample can be calculated. Moreover, in the wet sieving method, a sample of digesta is suspended in 50 ml of distilled water for 30 minutes prior to sieving to ensure adequate hydration (Lentle et al., 2006). The sample is then washed through a set of sieves and the content of each sieve is washed onto a dried pre-weighed filter paper. The retained contents on the filter papers are then dried for 24 hours in a forced draft oven at 80^{o}C. The GMD and GSD of the digesta and excreta samples can then be calculated.

2.3.2 Methods of particle size reduction

The two most commonly used equipment for particle size reduction of grains are the hammer mill and roller mill (Koch, 1996). Hammer mill comprises a set of hammers moving at high speed in a grinding chamber, which reduces the size of the grains until the particles are able to pass through a screen of designated size (Amerah et al., 2007a). The distribution of particle sizes will vary widely around the geometric mean such that there will be some large-sized and small-sized particles (Koch, 1996). Moreover, the efficiency of hammer mill is influenced by factors such as grain type, grain moisture content, screen size, screen area, peripheral speed, hammer width and design, number of hammers, hammer tip to screen clearance, feed rate, power of the motor and speed of airflow through the mill (Martin, 1985).

Roller mill comprises one or more pairs of horizontal rollers in a supporting frame where the distance between rollers may be varied according to the particle size required (Amerah et al., 2007a). Roller mills are more efficient and require less energy for grinding than hammer mill. Compared to the hammer mill, the roller mill produces a more uniform particle size distribution with a lower proportion of fines (Nir and Ptichi, 2001). As with the hammer mill, the particle size of roller mill product is influenced by grain type, such that different grains ground with the same conditions produce different particle sizes (Amerah et al., 2007a).

2.3.3 Effect of particle size and distribution on performance

The performance of birds is influenced, not only by the feed particle size but also the uniformity of the particle sizes (Amerah et al., 2007a). When the particle size of two ingredients are extremely different, they have a high chance to segregate, thus, particle size is the most important ingredient factor (Axe, 1995). Particle shape is another ingredient factor that influences properties such as flowability and packing ability (Axe, 1995). Birds select their feed according to particle sizes due to the mechanoreceptors located at the beak. Therefore, a more uniform diet will reduce the time spent searching and selecting larger particles, with beneficial effects on performance (Amerah et al., 2007a). Nir et al. (1994a) reported that the best performance of birds was observed with diets having a medium texture. The GMD of the medium texture of maize, wheat and sorghum diets varied from 1130 to 1230 µm and GSD from 1.19 to 1.35. Furthermore, the fine diets (GMD 500 to 670 µm) had the lowest performance while the coarse fraction (2010 to 2100 µm) was intermediate. Nir et al. (1994b) also reported that there was an improved performance with diets containing the medium particle size. This highlights that uniform feed particle size (smaller GSD) improves performance due to improved feed utilisation and to a certain extent, feed intake.

There are contradictory results regarding the effect of feed particle size in pelleted diets on broiler performance. Svihus et al. (2004a) showed no difference in any of the performance parameter when broilers were fed pelleted wheat-based diets made in the hammer and roller mills to a range of particle sizes and concluded that pelleting evened out the differences in particle size distribution. Amerah et al. (2007b) also reported improved performance in pelleted diets compared to mash diets, but no effect of particle size between treatments was observed pelleted diets, which suggest that pelleting evened out the differences in particle size distribution. In contrast, Lentle et al. (2006) reported that in three wheat varieties with different particle size spectra as a result of hammer milling, diets with a higher relative proportion of coarser particles resulted in better FCR. On the other hand, Reece et al. (1986) reported that broilers fed pelleted fine and coarse ground diets improved weight gain and FCR, compared to those fed pelleted medium ground diets, even though there was no difference in feed intake. This may suggest that the effect of particle size on performance may also be influenced by grain type.

2.3.4 Effect of particle size on GIT development and physiology

The development of the digestive tract of poultry, particularly the gizzard, has been shown to be influenced by feed particle size (Amerah et al., 2007a). Nir et al. (1994b) reported that feeding a coarse mash diet to broilers increased the gizzard weight compared to fine mash diets. The gizzard has adapted to grinding food particles in the digestive tract through the development of a very large mass of strongly myelinated smooth muscles (Duke, 1986; Svihus, 2011b). The main body of the gizzard comprises two thick, opposed lateral muscles and two thin anterior and posterior muscles. The gizzard is where the digestive action takes place by mixing ingested food with digestive enzymes and grinding to reduce feed particle sizes (Duke, 1986; Svihus, 2011b). The increase in the size of the gizzard is a logical consequence of an increased need for particle size reduction, due to the stimulative effect of the increased grinding activity on the size of the two pairs of gizzard muscles (Svihus, 2011b). However, the fine grinding of feed particles in diets negatively affects the gizzard size and function. As a result, the gizzard is underdeveloped, and the proventriculus is dilated and enlarged when fed finely ground diets (Jones and Taylor, 2001). In this condition, when the finely ground diet is fed to birds, the gizzard only acts as a transit point rather than performing its original function to grind.

The particle size is positively correlated to the gizzard development in that as feed particle size increases, the relative weight of gizzard also increases (Nir and Ptichi, 2001; Amerah et al., 2008; Svihus, 2011b). The medium and coarse particle sizes of feed result in increased gizzard weight compared to the fine particle size (Nir et al., 1994a; Naderinejad et al., 2016). In addition, regardless of the feed particle size, Abdollahi et al. (2018a) reported that feeding oat hulls and wood shavings in wheat diets increased the caudodorsal muscle of the gizzard. They concluded that a high proportion of coarse particles and GMD is not always associated with the most developed gizzard musculature. Gizzard mass and musculature response are driven more likely by the nature of structural components than particle size.

The gizzard has been described as the 'pace-maker' of gut motility in broiler chickens (Ferket, 2000). The major stimuli causing an increased enzyme secretion by the pancreas are the vagus nerve and cholecystokinin (CCK) in the duodenal region of birds (Duke, 1992; Li and Owyang, 1993; Svihus et al., 2004a; Svihus, 2011b). Large feed particles have been shown to influence the development and increased activity of the gizzard, thus, a stimulating effect of an increased gizzard activity on bile and enzyme secretion may be due to the gizzard-mediated vagovagal reflexes and increased CCK in the pyloric region (Svihus et al., 2004a; Hetland et al., 2003).

Feed particle size has been shown to influence the development of the lower segments of the digestive tract of birds (Amerah et al., 2007a). Interaction between feed form and particle size on the relative lengths of the gut components (duodenum, jejunum, ileum, and caeca) have been reported in Amerah et al. (2007b) and Naderinejad et al. (2016) studies. Feeding fine mash diets resulted in the hypertrophy of the small intestine as well as a reduction in pH (Nir et al., 1994b). Amerah et al. (2007b) reported that in birds fed mash diets, gut components were shorter with coarse particles than medium particles; however, in birds fed pelleted diets, there was no difference in gut lengths with coarse and medium particles. In addition, Amerah et al. (2008) reported an interaction between particle size and grain type where wheat-based diets generally had shorter relative lengths of the gut components compared to maize-based diets. In maize-based diets, the gut components were shorter in birds fed coarse particles compared to those fed fine particles; however, there was no difference between gut components with different particle sizes in wheat-based diets (Amerah et al., 2008).

2.3.5 Effect of particle size on digesta particle size

The gizzard has a remarkable ability to grind all organic constituents of feed to a very consistent particle size range regardless of the original particle size of the feed, with some as fine as 0.05 µm (Hetland et al., 2004). The digesta passing through the gizzard has a consistent particle size distribution, with majority of the particle in the duodenum being below 40 µm in size (Hetland et al., 2002). In a barley-based diet, the number of particles in the intestinal contents with sizes greater than 2400 µm was not significantly different between broilers fed whole barley and those fed ground barley; however, the number of particles with sizes between 2400 µm and 700 µm was lower when diets with whole barley were fed (Svihus et al., 1997). In contrast to Hetland et al. (2004), Amerah et al. (2007b) reported that feed form significantly influenced the duodenal particle size, whereas particle size had no effect. In their study, birds fed mash diets had a higher relative weight of gizzard, but a high proportion of large particles (1000-2000 µm) was found in the duodenal digesta than in birds fed pelleted diets. This indicated that the larger gizzard size was not efficient to reduce the size of all particles uniformly. It is noteworthy to mention that the method of particle size measurement determines the feed particle size spectrum, for example, wet sieving determines changes over the range from less than 75 µm to over 2000 µm (Lentle et al., 2006). It has been reported that although the enlarged gizzard could grind and reduce large particle size, some large particles can escape grinding into the duodenum (Amerah et al., 2007b).

2.3.6 Effect of particle size on nutrient digestibility and energy utilisation

Although particle size reduction improves digestion of nutrients by increasing the surface area available to digestive enzymes, studies investigating the effect of particle size on digestibility of nutrients are limited. It is generally believed that small particle size of feed has a larger surface area and might be better digested due to greater access of digestive enzymes on the substrate in the GIT (Goodband et al., 2002). However, it has been shown that large particle size of feed improves nutrient digestibility. Parsons et al. (2006) reported that the coarse particle size of maize increased nitrogen and lysine retention in broiler diets. Large maize particle size improved utilisation of calcium, total phosphorus and phytate phosphorus in broilers (Kasim and Edwards, 2000; Kilburn and Edwards, 2001). The retention of these minerals decreases with medium particle size maize and lowest with fine particle size of maize (Kasim and Edwards, 2000). On the other hand, fine grinding of wheat was reported to improve starch digestibility and AME compared to coarse grinding (Péron et al., 2005).

2.3.7 Effect of particle size on passage rate

O'Dell et al. (1959) reported that a purified basal diet with fine particle size passes through the GIT more rapidly than maize meal or maize grits (135 *vs* 165 minutes). The proventriculus of birds fed the diet with fine particles was filled with fluid rather than with food. In recent studies, the mean retention time in the proventriculus and gizzard has been estimated to vary between half an hour to an hour (Svihus, 2011b), with an average total retention time of 3-4 hours (Svihus, 2011a). Svihus et al. (2002) revealed that after 30 minutes of feeding, there was a significant amount of titanium dioxide (TiO2) that had passed through the gizzard regardless of the structure of the diet. Similarly, Amerah et al. (2008) reported that the digesta transit time is not influenced by grain type or particle size. Furthermore, Svihus et al. (2002) hypothesised that whole grain slows the passage rate through the upper digestive tract; however, the results in their study did not support this hypothesis because the passage rate for titanium through the gizzard was not different between diets with ground and whole wheat. Naderinejad et al. (2016) reported that the digesta transit time was not significantly different between fine, medium, and coarse particles in mash and pellet diets.

The passage rate of the digesta is measured using insoluble (solid phase) coloured markers such as chromic or ferric oxide or TiO_2. It is noteworthy that the results may be confounded by preferential retention of particles in particular segments of the gut, by adherence to other particles or by dissolution (Svihus et al., 2002; Amerah et al., 2007a). Svihus et al. (2002) stated that uncertainty exists if titanium flow is representative for the flow of the whole diet.

Several factors are known to affect the passage rate of solid-phase markers, including the strain of chicken, age of the bird, NSP content, insoluble NSP content, dietary fat level, and environmental temperature (Amerah et al., 2007a). In general, larger particles are retained longer than finer particles in the digestive tract through selective retention (Nir et al., 1994b; Svihus et al., 2002). This highlights the fact that regardless of diet structure, the retention time of feed may vary between the feed particle sizes, but the flow rate is not different. This may be attributed to a functional gizzard that is able to grind large particles to smaller particles, as well as an increased volume to contain the food ingested.

2.3.8 Pellet quality

The cost of poultry feed ingredients is always increasing; thus, decreasing the physical feed wastage through better pellet quality enhances the economic value of feed (Abdollahi et al., 2013b). Pelleting is the most prevalent heat treatment in poultry feed production, usually used to agglomerate smaller feed particles into larger particles as pellets (Abdollahi et al., 2013b).

Good pellet quality is defined as the ability to withstand mechanical handling (bagging, transport, etc.) without breaking up, and reaching feeders without generating a high proportion of fines (Amerah et al., 2007a). Pellet quality is determined by two physical parameters; the pellet durability index (PDI) and pellet hardness (Amerah et al., 2007a). The PDI is expressed as the ratio of pellets not passing through the sieve after the test to whole pellets at the start (Abdollahi et al., 2013b). It is typically measured using the two parameters, namely the Holman Pellet Tester and Tumbling can (Behnke, 2001). Pellet hardness is determined, in a spring hardness tester, as the static force in kilograms (kg) required to break the pellet (Amerah et al., 2007a).

2.3.8.1 Steps of pellet processing

The pelleting process undergoes four stages; i) grinding, proportioning and mixing, ii) steam conditioning, iii) pelleting process, and iv) cooling (Abdollahi et al., 2013b).

i) Grinding, proportioning and mixing

After receiving the ingredients, the majority of the feed ingredients, particularly cereal grains, are ground to reduce particle size before incorporation and mixing in the diet. The physical reduction of particle size increases the surface area, allowing for greater digestibility, improve blending ability and homogeneity of the mixed feed, decrease segregation and mixing problems, and facilitate pelleting (Behnke, 1996; Koch, 1996; Abdollahi et al., 2013b). The proportioning can be accomplished using two basic methods, namely, cyclical (batch) and continuous (Abdollahi et al., 2013b). In a cyclical or batching system, ingredients are individually weighed into batches, whereas a continuous system involves concurrent and continuous addition of ingredients; thus, to achieve a homogeneous mixture of proportioned ingredients, proper mixing of ingredients is essential.

ii) Steam conditioning

Steam conditioning of mash prior to pelleting is a major step in the pelleting process (Skoch et al., 1981). To optimise the steam conditioning process, a proper balance of heat and moisture must be obtained (Abdollahi et al., 2013b). The injection of heat during processing is essential to improve binding characteristics and eliminate feed-borne pathogens. Steam conditioning at temperatures of 65 and 78^{o}C increased pellet production by 250 and 275% respectively (Skoch et al., 1981). The PDI was also improved by steam-conditioning with 90.6 and 93.8% at temperatures 65 and 78^{o}C, respectively. High conditioning temperature to manufacture pellets is a major concern on the stability of exogenous feed enzymes (Abdollahi et al., 2013b). The conditioning temperatures employed worldwide generally range between 80 and 90^{o}C, and these high temperatures improve pellet quality and reduce potential levels of feed-borne pathogens, such as salmonella and campylobacter (Abdollahi et al., 2013b).

iii) Pelleting process

The agglomeration of small particles into large particles occur when passing the mash feed from the mash bin into the feeder and conditioner. Steam is injected to the feed inside the conditioner, and then the conditioned mash flows into the pelleting chamber, and the hot mash passes through a metal die, and pellets are formed (Abdollahi et al., 2013b).

iv) Cooling

Pellets leave the pellet mill at temperatures from 80 to 90^{o}C and contain about 150-170 g/kg of moisture. The high temperature must be reduced to about 8^{o}C ambient temperature, and the moisture to 100-120 g/kg (Abdollahi et al., 2013b).

2.3.8.2 Factors affecting pellet quality

Several factors have been shown to influence the quality of pellets and can be divided into several categories; i) feed formulation, ii) particle size, iii) conditioning temperature, iv) mash moisture, v) retention time in conditioner and conditioner design, and vi) die size (Behnke, 2001).

i) Feed formulation

The addition of fat to the mash pre-pellet diet usually results in decreased pellet quality (Behnke, 2001). Due to lubricating effects, fat could reduce friction force generated in the die holes, and often results in lower pellet quality. Dietary fat can partially cover feed particles and create a barrier for penetration of steam to feed particles, preventing starch gelatinisation and development of binding adhesions (Löwe, 2005; Abdollahi et al., 2013b).

ii) Particle size

Previous studies reported contradictory results on the effect of feed particle size on pellet quality. Reece et al. (1986) reported that pellets made from coarse maize particles (9.53 mm) were more durable compared to pellets from fine particles (3.18 mm). In contrast, some studies reported that the durability of pellets decreased as the screen size of the grinder increased (Angulo et al., 1995, Amerah et al., 2008, Chewning et al., 2012). Fine particles have more contact points with each other due to the larger surface area per unit volume which has shown to improve pellet quality (Behnke, 2001). Similarly, Svihus et al. (2004b) observed that pellets with coarse wheat particles had poorer quality than pellets with fine particles. Furthermore, Nathier-Dufour et al. (1995) reported that differences in particle size had no significant effect on the compacting behaviour of the pellet; however, the variation between the particle sizes was small and could have influenced the similar compacting behaviour. In addition, Amerah et al.

(2008) found that pellet durability was influenced by both grain type and particle size. In their study pellet quality showed different trend with different particle sizes in maize and in wheat.

iii) Conditioning

Steam-conditioning of mash pre-pelleting is a major factor in the pelleting process compared to dry pelleting (Skoch et al., 1981). Skoch et al. (1981) reported that steam conditioning improved pellet durability and production rates and decreased the amounts of fines generated, along with reduced energy consumption. Heat and moisture are two primary prerequisites for feed particles adhesion (Abdollahi et al., 2013b). Steam acts as a lubricant to reduce friction during pelleting (Behnke, 2001). Mash diet entering the conditioner comprises a wide variety of ingredients and each of these ingredients can affect the conditioning and eventual pellet quality (Behnke, 2001). According to Reimer (1992), pellet quality is proportionally dependent on the following factors: 40% diet formulation, 20% particle size, 20% conditioning, 15% die specification, and 5% cooling and drying. This shows that 60% of pellet quality is determined before entering the conditioner and increases to 80% after conditioning.

iv) Mash moisture

The initial moisture of mash entering the conditioner dictates the amount of steam that can be added to the mash (Behnke, 2001). No more than 6% can be added to the conditioner; thus, large variation in initial diet moisture will be reflected in the moisture of hot mash, which can cause varying pellet mill performances (Behnke, 2001) which eventually can affect the output of pellets, thus pellet quality.

v) Retention time and conditioner design

Retention time refers to the amount of time that mash feed spends in the conditioner; thus, it is a measure of the duration of exposure of mash to steam for heat and moisture absorption (Behnke, 2001). The retention time is affected by conditioner design, including physical dimensions and operating parameters. The design and dimensions of conditioners vary in diameter, length, type of picks, number and placement of picks, pick angles, steam inlet location, presence or absence of baffles, and baffle placement, thus changing any of these physical parameters will affect conditioner time (Behnke, 2001).

vi) Die selection

As suggested by Reimer (1992), die selection contributes about 15% on pellet quality; however, it is important to consider when dealing with issues relating to pellet quality. In general, a die with a greater thickness within a specific die diameter (greater L/d ratio) will result in improved pellet quality (Behnke, 2001). This is because of the greater flow resistance generated by a thicker die as well as long retention time under elevated pressure as the pellet passes through the die. It is generally suggested that the operator should choose the thinnest die possible to gain the greatest production rate at an acceptable pellet quality.

