INFLUENCE OF FEED PROCESSING AND ENZYME SUPPLEMENTATION ON PERFORMANCE, NUTRIENT UTILISATION AND GUT MORPHOLOGY OF POULTRY FED BARLEY-BASED DIETS

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ABSTRACT

The first experiment (Chapter 3) determined the metabolisable energy and standardised ileal digestible amino acids of two barley cultivars (NSH [normal starch hulled barley] and WSHL [waxy starch hull-less barley]) and wheat for broilers. These values were used to formulate the experimental diets in subsequent experiments that evaluated the optimum barley inclusion rate in wheat-based diets (Chapters 4 and 5), optimum barley particle size (Chapter 6) and conditioning temperature (CT; Chapter 7), and potential interaction of carbohydrases with each processing parameter.

In Chapter 3, wheat and WSHL had the highest and lowest metabolisable energy and digestible amino acid contents, respectively, with NSH being intermediate. Supplemental carbohydrases increased the energy utilisation with a pronounced effect in WSHL.

Data reported in Chapter 4 showed that optimum inclusion level of NSH was 283 g/kg of diet. Nutrient utilisation linearly improved with increasing inclusions of NSH. Carbohydrases improved feed per gain (F/G) and nutrient utilisation.

Chapter 5 suggested that WSHL could be safely included up to 260 g/kg in a wheat-based diet with no adverse effect on growth performance. Carbohydrases improved the F/G and, starch and energy utilisation.

In Chapter 6, particle size effect was preserved after pelleting and, coarse barley and carbohydrases improved the F/G and nutrient utilisation. The combination of carbohydrase and phytase produced no further improvements in nutrient utilisation.

The final experiment (Chapter 7) demonstrated that better pellet quality achieved by increasing CT to 88 °C failed to ameliorate the negative impacts of high CT on nutrient utilisation and broiler performance. Carbohydrases improved weight gain, F/G and, starch and energy utilisation. The lack of interaction between the carbohydrases and CT indicated that carbohydrase had similar efficacy at each CT.

The primary finding of this thesis research was that if cultivar-specific values for metabolisable energy and digestible amino acids are used in feed formulations, barley has the potential to substitute up to 50% of wheat in broiler diets. Coarse particle size (8.0

mm) and conditioning the diets up to 74 °C is recommended for the tested barley type. Supplemental carbohydrases improved the feeding value of barley for broilers.

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PUBLICATIONS

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Perera, W. N. U., Abdollahi, M. R., Zaefarian, F., Wester, T. J., & Ravindran, V. (2020). The effect of graded inclusions of waxy starch hull-less barley and a multi-component exogenous carbohydrase on the growth performance, nutrient digestibility and intestinal morphometry of broiler chickens. *British Poultry Science, 61*(4), 442-453.

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Conference proceedings

Perera, W. N. U., Abdollahi, M. R., Zaefarian, F., & Ravindran, V. (2017). Barley in poultry diets: Feed value and limitations. *Proceedings of 19th Massey Technical Update Conference* (pp. 70-83). Palmerston North, New Zealand.

Perera, W. N. U., Abdollahi, M. R., Zaefarian, F., & Ravindran, V. (2018). Influence of carbohydrase supplementation on metabolisable energy and ileal nutrient digestibility of two cultivars of barley for broilers. *Proceeding of Australian Poultry Science Symposium* (pp. 38). Sydney, Australia.

Abdollahi, R., Perera, N., Ravindran, V., & Zaefarian, F. (2018). Barley in broiler diets: Optimum inclusion and response to carbohydrase enzyme (Abstract No. 178). *Proceedings of European Poultry Conference* (pp. 33). Dubrovnik, Croatia.

Perera, W. N. U., Abdollahi, M. R., Ravindran, V., Zaefarian, F., Wester T. J., & Ravindran, G. (2018). Barley in broiler diets: The optimum inclusion level and response to carbohydrase enzyme. *Proceedings of 20th Massey Technical Update Conference* (pp. 102-114). Palmerston North, New Zealand.

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Perera, W. N. U., Abdollahi, M. R., Zaefarian, F., Wester T. J., & Ravindran, V. (2020). Barley particle size and supplemental enzymes: Influence on growth performance and nutrient utilisation of broiler starters. *Proceedings of the Australian Poultry Science Symposium* (pp. 40-43). Sydney, Australia.

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LIST OF ABBREVIATIONS

AA	Amino acids
ADF	Acid detergent fibre
AID	Apparent ileal digestibility
AME	Apparent metabolisable energy
AMEn	Nitrogen-corrected apparent metabolisable energy
ANOVA	Analysis of variance
AOAC	Association of Official Analytical Chemists
BGU	Unit of β-glucanase
BW	Body weight
C	Celsius
Ca	Calcium
CAID	Coefficient of apparent ileal digestibility
Carb	Carbohydrase
CP	Crude protein
cP	Centipoise
CSID	Coefficient of standardised ileal digestibility
CT	Conditioning temperature
CV	Coefficient of variation
DM	Dry matter
DMI	Dry matter intake
EAA	Endogeneous amino acids
FA	Fatty acids
F/G	Feed per gain
FI	Feed intake
FPD	Foot pad dermatitis
FYT	Phytase unit
g	Gram
GE	Gross energy
GIT	Gastrointestinal tract
GMD	Geometric mean diameter
GS	Gelatinised starch
GSD	Geometric standard deviation
ha	Hectare

HCl	Hydrochloric acid
IDE	Ileal digestible energy
I.NSP	Insoluble non-starch polysaccharides
IU	International units
kg	Kilogram
Mg	Magnesium
N	Nitrogen
Na	Sodium
NDF	Neutral detergent fibre
NSH	Normal starch hulled barley
NSP	Non-starch polysaccharides
P	Phosphorus
P	Probability
PDI	Pellet durability index
Phy	Phytase
RS	Resistant starch
SI	Small intestine
SID	Standardised ileal digestibility
S.NSP	Soluble non-starch polyschchrides
SEM	Pooled standard error of mean
SEM	Scanning electron microscopic
T.NSP	Total non-starch polysachchrides
Ti	Titanium
UV	Ultraviolet
WG	Weight gain
WSHL	Waxy-starch hull-less barley
XU	Unit of xylanase

CHAPTER ONE

GENERAL INTRODUCTION

The supply of conventional cereal grains, such as maize and wheat, will be a major constraint to the future growth of the poultry industry and will be further exacerbated by increased competition with human food. Different alternative feed ingredients are being continuously tested to replace conventional cereal grains in poultry diets. Barley (*Hordeum vulgare L.*) is one such feed ingredient, the use of which remains low in poultry diets due to the presence of anti-nutritive, soluble non-starch polysaccharides (Jacob and Pescatore, 2012). Moreover, the wide range of physical and chemical characteristics of barley cultivars make it one of the most variable cereal grains (Villamide *et al*., 1997; Choct *et al*., 2001). The different research methodologies used in published studies have also contributed to the inconsistent findings and prevented a clear understanding of the nutritional value of barley for poultry.

In studies evaluating barley in broiler diets, most studies have replaced other cereals with barley either on a weight to weight basis (Arscott *et al*., 1955; Petersen, 1969; Moss *et al*., 1983; Yu *et al*., 1998) or by using nutrient composition data for barley and the substituted grain from established data sources such as National Research Council (NRC; Moharrery, 2006) and tables published by Spanish Foundation for the Development of Animal Nutrition (FEDNA; de Blas *et al*., 2010; Lázaro *et al*., 2003), or chemical analysis (Brake at al., 1997). Limitations associated with these research methodologies have resulted in a wide range of barley inclusion levels being recommended for broiler diets. The fact that anti-nutritive components in barley play a key role in determining the availability of dietary components to poultry emphasises the importance of using cultivar-specific nutrient profiles to formulate barley-based diets, ensuring that birds' nutrient requirements are met. However, there are no published studies where barley-based diets were formulated using accurate nutrient profiles based on measured contents of metabolisable energy and digestible amino acids specific to barley cultivars.

The use of non-starch polysaccharides-degrading enzymes in diets based on viscous grains, such as barley, has become a norm to overcome the adverse effects of antinutritional factors on nutrient utilisation and bird performance. Responses to supplemental enzymes in terms of nutrient utilisation and bird performance are variable (Chesson, 1993; Bao *et al*., 2013). The factors contributing to these inconsistencies are complex, involving enzyme, diet and bird factors and their interactions. The potential for improving the efficacy of supplemental enzymes by optimising the physical characteristics of diets has been recognised (Amerah *et al*., 2011; Amerah, 2015).

The influence of feed processing on growth performance and nutrient utilisation of poultry fed maize- (Naderinejad et al., 2016; Abdollahi *et al*., 2010a,b) and wheat- (Lentle *et al*., 2006; Amerah *et al*., 2007b) based diets have been understood to a greater extent, but corresponding studies with barley are limited and contradictory due possibly to cultivar differences (Ankrah *et al*., 1999). Most previous studies have overlooked the cultivar differences, in terms of factors such as starch type and presence of hulls, when evaluating barley in poultry diets. Moreover, most of the research has not used barley as the sole cereal in the diet, which makes it difficult to reach definite conclusions. Consequently, more research is warranted to establish a scientific approach for the evaluation and application of barley in poultry diets by addressing the limitations in previous publications. Moreover, investigations on the potential interactive influence of feed processing and supplemental enzymes on nutrient utilisation and bird performance of broilers fed barley-based diets are also warranted to determine the optimum dietary conditions for a better enzyme efficacy.

This thesis consists of eight chapters. The first two chapters present the framework of the experimental research with Chapter 1 giving a general introduction to the thesis. Chapter 2 reviews the published literature on the chemical and physical characteristics of barley with special focus to the factors contributing to the variability of the nutritional composition. Moreover, the growth performance and nutrient utilisation responses in broilers fed barley-based diets are discussed in Chapter 2. In addition, Chapter 2 provides a discussion on some measures to minimise or eliminate the negative impact of barley antinutritional factors in poultry diets. Chapters 3 through 7 present the experimental work of this thesis. Each chapter includes an abstract, introduction, materials and methods, results, discussion and conclusions.

The specific objectives of the experiments conducted in this thesis research are,

1. To characterise the nutrient composition of two barley cultivars in comparison with wheat (control) and determine the content of nitrogen-corrected apparent metabolisable energy and standardised ileal digestible amino acids in the three grain types, without or with carbohydrase enzyme addition (Chapter 3).

2. To determine the optimum inclusion level of a normal-starch hulled barley in diets for broiler starters and to investigate the possible interaction between barley inclusion level and supplementation of a carbohydrase on the performance, nutrient utilisation and gut morphometry in broiler starters (Chapter 4).

3. To evaluate the influence of graded inclusions of a waxy starch hull-less barley cultivar and supplementation of carbohydrase on the performance, nutrient utilisation and intestinal morphometry in broiler starters (Chapter 5).

4. To evaluate potential interactive influences of barley particle size and carbohydrase and phytase addition, individually or in combination, on growth performance, nutrient utilisation and intestinal morphometry of broiler starters fed pelleted diets (Chapter 6).

5. To evaluate whether interactive effects between supplemental carbohydrases and conditioning temperature exist on the performance, nutrient utilisation, and gut morphometry in broiler starters fed barley-based diets (Chapter 7).

Chapter 8 is a general discussion of the experimental results, which addresses the major findings of this thesis research and draws some conclusions from the results.

CHAPTER TWO

LITERATURE REVIEW

2.1. Background and classification of barley

Barley (*Hordeum vulgare* L.), one of the first domesticated crop has played a role of multipurpose grain as both food and feed throughout the history. It is extensively cultivated, ranking fourth in world cereal production with an annual production of 128 million metric tonnes (Figure 2.1; FAO STAT, 2018). Characteristics such as resistance to drought and saline soils (Fayez and Bazaid, 2014) and ability to mature in climates with a short growing season (Svihus and Gullord, 2002) have encouraged the cultivation of barley over maize and wheat. In addition to the common usage of barley for malting and brewing (90% of total barley production; Li *et al*., 2001), it is also used as a feed ingredient in animal diets, especially in Europe where there is the highest concentration of cultivation in the world (McNab and Smithard, 1992; Jacob and Pescatore, 2014). According to latest available records on barley use in animal feeds, 40% of the barley was fed to feedlot cattle, 34% to dairy cows, 20% to pigs and 5% to grazing ruminants, and only less than 1% used for poultry (Black *et al*., 2005; Nikkhah, 2012).

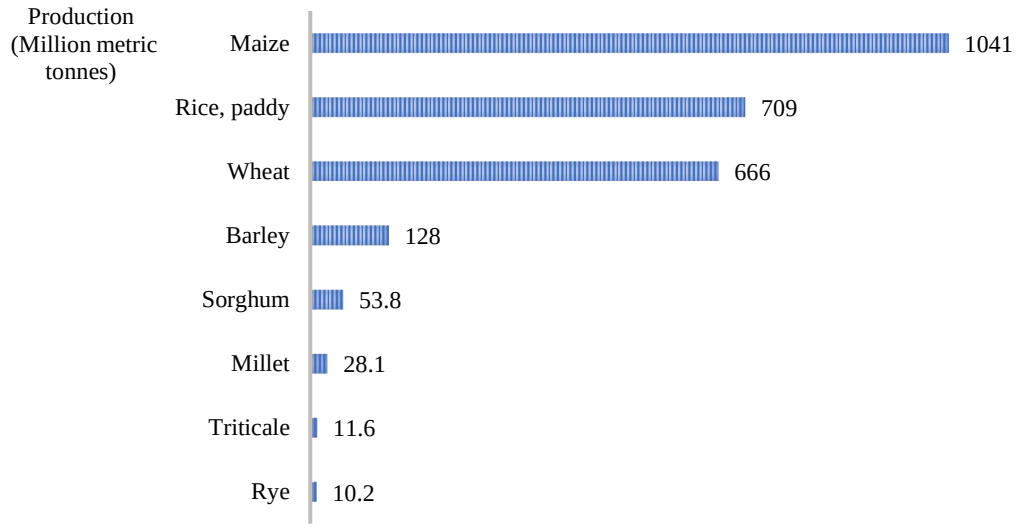

Figure 2.1. Worldwide production of cereal grains, by type. Source: Food and Agriculture Organisation (2018).

Morphological and physico-chemical characteristics have laid the foundation for classification of barley. As shown in Figure 2.2., barley cultivars are classified based on factors such as growing season, presence or absence of an awn (a bristle-like appendage), number of the seeds on the stalk, presence or absence of the hull, composition of the starch, aleurone colour and growth height.

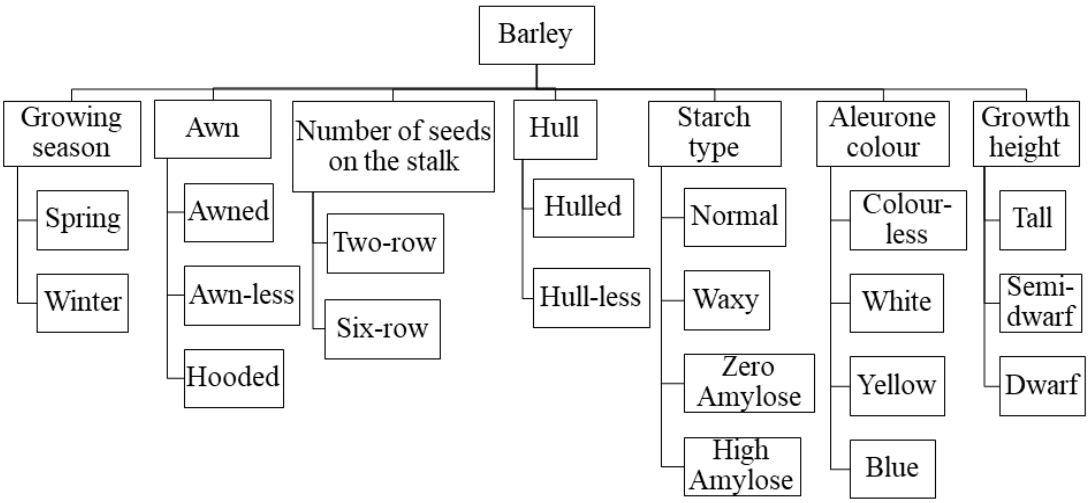

Figure 2.2. Classification of barley based on morphological and physio-chemical characteristics. Source: Jacob and Pescatore (2012).

Barley is classified according to the growing season as spring or winter cultivars. More genetic selection has been performed on spring barley cultivars, which contain greater energy value (Villamide *et al*., 1997) and higher resistance to extreme environmental conditions compared to the winter cultivars (Jeroch and Dänicke, 1995). Barley cultivars can also be classified based on the presence or absence of a bristle-like appendage which is called an awn or beard (Figures 2.2 and 2.3). Barley without awns (awn-less) or with short awns (hooded) have also been developed. Two-row and six-row barley cultivars differ in the number of seeds on the stalk of the plant (Figures 2.2 and 2.3). With the higher adaptation to drier climates, two-row cultivars are concentrated in Europe, while most of the six-row barley cultivars are grown in North America (Jacob and Pescatore, 2012). Classification of barley based on the presence or absence of a hull that contributes to the insoluble fibre fraction (Svihus and Gullord, 2002), is of particular interest to poultry nutritionists. Hull-less or naked barley appears similar to hulled barley until maturity and, then the hulls are loosened and detached during harvesting (Bhatty,

1999). In addition to hulled and hull-less barley cultivars, dehulled and pearl barley are produced by the processing of barley grain. Dehulled barley, which is often confused with hull-less barley, is formed by removing the hull from hulled barley. Pearl barley is developed from steam processed and polished (also known as abrading or pearling; Liu, 2011) dehulled barley. The major difference between dehulled and pearl barley is the presence of both bran and germ in dehulled barley, and absence of bran in pearl barley.

Figure 2.3. Classification of barley based on awn (left) and number of seeds on the stalk (right). Source: Terzi et al. (2017).

2.2. Composition

The composition and properties of barley grain are of interest in nutritional studies for their role in determining the availability of nutrients to humans or animals. The large variations in composition, structure and physico-chemical properties in different barley types can provide the basis for the differing responses observed among experiments. Extensive research on the composition of barley has recognised that the wide diversity is mainly associated with the differences in hull and starch type, which will be considered as the basis of comparison in this review.

2.2.1. Structural composition

As shown in Figure 2.4, barley grain is composed of a large endosperm (80% of the cereal grain), an embryo and a mass of maternal tissues. Mature endosperm consists of five types of cells, as aleurone, sub-aleurone, starchy endosperm, embryo-surrounding region and endosperm transfer cells. Endosperm cells are filled with starch granules embedded in a

protein matrix (Figures 2.4 and 2.5) and, therefore, possess a greater nutritional value compared to other parts of barley grain. The embryo is rich in lipids and enzymes while the aleurone layer is rich in soluble protein (about 50%) and is a source of enzymes, lipids and vitamins (Li *et al*., 2013). Endosperm cell walls are thinner than cell walls of other regions from barley grain and are mainly composed of β-glucans (70%) and smaller amount of arabinoxylans (20%; Andriotis *et al*., 2016). While aleurone cell walls are mainly composed of arabinoxylans (67-71%) and smaller amounts of β-glucans (26%; Izydorczyk and Dexter, 2008), maternal tissues such as testa (fruit and seed coat) surround the embryo and endosperm.

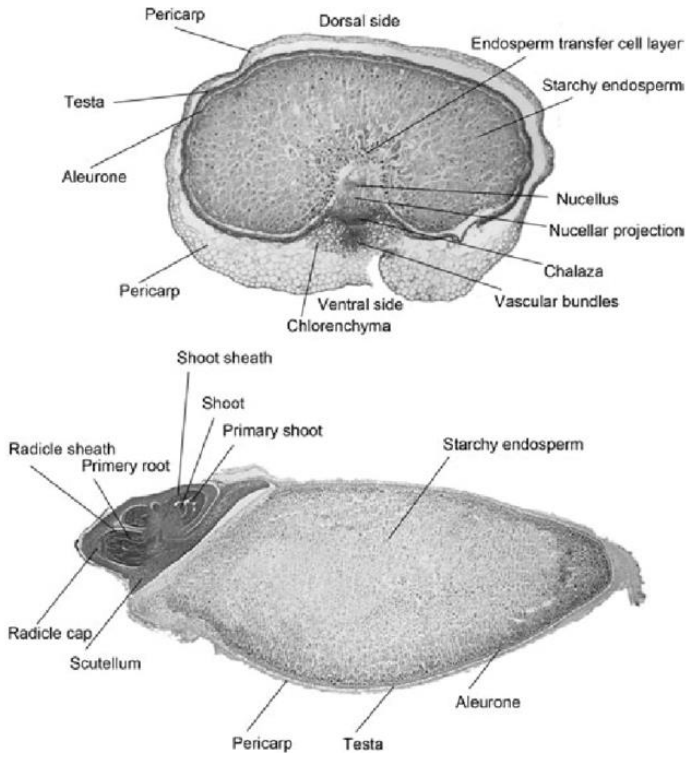

Figure 2. 4. Transverse (above) and longitudinal (below) sections of barley grain. Source: Li *et al*. (2013).

Oscarsson *et al*. (1997), Izydorczyk and Dexter (2008) and Shaik *et al*. (2014) compared cross-sections of different barley types with different levels of β-glucan and, reported thicker endosperm cell walls in barley genotypes with high content of β-glucan. Histochemical images of three barley types (wild, hyperphosphorylated and amylose only) with three different levels of β-glucan (59.1, 54.1 and 66.4 g/kg DM, respectively) are shown in Figure 2.5 (Shaik *et al*., 2014). A thicker endosperm cell wall was observed for amylose only type with high occurrence of β-glucan.

As shown in Figure 2.5., starch granules (pink-purple) are embedded in the protein matrix (blue) inside endosperm cells and have a bimodal size distribution with large disc-shaped A-granules and small spherical B-granules (Song and Jane, 2000; Li *et al*., 2001; Ao and Jane, 2007).

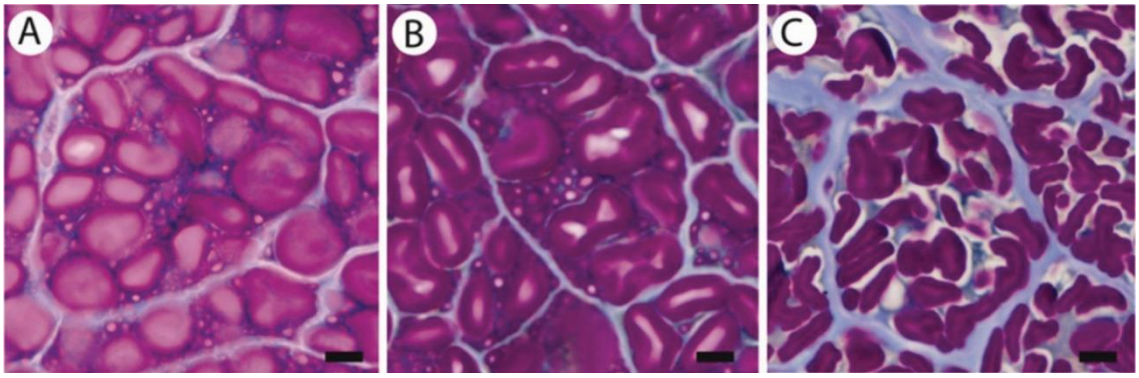

Figure 2. 5. Histochemical analysis of starch granules by periodic acid-Schiff's on sectioned endosperm tissue; localisation of starch (pink-purple) and protein (blue) near the embryo-endosperm junction of pre-germinated dry grain at ×4000 magnification. A, Wild-type; B, Hyperphosphorylated line; C, Amylose-only. Source: Shaik *et al.* (2014).

2.2.2. Chemical composition

Wide range of chemical composition of different barley cultivars has been reported in the literature (Bhatty *et al*., 1975; Villamide *et al*., 1997; Helm and Francisco, 2004), while considerable variation was observed even among similar cultivars (Oscarsson *et al*., 1996; Izydorczyk *et al*., 2000). Minor changes in chemical composition may result in significant changes in nutrient availability, creating remarkable effects on the nutritional quality of barley for poultry (Almirall *et al*., 1995; Hughes and Choct, 1999).

Environmental factors such as year of harvest, rainfall, soil conditions, fertilisation and other agronomic conditions can affect the chemical characteristics of cereal grains. A wide range of variability that exists between barley types grown in different geographical locations has been reported (Jeroch and Dänicke, 1995; Hughes and Choct, 1999; Helm and Francisco, 2004). Svihus and Gullord (2002) compared five varieties of barley grown in two different locations during two growing years in terms of chemical composition and, reported that starch and fat contents were affected by barley variety and year, respectively. The protein content was affected by both the year and

location. Varying effects caused by environmental factors on chemical composition of barley, highlight the need for standardising the environmental conditions, when comparing the chemical composition of different barley types.

2.2.2.1. Starch

As the main component in cereals, starch is present in barley as well (513 to 642 g/kg DM; Holtekjølen *et al*., 2006) and serves as the primary source of energy for poultry fed barley diets. Starch accumulates in granules in the endosperm and consists of two glucose polymers, amylose and amylopectin (Bewley and Black, 1978), which differ in terms of degree of branching, where amylose is more linear compared to the branched amylopectin (Aberle *et al*., 1994). Barley starches differ widely in amylose to amylopectin ratios resulting four different barley types as normal, high amylose, waxy and zero amylose waxy barley types (Table 2.1). The starch in normal barley genotypes consists of 650-840 g/kg amylopectin, and waxy starch consists of 850-1000 g/kg amylopectin (Ullrich *et al*., 1986; Tester *et al*., 2004). Barley types with 1000 g/kg of amylopectin are termed as zero amylose waxy and, high amylose barley cultivars contain around 550 g/kg amylopectin (Li *et al*., 2001; Table 2.1). Waxy gene originated from natural mutations affecting the synthesis of amylose (Svihus *et al*., 2005), was originally found in maize and later incorporated into barley (Jacob and Pescatore, 2012). In addition to waxy maize and barley types, waxy wheat types are also available (Table 2.1; Abdel-Aal *et al*., 2002); however, studies evaluating waxy wheat for poultry are limited (Pirgozliev *et al*., 2002).

Even within the same starch type, amylose to amylopectin ratio can vary widely (Table 2.1). Nevertheless, some studies evaluating the feeding value of different barley types for poultry have only reported the starch type with no information on the amylose to amylopectin ratio (Bergh *et al*., 1999). As even a minor change in amylose to amylopectin ratio can affect the utilisation of starch by birds (Pirgozliev *et al*., 2010), it is recommended to consider the starch characteristics beyond already established classifications on starch types. Moreover, in most studies with barley, despite of being the major energy source, no attempt was made to identify starch type and to quantify the starch contents, highlighting a major limitation in barley-related nutritional studies.

Table 2. 1. Comparison of starch in maize, wheat, hulled barley and hull-less barley types (g/kg, DM basis).

Reference	Grain type	Hull/ Hull-less	Starch type	n[1]	Starch	Amylose[2]	Amylopectin[2]
Li *et al*. (2001)	Barley	Hull-less	Normal	2	642	158 (25)	483 (75)
			Normal (CG[3])	2	605	171 (28)	433 (72)
			High amylose	2	563	243 (43)	320 (57)
			Waxy	2	622	33 (5.0)	589 (95)
			Waxy (CG)	1	582	27 (5.0)	555 (95)
			Zero amylose	1	585	0 (0)	585 (100)
	Maize[4]		Normal	1	-	- (25)	- (75)
			Waxy	1	-	- (1.0)	- (99)
Abdel-Aal *et al*. (2002)	Wheat		Normal	1	605	163 (27)	442 (73)
			Waxy	1	563	18 (3.0)	545 (97)
	Maize[4]		Normal	1	-	- (21)	- (79)
			Waxy	1	-	- (3.0)	- (97)
Storsley *et al*. (2003)	Barley	Hull-less	Normal	2	616	248 (40)	368 (60)
			High amylose	2	537	416 (77)	121 (23)
			Waxy	2	561	51 (9.0)	510 (91)
			Zero amylose	2	533	0 (0.0)	533 (100)
Holtekjølen *et al*. (2006)	Barley	Hulled	Normal	2	588	147 (25)	441 (75)
		Hull-less	Normal	6	609	152 (25)	457 (75)
		Hulled	Waxy	1	552	44 (8.0)	508 (92)
		Hull-less	Waxy	3	582	29 (5.0)	553 (95)
		Hull-less	High amylose	1	535	193 (36)	342 (64)
Ravindran *et al*. (2007)	Barley	Hulled	Normal	1	598	168 (28)	430 (72)
		Hull-less	Normal	1	655	164 (25)	491 (75)
		Hull-less	Waxy	2	614	37 (6.0)	577 (94)

[1]Number of analysed grain types.
[2]Values in the parenthesis are amylose or amylopectin as a percentage of starch content.
[3]CG, compound starch granules that exist in clusters of individual granules.
[4]Total starch content was not reported.

In addition to the key role of chemical characteristics in determining the contribution of barley starch to feeding value, functional properties of starch such as granule structure, size, shape, surface area and interactions with other nutrients (proteins and lipids) can affect the accessibility of starch granules by digestive enzymes and thus the rate and extent of starch digestion. Starch granules in both wheat and barley are known to have a bimodal size distribution with small (≤ 10 μm of diameter) spherical B-granules and large (> 10 μm of diameter) disc-shaped A-granules (Song and Jane, 2000; Li *et al*., 2001; Ao and Jane, 2007). Li *et al*. (2001) reported a wide range of starch granule sizes

(4.0 to 18.8 µm) in barley compared to maize (6.3 to 13.2 µm), and a negative correlation between starch granule diameter and total amylose content. Moreover, the ratio of number of small granules to large granules in barley starches vary widely compared to maize starch and, the proportion of small granules was correlated with total amylose content (Li *et al*., 2001).

Jane (2006) described that disc-shaped starch granules in wheat and barley contain starch granules perpendicular to the flat surface of starch granules, allowing more contact with digestive enzymes. The size and shape of starch granules have been recognised as important functional properties that can control the accessibility of the enzyme to the interior of the granule and regulate enzymatic hydrolysis (Svihus *et al*., 2005; Tester *et al*., 2006). Different shapes of starch granules can affect the surface area to volume ratio and, hence, the potential for enzymatic digestion. The larger the granules, the smaller the surface area to volume ratio and the lower potential surface to be attacked and hydrolysed by digestive enzymes. Moreover, some starch granules were clustered and present as compound granules reducing the capacity of enzymes to attach to starch granule surfaces (Li *et al*., 2001; Tester *et al*., 2006).

Non-starch components associated with starch granules such as fat and protein were found on the surface of isolated starch granules from barley and maize (Li *et al*., 2001), and wheat (Abdel-Aal *et al*., 2002). Fats and proteins can impair starch digestion both directly by reducing the contact between digestive enzymes and starch granules, and indirectly by reduced swelling of the starch granules and interactions with milling and gelatinisation properties during feed processing (Svihus *et al*., 2005).

Whilst starch granule properties vary between different barley types, environmental factors such as temperature during grain filling can also have a huge impact. High temperature (> 35 °C) during grain filling is not favorable as it impairs starch synthesis and results in less starch per endosperm and smaller starch granules (Fox *et al*., 2003). Tester *et al*. (1991) tested the response of starch isolated from four genotypes of barley (one waxy, two normal and one high-amylose) grown at constant ambient temperatures of 10, 15, and 20 °C and, reported that higher temperatures above the optimum temperature for a particular barley type can result in reduced starch accumulation, smaller A- and B-starch granules and fewer B-granules. However, the

reduced starch granule dimensions resulted in increased surface area per granule. Increasing N fertilisation from 45 to 135 N/ha reduced starch contents in hulled normal, hull-less high amylose, hulled high amylose and hulled waxy barley types by 12.5, 11.8, 6.8 and 5.7%, respectively (Oscarsson *et al*., 1997), highlighting the variation in response of starch from different barley types to management practices.

The ratio between amylose and amylopectin has been given special attention as an important factor determining the nutritive value of barley for monogastric animals. Even relatively small variations in total dietary starch supply and changes in starch amylose: amylopectin ratio can affect the growth performance of poultry (Pirgozliev *et al*., 2010). In comparison to amylopectin rich starch, high amylose starch is less susceptible to enzymatic degradation by α-amylase in small intestine, highlighting that waxy starch may be more digestible than the normal starch type (Björck *et al*., 1990). However, most of the information on effect of structure and integrity of dietary starch granule and changes of amylose: amylopectin ratio on starch digestion is based on starch from wheat and maize (Svihus *et al*., 2005; Pirgozliev *et al*., 2010) and conducted *in vitro* (Li *et al*., 2004a; Al-Rabadi, 2014; Bdour *et al*., 2014). Therefore, careful attention should be given when drawing conclusions from those studies for the barley diets especially due to the interference of non-starch polysaccharides (NSP) in barley.

2.2.2.2. Protein and amino acids

In contrast to plant-based protein sources commonly used in poultry diets, cereals contain lower amounts of crude protein (CP) and amino acids (AA). Nevertheless, owing to the high inclusion of cereal grains in poultry diets, cereal proteins make a substantial contribution to the supply of dietary AA. The CP content of barley can vary between cultivars and cultivation practices, while nitrogen (N) fertilisation can have a huge impact. Nitrogen fertilisation was shown to increase the CP content in different barley types irrespective of hull and starch type (Oscarsson *et al*., 1997, 1998). Increasing N fertilisation from 45 to 135 N/ha increased CP in hulled normal, hull-less high amylose, hulled high amylose and hulled waxy barley types at 39, 29, 21 and 20%, respectively (Oscarsson *et al*., 1997). The relative levels of essential AA to CP in barley were decreased with the increased level of CP content due to N fertilisation (Jeroch and Dänicke, 1995; Jacob and Pescatore, 2012). Rodehutscord *et al*. (2016) analysed the

composition of different cereal grain genotypes grown in the same site, thereby excluding the influence of location, management and fertilisation on nutrient composition. However, despite of standardised growing conditions, wide range of CP (from 108 to 136 g/kg DM; 6.0% coefficient of variation) was reported for eight winter barley types.

Similar to other grains, barley protein is low in lysine, threonine, methionine and histidine. However, compared with maize and wheat proteins, barley protein has more favourable AA composition (Table 2.2). According to Bryden *et al*. (2009) and Rodehutscord *et al*. (2016), barley has more protein compared to maize, indicating the nutritional potential of barley in poultry diets. In barley, maize and wheat, methionine concentration was the lowest followed by histidine and cysteine, while glutamic acid was the highest (Rodehutscord *et al*., 2016). Maize protein is higher in leucine and lower in lysine concentrations, compared to wheat and barley proteins (Bryden *et al*., 2009; Rodehutscord *et al*., 2016). A negative correlation between starch and protein contents has been observed in studies on chemical composition of different barley types (Li *et al*., 2001; Holtekjølen *et al*., 2006). It has been commonly observed that when the content of starch increases, all other main constituents decrease.

The absence of hull was known to influence the protein content (Andersson *et al*., 1999; Bhatty, 1999). However, CP seems to be independent of hull, due to both lower (Ravindran *et al*., 2007) and higher (Holtekjølen *et al*., 2006) CP contents reported for hull-less barley compared to hulled barley (Table 2.2). The lack of attempts to distinguish between different barley types evaluated in some extensive studies (Bandegan *et al*., 2011; Rodehutscord *et al*., 2016) has narrowed the opportunity to interpret the influence of starch type and hull on CP and AA concentration. However, according to limited literature on AA comparison in different barley types (Ravindran *et al*., 2007), the differences in AA composition seems to be influenced by the CP content, rather than the starch type or hull.

Table 2. 2. Crude protein and amino acid (AA) composition of barley, maize and wheat (g/kg, DM).

Reference	Steenfeldt (2001)	Ravindran *et al*. (2007)				Bryden *et al*. (2009)			Bandegan *et al*. (2011)		Rodehutscord *et al*. (2016)		
Grain type	Wheat	Barley				Barley	Maize	Wheat	Wheat	Barley	Barley	Maize	Wheat
Starch type		Normal		Waxy									
Hulled (H)/Hull-less (HL)		H	HL	HL									
n[1]	16	1	1	1	1	1	7	7	6	7	21	27	29
DM	879-889	890	899	894	903	896	895	898	940	921	882	903	877
CP	120	116	104	105	137	94.9	89.4	103	162	143	123	93.5	137
Indispensable AA													
Arginine	5.8	5.55	4.91	4.08	6.39	5.47	4.44	5.19	7.6	6.8	5.99	4.33	6.56
Histidine	2.9	3.11	2.58	2.3	3.45	2.57	2.71	2.85	3.8	3.0	2.9	2.87	3.47
Isoleucine	4.2	4.18	3.89	3.54	5.24	3.79	3.59	4.15	5.3	4.8	3.85	3.07	4.25
Leucine	7.6	8.15	7.24	6.47	10.1	7.7	12.1	7.77	10.5	9.9	8.3	11.78	9.14
Lysine	3.4	4.06	3.43	3.07	5.23	4.02	2.83	3.44	4.4	4.9	4.29	2.79	3.73
Methionine	1.8	1.85	1.69	1.66	1.89	1.45	1.63	1.45	2.5	2.4	1.93	1.93	2.01
Phenylalanine	5.1	6.56	5.13	4.51	8.16	5.36	4.77	5.08	7.4	7.6	6.3	4.63	6.37
Threonine	3.3	3.77	3.55	3.1	4.68	3.46	3.83	3.47	4.5	4.7	4.17	3.41	3.92
Valine	5.2	5.95	5.46	4.88	7.08	5.36	4.83	5.02	6.6	7.0	5.44	4.2	5.26
Tryptophan	-	-	-	-	-	1.23	0.46	0.54	-	-	1.51	0.7	1.58
Dispensable AA													
Alanine	4.2	4.54	4.12	3.69	5.79	4.58	7.39	4.23	5.5	5.5	4.82	7.38	4.71
Aspartic acid	6	7.73	6.72	6.37	10.9	6.36	6.37	5.93	8.0	8.1	7.11	6.26	6.84
Cysteine[2]	2.6	2.33	2.26	2.21	2.41	-	-	-	3.5	2.8	2.57	2.09	3.03
Glutamic acid	31.4	31.8	27.5	24.2	37.9	25.2	18.2	31	46.5	35.8	29.9	17.4	40.4
Glycine[2]	4.8	4.62	4.02	3.56	5.54	4.35	3.83	4.8	6.5	5.4	4.74	3.47	5.53
Proline	11	14.2	11.4	10.37	18.3	-	-	-	16.4	15.9	15.62	9.82	15.76
Serine	5.5	4.53	4.26	3.63	5.25	4.91	4.64	5.86	7.2	6.1	5.4	4.74	6.67
Tyrosine	-	-	-	-	-	3.01	3.00	2.54	-	-	3.47	3.46	3.66

[1]Number of analysed grain types.
[2]Semi-indispensable AA for poultry.

2.2.2.3. Non-starch polysaccharides

Non-starch polysaccharides belong to the fibre component in cereal grains, which is mainly from the cell wall structure (Choct, 1997). Encapsulation of nutrients within endosperm cells and increased intestinal digesta viscosity are two major mechanisms whereby NSP impair digestion and absorption of nutrients in birds fed diets based on viscous grains. Water solubility of NSP is an important measure of the physiological characteristics and properties of NSP for monogastric animals and, based on the solubility in water, NSP are categorised into two main fractions namely insoluble (I.NSP) and soluble NSP (S.NSP; Choct, 2015). Compared to barley and wheat, maize contains only negligible amounts of S.NSP (Table 2.3; Choct, 2015). In contrast to the relatively constant S.NSP proportion in wheat (Zijlstra *et al*., 1999; Choct, 2015), a wide range of barley NSP solubility has been reported (Table 2.3; Andersson *et al*., 1999). The proportions of I.NSP and S.NSP can be greatly dependent on morphological and physio-chemical characteristics of different barley types.

2.2.2.3.1. Insoluble non-starch polysaccharides

Insoluble fibre creates a cage effect by encapsulating nutrients (starch and protein) in barley endosperm cells. Intact cell wall structures enclose the nutrients in the endosperm cells, and act as a physical barrier interfering the contact with digestive enzymes, and consequently limit the feeding value of barley in poultry diets. It has been demonstrated that the cell walls in the endosperm of barleys with high levels of β-glucans were thicker than in barleys with low levels of β-glucans (Oscarsson *et al*., 1997; Izydorczyk and Dexter, 2008). It can be therefore speculated that waxy and high amylose barley types with a higher content of β-glucan may more affected by the cage effect due probably to the thicker endosperm cell walls than other barley types.

Insoluble NSP was historically known as a nutrient diluent with little or no effect on nutrient utilisation (Carré *et al*., 1990). It was observed later that I.NSP can assist gut motility by absorbing large amounts of water (Smits and Annison, 1996) and, thus controlling excessive NSP solubilisation. Moreover, I.NSP can influence the gut development and health, digesta transit time (Choct, 1997), nutrient digestion (Svihus and Hetland, 2001) and birds' behaviour (Hetland *et al*., 2004). Consequently, it is now recommended to include moderate amounts of coarse I.NSP, such as wood shavings

(Hetland *et al*., 2003; Amerah *et al*., 2009) and oat hulls (Rogel 1987a, b; Sacranie *et al*., 2012), at levels between 2 and 3% to modern low fibre broiler diets (Mateos *et al*., 2012).

Majority of the benefits of I.NSP on enhanced nutrient utilisation and growth performance is a consequence of improved gizzard functionality. The effect of I.NSP on gizzard development and consequently on nutrient digestibility is more pronounced for starch. Svihus (2001) observed greater starch digestibility for a barley-based diet (0.96), compared to four wheat types (0.80, 0.76, 0.83 and 0.73), a finding that was attributed to gizzard development influenced by I.NSP available in barley (Svihus and Hetland, 2001). A surplus of starch in the digestive tract can result in low starch digestibility in broiler chickens and, Svihus and Hetland (2001) identified gizzard as the key site for preventing starch overload in the digestive tract by regulating the digesta passage rate (Hetland *et al*., 2004).

Literature on the relationship between cellulose content and hull type offers contradictory findings as Oscarsson *et al*. (1996) and Andersson *et al*. (1999) observed higher contents of cellulose in hulled barley types wherein Holtekjølen *et al*. (2006) suggested that cellulose content is not affected by the hull. Moreover, Holtekjølen *et al*. (2006) suggested that cellulose content seemed to be influenced by the starch type, as normal starch barley contained less cellulose (91.8 g/kg DM) than waxy (127 g/kg DM) and high amylose (140 g/kg DM) hull-less barley types. However, a higher level of I.NSP has been reported in hulled barley types compared to hull-less barley types, due to the presence of hulls (Table 2.3; Beames *et al*., 1996; Holtekjølen *et al*., 2006), suggesting the more occurrence of I.NSP in the hull compared to the barley kernel. Comparing 18 barley types (12 and six hulled and hull-less barley types, respectively), Beames *et al*. (1996) reported that hulled and hull-less barley types differed mainly in the I.NSP (11.5-17.3 vs. 6.6-8.7% DM, respectively) and lignin (1.7-4.5 vs. 0.7-l.3% DM, respectively) contents.

The stimulatory effect of hulled barley with a higher proportion of I.NSP on starch digestibility and thereby on energy utilisation can eventually result in improved growth performance compared with hull-less barley types. However, these positive effects of I.NSP are dependent on grain physical characteristics such as particle size, as fine grinding of barley can diminish its stimulatory effect on gizzard musculature development (Hetland *et al*., 2004).

2.2.2.3.2. Soluble non-starch polysaccharides

Eliciting anti-nutritive properties, S.NSP causes a distinct negative effect on the nutritive value of cereal grains used in poultry diets (Hughes and Choct, 1999). Due to its chemical composition highlighted with a higher level of S.NSP, barley is categorised as a viscous cereal together with rye, wheat, triticale, and oats. Partially soluble mixed linkage (1→3), (1→4)-β-D-glucan and arabinoxylans have been identified as main NSP present in both wheat and barley compared to maize. While β-glucan is prominent in barley, arabinoxylans are the predominant NSP in wheat. Though both wheat and barley have higher levels of NSP compared to maize, barley NSP mainly consists of the soluble fraction compared to wheat (Messia *et al*., 2017; Table 2.3).

Soluble NSP form a gel that interferes in the interaction of nutrient substrates with endogenous enzymes (Svihus *et al*., 2000). Greater intestinal viscosity in chickens fed barley diets was reported as the major anti-nutritive mechanism by S.NSP, resulting in reduced accessibility of digestive enzymes to nutrients (Choct *et al*., 1996; Classen, 1996; Svihus and Gullord, 2002). Moreover, increased digesta viscosity can modify gut physiology (Viveros *et al*., 1994; Iji, 1999) and interaction with gut microflora (Józefiak *et al*., 2006, 2010), consequently lowering the feeding value of barley for poultry.

2.2.2.3.2.1. β-glucans

Barley β-glucan consists of D-glucose molecules joined by (1→3) and (1→4) glycosidic bonds and the structure of the glucose chain depends on the relative number of (1→3) and (1→4) β-glycosidic bonds between the repeating glucose units (Jacob and Pescatore, 2014). β-glucan makes up 70% of the endosperm cell wall that surrounds starch granules and about 25% of the aleurone cell walls (Åman and Graham, 1987; Choct, 2015).

Table 2. 3. The type and content of non-starch polysaccharides in barley, maize and wheat (g/kg, DM basis).

Reference	Grain type	n[1]	Starch type	Hulled/ Hull-less		NSP[2]										Klason lignin	Proportion of total NSP
						AX	A	X	BG	CEL	MA	GAL	UA	GLU	Total		
Choct (2015)	Wheat	-			Soluble	18	-	-	4.0	-	t[5]	2.0	t	-	24	-	21
			-	-	Insoluble	63	-	-	4.0	20	t	1.0	2.0	-	90	-	79
	Barley[3]	-			Soluble	8.0	-	-	36	-	t	1.0	t	-	45	-	27
			-	-	Insoluble	71	-	-	7.0	39	2.0	1.0	2.0	-	122	-	73
	Maize	-			Soluble	1.0	-	-	t	-	t	t	t	-	1.0	-	1.0
			-	-	Insoluble	51	-	-	-	20	2.0	6.0	t	-	80	-	99
Zijlstra *et al*. (1999)	Wheat	16			Soluble	-	10	7.0	-	-	0.4	1.8	-	3.0	23	-	18
			-	-	Insoluble	-	41	25	-	-	1.3	1.4	-	34	103	-	82
Andersson *et al*. (1999)[4]	Barley	1	Normal	Hulled	Soluble	77	2.4	3.2	22	40	0.7	0.7	1.5	32	40	-	17
					Insoluble		21	50	25		3.6	2.0	2.9	55	200	15	83
		1	High Amylose	Hulled	Soluble	90	4	5.6	26	47	1.4	0.8	1.7	49	63	-	20
					Insoluble		23	57	43		6.7	2.3	3.4	67	249	15	80
		1	Waxy	Hulled	Soluble	75	3.3	4.6	31	35	0.9	0.8	2.1	46	58	-	23
					Insoluble		22	45	30		3.7	2.0	3.1	50	191	14	77
		1	Normal	Hulled	Soluble	83	2.6	3.2	15	42	0.8	0.7	1.1	21	29	-	13
					Insoluble		23	55	13		6.7	2.1	3.5	49	194	17	87
		1	Normal	Hull-less	Soluble	52	3.5	4.9	24	19	1.0	1.1	1.5	32	44	-	26
					Insoluble		17	27	22		3.9	1.6	1.9	33	125	7.4	74
		1	High amylose	Hull-less	Soluble	57	4.5	6.6	26	16	1.4	0.8	1.7	48	63	-	28
					Insoluble		18	28	48		4.7	1.5	1.9	42	160	11	72
		1	Waxy	Hull-less	Soluble	48	2.8	3.6	30	14	0.9	0.7	1.9	37	46	-	27
					Insoluble		18	24	26		4.2	1.8	1.7	33	123	6.9	73
		1	Waxy	Hull-less	Soluble	120	7.8	13	12	41	3.7	1.8	2.4	123	152	-	30
					Insoluble		38	61	137		10	2.9	3.3	67	360	10	70
Holtekjølen *et al*. (2006)	Barley	28	Normal	Hulled	Soluble	13.7	-	-	-	127	-	-	-	-	106	-	31
					Insoluble	116	-	-	-		-	-	-	-	232	-	69
		1	Waxy	Hulled	Soluble	15.5	-	-	-	177	-	-	-	-	184	-	45
					Insoluble	109	-	-	-		-	-	-	-	223	-	55
		6	Normal	Hull-less	Soluble	22.6	-	-	-	91.8	-	-	-	-	125	-	49
					Insoluble	66.1	-	-	-		-	-	-	-	128	-	51
		3	Waxy	Hull-less	Soluble	24.1	-	-	-	127	-	-	-	-	200	-	64
					Insoluble	62.9	-	-	-		-	-	-	-	114	-	36
		1	High amylose	Hull-less	Soluble	20.5	-	-	-	140	-	-	-	-	222	-	64
					Insoluble	60.8	-	-	-		-	-	-	-	125	-	36

[1]n, number of analysed samples.
[2]AX, arabinoxylan; A, arabinose; X, xylose; BG, β-glucan; CEL, cellulose; MA, mannose; GAL, galactose; UA, uronic acid; GLU, glucose.
[3]Englyst (1989).
[4]Total insoluble NSP = The sum of insoluble A, X, BG, MA, GAL, UA, GLU and total CEL.
t, Trace amounts.

High β-glucan content is probably the most detrimental anti-nutritional factor in barley, which causes the unpopularity of barley as a constituent of poultry diets. The content and properties of β-glucan play a key role in determining the potential of barley utilisation in poultry diets (Burnett, 1966). Conversely, the presence of β-glucan has become the primary factor for a growing interest in barley for human consumption. While high molecular weight, extractable β-glucan negatively affects nutrient digestion and absorption in monogastric animals, and interferes with filtration in the brewing industry which results in reduced clarity in beer (Andersson *et al*., 1999), it enhances human health by lowering cardiovascular risk through decreasing plasma cholesterol and improving lipid metabolism (Behall *et al*., 2004; Izydorczyk and Dexter, 2008; Talati *et al*., 2009).

High occurrence of β-glucan in waxy and high amylose types compared to normal starch, irrespective of the absence or presence of hull, was reported (Oscarsson *et al*., 1996; Andersson *et al*., 1999; Izydorczyk *et al*., 2000; Holtekjølen *et al*., 2006). Izydorczyk *et al*. (2000) compared the total and soluble β-glucan contents in different hull-less barley types and, reported significant differences in total β-glucan, with average values of 74.9, 68.6, 63.0, and 43.8 g/kg DM for high amylose, waxy, zero amylose waxy, and normal starch barley, respectively. The solubility of β-glucan in high amylose barley was relatively low (20.6-29.7%) compared to that in normal (29.8-44.3%), zero amylose waxy (34.0-52.5%), and waxy (36.7-52.7%) barley types. On the other hand, Beames *et al*. (1996) demonstrated that neither S.NSP nor β-glucan contents differed in hulled and hull-less barley types. The wide range of solubility of β-glucan in different barley types (Andersson *et al*., 1999, Table 2.3) suggest that anti-nutritive properties generated by β-glucan cannot be predicted if only the total content is analysed.

Though the influence of genetic (Lee *et al*., 1997) and environmental (Güler, 2003) factors on levels of β-glucan have been established to a great extent, the relationship between the β-glucan and other constituents of barley grain is yet to be understood. Literature on the relationship between β-glucan and other components has been inconsistent. Holtekjølen *et al*. (2006) reported a negative correlation of β-glucan with starch, cellulose, arabinoxylans and amylose contents and, a strong positive correlation with protein and soluble NSP. Izydorczyk *et al*. (2000) also observed an inverse relationship between total β-glucan and starch contents. Bhatty (1999) observed that β-

glucan content is positively correlated with total NSP content in the barley. Li *et al*. (2001) reported no correlation between β-glucan and amylose contents.