An important parameter in describing the pellet die is the L/d ratio, which is the effective length of the die hole over the minimum die opening diameter (Behnke, 2001). For example, a 6 mm die with an effective hole length of 60 mm would have an L/d ratio of 10:1. For most common livestock diets, L/d ratios should be between 8:1 and 12:1. It must be noted that a greater L/d ratio will result in improved pellet quality but will sacrifice production rate while lower L/d ratios can result in increased production but lower pellet quality (Behnke, 2001).

2.4 Effects of whole-grain feeding in broiler diets

Whole-grain feeding in broilers has gained interest as it provides an economic advantage by effectively reducing the cost of grinding and processing as well as positive effects on the digestive functions (Ravindran et al., 2006; Singh et al., 2014a; Liu et al., 2015). The cost of feed accounts for up to 65% of the production cost of chicken meat and eggs thus eliminating the grinding mechanism lowers the cost of production, hence the feed cost. Therefore, the primary role of whole-grain feeding is to lower the costs of feed by eliminating the grinding and processing step (Singh et al., 2014a) as well as meeting consumer demand for a natural feeding system (Gabriel et al., 2003, 2008).

2.4.1 Methods of whole-grain feeding

The methods of whole-grain inclusion in broiler diets are classified into three main feeding methods, namely the free-choice feeding (FCF), mixed feeding (MF) and sequential feeding (SF, Rose, 1996; Singh et al., 2014a). In these feeding strategies, whole grain can be given along with another feed, either in mash, crumble or pellet form.

2.4.1.1 Free choice feeding

In FCF, birds are usually offered a choice among three types of ingredients; an energy source (e.g. cereal grains), a protein source (e.g. soybean meal, fish meal, meat meal, etc.) plus supplemental vitamins and minerals (Singh et al., 2014a). The basic principle behind FCF is that birds are capable of consuming the required nutrients from various feed ingredients to compose their diets according to their actual needs and production capacity (Hughes, 1984, Cumming, 1994). The theory behind this method is that birds if given a choice, can formulate a balanced ration on their own using nutritional wisdom (Singh et al., 2014a).

2.4.1.2 Mixed feeding

Mixed feeding is the commonly used method of feeding where whole grain is either substituted for a part of the ground grain in a complete diet or added to a complete diet in the same feeder at the same time in pellet or mash form (Singh et al., 2014a). There are two categories of mixed feeding method; the pre-pelleting (PRP) inclusion and post-pelleting (PP) inclusion of whole grain. In PRP, whole grain is first mixed with other feed components and then pelleted whereas in PP, other components of feed are mixed and pelleted, and whole grain is then mixed with the pelleted feed (Singh et al., 2014a).

2.4.1.3 Sequential feeding

In SF, birds are given time-limited *ad libitum* access to the whole grain, followed by time-limited *ad libitum* access to a complete or balancer diet (Rose et al., 1995). The birds are offered whole grains with a complete or balanced feed in the same feeder, but at different times (Singh et al., 2014a). This feeding regime is based on the principle of choice feeding and allows birds to exercise their freedom of choice between whole grains and complete or balanced feed, but with restricted time. When compared to FCF, SF is economical and efficient as it does not require offering food in two separate feeders, and more control on the amount of whole grain to be ingested by birds due to the time restriction (Singh et al., 2014a). However, this system needs more validation to direct it towards developing a commercial feeding system.

2.4.2 Effects of whole-grain feeding on digestive tract characteristics

A rapid and conspicuous enlargement and increased weight of the gizzard is usually observed when whole grains are included in broiler diets, which indicates that the development and physiology of the

GIT can be manipulated by dietary means (Svihus et al., 1997; Nahas and Lefrancois, 2001; Svihus and Hetland, 2001; Hetland et al., 2002; Taylor and Jones, 2004a; Wu and Ravindran, 2004; Amerah and Ravindran, 2008). The two major muscles in the gizzard reduce the particle size of ingested feed by grinding and mixing them with digestive enzymes (Duke, 1986). In the conventional feeding regime, whole grain is ground before incorporation into feed, and as a result, the gizzard is underdeveloped and functions as a transit rather than a grinding organ (Cumming, 1994). There is mounting evidence that gizzard responds rapidly to changes in the diet, particularly to changes in insoluble fibre and particle size, resulting in increased organ size or muscularity or both (Svihus, 2011b). The reduction of particle size to critical size must be consistent before leaving the gizzard (Hetland et al., 2002), therefore when feeding whole grain, the gizzard must be functional to achieve this. A comprehensive review by Svihus (2011b) recommended that at least 200 g/kg grain particles larger than 1.5-2.0 mm in size in the diet are needed to stimulate gizzard development.

Compared to the gizzard, when birds are offered ground grain, the average weight of proventriculus is increased due to proventricular dilation (Taylor and Jones, 2004b). However, the hypertrophy of the proventriculus due to the conventional pelleted diets was eliminated when whole grains were fed to broilers (Taylor and Jones, 2004a). In contrast, Ravindran et al. (2006) reported that whole wheat inclusion had no effect on the relative weight of proventriculus. Moreover, the weight of the crop and relative lengths and weights of the small intestine were not affected by whole grain inclusion in the diet (Wu and Ravindran, 2004; Ravindran et al., 2006; Amerah and Ravindran, 2008).

Furthermore, birds fed coarse ground diets and whole-grain diets resulted in a reduction in pH of the gizzard contents (Nir et al., 1994a; Gabriel et al., 2003). However, Hetland et al. (2002) experimented with different rate of replacement of ground wheat with whole wheat, barley and oats during 10 to 24 and 24 to 38 days of age (very high, 500 and 600; high 300 and 400 and moderate 125 and 300 g/kg, respectively). They reported that the pH of gizzard contents was not conclusively affected by grain type or form of the grain.

2.4.3 Effects of whole-grain feeding on feed passage rate, digesta particle size and viscosity

The feed passage rate is affected by particle size where larger particles are retained longer than finer particles in the gizzard (Svihus, 2011b). Svihus et al. (2002) hypothesised that rapid passage rate reduces the time available for digestion and absorption, whilst slower rate limits feed intake. Since the coarser particles need to be ground to a certain critical size before leaving the gizzard (Svihus, 2011b), longer transit time is expected with whole-grain feeding. However, surprisingly, several studies revealed that the overall retention time does not change when birds are fed whole grains (Svihus et al., 2002; Wu et al., 2004; Amerah et al., 2007a; Amerah and Ravindran, 2008). Hetland et al. (2005) reported that the lack of effect on passage rate may be due to the rapid dissolution of starch granules and protein from whole grain in the low pH environment of the gizzard which results in a rapid reduction of particle size.

Svihus et al. (1997) found that there was no difference in digesta particle size distribution in the duodenum of birds fed either whole or ground barley. Similarly, Hetland et al. (2003) reported that the particle size distribution in the duodenal digesta is not affected by whole wheat inclusion in the diet. Hetland et al. (2002) reported that the digesta passing through the gizzard had a consistent particle size distribution, with most particles being smaller than 40 µm regardless of the original particle size, highlighting the ability of gizzard as a grinding organ.

Studies investigating the influence of whole grain on digesta viscosity are limited. The degree of digesta viscosity is dependent upon the amount of NSP, which varies amongst cereal grains (Amerah et al., 2007). In wheat-based diets, fine particle size was found to increase digesta viscosity compared to whole wheat diets (Yasar, 2003). In contrast, some studies reported that whole wheat feeding resulted in an increased digesta viscosity (Engberg et al., 2004; Taylor and Jones, 2004b; Wu et al., 2004). In addition, the inclusion of 200 g/kg of whole barley did not alter the viscosity of the digesta beyond the duodenum (Taylor and Jones, 2004a). This highlights that viscosity may be dependent on the NSP content rather than the method of inclusion in the diet.

2.4.4 Effects of whole-grain feeding on performance

Whole barley is used as an alternative feed ingredient but compared to wheat and maize it is least preferred due to its relatively low protein and energy, and high fibre content. Svihus et al. (1997) reported that the inclusion of 200 g/kg whole barley and up to 350 g/kg whole wheat resulted in similar FCR compared with birds fed the maize-soybean diets (Nahas and Lefrancois, 2001). In contrast, broilers fed diets containing 80 to 150 g/kg of whole barley grains had lower body weight gains and a further decrease in body weights was observed when whole barley inclusion increased from 120 to 250 g/kg (Kliševičiūtė et al., 2012). Similarly, in a wheat-based diet, the inclusion of 200 g/kg whole wheat in a MF system had no effect on the weight gain and FCR of broilers (Amerah and Ravindran, 2008). Moss et al. (2018) also found that whole wheat feeding did not influence weight gain, but feed intake was depressed by whole wheat feeding. This highlights that in cases where whole-grain feeding had no effect on weight gain and FCR compared to conventional feed, the economics will favour whole-grain feeding due to lower processing costs. To optimise the benefits of whole-grain feeding, Forbes and Covasa (1995) suggested that whole-grain feeding should be introduced to broiler chicks from the first week of life.

2.4.5 Effects of whole-grain feeding on energy and nutrient utilisation.

As previously discussed, whole-grain feeding is generally associated with an increase in the size of gizzard, and it has been hypothesised that the resultant increase in grinding activity will favourably increase the bird's ability to better utilise nutrients (Svihus and Hetland, 2001). In addition to the grinding effect, an active gizzard also serves as a mixing compartment for digestive juices and substrates (Svihus, 2011b).

Wu et al. (2004) compared PRP and PP inclusion of 200 g/kg whole wheat in broiler diets and reported improvements in AME irrespective of the method of whole wheat inclusion, however, PP inclusion resulted in 6% greater improvement in AME than PRP inclusion. Svihus et al. (2004a) also reported that AME was increased at day 14 and 20 when 375 g/kg whole wheat was included PRP. In addition, Preston et al. (2000) reported an increase in AME when ground wheat was replaced by whole wheat. However, Uddin et al. (1996) found no difference when two wheat cultivars were fed ground or whole at different levels (100-400 g/kg) to broiler chickens at 19 to 27 days of age. Amerah et al. (2011) observed that PRP inclusion of whole wheat (100 and 200 g/kg) increased ileal protein digestibility but had no effect on apparent ileal digestible energy.

Some studies have shown that starch digestibility improved in birds fed whole wheat (Svihus and Hetland, 2001; Hetland et al., 2003; Svihus et al., 2004a; Wu et al., 2004) or whole barley (Hetland et al., 2002). Svihus et al. (2004a) reported that PP inclusion of 375 g/kg whole wheat increased starch digestibility at both ileal and excreta levels; however, a subsequent experiment using PRP replacement of ground wheat with 500 g/kg whole wheat failed to show any improvement in starch digestibility. This highlights that inclusion of whole grain in the diet for optimal starch digestion can be achieved at recommended optimum levels.

2.5 Exogenous enzymes in broiler diets

The use of exogenous feed enzymes in poultry diets is becoming a norm to overcome the adverse effects of anti-nutritional factors and improve digestion of dietary components and bird performance (Ravindran, 2013). The responses to supplementation of enzymes are often variable, with some limitations caused by pH and digesta retention time within the digestive tract. The largest user of feed enzymes is the poultry industry (Ravindran, 2013). The highly integrated nature of the poultry sector has enabled the faster uptake of these new technologies, and the inclusion of exogenous enzymes has now become the norm to improve the digestibility and efficiency of utilisation of nutrients. During the past three decades, the chemistry of target substrates in feed ingredients has been better understood, and it has become possible to fine-tune the production of enzymes that are specific for individual substrates (Ravindran, 2013). Almost all wheat- and barley-based diets for broilers worldwide are now supplemented with NSP-degrading enzymes such as xylanase and β-glucanase (Ravindran, 2013). During the past decade, the use of microbial phytase in poultry diets has increased in response to concerns over phosphorus pollution of effluents from intensive animal production and the increased price of inorganic phosphate (Ravindran, 2013). As a result, microbial phytase has overtaken xylanase and β-glucanase as the primary feed enzyme worldwide.

Protease enzyme supplementation has been of interest to improve protein and amino acid digestibility, particularly in very young animals where the relative activity of endogenous proteases may not be optimal (Lewis et al., 1955; Mahagna et al., 1995). A wide range of endogenous proteases are synthesised and released in the GIT of birds, and these are generally considered sufficient to optimise feed protein utilisation (Nir et al., 1993; Le Huerou-Luron et al., 1993). However, in the literature on protein digestibility, it shows that valuable amounts of protein pass through the GIT without being completely digested (Lemme et al., 2004), therefore this undigested protein presents an opportunity for the addition of specific exogenous proteases (Freitas et al., 2011). The degree of response is also governed by the existing level of performance in animals. Exogenous enzymes markedly influence ingredient and diet digestibility of younger birds which have deficient endogenous enzymes and high intestinal viscosity that compromise the digestive efficiency of the GIT (Bedford, 1996).

2.5.1 Mode of action of NSP-degrading enzymes and microbial phytase.

The enzyme technology has progressed greatly over the years with respect to the efficacy and matching activities of enzymes with their target substrates. The application of enzymes on a practical scale in the poultry industry was possible due to the recognition that the soluble NSPs present in viscous cereals (barley, wheat, triticale, and rye) impair nutrient digestion and absorption. Xylanase and β-glucanase enzymes target the degradation of the arabinoxylans and β-glucans in viscous cereal grains (Choct, 2006).

The supplementation of β-glucanases became a practical solution for improving the nutritive value of barley for poultry (Chesson, 1992; Choct, 2006). The β-glucanase is able to attack the mixed linked (1→3), (1→4) β-D-glucans and rapidly destroy the integrity of the polymer causing substantial disruption of the cell wall structure of the endosperm and this allows rapid access of the birds' endogenous amylases and proteases to the cell contents (De Silva et al., 1983; Hesselman and Åman, 1986; Chesson, 1992). β-glucanase supplementation in barley-based diets results in more rapid and complete digestion of nutrients which subsequently improves bird's performance.

Xylanase enhances nutrient digestion and utilisation mostly in wheat-based diets by the breakdown of arabinoxylans in the endosperm cell walls, resulting in reduced digesta viscosity due to increased depolymerisation of arabinoxylans to low-molecular-weight compounds (Ravindran et al., 1999b). In wheat-based diets, xylanase supplementation improved weight gain and decreased FCR and intestinal viscosity (Svihus et al., 1997). Similarly, Wu and Ravindran (2004) found that xylanase supplementation improved weight gains in wheat-based diets regardless of wheat form. Therefore, there are considerable amounts of nutrients such as starch remain encapsulated in the cell walls and usually end up in the small intestine of chickens, and this anti-nutritive effect is removed upon xylanase supplementation (Bedford, 2002).

Microbial phytase supplementation in poultry diets has been shown to improve phosphorus (P) utilisation. It increases the digestibility of phytate from around 25-70% in poultry diets (Choct, 2006). The magnitude of response of phytase is generally greater in diets containing low non-phytate P compared to those added adequate non-phytate P diets (Cabahug et al., 1999). Phytase supplementation resulted in improved P digestibility and retention (Juanpere et al., 2005; Tiwari et al., 2010), improved growth performance (Ravindran et al., 1999c) and increased utilisation of dietary amino acids and proteins (Selle et al., 2000).

2.5.2 Mode of action of protease enzyme

Endogenous proteases are synthesised and released in the GIT; however, CP and amino acid digestibility reported for poultry indicated that high amounts of proteins end up being secreted in excreta (Wang and Parsons, 1998; Angel et al., 2011). The undigested protein represents an opportunity for the use of supplemental exogenous proteases in broiler feeds to improve protein digestibility (Angel et al., 2011; Freitas et al., 2011). An effective protease may reduce, not only the production costs for farmers but also the total nitrogen content in manure being excreted into the environment (Ravindran et al., 1999a). Most commercial enzyme products exist as enzyme blends, containing enzymes such as xylanase, amylase and protease, and the benefits elicited by this combination is that NSP-degrading enzymes target cell wall substrates and viscosity allowing other enzymes such as amylase and protease to rapidly access the cell contents (Freitas et al., 2011). Furthermore, Liu et al. (2015) suggested that a decline in gizzard pH in the whole-grain feeding regime could be problematic due to an increase in protein solubility thus enhancing the digestion of proteins and amino acids, which could result in a decreased response of exogenous proteases. However, further studies need to evaluate the supplementation of proteases in whole-grain feeding to support this conclusion

2.5.2.1 Effect of exogenous protease on growth performance

The supplementation of exogenous protease has been reported to improve weight gain, FCR and feed intake (Ravindran et al., 1999a; Cowieson and Adeola, 2005). Simbaya et al. (1995) reported a synergistic response of protease, carbohydrase and phytase enzymes in growth of young broiler chickens (4 to 11 days of age) where all enzyme combination resulted in highest weight gain and better FCR compared to individual or combination of two enzymes in a wheat/canola meal-based diet. Cowieson and Ravindran (2008) found that higher doses of enzyme cocktail (500 g/tonne) consisting of a xylanase, amylase and protease, resulted in higher weight gain and better FCR. Furthermore, Hu et al. (2019) reported that an acid protease supplementation increased weight gain and decreased FCR over 18 to 35 days. In contrast, Freitas et al. (2011) found that protease supplemented diets had no effect on body weight gain, regardless of protease concentration. In addition, a recent study by Walk et al. (2019) reported that protease supplementation did not improve performance in nutrient adequate diets compared to phytase supplementation. When higher doses were supplemented, the growth performance was significantly reduced. These findings of previous studies may suggest that some improvements in protease supplementation might be attributed to the synergistic effect of enzyme blends rather than mono-component protease enzyme, however, further evaluation is needed for justification.