The wide variability of β-glucan content and solubility, and inconsistent and unpredictable relationship with other components of barley, suggest the importance of assessing the anti-nutritive components of barley prior to feed formulation. The established crucial role of β-glucan in determining the feeding value of barley for broilers (Bergh *et al*., 1999; Ravindran *et al*., 2007) emphasises the need of considering β-glucan content in selecting barley cultivars for use in poultry diets.

2.2.2.3.2.2. Arabinoxylans

In contrast to β-glucan, arabinoxylans are mainly located in aleurone cell walls, outer layers of barley kernel and husk, and only a small amount is present in endosperm cell walls. The structure of arabinoxylan is composed of two pentosans, arabinose and xylan (Choct, 1997). Holtekjølen *et al*. (2006) observed high occurrence of arabinoxylan in hulled barley types with a greater insoluble portion (89% of total arabinoxylan), compared to hull-less barley types, and confirmed the presence of arabinoxylans mainly in the hull. Generally, arabinoxylans constitute only a minor portion of water-extractable polysaccharides in barley (Izydorczyk *et al*., 1998; Choct, 2015; Table 2.3) and consequently have received less attention from poultry nutritionists compared to β-glucan. Choct (1997) illustrated that most of the arabinoxylans in cereal grains are insoluble in water because they are anchored in cell walls by strong cross-links and, arabinoxylans not bound to the cell walls can form highly viscous solutions. Therefore, the influence from arabinoxylans cannot be totally disregarded in case of barley and measures should be taken to minimise the anti-nutritive effects generated by arabinoxylans as well.

The molecular characteristics of β-glucan and arabinoxylans play a critical role in determining their physical properties (extractability, viscosity and gelation) and their behaviour in the gastrointestinal tract (Izydorczyk and Dexter, 2008). After studying the structure and physicochemical properties of β-glucans and arabinoxylans isolated from hull-less barley, Storsley *et al*. (2003) highlighted that molecular differences of NSP affect their physiological properties and result in different nutritional characteristics, even when overall amounts of S.NSP were equal. Digesta viscosity is dependent not only on

the concentration of NSP, but also on molecular weight (Saulnier *et al*., 1995; Dusel *et al*., 1997), therefore, a grain with a low content of S.NSP might result in high viscosity if the NSP is of a higher molecular weight (Bedford, 1995; Cowieson *et al*., 2005).

2.2.2.4. Other components (fat and minerals)

Fats or lipids can be considered as the third storage materials in barley grain after starch (513 to 642 g/kg DM; Holtekjølen *et al*., 2006) and proteins (108 to 136 g/kg DM; Rodehutscord *et al*., 2016) with an average lower content of 32.6 g/kg DM (Liu, 2011). Moreover, barley fats show a little variability according to Svihus and Gullord (2002), who reported a narrow range (26-32 g/kg DM) of crude fat for five barley types. Earlier studies on improving the feeding value of barley for poultry birds have emphasised the potential of increasing the intrinsic energy content by increasing storage fat content of the barley grains (Bhatty *et al*., 1974; Fedak and Roche, 1977). However, no significant improvement of fat content was observed over the years according to Fedak and Roche (1977) and Liu (2011), who reported fat contents of 30.8 and 32.6 g/kg DM, respectively.

Higher content of fat in hull-less barley types compared to hulled types attributed to the concentration effect caused by the absence of hull was reported (Pettersson and Lindberg, 1997; Andersson *et al*., 1999; Ravindran *et al*., 2007; Liu, 2011). Regardless of hull type, a higher fat content in high amylose barley followed by waxy and normal starch types has been reported (Oscarsson *et al*., 1996; Pettersson and Lindberg, 1997). Pettersson and Lindberg (1997) reported 38, 34 and 29 g/kg DM of crude fat for high amylose, waxy and normal starch hulled barley types, respectively. Compared to other nutrients, the relationship between fat and other compositional constituents in barley grain is relatively unexplored, which might be due to the narrow range of fat content resulting in poor chance of significant differences in comparisons.

The major fatty acids (FA) in barley grain is linoleic (518 g/kg of total FA), followed by palmitic (248 g/kg of total FA) and oleic acid (142 g/kg of total FA). The corresponding values in a wheat sample with 22.2 g/kg DM fat and 597, 203 and 123 g/kg of total FA of linoleic, palmitic and oleic acids, respectively (Liu, 2011). The high concentration of linoleic acid as an essential FA can be considered as one of nutritional importance in barley grain. In contrast to the relatively constant fat content in different barley types, a wide range of barley FA composition has been reported. Fedak and Roche

(1977) reported that linoleic (507-579 g/kg of total FA), palmitic (183-270 g/kg of total FA), oleic (122-212 g/kg of total FA) and linolenic acids (43-71 g/kg of total FA) of 21 barley types ranged widely. Welch (1978) analysed 27 barley types for FA composition and reported widely ranging palmitic (214-287 g/kg of total FA), stearic (6.0-18 g/kg of total FA), oleic (104-169 g/kg of total FA), linoleic (524-583 g/kg of total FA) and linolenic acids (45-73 g/kg of total FA). The variability of FA concentration in different studies can be mainly attributed to oxidation and thus, differences in the sample storage periods and analytical methodologies. The varietal differences also can play a significant role in observed variability. Reports on the effect of environmental factors on FA composition, however, have been inconsistent, which may be related *inter alia* to cultivar differences (Fedak and Roche, 1977; Welch, 1978).

Liu (2011) studied the distribution of fat within the barley grain and, reported that fat is largely concentrated in germ and bran region, while inner endosperm has much less fat. This observation provides scientific basis for the pearling of barley as the removal of surface layers (bran) of grains, thus reducing the lipid contents can improve storage stability of pearl barley. In addition, Liu (2011) proved that removing surface layers improve the stability of FA composition of the remaining kernels by increasing saturated FA while decreasing unsaturated FA.

Most of the studies providing the mineral composition of different barley types lack information on hull and starch type. Rodehutscord *et al*. (2016) reported potassium as the major mineral followed by phosphorus. Rodehutscord *et al*. (2016), reported a higher content of calcium in barley (ranged from 0.35 to 0.6 g/kg DM) compared to maize (0.04 g/kg DM) and wheat (0.4 g/kg DM). Moreover, barley has a higher sodium content compared to wheat and maize (Table 2.4; Rodehutscord *et al*., 2016). Except for calcium and sodium, the patterns of differences in other minerals in barley, maize and wheat seemed to be inconsistent. Both low-phytate barley and maize types contained less amount of phytate-phosphorus compared to wild type (Jang *et al*., 2003).

Table 2. 4. Mineral composition of maize, barley and wheat grains (g/kg, DM basis).

	Jang *et al*. (2003)				Linares *et al*. (2007)		Rodehutscord *et al*. (2016)		
	Barley		Maize		Barley		Barley	Maize	Wheat
	Wild[1]	LP[2]	Wild	LP	Wild	LP			
n[3]	1	1	1	1	1	1	21	27	29
Calcium	0.6	0.6	0.02	0.03	0.47	0.49	0.59	0.04	0.4
Phosphorus (P)	4.1	3.3	3.2	3.2	3.63	3.52	4.3	3.17	3.67
Phytate P	2.3	1.1	2.2	0.9	2.38	0.05	2.81	2.26	1.92
Non-phytate P	1.8	2.2	1	2.3	1.25	3.47	1.49	0.91	1.75
Magnesium	1.3	1.3	1.3	1.2	1.2	1.2	1.63	1.45	1.56
Potassium	-	-	-	-	-	-	5.53	3.96	4.33
Sodium	-	-	-	-	-	-	0.05	0.003	0.005
Iron	-	-	-	-	0.062	0.071	0.04	0.02	0.04
Chloride	-	-	-	-	-	-	-	-	-
Manganese	0.017	0.02	0.007	0.007	0.016	0.015	0.015	0.005	0.032
Zinc	0.030	0.04	0.010	0.010	0.023	0.024	0.024	0.021	0.022
Copper	0.009	0.01	0.006	0.006	0.003	0.004	0.005	0.002	0.004

[1]Wild-type barley with normal phytate P contents.
[2]Low-phytate.
[3]Number of analysed samples.

The huge variation in chemical composition in cereals are attributed to the differences in grain types, variety, growing locations, seasonal effects, crop treatments and grain fumigants, conditions and duration of storage. Numerous attempts have been made to predict the nutritive value in grains for poultry from the chemical and physical composition values individually or in combination. Villamide *et al*. (1997) developed an equation to determine the N-corrected apparent metabolisable energy (AMEn) of enzyme supplemented barley from chemical parameters, mainly crude fibre and NSP. The practicality of this prediction equation can be questioned due to the variability of response observed in enzyme supplemented barley-based diets. Besides, nutritive value of grains for poultry is usually determined not only by the chemical and physical properties of the grains but also by interactions of nutritive components during the process of ingestion, digestion and metabolism in birds (Hughes and Choct, 1999). The concentration and the extent of solubility of NSP also play a significant role in determining how efficient dietary components are utilised by animals. Therefore, the reliability of using the chemical composition data in poultry diet formulation should be a matter of concern, at least for NSP rich grains.

2.3. Barley in poultry nutrition

Research into barley use in poultry diets has a long history. According to available literature, around 1930s, studies began to emerge comparing barley with other cereal

grains for poultry nutrition (Crampton, 1936). The occurrence of wet litter and sticky droppings was first to be noticed as problems associated with feeding barley-based diets, leading to poor quality in meat and eggs. In addition, depressed growth performance and nutrient utilisation of birds fed barley-based diets were observed (Jeroch and Dänicke, 1995). Earlier research acknowledged a close relationship between extract viscosity of barley and growth impairment of birds fed barley-based diets and, the greater digesta viscosity in birds fed barley-based diets was attributed to the NSP present in barley grain (Burnett, 1966; White *et al*., 1981). Feed enzyme preparations were proven to be effective in ameliorating the depressions in growth and nutrient utilisation in birds fed barley-based diets (Hesselman and Aman, 1986; Rotter *et al*., 1990). However, the increased interest of the barley usage in poultry feed due to the development of feed enzymes was challenged by the variable responses of birds fed enzyme supplemented barley-based diets (Chesson, 1993). Moreover, the demand for barley for poultry feed has been inconsistent throughout the history, presumably be driven by changes in economic circumstances (Jeroch and Dänicke, 1995; Tricase *et al*., 2018). In consequence, the choice of other cereals that are less problematic and more economical, has restricted the proportion of barley used in poultry diets to less than 1.0% of total barley utilised as animal feed (Black *et al*., 2005). In this section of the review, the aim is to understand the impact of barley in broiler diets on growth performance, nutrient utilisation and gut morphometric parameters, and contribution of each parameter to the feeding value of barley in broiler diets, with emphasis on strengths and weaknesses of previous studies.

2.3.1. Intestinal digesta viscosity

It is well recognised today that inclusion of barley in poultry diets impedes the nutrient digestion through increasing intestinal viscosity. Elevated viscosity of the intestinal contents can cause inefficient mixing of digesta and enzymes, limiting the room for an efficient nutrient digestion. Transport properties of nutrients at mucosal surface can also be adversely affected, lowering the efficiency of the nutrient absorption (Jacob and Pescatore, 2012).

White *et al*. (1981) isolated β-glucan from barley and added it to a maize-based diet and the resultant increase in the intestinal digesta viscosity supported the fact that the β-glucans of barley are the primary cause of poor growth performance. Moreover, it was

recognised that not only the concentration but also the structure and molecular weight of NSP is responsible for increased viscosity of the intestinal contents of birds fed barley-based diets (Bengtsson *et al*., 1990).

Carré *et al*. (1994, cited in Carré, 2004) reported that rye resulted in the highest viscosity of gut contents, followed by barley, triticale, wheat, maize, and sorghum. In agreement, majority of the studies has reported more viscous intestinal contents in birds fed barley-based diets compared to birds fed maize-, wheat- and sorghum-based diets (Table 2.5). In contrast, Shakouri *et al*. (2009) reported higher digesta viscosity in the broilers fed wheat-based diets (5.74 cP) compared to barley-based diets (2.92 cP) speculating that the used wheat to be a viscous cultivar*.

In addition to the proven influence of barley S.NSP, it has been shown that a variety of factors can influence barley viscosity: a) grain-related factors such as growing location (Campbell *et al*., 1989), storage time (Fuente *et al*., 1998), b) dietary factors such as barley inclusion level (Fuente *et al*., 1995; Yu *et al*., 1998), heat processing of grain (Gracia *et al*., 2003), conditioning temperature of the diet (Samarasinghe *et al*., 2000), and c) bird-related factors such as the age of the bird (Petersen *et al*., 1999) and sampling point in gastrointestinal tract (GIT; Table 2.5; Ankrah *et al*., 1999; Petersen *et al*., 1999).

Campbell *et al*. (1989) compared 16 barley cultivars selected for variation in extract viscosity and grown at five different locations and reported that differences in extract viscosity among locations were most apparent for high viscosity genotypes while low viscosity genotypes were more uniform across locations. Fuente *et al*. (1998) stored a two-rowed winter barley (Beka cultivar) at room temperature for 0, 3, 6, 16, and 32 weeks after harvesting and, reported that viscosity of the intestinal contents of broilers decreased with the barley storage time. Moreover, a storage time × enzyme interaction was reported with a greater decrease in digesta viscosity in birds fed non-supplemented barley diets than in enzyme supplemented diets.

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*Viscosity is the internal friction in fluids that results in resistance to flow. It is measured in poise (P), 0.100 kg m^{-1} s^{-1}, but usually expressed as centipoise (cP), 0.001 kg m^{-1} s^{-1} (Dembicki, 2017).

Table 2. 5. Comparison of different cereal types for intestinal digesta viscosity of broilers.

Reference	Grain	Inclusion level (g/kg of diet)	Sampling point	Major NSP[a]	Bird age (d)	Viscosity (cP)
Wang *et al*. (1992)	Maize	452	Small intestine	Soluble BG: 0.2	14	1.7
	Barley	698		Soluble BG: 17.2		2.4
Almirall *et al*. (1995)	Maize	600	PSI[b]	-	22	1.0
	Low viscosity barley			Total BG: 32.3		13
	High viscosity barley			Total BG: 38.7		29
Ankrah *et al*. (1999)	Hull-less normal starch barley	610	PSI[b]	Total BG: 60	21	178
			DSI[b]			353
	Hull-less waxy starch barley		PSI	Total BG: 73		376
			DSI			440
Józefiak *et al*. (2007)	Triticale	686/719[c]	Ileum	Soluble AX:12.3	35	6.0
	Rye	621/652[c]		Soluble AX: 27.3		140
	Wheat	745/740[c]		Soluble AX:10.6		3.0
Shakouri *et al*. (2009)	Barley	600	Ileum	-	28	3.2
	Sorghum	623		-		2.2
	Wheat			-		7.3
	Maize			-		2.4
Petersen *et al*. (1999)	Wheat	657	Foregut[d]	-	Average value of 20, 25, 30, 35	2.7
			Hindgut[d]	-		8.0
	Barley	660	Foregut	-		21
			Hindgut	-		28

[a]NSP, non-starch polysaccharides; BG, β-glucan; AX, Arabinoxylan.
[b]PSI, proximal small intestine (from gizzard to Meckel's diverticulum); DSI, distal small intestine (from Meckel's diverticulum to the ileo-caecal junction).
[c]Starter (1-14 d)/finisher (15-35 d) diet composition.
[d]Foregut, duodenum to Meckel's diverticulum; Hindgut, from Meckel's diverticulum to the ileo-caecal junction.

Increasing intestinal digesta viscosity of broilers in response to increasing inclusion of barley in maize-based diets has been reported in the literature. Increasing barley inclusion by 300 g/kg (from 300 to 600 g/kg) in diets with no enzyme supplementation has been shown to increase the digesta viscosity by 222% (from 4.68 to 15.08 cP; Fuente *et al*., 1995). However, when a combination of β-glucanase and xylanase was added to the diets, the viscosity increased only by 62% (2.44 to 3.95 cP) over a similar increment of barley in the diet. Yu *et al*. (1998, 2002) reported increases in duodenal digesta viscosity in response to complete replacement of maize with barley, with greater magnitude of response to complete replacement of maize in younger broilers (d 21) than broilers aged 42 d.

Ankrah *et al*. (1999) reported that intestinal digesta viscosity increased from the proximal to the distal small intestine in broilers fed non-supplemented barley diets and mainly attributed to the increased β-glucan solubilisation along the GIT. Supplemental enzyme lowered the digesta viscosity in both proximal and distal small intestine. Moreover, comparing mash and reground pellets of both waxy and normal starch hull-less barley types, a pelleting-induced 45% reduction in digesta viscosity was also observed in both barley types, due likely to the shearing effect on β-glucan during pelleting (Ankrah *et al*., 1999). In contrast, an increase of intestinal digesta viscosity in response to heat processing of barley grain was reported by Gracia *et al*. (2003), and the reduction of digesta viscosity in response to the added enzyme was greater in heat-processed barley diets. Samarasinghe *et al*. (2000) reported greater dietary viscosity due to high conditioning temperatures (75 and 90 °C) during pelleting a barley-maize-soybean meal diet compared to 60 °C. Supplemental enzyme reduced the dietary viscosity by 11, 14 and 17% in diets conditioned at 60, 75 and 90 °C, respectively, showing greater magnitudes of response at higher temperatures.

Decreasing intestinal viscosity with increasing age of the broilers fed barley-based diets has been reported in some studies (Salih *et al*., 1991; Petersen *et al*., 1999; Gracia *et al*., 2003). Rotter *et al*. (1990) showed that adult cockerels fed barley diets had sufficiently developed GIT to avoid the negative effects of high β-glucan-induced digesta viscosity. Therefore, intestinal viscosity is not as great limiting factor in adult birds as it is in young birds (Almirall *et al*., 1995). According to Salih *et al*. (1991), who evaluated a high viscosity hull-less barley, the relative intestinal digesta viscosity dropped from

2.59 at two-weeks to 1.74 at eight-weeks in broilers. The non-supplemented high viscosity barley type used by Almirall *et al*. (1995) resulted in 29 and 19 cP digesta viscosity when fed to three-week old broilers and one-year old cockerels, respectively. In non-supplemented low viscosity barley, the values were 13 and 7 cP for three-week old chicks and one-year old cocks, respectively. Petersen *et al*. (1999) reported that foregut digesta viscosity of broilers fed barley-based diets reduced with age by 51%, from 16.7 cP at 25 d to 8.2 cP at 45 d. These observations support the suggestion by Bedford (2018) that the mechanisms of viscosity needed to be re-evaluated as being a function not only of the cereal being fed, but also of the age of the bird.

A better understanding of the influence of chemical and physical characteristics of barley grain, different feed processing factors and enzyme supplementation on the response of intestinal digesta viscosity would allow poultry nutritionists to increase the barley inclusion in poultry diets by strategically minimising the viscosity related negative consequences.

2.3.2. Growth performance

Poor growth performance in broilers fed barley-based diets has been reported compared to maize (Moharrery, 2006; Onderci *et al*., 2008), wheat (Salih *et al*., 1991; Friesen *et al*., 1992) and sorghum (Tang *et al*., 2017), and commonly attributed to the increased digesta viscosity in barley-fed birds.

The effect of barley inclusion in poultry diets on feed efficiency has been inconsistent, as both improvements (Friesen *et al*., 1992) and declines (Moss *et al*., 1983) were reported in the literature. Shakouri *et al*. (2009) and Tang *et al*. (2017) evaluated barley as the sole cereal in the broiler diets in comparison to maize, sorghum and wheat and reported that birds fed barley-based diets had the poorest weight gain (WG), feed intake (FI) and feed to gain ratio (F/G). In contrast, Brenes *et al*. (1993), who compared barley (cultivar, Scout) with wheat in broilers, reported 58 g superior WG for barley-fed birds, however, F/G was not differed between grain types. The WG differences caused by the grain type were minimised by the supplemental carbohydrases.

Bergh *et al*. (1999) evaluated mash diets based on hulled barley cultivars (696 g/kg) with normal, high amylose and waxy starch types, without or with supplementation

of a β-glucanase enzyme for broiler starters (1-18 d). Growth performance was determined at d 13 and birds offered normal starch barley had a better BW, FI and F/G. The magnitude of improvement in growth performance due to supplemental enzyme was greater in birds fed high amylose and waxy barley types. The increases of WG in response to supplemental enzyme were 22, 44, and 38 g/bird for normal, high amylose and waxy barley types, respectively, and the corresponding improvements in F/G were 7, 24 and 21 points, respectively. In contrast, Ankrah *et al*. (1999) who compared two hull-less barley types with normal or waxy starch, without and with β-glucanase, in two feed forms (mash and reground pellets), reported no differences in growth performance parameters due to barley type, and no interactions of barley type with supplemental enzymes or feed form. Supplemental enzymes enhanced WG and F/G in both barley types and the feed form had no effect.

Due to the low metabolisable energy content of barley (Black *et al*., 2005; Table 2.6), birds need to consume more feed to maintain a constant energy intake (Classen, 2017). However, reduced feed passage associated with higher digesta viscosity caused by NSP (Salih *et al*., 1991) can depress the FI, especially in younger birds (Almirall and Esteve-Garcia, 1994), resulting in birds not being able to meet their nutritional requirements (McNab and Smithard, 1992). Moreover, barley is less palatable to poultry compared to maize (Ravindran and Blair, 1991) and wheat (Hughes, 1984). The removal of the hull is believed to increase the palatability of barley (Yu *et al*., 2002) and this perception was one of incentives for the development of hull-less barley types.

Moss *et al*. (1983), replaced wheat (w/w basis) with 0, 272, 408 and 544 g/kg of waxy starch hulled barley (cultivar, Wapana) in a broiler diet with no enzyme addition and reported that increasing levels of barley consistently decreased WG, but had no effect on F/G. Classen *et al*. (1988) substituted hull-less barley (cultivar, Scout; starch type, unidentified) on weight basis (0, 200, 400 and 600 g/kg) for wheat in a broiler starter diet and reported a linear decrease in BW with increasing levels of barley, while no depression was reported for F/G. Friesen *et al*. (1992) evaluated the influence of different inclusion levels of hull-less barley (0, 350 and 700 g/kg) in a wheat diet and supplementation of a cellulase enzyme on growth performance, energy and nutrient utilisation in 14-d old broilers. Weight gain and F/G of birds fed the non-supplemented hull-less barley at 350 g/kg was similar to those fed the control wheat diet, wherein barley inclusion at 700 g/kg

resulted in the lowest WG and highest F/G. The deterioration of growth performance associated with barley inclusion reported in previous studies may partly be explained by weight-to-weight substitution of barley for the major cereal in the diets (Moss *et al*., 1983; Friesen *et al*.,1992), resulting in lower metabolisable energy and digestible AA content than the corresponding cereal-based diets.

Yu *et al*. (2002) evaluated the inclusion of de-hulled barley at inclusion levels of 0, 400 and 800 g/kg, and supplementation of β-glucanase in iso-nitrogenous and iso-caloric maize-based diets and reported improved FI and WG with no effect on feed efficiency in response to the increasing inclusion of barley. The improvement in WG and FI was mainly attributed to the greater amount of fat added to the diets with higher inclusion of dehulled barley with a low energy value. Both greater amount of fat and the removal of fibrous hull of barley were believed to increase the palatability of the diets, improving the FI and WG.

2.3.3. Energy utilisation

The nutritive value of a particular grain for poultry can be best interpreted by the availability of energy and protein to the bird. Metabolisable energy of a cereal grain is dependent on the energy contained, the availability of the energy to the bird, and the presence or absence of anti-nutritive factors such as S.NSP (Scott *et al*., 1998). Wide variation in the apparent metabolisable energy (AME) within and between grain types is primarily attributed to a variable chemical and physical characteristics (Villamide *et al*., 1997) and grain specific anti-nutritive factors (Hughes and Choct, 1999). Kocher *et al*. (1997) reported the AME of Australian barley types to range from 10.4 to 12.2 MJ/kg DM. In addition, Choct *et al*. (2001), who analysed 11 barley cultivars, reported ranges of 11.6 to 13.8 and 12.5 to 13.58 MJ/kg DM for AME of barley in broilers and layers, respectively. Among all cereal grains used in poultry feed, barley has been identified as one of the most variable cereal grains in terms of its energy value (Choct *et al*., 2001) and this variability is not reflected in table values (Scott *et al*., 1998; Jacob and Pescatore, 2012).

Table 2. 6. Comparison of apparent metabolisable energy (AME; MJ/kg DM) and nitrogen-corrected AME (AMEn; MJ/kg DM) of different cereal grains for broilers.

Reference	Grain type	AME	AMEn
Choct and Annison (1990)	Pearled rice	17.36	
	Maize	15.83	
	Sorghum	15.77	
	Wheat	14.32	
	Triticale	13.83	
	Barley	11.92	
	Rye	11.34	
Moharrery (2006)	Maize	14.01	
	Hull-less barley	11.12	
	Hulled barley	10.05	
Ravindran *et al*. (2007)	Hull-less normal starch barley		12.97
	Hulled normal starch barley		12.72
	Hull-less waxy starch barley		11.23
Choct *et al*. (2001)	Sorghum	15.0	
	Barley	12.5	
Tang *et al*. (2017)	Maize	10.75	
	Wheat	10.74	
	Sorghum	10.64	
	Barley	9.91	

The early studies to evaluate the feeding value of barley for poultry attributed its lower energy content to the presence of fibrous hull (Jeroch and Danicke, 1995). However, Scott *et al*. (1998) analysed 14 barley types characterised for hull type, starch type, malting and row (two- or six-row) and reported the lack of effect from hull type on AME in non-supplemented barley diets. It was speculated that the adverse effects of the higher fibre content of hulled cultivars on AME were confounded by the higher β-glucan levels of the hull-less cultivars. In carbohydrase supplemented diets, however, hull-less barley cultivars showed greater AME content due to the carbohydrase enzyme action on minimising NSP-induced anti-nutritive effects.