2.5.2.2 Effect of exogenous protease on energy and nutrient utilisation

The supplementation of exogenous protease has shown little effect on AME (2.3%) (Ravindran et al., 1999c), digestibility of amino acids (Angel et al., 2011; Walk et al., 2018), and energy digestibility as a cumulative effect of increased protein and total fat digestibility (Fru-Nji et al., 2011). The improvement in total fat digestibility was assumed to be due to a secondary effect of the protein digestibility (Fru-Nji et al., 2011). Fru-Nji et al. (2011) highlighted that by degrading large protein molecules in a chyme complex, there might be better access to the total surface area of the lipid molecules for micelle formation. In addition, Ghazi et al. (2003) reported that the use of one mono-component protease resulted in increased body weight gain and feed intake, but that feed efficiency was either negatively affected or not affected at all, depending on the protease concentration. In contrast, Walk et al. (2019) evaluated three novel proteases and observed that the apparent N digestibility was influenced by main effect of protease source or protease dose. They reported that protease supplementation reduced N digestibility with further reduction with higher doses (3x). In addition, supplementation of protease had no effect on the apparent ileal digestibility of amino acids (Ravindran et al., 1999c; Rada et al., 2016; Walk et al., 2019).

2.6 Conclusion

Barley is a good source of energy for poultry, but due to the presence of NSPs, the nutrient digestibility in barley is low compared to maize and wheat; therefore, it is least preferred among other cereals. However, the antinutritive effects caused by the NSPs can be eliminated by the supplementation of NSP degrading enzymes which improve the digestibility of nutrients by reducing the viscosity of the digesta. This results in improved performance, energy and nutrient utilisation. The methods of inclusion of cereal grains have been shown to affect performance, energy and nutrient utilisation, and GIT development in broiler chickens. Therefore, the method of inclusion of cereal grain, as well as the exogenous enzymes, are important to consider in barley-based diet formulations to exploit the potential benefits of barley as a poultry feed.

CHAPTER 3

Influence of barley inclusion method and protease supplementation on growth performance, energy and nutrient utilisation and gastrointestinal tract development in broiler starters

3.1 Introduction

Barley is an alternative to maize in the production of ethanol and with its relative lower price over maize and wheat, there is an opportunity to utilise it as an alternative feed ingredient to conventional cereal grains (Jacob and Pescatore, 2012). Barley is a good source of energy for poultry feed; however, its carbohydrates are not easily digested compared to those in maize, due to the presence of non-starch polysaccharides (NSP, Jacob and Pescatore, 2012). The dominant NSP in barley is β-glucan and it impedes digestion by binding with water and forming viscous gels (McNab and Smithard, 1992; Jacob and Pescatore, 2012). This increase in digesta viscosity interferes with digestion and absorption of nutrients (McNab and Smithard, 1992), thus supplementation of NSP-degrading enzymes, namely β-glucanase, reduces the viscosity, therefore improves the nutritive value of barley (Jacob and Pescatore, 2012).

Cereal grains for poultry feed are usually ground. The grinding can be categorised into three classes, namely fine, medium and coarse according to the screen size that the ground grains pass through. Fine grinding results in a greater surface area and consequently a greater substrate availability for enzymatic digestion. On the other hand, coarse grinding results in a well-developed gizzard with enhanced grinding activity and facilitates digestion of nutrients with increased gut motility (Amerah et al., 2007a). Grinding of whole grains is the second largest energy cost after pelleting in broiler production (Reece et al., 1985).

Whole-grain feeding has received renewed attention in the commercial poultry industry and is being increasingly used in many parts of the world. Whole grain feeding, through the reduction of energy consumption for grinding, could significantly lower the cost of feed. Furthermore, it has shown positive effect on performance, nutrient digestibility and gut development and functionality of gizzard. The most common method of whole-grain feeding is the mixed feeding system where whole grains are added pre-pellet (PRP) or post-pellet (PP) in a complete diet (Singh et al., 2014a).

The increase in population growth, climatic changes, limited land available for crop production and increase in fuel prices have affected the supply of global feed ingredients for poultry. The traditional feed ingredients such as maize, wheat and soybean meal may not meet future demand (Abdollahi and Ravindran, 2019), thus alternative feed ingredients are required to overcome the global problem. With the realisation of barley as an alternative feed ingredient, it presents an opportunity to explore the use of whole barley grain and coarse-ground barley grain in broiler diets. The influence of grain particle size and whole grain inclusion on growth performance and nutrient utilisation in birds fed wheat-based diets have been understood (Plavnik et al., 2002; Amerah et al., 2007b), but corresponding studies with barley are limited. Moreover, the effects of protease enzyme with different barley inclusion methods on broiler performance and nutrient digestibility have been evaluated before and merit further investigation. Therefore, the present study was designed to investigate the possible interactions between finely-ground, coarsely-ground and PRP whole barley inclusion, and protease supplementation on the performance, nutrient digestibility and energy utilisation, and gastrointestinal tract development in broiler starters fed wheat-based diets.

3.2 Materials and methods

3.2.1 Enzymes

A multi-component NSP-degrading enzyme, Ronozyme® Multigrain (produced by *Trichoderma reesei*, also known as *Trichoderma longiabrachiatum)*, Ronozyme® HiPhos (a granular 6-phytase expressed in a strain of *Aspergillus oryzae*) and Ronozyme® ProAct (a serine protease from *Nocardiopsis prasina*) were obtained from DSM Nutritional Products, Kaiseraugst, Switzerland. The activities of endo-1, 4-β-glucanase, endo-1, 3 (4)-β-glucanase and endo-1, 4-β-xylanase in Ronozyme® Multigrain were 800 BGU/g, 700 BGU/g and 2,700 XU/g, respectively. One unit of β-glucanase (BGU) is defined as the quantity of enzyme that releases 1.0 µmol of reducing moieties from 1.5% of β-glucan per minute at pH 5.0 at an incubation temperature of 40ºC for 20 minutes. One unit of xylanase (XU) is defined as the quantity of enzyme that releases 1.0 µmol of reducing moieties from 1.5% arabinoxylan per minute at pH 5.0 at incubation temperature of 40°C for 20 minutes. Ronozyme® HiPhos is a granular 6-phytase preparation expressed by submerged fermentation of *Aspergillus oryzae* and contains >10,000 units (FYT)/g. One FYT is defined as the activity of enzyme that releases 1.0 µmol of inorganic phosphorus per minute from 5.0 µmol/l sodium phytate at pH 5.5 and 37 ºC (DSM Nutritional Products Ltd., 2013). One protease unit (PROT) of Ronozyme® ProAct is defined as the amount of enzyme that releases 1.0 mmol of p-nitroaniline from 1.0 mM substrate (Suc-Ala-Ala-Pro-Phe-pNA) per minute at pH 9.0 and 37ºC. The activities of endo-1, 3 (4)-β-glucanase, endo-1, 4-

xylanase, phytase and protease in samples of mash and pelleted diets were measured at Biopract GmbH, Berlin, Germany. The enzyme recovery was calculated as the percentage of measured enzyme activity in the diet to the expected enzyme activity estimated from the amount and minimum activity (DSM Nutritional Products Ltd., 2013) of enzymes added to the diets.

3.2.2 Diets

A normal starch hulled barley (cultivar, Fortitude) was obtained from a seed multiplication company (Luisetti seeds, Rangiora, New Zealand) and ground in a hammer mill to pass through screen sizes of 2.5 and 8.0 mm for fine and coarse particle sizes, respectively. Wheat was obtained from a local commercial supplier ground to a size of 3.0 mm. A completely randomised design was used in this study, with a 3 x 2 factorial arrangement of 6 treatments. Three basal diets were prepared either without or with protease enzyme resulting in 6 dietary treatments: (1) Finely-ground barley without protease; (2) Finely-ground barley with protease; (3) Coarsely-ground barley without protease; (4) Coarsely-ground barley with protease; (5) whole barley without protease; and (6) whole barley with protease. The diets were formulated to meet the Ross 308 strain recommendations for major nutrients (Ross 2014; Table 3.1). The nutrient composition, optimum inclusion level, apparent metabolisable energy (AME) and standardised digestible amino acid (AA) contents of barley were determined in a previous study at Massey University (Perera et al., 2019b). The three basal diets were used to develop six dietary treatments using two levels of enzyme; without and with protease enzyme (Ronozyme® ProAct (GT)) at a rate of 0.2 g/kg of the diet. To reduce the negative effects of NSPs in barley and wheat, NSP-degrading enzymes (Ronozyme® Multigrain) was added in all diets at a rate of 0.15 g/kg of each diet. Phytase (Ronozyme® HiPhos) was also used in all diets at a rate of 0.1 g/kg of each diet. To determine the apparent metabolisable energy (AME) and the ileal nutrient digestibility, the indigestible marker, TiO2 (Merck KGaA, Darmstadt, Germany) was added at the rate of 5.0 g/kg in the diet. All diets were steam-conditioned at 60^{o}C for 30 seconds and pelleted in the pellet mill (Model Orbit 15; Richard Sizer Ltd., Kingston-upon-Hull, UK) into small size pellets (screen size, 3.0 mm). Samples of each diet in mash and pelleted form were sent for measurement of enzyme activity at Biopract GmbH, Berlin, Germany One sample of the basal diet was ground to pass through a 0.5 mm sieve and stored in an airtight container at 4^{o}C for laboratory analysis. The basal diet sample was analysed for DM, crude protein.

Table 3.1. Composition, calculated and analysed values (g/kg, as fed) of the basal broiler starter diet (day 1-21)

Item	Inclusion (g/kg)
Wheat	314
Normal (hulled) barley	283
Soybean meal	297
Maize gluten meal	50.0
Soybean oil	16.4
Di-calcium phosphate	11.0
Limestone	8.70
L-Lysine HCl	3.45
DL-Methionine	2.20
L-Threonine	1.30
Sodium chloride	2.10
Sodium bicarbonate	3.60
Titanium dioxide[1]	5.00
Vitamin premix[2]	1.00
Mineral premix[2]	1.00
Ronozyme Multigrain[3]	0.15
Ronozyme HiPhos[4]	0.10
Calculated analysis	
Nitrogen-corrected apparent metabolisable energy (AMEn, MJ/kg)	11.90
Digestible methionine	5.80
Digestible methionine + cysteine	9.00
Digestible lysine	12.2
Digestible threonine	8.20
Crude fat	30.5
Crude fibre	37.8
Calcium	9.60
Non-phytate phosphorus	4.80
Sodium	2.00
Chloride	2.00
Analysed values	
Dry matter	908
Gross Energy (MJ/kg)	16.8
Crude protein (N x 6.25)	255
Fat	37.2
Starch	326
Calcium	7.35
Phosphorus	5.90

[1]Merck KGaA, Darmstadt, Germany.
[2]Supplied per kilogram of diet: antioxidant, 100 mg; biotin, 0.2 mg; calcium pantothenate, 12.8 mg; cholecalciferol, 60 µg; cyanocobalamin, 0.017 mg; folic acid, 5.2 mg; menadione, 4 mg; niacin, 35 mg; pyridoxine, 10 mg; trans-retinol, 3.33 mg; riboflavin, 12 mg; thiamine, 3.0 mg; dl-α-tocopheryl acetate, 60 mg; choline chloride, 638 mg; Co, 0.3 mg; Cu, 3.0 mg; Fe, 25 mg; I, 1 mg; Mn, 125 mg; Mo, 0.5 mg; Se, 200 µg; Zn, 60 mg.

[3]Ronozyme® Multigrain (800 BGU/g endo-1,4-β- glucanase, 700 BGU/g endo-1,3 (4)-β-glucanase and 2700 XU/g endo-1,4-β-xylanase. One unit of xylanase (XU) is defined as the quantity of enzyme that releases 1µmol of reducing moieties from 1.5% arabinoxylan per minute at pH 5.0 and incubation temperature of 40°C for 20 minutes. One unit of β-glucanase (BGU) is defined as the quantity of enzyme that releases 1µmol of reducing moieties from 1.5% β-glucan per minute at pH 5.0 at incubation temperature of 40°C for 20 minutes.
[4]Ronozyme® HiPhos, DSM Nutritional Products, Kaiseraugst, Switzerland (1000 phytase units (FYT)/kg diet). One FYT is defined as the activity of enzyme that releases 1.0 µmol of inorganic phosphorus per minute from 5.0 µmol/l sodium phytate at pH 5.5 at 37 ºC.

(CP), calcium (Ca), phosphorus (P), fat and starch at the Nutrition Laboratory of the Institute of Food Science and Technology, Massey University, Palmerston North.

3.2.3 Determination of particle size distribution

Dry sieving method was used to determine the particle size distribution of ground barley (2.5 and 8.0 mm) using the method described by Baker and Herrman (2002). Ground barley samples (100g; two replicates per particle size) were passed through a set of six steel sieves (Endecotts Ltd., London, UK) stacked with the coarsest on the top and finest on the bottom (2000, 1000, 500, 250, 125, 63 µm), and allowed on the shaker for 10 minutes. The amount of sample retained in each sieve was weighed and the geometric mean diameter (GMD) and geometric standard deviation (GSD) was calculated for each sample. Particles retained in each sieve cannot be counted individually, therefore the average particle size was determined on a weight basis. Thus, the GMD and GSD were calculated with the following equations.

$$di = (d_u \times d_0)^{0.5}$$

$$GDM = \log^{-1}[\sum (W_i \log d_i) / \sum W_i]$$

$$GSD = \log^{-1}[\sum W_i(\log d_i - \log GMD)^2 / \sum W_i]^{0.5}$$

Where,

di = diameter of i^{th} sieve on stack

du = diameter opening through which particles will pass (sieve proceeding i^{th})

d_0 = diameter opening through which particles will not pass (i^{th} sieve)

W_i = weight fraction of sample on i^{th} sieve

The particle size distribution of three basal diets, both in mash and pellet forms (A, C and E consisting of fine and coarse ground, and whole barley, respectively) were determined by wet sieving method described by Lentle et al. (2006). Two samples of each diet in both mash and pellet forms (100g; two replicates per diet) were weighed. The first sample of each diet was dried at 80°C in a forced draft oven for 3 days to determine the DM content, and the second sample was soaked in water (100 g diet in 400 ml water) and was allowed to stand for two hours prior to sieving to ensure adequate hydration. The set of steel sieve stack (Endecotts Ltd., London, UK) sizes were 2000, 1000, 500, 250, 125, and 63 µm. The sample was then washed through the set of sieves, and the contents of each sieve were washed onto a dried, pre-weighed filter paper. The sample on the filter paper was then dried for 24 hours in a forced draft oven at 80^{o}C and re-weighed. The dry weight of particles retained by each sieve will be expressed as a proportion of the total DM recovered.

3.2.4 Pellet durability

The pellet durability of three basal diets (A, C and E) were determined using the Holmen pellet tester (New Holmen Pellet Tester, TekPro Ltd., Norfolk, UK) as described by Abdollahi et al. (2010). Clean pellet samples with no fines (100 g) were rapidly circulated in an air stream around a perforated test chamber for 30 seconds and fines were removed continuously through the perforations (screen size of 2 mm diameter) during the test cycle. After the test cycle, the subject pellets were ejected and weighed manually. The pellet durability index (PDI) was calculated as the ratio of the pellets not passing through the perforations after the test to the whole pellets at the start.

3.2.5 Pellet hardness

The pellet hardness was tested in a Stable micro System Texture Analyser (TA-XT Plus, Godalming, Surrey, UK) as described by Abdollahi et al. (2013c). 15 pellets of similar size were selected from three basal diets (A, C and E), and each individual pellet was inserted between a pressure piston and a bar, and by increasing pressure applied by means of the pressure piston, the force (Newton) needed to break the pellets was determined.

3.2.6 Birds and housing

The experimental procedures employed were approved and in accordance with the guidelines of Massey University Animal Ethics Committee and conducted at the Massey University poultry unit farm. A total of 288 one-day old male broilers (Ross 308) were obtained from a commercial hatchery. Each bird was weighed individually and allocated into 36 cages in electrically heated battery brooders so that the average weight per cage was similar. Each of the six dietary treatments were randomly assigned into six cages, with eight birds per cage. A total of 6 cages were assigned to one dietary treatment. On day 1, the temperature was set to 31^{o}C and was gradually reduced to 24 ^{o}C by 21 days of age. The battery brooders and grower cages, with wired floors, were housed in an environmentally controlled room with 20 h of fluorescent illumination per day. The diets were offered *ad libitum* and water was available at all times throughout the experimental period. The birds were transferred into grower cages at 11 days of age and were fed the same diets until day 21.

3.2.7 Performance data

Throughout the experimental period, the body weights and feed intake were recorded on a cage basis at weekly intervals. In the first 2 days of life, mortality from each cage was replaced by birds from a spare cage, and after that, daily mortality was recorded. The FCR values were corrected for the body weights of any bird that died during the experimental period.

3.2.8 Determination of nitrogen-corrected apparent metabolisable energy (AMEn)

The AMEn value of the assay diets was determined by using the classical total excreta collection method. On day 17, feed was weighed, and excreta was collected from each cage over four consecutive days, starting on day 18 to 21, and feed intake was recorded on day 21. The pooled excreta were mixed well in a blender and subsampled as representative samples and were freeze-dried (Model 0610m Cuddon Engineering, Blenheim, New Zealand). The dried excreta samples were ground through a 0.5 mm sieve and stored in airtight plastic containers at 4 ^{o}C for analysis of DM, N, Ti and GE.

3.2.9 Determination of coefficient of apparent ileal digestibility (CAID) of nutrients

On day 21, six broilers per cage were euthanised by an intracardial injection (1 ml per 2 kg live weight) of pentobarbitone solution (Provet NZ Pty Ltd, Auckland, New Zealand). The ileal digesta was collected from the lower half of the ileum according to procedures described by Ravindran et al. (2005). Distilled water was used to gently flush the contents of the lower half of the ileum into airtight plastic containers. The ileum is defined as the portion of the small intestine extending from the Meckel's diverticulum to a point approximately 40 mm proximal to the ileo-caecal junction. The ileum was divided into two halves, and the digesta was collected from the lower half towards the ileo-caecal junction. The collected ileal digesta from birds of respective cages were pooled, freeze-dried, ground to pass through a 0.5 mm sieve and then stored in airtight containers at 4 ^{o}C for laboratory analysis. The ileal digesta samples were analysed for DM, Ti, GE, N, starch, fat, Ca and P at the Nutrition Laboratory of the Institute of Food Science and Technology, Massey University, Palmerston North.