As shown in Table 2.6, comparing two hull-less barley types that differed in starch type, Ravindran *et al*. (2007) reported 1.74 MJ/kg higher AMEn content for the normal starch barley than waxy starch barley. On the other hand, comparing two normal starch barley types differing in the presence of hull, only 0.25 MJ/kg difference in AMEn was reported. This finding suggests that starch characteristics of barley cultivars are probably

more important than fibre contents in determining the available energy content of barley for broilers. In contrast, Villamide *et al*. (1997) compared the energy content of eight barley cultivars, without and with a multi-component enzyme complex, and reported no relationship between AMEn of non-supplemented barley cultivars and chemical composition.

As shown in Figure 2.6, the available energy of cereal grains has a strong negative correlation with NSP concentrations in each grain type. In the case of barley, especially in non-supplemented diets, the bioavailable energy depends on its content of soluble β-glucan and consequent higher digesta viscosity (Rotter *et al*., 1990). A linear reduction of AMEn with the increasing inclusion of barley in wheat- (Friesen *et al*., 1992) and maize- (Fuente *et al*., 1995) based diets was reported and attributed to the increasing digesta viscosity. Villamide *et al*. (1997) demonstrated about 0.14 MJ decline in dietary AMEn for each 10% units increase in barley inclusion. Fuente *et al*. (1995) reported 0.06 MJ decline in AMEn per unit (cP) increase in digesta viscosity, suggesting that digesta viscosity accounts for 97% of the variation in AMEn among barley-based diets.

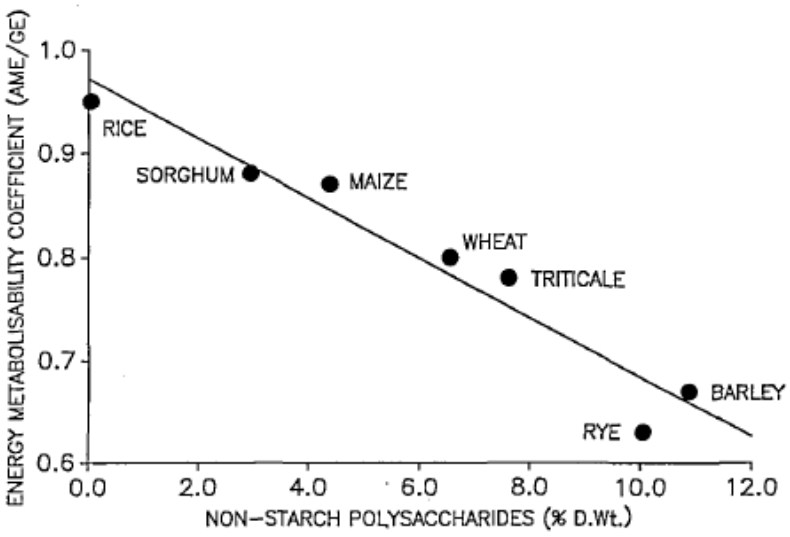


Figure 2. 6. The relationship between energy metabolisability (apparent metabolisable energy/gross energy) of cereals and their non-starch polysaccharide composition (pentosans+β-glucans; % dry weight), (r = -0.97). Source: Choct and Annison (1990).

2.3.4. Nutrient digestibility

In viscous grains such as barley, a small change in S.NSP can have a significant impact on nutrient utilisation. The prediction of resultant digestibility from the bird's capacity to utilise the nutrients solely from the nutrient composition data is challenging, justifying the need for using digestible nutrient values in barley-based diet formulation.

2.3.4.1. Amino acids

Owing to the high inclusion of cereal grains in poultry diets, the proportion of cereal protein represent above 30% of the total dietary CP and have a substantial contribution to the supply of dietary AA. In order to increase the dietary inclusion of barley without any adverse effect on AA utilisation, the factors affecting AA digestibility of birds fed barley-based diets should be well-understood.

The digestibility of barley AA has been determined either as apparent or standardised. The apparent ileal digestibility (AID) does not consider the endogenous AA losses, and the correction of AID values for diet-independent and inevitable endogenous AA flow (Lemme *et al*., 2004), yields more precise values as standardised ileal digestibility (SID). Several studies have evaluated the AID of AA in different barley types (Perttilä *et al*., 2005; Ravindran *et al*., 2005, 2007; Al-Marzooqi *et al*., 2010). However, studies evaluating the SID of barley types are limited (Szczurek, 2009; Bandegan *et al*., 2011; Barua *et al*., 2019; Szczurek *et al*., 2020).

As shown in Table 2.7, the SID of AA of maize, triticale, sorghum and wheat are higher than that of barley. Barua *et al*. (2019) reported average SID of AA for maize, sorghum, wheat and barley as 0.838, 0.804, 0.778 and 0.723, respectively. The AA digestibility of barley ranged from 0.639 for lysine to 0.815 for cysteine (Barua *et al*., 2019). The incomplete digestion of the AA justifies the use of digestible AA values, instead of total AA values, for broiler feed formulations. The AID of AA, however, is not recommended to be used in diet formulations due mainly to the underestimation of AA digestibility caused by endogenous AA flow and lack of additivity in complete diets. These concerns are critical in particular for low-protein feed ingredients (Stein *et al*., 2005; Xue *et al*., 2014) such as barley and, use of SID values with higher precision and additivity is, therefore, recommended for barley-based diet formulation.

In addition to the differences of AA content and digestibility between different grain types, inconsistent AA digestibility in different barley cultivars has been observed (Ravindran *et al*., 2007), and partly attributed to the different concentration of NSP. The average AID values reported by Ravindran *et al*. (2007) for non-supplemented hulled normal starch, hull-less normal starch, and hull-less waxy barley-1 and hull-less waxy barley-2 were 0.67, 0.66, 0.63 and 0.71, respectively, with corresponding CP contents of 116, 104, 105 and 137 g/kg DM, respectively. The inter-cultivar variability of AA digestibility justifies the use of individual SID values for each AA, specific for barley types, for formulating balanced barley-based diets, ensuring an adequate supply of AA for maintenance and growth functions.

Significant improvements in AA digestibility of barley due to exogenous carbohydrases have been reported (Bedford, 1995; Perttilä *et al*., 2001; Ravindran *et al*., 2007). However, the effect of enzyme supplementation on individual AA has also been inconsistent, which may be related to variations in chemical and physical characteristics of grains and different efficacies of supplemented enzymes. Other factors that contribute to the variation of AA digestibility in barley-based diets include; bird type (Al-Marzooqi *et al*., 2010), age of birds (Szczurek *et al*., 2020), barely particle size and feed form (Barua *et al*., 2019) and thus warrants consideration of these factors when determining AA digestibility in barley grain.

Table 2. 7. Comparison of standardised ileal digestibility of amino acids (AA) in different cereal grains.

Reference	Bandegan *et al*. (2011)		Barua *et al*. (2019)				Szczurek *et al*. (2020)					
Age of the birds (d)	21		24				14			28		
Grain type	Wheat	Barley	Maize	Sorghum	Wheat	Barley	Wheat	Triticale	Barley	Wheat	Triticale	Barley
Crude protein	0.872	0.797	0.829	0.808	0.797	0.711	-	-	-	-	-	-
Indispensable AA												
Arginine	0.852	0.804	0.873	0.841	0.746	0.715	0.89	0.79	0.77	0.87	0.88	0.82
Histidine	0.870	0.807	0.841	0.737	0.775	0.714	0.90	0.86	0.76	0.89	0.93	0.91
Isoleucine	0.904	0.839	0.825	0.809	0.769	0.684	0.91	0.85	0.81	0.89	0.93	0.92
Leucine	0.905	0.848	0.898	0.843	0.805	0.736	0.92	0.88	0.83	0.88	0.96	0.88
Lysine	0.837	0.805	0.767	0.758	0.635	0.639	0.85	0.79	0.78	0.82	0.82	0.82
Methionine	0.914	0.883	0.890	0.846	0.813	0.760	0.92	0.85	0.76	0.90	0.90	0.82
Phenylalanine	0.938	0.909	-	-	-	-	0.9	0.84	0.8	0.89	0.91	0.85
Threonine	0.854	0.806	0.809	0.794	0.727	0.701	0.79	0.81	0.75	0.79	0.88	0.87
Valine	0.877	0.825	0.833	0.808	0.755	0.722	0.86	0.86	0.79	0.88	0.93	0.86
Tryptophan	-	-	0.719	0.799	0.747	0.667	0.90	0.90	0.86	0.89	0.95	0.92
Dispensable AA												
Alanine	0.838	0.781	0.878	0.843	0.692	0.671	0.83	0.82	0.76	0.79	0.91	0.79
Aspartic acid	0.838	0.781	0.818	0.814	0.682	0.674	0.87	0.84	0.75	0.76	0.96	0.81
Cysteine	0.908	0.839	0.857	0.781	0.862	0.815	0.88	0.82	0.75	0.84	0.82	0.79
Glutamic acid	0.966	0.876	0.895	0.847	0.914	0.804	0.96	0.92	0.85	0.95	0.93	0.91
Glycine	0.841	0.767	0.745	0.713	0.731	0.652	0.83	0.82	0.72	0.79	0.84	0.85
Proline	0.954	0.866	0.864	0.797	0.912	0.808	0.95	0.89	0.86	0.96	0.94	0.91
Serine	0.891	0.822	0.858	0.831	0.824	0.736	0.87	0.83	0.76	0.86	0.91	0.83
Tyrosine	-	-	-	-	-	-	0.89	0.85	0.76	0.91	0.95	0.87

2.3.4.2. Starch

Supported by the similar trends between starch and energy utilisation of birds fed barley-based diets (Wu *et al*., 2004a; Ravindran *et al*., 2007), digestible starch is considered as the primary contributor to metabolisable energy in barley-based diets. Table 2.8 shows the comparison of ileal starch digestibility between different grain types and different barley types fed to broilers. While starch in maize is almost completely digested in broiler chickens (Zaefarian *et al*., 2015), other cereal grains show comparatively lower starch digestibility and greater variability than maize. Reasons for this variability include starch granule structure variations, anti-nutritional factors and access problems in coarse particles and extensively reviewed by Carré (2004), Svihus *et al*. (2005) and Zaefarian *et al*. (2015).

As discussed in section 2.2.2.1, barley grains can be categorised based on the starch type and, in contrary to the expectation that waxy barley starch with more amylopectin (970-1000 g/kg of starch, Ullrich *et al*., 1986) is more digestible (Björck *et al*., 1990), poor starch digestibility has been observed in birds fed waxy barley-based diets, regardless of the hull type (Bergh *et al*., 1999; Ravindran *et al*., 2007). Bergh *et al*. (1999) and Ravindran *et al*. (2007) thus proposed the contribution of factors other than hull type, in particular β-glucan content, affecting starch digestibility of broilers fed barley-based diets.

Ankrah *et al*. (1999) evaluated the starch digestibility in birds fed hull-less barley cultivars of normal or waxy starch (722 and 945 g/kg amylopectin, respectively) and, despite the higher digesta viscosity of birds fed waxy starch barley compared to the normal starch barley (276 vs. 102 cP), reported similar starch digestibility for the different starch types. Poor response of starch digestibility to variations in digesta viscosity in other grains has been previously reported (Carré *et al*., 2002) and among the three main nutrients (nitrogen, starch and fat), the extent of digestibility reduction due to viscosity seems to be the lowest for starch (Choct and Annison, 1992a,b; Smits *et al*., 1997). However, Carré *et al*. (2004) suggested that viscosity may induce a noticeable effect on starch digestibility in high viscosity barley types.

Table 2. 8. Ileal starch digestibility of different grain types fed to broilers.

Reference	Grain type	H/HL[a]	Starch type	Total β-glucans (g/kg)	Starch digestibility coefficient
Bergh *et al*. (1999)	Barley	H	Normal	31[b]	0.91
	Barley	H	Waxy	40[b]	0.87
	Barley	H	High	39[b]	0.89
Svihus (2001)	Wheat	-	-	-	0.79
	Barley	-	-	-	0.96
	Oat	-	-	-	0.99
Weurding *et al*. (2001)	Wheat	-	-	-	0.944
	Maize	-	-	-	0.970
	Barley	-	-	-	0.981
	Sorghum	-	-	-	0.953
Ravindran *et al*. (2007)	Barley	H	Normal	50	0.804
	Barley	HL	Normal	40	0.837
	Barley	HL	Waxy	64	0.587
Shakouri *et al*. (2009)	Maize	-	-	-	0.95
	Wheat	-	-	-	0.97
	Sorghum	-	-	-	0.93
	Barley	-	-	-	0.93

[a]Hulled (H) or hull-less (HL)
[b]Soluble β-glucans for normal, waxy and high amylose barley types were 14, 22 and 12 g/kg, respectively.

Enhanced starch digestibility of barley-based diets in response to the supplemental β-glucanase has been commonly observed in studies with broilers (Almirall *et al*., 1995; Bergh *et al*., 1999; Ravindran *et al*., 2007). According to Ravindran *et al*. (2007), magnitude of improvement in starch digestibility varied depending on barley type and was markedly greater in waxy genotypes (41 and 73%) compared to the normal starch genotypes (18 and 15%). Owing to the lack of sensitivity of starch digestibility to the digesta viscosity, it was hypothesised that that the effect of enzymes on starch digestion is not only through the reduction of intestinal digesta viscosity (Carré, 2004). With the recent finding by Andriotis *et al*. (2016) that endosperm cell wall degradation is an important determinant of the starch degradation rate in barley grains, it can be speculated that the supplemental carbohydrases enhance the starch digestibility primarily by

breaking down the barley endosperm cell walls and releasing the encapsulated starch granules.

Ankrah *et al*. (1999) reported enhanced starch digestibility in reground pellets compared to mash (0.860 vs. 0.774) in broilers fed barley-based diets, irrespective of the starch type and enzyme supplementation. Hetland *et al*. (2002) reported enhanced starch digestibility in response to replacing ground barley with whole barley. The limited number of studies evaluating the influence of different feed processing parameters on starch digestibility of barley-based diets is discussed in section 2.4.3. The fact that feed processing techniques can have variable outcomes on starch digestibility depending on the grain type (Zaefarian *et al*., 2015) warrants further studies evaluating the impact of different processing parameters such as barley particle size and thermal treatment on starch digestion in barley-fed broilers.

2.3.4.3. Fat

Increased intestinal digesta viscosity in birds fed barley-based diets has been reported to be more detrimental to fat digestion (Edney *et al*., 1989; Almirall *et al*., 1995), making fat digestion to be most affected by the presence of S.NSP in the diet (Choct and Annison, 1992a). High digesta viscosity results in difficult diffusion and passage of droplets of emulsion, fatty acids, mixed micelles, bile salts and lipase within the gastrointestinal tract, leading to reduced transport of micelles to the mucosal surface (Smulikowska, 1998; Smulikowska *et al*., 2002). Martinez *et al*. (1992) suggested that in addition to S.NSP, fat-soluble tocotrienol (subclass of vitamin E) present in barley can inhibit the cholesterol synthesis exacerbating the bile acid shortage created by S.NSP. In addition to the adverse impact by higher intestinal digesta viscosity, stimulation of gut microbial growth (Salih *et al*., 1991; Viveros *et al*., 1994) leading to higher bacterial activity may reduce the recycling of bile acids and the resultant low concentration of bile salts in birds fed barley-based diets, leading to poor digestibility of fat.

Bergh *et al*. (1999), who compared three hulled barley types differed in starch type, reported no differences in ileal fat digestibility between barley types, despite the different amounts of S.NSP. However, supplementation of β-glucanase enhanced the digestibility of fat with the greatest magnitude of response observed for waxy barley types. Friesen *et al*. (1992) evaluated the impact of increasing inclusion of hulled and

hull-less barley cultivars in a wheat-based diet (on a w/w basis and similar fat inclusion) and reported decreasing fat digestibility only in broilers fed hull-less barley. The depressed fat digestibility was, however, restored with supplemented carbohydrases.

Indicating an age-related variation in fat digestibility in broilers fed barley-based diets, Viveros *et al*. (1994) reported a lower fat digestibility in 12-d old broilers compared to 28-d old broilers (73.2 vs. 83.2%). Limited production of lipase (Al-Marzooqi and Leeson, 2000) and bile salts (Viveros *et al*., 1994) that causes lower fat digestibility has been reported in broiler starters fed barley-based diets. Supplemental β-glucanase to barley-based diet elevated the lipase activity in both broiler starters and adult roosters with a greater magnitude in the young birds (Almirall *et al*., 1995).

The nutrient digestibility response to barley inclusion in broiler diets seems to be nutrient-dependent due to variable sensitivity of nutrients to digesta viscosity, the storage location of nutrients and interactions with other nutrients. Determination of the rate and extent of nutrient digestion in barley-based diets will enable the manipulation of diet formulation, feed processing practices and strategic determinations of enzyme dosages to achieve the optimum inclusion of barley in poultry diets.

2.3.5. Morphology

Greater digesta viscosity can cause significant influence on the intestinal morphometry of birds fed barley-based diets. Viveros *et al*. (1994) reported shortening, thickening and atrophy of villi, and increased number (hypertrophy) and size (hyperplasia) of goblet cells in jejunum of birds fed barley-based diets (600 g/kg) compared to those fed maize-based diets. These effects were minimised, however, by supplementation with β-glucanases. Onderci *et al*. (2008) also reported shorter and narrower villus in birds fed barley- compared to maize-based diets. Shakouri *et al*. (2009) reported decreased jejunal villus height and villus: crypt ratio in birds fed diets with 600 g barley/kg compared to the diets containing maize, wheat and sorghum (623 g grain/kg). Kalantar *et al*. (2016) observed shorter villus height in birds fed diets with barley included as low as 150 g/kg in a maize-based diet. The poor growth performance of broilers fed barley compared to other grain types was attributed to alterations of intestinal morphology induced by barley inclusion (Viveros *et al*., 1994; Yasar and Forbes, 1999; Mathlouthi *et al*., 2002). Comparative studies based on different barley cultivars on intestinal morphometry are limited.

Comparing barley with wheat for relative lengths and weights of the GIT segments, birds fed barley-based diets were reported to have longer duodenum, jejunum, ileum and caeca and lighter gizzard than those birds fed wheat-based diets (Brenes *et al*., 1993). While supplemental enzymes did not impact the gut morphometry of birds fed wheat-based diets, it reduced the lengths of intestinal segments in barley-fed birds. Comparing two hull-less and hulled barley cultivars, heavier proventriculus and gizzard and shorter jejunum and ileum were reported in birds fed hulled barley than those fed hull-less cultivar (Brenes *et al*., 1993). As discussed in section 2.2.2.3.1, the I.NSP from hulled barley can facilitate gizzard development causing a substantial impact on nutrient utilisation.

2.3.6. Welfare

Incorporation of viscous cereals such as rye, barley, triticale and wheat into the poultry diets have been associated with litter problems caused by elevated excreta moisture or increased occurrence of sticky droppings. Roberts *et al*. (1998) compared the effect of sorghum, barley, wheat and triticale, on excreta moisture content in laying hens and reported that barley diets resulted in the wettest litter (77.5 vs. 74.5% moisture, on average), a finding primarily attributed to increased digesta viscosity of birds fed barley-based diets that lowers the water absorption and thus increases water loss through the excreta. This situation has led to welfare and management problems in barley-fed birds. Dirty eggs in layers and breast muscle damage in broilers resulting from sticky droppings reduce the marketability of eggs and chicken meat (Gohl *et al*., 1978; Chesson, 1992, 1993; McNab and Smithard, 1992; Classen, 1996). The occurrence of foot pad dermatitis (FPD) can also be encouraged by moist litter and is considered as a major welfare issue in birds fed barley-based diets. Moreover, increasing litter moisture caused by the sticky droppings can reduce the air quality of the poultry house (Jacob and Pescatore, 2012). Francesch *et al*. (1989, cited in Francesch and Brufau, 2004) reported increasing water consumption and the incidence of sticky droppings in response to increasing inclusion of barley in the diet whereas the effects of barley inclusion were diminished with the supplemental enzymes.

2.3.6.1. Foot pad dermatitis

Foot pad dermatitis is a disease characterised by necrotic lesions on the plantar surface of feet in growing broilers and turkeys. The FPD can impair the health and productivity of birds and reduce the quality of chicken feet as human food resulting in economic losses (Mayne *et al*., 2007; Cengiz *et al*., 2012). Litter moisture less than 30% is usually recommended as optimal for footpad health (Mayne *et al*., 2007). The major cause of FPD is the wet litter which can depend on diverse factors such as composition of diet, sex, breed, body weight, initial litter moisture, environmental temperature, stocking density and litter type. Among these factors, the composition of diet plays a major role in grains with high levels of NSP such as barley. The occurrence of sticky droppings due to highly viscous digesta in barley-fed broilers, and the continuous sticking of excreta can deteriorate the epidermis and keratin layers in the footpad causing FPD (Shepherd and Fairchild, 2010).

Cengiz *et al*. (2012) evaluated barley inclusion at 250 g/kg in a maize-based diet, without and with enzyme supplementation, on FPD in broiler chickens exposed to early high-moisture litter from d 1 to 5, and reported no influence of barley inclusion on development of FPD, litter moisture level, or litter pH. In a follow-up study, Cengiz *et al*. (2017) provided hulled barley at 300 g/kg in a maize-based diet and observed high litter moisture (32 vs. 19%), high incidence and severity of FPD in barley-fed birds in comparison to the birds fed maize-based diets at 42 d of age. The occurrence of FPD, however, cannot be solely attributed to the inclusion of NSP rich ingredients in the diet and, seemed to be influenced by the litter properties and management conditions as well. Predispose factors created by inclusion of barley in the diets can be managed through proper management practices and dietary modifications. However, literature on the efficacy of nutritional approaches on the litter quality and FPD incidence are inconsistent.

2.3.6.2. Diseases

Barley β-glucans can modify the intestinal microflora composition leading to increased susceptibility to disease (Jacob and Pescatore, 2014). Chickens fed barley-based diets have been reported with an increased incidence of necrotic enteritis associated with increased levels of *Clostridium perfringens*. Kaldhusdal and Hofshagen (1992) reported a higher occurrence of sub-clinical necrotic enteritis and associated depression in growth

rate in birds on a diet containing 270 g/kg barley in an oat-wheat-based diet, compared to birds fed 360 g/kg maize in the same oat-wheat-based diet. Riddell and Kong (1992) challenged broiler chickens at 18 d of age with *C. perfringens* and fed diets based on maize, wheat, rye and barley up to 42 d of age. While zero mortality occurred in birds fed maize-based diets, a 26.7% mortality was recorded in birds fed either wheat, rye or barley diets. It is reasonable to assume that a slower passage rate caused by high intestinal viscosity can facilitate the colonisation of potentially pathogenic bacteria (Yegani and Korver, 2008), deteriorating the health of barley-fed birds.

2.3.7. Bird age

Bird age is a determining factor for feeding value of barley primarily because of the influence on intestinal digesta viscosity. The changes in digesta viscosity of birds fed barley-based diets in response to birds' age are discussed in section 2.3.1. The reduction in digesta viscosity with increasing birds' age suggests that impact of barley antinutrients seem to be age-dependent due to the changes in birds' digestive system. According to Almirall *et al*. (1995), the limited production of pancreatic enzymes and limited functionality of digestive enzymes are disturbed by intestinal viscosity in young birds. However, when diets were supplemented with enzymes, young birds had a greater response to β-glucanase (Almirall *et al*., 1995). It has been suggested that mature birds have a sufficiently developed GIT to counteract the negative effects of the β-glucans (Salih *et al*., 1991; Almirall and Esteve-Garcia, 1994). Petersen *et al*. (1999) speculated that the decrease in foregut viscosity with broilers age may be a consequence of acclimatisation to diet, while the reduction of digesta viscosity in the hindgut with age can be attributed to an alteration in the intestinal microflora composition.

Salih *et al*. (1991) reported that WG and feed efficiency of broilers fed three different diets (wheat control, hull-less barley and enzyme supplemented hull-less barley) were not influenced beyond four weeks of age. Viveros *et al*. (1994) also reported lower fat and starch digestibility in 12 d-old-broilers compared to 28 d-old broilers fed barley-based diets. These observations highlight the importance of considering bird age when determining the optimum barley inclusion and enzyme dosage rates in broiler diets as an important factor that can influence digestibility and performance responses in barley-based diets.

2.3.8. Recommended inclusion of barley in poultry diets

A wide range of inclusion levels of barley has been recommended for broiler diets. However, recommendations on the optimum inclusion of barley have been contradictory due to confounding factors such as starch type, presence of hull and cultivar differences, being overlooked in most previous studies. As shown in Table 2.9, most studies have replaced other cereals with barley either on a weight to weight basis (Arscott *et al*., 1955; Petersen, 1969; Moss *et al*., 1983; Yu *et al*., 1998) or by using nutrient composition data for barley and the substituted grain from established data sources such as National Research Council (Moharrery, 2006) and tables published by Spanish Foundation for the Development of Animal Nutrition (FEDNA; de Blas *et al*., 2010; Lázaro *et al*., 2003), or chemical analysis (Brake at al., 1997). There are apparently no studies where barley-based diets were formulated using accurate nutrient profiles specific to the barley cultivar based on AMEn and digestible AA contents determined in assays using broilers.

According to previous studies, Arscott *et al*. (1955) suggest that barley can be included in broiler diets up to 153 g/kg without affecting growth performance. Jeroch and Danicke (1995) recommended up to 200-300 g barley/kg for broiler finishers. Brake *et al*. (1997) suggested that 200 g barley/kg can be included in both broiler grower and finisher diets without compromising growth, feed efficiency or litter conditions. According to Yu *et al*. (1998) and Bergh *et al*. (1999), 140 g barley/kg can be included in β-glucanase supplemented broiler diets. This discrepancy of recommendations for barley inclusion in broiler diets can be partly attributed to the lack of characterisation of tested barley types and inconsistency of research methodology, as shown in Table 2.9.

Table 2. 9. Comparison of previous studies evaluating barley inclusion in broiler diets.

Reference	Barley type		Replaced or compared with	Inclusion levels of barley (g/kg diet)	Method of determination				Diets are balanced for	
	Starch type	H/HL/ DH[a]			Weight-to-weight basis	Grain chemical composition	Table values	Digestible AA[b]	Energy	Protein
Moss *et al*. (1983)	Waxy	H	Wheat	Starter,0, 272, 408 and 544; Grower, 0, 323, 485, 646	Yes	No	No	No	No	No
	Normal	H			Yes	No	No	No	No	No
Friesen *et al*. (1992)	-	H	Wheat	0,350,700	Yes	No	No	No	No	No
	-	HL	Wheat	0,350,700	Yes	No	No	No	No	Yes
Fuente *et al*. (1995)	-	-	Maize	300,400, 500, 600	Yes	Yes	No	No	No	No
Yu *et al*. (1998)	-	-	Maize	0, 70, 140, 278, 557	No	No	Yes	No	Yes	Yes
				0, 79, 157, 314, 627				No	Yes	Yes
Yu *et al*. (2002)	-	DH	Maize	0, 400, 800	Yes	Yes	No	No	Yes	Yes
Shakouri *et al*. (2009)	-	-	Wheat, Maize, Sorghum	600.2	No	No	Yes	No	Yes	Yes
Tang *et al*. (2017)	-	-	Wheat, Maize, Sorghum	Starter diet, 652; Finisher diet,669	No	No	Yes	No	Yes	Yes

[a]Hulled (H), hull-less (HL), de-hulled (DH).
[b]Using digestible amino acid contents.

The nutritive value of grains for poultry is determined not only by the chemical and physical properties of grains but also by the interactions of ingestion, digestion, absorption, and metabolism in birds (Hughes and Choct, 1999). As discussed in this section, a minor change in NSP content and composition can have a substantial impact on performance and nutrient utilisation of birds with a considerable variation between barley types. In order to minimise the impact of barley variation and to meet birds' nutrient requirements based on their nutrient utilisation capacity, the use of grain-specific metabolisable energy and digestible nutrients, in particular AA contents in formulating barley-based diets, therefore, can be strongly recommended.

2.4. Measures to overcome the limitations of barley in poultry diets

With growing knowledge of physical and chemical characteristics of barley grain and mechanisms of anti-nutritive action, measures to minimise or eliminate the anti-nutritive impact of barley NSP in poultry diets have evolved over the years. These measures can be categorised as (i) morphological and compositional changes in barley grains using genetic selection and breeding (ii) regulation of NSP-induced anti-nutritive conditions by feed additives and (iii) physical manipulations of barley grains by feed processing methods. This section intends to provide a comprehensive review of each measure highlighting the specific objectives, mechanisms and outcomes.

2.4.1. Genetic development

2.4.1.1. Hull-less barley

The established perception around the 1970s that the fibrous hull of barley had a significant anti-nutritive influence on digestible energy in poultry feeding (Bhatty *et al*., 1975) led to the development of hull-less barley and raised the acceptance of barley as poultry feed ingredient (Bhatty, 1999; Jacob and Pescatore, 2012). Use of hull-less over hulled barley in poultry feed eliminates the cost and labour associated with dehulling, resulting in a cereal that is more compatible with nutrient-dense feeds preferred by the poultry industry (Campbell *et al*., 1993).

As discussed in section 2.2.2., both hulled and hull-less barley types have been reported with variable amounts of nutrients suggesting an inconsistent effect by the hull type on nutrient content. Nevertheless, constant lower concentrations of I.NSP in hull-

less barley compared to hulled barley have been reported in different studies (Oscarsson *et al*., 1996; Ravindran *et al*., 2007), which eventually equalised hull-less barley to wheat, in terms of fibre content (Li *et al*., 1996).

As shown in Table 2.3, different β-glucan contents have been reported for hull-less varieties indicating the influence of factors other than presence of absence of hull. Moreover, majority of these studies have neglected the other physico-chemical differences, such as starch type, associated with different hull-less barley cultivars. Ravindran *et al*. (2007) emphasised the need for considering the characteristics of starch and β-glucan content over the fibre content, when selecting barley cultivars for poultry diets.

With the recent recognition on value of fibre in poultry diets to restore the gut integrity of birds fed highly processed diets, the tendency is to incorporate insoluble and functional fibre, such as hulls, into the poultry diets. The impact of barley hulls on gizzard development has been discussed in the literature (Sacranie *et al*., 2012; Adibmoradi *et al*., 2016). Instead of separate hull inclusion, direct use of hulled barley in poultry diets can be considered as a cost- and labour-effective approach.

2.4.1.2. Waxy-starch and high amylose-starch barley

In addition to the conventional barley composed of normal starch (650-840 g/kg amylopectin), both hulled and hull-less barley have been developed into waxy (850-1000 g/kg amylopectin) and high amylose (450 g/kg amylose; 550 g/kg amylopectin) barley types (Ullrich *et al*., 1986; Tester *et al*., 2004). These cultivars vary not only in starch composition but also with morphology and physico-chemical characteristics of starch granule, as discussed in section 2.2.2.1.

From a poultry nutrition perspective, development of waxy starch barley was considered advantageous primarily for starch digestion. According to *in vitro* enzyme hydrolysis of barley starches, waxy barley starch has a higher susceptibility to α-amylase, compared to normal or high amylose barley starch (Björck *et al*., 1990; Li *et al*., 2004a). However, when analysed *in vivo*, waxy barley-based diets were reported with a lower starch digestibility (Table 2.8, Bergh *et al*., 1999; Ravindran *et al*., 2007). In addition, as discussed in section 2.2.2.1., birds fed waxy starch barley diets had a poor growth performance compared to those fed other barley types (Bergh *et al*., 1999). The impaired

growth performance and nutrient utilisation in birds fed waxy starch barley has been attributed to soluble β-glucan with high molecular weights, which occur in greater amounts in waxy starch barley types (Storsley *et al*., 2003).

Nevertheless, waxy starch barley benefits the feed production in pellet form due to lower starch gelatinisation temperature, resulting higher physical pellet quality and reduced energy input in pellet production. According to Ankrah *et al*. (1999), equivalent pellet hardness in waxy starch hull-less barley was achieved at a lower temperature (by 14.2 °C) than in normal starch hull-less barley. However, waxy starch barley, with higher soluble β-glucan content, also increased digesta viscosity compared to the normal starch barley. With a comparatively greater efficacy in waxy starch barley types (Table 2.10), exogenous enzymes are proven to mitigate the anti-nutritive effects of soluble NSP, making waxy starch barley an attractive feed ingredient for poultry.

New barley varieties with different starch types, varying β-glucan content and diverse solubility characters are being continuously developed to minimise the anti-nutritional effects of barley-based diets. However, large variations in the chemical and physical characteristics due probably to genetic and climatic factors, maturity stage and the storage time of barley grain (Jeroch and Dänicke, 1995; Hughes and Choct, 1999; Helm and Francisco, 2004) exist even in similar cultivars (Izydorczyk *et al*., 2000; Black *et al*., 2005). Due to this variability, Hughes and Choct (1999) highlighted the need for an assessment of nutritive value prior to incorporation of barley in poultry diets and deciding the type of treatment to be implemented with barley-based diets. Yu *et al*. (1998) proposed to measure the β-glucan content in the barley or in the poultry diet prior to the determination of enzyme dosage.

2.4.2. Feed enzymes

With the developing knowledge on the anti-nutritive impact of barley NSP in poultry diets, the research on the use of feed enzymes in barley-based diets has evolved over the years. Initially, supplementation of amylolytic enzymes to barley-based broiler diets was reported to be effective in reducing the sticky droppings and enhancing the growth performance (Fry *et al*., 1958; Arscott and Rose, 1960; Rose and Arscott, 1962). At this time, only rudimentary knowledge was available on substrate specificity of exogenous enzymes. However, with the finding by Burnett (1966) that viscous β-glucans present in

barley is the main reason for its low nutritive value, the observed improvement in birds fed barley-based diets by amylolytic enzyme product was attributed to a contaminant side activity of β-glucanase and its action of reducing digesta viscosity (O'Neill *et al*., 2014).

Following this recognition (Gohl *et al*., 1978; Hesselman *et al*., 1982), the first β-glucanase for barley-based poultry diets was commercialised in 1984 (Danisco Animal Nutrition, 2014). When supplementing barley-based diets with exogenous enzyme, the rule of thumb adopted by the poultry industry was "barley + β-glucanase = wheat" (Sheppy, 2001). Currently, almost all barley-based broiler diets worldwide are supplemented with glycanases (xylanases and β-glucanases; Ravindran, 2013). Three major modes of action of NSP-degrading enzymes have been recognised in the literature; (i) reduction of digesta viscosity via partial depolymerisation of NSP (Almirall *et al*., 1995), (ii) releasing the encapsulated nutrients via cell wall degradation (Hesselman and Åman, 1986; Bedford, 1996) and, (iii) improving the gut microflora through the supply of prebiotic oligosaccharides (González-Ortiz *et al*., 2017; Bedford, 2018). However, the improvement in growth performance and nutrient utilisation in response to the supplementation of carbohydrase in barley-based diets has been commonly attributed to the viscosity reduction caused by the partial degradation of S.NSP (Almirall *et al*., 1995; Ankrah *et al*., 1999). Moreover, NSP-degrading enzymes can degrade endosperm cell walls, enabling more rapid access of endogenous proteases and amylases to the previously encapsulated protein and starch (Hesselman and Åman, 1986; Bedford, 1996, 2018). Supporting this hypothesis of cell wall solubilising effects of added carbohydrase, Ravn *et al*. (2017) has shown the *in vitro* destruction of the cell walls taking place in barley by supplemental xylanase.

Depolymerisation of S.NSP by supplemental carbohydrases can generate fermentable oligosaccharides that can act as prebiotic compounds in the chicken GIT. Prebiotic oligosaccharides can encourage proliferation of beneficial bacteria such as *Lactobacillius* and *Bifidobacteria* (Józefiak *et al*., 2010; Rodriguez *et al*., 2012) preventing the growth of pathogenic bacteria such as *Escherichia coli* and *Salmonella* through competitive exclusion (Mathlouthi *et al*., 2002; Gabriel *et al*., 2006). Fermentation of oligosaccharides by caecal microbes stimulate the production of short-chain fatty acids that may contribute a certain amount of energy to the host bird (Jamroz *et al*., 2002). A substantial increase in *Bifidobacteria* counts in the caecal digesta (from

3.92 to 9.69 log cfu/ml of digesta; Józefiak *et al*., 2010) and 61% increase in lactic acid production in the crop (Józefiak *et al*., 2006) of broilers fed barley-based diet in response to β-glucanase supplementation has been reported. Suggesting the positive contribution towards nutrient utilisation, improved protein and fat digestibility due to supplemental carbohydrases in a wheat-barley-based diet, has been partly attributed to the reduction of total anaerobic bacteria (Mathlouthi *et al*., 2002).

As shown in Table 2.10, majority of studies with barley-based diets have confirmed the efficacy of dietary carbohydrase supplementation in enhancing the feeding value of barley for broilers through improved growth performance, enhanced nutrient utilisation and flock uniformity. In addition, supplemental carbohydrases minimise the variability in nutritional value of barley grains. Villamide *et al*. (1997) reported that supplementing a multi-enzyme containing β-glucanase, xylanase, and protease, reduced the range of AMEn by 23.9% by minimising the variability of AMEn in eight barley cultivars, with a greater effect on highly viscous barley types. Kocher *et al*. (1997) reported that variability of AME of 11 different barley cultivars was reduced by 55% due to supplemental β-glucanase.

Combinations of different exogenous enzymes have also been evaluated in barley-based diets (Table 2.10). Phytase has been commonly used in combination with carbohydrases in barley-based diets (Ravindran *et al*., 1999; Wu *et al*., 2004a). In addition to primary objectives of adding phytase to facilitate the release of phytate-bound P and to reduce the P effluents from intensive animal production (Ravindran, 1995), the supplementation of phytase to barley-based diets is justified by the fact that phytate is an integral part of barley cell wall matrix (Eeckhout and De Paepe, 1994). The combination of enzymes in barley-based diets is believed to facilitate each other's substrate access. Nevertheless, when a combination of different enzymes is used, the response of barley to enzyme mixtures is largely dependent on content of carbohydrase, especially β-glucanase, over other enzymes (Yin *et al*., 2001).

Table 2. 10. Response of growth performance, nutrient utilisation and intestinal digesta viscosity of broilers fed barley-based diets to supplemental enzymes.

Reference	Barley		Inclusion level (g/kg of diet)	Feed form (M/P)[3]	Components in carbohydrase (BG/XY)[4]	Phytase	Bird age (d)	Growth performance[5]			Nutrient utilisation[6]						Reduction in digesta viscosity (cP)
	Hull type[1]	Starch type[2]						WG (%)	FI (%)	F/G (points)	N (%)	Starch (%)	Fat (%)	P (%)	AME (%)	AMEn (%)	
Almirall *et al*. (1995)	-	-	600	M	BG	-	24	8.8	3.2	4	12.6	6.91	5.35	-	-	-	11
	-	-				-		13.2	6.0	3	16.6	2.01	3.14	-	-	-	26
Bergh *et al*. (1999)	H	N	696	M	BG + XY	-	13/ 18[7]	8.0	4.6	7	8.2	7.7	22.1	-	-	-	-
		HA				-		18.6	6.3	24	6.8	7.9	14.1	-	-	-	-
		W				-		17.6	9.3	21	6.9	12.6	23.4	-	-	-	-
Ankrah *et al*. (1999)	HL	N	610	M	BG	-	21	54.6	18.3	50	-	52.4	-	-	-	-	245
		N		P		-		37.6	5.6	50	-	29.6	-	-	-	-	91
		W		M		-		44.0	7.4	56	-	87.3	-	-	-	-	466
		W		P		-		51.5	19.4	45	-	21.3	-	-	-	-	267
Ravindran *et al*. (2007)	H	N	963	M	BG	-	28	-	-	-	17.3	17.9	-	-	-	9.2	-
	HL	N				-		-	-	-	20.7	15.2	-	-	-	5.5	-
		W				-		-	-	-	16.5	41.0	-	-	-	22.2	-
		W				-		-	-	-	14.7	73.0	-	-	-	23.1	-
Ravindran *et al*. (1999)	-	-	820	M	BG + XY	-	42	-	-	-	-	-	-	-	- 0.5	-	-
					-	+		-	-	-	-	-	-	-	2.7	-	-
					BG + XY	+		-	-	-	-	-	-	-	3.8	-	-
Wu *et al*. (2004a)	-	-	990	M	BG + XY	-	35	-	-	-	13.8	9.0		9.8	8.8	8.6	-
					-	+		-	-	-	10.8	5.6		23.0	7.8	7.4	-
					BG + XY	+		-	-	-	13.8	10.1		26.2	13.2	12.9	-

[1]Hulled (H) or hull-less (HL).
[2]Normal (N), high amylose (HA) or waxy (W).
[3]Mash (M) or pellets (P).
[4]β-glucanase (BG) or xylanase (XY).
[5]WG, weight gain; FI, feed intake; F/G, feed per gain.
[6]N, nitrogen; P, Phosphorus; AME, apparent metabolisable energy; AMEn, N-corrected AME.
[7]Growth performance determined at d 13. Nutrient utilisation and viscosity values determined at d 18.

The variable response to supplemental enzymes in birds fed barley-based diets (Table 2.10) can be attributed to the variable survival of enzymes during feed processing (Lamp *et al*., 2015), variations in barley nutritional composition mainly NSP and starch (Ravindran *et al*., 2007) and interactions with grain physical characteristics (e.g. particle size; Amerah *et al*., 2007a).

2.4.3. Feed processing

Different feed processing practices evaluated in barley-based diets primarily aim to liberate the encapsulated nutrients by modifying the physical characteristics of barley grain.

2.4.3.1. Particle size

Cereal grains are ground during feed manufacture to modify their physical characteristics by reducing the particle size. Grinding of whole grains can be categorised into three classes as fine, medium, and coarse according to the screen size in a hammer mill or distance between horizontal rollers in a roller mill that the grains are ground to pass through (Amerah *et al*., 2007a). Morel and Cottam (2007) achieved three different classes of barley particles by grinding barley through a hammer mill (7.0, 4.0 and 1.0 mm sieve openings for coarse, medium and fine grinds, respectively) and, reported average particle sizes of 1100 for coarse, 785 for medium, and 434 μm for fine grinds in barley-based pig diets. Fine grinding results in a greater surface area leading to greater substrate availability for enzymatic digestion and decreases segregation, ensuring the homogeneity of mixed feed. Coarse grinding, on the other hand, stimulates gizzard development and functionality, facilitating digestion of nutrients through enhanced grinding activity and gut motility (Amerah *et al*., 2007a).

The particle size of a milled product can be influenced by grain type and, grinding different grains in the same mill under similar conditions can result in different particle sizes due mainly to the variations in endosperm hardness (Amerah *et al*., 2007a). In accordance, it has been speculated that variation in barley kernel hardness is responsible for the differences in particle size distribution observed between hard and soft barley lines (Nair *et al*., 2011). Nair *et al*. (2011), compared the microscopic images of endosperm from hard and soft-hulled spring barley lines and reported thicker endosperm cell walls

in hard barley lines. Moreover, Gamlath *et al*. (2008) reported that both β-glucan and arabinoxylan in barley endosperm positively correlated with kernel hardness. It is therefore reasonable to speculate that barley NSP may indirectly influence the particle size distribution in different barley types.

Al-Rabadi *et al*. (2012), comparing ground barley fractions for *in vitro* starch digestion (Figure 2.7), reported that the extent of starch digestion varied between different barley particles sizes. Barley particles < 1.0 mm achieved a complete starch digestion (1.00) after 24 h, as confirmed by Fig. 2.7a showing the residual endosperm structure with holes where starch granules used to be. Figure 2.7b with 10-15 µm partly-digested granules showing amylase-mediated pits and channels confirms the incomplete starch digestion (0.63) in barley particles > 1.0 mm. However, these results obtained from *in vitro* studies may not be totally applicable to *in vivo* conditions due to the absence of the effects caused by NSP-induced digesta viscosity and mechanical grinding in gizzards.

The grinding extent of barley has been compared with other physical manipulations such as whole barley feeding, pelleting and grit supplementation (Mcintosh *et al*., 1962; Svihus *et al*., 1997a) in poultry diets. However, there are no studies comparing the effect of different particle sizes of barley on broiler performance and nutrient digestibility to determine the optimum barley particle size in poultry diets.

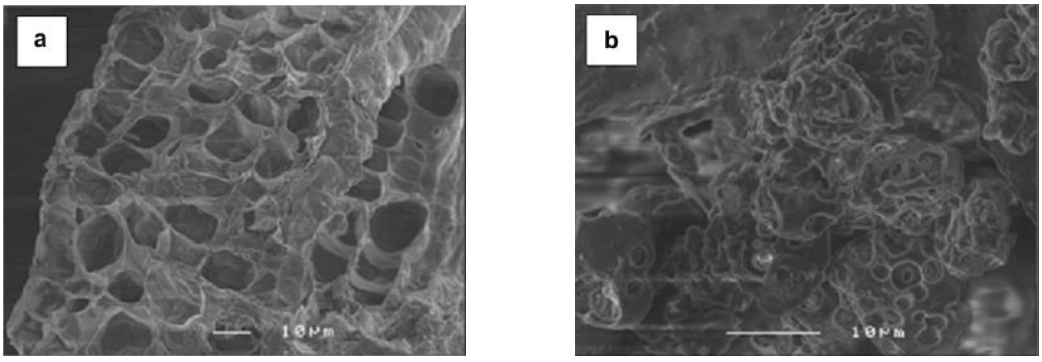


Figure 2.7. (a) Complete starch digestion in 0.125 mm barley particles (residual endosperm structure with holes where starch granules used to be) (b) Incomplete starch digestion in 1.0 mm barley particles (10-15 µm partly digested granules showing amylase-mediated pits and channels). Source: Al-Rabadi *et al*. (2012)

2.4.3.2. Feed form

Supporting the established fact that pelleting enhances the economics of production by improving the growth and feed efficiency responses in broilers (Abdollahi *et al*., 2013a), pelleted barley-based diets have been also reported to improve growth performance over mash diets (Arscott *et al*., 1957; Al Bustany, 1996; Lamp *et al*., 2015). Al Bustany (1996) reported that pelleting a barley-based diet (500 g barley and 200 g maize/kg of diet) enhanced BW, FI and feed efficiency of 21-d broilers by 36 g/bird, 40 g/bird and 6 points, respectively. Comparing barley-based diets (450 g/kg) fed as either unprocessed mash or ground pellets, Lamp *et al*. (2015) reported that broilers (d 21) fed ground pellets resulted in greater WG (611 vs. 665 g/bird) and FI (879 vs. 954 g/bird) compared to the birds fed unprocessed mash diets. The feed efficiency, however, was not affected by the feed form. Ankrah *et al*. (1999) reported no effect of pelleting of either normal or waxy starch hull-less barley on growth performance of 21-d old broilers. The discrepancies in the extent of growth performance responses of broilers fed barley-based diets to the feed form presumably be driven by, *inter alia,* the variability in barley types and different conditions employed during the pelleting process.

Physical stress of pelleting can break the cell walls releasing the encapsulated nutrients leading to a greater accessibility by digestive enzymes. In agreement, Ankrah *et al*. (1999) reported a 17% increase in starch digestibility in broilers fed barley-based diets as a result of pelleting. Conversely, no effect of feed from on AA digestibility (Barua *et al*., 2019) or AMEn, DM or N retention (Khalil *et al*., 2019) in broilers fed barley-based diets has been reported.

It has been hypothesised that pelleting can increase soluble carbohydrate concentrations or change the molecular weight of S.NSP, leading to an increase in digesta viscosity (Abdollahi *et al*., 2013a). Lending support to that thesis, Al Bustany (1996) reported that pelleting a barley-based diet increased the occurrence of sticky droppings of broilers (d 1-7) by 223%, due probably to an increase in digesta viscosity. However, Lamp *et al*. (2015) reported no difference in digesta viscosity in broilers fed barley-based diets either as unprocessed mash or ground pellets. Ankrah *et al*. (1999) reported 45% reduction in viscosity of barley after pelleting, an observation that was attributed to the shearing effect of the pelleting process that facilitated β-glucan degradation.

2.4.3.3. Heat processing

Different heat processing methods such as autoclaving (Classen *et al*., 1985; Campbell *et al*., 1986; Viveros *et al*., 1994), steam-cooking (Gracia *et al*., 2003), steam-conditioning (Al Bustany, 1996; Lamp *et al*., 2015), expansion, micronisation (García *et al*., 2008) and extrusion (Vranjes and Wenk, 1995) have been evaluated to enhance the feeding value of barley in poultry diets. Heat processing is believed to disrupt the cell structures and to release the encapsulated nutrients (Gracia *et al*., 2003; García *et al*., 2008) facilitating the nutrient utilisation. However, thermal processing can increase the solubilisation of NSP in cereal grains (Silversides and Bedford, 1999), leading to higher viscosity in both feed and intestinal contents (Svihus *et al*., 2000; García *et al*., 2008) with an exacerbated effect on diets based on viscous grains such as barley (Cowieson *et al*., 2005). In addition, other common drawbacks of employing extreme heat treatments such as; formation of resistant starch (Abdollahi *et al*., 2010b, 2011), degradation of heat-labile AA (Papadopoulos, 1989), inactivation of synthetic vitamins (Jensen, 2000) and exogenous enzymes (Inborr and Bedford, 1994) also apply to cereal-based diets.

Impaired WG, feed efficiency and nutrient utilisation in birds fed autoclaved barley (121 °C for 20 min) compared to those fed non-treated barley has been reported in the literature (Classen *et al*., 1985; Campbell *et al*., 1986). According to Vranjes and Wenk (1995), feeding extruded barley deteriorated F/G and dietary AME in broilers by 3.9 points and 0.82 MJ/kg, respectively. These researchers reported an increased viscosity of barley extract (1.3 vs. 3.7 cP) due to an increase in concentrations of S.NSP (28.4 vs. 36.2 g/kg) induced by extrusion (120-130 °C for 20 s). In contrast, applying comparatively mild conditions, Viveros *et al*. (1994) demonstrated that autoclaving (70 and 90 °C for 10 mins) of enzyme-supplemented barley-based diet improved the growth performance of young broilers compared to the unprocessed control diet.

Gracia *et al*. (2003), using broiler starters (d 1-21), evaluated steam-cooked barley grains in mash diets, without or with a multi-component enzyme. An interaction between steam cooking (99 ± 2° C for 50 mins) and enzyme addition was reported for intestinal digesta viscosity with a greater response to enzyme in steam-cooked barley. Broilers fed steam-cooked barley grew faster than broilers fed unprocessed barley only up to 8 d of age. The F/G of broilers fed steam-cooked barley at 21 d of age was deteriorated by 8

points due likely to the 82% increase in intestinal digesta viscosity of broilers due to steam-cooking.

García *et al*. (2008) reported that heat processing of barley increased the intestinal digesta viscosity at 7-d of age resulting 270, 121, and 89 cP for micronised, expanded, and non-processed barley, respectively. The effect of heat processing on intestinal digesta viscosity, however, disappeared at d 42 resulting 11, 6, and 11 cP for micronised, expanded, and non-processed barley, respectively. Micronisation and expansion, however, improved the NSP digestibility by 14.5 and 27.8%, respectively, confirming the heat induced NSP solubilisation. Comparing two heat processing methods, birds fed micronised barley gained less weight and had poorer F/G than broilers fed expanded barley, suggesting that micronisation might have a more severe impact on barley compared to the mild heating by expansion. Moreover, benefits of heat processing on barley seemed to be limited to broilers' first week of age (Viveros *et al*., 1994; Gracia *et al*.; 2003; García *et al*., 2008).

Inborr and Bedford (1994) reported that WG and feed efficiency in broilers decreased following conditioning a barley-based diet at 95 °C compared to diets conditioned at 75 and 85 °C. Samarasinghe *et al*. (2000) reported that conditioning temperature of 90 °C compared to 60 °C, in a non-supplemented barley-maize-soybean meal diet numerically impaired WG, daily FI and F/G of broilers (d 7-21). Moreover, conditioning non-supplemented barley-maize-soy diet at 75 and 90 °C increased the dietary viscosity by 0.11 and 0.29 cP, respectively, compared to the diet conditioned at 60 °C.