3.2.10 Gizzard pH

The gizzard pH was measured as described by Singh et al. (2014b). On day 21, 2 birds per cage were euthanised by intracardial injection of pentobarbitone solution, and the gastrointestinal tract was eviscerated immediately on a stainless-steel dissection tray at room temperature and the gizzard and proventriculus were excised. The gizzard pH of each bird was measured by inserting a calibrated digital pH meter (Model IQ120, ISFET pH Meter, Shindengen, Japan) at three different regions (proximal, middle and distal) of the gizzard. The readings were recorded after stabilisation of each value, and the average of the three readings was considered as the final pH value.

3.2.11 Digestive tract measurement

The same birds euthanised for gizzard pH measurements were used for the measurements of the digestive tract. The digestive tract, from the crop to caeca was carefully excised and adherent fat was removed. The relative lengths (cm) of the duodenum, jejunum, ileum, and caeca were determined and reported as cm/kg liveweight. The relative empty weights of the crop, proventriculus, gizzard, duodenum, jejunum, ileum, and caeca in the individual birds were determined and reported as g/kg of body weights. The absolute weight (g) of the crop, proventriculus and gizzard was also determined.

3.2.12 Viscosity

The jejunal digesta was obtained from the lower jejunum of two birds from each of the replicate cages euthanised for ileal collection and was collected into a centrifuge tube according to methods described by Perera et al. (2019a). The samples were centrifuged at 3000 g at 20 ^{o}C for 15 minutes. A 0.5 ml aliquot was obtained from the supernatant solution and was used in a viscometer (Brookfield digital viscometer, Model DV2TLV; Brookfield Engineering Laboratories Inc., Stoughton, MA) fitted with CP-40 cone spindle with shear rates of 5 to 500/s to measure the viscosity.

3.2.13 Determination of duodenal particle size distribution

The determination of particle size distribution of the duodenal digesta was determined according to methods described by Amerah et al. (2007b). The duodenal digesta was obtained from the duodenum of two birds from each of the replicate cages euthanised for ileal collection. The digesta was removed from the duodenum by simple drainage. A sample from two birds within each pen was pooled, giving a total of 6 digesta samples per treatment. The particle size spectra of various samples were determined by wet sieving as described by Lentle et al. (2006). The samples were weighed and equally divided into two sub-samples. One will be oven-dried at 80 ^{o}C in a forced draft oven for 3 days to determine the DM content, and the other sample was suspended in 50 ml of distilled water for 15 minutes. The sample was then washed through a set of steel sieves stacks (2000, 1000, 500, 250, 125 and 63 µm). The content of each sieve was then subsequently washed onto a dried, pre-weighed filter paper, and dried for 24 hours in a forced draft oven at 80 ^{o}C and was reweighed. The dry weights of the particles retained in each sieve and of fines remaining in the bottom of the pan were expressed as the percentage of the total DM recovered.

3.2.14 Chemical analyses

Dry matter was determined using standard procedures (Methods 925.10 and 930.16; AOAC, 2016). Nitrogen was determined by combustion (Method 968.06; AOAC, 2016) using a CNS-200 carbon, N and Sulphur auto-analyser (LECO Corporation, St. Joseph, MI). An adiabatic bomb calorimeter (Gallenkamp Autobomb, London, UK) standardised with benzoic acid was used for the determination of GE. Starch was measured using a Megazyme kit (method 996.11; AOAC, 2016) based on thermostable α-amylase and amyloglucosidase (McCleary et al., 1997). Ash was determined by standard procedures (Method 942.05; AOAC, 2005) using a furnace at 550 ^{o}C for 16 hours. Ca and P were determined by colorimetric methods after ashing the samples at 550 ^{o}C and acid digestion in 6.0 M HCl using standard procedures (Method 968.08D; AOAC, 2005). The samples were tested for Ti on a UV spectrometer in accordance with the method described by Short et al. (1996).

3.2.15 Calculations

The AMEn values of the diet (without and with enzymes) were calculated using the following formula:

$$AME_{diet}\ (MJ/kg) = \frac{(\text{Feed intake x } GE_{diet}) - (\text{Excreta output x } GE_{excreta})}{\text{Total feed intake}}$$

The correction for zero nitrogen retention was calculated using a factor of 36.54 kj per gram nitrogen retained in the body as described by Hill and Anderson (1958).

The following formula was used to calculate the coefficient of apparent ileal digestibility (CAID):

$$\text{CAID of diet nutrient} = \frac{(\text{Nutrient / Ti})_{diet} - (\text{Nutrient /Ti})_{ileal}}{\text{Nutrient / Ti})_{diet}}$$

Where,

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

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

3.2.16 Statistical analyses

The data were analysed by two-way analysis of variance (ANOVA) to determine the main effects (barley inclusion method and protease) and their interaction using the General Linear Models procedure of SAS (version 9.4; SAS Institute., Cary, NC). The cage means were served as the experimental unit and the differences were considered to be significant at $P < 0.05$. The significant differences between means were separated by the Least Significant Difference (LSD) test.

3.3 Results

3.3.1 Enzyme recovery

The average recovery of endo-1,3 (4)-glucanase, endo-1, 4-β-xylanase, phytase, and protease from enzyme-supplemented diets were 87.8, 155.3, 200.9 and 77.9%, respectively.

3.3.2 Particle size distribution, pellet durability index and pellet hardness

The particle size distribution of the ground barley and mash and pelleted diets is shown in Table 3.2. The GMD of barley ground through 2.5 and 8.0 mm screen sizes were determined to be 635 and 1274 µm, respectively, with corresponding GSD values of 2.1 and 1.8. The GMD values of mash diets based on fine and coarse ground and whole barley were 399, 478 and 515, with corresponding GSD values of 4.1, 4.4 and 4.5, respectively. However, the GMD values of pellets made from diets based on fine and coarse ground and whole barley were 190, 217 and 236, with corresponding GSD values of

3.9, 4.1. and 4.3, respectively. Pelleting reduced the relative proportion of coarse particles (>2000 and 1000 µm) and increased that of fine particles < 63 µm with fine, coarse and whole barley.

Graphic comparisons of the particle size distribution of mash and pelleted diets (Figure 3.1) obtained by wet sieving showed that pelleting reduced the relative proportion of particles > 500 µm, and increased the proportion of fines < 63 µm so that the variation of average particle size distribution of the three methods of barley inclusion was small in pellet diets compared to the mash diets.

Table 3.2. Determined particle size distribution (percentage of retained particles on sieve)[1] and geometric mean diameter ± geometric standard deviation (GMD ± GSD, µm) of ground barley, mash and pelleted diets, and the influence of fine, coarse and whole barley inclusion on pellet durability index (PDI, %)[2] and pellet hardness[3] (Newton)

Barley inclusion method	Openings (µm)								Pellet quality	
	2000	1000	500	250	125	63	<63	GMD±GSD	PDI	Pellet hardness
Ground barley										
Fine	0.00	28.70	42.56	17.92	6.91	3.16	0.75	635 ± 2.1	-	-
Coarse	30.00	47.48	14.62	5.28	1.90	0.60	0.13	1274 ± 1.8	-	-
Mash diets										
Fine	3.36	38.11	16.77	8.37	4.56	3.55	25.28	399 ± 4.1	-	-
Coarse	20.57	26.99	14.21	7.24	3.99	3.21	23.79	478 ± 4.4	-	-
Whole barley	27.85	21.86	13.57	6.66	4.12	3.11	22.83	515 ± 4.5	-	-
Pelleted diets										
Fine	0.24	13.95	20.67	11.41	6.89	2.78	44.07	190 ± 3.9	79.5a	19.2
Coarse	2.02	17.36	20.31	10.66	5.24	2.16	42.24	217 ± 4.1	76.7b	20.0
Whole barley	6.78	16.92	17.90	9.74	5.94	2.82	39.90	239 ± 4.3	75.7b	19.5
SEM[4]									0.57	0.79
Probabilities, P ≤									0.001	0.758

Fine and coarse grades were achieved using screen sizes of 2.5 and 8.0 mm, respectively. Means in a column not sharing a common letter (a-b) are significantly different (P < 0.05).
[1]Each value represents the mean of two replicates.
[2]Each value represents the mean of 6 replicates.
[3]Each value represents the mean of 15 replicates.
[4]Pooled standard error of mean.

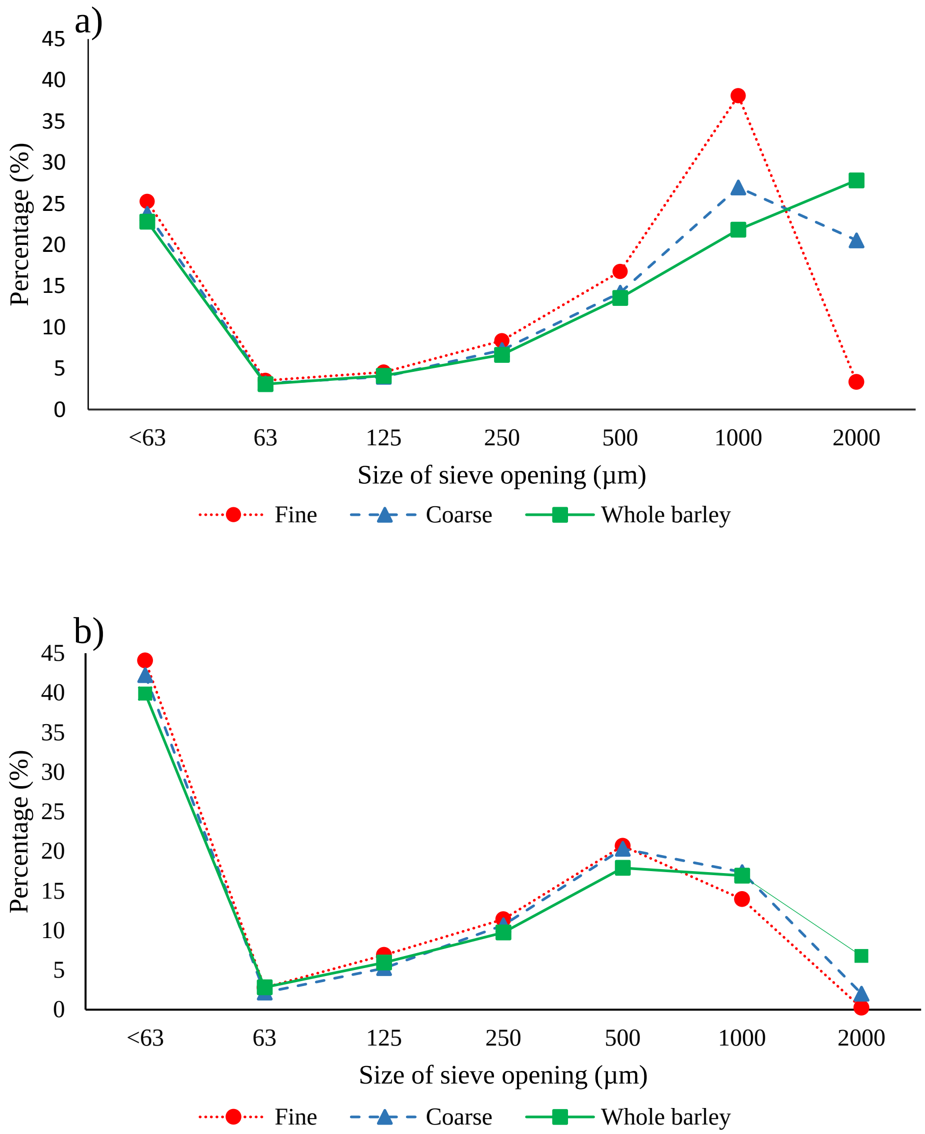


Figure 3.1. Particle size distribution of mash (a) and pelleted (b) diets.

The pellet durability index was significantly ($P < 0.05$) higher in the pellets made from diets based on finely ground barley than those made from coarsely ground or whole barley (Table 3.2). There was no difference ($P > 0.05$) in pellet hardness between pellets made from finely and coarsely ground barley, and whole barley.

3.3.3 Growth performance

Mortality of 4.9% was recorded over the experimental period and was not influenced ($P > 0.05$) by any dietary treatment (Table 3.3).

The main effect of barley inclusion method was significant for feed intake ($P < 0.01$) and FCR ($P < 0.001$) during day 1 to 7 post-hatch (Table 3.3). Birds fed diets containing fine particles showed higher ($P < 0.05$) feed intake compared to those fed whole barley. Feed conversion ratio was higher ($P < 0.05$) in birds fed diets containing fine and coarse particles compared to those fed whole barley.

During day 8 to 14 post-hatch, barley inclusion method was significant for weight gain ($P < 0.05$) and FCR ($P < 0.001$). Fine grinding resulted in lower ($P < 0.05$) weight gain and higher FCR ($P < 0.05$) compared to coarse grinding and whole barley inclusion. Main effects of barley inclusion method for feed intake tended ($P = 0.059$) to be significant.

During day 15 to 21 post-hatch, effect of barley inclusion method was significant for weight gain ($P < 0.05$) and feed intake ($P < 0.01$). Birds fed diets containing whole barley showed higher ($P < 0.05$) weight gain than birds fed fine particles. Fine grinding resulted in lower ($P < 0.05$) feed intake compared to coarse grinding and whole barley inclusion.

Over the whole experimental period (day 1 to 21), the main effect of barley inclusion method was significant ($P < 0.01$) for weight gain and FCR. Coarse grinding and whole barley inclusion resulted in higher ($P < 0.05$) weight gain compared to fine grinding. Feed conversion ratio was higher ($P < 0.05$) in birds fed diets containing fine particles compared to those fed diets containing whole barley. Barley inclusion method tended ($P = 0.078$) to be significant for feed intake during the whole trial period.

The main effect of protease supplementation and interaction between barley inclusion method and protease supplementation were not significant ($P > 0.05$) for weight gain, feed intake and FCR during each and whole experimental periods.

3.3.4 Nutrient digestibility and energy utilisation

The effects of dietary treatments on CAID of nutrients and GE and energy utilisation parameters are shown in Table 3.4. The main effect of barley inclusion method was significant ($P < 0.01$-0.05) for the CAID of DM, N, fat, Ca, P and GE, with coarsely ground and whole barley inclusion resulting in higher ($P < 0.05$) DM, N, Ca and GE digestibility than finely ground barley. Coarse grinding of barley resulted in higher ($P < 0.05$) CAID of fat compared to fine grinding and whole barley inclusion. For CAID of P, birds fed diets containing finely and coarsely ground particles had higher ($P < 0.05$) values compared to birds fed whole barley

The barley inclusion method was significant ($P < 0.05$) for ileal digestible energy (IDE). Diets made from coarse particle and whole barley had higher ($P < 0.05$) IDE values compared to diets made from fine particles.

The main effect of protease supplementation and interaction between barley inclusion method and protease supplementation were not significant ($P > 0.05$) for nutrient digestibility and energy utilisation parameters.

3.3.5 Digestive tract measurements and gizzard pH

Barley inclusion method had significant ($P < 0.001$ to 0.05) effects on absolute weight of crop, proventriculus and gizzard (Table 3.5). Fine grinding of barley resulted in higher ($P < 0.05$) absolute weight of crop compared to whole barley inclusion. Birds fed diets containing finely and coarsely ground barley had higher ($P < 0.05$) absolute weight of proventriculus and lower ($P < 0.05$) absolute weight of gizzard compared to birds fed whole barley.

The main effect of barley inclusion method was also significant ($P < 0.05$ to 0.001) for the relative weight of crop, proventriculus, gizzard, jejunum and ileum; with relative weight of crop, jejunum and ileum being heavier ($P < 0.05$) in birds fed finely ground barley compared to those fed whole barley diets. Birds fed diets containing finely and coarsely ground particles had higher ($P < 0.05$) relative weight of proventriculus and lower ($P < 0.05$) relative weight of gizzard compared to birds fed whole barley.

The main effect of barley inclusion method was not significant ($P > 0.05$) for relative weight of duodenum. The main effect of protease supplementation and interaction between barley inclusion method and protease supplementation were not significant ($P > 0.05$) for absolute and relative weight of digestive tract compartments.

Significant ($P < 0.05$) barley inclusion method x enzyme interaction was observed for the relative weights of caeca. In finely and coarsely ground diets, relative weights of caeca numerically reduced by protease supplementation, whereas, in whole barley diets, protease supplementation numerically increased relative weights of caeca compared to diets without protease.

The main effect of barley inclusion method was significant ($P < 0.001$) for the gizzard pH with whole barley having lower ($P < 0.05$) gizzard pH compared to finely and coarsely ground barley.