While most studies have compared different methods of heat processing, studies evaluating the optimum pelleting conditions for barley-based diets are limited (Inborr and Bedford, 1994; Samarasinghe *et al*., 2000). Based on the limited available literature, it can be hypothesised that the conditions (heat, moisture and mechanical pressure) applied during the heat processing, rather than the heat processing method are of higher importance in barley-based diets. It, therefore, necessitates the determination of optimum conditions for each heat treatment, particularly pelleting process, used for manufacturing barley-based broiler diets. Moreover, thermal processing conditions can also interact with exogenous carbohydrases in barley-based diets, due to high temperature-induced

viscosity increase and partial inactivation of enzymes during heat processing (Inborr and Bedford, 1994; Gracia *et al*., 2003). A better understanding of the interactions between exogenous enzymes and heat processing conditions, particularly on intestinal digesta viscosity and nutrient utilisation, is vital to minimise the viscosity related negative consequences and to facilitate increased use of barley in contemporary highly processed poultry diets.

2.4.3.4. Whole barley feeding

Feeding whole grain has traditionally been a part of backyard poultry operations. The importance of whole grains in poultry nutrition has been recognised due to its benefits associated with a better developed and more functional gizzard. Moreover, whole grain feeding can lower the feed milling cost and enhance the gut integrity of broilers fed highly processed diets. Different methods of whole-grain feeding have been reported in the literature as extensively reviewed in Singh *et al*. (2014).

Wheat was usually considered as the whole grain of choice, and barley has been used as an alternative only when the cost or supply discourages the use of wheat (Singh *et al*., 2014). Whole barley has been recognised less preferred in free-choice feeding method when chickens were offered alternatives (Adret-Hausberger and Cumming, 1985). Nevertheless, barley has been used in mixed feeding method, for its greater impact on gizzard development compared to other whole grain types (Biggs and Parsons, 2009). Whole barley has been investigated in maize- (Nahas and Lefrancois, 2001; Biggs and Parsons, 2009), wheat- (Hetland *et al*., 2002; Moss *et al*., 2017) and sorghum- (Taylor and Jones, 2004; Moss *et al*., 2017) based diets for determination of optimum inclusion level and possible interactions with supplemental enzymes (Svihus *et al*., 1997a,b; Moss *et al*., 2017).

Reduced incidence of dilated proventriculus in response to whole barley has been evident (Taylor and Jones, 2004; Moss *et al*., 2017), confirming barley potential for enhancing gut integrity. Even though enhanced gizzard development and functionality is the motivation for whole grain feeding, the effect of whole barley on gizzard development seemed to be inconsistent. While a majority of studies (Svihus *et al*., 1997a; Hetland *et al*., 2002; Taylor and Jones, 2004; Moss *et al*., 2017) reported increased gizzard weight in response to replacing ground grain fractions with whole barley, Nahas and Lefrancois

(2001) reported no effect of whole barley inclusion on gizzard development. Furthermore, gizzard development response to whole barley can be confounded by the inclusion level, type, quality and hardness of the grain, age of birds, and whole grain feeding method. Nevertheless, with no difference in duodenal particle size distribution in broilers fed whole vs. ground barley-based diets, Svihus *et al*. (1997a) suggested the better grinding function by well-developed gizzards in broilers fed whole barley.

The effect of whole barley feeding on growth performance has been contradictory. Hetland *et al*. (2002) reported that both WG and FI were impaired in broilers offered whole barley in wheat-based diets at inclusion levels of 125, 300 and 440 g/kg. Moss *et al*. (2017) reported that post-pelleting inclusion of whole barley depressed WG by 74 g/bird, FI by 48 g/bird and FCR by 3.2 points compared to the ground barley fed birds at 28-d of age. In contrast, higher WG (744 vs. 693) and FI (1113 vs. 1037) in birds fed whole barley diets compared to those fed ground barley diets was reported by Svihus *et al*. (1997a). The F/G, however, was not affected by the form of barley. According to Nahas and Lefrancois (2001), inclusion of whole barley (150 and 200 g/kg in the grower and finisher diets, respectively) in an enzyme supplemented maize-based diet, improved the WG and FI of broilers by 83 and 126 g/bird, respectively, compared to a non-supplemented maize-based diet without whole barley. Moreover, the inclusion of 150 g/kg whole barley in non-supplemented maize-based diet enhanced the F/G by 1.9 points, confirming the beneficial effects of whole barley inclusion in conventional maize-soybean broiler diets. However, Biggs and Parsons (2009) reported similar WG in 21-d old broilers fed 100 and 200 g/kg whole barley to those fed a ground maize-soybean mash diet. The discrepancy in growth responses has resulted in varying whole barley inclusion levels being recommended for broiler diets. An inclusion of 300 g/kg (Hetland *et al*., 2002) and 350 g/kg (Bennett *et al*., 2002) of whole barley in broiler diets has been suggested without any adverse effects on bird performance. However, Nahas and Lefrancois (2001) recommended a lower inclusion of up to 200 g/kg whole barley as an optimum level.

The beneficial effects of whole barley feeding on gizzard development favourably influence the nutrient utilisation of birds. The enhanced starch digestibility (0.96 vs. 0.92) reported by Hetland *et al*. (2002) in response to replacing ground barley with whole barley (440 g/kg) was attributed to 79.0% increase in relative gizzard weight (34 vs. 19 g/kg).

Moss *et al*. (2017) also reported a 1.05% increase in ileal starch digestibility parallel to the 20.7% increase in the relative gizzard weight in broilers fed 125 g/kg whole barley.

Density of whole barley grain can prevent the proper mixing with concentrate portion of the mash feed and consequently induce segregation in the mixed feed. When whole barley is added post-pelleting, separation and floating of whole barley on the top of the feed bins can result in incomplete distribution. Moreover, whole barley from awned cultivars can be hazardous to young broilers resulting in perforation or impaction of the crop (Singh *et al*., 2014). However, these limitations can probably be avoided by pre-pelleting inclusion of whole barley, with whole barley cracked and embedded in intact pellets. The possible interactions of whole barley feeding with supplemental enzymes (Moss *et al*., 2017), particularly carbohydrases that are commonly added to barley-based diets, merits further investigation.

2.4.4. Other strategies to enhance barley nutritional value

Different pre-treatments such as soaking (Fry *et al*., 1958) and germination (Fengler *et al*., 1990; Svihus *et al*., 1997b; Afsharmanesh *et al*., 2013) have been investigated as possible strategies to enhance the nutritional value of barley for poultry. These treatments mainly focus on the activation of endogenous enzymes, mainly, β-glucanase (Fry *et al*., 1958). Germinated barley was reported to have lower total and soluble β-glucan contents and digesta viscosity and, consequently improved growth performance and nutrient utilisation in broilers (Fengler *et al*., 1990; Svihus *et al*., 1997b). In comparison, the positive effect of soaking was not consistent and seemed to be dependent on the conditions (water temperature, time) employed during soaking (Fry *et al*., 1958; Svihus *et al*., 1997b).

Beyond the aim of sterilising the feed ingredients, gamma irradiation has been evaluated in barley grains prior to dietary inclusion to induce depolymerisation of β-glucan and consequent reduction in viscosity (Classen *et al*., 1985; Campbell *et al*., 1986). A 63% reduction in viscosity of a β-glucan solution in response to gamma irradiation was reported by Classen *et al*. (1985). When fed to broilers (d 1-21), irradiated hull-less barley improved WG and fat absorption compared to the non-treated barley (Classen *et al*., 1985). Deteriorated growth performance and nutrient utilisation of broilers fed autoclaved barley was restored by subsequent irradiation of autoclaved barley (Campbell *et al*.,

1986). Comparing two barley types subjected to gamma irradiation, Al-Kaisey *et al*. (2002) reported a gradual decrease in viscosity of barley extract in response to increasing dose of gamma irradiation. However, the magnitude of the reduction in extract viscosity to irradiation dose was dependent on the barley type. The reduction in barley extract viscosity was attributed to depolymerisation of β-glucans, leading to lower β-glucan content and viscosity. In contrast, Campbell *et al*. (1986) reported an increased soluble β-glucan content in barley in response to increasing levels of irradiation. Despite the higher soluble β-glucan content, these researchers reported a decline in barley extract viscosity, due probably to an irradiation-induced reduction in molecular size.

However, most of these strategies are not economically attractive and their large-scale applications have been proven to be logistically difficult due to high cost and labour. Comparatively, supplementation of exogenous enzymes remains the most attractive approach because of its' easy practice and lesser variability in response. A combination of compatible measures would facilitate each other mechanism enabling maximum efficacy in improving the feeding value of barley for poultry diets.

2.5. Conclusions

With the developing knowledge of physical and chemical characteristics of barley grain, the understanding of anti-nutritive effects of barley in poultry diets has evolved over the years. The fact that the nutritive value of barley for poultry is determined not only by the chemical and physical properties but also by the interactions of the nutrient and anti-nutrient components highlights the need for the application of grain-specific metabolisable energy and digestible nutrients, in particular AA, in formulating barley-based diets. In order to minimise the negative impact caused by the inherent variability of barley in poultry diets, grain specific determination of inclusion levels and processing conditions should be encouraged. The combination of enzyme supplementation with an appropriate feed processing practice may enable achieving maximum efficacy of supplemental enzymes by optimising the physical characteristics in barley-based diets.

CHAPTER THREE

Nutritional evaluation of two barley cultivars, without and with carbohydrase supplementation, for broilers: Metabolisable energy and standardised amino acid digestibility[1]

3.1. Abstract

Two experiments were conducted to assess the nitrogen-corrected apparent metabolisable energy (AMEn; Exp. 1; 288 Ross 308 male broilers at d 14; six cages/treatment; eight birds/cage) and coefficient of standardised ileal digestibility (CSID) of amino acids (AA; Exp. 2; 336 Ross 308 male broilers at d 21; six cages/treatment; eight birds/cage) of two barley cultivars for broilers in comparison to wheat, without or with a multi-component non-starch polysaccharide (NSP) degrading enzyme. A 3 × 2 factorial arrangement of treatments was used in both experiments with three types of grains (normal starch hulled barley [NSH], waxy starch hull-less barley [WSHL], and wheat) and two levels of enzyme supplementation (0 and 200 g/tonne of feed). Enzyme supplemented diets contained 406 and 128 of endo-1, 4-β-xylanase and endo-1, 3 (4)-β-glucanase units per kg of feed, respectively. Analysis showed that the starch content was higher in NSH (610 g/kg) than in wheat (537 g/kg) and WSHL (554 g/kg), and the composition of starch differed markedly among the grain types. The β-glucan content was considerably higher in WSHL (68.6 g/kg) compared to NSH (38.5 g/kg) and wheat (7.74 g/kg). The contribution of soluble fraction to the total non-starch polysaccharides was higher in WSHL (38.2%) compared to NSH and wheat (17.1 and 13.3%, respectively). A significant ($P < 0.01$) interaction was observed between the grain type and enzyme supplementation for AMEn. The WSHL, with the highest content of β-glucan, showed the greatest response to enzyme supplementation for AMEn. Birds fed wheat- and WSHL-based diets had the highest and lowest CSID of nitrogen and most of AA, respectively, with NSH diets being intermediate Regardless of grain type, enzyme supplementation increased ($P < 0.05$) the CSID of nitrogen. These data suggest that β-glucan content plays an important role in determining the digestibility of nutrients in barley for broilers, resulting in a better feeding value for NSH over WSHL. Supplementation of a multi-component NSP-degrading enzyme can improve the feeding value of barley in broiler diets by increasing the digestibility with the effect being more pronounced in WSHL barley.

[1]*British Poultry Science, 60*(4), 404-413.

3.2. Introduction

Development of new cultivars and feed enzymes has received attention as a potential strategy to mitigate the negative effects of anti-nutritional factors present in barley for poultry. Barley cultivars with varying starch and β-glucan contents with diverse solubility characters are being continuously developed, while combinations of different enzymes are also being tested to enhance the nutrient utilisation. However, large variations in the chemical and physical characteristics of barley exist even among similar types of barley (Izydorczyk *et al.*, 2000) and wide variability in responses to enzyme supplementation has been reported (Bao *et al.*, 2013).

Hull-less barley has gained more attention over conventional hulled barley with the perception that reduction of fibre components will increase the nutritive value for poultry. The fibre content in hull-less barley is lower than in hulled barley (Oscarsson *et al.*, 1996; Ravindran *et al.*, 2007). Receiving the same attention as hull-less barley, waxy barley with high contents of amylopectin is thought to result in higher starch digestibility compared to normal starch and high amylose barley types (Björck *et al.*, 1990; Li *et al.*, 2004a). However, available reports indicate that starch digestion in waxy barley is lower compared to normal starch or high amylose barleys (Bergh *et al.*, 1999; Ravindran *et al.*, 2007). These observations are suggestive of contribution of factors other than starch composition and hulls to the feed value of barley for poultry.

It is important to determine the nutrient composition, metabolisable energy and digestible nutrient contents of ingredients prior to feed formulation for efficient utilisation. The objectives of the present study were to (i) characterise the nutrient composition of two barley cultivars in comparison with a sample of wheat (control) and (ii) determine the nitrogen-corrected apparent metabolisable energy (AMEn) and coefficient of standardised ileal digestibility (CSID) of amino acids (AA) in the three grain types, without or with carbohydrase enzyme addition.

3.3. Materials and methods

Two barley types namely normal starch hulled barley (NSH; cultivar, Fortitude) and waxy starch hull-less barley (WSHL; cultivar, Streaker) were obtained from a seed company (Luisetti Seeds Ltd, Rangiora, New Zealand), and ground in a hammer mill to pass

through the screen size of 3.0 mm. Wheat was obtained from a local commercial source and ground through the same screen size. The nutritional evaluation of the barley cultivars and wheat was carried out in three phases: (i) proximate and nutrient composition analysis, (ii) metabolisable energy evaluation and (iii) ileal nutrient digestibility assay. The experimental procedures were approved by the Massey University Animal Ethics Committee (MUAEC protocol 17/13) and complied with the New Zealand Code of Practice for the Care and Use of Animals for Scientific Purposes.

3.3.1. Proximate and nutrient composition

All analyses were conducted in an ISO17025 accredited laboratory (Nutrition Laboratory, Massey University). Representative samples of grains were analysed, in duplicate, for dry matter (DM), gross energy (GE), nitrogen (N), AA, starch, fat, ash, calcium (Ca), phosphorus (P) and other minerals. The samples were also analysed for neutral detergent fibre (NDF), acid detergent fibre (ADF), non-starch polysaccharides (soluble [S.NSP], insoluble [I.NSP] and total [T.NSP]), amylose, amylopectin and β-glucan contents.

3.3.2. Scanning electron microscopic (*SEM*) imaging of grains

Samples of whole grains of barley and wheat were placed in primary fixative (Modified Karnovsky's fixative [3% gluteraldehyde, 2% formaldehyde in 0.1M phosphate buffer, pH 7.2]) for 8 h at room temperature. Samples were then washed three times (15 min each) in phosphate buffer (0.1M, pH 7.2) followed by dehydration in graded ethanol series (25, 50, 75, 95 and 100%) for 5 min each, with a final 100% ethanol wash for 1 h. Samples were critical-point dried using liquid carbon dioxide as the critical-point fluid and 100% ethanol as the intermediary (Polaron E3000 series II critical point drying apparatus). Dried grains were manually broken along the cross section and mounted on aluminium stubs using double-sided tape, coated with approximately 100 nm of gold (Baltec SCD 050 sputter coater), and viewed in the FEI Quanta 200 Environmental Scanning Electron Microscope at an accelerating voltage of 20 kV at magnifications of ×400, ×1300 and ×5000.

3.3.3. Carbohydrase enzyme

A multi-component non-starch polysaccharide (NSP) degrading enzyme, Ronozyme® Multigrain (produced by *Trichoderma reesei*, also known as *Trichoderma longiabrachiatum*), was obtained from the DSM Nutritional Products, Australia. The activities of endo-1,4-β- glucanase, endo-1,3(4)-β-glucanase and endo-1,4-β-xylanase in this product were 800 BGU/g, 700 BGU/g and 2700 XU/g, respectively. Endo-1,3 (4)-β-glucanase and endo-1,4-β-xylanase activities in diet samples were measured at Biopract GmbH, Berlin, Germany. One unit of β-glucanase (BGU) was defined as the quantity of enzyme that released 1 µmol of reducing moieties from 1.5% β-glucan per minute at pH 5.0 at an incubation temperature of 40 °C with an incubation time of 20 min. One unit of xylanase (XU) was defined as the quantity of enzyme that released 1 µmol of reducing moieties from 1.5% arabinoxylan per minute at pH 5.0 and incubation at 40 °C for 20 min (DSM Nutritional Products Ltd., 2013).

3.3.4. Experiment 1- Evaluation of metabolisable energy

Apparent metabolisable energy (AME) was determined using the direct method and total excreta collection. Six dietary treatments were developed from the three grains, with two levels of enzyme supplementation (0 and 200 g/tonne of feed). The assay diets contained 962 g/kg of either barley or wheat as the only source of energy in the diet (Table 3.1).

Day-old male broilers (Ross 308), obtained from a commercial hatchery, were raised in floor pens and fed a commercial broiler starter diet until d 14 of age. The temperature was maintained at 32 °C during the first week and gradually decreased to approximately 23 °C by the end of the third week of the entire experiment. Ventilation was controlled by central ceiling extraction fans and wall inlet ducts. On d 14, 288 birds of uniform body weights (closest to mean body weight) were selected and randomly assigned to 36 cages (eight birds per cage). The floor pens and grower cages were housed in an environmentally controlled room with 20 h of fluorescent illumination per d. Each diet was supplied to six replicate cages for seven days (14-21 d) with the first three days serving as an adaptation period. The diets, in mash form, were offered *ad libitum* and water was available at all times. During the last four days, feed intake was monitored and excreta was collected daily, weighed and pooled within a cage. Pooled excreta were mixed well in a blender and representative samples were obtained and lyophilised (Model

0610, Cuddon Engineering, Blenheim, New Zealand). Diets and excreta samples were ground to pass through a 0.5 mm sieve and stored in airtight plastic containers at 4 °C in preparation for laboratory analysis. The DM, GE and N contents of the diet and excreta samples were determined.

Table 3. 1. Composition of the basal diets (g/kg, as fed basis) used in metabolisable energy (Experiment 1) and ileal nutrient digestibility (Experiment 2) assays.

Item	Experiment 1	Experiment 2	
	Basal diet	Basal diet	Nitrogen-free diet
Test grain	962	917	-
Dextrose	-	-	842
Sodium bicarbonate	2.0	2.0	2.0
Sodium chloride	2.0	2.0	2.0
Dicalcium phosphate	19.0	19.0	19.0
Limestone	13.0	13.0	13.0
Vitamin premix[1]	1.0	1.0	2.0
Mineral premix[1]	1.0	1.0	3.0
Soybean oil	-	40.0	50.0
Titanium dioxide	-	5.0	5.0
Solkafloc (cellulose)	-	-	50.0
Dipotassium hydrogen phosphate	-	-	12.0

[1]Supplied per kg of diet: antioxidant, 125 mg; biotin, 0.2 mg; calcium pantothenate, 20 mg; cholecalciferol, 5000 IU; cyanocobalamin, 0.02 mg; folic acid, 2.0 mg; menadione, 4 mg; niacin, 80 mg; pyridoxine, 5.0 mg; trans-retinol, 15000 IU; riboflavin, 9.0 mg; thiamine, 4.0 mg; dl-α-tocopheryl acetate, 80 IU; choline, 0.45 mg; ascorbic acid, 100 mg; Co, 1.0 mg; Cu, 20 mg; Fe, 40 mg; I, 2.0 mg; Mn, 100 mg; Mo, 1.0 mg; Se, 0.15 mg; Zn, 100 mg.
[1]Image Holdings Ltd., Auckland, New Zealand.

All data were expressed on a DM basis, and the AME and AMEn values of assay diets and grains, without and with enzyme supplementation, were calculated using the following formula:

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

Nitrogen-corrected AME was determined by correction for zero N retention by multiplication using a factor of 36.54 kJ per gram N retained in the body (Hill and Anderson, 1958).

$$AMEn_{grain\ (without\ and\ with\ enzyme)}\,(MJ/kg) = (\text{AMEn of the assay diet} \times 100)/96.2$$

3.3.5. Experiment 2- Ileal digestibility assay

The coefficient of apparent ileal digestibility (CAID) of DM, N, AA and starch of two barley cultivars and one wheat cultivar was determined using the direct method. Six dietary treatments were developed from combination of the three grains and two levels of enzyme supplementation (0 and 200 g/tonne of feed). The assay diets contained 917 g/kg of either barley or wheat as the only source of AA and starch in the diet (Table 3.1). A N-free diet was developed to determine the endogenous N and AA losses for the calculation of standardised digestibility values. Titanium dioxide (TiO_2; 5 g/kg; Merck KGaA, Darmstadt, Germany) was added to all diets as an indigestible marker to determine ileal digestibility.

A total of 336, 21-d old male broilers (Ross 308), with body weights closest to mean body weight were selected and randomly assigned to 42 cages (eight birds per cage). Each diet was fed to six replicate cages for four days from d 21 to 24. The diets, in mash form, were offered *ad libitum* and water was available at all times.

On d 24, all the birds in each cage were euthanised by intravenous injection (1 ml per 2 kg live weight) of sodium pentobarbitone (Provet NZ Pty Ltd., Auckland, New Zealand) and eviscerated. The small intestine was isolated, and the ileum was defined as that portion of the small intestine extending from the Meckel's diverticulum to a point ~40 mm proximal to the ileo-caecal junction, to avoid potential contamination from caecal fermentation products. The ileum was then divided into two halves and the digesta was collected from the lower half towards the ileo-caecal junction by gently flushing with distilled water, as described by Ravindran *et al*. (2005). Digesta from birds within a cage were pooled, lyophilised, ground to pass through a 0.5 mm sieve and stored at 4 ºC until laboratory analysis. The diets and digesta samples were analysed for DM, titanium (Ti), N, AA and starch.

The CAID of nutrients were calculated from the dietary ratio of nutrient to Ti relative to the corresponding ratio in the ileal digesta.

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

where, $(Nutrient /Ti)_d$ = ratio of nutrient to Ti in diet and $(Nutrient /Ti)_i$ = ratio of nutrient to Ti in ileal digesta.

The basal endogenous AA (EAA) flow at the terminal ileum was calculated as grams lost per kilogram of DM intake (DMI; Moughan *et al*., 1992).

$$\text{Basal EAA flow (g/kg DMI)} = [\text{AA in ileal digesta (g/kg)} \times Ti_d \text{ (g/kg)}]/\ Ti_i \text{ (g/kg)}$$

where, Ti_d= titanium in diet and Ti_i = titanium in ileal digesta.

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

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

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

3.3.6. Chemical analysis

Dry matter was determined using standard procedures (Methods 930.15; AOAC, 2016). Ash was determined by a standard procedure (Method 942.05; AOAC, 2016) using a muffle furnace at 550 ºC for 16 hours. 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). The crude protein (CP) content was calculated as N × 6.25. The adiabatic bomb calorimeter (Gallenkamp Autobomb, London, UK) standardised with benzoic acid was used for the determination of GE. The NDF (Method 2002.04; AOAC, 2016) and ADF (Method 973.18; AOAC, 2016) were determined using Tecator Fibertec™ (FOSS Analytical AB, Höganäs, Sweden).

Amino acids were determined as described by Ravindran *et al*. (2008). Briefly, the samples were hydrolysed with 6N HCl (containing phenol) for 24 h at 110 ± 2 °C in glass tubes sealed under vacuum. Amino acids were detected on a Waters ion-exchange HPLC system, and the chromatograms were integrated using dedicated software

(Millennium, Version 3.05.01, Waters, Millipore, Milford, MA), with the AA identified and quantified using a standard AA mixture (Product no. A2908, Sigma, St. Louis, MO). The HPLC system consisted of an ion-exchange column, two 510 pumps, Waters 715 ultra WISP sample processor, a column heater, a post column reaction coil heater, a ninhydrin pump and a dual wavelength detector. Amino acids were eluted by a gradient of pH 3.3 sodium citrate eluent to pH 9.8 sodium borate eluent at a flow rate of 0.4 ml/min and a column temperature of 60 °C. Cysteine and methionine were analysed as cysteic acid and methionine sulphone, respectively, by oxidation with performic acid for 16 h at 0 °C and neutralisation with hydrobromic acid prior to hydrolysis.

Total, soluble and insoluble NSP were determined using an assay kit (Megazyme International Ireland Ltd., Wicklow, Ireland) based on thermostable α-amylase, protease and amyloglucosidase (Englyst *et al*., 1994). Starch was measured using a Megazyme kit (Method 996.11; AOAC, 2016) based on thermostable α-amylase and amyloglucosidase (McCleary *et al*., 1997). Fat was determined using Soxtec extraction procedure for animal feed, forage and cereal grains (Method 2003.06; AOAC, 2016). Samples were assayed for Ti on a UV spectrophotometer following the method of Short *et al*. (1996).

For mineral analysis, the samples were wet digested in a nitric and perchloric acid mixture, and concentrations of P, Ca, potassium (K), magnesium (Mg), sodium (Na), chloride and iron were determined by Inductively Coupled Plasma-Optical Emission Spectroscopy (ICP-OES) using a Thermo Jarrell Ash IRIS instrument. Phytate phosphorus was analysed by the colorimetric procedure of Caldwell (1992). Phytate was extracted using hydrochloric acid and sodium sulphate solution and precipitated as ferric phytate. The precipitate was hydrolysed, and the P content was determined colorimetrically using the phosphomolybdate method (Selle *et al*., 2003a).

3.3.7. Statistical analysis

The data were analysed as a 3 × 2 factorial arrangement of treatments using the General Linear Models procedure of SAS (version 9.4; SAS Institute Inc., Cary, NC.). Cages served as the experimental unit and differences were considered to be significant at $P < 0.05$. Significant differences between means were separated by Least Significant Difference test.