Table 3.3. Influence of barley inclusion method and protease supplementation on weight gain (g/bird), feed intake (g/bird) and feed conversion ratio (FCR; g feed/g gain) of broiler starters[1] and mortality (%)

		day 1 to 7			day 8 to 14			day 15 to 21			day 1 to 21			
Barley inclusion method	Protease	Weight gain	Feed intake	FCR	Weight gain	Feed intake	FCR	Weight gain	Feed intake	FCR	Weight gain	Feed intake	FCR	Mortality
Fine	-	197	182	0.925	389	494	1.272	553	728	1.334	1138	1404	1.241	2.1
	+	192	183	0.953	393	495	1.280	567	728	1.314	1152	1406	1.238	8.3
Coarse	-	190	178	0.940	403	508	1.259	585	759	1.300	1178	1444	1.226	4.2
	+	192	180	0.938	407	509	1.253	575	738	1.297	1173	1427	1.221	6.3
Whole barley	-	193	176	0.909	406	496	1.228	584	761	1.308	1183	1433	1.212	6.3
	+	193	175	0.904	399	493	1.234	597	764	1.299	1190	1431	1.211	2.1
SEM[2]		2.4	2.1	0.0084	5.2	6.5	0.0076	11.1	10.0	0.0164	12.8	14.4	0.0083	3.14
Main effects														
Barley inclusion method														
Fine		194	182a	0.939a	391b	495	1.276a	560b	728b	1.324	1145b	1405	1.239a	5.2
Coarse		191	179ab	0.939a	405a	508	1.256b	580ab	748a	1.298	1176a	1436	1.223ab	5.2
Whole barley		193	175b	0.906b	403a	494	1.231c	591a	763a	1.303	1187a	1432	1.212b	4.2
Protease	-	193	178	0.924	399	499	1.253	574	749	1.314	1167	1427	1.226	4.2
	+	192	179	0.932	400	499	1.256	580	743	1.303	1172	1421	1.223	5.6
Probabilities, P ≤														
Barley inclusion method		0.302	0.010	0.001	0.024	0.059	0.001	0.032	0.006	0.269	0.009	0.078	0.009	0.929
Protease		0.705	0.667	0.295	0.898	0.977	0.652	0.546	0.449	0.428	0.614	0.633	0.639	0.592
Barley inclusion method x Protease		0.367	0.734	0.107	0.504	0.925	0.611	0.459	0.451	0.867	0.765	0.779	0.960	0.263

Fine and coarse grades were achieved using screen sizes of 2.5 and 8.0 mm, respectively. Means in a column not sharing a common letter (a-c) are significantly different ($P < 0.05$).

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

[2]Pooled standard error of mean.

Table 3.4. Influence of barley inclusion method and protease supplementation on the coefficient of apparent ileal digestibility (CAID) of dry matter (DM), nitrogen (N), starch, fat, calcium (Ca), phosphorus (P) and gross energy (GE); ileal digestible energy (IDE), apparent metabolisable energy (AME, MJ/kg DM) and N-corrected AME (AMEn, MJ/kg DM) in broilers

		CAID[1]							Energy utilisation		
Barley inclusion method	Protease	DM	N	Starch	Fat	Ca	P	GE	IDE	AME	AMEn
Fine	-	0.578	0.713	0.957	0.715	0.345	0.648	0.606	11.20	13.75	12.70
	+	0.597	0.726	0.954	0.749	0.353	0.664	0.623	11.52	13.51	12.49
Coarse	-	0.628	0.752	0.946	0.829	0.456	0.672	0.653	12.07	13.71	12.65
	+	0.633	0.762	0.955	0.841	0.451	0.656	0.660	12.20	13.62	12.61
Whole barley	-	0.634	0.778	0.957	0.725	0.455	0.595	0.659	12.18	13.86	12.77
	+	0.632	0.786	0.956	0.728	0.453	0.591	0.659	12.18	13.90	12.81
SEM[2]		0.0173	0.0160	0.0046	0.0410	0.0374	0.0195	0.0173	0.3200	0.104	0.126
Main effects											
Barley inclusion method											
Fine		0.588b	0.720b	0.956	0.732b	0.349b	0.656a	0.615b	11.36b	13.63	12.60
Coarse		0.630a	0.757a	0.950	0.835a	0.453a	0.664a	0.657a	12.13a	13.67	12.63
Whole barley		0.633a	0.782a	0.957	0.726b	0.454a	0.593b	0.659a	12.18a	13.88	12.79
Protease											
	-	0.613	0.748	0.953	0.756	0.418	0.638	0.640	11.82	13.77	12.71
	+	0.620	0.758	0.955	0.772	0.419	0.637	0.647	11.96	13.68	12.64
Probabilities, P ≤											
Barley inclusion method		0.023	0.002	0.340	0.020	0.011	0.002	0.026	0.025	0.171	0.293
Protease		0.608	0.446	0.660	0.628	0.986	0.926	0.579	0.585	0.413	0.490
Barley inclusion method x Protease		0.823	0.984	0.388	0.929	0.983	0.714	0.885	0.887	0.623	0.614

Fine and coarse grade were achieved using screen sizes of 2.5 and 8.0 mm, respectively. Means in a column not sharing a common letter (a-b) are significantly different ($P < 0.05$).

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

[2]Pooled standard error of mean.

Table 3.5. Influence of barley inclusion method and protease supplementation on the absolute weights (g) of crop, proventriculus and gizzard, and relative weights (g/kg of bird live weight) of the crop, proventriculus, gizzard, duodenum, ileum, jejunum and caeca, and the gizzard pH of broilers[1]

Barley inclusion method	Protease	Absolute weights (g)			Relative weights (g/kg body weight)							Gizzard pH[2]
		Crop	Proventriculus	Gizzard	Crop	Proventriculus	Gizzard	Duodenum	Jejunum	Ileum	Caeca	
Fine	-	3.33	4.89	9.68	2.73	4.10	7.89	3.37	7.02	6.62	2.26ab	3.70
	+	3.31	4.51	9.94	2.75	3.72	8.23	3.48	7.20	7.00	2.12ab	3.72
Coarse	-	3.14	5.07	10.29	2.57	4.14	8.39	3.10	6.51	6.14	2.26ab	3.48
	+	3.09	4.82	9.80	2.49	3.87	7.88	3.32	6.65	6.45	2.03b	3.59
Whole barley	-	2.86	3.77	12.18	2.31	3.03	9.76	3.13	6.47	6.05	2.13ab	3.02
	+	3.03	4.10	13.29	2.42	3.27	10.60	3.13	6.39	6.02	2.37a	3.03
SEM[3]		0.147	0.339	0.440	0.146	0.270	0.336	0.164	0.275	0.264	0.093	0.151
Main effects												
Barley inclusion method												
Fine		3.32a	4.70a	9.81b	2.74a	4.01a	8.13b	3.43	7.11a	6.81a	2.19	3.71a
Coarse		3.12ab	4.95a	10.04b	2.53ab	3.91a	8.06b	3.21	6.58ab	6.29ab	2.15	3.54a
Whole barley		2.95b	3.94b	12.73a	2.36b	3.15b	10.18a	3.13	6.43b	6.04b	2.25	3.02b
Protease												
	-	3.11	4.58	10.71	2.54	3.76	8.68	3.20	6.67	6.27	2.22	3.40
	+	3.15	4.48	11.01	2.55	3.62	8.90	3.31	6.75	6.49	2.18	3.45
Probabilities, P ≤												
Barley inclusion method		0.050	0.016	0.001	0.049	0.006	0.001	0.195	0.045	0.020	0.550	0.001
Protease		0.785	0.724	0.414	0.895	0.546	0.424	0.403	0.723	0.313	0.592	0.694
Barley inclusion method x Protease		0.709	0.547	0.208	0.818	0.477	0.144	0.799	0.877	0.710	0.041	0.930

Fine and coarse grades were achieved using screen sizes of 2.5 and 8.0 mm, respectively. Means in a column not sharing a common letter (a-b) are significantly different ($P < 0.05$).

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

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

[3]Pooled standard error of mean.

The influence of dietary treatments on relative length of digestive tract compartments and jejunal digesta viscosity are shown in Table 3.6. A tendency (P = 0.09) was observed for the interaction between barley inclusion method and protease supplementation on relative length of duodenum. Protease supplementation in coarsely ground barley tended to reduce relative length of duodenum while in finely ground and whole barley protease supplementation tended to have no effect on relative length of duodenum.

Table 3.6. Influence of barley inclusion method and protease supplementation on the relative lengths (cm/kg of live body weight) of duodenum, jejunum, ileum and caeca and jejunal digesta viscosity (cP) of broilers[1]

Barley inclusion method	Protease	Relative lengths (cm/kg body weight)				Jejunal digesta viscosity[2]
		Duodenum	Jejunum	Ileum	Caeca	
Fine	-	21.6	55.6	62.6	14.0	3.77
	+	22.4	56.2	64.4	14.1	3.51
Coarse	-	22.7	58.3	64.7	14.5	3.55
	+	21.1	56.7	65.0	13.8	3.40
Whole barley	-	21.8	53.7	61.1	13.2	3.53
	+	21.6	55.1	61.0	13.6	3.58
SEM[3]		0.52	1.65	1.89	0.34	0.188
Main effects						
Barley inclusion method						
Fine		22.0	55.9	63.5	14.1	3.64
Coarse		21.9	57.5	64.9	14.2	3.47
Whole barley		21.7	54.4	61.0	13.4	3.56
Protease						
	-	22.0	55.9	62.8	13.9	3.62
	+	21.7	56.0	63.4	13.8	3.50
Probabilities, P ≤						
Barley inclusion method		0.800	0.195	0.137	0.065	0.680
Protease		0.460	0.920	0.676	0.791	0.460
Barley inclusion method x Protease		0.093	0.655	0.875	0.272	0.708

Fine and coarse grades were achieved using screen sizes of 2.5 and 8.0 mm, respectively. Means in a column not sharing a common letter (a-b) are significantly different (P < 0.05).

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

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

[3]Pooled standard error of mean

A tendency (P = 0.065) was observed for the main effect of barley inclusion method on the relative length of caeca (Table 3.6). Relative length of caeca tended to be lower in birds fed whole barley compared to those fed fine barley.

The main effect of protease supplementation and interaction between barley inclusion method and protease supplementation were not significant (P > 0.05) for relative lengths of duodenum, jejunum and ileum and jejunal viscosity.

3.3.6 Determination of particle size distribution in duodenal digesta

Table 3.7 shows that there is no distinction between particle size distributions of the duodenal digesta of birds fed fine and coarse ground and whole barley.

The proportion of particles less than 500 µm in the duodenal digesta were 94.5, 92.5 and 93.2% for fine and coarse ground and whole barley diets, respectively. The percentage of particles over 1000 µm were 0, 2.4 and 0.9% for fine and coarse ground and whole barley diets, respectively. The GMD of particles in duodenal digesta of birds fed fine and coarse ground and whole barley showed that the average particle size is less than 250 µm for the three different barley inclusion method.

Table 3.7. Influence of barley inclusion method on the proportion of particle size classes (mean) in the duodenal digesta (% based on dry weight basis) of broilers[1]

Duodenal particle size (µm)	Fine	Coarse	Whole barley
< 63	78.61 ± 2.47	73.22 ± 3.64	72.81 ± 4.97
63 to 125	6.57 ± 1.02	7.33 ± 1.02	8.36 ± 1.57
125 to 250	4.77 ± 0.70	6.01 ± 0.84	6.49 ± 1.19
250 to 500	4.53 ± 0.56	5.99 ± 0.61	5.49 ± 1.24
500 to 1000	5.52 ± 0.60	5.10 ± 0.92	5.94 ± 1.10
1000 to 2000	0.00	2.35 ± 1.03	0.91 ± 0.91
GMD ± GSD	190 ± 3.9	217 ± 4.1	238 ± 4.3

[1]Each value represents the mean ± SE of 6 replicate samples.

3.4 Discussion

Data from the current study showed that coarse grinding of barley increased the relative proportion of particles > 1000 µm compared to fine grinding (77.5 and 28.7%, respectively). The relative proportion of large particles (> 1000 µm) in fine and coarse ground and whole barley diets were reduced by 65.8 (from 41.5 to 14.2), 59.2 (from 47.6 to 19.4) and 52.3% (from 49.7 to 23.7), respectively, as a result of pelleting. Pelleting-induced particle size reduction was more pronounced in particles > 2000 µm, with corresponding reductions of 92.9, 90.2 and 75.7% in fine and coarse ground, and whole barley diets, respectively. This shows that the pelleting process further reduced large feed particles and whole barley and increased the proportion of fines < 63 µm, thus minimizing the differences in the particle size distribution between fine and coarse ground, and whole barley diets. These findings are in accordance with previous results of Engberg et al. (2002), Svihus et al. (2004b), Péron et al. (2005), Amerah et al. (2007b), Abdollahi et al. (2011, 2013a), and Naderinejad et al. (2016) who reported that pelleting reduced feed particle size. Large feed particles are prone to grinding due to the narrow distance between the pellet rolls and the pellet die, thus, adding further grinding which reduces the proportion of large particles and even out the differences in the particle size distribution (Engberg et al., 2002; Svihus et al., 2004b; Abdollahi et al., 2011).

The pellet quality values, as determined by the PDI test, showed that PDI for finely ground barley is higher compared to coarsely ground and whole barley diets. This is consistent with the results by Angulo et al. (1996), Svihus et al. (2004b) and Chewning et al. (2012). The smaller particles produce more durable pellets due to more contact points to each other owing to a larger surface area to volume ratio (Behnke, 2001). In contrast to these findings, Reece et al. (1986) and Naderinejad et al. (2016) found that pellets made from coarse grinding of maize were more durable than fine grinding. In addition, Singh et al. (2014b) found that increasing the inclusion of pre-pelleting of whole maize resulted in increased PDI. In the present study, the normal starch hulled (NSH) barley has considerable amounts of hulls, however, the hulls are finely ground in the fine diets while coarse grinding and whole barley have large size of hulls and this may have contributed to higher PDI of pellets made from finely ground barley compared to those made from coarse ground and whole barley.

According to Nir et al. (1995), better PDI improved feed intake. In the present study, on day 1 to 7 post-hatch, feed intake was 4% higher in finely ground compared to whole barley diets (182 *vs* 175 g/bird). But this increase transformed to 4.6 (728 *vs* 763 g/bird) and 1.9% (1405 *vs* 1432 g/bird) decrease on day 15 to 21 and whole trial period, respectively. These results are in agreement with Singh et al. (2014b) who reported that despite the improved PDI, feed intake was reduced in broiler chickens from day 1 to 21 of age compared to the diets with lower PDI. Svihus et al. (1997) found that feed intake was 7.33% higher in diets containing whole barley compared to ground barley and this increase was not influenced by NSP-degrading enzyme supplementation. Nahas and Lefrancois (2001) found that inclusion of 20% whole barley in a maize-soybean meal-based diet resulted in an increased feed intake. They reported that higher feed intake could be a consequence of a reduction in digesta viscosity. In contrast, Liu et al. (2015) reported that whole-grain feeding is associated with a reduction in feed intake due to increased retention of large particles of digesta in the gizzard and slower passage rates along the digestive tract. Hetland et al. (2002) found that feed intake was reduced when ground barley was replaced by whole barley with moderate inclusion rate (12.5-30%). Moss et al. (2017) found that wheat- and sorghum-based diets and a blend of a wheat-sorghum-based diet containing post-pelleted whole barley depressed feed intake by 2.1% compared to ground barley (2297 *vs* 2345).

The lower feed intake observed in first week in birds fed diets containing whole barley may have been caused by physical limitations of digestive tract of newly hatched chicks. Chickens in early life have limited gizzard capacity to grind coarse particles along with slower feed passage rate through the digestive tract (Hetland et al., 2002; Biggs and Parsons, 2009). The higher feed intake reported at d 15 to 21 in birds fed diets containing coarse ground and whole barley can be attributed to the ability of birds to grind larger feed particles after week two. Svihus et al. (1997) found that broiler chickens at 14 to 28 days of age are able to grind diets containing whole barley efficiently even when no grit was added.

Feed intake is the primary factor driving the growth rate of broilers (Abdollahi et al., 2018b). In the present study, over the first week (day 1-7), there was no improvement in weight gain between the treatments; however, during the second week, coarsely ground and whole barley improved weight gain by 3.6 and 3.1%, respectively. On the third week, coarse ground and whole barley improved weight gain by 3.6 and 5.5% compared to fine diets. During the whole experimental period, coarsely ground and whole barley inclusion improved weight gain by 2.2 and 3.7%, respectively. Improvements in weight gain with inclusion of whole barley in boiler diets have been reported in some studies (Svihus et al., 1997; Bennett et al., 2002; Biggs and Parsons, 2009). Svihus et al. (1997) found that the increase in weight gain was attributed to higher feed intake. Coarse grinding of maize also improved weight gain compared to fine grinding in Amerah et al. (2008) study. In contrast, Hetland et al. (2002) and Kliševičiūtė et al. (2012) found that moderate or high inclusion of whole grains (wheat, oats, and barley) in broiler diets resulted in lower weight gains. They suggested that lower feed intake is the reason for lower weight gain in birds fed moderate or high inclusion of whole grains. Hetland et al. (2002) also reported that grinding process of the gizzard requires energy, and as a consequence, there would be less energy expected to be available for growth. In addition, Lott et al. (1992) reported that broilers fed pelleted feeds with coarse maize particles (GMD, 1196 µm) had significantly lower weight gain and higher FCR at day 21 compared to those fed pellets with fine maize particles (GMD, 679 µm).

In the present study, overall, birds fed coarse particles or whole barley outperformed those fed fine particles. Regardless of enzyme supplementation, whole barley feeding improved weight gain, FCR by 3.5, 3.5 and 2.2%, during day 1 to 7, 8 to14 and 1 to 21 post-hatch, respectively.

The greater proportion of coarse particles that are retained longer within the gizzard may have enhanced digestion and thus improve feed efficiency. Coarse particles or whole grains might stimulate greater gizzard activity leading to a more efficient grinding and greater production of finer particles that are more readily digested (Amerah et al., 2008). Hetland et al. (2002) found that inclusion of moderate (300 g/kg) amount of whole barley did not affect FCR compared to diet containing ground wheat. Furthermore, Moss et al. (2017) found that whole barley diets supplemented with phytase enzyme improved FCR by 3.2% (1.362 *vs* 1.407) compared to ground barley diets. However, FCR was compromised in birds fed ground barley supplemented with phytase enzyme.

The main intention of the present study, however, was to examine whether the manipulation of barley particle size will have similar effects on nutrient digestibility of broilers fed diets with or without protease enzyme. Barley inclusion method had no effect on the CAID of starch, AME and AMEn, but affected those of DM, N, fat, Ca, P, GE and IDE. In general, coarse grindings and whole barley inclusion appeared to increase DM, N, fat and GE digestibility and IDE. A similar finding which is consistent with that of Biggs and Parsons (2009), who reported an increase in nutrient digestibility and AMEn when broiler chicks were fed whole grains (barley, wheat and sorghum). In the present study, despite the improved CAID of GE and IDE in birds fed coarsely ground barley and whole barley, the AME and AMEn were not affected. In contrast to our results, Moss et al. (2017) found that whole barley inclusion with phytase supplementation improved AME and AMEn of the diets compared to ground barley diets. Amerah et al. (2011) observed that PRP inclusion of whole wheat (100 and 200 g/kg) had no effect on apparent IDE compared to ground wheat diet.

The results from the present study regarding the improvement of CAID of Ca with coarse and whole barley are in agreement with Qaisrani et al. (2015) who reported that a more acidic gizzard pH might have contributed, at least in part, to the increased digestibility of Ca. Guinotte et al. (1995) also highlighted that a lower pH enhances the solubility and absorption of mineral salts. It was also previously reported that large feed particle size of maize diets also improved the CAID of Ca (Kasim and Edwards, 2000; Kilburn and Edwards, 2001). Furthermore, Abdollahi et al. (2018a) found that insoluble fibre increased CAID of Ca by 45% and 61% in coarsely ground and whole wheat diets, respectively. This could be attributed to the effect of low pH due to structural components and large feed particle sizes. In the current study, diets containing fine and coarse particles had higher CAID of P compared to whole barley. Improved CAID of P with coarse feed particles has previously been reported by Kasim and Edwards (2000) and Kilburn and Edwards (2001).

Changes in the gastrointestinal morphology associated with inclusion of whole grain, coarse particles and dietary fibre have been reported with special emphasis to the gizzard morphology (Hetland et al., 2002; Bennett et al., 2002; Hetland et al., 2003; Engberg et al., 2004; Amerah et al., 2008; Abdollahi et al., 2018a). A more developed gizzard musculature, as an adaptive response to inclusion of whole grain, coarse particles, and dietary fibre, can lead to increase in gizzard weight. In the present study, the inclusion of whole barley in the diet resulted in higher relative and absolute weights of the gizzard compared to finely and coarsely ground barley. The increase in gizzard weight as a response to large particle size and whole grain stimulates its grinding efficiency, and this might contribute to the higher digestibility of nutrients observed in the current study which subsequently resulted in an improved FCR. The increased gizzard grinding activity and development by feeding coarse particles and whole grain can increase reverse peristalsis of digesta, and subsequently retention time leading to better mixing of substrate and enzymes (Duke, 1992; Li and Owyang, 1993; Ferket, 2000; Singh et al., 2014b).

Finely ground pelleted diets have been related to an enlargement of the proventriculus and atrophy of the gizzard (Singh et al., 2014a). However, whole-grain feeding has been associated with an increase in relative weight of the gizzard but a reduction in the relative weight of proventriculusand reduced gizzard pH (Forbes and Covasa, 1995; Gracia et al., 2016). The proventriculus is the glandular stomach where digestion primarily begins and is anterior to the muscular gizzard (ventriculus). The proventriculus secrets pepsinogen and hydrochloric acid (HCl) and the small volume of the proventriculus results in a short retention time (Svihus, 2011b). However, finely ground pelleted diets may contribute to a non-functional gizzard resulting in the hypertrophy of the proventriculus. The proventriculus is the initial site of protein digestion in chickens where proteins are exposed to HCl, which denatures the proteins and exposes the peptide bonds for enzyme hydrolysis (Perera et al., 2019a).

Coarse feed particles need to be ground to a certain critical size before they can leave the gizzard (Clemens et al., 1975; Moore, 1999). As a result, the contraction of the large gizzard muscles to grind large feed particles cause refluxes of feed materials into the proventriculus allowing for additional secretions to be added to the substrate and also moves smaller particles that are able to pass through the pylorus into the duodenum (Duke, 1992). In the present study, there was no difference observed in duodenal digesta particle size distribution between treatments which suggests that the grinding activity of the gizzard evened out the differences in particle size distribution. This is in agreement with the results by Amerah et al. (2007b, 2008). In three different barley inclusion methods, over 70% of the duodenal particle size was below 63 µm and this highlights the ability of the gizzard to grind large feed particles to fine particles. Furthermore, the longer retention time to grind large feed particle sizes and continuous refluxes allows for more secretion of HCl which contributed to a lower pH in birds fed diets containing whole barley compared to those fed finely ground barley. As a result, a heavier gizzard in birds fed coarsely ground barley and whole barley might have aided in initial protein hydrolysis and subsequently resulted in greater CAID of N. Amerah et al. (2011) found that PRP inclusion of whole wheat (100 and 200 g/kg) increased ileal protein digestibility.

In the present study, all dietary treatments were supplemented with phytase and NSP-degrading enzymes and this might have contributed to the lack of effect of protease on growth performance and nutrient digestibility. Supplementation of NSP-degrading enzymes have been reported to reduce the intestinal viscosity and empty weights of the small intestine (Simon, 1998; Dänicke et al., 2000). However, the lack of effect of dietary treatments on the jejunal digesta viscosity in current study can be explained by supplementation of NSP-degrading enzymes, in all diets.

In the present study, the relative weight of jejunum and ileum was lower in birds fed whole barley compared to birds fed finely ground barley. Previous studies have shown that relative weight and length of intestinal segments were not affected by the substitution of ground wheat with whole wheat (Jones and Taylor, 2001; Engberg et al., 2004; Wu et al., 2004; Ravindran et al., 2006). However, according to Brenes et al. (1993), a decrease in weights of pancreas and the GIT by whole-grain feeding may presumably be a result of an adaptive response to increased nutrient digestibility and availability which has also contributed to an increase in carcass yield. The present study showed that the main effect of barley inclusion method did not have any effect on the relative lengths of duodenum, jejunum and ileum. These results are in agreement with the results of Naderinejad et al. (2016) study, who reported that maize particle size had no effect on the relative length of GIT component. However, Amerah et al. (2007b) found that the gut components of birds fed coarse particles were relatively shorter than those fed medium particles. In a subsequent study, Amerah et al. (2008) reported that all gut components (duodenum, ileum, jejunum and caeca) were shorter in birds fed coarse particles compared to those fed fine particles in maize-based diets, but no difference was observed between particle sizes in wheat-based diets.

In the current study, whole barley inclusion tended to decrease the length of the caeca compared to fine and coarsely ground barley. This could be attributed to the lower undigested feed materials being delivered to the caeca for fermentation as active gizzard might have contributed to better utilisation of nutrients and lower fermentation products in the caeca. Amerah et al. (2008) found that birds fed coarse ground maize had shorter caeca compared to fine ground maize diets.

Singh et al. (2014b) found that the length of caeca was unaffected when pre-pelleting inclusion of whole maize was increased from 0 to 450 g/kg; however, increasing up to 600 g/kg increased the relative length of the caeca. An interaction of barley inclusion method x protease was also observed for the relative weight of caeca where protease supplementation numerically reduced the relative weight of caeca in finely and coarsely ground barley diets whereas in whole barley diets, protease supplementation numerically increased the relative weights of caeca compared to diets without enzyme. Gracia et al. (2016) found that there was no significant effect of whole wheat on caecal weight. The higher relative weight of caeca in birds fed whole barley with enzyme might have contributed to lower water loss through excreta than those fed no enzyme.

In the current study, protease supplementation did not have any effect on performance and nutrient digestibility. Reviewing the literature shows that the results of protease supplementation on performance and nutrient digestibility of broiler chickens are not consistent. Kocher et al. (2003) suggested that the efficacy of proteases might be affected by the type of the ingredients used in the diet. Some researchers have reported that the benefit of protease may be influenced by the presence of other enzymes such as xylanase and/or phytase (Sultan et al., 2011; Kalmendal and Tauson, 2012). Kocher et al. (2003) have stated that various functions of proteases may also depend on dietary formulation and ingredients used in the diet. With carbohydrases supplementation in the diet, two separate hypotheses on the mode of action have been suggested: the viscosity theory and the cell wall encapsulation theory (Bedford, 2002). Independent of which theory best describes the action of carbohydrases, it may be expected that an elevated accessibility to nutrients following the addition of xylanases would increase the potential of exogenous proteases to digest proteinous components in the feed. It has been reported that the addition of protease into the diet can significantly improve the digestibility of amino acids (Angel et al., 2011; Liu et al., 2013). But this effect has not been observed in the current study. Cowieson and Roos (2014) suggested that inherent digestibility of the control diet is important to see the protease effect. They reported that when the inherent digestibility of amino acids in the diet was less than 70%, protease supplementation improved amino acid digestibility around 10%. However, when the inherent digestibility of amino acids in the diet was more than 90% there was 2% improvement in digestibility of amino acids by protease supplementation.

In summary, the current study demonstrated that when coarsely ground and pre-pelleting whole barley were fed to broilers, growth performance started to improve from early stage of life. The CAID of nutrients were also improved when coarsely ground and whole barley were fed. Both the increased weight of the gizzard and the inclusion of whole barley had a positive effect on the gizzard pH and nutrient digestibility and energy utilisation.

3.5 Conclusion

In conclusion, the findings of the present study suggest that coarse grinding and PRP whole barley inclusion resulted in enhanced nutrient digestibility and gizzard development and subsequently improved performance in broilers. Exogenous protease supplementation had no effect on broiler performance and nutrient digestibility. The lack of protease effects may hypothetically mirror a low potential for further improvements, due to the phytase and carbohydrase enzymes and high digestible components in the feed.

REFERENCES

Abdollahi, M. R. and Ravindran, V. (2019) Alternative feed ingredients for poultry diets: Challenges and prospects. Proceedings of the Australian Poultry Science Symposium 30: 102-108.

Abdollahi, M. R., Ravindran, V. and Svihus, B. (2013a) Influence of feed form on growth performance, ileal nutrient digestibility, and energy utilisation in broiler starters fed a sorghum-based diet. Livestock Science 165: 80-86.

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

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

Abdollahi, M. R., Ravindran, V., Wester, T. J., Ravindran, G. and Thomas, D. V. (2010) Influence of conditioning temperature on the performance, nutrient utilisation and digestive tract development of broilers fed on maize-and wheat-based diets. British Poultry Science 51: 648-657.

Abdollahi, M. R., Ravindran, V., Wester, T. J., Ravindran, G. and Thomas, D. V. (2013c) The effect of manipulation of pellet size (diameter and length) on pellet quality and performance, apparent metabolisable energy and ileal nutrient digestibility in broilers fed maize-based diets. Animal Production Science 53: 114-120.

Abdollahi, M. R., Zaefarian, F., Hunt, H., Anwar, M. N., Thomas, D. G. and Ravindran, V. (2018a) Wheat particle size, insoluble fibre sources and whole wheat feeding influence gizzard musculature and nutrient utilisation to different extents in broiler chickens. Journal of Animal Physiology and Animal Nutrition 103: 146-161.

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

Alberle, T., Burchard, W., Vorwerg, W. and Radosta, S. (1994) Conformational contributions of amylose and amylopectin to the structural properties of starches from various sources. Starch- Stärke 46: 329-335.

Åman, P. and Graham, H. (1987) Mixed-linked β-(1→ 3), (1→ 4)-D-glucans in the cell walls of barley and oats-chemistry and nutrition. Scandinavian Journal of Gastroenterology 22: 42-51.

Åman, P., Hesselman, K. and Tilly, A. C. H. (1985) The variation in chemical composition of Swedish barleys. Journal of Cereal Science 3: 73-77.

Amerah, A. M., Péron, A., Zaefarian, F. and Ravindran, V. (2011) Influence of whole wheat inclusion and a blend of essential oils on the performance, nutrient utilisation, digestive tract development and ileal microbiota profile of broiler chickens. British Poultry Science 52: 124-132.

Amerah, A. M. and Ravindran, V. (2008) Influence of method of whole-wheat feeding on the performane, digestive tract development and carcass traits of broiler chickens. Animal Feed Science and Technology 147: 326-339.

Amerah, A. M., Ravindran, V. and Lentle, R. G. (2009) 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: 366-375.

Amerah, A. M., Ravindran, V., Lentle, R. G. and Thomas, D. G. (2007a) Feed particle size: Implications on the digestion and performance of poultry. World's Poultry Science 63: 439-455.

Amerah, A. M., Ravindran, V., Lentle, R. G. and Thomas, D, G. (2008) Influence of feed particle size on the performance, energy utilization, digestive tract development, and digesta parameters of broiler starters fed wheat- and corn-based diets. Poultry Science 87: 2320-2328.

Amerah, A. M., Ravindran, V., Lentle, R. G. and Thomas, D. G. (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: 2615-2623.

Anderson, P. M., Oelke, E. A. and Simmons, S. R. (1995) Growth and development guide for spring barley. University of Minnesota Extension Service, viewed 8th August 2019: https://conservancy.umn.edu/bitstream/handle/11299/165839/Growth%20and%20Development%20Guide%20for%20Spring%20Barley.pdf?sequence=1.

Andersson, A. A. M., Elfverson, C., Andersson, R., Regner, S. and Åman, P. (1999) Chemical and physical characteristics of barley samples. Journal of the Science of Food and Agriculture 79: 979-986.

Angel, C. R., Saylor, W., Vieira, S. L. and Ward, N. (2011) Effects of a monocomponent protease on performance and protein utilization in 7- to 22-day-old broiler chickens. Poultry Science 90: 2281-2286.

Angulo, E., Brufau, J. and Esteve-Garcia, E. (1995) Effect of sepiolite on pellet durability in feeds differing in fat and fibre content. Animal Feed Science and Technology 53: 233-241.

Angulo, E., Brufau, J. and Esteve-Garcia, E. (1996) Effect of sepiolite product on durability in pig diets differing in particle size and in broiler starter and finisher diets. Animal Feed Science and Technology 63: 25-34.

Anker-Nilssen, K., Færgestad, E., Sahlstrøm, S. and Uhlen, A. K. (2006) Interaction between barley cultivars and growth temperature on starch degradation properties measured in vitro. Animal Feed Science and Technology 130: 3-22.

Ankrah, N. O., Campbell, G. L., Tyler, R. T., Rossnagel, B. G. and Sokhansanj, S. R. T. (1999) Hydrothermal and β-glucanase effects on the nutritional and physical properties of starch in normal and waxy hull-less barley. Animal Feed Science and Technology 81: 205-219.

Ao, Z. and Jane, J. (2006) Characterization and modelling of the A- and B-granule of starches of wheat, triticale, and barley. Carbohydrate Polymers 67: 46-55.

Axe, D. E. (1995) Factors affecting uniformity of a mix. Animal Feed Science and Technology 53: 211-220.

Badr, A., Muller, K., Schafer-Pregl, R., El Rabey, H., Effgen, S., Ibrahim, H. H., Pozzi, C., Rohde, W. and Salamini, F. (2000) On the origin and domestication history of barley (Hordeum vulgare). Molecular Biology and Evolution 17: 499-510.

Baker, S. and Herrman, T. (2002) Evaluating Particle Size. Department of Grain Science and Industry: Kansas State University.

Bedford, M. R. (1996) The effect of enzymes on digestion. Journal of Applied Poultry Research 5: 370-378.

Bedford, M. R. (2002) The role of carbohydrases in feedstuff digestion. Pages 319-336 in Poultry Feedstuffs: Supply, Composition and Nutritive Value. J. MacNab and N. Boorman, ed. CAB International, Wallingford, UK.

Behall, K. M., Scholfield, D. J., and Hallfrisch, J. (2004) Diets containing barley significantly reduce lipids in mildly hypercholesterolemic men and women. The American Journal of Clinical Nutrition 80: 1185-1193.

Behnke, K. C. (1996) Feed manufacturing technology: current issues and challenges. Animal Feed Science and Technology 62: 49-57.

Behnke, K. C. (2001) Factors influencing pellet quality. Feed Technology 5: 19-22.

Benech-Arnold, R. L., Giallorenzi, M. C., Frank, J. and Rodriguez, V. (1999) Termination of hull-imposed dormancy in developing barley grains is correlated with the changes in embryonic ABA levels and sensitivity. Seed Science Research 9: 39-47.

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

Bhatty, R. S., Christison, G. I., Sosulski, F. W., Harvey, B. L., Hughes, G. R. and Berdahl, J. D. (1974) Relationships of various physical and chemical characteristics to digestible energy in wheat and barley cultivars. Canadian Journal of Animal Science 54: 419-427.

Bhatty, R. S. (1986) The potential of hull-less barley, a review. American Association of Cereal Chemists 63: 97-103.

Biel, W. and Jacyno, E. (2013) Chemical composition and nutritive value of spring hulled barley varieties. Bulgarian Journal of Agricultural Science 19: 721-727.

Biggs, P. and Parsons, C. M. (2009) The effect of whole grains on nutrient digestibilities, growth performance, and cecal short-chain fatty acid concentrations in young chicks fed ground corn-soybean meal diets. Poultry Science 88: 1893-1905.

Black, J. L., Tredrea, A. M., Nielsen, S. G., Flinn, P. C., Kaiser, A. G. and van Barneveld, R. J. (2005) September. Feed uses for barley. 12th Australian Barley Technical Symposium: 1-4. Australian Barley Association.

Bleidere, M. and Gaile, Z. (2012) Grain quality traits important in feed barley. *Proceedings of the* Latvian Academy of Sciences 66: 1-9.

Brenes, A., Smith, M., Guenter, W. and Marquardt, R. R. (1993) Effect of enzyme supplementation on the performance and digestive tract size of broiler chickens fed wheat- and barley-based diets. Poultry Science 72: 1731-1739.

Burnett, G. S. (1966) Studies of viscosity as the probable factor involved in the improvement of certain barleys for chickens by enzyme supplementation. British Poultry Science 7: 55-75.

Cabahug, S., Ravindran, V., Selle, P. and Bryden, W. (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: 660-666.

Chesson, A. (1992) Effects of supplementary enzymes in barley diets. Options Méditerranéennes, Série Séminaires 20: 555-62.

Chewning, C. G., Stark, C. R. and Brake, J. (2012) Effects of particle size and feed form on broiler performance. Journal of Applied Poultry Research 21: 830-837.

Choct, M. (2006) Enzymes for the feed industry: past, present and future. World's Poultry Science Journal 62: 5-16.

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

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

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

Classen, H. L. (1996) Cereal grain starch and exogenous enzymes in poultry diets. Animal Feed Science and Technology 62: 21-27.

Clemens, E. T., Stevens, C. E. and Southworth, M. (1975) Sites of organic acid production and pattern of digesta movement in the gastrointestinal tract of geese. Journal of Nutrition 105: 1341-1350.