3.4. Results and discussion

3.4.1. Proximate and nutrient compositions

The proximate and nutrient compositions of the two barley cultivars and wheat are shown in Table 3.2. The results, in general, were within the range reported in the literature (Beames *et al.*, 1996; Jensen *et al.*, 1998; Andersson *et al.*, 1999; Izydorczyk *et al.*, 2000; Ravindran *et al.*, 2007). Values outside the range have been reported by some researchers (Hew *et al.*, 1998; Li *et al.*, 2004b; Rebolé *et al.*, 2010), which highlighted the variability that exists between barley cultivars grown in different geographical locations (Jeroch and Dänicke, 1995; Hughes and Choct, 1999; Helm and Francisco, 2004).

The composition of starch differed markedly among the grain types. A higher proportion of amylopectin, which is beneficial in terms of starch digestibility, was reported in the present study for WSHL than for NSH (860 vs. 562 g/kg starch). *In vitro* enzyme hydrolysis of barley starches has shown that the waxy form has a higher susceptibility to α-amylase, compared to normal or high amylose barley starch (Björck *et al.*, 1990; Li *et al.*, 2004a).

Starch was the main chemical component followed by T.NSP in all three grains. The observation of higher content of starch in NSH compared to WSHL is in contrast to some previous studies that reported higher contents of starch in WSHL compared to NSH barley (Oscarsson *et al.*, 1996; Holtekjølen *et al.*, 2006). Knudsen (1997), with no reference to starch type, reported that the concentration of starch was higher in hull-less barley (645 g/kg DM) compared to hulled barley (587 g/kg DM) owing to a strong influence of hulls on the starch concentration. However, in agreement with the present findings, Andersson *et al.* (1999) reported higher contents of starch in NSH barley compared to WSHL barley. Asare *et al.* (2011) compared hull-less barley types with different starch composition and reported higher starch content in normal starch barley compared to waxy and high amylose barley. Ravindran *et al.* (2007) compared two WSHL barley cultivars with an NSH cultivar and found no consistent differences in starch content.

Table 3. 2. Proximate, carbohydrate, mineral and amino acid composition (g/kg) of normal starch hulled barley (NSH), waxy starch hull-less barley (WSHL) and wheat (dry matter basis).

	NSH	WSHL	Wheat		NSH	WSHL	Wheat
Proximate and carbohydrate composition				*Amino acid concentration*			
Dry matter	893	907	892	*Essential amino acids*			
Ash	17.6	17.8	18.4	Arginine	5.28	6.44	6.79
Nitrogen	16.2	21.2	22.6	Histidine	2.35	2.82	3.46
Crude protein (N×6.25)	101	133	141	Isoleucine	3.69	4.87	4.94
Starch	610	554	537	Leucine	7.02	8.99	9.82
Amylopectin	343	477	308	Lysine	3.84	4.55	3.95
Amylose	267	77.2	229	Methionine	2.16	2.23	2.52
Fat	21.2	27.3	21.0	Phenylalanine	5.13	7.31	6.99
NDF[1]	129	89	112	Threonine	3.67	4.18	4.14
ADF[1]	43.7	17.6	27.8	Valine	5.54	6.82	6.49
Gross energy (MJ/kg)	18.1	18.4	18.3				
I.NSP[1]	142	110	119	*Non-essential amino acids*			
S.NSP[1]	29.2	68.0	18.3	Alanine	4.28	4.98	4.99
T.NSP[1]	171	178	138	Aspartic acid	6.82	8.09	7.46
β-glucan	38.5	68.6	7.74	Cysteine[2]	2.65	3.00	3.50
				Glycine[2]	4.38	4.99	5.95
Minerals				Glutamic acid	23.6	34.4	45.1
Calcium	0.39	0.36	0.35	Proline	10.6	16.1	15.2
Total phosphorus (P)	3.25	3.86	4.26	Serine	4.50	5.23	7.10
Phytate P	1.32	1.79	2.22	Tyrosine	3.41	4.36	4.68
Non-phytate P	1.93	2.07	2.04				
Magnesium	1.28	1.39	1.45				
Potassium	4.25	5.62	4.93				
Sodium	0.20	0.10	< 0.06				
Iron	0.06	0.06	0.06				
Chloride	1.31	1.27	0.71				

[1]NDF, neutral detergent fibre; ADF, acid detergent fibre; T.NSP, total non-starch polysaccharides; I.NSP, insoluble non-starch polysaccharides; S.NSP, soluble non-starch polysaccharides.
[2]Semi-essential amino acids for poultry.

Higher contents of fat, CP and AA in WSHL compared to NSH were in agreement with published data (Edney *et al.*, 1992; Pettersson and Lindberg, 1997; Andersson *et al.*, 1999), and this was attributed to a concentration effect caused by the absence of hulls.

Higher contents of NDF and ADF were observed in NSH followed by wheat, which is in agreement with that reported by Knudsen (1997). In contrast, Li *et al.* (1996) observed similar contents of NDF and ADF in hull-less barley and wheat. A similar content of T.NSP observed in two barley cultivars disagrees with those reported by Beames *et al.* (1996) who determined higher contents of T.NSP in hulled barley cultivars. However, the contribution of the soluble fraction to the T.NSP was higher in WSHL

(38.2%) compared to NSH and wheat (17.1 and 13.3% of T.NSP, respectively). Beames *et al.* (1996) and Jensen *et al.* (1998) reported a higher level of I.NSP in NSH due to the presence of hulls. β-glucan content was considerably higher in WSHL (68.6 g/kg DM) compared to NSH (38.5 g/kg DM) and wheat (7.74 g/kg DM), which was in agreement with previous studies (Oscarsson *et al.*, 1996; Izydorczyk *et al.*, 2000; Li *et al.*, 2001; Izydorczyk and Dexter, 2008; Knudsen, 2014). However, β-glucan content observed in WSHL was outside the range of values reported in some studies (Beames *et al.*, 1996; Li *et al.*, 1996). Asare *et al.* (2011), who compared ten hull-less barley types with different starch composition, reported higher CP, β-glucan and fat contents in waxy starch types compared to a normal starch barley cultivar, which is in agreement with the current study.

The differences in CP content were reflected in AA contents, with WSHL having higher concentration of N and each AA compared to NSH. In all three grains evaluated, methionine concentration was the lowest followed by histidine and cysteine, while glutamic acid was the highest. In comparison with wheat, WSHL contained higher contents of lysine, phenylalanine, valine, proline and aspartic acid, while the other AA showed comparable values. Compared to the variable AA contents of barley and wheat reported in the literature, AA concentrations in the current study were within the range of AA of barley and wheat with similar CP contents reported by Ravindran *et al.* (2005) and Bandegan *et al.* (2011). Moreover, differences of AA contents between the current study and literature were consistent with the differences in CP content (Short *et al.*, 1999; Ravindran *et al.*, 2005).

Potassium was the major mineral in all tested grains followed by P. In the current study, higher contents of Mg and K were observed in WSHL than NSH, while K content was higher in WSHL than wheat. Normal starch hulled barley contained markedly higher content of Na compared to WSHL and wheat. In agreement with Bartnik and Szafrańska (1987) results, both total and phytate P were higher in wheat compared to two barley types. The reported total P content for NSH (3.25 g/kg DM) was lower than the range reported for hulled barley types (3.5-4.3 g/kg DM) by Fairbairn *et al.* (1999) and Salarmoini *et al.* (2008). However, the total P content of WSHL (3.86 g/kg DM) fell within the range (3.8-4.6 g/kg DM) reported for two hull-less barley types by Salarmoini *et al.* (2008). The determined level of phytate for NSH and WSHL (1.32 and 1.79 g/kg DM, respectively) were below the range reported by Salarmoini *et al.* (2008) for hulled

low-phytate barley (3.3-5.5 g/kg DM) and hull-less low-phytate barley (6.1 g/kg DM). These observations indicated that both barley cultivars used in the present study were low-phytate types. In common with other cereals, all three ingredients contained only negligible amounts of Ca (0.35-0.39 g/kg DM).

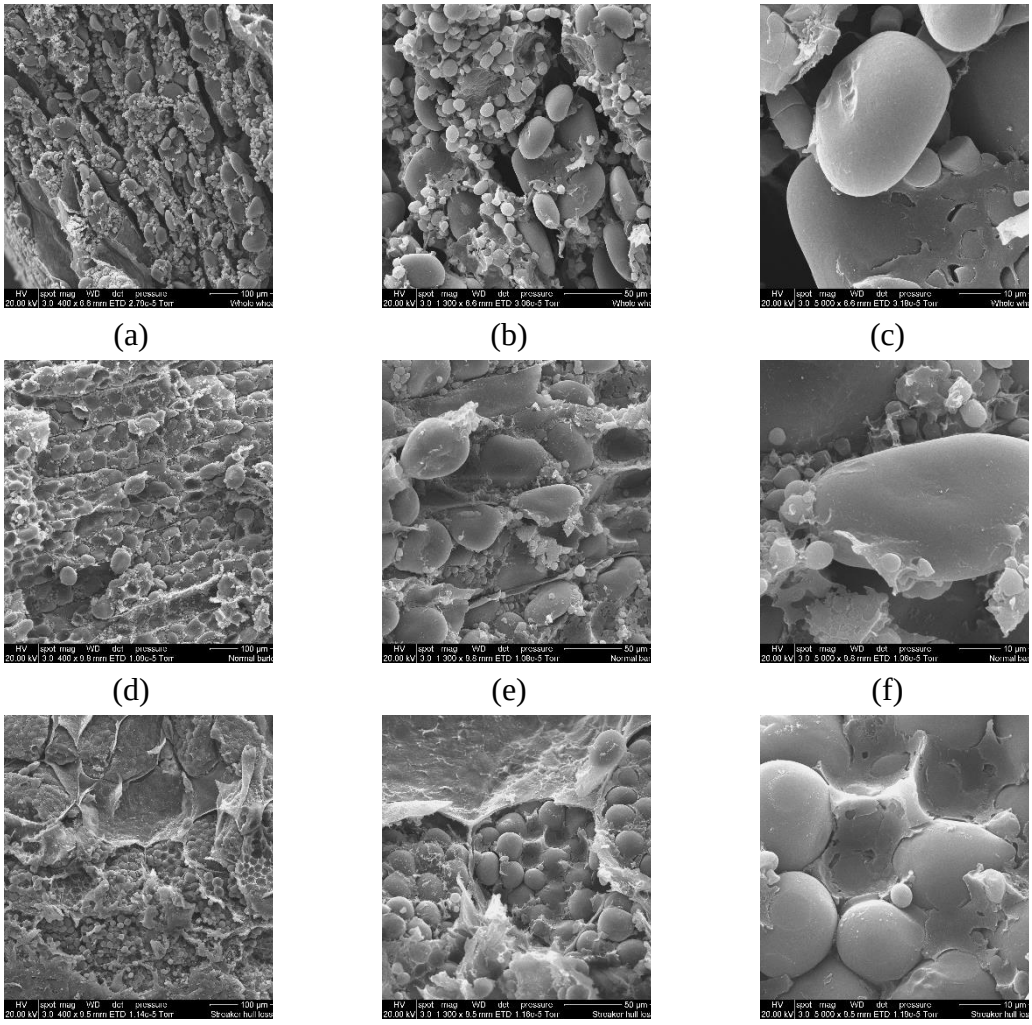
3.4.2. Microscopic characterisation of barley and wheat

The *SEM* images showing cross section of the grains are shown in Figure 3.1. Starch granules in both wheat and barley are known to have a bimodal size distribution with large disc-shaped A-granules and small spherical B-granules (Song and Jane, 2000; Li *et al.*, 2001; Ao and Jane, 2007). Starch granules from wheat endosperm (Figure 3.1 a, b and c) were mainly composed of a mixture of elliptical- and oval-shaped large starch granules and irregular shaped small starch granules. Moreover, starch endosperm of NSH (Figure 3.1 d, e and f) mainly consisted of elliptical-shaped large starch granules and spherical-shaped small starch granules. Conversely, starch granules from WSHL (Figure 3.1 g, h and i) were mainly composed of spherical-shaped large and small granules of starch, and large starch granules were more uniform in shape compared to other two grain types. It has been suggested that starch granule shape depends on the amylose content and that the less angular, rounded starch granules have relatively higher amylose levels (Bewley and Black, 1978; Waldron, 1997).

Wheat and NSH that had disc-shaped starch granules had a higher starch digestibility compared to WSHL (Table 3.3), which contained spherical-shaped starch granules. Jane (2006) described that disc-shaped starch granules in wheat and barley contain starch granules perpendicular to the flat surface of starch granules, allowing more contact with digestive enzymes.

The size and shape of starch granules have been recognised as important functional properties that can control the accessibility of the enzyme to the interior of the granule and regulate enzymatic hydrolysis (Svihus *et al.*, 2005; Tester *et al.*, 2006). Different shapes of starch granules, as observed in the current study, can affect the surface area to volume ratio and, hence, the potential for enzymatic digestion (Waldron, 1997). The larger the granules, the smaller the surface area to volume ratio and the lower potential surface to be attacked and hydrolysed by digestive enzymes. Moreover, some

starch granules present as compound granules made from individual granules which reduce the capacity of enzymes to attach to starch granule surfaces (Tester *et al.*, 2006).



(a) (b) (c)

(d) (e) (f)

(g) (h) (i)

Figure 3. 1. Scanning electron microscopic images of cross sections of whole grains of wheat (a, b, c), normal starch hulled barley (d, e, f) and waxy starch hull-less barley (g, h, i) under magnifications of ×400 (a, d, g), ×1300 (b, e, h) and ×5000 (c, f, i)

Izydorczyk and Dexter (2008) compared cross sections of an NSH genotype with a WSHL genotype with two levels of β-glucan, (45 and 97 g/kg β-glucan, respectively) and reported thicker endosperm cell walls in barley genotypes with high level of β-glucan. Accordingly, endosperm cell walls were more visible in WSHL (Figure 3.1; g and h) compared to NSH (Figure 3.1; d and e).

3.4.3. Nutrient utilisation

The average recovery of endo-1, 3 (4)-β-glucanase and endo-1, 4-β-xylanase from enzyme-supplemented diets from Experiments 1 and 2 were 91.3 and 75.1%, respectively (data not shown).

The AME, AMEn and nutrient digestibility in the two barley cultivars and wheat, with and without enzyme supplementation, are summarised in Table 3.3. A significant ($P < 0.01$) interaction between grain type and enzyme supplementation was observed for both AME and AMEn. The greatest energy responses to enzyme supplementation were observed in WSHL, which contained the highest content of β-glucan. The higher magnitude of response of AMEn by WSHL to added enzyme was in agreement with Ravindran *et al.* (2007), but the 9.6% improvement was considerably lower than the average increase of 22.8% reported by these researchers. This lends support to the variability in responses of barley grains to enzyme supplementation, which has been reported in the literature (Bao *et al.*, 2013). The finding of lower AME and AMEn of WSHL compared to NSH is in agreement with Ravindran *et al.* (2007), but contrary to the study by Moharrery (2006) who reported a higher AME value for hull-less barley (11.17 MJ/kg DM) compared to hulled barley (10.05 MJ/kg DM). The AME value determined for WSHL (10.87 MJ/kg DM) fall within the range of AME (10.4 to 12.2 MJ/kg DM) reported for Australian barley types (Kocher *et al.*, 1997), while NSH showed a greater AME value (13.67 MJ/kg DM). Moreover, the AME value of wheat (14.71 MJ/kg DM) was within the range reported for wheat (10.20 to 15.95 MJ/kg DM) cultivated in New Zealand (Ravindran *et al.*, 2001).

Table 3. 3. Influence of grain type and enzyme supplementation on apparent metabolisable energy (AME, MJ/kg DM basis[2]), nitrogen-corrected AME (AMEn, MJ/kg DM basis[2]) and coefficient of apparent ileal digestibility (CAID) of dry matter (DM) and starch.

Grain type	Enzyme	Energy[3]		CAID[4]	
		AME	AMEn	DM	Starch
NSH[1]	-	13.67c	13.39c	0.706b	0.985a
	+	14.14b	13.87b	0.717b	0.990a
WSHL[1]	-	10.87e	10.60e	0.594d	0.839c
	+	11.89d	11.62d	0.653c	0.901b
Wheat	-	14.67a	14.38a	0.731ab	0.986a
	+	14.75a	14.43a	0.745a	0.988a
SEM[5]		0.132	0.131	0.0095	0.0121
Main effects					
Grain type					
NSH		13.90	13.63	0.711	0.987
WSHL		11.38	11.11	0.624	0.870
Wheat		14.71	14.40	0.738	0.987
Enzyme					
	-	13.07	12.79	0.677	0.936
	+	13.59	13.31	0.705	0.960
Probabilities, $P \leq$					
Grain type		0.001	0.001	0.001	0.001
Enzyme		0.001	0.001	0.001	0.025
Grain type × Enzyme		0.005	0.004	0.027	0.031

Means in a column not sharing a common letter (a-e) are different ($P < 0.05$).
[1]NSH, normal starch hulled barley; WSHL, waxy starch hull-less barley.
[2]DM content of the grains: NSH, 893 g/kg; WSHL, 907 g/kg; Wheat, 892 g/kg.
[3]Each value represents the mean of six replicates (eight birds per replicate), measured over the last four days (d 18 to 21), Experiment 1.
[4]Each value represents the mean of six replicates (eight birds per replicate), measured after 4 days on assay diets (d 24), Experiment 2.
[5]Pooled standard error of mean.

A significant ($P < 0.05$) interaction between grain type and enzyme supplementation was observed for the CAID of starch. The greatest digestibility response (7.4%) to enzyme supplementation was observed in WSHL, which contained the highest level of β-glucan. Contrary to the general belief that waxy starch barley with higher contents of amylopectin is more digestible than the normal starch barley (Björck *et al.*, 1990), a lower ($P < 0.05$) starch digestibility was found in WSHL compared to the other two grains, an observation that was in agreement with the findings of Bergh *et al.* (1999) and Ravindran *et al.* (2007). The anti-nutritional nature of β-glucan and its effect on ileal starch digestibility has been previously discussed by Bergh *et al.* (1999) and Ravindran *et al.* (2007). Bergh *et al.* (1999) compared the ileal starch digestibility of three hulled barley cultivars with different starch composition (normal, high amylose and waxy) and with different contents of soluble β-glucan (14.5, 14.5 and 20.0 g/kg DM, respectively),

and reported a lower CAID of starch for non-supplemented waxy barley diet (0.87), compared to the non-supplemented normal starch barley diets (0.91). However, no significant difference in CAID of starch was observed between enzyme supplemented normal and waxy barley diets, confirming the efficacy of the β-glucanase enzyme. Ravindran *et al.* (2007) also reported poor starch digestibility in non-supplemented hull-less waxy barley types (0.53 and 0.65) compared to a non-supplemented normal barley (0.80).

The similar treatment trends in the AME, AMEn and CAID of starch in the current study are in agreement with Wu *et al.* (2004a) and Ravindran *et al.* (2007), suggesting that digestible starch is the major contributor to metabolisable energy in barley. The relationship between the AME and starch digestibility in wheat has been identified by Mollah *et al.* (1983), who analysed 22 samples of 13 wheat cultivars for energy and nutrient utilisation, where low-AME wheats exhibited relatively low starch digestibilities. Despite the high content of starch present in wheat (659 g/kg DM) compared to barley (630 g/kg DM), a similar level of AME (11 MJ/kg DM) for barley and wheat was reported by Perttilä *et al.* (2005). However, starch composition of the barley type used was not identified. Moss *et al.* (1983), who reported comparatively similar AME values for hulled normal starch and hulled waxy starch barley types, suggested that metabolisable energy is not affected by type of starch.

Shakouri *et al.* (2009), who compared the main cereal grains (maize, barley, sorghum and wheat) in terms of nutrient digestibility and ileal digesta viscosity, reported an improvement in ileal starch digestibility due to the addition of NSP-degrading enzymes. However, based on the non-significant effect of the enzyme supplementation on digesta viscosity (except in wheat), the improvement of starch digestibility was not attributed to a change in digesta viscosity alone, but believed to be associated with degradation of cell wall which consequently released encapsulated starch.

A significant ($P < 0.05$) interaction between grain type and enzyme supplementation was observed for the CAID of DM. The responses to enzyme supplementation were markedly higher in WSHL (9.9%) which contained the highest β-glucan content, compared to NSH and wheat (1.6 and 1.9%, respectively). With the improved DM digestibility due to supplemental enzyme, the excretion of undigested

materials is reduced and, therefore, environmental and management problems would be minimised. Moharrery (2006) reported a higher DM digestibility for hull-less barley (0.73) compared to hulled barley (0.66). The values observed for CAID of DM for barley (0.711 and 0.624 for NSH and WSHL, respectively) and wheat (0.738) were in general agreement with values (0.67 and 0.72 for barley and wheat, respectively) reported by Shakouri *et al.* (2009).

Influence of grain type and enzyme supplementation on CSID of AA is presented in Table 3.4. No interaction ($P > 0.05$) between grain type and enzyme supplementation was observed for the CSID of N or any AA. Grain type had significant ($P < 0.001$) effects on the CSID of N and average AA digestibility, whereas enzyme effect was significant ($P < 0.05$) only for N digestibility. Birds fed wheat- and WSHL-based diets had the highest and lowest CSID for N and AA respectively, with NSH diets being intermediate. Despite the fact that contents of N and AA were higher in WSHL compared to NSH (Table 3.2), CSID values were lower for WSHL, indicating poorer digestion. The improved N digestibility due to the enzyme supplementation was in agreement with Wu *et al.* (2004a), who evaluated the effect of β-glucanase and xylanase on nutrient digestibility in barley and wheat, respectively. However, the response of improvement in N digestibility (1.9, 4.1 and 2.5% increase for NSH, WSHL and wheat, respectively) was comparatively lower than the responses in CAID of N for wheat and barley (6.6 and 13.8%, respectively) reported by Wu *et al.* (2004a).

Similar to the pattern observed for the CSID of N, grain type had significant ($P < 0.001$) effects on the CSID of all AA, except for cysteine. In general, wheat and WSHL showed the highest and lowest CSID, respectively, with NSH being intermediate. Lower CSID values for N and AA for two barley types compared to wheat was in agreement with Bandegan *et al.* (2011), who compared CSID of six wheat and seven barley samples. These researchers reported threonine (0.854 and 0.806), lysine (0.837 and 0.805) and arginine (0.852 and 0.804) as the least digestible indispensable AA in wheat and barley, respectively. Moreover, methionine (0.914 and 0.883) and phenylalanine (0.938 and 0.909) in wheat and barley respectively had the highest CSID values. Comparing CSID for individual AA in NSH, all digestibility coefficients except phenylalanine, threonine and serine were within the range of values reported by Bandegan *et al.* (2011). In comparison to CSID for individual AA in WSHL, all CSID values except arginine,

histidine and alanine were below the range reported by Bandegan *et al.* (2011). The range of CSID (0.837 for lysine to 0.938 for phenylalanine) for the indispensable AA in wheat reported by Bandegan *et al.* (2011) was higher than the range reported in the present study (0.784 for lysine to 0.914 for methionine). This is to be expected as wheat cultivars evaluated by Bandegan *et al.* (2011) had a higher CP content (162 g/kg DM) compared to wheat (141 g/kg DM) in the current study.

Short *et al.* (1999), who compared true ileal digestibility of four wheat cultivars with two levels of protein, suggested that AA digestibility coefficients were higher for the cultivars with higher protein level. Conversely, in the current study, WSHL with a higher CP and AA content resulted in lower CSID values compared to NSH with lower CP and AA content. This suggests that the observation by Short *et al.* (1999) on higher CP and AA digestibility coefficients for wheat cultivars with a higher protein contents might be valid only for grains with a lower anti-nutritive NSP contents, such as wheat. Szczurek (2009), who compared two wheat and barley types (CP; 135.9 and 120.4 g/kg DM, respectively) for standardised ileal digestibility of AA, reported a similar average CSID for indispensable AA in both grain types. Even though CP content of wheat was higher in wheat compared to barley, no significant difference was observed in CSID of individual AA except cysteine, leucine and serine.

Table 3. 4. Influence of grain type and enzyme supplementation on the coefficient of standardised ileal digestibility[2] (CSID) of nitrogen (N) and amino acids[3], Experiment 2.

Grain type	Enzyme	N	AA[4]	Met	Cys	Lys	Thr	Arg	Ile	Leu	Val	His	Phe	Gly	Ser	Pro	Ala	Asp	Glu	Tyr
NSH[1]	-	0.781	0.785	0.849	0.808	0.757	0.702	0.784	0.789	0.810	0.783	0.799	0.811	0.719	0.725	0.849	0.742	0.759	0.870	0.788
	+	0.796	0.790	0.842	0.831	0.741	0.714	0.788	0.794	0.812	0.788	0.795	0.817	0.724	0.750	0.862	0.738	0.748	0.875	0.801
WSHL[1]	-	0.732	0.727	0.744	0.754	0.698	0.658	0.738	0.731	0.744	0.724	0.734	0.763	0.671	0.678	0.791	0.687	0.717	0.801	0.720
	+	0.762	0.754	0.770	0.772	0.715	0.684	0.758	0.758	0.776	0.755	0.766	0.797	0.693	0.708	0.831	0.712	0.734	0.837	0.754
Wheat	-	0.838	0.852	0.916	0.793	0.831	0.765	0.833	0.858	0.880	0.820	0.896	0.892	0.809	0.809	0.917	0.820	0.810	0.956	0.884
	+	0.859	0.868	0.912	0.839	0.833	0.803	0.843	0.877	0.890	0.844	0.895	0.903	0.826	0.846	0.933	0.831	0.829	0.959	0.895
SEM[5]		0.0112	0.0143	0.0128	0.0289	0.0183	0.0205	0.0151	0.0145	0.0133	0.0156	0.0122	0.0126	0.0168	0.0229	0.0119	0.0155	0.0152	0.0098	0.0126
Main effects																				
Grain type																				
NSH		0.788b	0.787b	0.846b	0.819	0.749b	0.708b	0.786b	0.791a	0.811b	0.786b	0.797b	0.814b	0.722b	0.738b	0.856b	0.740b	0.753b	0.873b	0.795b
WSHL		0.747c	0.74c	0.757c	0.763	0.707c	0.671b	0.748c	0.745c	0.760c	0.740c	0.750c	0.780c	0.682c	0.693b	0.811c	0.699c	0.726b	0.819c	0.737c
Wheat		0.849a	0.86a	0.914a	0.816	0.832a	0.784a	0.838a	0.868a	0.885a	0.832a	0.896a	0.897a	0.818a	0.828a	0.925a	0.826a	0.820a	0.957a	0.889a
Enzyme																				
	-	0.784b	0.788	0.842	0.785	0.763	0.709	0.785	0.793	0.811	0.776	0.810	0.822	0.733	0.737	0.852b	0.749	0.762	0.876	0.798
	+	0.806a	0.804	0.836	0.814	0.762	0.734	0.796	0.810	0.826	0.796	0.819	0.839	0.748	0.768	0.875a	0.760	0.771	0.891	0.817
Probabilities, P ≤																				
Grain type		0.001	0.001	0.001	0.107	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001
Enzyme		0.023	0.179	0.610	0.226	0.921	0.143	0.371	0.153	0.184	0.120	0.385	0.112	0.299	0.105	0.025	0.395	0.489	0.074	0.079
Grain type × Enzyme		0.805	0.725	0.359	0.873	0.659	0.820	0.876	0.744	0.521	0.718	0.270	0.480	0.875	0.966	0.456	0.660	0.556	0.184	0.608

Means in a column not sharing a common letter (a-c) are different ($P < 0.05$).
[1]NSH, normal starch-hulled barley; WSHL, waxy starch hull-less barley.
[2]Apparent digestibility values were standardised using the following basal ileal endogenous flow values (g/kg DM intake), determined by feeding N-free diet: N, 1.13; Met, 0.13; Cys, 0.22; Lys, 0.31; Thr, 0.51; Arg, 0.31; Ile, 0.27; Leu, 0.44; Val, 0.37; His, 0.13; Phe, 0.27; Gly, 0.35; Ser, 0.48; Pro, 0.41; Ala, 0.30; Asp, 0.60, Glu, 0.77 and Tyr, 0.25.
[3]Each value represents the mean of six replicates (eight birds per replicate), measured after 4 days on assay diets (d 24).
[4]Average standardised ileal digestibility of 17 amino acids.
[5]Pooled standard error of mean.