Copeland, L., Blazek, J., Salman, H. and Tang M. C. (2009) Form and functionality of starch. Food Hydrocolloids 23(6), 1527-1534.

Cowieson, A. J. and Adeola, O. (2005) Carbohydrases, protease, and phytase have an additive beneficial effect in nutritionally marginal diets for broiler chicks. Poultry Science 84: 1860-1867.

Cowieson, A. J. and Ravindran V. (2008) Sensitivity of broiler starters to three doses of an enzyme cocktail in maize-based diets. British Poultry Science 49: 340-346.

Cumming, R. B. (1994) Opportunities for whole grain feeding. Proceedings 9th European Poultry Conference, Glasgow, U.K., 219-223.

Dahleen, L. S., Vander Wal, L. J., and Frankowiak, J. D. (2005) Characterization and molecular mapping of genes determining semidwarfism in barley. Journal of Heredity 96: 654-662.

Dänicke, S., Jeroch, H., Böttcher, W. and Simon, O. (2000) Interactions between dietary fat type and enzyme supplementation in broiler diets with high pentosan contents: effects on precaecal and total tract digestibility of fatty acids, metabolizability of gross energy, digesta viscosity and weights of small intestine. Animal Feed Science and Technology 84: 279-294.

De Man, W. (1985) The effect of genotype and environment on the fatty acid content of barley (Hordeum vulgare L.) grains. Plant, Cell and Environment 8: 571-577.

De Paula, R., Abdel-Aal, E. S. M., Messia, M. C., Rabalski, I. and Marconi, E. (2017) Effect of processing on the beta-glucan physicochemical properties in barley and semolina pasta. Journal of Cereal Science 75: 124-131.

De Silva, S., Hesselman, K. and Åman P. (1983) Effects of water and β-glucanase treatment of non-starch polysaccharides in endosperm of low and high viscous barley. Swedish Journal of Agricultural Research 12: 211-219.

DSM Nutritional Products Ltd. (2013) Ronozyme® Multigrain (GT)-Specification and Tests-Basel, Switzerland.

Duke, G. E. (1986) Alimentary canal: anatomy, regulation of feeding, and motility. In Avian Physiology: 269-288. Springer, New York, NY.

Duke, G. E. (1992) Recent studies on regulation of gastric motility in turkeys. Poultry Science 71: 1-8.

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

Engberg, R. M., Hedemann, M. S., Steenfeldt, S. and Jensen, B. B. (2004) Influence of whole wheat and xylanase on broiler performance and microbial composition and activity in the digestive tract. Poultry Science 83: 925-938.

Evers, A. D., O'Brien, L. and Blakeney, A. B. (1999) Cereal structure and composition. Australian Journal of Agricultural Research 50: 629-650.

Fayez, K. A. and Bazaid, S. A. (2014) Improving drought and salinity tolerance in barley by application of salicylic acid and potassium nitrate. Journal of the Saudi Society of Agricultural Science 13: 45-55.

Fedak, G. and Roche, I. D. L. (1977) Lipid and fatty acid composition of barley kernels. Canadian Journal of Plant Science 57: 257-260.

Fengler, A. I. and Marquardt, R. R. (1988) Water-soluble pentosans from rye: II. Effect on rate of dialysis and on the retention of nutrients by the chick. Cereal Chemistry 65: 298-302.

Ferket, P. (2000) Feeding whole grains to poultry improves gut health. Feedstuffs USA September 04: 12-14.

Food and Agriculture Organisation, (2019) FAOSTAT, Viale delle Terme di Carcalla, Rome, Italy, viewed 6th August 2019: http://www.fao.org/faostat/en/#data/QC.

Forbes, J. M. and Covasa, M. (1995) Application of diet selection by poultry with particular reference to whole cereals. World's Poultry Science Journal 51: 149-165.

Freitas, D. M., Vieira, S. L., Angel, C. R., Favero, A. and Maiorka, A. (2011) Performance and nutrient utilization of broilers fed diets supplemented with a novel mono-component protease. Journal of Applied Poultry Research 20: 322-334.

Fru-Nji, F., Kluenter, A. M., Fischer, M. and Pontoppidan, K. (2011) A feed serine protease improves broiler performance and increases protein and energy digestibility. The Journal of Poultry Science 48: 239-246.

Gabriel, I., Mallet, S., and Lconte, M. (2003) Differences in the digestive tract characteristics of broiler chickens fed on complete pelleted diets or on whole wheat added to pelleted protein concentrate. British Poultry Science 44: 283-290.

Gabriel, I., Mallet, S., Leconte, M., Travel, A. and Lalles, J. P. (2008) Effects of whole wheat feeding on the development of the digestive tract of broiler chickens. Animal Feed Science and Technology 142: 144-162.

Gajdošová, A., Petruláková, Z., Havrlentová, M., Červená, V., Hozová, B., Šturdík, E. and Kogan, G. (2007) The content of water-soluble and water-insoluble β-D-glucans in selected oats and barley varieties. Carbohydrate Polymers 70: 46-52.

Goodband, R. D., Tokach, M. D. and Nelssen, J. L. (2002) The effects of diet particle size on animal performance. MF-2050 Feed Manufacturing. Kansas State, Manhattan.

Gracia, M. I., Sánchez, J., Millán, C., Casabuena, Ó., Vesseur, P., Martín, Á., García-Peña, F. J. and Medel, P. (2016) Effect of feed form and whole grain feeding on gastrointestinal weight and the prevalence of Campylobacter jejuni in broilers orally infected. PloS One 11: 1-16.

Grundbacher, F. (1963) The physiological function of the cereal awn. The Botanical Review 29: 366-381.

Guinotte, F., Gautron, J., Nys, Y. and Soumarmon, A. (1995) Calcium solubilization and retention in the gastrointestinal tract in chicks (Gallus domesticus) as a function of gastric acid secretion inhibition and calcium carbonate particle size. British Journal of Nutrition 72: 125-139.

Henry, R. J. (1988) The carbohydrates of barley grains – A review. Journal of the Institute of Brewing 94: 71-78.

Hesselman, K. and Åman, P. (1986) The effect of β-glucanase on the utilization of starch and nitrogen by broiler chickens fed on barley of low- and high-viscosity. Animal Feed Science and Technology 15: 83-93.

Hetland, H., Choct, M., and Svihus, B. (2004) Role of insoluble non-starch polysaccharides in poultry nutrition. World's Poultry Science Journal 60: 415-422.

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

Hetland, H., Svihus, B. and Krogdahl, Å. (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: 275-282.

Hetland, H., Svihus, B. and Olaisen, V. (2002) Effect of feeding whole cereals on performance, starch digestibility and duodenal particle size distribution in broiler chickens. British Poultry Science 43: 416-423.

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

Holtekjølen, A. K., Uhlen, A. K., Bråthen, E., Sahlstrøm, S. and Knutsen, S. H. (2006) Contents of starch and non-starch polysaccharides in barley varieties of different origin. Food Chemistry 94: 348-358.

Hu, J., Ingale, S., Rathi, P., Zhang, J. Y. and Kim, I. H. (2019) Influence of exogenous acid protease in broiler chickens fed corn-soybean meal-based diets. Journal of Animal Physiology and Animal Nutrition (accepted): 1-8.

Hughes, B. O. (1984) The principles underlying choice feeding behaviour in fowls – with special reference to production experiments. World's Poultry Science Journal 40: 141-150.

Hughes, R. J. and Choct, M. (1999) Chemical and physical characteristics of grains related to variability in energy and amino acid availability in poultry. Australian Journal of Agricultural Research 50: 689-701.

Iji, P. A. (1999) The impact of cereal non-starch polysaccharides on intestinal development and function in broiler chickens. World Poultry Science Journal 55: 375-387.

Izydorczyk, M. S. and Dexter, J. E. (2008) Barley β-glucans and arabinoxylans: Molecular structure, physicochemical properties, and uses in food products – a review. Food Research International 41: 850-868.

Izydorczyk, M. S., Macri, L. J. and MacGregor, A. W. (1998) Structure and physicochemical properties of barley non-starch polysaccharides I. Water-extractable β-glucans and arabinoxylans. Carbohydrate Polymers 35: 249-258.

Izydorczyk, M. S., Storsley, J., Labossiere, D., MacGregor, A. W. and Rossnagel, B. G. (2000) Variation in total and soluble β-glucan content in hulless barley: effects of thermal, physical and enzymic treatments. Journal of Agricultural and Food Chemistry 48: 982-989.

Jacob, J. P. and Pescatore, A. J. J. (2012) Using barley in poultry diets - a review. Journal of Applied Poultry Research 21: 915-940.

Jacob, J. P. and Pescatore, A. J. J. (2014) Barley β-glucan in poultry diets. Annals of Translational Medicine 2: 1-7.

Jang, D. A., Fadel, J. G., Klasing, K. C., Mireles Jr, A. J., Ernst, R. A., Young, K. A., Cook, A. and Raboy, V. (2003) Evaluation of low-phytate corn and barley on broiler chick performance. Poultry Science 82: 1914-1924.

Jeroch, H. and Dänicke, S. (1995) Barley in poultry feeding: a review. World's Poultry Science Journal 51: 271-291.

Jones, G. P. D. and Taylor, R. D. (2001) The incorporation of whole grain into pelleted broiler chicken diets: production and physiological responses. British Poultry Science 42: 477-483.

Juanpere, J., Perez-Vendrell, A. M., Angulo, E. and Brufau, J. (2005). Assessment of potential interactions between phytase and glycosidase enzyme supplementation on nutrient digestibility in broilers. Poultry Science 84: 571-580.

Kalmendal, R. and Tauson, R. (2012) Effects of a xylanase and protease, individually or in combination, and an inophore coccidiostat on performance, nutrient utilization, and intestinal morphology in broiler chickens fed a wheat-soybean meal-based diet. Poultry Science 91: 1387-1393.

Kasim, A. B. and Edwards, H. M. (2000) Effect of sources of maize and maize particles on the utilization of phytate phosphorus in broiler chicks. Animal Feed Science and Technology 86: 15-26.

Khan, M. (1988) Correlation of awns/hoods and two rowed vs six rowed characters with yield in barley. Pakistan Journal of Agricultural Research 9: 25-27.

Kilburn, J. and Edwards, H. M. (2001) The response of broilers to the feeding of mash or pelleted diets containing maize of varying particle sizes. British Poultry Science 42: 484-492

Kliševičiūtė, V., Gruzauskas, R., Dauksiene, A., Raceviciute-Stupeliene, A., Svirmickas, G. J. and Bliznikas, S. (2012) Influence of different amount of whole barley in diets on productivity and digestive processes of broiler chickens. Veterinarija ir Zootechnika 58: 26-34.

Knudsen, K. E. B. (1997) Carbohydrate and lignin contents of plant materials used in animal feeding. Animal Feed Science and Technology 67: 319-338.

Koch, K. (1996) Hammermills and rollermills. MF-2048 Feed Manufacturing, Department of Grain Science and Industry, Kansas State University.

Kocher, A., Choct, M., Ross, G., Broz, J. and Chung, T. K. (2003) Effects of enzyme combinations on apparent metabolizable energy of corn-soybean meal-based diets in broilers. Journal of Applied Poultry Research 12: 275-283.

Kocher, A., Hughes, R. J. and Barr, A. R. (1997) B-glucanase reduces but does not eliminate variation in AME of barley varieties. Proceedings of the Australian Poultry Science Symposium 9: 142-145.

Komatsuda, T., Pourkheirandish, M., He, C., Azhaguvel, P., Kanamori, H., Perovic, D., Stein, N., Graner, A., Wicker, T., Tagiri, A. and Lundqvist, U. (2007) Six-rowed barley originated from a mutation in a homeodomain-leucine zipper I-class homeobox gene. Proceedings of the National Academy of Sciences 104: 1424-1429.

Le Huerou-Luron, I., Lhoste, E., Wicker-Planquart, C., Dakka, N., Toullec, R., Corring, T., Guilloteau, P. and Puigserver, A. (1993) Molecular aspects of enzyme synthesis in the exocrine pancreas with emphasis on development and nutritional regulation. Proceedings of the Nutrition Society 52: 301-313.

Lemme, A., Ravindran, V. and Bryden, W. L. (2004) Ileal digestibility of amino acids in feed ingredients for broilers. World's Poultry Science Journal 60: 423-438.

Lentle, R. G., Ravindran, V., Ravindran, G. and Thomas, D. V. (2006) Influence of feed particle size on the efficiency of broiler chickens fed wheat-based diets. The Journal of Poultry Science 43: 135-142.

Lewis, C. J., Catron, D. V. Liu, C. H., Speer, V. C. and Ashton, G. C. (1955) Swine nutrition, enzyme supplementation of baby pig diets. Journal of Agricultural and Food Chemistry 3: 1047-1050.

Li, J. H., Vasanthan, T., Rossnagel, B. and Hoover, R. (2001) Starch from hull-less barley: I. Granule morphology, composition and amylopectin structure. Food Chemistry 74: 395-405.

Li, Y. and Owyang, C. (1993) Vagal afferent pathway mediates physiological action of cholecystokinin on pancreatic enzyme secretion. The Journal of Clinical Investigation 92: 418-424.

Liu, K. (2011) Comparison of lipid content and fatty acid composition and their distribution within seeds of 5 small grain species. Journal of Food Science 76: C334-C342.

Liu, S. Y., Sell, P. H., Court, S. G. and Cowieson, A. J. (2013) Protease supplementation of sorghum-based diets enhances amino acid digestibility coefficients in four small intestinal sites and accelerates their rates of digestion. Animal Feed Science and Technology 183: 175-183.

Liu, S. Y., Truong, H. H. and Selle, P. H. (2015) Whole-grain feeding for chicken-meat production: possible mechanisms driving enhanced energy utilisation and feed conversion. Animal Production Science 55: 559-572.

Lott, B. D., Day, E. J., Deaton, J. W. and May, J. D. (1992) The effect of temperature, dietary energy level, and corn particle size on broiler performance. Poultry Science 71: 618-624.

Löwe, R. (2005) Judging Pellet Stability as Part of Pellet Quality. Feed Technology 9: 15-19.

Macewicz, J., Orzechowski, S. and Haebel, S. (2006) Is quantity of protein in barley forms determined by proteins localized in the subaleurone layer? Acta Physiologiae Plantarum 28: 409-416.

MacGregor, A. W. and Fincher, G. B. (1993) Carbohydrates of barley grain. Barley: Chemistry and Technology (eds. W. Mac-Gregor, RS Bhatty). AACC, St. Paul, USA: 73-130.

Macleod, L. C. and Duffus, C. M. (1988) Reduced starch content and sucrose synthase activity in developing endosperm of barley plants grown at elevated temperatures. Australian Journal of Plant Physiology 15: 367-375.

Mahagna, M., Nir, I., Larbier, M. and Nitsan, Z. (1995) Effect of age and exogenous amylase and protease on development of the digestive tract, pancreatic enzyme activities and digestibility of nutrients in young meat-type chicks. Reproduction Nutrition Development 35: 201-212.

Mano, Y., Nakazumi, H. and Takeda, K. (1996) Varietal variation in and effects of some major genes on salt tolerance at the germination stage of barley. Breeding Science 46: 227-233.

Mano, Y. and Takeda, K. (1998) Genetic resources of salt tolerance in wild Hordeum species. Euphytica 103: 137-141.

Martin, S. (1985) Comparison of hammer mill and roller mill grinding and the effect of grain particle size on mixing and pelleting. Master Thesis, Kansas State University, Kansas.

Mateos, G. G., Jiménez-Moreno, E., Serrano, M. P. and Làzaro, R. P. (2012) Poultry response to high levels of dietary fiber sources varying in physical and chemical characteristics. Journal of Applied Poultry Research 21: 156-174.

McCleary, B. V., Gibson, T. S. and Mugford, D. C. (1997) Measurement of total starch in cereal products by amyloglucosidase vs α-amylase method: collaborative study. Journal of Association of Official Analytical Chemists 80: 571-579.

McNab, J. M. and Smithard, R. R. (1992) Barley β-glucan: An antinutritional factor in poultry feeding. Nutrition Research Reviews 5: 45-60.

Messia, M. C., Candigliota, T., De Arcangelis, E. and Marconi, E. (2016) Arabinoxylans and β-glucans assessment in cereals. Italian Journal of Food Science 29: 112-122.

Michard, J. (2011) Dietary fibre – the forgotten nutrient. Hubbard Technical Bulletin. Viewed 29th August, 2019, https://www.hubbardbreeders.com/media/dietary_fibre_the_forgotten_nutrienten_041329800_1521_26062017.pdf.

Moore, S. J. (1999) Food breakdown in an avian herbivore; who needs teeth? Australian Journal of Zoology 47: 625-632.

Moss, A. F., Sydenham, C. J., Truong, H. H., Liu, S. Y. and Selle, P. H. (2017) The interactions of exogenous phytase with whole grain feeding and effects of barley as the whole grain component in broiler diets based on wheat, sorghum and wheat-sorghum blends. Animal Feed Science and Technology 227: 1-12.

Moss, A. F., Truong, H. H., Liu, S. Y. and Selle, P. H. (2018) Inclusion levels and modes of whole grain incorporation into wheat-based rations differentially influence the performance of broiler chickens. British Poultry Science 59: 110-120.

Naderinejad, S., Zaefarian, F., Abdollahi, M. R., Hassanabadi, A., Kermanshahi, H. and Ravindran, V. (2016) Influence of feed form and particle size on performance, nutrient utilisation, and gastrointestinal tract development and morphometry in broiler starters fed maize-based diets. Animal Feed Science and Technology 215: 92-104.

Nahas, J. and Lefrancois, M. R. (2001) Effects of feeding locally grown whole barley with or without enzyme addition and whole wheat on broiler performance and carcass traits. Poultry Science 80: 195-202.

Nathier-Dufour, N., Angue, Y., Devaux, M. F., Bertrand, D. and de Monredon, F. L. D. (1995) Influence of wheat meal variability upon compacting behaviour during pelleting. Animal Feed Science and Technology 51: 255-268.

Nir, I., Hillel, R., Ptichi, I. and Shefet, G. (1995) Effect of particle size on performance. 3. Grinding pelleting interactions. Poultry Science 74: 771-783.

Nir, I., Hillel, R., Shefet, G. and Nitsan, Z. (1994a) Effect of grain particle size on performance. 2. Grain texture interactions. Poultry Science 73: 781-791.

Nir, I., Nitsan, Z. and Mahagna, M. (1993) Comparative growth and development of the digestive organs and of some enzymes in broiler and egg type chicks after hatching. British Poultry Science 34: 523-532.

Nir, I. and Ptichi, I. (2001) Feed particle size and hardness: influence on performance, nutritional, behavioural and metabolic aspects. In:Advances in Nutritional Technology 2001 (van der Poel, A. F. B.,Vahl, J. L. and Kwakkel, R. P. eds), pp. 157-186. Proceedings of the1st World Feed Conference, Utrecht, Netherlands, 7-8 November. Wageningen Pers, Wageningen.

Nir, I., Shefet, G. and Aaroni, Y. (1994b) Effect of particle size on performance. 1. Corn. Poultry Science 73: 45-49.

O'Dell, B. L., Newberne, P. M. and Savage, J. E. (1959) An abnormality of the proventriculus caused by feed texture. Poultry Science 38: 296-301.

Olkku, J., Kotaviita, E., Salmenkallio-Marttila, M., Sweins, H. and Home, S. (2005) Connection between structure and quality of barley. Journal of the American Society of Brewing Chemists 63: 17-22.

Oliveira, P. M., Mauch, A., Jacob. F., Waters, D. M., and Arendt, E. K. (2012) Fundamental study on the influence of fusarium infection on quality and ultrastructure of barley malt. International Journal of Food Microbiology 156: 32-43.

Olsen, O. A. (2001) Endosperm development: cellularization and cell fate specification. Annual review of plant biology 52: 233-267.

Olsen, O. A. (2004) Nuclear endosperm development in cereals and Arabidopsis thaliana. The Plant Cell 16: 214-227.

Oscarsson, M., Andersson, R., Åman, P., Olofsson, S. and Jonsson, A. (1998) Effects of cultivar, nitrogen fertilization rate and environment on yield and grain quality of barley. Journal of the Science of Food and Agriculture 78: 359-366.

Oscarsson, M., Andersson, R., Salomonsson, A. C. and Åman, P. (1996) Chemical composition of barley samples focusing on dietary fibre components. Journal of Cereal Science 24: 161-170.

Oscarsson, M., Parkkonen, T., Autio, K. and Åman, P. (1997) Composition and microstructure of waxy, normal and high amylose barley samples. Journal of Cereal Science 26: 259-264.

Osman, R. O., El-Gelil, F. A., El-Noamany, H. M. and Dawood, M. G. (2000) Oil content and fatty acid composition of some varieties of barley and sorghum grains. Grasas y Aceites 51: 157-162.

Palmer, G. H., and Bathgate, G. H. (1976) Malting and brewing. Advances in Cereal Science and Technology 1: 237-324.

Perera, W. N. U., Abdollahi, M. R., Zaefarian, F., Wester, T. J., Ravindran, G. and Ravindran, V. (2019a) Influence of inclusion level of barley in wheat-based diets and supplementation on carbohydrase on growth performance, nutrient utilisation and gut morphometry in broiler starters. British Poultry Science (accepted).

Perera, W. N. U., Abdollahi, R. R., Ravindran, V., Zaefarian, F., Wester, T. J. and Ravindran, G. (2019b) Nutritional evaluation of two barley cultivars, without and with carbohydrase supplementation, for broilers: Metabolisable energy and standardised amino acid digestibility. British Poultry Science 60: 404-413.

Perlin, A. S. (1951) Isolation and composition of the soluble pentosans of wheat flours. Cereal Chemistry 28: 370-381.

Péron, A., Bastianelli, D., Oury, F. X., Gomez, J. and Carré, B. (2005) Effects of food deprivation and particle size of ground wheat on digestibility of food components in broilers fed on a pelleted diet. British Poultry Science 46: 223-230.

Pirgozliev, V. R., Rose, S. P. and Bedford, M. R. (2010) The effect of amylose:amylopectin ratio in dietary starch on growth performance and gut morphology in broiler chickens. Archiv fur Geflugelkunde 74: S21-S29.

Plavnik, I., Macovsky, B. and Sklan, D. (2002) Effect of feeding whole wheat on performance of broiler chickens. Animal Feed Science and Technology 96: 229-236.

Preston, C. M. McCracken, K. J. and McAllister, A. (2000) Effect of diet form and enzyme supplementation on growth, efficiency and energy utilisation of wheat-based diets for broilers. British Poultry Science 41: 324-331.

Price, P. B. and Parsons, J. G. (1975) Lipids of seven cereal grains. Journal of the American Oil Chemists' Society 52: 490-493.

Qaisrani, S. N., van Krimpen, M. M., Kwakkel, R. P., Verstegen, M. W. A. and Hendriks, W. H. (2015) Diet structure, butyric acid, and fermentable carbohydrates influenced growth performance, gut morphology, and cecal fermentation characteristics in broilers. Poultry Science 94: 2152-2164.

Rada, V., Lichovníková, M., Foltyn, M. and Šafařík, I. (2016) The effect of exogenous protease in broiler diets on the apparent ileal digestibility of amino acids and on protease activity in jejunum. Acta Universitatis Agriculturae et Silviculturae Mendelianae Brunensis 64: 1645-1652

Rasmusson, D. C. (1985) Barley, American Society of Agronomy, Crop Science Society of America, Soil Science Society of America, Publishers Madison, Wisconsin.

Ravindran, V. (2013) Feed enzymes: The science, practice, and metabolic realities. Journal of Applied Poultry Research 22: 628-636.

Ravindran, V., Cabahug, S., Ravindran, G. and Bryden, W. L. (1999a) Influence of microbial phytase on apparent ileal amino acid digestibility of feedstuffs for broilers. Poultry Science 78: 699-706.

Ravindran, V., Hew, L. I., Ravindran, G. and Bryden, W. L. (2005) Apparent ileal digestibility of amino acids in dietary ingredients for broiler chickens. Animal Science 81: 85-97.

Ravindran, V., Hew, L. I., Ravindran, G., Gill, R. J., Pittolo, P. H. and Bryden, W. L. (1999b) Influence of xylanase supplementation on the apparent metabolisable energy and ileal amino acid digestibility in a diet containing wheat and oats, and on the performance of three strains of broiler chickens. Australian Journal of Agricultural Research 50: 1159-1163.

Ravindran, V., Selle, P. and Bryden, W. (1999c) Effects of phytase supplementation, individually and in combination, with glycanase, on the nutritive value of wheat and barley. Poultry Science 78: 1588-1595.

Ravindran, V., Tilman, z., Morel, P. C. H., Ravindran, G. and Coles, G. D. (2007) Influence of β-glucanase supplementation on the metabolisable energy and ileal nutrient digestibility of normal starch and waxy barleys for broiler chickens. Animal Feed Science and Technology 134: 45-55.

Ravindran, V., Wu, Y., Thomas, D. and Morel., P. C. H. (2006) Influence of whole wheat on the development of gastrointestinal tract and performance of broiler chickens. Australian Journal of Agricultural Research 57: 21-26.

Reece, F. N., Lott, B. D. and Deaton, J. W. (1985) The effects of feed form, grinding method, energy level, and gender on broiler performance in a moderate (21 C) environment. Poultry Science 64: 1834-1839.

Reece, F. N., Lott, B. D. and Deaton, J. W. (1986) The effects of hammer mill screen size on ground corn particle size, pellet durability, and broiler performance. Poultry Science 65: 1257-1261.

Reimer, L. (1992) Northern Crops Institute feed mill management and feed manufacturing technology short course. California Pellet Mill Co.: Crawfordsville, IN.

Rodehutscord, M., Rückert, C., Maurer, H. P., Schenkel, H., Schipprack, W., Bach Knudsen, K. E., Schollenberger, M., Laux, M., Eklund, M., Siegert, W. and Mosenthin, R. (2016) Variation in chemical composition and physical characteristics of cereal grains from different genotypes. Archives of Animal Nutrition 70: 87-107.

Rogel, A. M., Annison, E. F., Bryden, W. L. and Balnave, D. (1987) The digestion of wheat starch in broiler chickens. Australian Journal of Agricultural Research 38: 639-649.

Rose, S. P. (1996) The use of whole wheat in poultry diets. World's Poultry Science Journal 52: 59-60.

Rose, S. P., Fielden, M., Foote, W. R. and Gardin, P. (1995) Sequential feeding of whole wheat to growing broiler chickens. British Poultry Science 36: 97-111.

Ross. (2014) Ross 308 Broiler: Nutrition Specification, Ross Breeders Limited, Newbridge, Midlothian, Scotland, UK.

Sacranie, A., Svihus, B., Denstadli, V., Moen, B., Iji, P. A. and Choct, M. (2012) The effect of insoluble fiber and intermittent feeding on gizzard development, gut motility, and performance of broiler chickens. Poultry Science 91: 693-700.

SAS Institute (2018) SAS® Qualification Tools User’s Guide. Version 9.4. SAS Institute Inc., Cary, NC.

Savin, R. and Nicolas, M. E. (1996) Effects of short periods of drought and high temperature on grain growth and starch accumulation of two malting barley cultivars. Australian Journal of Plant Physiology 23: 201-210.

Selle, P. H., Ravindran, V., Caldwell, A. and Bryden, W. (2000) Phytate and phytase: consequences for protein utilisation. Nutrition Research Reviews 13: 255-278.

Shewry, P. R. and Halford, N. G. (2002) Cereal seed storage proteins of cereal seeds: structure, properties and role in grain utilization. Journal of Experimental Botany 53: 947-958.

Simon, O. (1998) The mode of action of NSP hydrolysing enzymes in the gastrointestinal tract. Journal of Animal Feed Science 7: 115-123.

Short, F. J., Gorton, P., Wiseman, J. and Boorman, K. N. (1996) Determination of titanium dioxide added as an inert marker in chicken digestibility studies. Animal Feed Science and Technology 59: 215-221.

Simbaya, J. Slominski, B. A., Guenter, W., Morgan, A. and Campbell, L. D. (1995) The effects of protease and carbohydrase supplementation on the nutritive value of canola meal for poultry: in vitro and in vivo studies. Animal Feed Science and Technology 61: 219-234.

Singh, Y., Amerah, A. R. and Ravindran, V. (2014a) Whole grain feeding: Methodologies and effects on performance, digestive tract development and nutrient utilisation of poultry. Animal Feed Science and Technology 190: 1-18.

Singh, Y., Ravindran, V., Wester, T. J., Molan, A. L. and Ravindran, G. (2014b) Influence of prepelleting inclusion of whole corn on performance, nutrient utilization, digestive tract measurements, and cecal microbiota of young broilers. Poultry Science 93: 3073-3082.

Skoch, E. R., Behnke, K. C., Deyoe, C. W. and Binder, S. F. (1981) The effect of steam-condition rate on the pelleting process. Animal Feed Science and Technology 6: 83-90.

Smits, C. H. M., and Annison, G. (1996) Non-starch plant polysaccharides in broiler nutrition – Towards a physiological valid approach to their determination. World’s Poultry Science 52: 203-221.

Song, Y. and Jane, J. (2000) Characterization of barley starches of waxy, normal, and high amylose varieties. Carbohydrate Polymers 41: 365-377.

Sultan, A., Gan, C. Y., Li, X., Zhang, D. and Bryden W. L. (2011) Dietary enzyme combinations improve sorghum ileal protein and starch digestibility during the broiler starter phase. Proceedings, Australian Poultry Science Symposium 22: 82.

Svihus, B. (2011a) Effect of digestive tract conditions, feed processing and ingredients on response to NSP enzymes. 129-159 in Enzymes in Farm Animal Nutrition. 2nd ed, M. Bedford and G. Partridge, ed. CAB International, Wallingford, UK.

Svihus, B. (2011b) The gizzard: function, influence of diet structure and effects on nutrient availability. World's Poultry Science Journal 67: 207-224.

Svihus, B., Edvardsen, D. H., Bedford, M. R. and Gullord, M. (2000) Effect of methods of analysis and heat treatment on viscosity of wheat, barley and oats. Animal Feed Science and Technology 88: 1-12.

Svihus, B. and Gullord, M. (2002) Effect of chemical content and physical characteristics on nutritional value of wheat, barley and oats for poultry. Animal Feed Science and Technology 102: 71-92.

Svihus, B. and Hetland, H. (2001) Ileal 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: 633-637.

Svihus, B., Hetland, H., Choct, M. and Sundby, F. (2002) Passage rate through the anterior digestive tract of broiler chickens fed on diets with ground and whole wheat. British Poultry Science 43: 662-668.

Svihus, B., Juvik, E., Hetland, H. and Krogdahl, Å. (2004a) Causes for improvement in nutritive value of broiler chicken diets with whole wheat instead of ground wheat. British Poultry Science 45: 55-60.

Svihus, B., Kløvstad, K., Perez, V., Zimonja, O., Sahlström, S., Schüller, R., Jeksrud, W. and Prestløkken, E. (1997) Comparison of performance and intestinal characteristics of broiler chickens fed on diets containing whole, rolled or ground barley. British Poultry Science 38: 524-529.

Svihus, B., Kløvstad, K., Perez, V., Zimonja, O., Sahlström, S., Schüller, R., Jeksrud, W. and Prestløkken, E. (2004b) Physical and nutritional effects of pelleting of broiler chicken diets made from wheat ground to different coarsenesses by the use of roller mill and hammer mill. Animal Feed Science and Technology 117: 281-293

Svihus, B., Uhlen, A. K. and Harstad, O. M. (2005) Effect of starch granule structure, associated components and processing on nutritive value of cereal starch: a review. Animal Feed Science and Technology 122: 303-320.

Talati, R., Baker, W. L., Pabilonia, M. S., White, C. M. and Coleman, C. I. (2009) The effects of barley-derived soluble fiber on serum lipids. The Annals of Family Medicine 7: 157-163.

Tang, H., Wantanabe, K. and Mitsunaga, T. (2002) Structure and functionality of large, medium and small granule starches in normal and waxy barley endosperms. Carbohydrate Polymers 49: 217-224.

Taylor, R. D. and Jones, G. P. D. (2004a) The incorporation of whole grain into pelleted broiler chicken diets. II. Gastrointestinal and digesta characteristics. British Poultry Science 45: 237-246.

Taylor, R. D. and Jones, G. P. D. (2004b) The influence of whole grain inclusion in pelleted broiler diets on proventricular dilation and ascites mortality. British Poultry Science 45: 247-254.

Tester, R. F., South, J. B., Morrison, W. R. and Ellis, R. P. (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: 113-127.

Tester, R. F. and Karkalas, Qi. J. (2006) Hydrolysis of native starches with amylases. Animal Feed Science and Technology 130: 39-54.

Tsvetkov, St. M. and Tsvetkov, K. St. (2007) An attempt to develop two-rowed awnless barley (vvLkLk) by using genetic awnless plasma of cv. Tsvetelina (Hordeum sativum Jess., ssp. Vulgare, var. Dundar Bey Zhuk) Bulgarian Journal of Agricultural Science 13: 529-533.

Uddin, M. S., Rose, S. P., Hiscock, T. A. and Bonnet, S. (1996) A comparison of the energy availability for chickens of ground and whole grain samples of two wheat varieties. British Poultry Science 37: 347-357.

Ullrich, S. E., Clancy, J. A., Eslick, R. F. and Lance, R. C. M. (1986) B-Glucan content and viscosity of extracts from waxy barley. Journal of Cereal Science 4: 279-285.

Ullrich, S. E. (2010) Barley: Production, improvement, and uses. Wiley Blackwell, Hoboken, NJ.

Valaja, J., Suomi, K., Alaviuhkola, T. and Mela, T. (1997) Effects of variety, soil type and nitrogen fertilizer supply on the nutritive value of barley for growing pigs. Agricultural and Food Science 6: 295-303.

Walk, C., Juntunen, K., Paloheimo, M. and Ledoux, D. (2019) Evaluation of novel protease enzymes on growth performance and nutrient digestibility of poultry: enzyme dose response. Poultry Science (accepted).

Walk, C., Pirgozliev, V., Juntunen, K., Paloheimo, M. and Ledoux, D. (2018) Evaluation of novel protease enzymes on growth performance and apparent ileal digestibility of amino acids in poultry: enzyme screening. Poultry Science 97: 2123-2138.

Wang, X. and Parsons, C. M. (1998) Effect of raw material source, processing systems, and processing temperatures on amino acid digestibility of meat and bone meals. Poultry Science 77: 834-841.

White, W. B., Bird, H. R., Sunde, M. L. and Marlett, J. A. (1983) Viscosity of β-D-glucan as a factor in the enzymatic improvement of barley for chicks. Poultry Science 62: 853-862.

Wu, Y. B. and Ravindran, V. (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: 129-139.

Wu, Y. B., Ravindran, V., Thomas, D. G., Birtles, M. J. and Hendriks, W. H. (2004) Influence of method of whole wheat inclusion and xylanase supplementation on the performance, apparent metabolisable energy, digestive tract measurements and gut morphology of broilers. British Poultry Science 45: 385-394.

Yasar, S. (2003) Performance, gut size and ileal digesta viscosity of broiler chickens fed with a whole wheat added diet and the diets with different wheat particle sizes. International Journal of Poultry Science 2: 75-82.

Zaefarian, F., Abdollahi, M. R. and Ravindran, V. (2015) Starch digestion in broiler chickens fed cereal diets. Animal Feed Science and Technology 209: 16-29.

Zaefarian, F., Abdollahi, M. R. and Ravindran, V. (2016) Particle size and feed form in broiler diets: impact on gastrointestinal tract development and gut health. World's Poultry Science Association 72: 277-290.

Zhang, G. and Li, C. (2010) Genetics and improvement of barley malt quality. Spriner Science and Business Media, Zhejiang University Press, Hangzhou.

Zhang, W. J., Campbell, L. D. and Stothers, S. C. (1994) An investigation of the feasibility of predicting nitrogen-corrected true metabolizable energy (TMEn) content in barley from chemical composition and physical characteristics. Canadian Journal of Animal Science 74: 355-360.