Enzyme addition improved the ileal digestibility of proline ($P < 0.05$) and tended to improve that of glutamic acid ($P = 0.07$) and tyrosine ($P = 0.08$) but had no effect ($P > 0.05$) on the other AA. There are apparently no studies which evaluated the influence of NSP-degrading enzyme on CSID of AA in barley and wheat in poultry diets. However, available reports on the effect of NSP-degrading enzymes on CAID of AA in barley- (Bedford, 1995; Perttilä *et al.*, 2001; Ravindran *et al.*, 2007) and wheat-based diets (Hew *et al.*, 1998; Ravindran *et al.*, 1999) have shown significant improvements in CAID of AA due to supplementation of enzymes. However, the effect of enzyme supplementation on individual AA has been inconsistent, which may be related to variations in chemical and physical characteristics of grains and different efficacies in supplemented enzymes.

3.5. Conclusions

It can be concluded that, in addition to amylose: amylopectin ratio, the level of β-glucan plays a crucial role in determining the feeding value of barley for broilers. Normal starch hulled barley had a better nutritive value compared to WSHL, showing higher metabolisable energy and, DM, starch, N and AA digestibility. The current work confirms that the feeding value of barley in broiler diets can be improved through multi-component carbohydrase supplementation, with the effect being more pronounced in WSHL.

CHAPTER FOUR

Influence of inclusion level of barley in wheat-based diets and supplementation of carbohydrase on growth performance, nutrient utilisation and gut morphometry in broiler starters[2]

4.1. Abstract

The influence of barley inclusion level and supplementation of a multi-component non-starch polysaccharide degrading enzyme on performance and nutrient utilisation in broilers was investigated. Normal-starch hulled barley was evaluated with five levels of inclusion (0, 141, 283, 424 and 565 g/kg) in a wheat-based diet and two levels of enzyme supplementation (0 and 150 g/tonne of feed; a 5 × 2 factorial arrangement of ten dietary treatments). All diets were equivalent in metabolisable energy and digestible amino acid contents. A total of 400, one-d-old male broilers (five cages/treatment; eight birds/cage) were used in the experiment. Regardless of enzyme supplementation, weight gain (WG) increased up to 283 g/kg of barley and reduced at higher inclusion levels ($P < 0.01$). Increasing levels of barley resulted in greater ($P < 0.001$) feed per gain (F/G). Enzyme addition increased WG ($P < 0.05$) and F/G ($P < 0.001$) at each barley inclusion level. Birds fed diets with 0 and 565 g/kg barley showed the lowest and highest ($P < 0.001$ to 0.05) digestibility for all nutrients measured, respectively. Digestibility of all nutrients was improved by enzyme supplementation at each barley inclusion level ($P < 0.05$). The nitrogen-corrected apparent metabolisable energy improved with increasing inclusion of barley ($P < 0.001$) and supplemental enzyme ($P < 0.01$). Increasing inclusion of barley increased the relative weight of gizzard ($P < 0.001$) and reduced jejunal digesta viscosity ($P < 0.001$). Supplemental enzyme ($P < 0.001$) reduced the digesta viscosity. The optimum inclusion level of barley, with respect to growth performance, was 283 g/kg of diet. Increasing barley inclusion improved nutrient and energy utilisation, possibly through lowered digesta viscosity and better function of the gizzard. Feed efficiency and nutrient and energy utilisation can benefit from carbohydrase supplementation in barley-based diets, regardless of barley inclusion level.

[2]*British Poultry Science*, *60*(6), 736-748.

4.2. Introduction

The proportion of barley (*Hordeum vulgare* L.) used in poultry diets remains low (less than 1.0% of total barley utilised as animal feed; Black *et al*., 2005) due to its low energy, relatively high fibre content (220 g/kg), and high content of non-starch polysaccharides (NSP; Jacob and Pescatore, 2012). β-glucans, the dominant NSP present in barley, are recognised as the main anti-nutritional factor that limits the nutritive value for poultry. The NSP encapsulates the nutrients within endosperm cells (Åman and Graham, 1987), known as the cage effect, and increases digesta viscosity in birds fed barley-based diets (Wang *et al*., 1992; Almirall *et al*., 1995). Ways to improve the feeding value of barley in poultry diets has been studied over the years, however, the published data have been contradictory, resulting in variable range of inclusion levels being recommended in broiler diets. Arscott *et al*. (1955) suggested that barley can be included in broiler diets up to 153 g/kg without affecting growth performance. According to Brake *et al*. (1997), 200 g barley/kg can be included in both broiler grower and finisher diets without compromising growth, feed efficiency or litter condition. Jeroch and Danicke (1995) recommended 200-300 g barley/kg for broiler finishers. According to Yu *et al*. (1998) and Bergh *et al*. (1999), 140 g barley/kg can be included in β-glucanase supplemented broiler diets.

This discrepancy of recommendations for barley inclusion in broiler diets is partly because most studies replaced other cereals with barley either on a weight to weight basis (Arscott *et al*., 1955; Petersen, 1969; Moss *et al*., 1983; Yu *et al*., 1998) or by using nutrient composition data for barley and the substituted grain from established sources such as National Research Council (1994; Moharrery, 2006) and Spanish Foundation for the Development of Animal Nutrition (FEDNA; De Blas *et al*., 2010; Lázaro *et al*., 2003), or chemical analysis (Brake *et al*., 1997). To the authors' knowledge, there are no published studies that formulated barley-based diets using nutrient profiles for the specific barley cultivar based on apparent metabolisable energy (AME) and digestible amino acids (AA) contents. Moreover, most of the available recommendations on inclusion levels of barley have overlooked the influence of the hull, NSP and starch type on the feeding value of barley for poultry.

Non-starch polysaccharide degrading enzymes can reduce the intestinal digesta viscosity through partial depolymerisation of NSP in cereal grains (Almirall *et al*., 1995; Józefiak *et al*., 2006), wherein cell wall integrity is disrupted by the enzyme action and encapsulated nutrients are exposed to the digestive enzymes (Hesselman and Åman, 1986; Bedford, 1996), leading to better interaction of endogenous digestive enzymes with their respective substrates. Extensive research evaluating the effect of enzyme supplementation on the feeding value of barley for broilers with special reference to growth performance and nutrient digestibility has been conducted (Hesselman and Åman, 1986; Marquardt *et al*., 1994; Almirall *et al*., 1995; Bergh *et al*., 1999). The findings have shown the capability of exogenous enzymes in poultry fed barley-based diets through increased feed consumption, weight gain, improved feed efficiency, enhanced nutrient utilisation and flock uniformity.

Only minimal attempts have been made to elucidate the possible interaction between barley inclusion level and enzyme addition on the utilisation of nutrients and performance of broilers and this aspect merits further evaluation. The present experiment was designed to investigate the possible interaction between inclusion level of a normal-starch hulled barley (NSH), previously evaluated for nutrient composition, nitrogen-corrected AME (AMEn) and digestible AA content (Chapter 3) and supplementation of a carbohydrase on the performance, nutrient and energy utilisation and gut morphometry in broiler starters.

4.3. Materials and methods

4.3.1. Enzymes

A multi-component NSP-degrading enzyme, Ronozyme® Multigrain, (produced by *Trichoderma reesei,* also known as *Trichoderma longiabrachiatum*) and Ronozyme® HiPhos were obtained from DSM Nutritional Products, Australia. 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 2700 XU/g, respectively. 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 min. 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 min. Ronozyme® HiPhos was a granular 6-phytase preparation expressed by submerged fermentation of *Aspergillus oryzae* and contained > 10,000 phytase units (FYT)/g. One FYT is defined as the activity of enzyme that releases 1.0 µmole of inorganic phosphorus/min from 5.0 mM sodium phytate at pH 5.5 at 37 ºC (DSM Nutritional Products Ltd., 2013). The activities of phytase, endo-1,3 (4)-β-glucanase and endo-1,4-β-xylanase in samples of final, 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.

4.3.2. Diets

Normal-starch hulled barley (cultivar, Fortitude) was obtained from a seed multiplication company (Luisetti Seeds Ltd., Rangiora, New Zealand) and ground in a hammer mill to pass through the screen size of 3.0 mm. Wheat was obtained from a commercial supplier and ground through the same screen size. Nutrient composition, AMEn and standardised digestible AA contents of same batches of non-supplemented barley and wheat, determined in Chapter 3, were used in formulating the experimental diets.

Five levels of inclusion of barley (0, 141, 283, 424 and 565 g/kg) in a wheat-based diet and two levels of enzyme supplementation (0 and 150 g/tonne of feed) was evaluated in a 5 × 2 factorial arrangement of ten dietary treatments. Five basal diets, with different inclusion levels of barley, were formulated to meet the Ross 308 strain recommendations for major nutrients for broiler starters (Ross, 2014; Table 4.1).

All diets were formulated to be equivalent in respect of AMEn and digestible AA contents. Ronozyme® HiPhos was added (1000 FYT/kg diet) across all basal diets. Each mixed diet was then divided into two equal batches, with one of the batches supplemented with Ronozyme® Multigrain (150 g/tonne of feed), resulting in ten dietary treatments. The diets contained 5.0 g/kg of titanium dioxide (TiO_2, Merck KGaA, Darmstadt, Germany) as an indigestible marker to determine ileal nutrient digestibility. A pellet binder (KEMBIND®, Kemin Industries [Asia] Pte Ltd, Singapore), at an inclusion rate

of 2.0 g/kg, was added on top of all diets. Diets were mixed in a single-screw paddle mixer. Following mixing, all diets were steam-conditioned to 70 °C for 30 seconds and pelleted using a pellet mill (Model Orbit 15; Richard Sizer, Kingston-upon-Hull, UK) capable of manufacturing 180 kg of feed/h and equipped with a die ring with 3.0 mm holes and 35 mm thickness. Representative samples of all diets were collected after pelleting for chemical analysis.

4.3.3. Pellet durability

Pellet durability was determined in a Holmen Pellet Tester (New Holmen NHP100 Portable Pellet Durability Tester, TekPro Ltd., Willow Park, North Walsham, Norfolk, UK) using the method described by Abdollahi *et al*. (2013b). Briefly, clean pellet samples (100 g; ten replicates per diet), with no fines, were rapidly circulated in an air stream around a perforated test chamber for 30 seconds. Resulting fines were removed continuously through the perforations using the test cycle. After the test cycle, the subject pellets were ejected and weighed manually. The pellet durability index (PDI) was calculated as the percentage of weight of pellets not passing through the perforations at the end of the test to weight of whole pellets at the start.

4.3.4. Birds and housing

The experimental procedures were approved by the Massey University Animal Ethics Committee (MUAEC protocol 17/13) and complied with the New Zealand Code of Practice for the Care and Use of Animals for Scientific Purposes. A total of 400, one-d-old male broilers (Ross 308), obtained from a commercial hatchery, were individually weighed and allocated to 50 cages in electrically heated battery brooders so that the average bird weight per cage was similar. Each of the ten dietary treatments was randomly assigned to five cages, each housing eight birds. The birds were transferred to grower cages on d-12 and continued on the same starter diets until the end of the trial (d-21). The battery brooders and grower cages were housed in an environmentally controlled room with 20 h of fluorescent illumination per d. The temperature was maintained at 31 °C on d-1 and was gradually reduced to 22 °C by 21 d of age. The diets, in pellet form, were offered *ad libitum* and water was available at all times.

Table 4. 1. Composition, calculated analysis, analysed values (g/kg, as fed) and pellet durability index (PDI; %) of the experimental diets based on wheat and normal-starch hulled barley.

	Barley inclusion level (g/kg)				
Item	0	141	283	424	565
Wheat	629	472	314	157	0.0
Normal-starch hulled barley	0.0	141	283	424	565
Soybean meal	278	288	297	307	316
Maize gluten meal	50.0	50.0	50.0	50.0	50.0
Soybean oil	3.4	9.9	16.4	22.9	29.4
Di-calcium phosphate	10.4	10.7	11.0	11.2	11.5
Limestone	9.3	9.0	8.75	8.5	8.2
L-Lysine HCl	3.8	3.6	3.45	3.3	3.1
DL-Methionine	2.0	2.1	2.2	2.3	2.4
L-Threonine	1.3	1.3	1.3	1.2	1.2
Sodium chloride	2.3	2.2	2.1	1.9	1.8
Sodium bicarbonate	3.4	3.5	3.6	3.7	3.8
Titanium dioxide[1]	5.0	5.0	5.0	5.0	5.0
Pellet binder[2]	2.0	2.0	2.0	2.0	2.0
Vitamin premix[3]	1.0	1.0	1.0	1.0	1.0
Mineral premix[3]	1.0	1.0	1.0	1.0	1.0
Phytase[4]	0.1	0.1	0.1	0.1	0.1
Calculated analysis					
Apparent metabolisable energy, MJ/kg	11.9	11.9	11.9	11.9	11.9
Digestible methionine	5.5	5.6	5.8	5.7	5.8
Digestible methionine + cysteine	9.0	9.0	9.0	9.0	9.0
Digestible lysine	12.2	12.2	12.2	12.2	12.2
Digestible threonine	8.2	8.2	8.2	8.2	8.2
Crude fat	18.9	24.7	30.5	36.3	42.1
Crude fibre	31.0	34.4	37.8	41.2	44.7
Calcium	9.6	9.6	9.6	9.6	9.6
Non-phytate phosphorus	4.8	4.8	4.8	4.8	4.8
Sodium	2.0	2.0	2.0	2.0	2.0
Chloride	2.0	2.0	2.0	2.0	2.0
Potassium	8.3	8.3	8.4	8.4	8.4
Analysed values					
Dry matter	883	880	883	879	884
Gross energy, MJ/kg	16.4	16.4	16.6	16.6	16.8
Crude protein (Nitrogen × 6.25)	250	246	246	236	231
Starch	343	337	330	324	317
Fat	19.1	24.1	28.5	35.4	37.9
PDI[5]	87.9a	86.9ab	85.6b	82.4c	82.1c

[1]Merck KGaA, Darmstadt, Germany.
[2]KEMBIND® (Kemin Industries [Asia] Pte Ltd) pellet binder, which contained modified lignosulphonate, guar gum, edible fatty acids and mineral oil, was added on top of each diet.
[3]Supplied per kg of diet: antioxidant, 125 mg; biotin, 0.2 mg; calcium pantothenate, 20 mg; cholecalciferol, 5000 IU; cyanocobalamin, 0.02 mg; folic acid, 2.0 mg; menadione, 4 mg; niacin, 80 mg; pyridoxine, 5.0 mg; trans-retinol, 15000 IU; riboflavin, 9.0 mg; thiamine, 4.0 mg; dl-α-tocopheryl acetate, 80 IU; choline, 0.45 mg; ascorbic acid, 100 mg; Co, 1.0 mg; Cu, 20 mg; Fe, 40 mg; I, 2.0 mg; Mn, 100 mg; Mo, 1.0 mg; Se, 0.15 mg; Zn, 100 mg.
[3]Image Holdings Ltd., Auckland, New Zealand.
[4]Ronozyme® HiPhos (1000 phytase units (FYT)/kg diet). One FYT is defined as the activity of enzyme that releases 1.0 µmole of inorganic phosphorus/min from 5.0 mM sodium phytate at pH 5.5 at 37 ºC. Nutrient matrix values (1.5 g/kg non-Phytate P and 1.8 g/kg Ca) were used in basal diet formulation.
[5]Each value represents the mean of ten replicate samples. Means not sharing common letters (a, b, c) are different ($P < 0.05$).

4.3.5. Performance data

Body weights and feed intake (FI) were recorded on a cage basis at weekly intervals. Mortality was recorded daily. Feed per gain (F/G) values were corrected for the body weight (BW) of any bird that died during the course of the experiment.

4.3.6. Energy and nutrient utilisation

4.3.6.1. Nitrogen-corrected apparent metabolisable energy (AME_n)

The AME_n was determined using the classical total excreta collection method. Feed intake and total excreta output of each cage were quantitatively measured from d-17 to 20 post-hatch. Daily collections from each cage were pooled, mixed in a blender and sub-sampled. Sub-samples were lyophilised (Model 0610, Cuddon Engineering, Blenheim, New Zealand), ground to pass through a 0.5 mm sieve and stored in airtight plastic containers at 4ºC pending analysis. The diets and excreta samples were analysed for dry matter (DM), gross energy (GE) and nitrogen (N).

4.3.6.2. Coefficient of apparent ileal digestibility (CAID) of nutrients

On d-21, six broilers per cage were euthanised by intravenous injection (0.5 mL per kg live weight) of sodium pentobarbitone (Provet NZ Pty Ltd., Auckland, New Zealand), and digesta were collected from the lower half of the ileum by gently flushing with distilled water, as described by Ravindran *et al*. (2005). The ileum was defined as that portion of the small intestine extending from the Meckel's diverticulum to a point ~40 mm proximal to the ileo-caecal junction. The ileum was then divided into two halves and the digesta was collected from the lower half towards the ileo-caecal junction.

Digesta from birds within a cage were pooled, frozen immediately after collection and subsequently lyophilised. Diet and lyophilised digesta samples were ground to pass through a 0.5 mm sieve and stored at 4 ˚C until laboratory analysis. The diets and digesta samples were analysed for DM, titanium (Ti), N, starch and fat.

4.3.7. Intestinal morphology

Two birds from each replicate cage (euthanised for ileal collection) were used for intestinal morphological examinations using the method described by Naderinejad *et al*. (2016). Sections from the middle of the duodenum and jejunum (about 5 cm in length) were excised and flushed with cold saline and immediately placed in 10% formalin solution. Samples were transferred to 70% ethanol after 72 h. Each fixed sample was then processed on a tissue processor. The samples were dehydrated through graded alcohol concentrations (70%, 95% and absolute alcohol) at ambient temperature, cleared in graded concentrations of isopropyl alcohol to remove any residual alcohol and then impregnated with Histosec pastilles under pressure at 60 °C. The samples were embedded in wax and cut using a rotary Microtome using Feather S35 disposable blades to a thickness of 5 µm. Samples were then stained with alcian blue and hematoxylin-eosin and examined by light microscopy. Four segments were fixed in each slide and the slides were viewed on an Olympus microscope (BX51TF, Olympus, Tokyo, Japan). The following variables were measured:

- Villus height (the distance from the apex of the villus to the junction of the villus and crypt)
- Crypt depth (the distance from the junction to the basement membrane of the epithelial cell at the bottom of the crypt)
- Epithelial thickness (the distance from the epithelial surface to the basement membrane of the epithelial cell)
- Goblet cell numbers (per 100 µm villus height)

Measurements of villus height and crypt depth were made on 10 villi at 4× magnification while epithelium thickness and goblet cell number were made at 40× magnification using microscopy imaging software (cellSens Standard [Ver.1.18] Olympus, Tokyo, Japan).

4.3.8. Relative weight of the proventriculus and gizzard

On d-22, two additional birds per cage with body weights closest to the mean weight of the cage were weighed and euthanised by intravenous injection (0.5 mL per kg live weight) of sodium pentobarbitone. The proventriculus and gizzard were carefully excised

and adherent fat was removed. The empty weight of these organs in individual birds were determined and reported as g/kg of BW.

4.3.9. Gizzard pH

Gizzard pH was measured in the same two birds using a pH meter (pH spear, Oakton Instruments, Vernon Hill, IL). The glass probe was inserted directly through an opening made in the gizzard and placed in the digesta. Three values were taken from the proximal, middle and distal areas of gizzard and the average value was considered as the final pH value.

4.3.10. Viscosity

Viscosity of jejunal digesta from these two birds was also measured. Digesta obtained from the lower jejunum was centrifuged at 3000 × g at 20 ^{o}C for 15 min. A 0.5 mL aliquot of the supernatant was used in a viscometer (Brookfield digital viscometer, Model DV2TLV; Brookfield Engineering Laboratories Inc., Stoughton, MA) fitted with CP-40 cone spindle with shear rates of 5 to 500/s to measure the viscosity.

4.3.11. Chemical analysis

Dry matter was determined using standard procedures (Method 930.15; AOAC, 2016). Nitrogen was determined by combustion (Method 968.06; AOAC, 2016) using a 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). Fat was determined using Soxtec extraction procedure for animal feed, forage and cereal grains (Method 2003.06; AOAC, 2016). Samples were assayed for Ti on a UV spectrophotometer following the method of Short *et al*. (1996).

4.3.12. Calculations

The AMEn of diets was calculated using the following formula:

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

Correction for zero N retention was made using a factor of 36.54 kJ per gram N retained in the body (Hill and Anderson, 1958).

$$AMEn_{diet}\ (MJ/kg) = AME_{diet} - (36.54 \times N\ retention)/1000$$

Apparent digestibility coefficients of nutrients were calculated from the dietary ratio of nutrients to Ti relative to the corresponding ratio in the ileal digesta.

$$CAID\ of\ nutrient = [(Nutrient / Ti)_d - (Nutrient / Ti)_i] / (Nutrient / Ti)_d$$

where, $(Nutrient / Ti)_d$ = ratio of nutrient to Ti in diet and $(Nutrient / Ti)_i$ = ratio of nutrient to Ti in ileal digesta.

4.3.13. Statistical analysis

The data were analysed as a 5 × 2 factorial arrangement of treatments using the general linear model procedure of SAS (version 9.4; SAS Institute Inc., Cary, NC.). Cage served as the experimental unit. Significant differences between means were separated by Least Significant Difference test. In addition, data on inclusion level of barley in the diet (as an average for without and with enzyme supplementation) were subjected to orthogonal polynomial contrasts using the general linear model procedure of SAS to study whether responses to increasing levels of barley had any linear or quadratic nature. Significance of effects was declared at $P < 0.05$.

4.4. Results

4.4.1. Pellet durability

The PDI of the experimental diets are shown in Table 4.1. A significant effect of inclusion level of barley ($P < 0.001$) was observed for PDI, with pellet durability deteriorating with increasing inclusion of barley in wheat-based diets.

4.4.2. Enzyme recovery

The average recovery of phytase, endo-1,3 (4)-β-glucanase and endo-1,4-β-xylanase from enzyme-supplemented diets were 113.5, 97.0 and 96.2%, respectively.

4.4.3. Growth performance

Mortality during the experiment was negligible. Only 11 out of the 400 birds died and the deaths were not related to any dietary treatment.

Influence of inclusion level of barley and enzyme supplementation on the weight gain (WG), FI and F/G of broiler starters fed diets with increasing levels of barley is summarised in Table 4.2. Neither the WG, FI nor F/G was subject to an interaction ($P > 0.05$). Inclusion level of barley had a significant effect on WG ($P < 0.01$), FI and F/G ($P < 0.001$). Barley inclusion tended to have a quadratic effect ($P = 0.06$) for WG; WG increased up to 283 g/kg of barley inclusion and then decreased with further inclusion. A linear reduction in FI ($P < 0.001$) and a quadratic improvement in F/G ($P < 0.05$) was observed with increasing inclusion of barley in the diet. Feed intake was similar up to 283 g/kg and then declined. The addition of enzyme increased the WG ($P < 0.05$) and F/G ($P < 0.001$) at each level of barley inclusion.

4.4.4. Nutrient digestibility

The CAID of DM, starch, N and fat in broiler starters fed diets with different inclusion levels of barley, without and with enzyme supplementation, are presented in Table 4.3. Inclusion level of barley and enzyme supplementation did not show any interaction ($P > 0.05$) for any nutrient, indicating similar impact of enzyme supplementation at each level of barley inclusion. However, significant effects of inclusion level of barley ($P < 0.05$ to 0.001) and enzyme supplementation ($P < 0.01$ to 0.001) were found for all nutrients. The CAID of DM, starch, N and fat, regardless of enzyme supplementation, was progressively improved with increasing inclusion of barley in the diet (linear effects, minimum $P < 0.003$). The lowest CAID of each nutrient was observed at 0 g/kg inclusion of barley, while the highest digestibility of each nutrient obtained for complete replacement of wheat with barley. Digestibility of all nutrients was improved ($P < 0.05$) by enzyme supplementation, regardless of the barley inclusion level.

Table 4. 2. Influence of barley inclusion (g/kg) and enzyme supplementation on weight gain (WG; g/bird), feed intake (FI; g/bird) and feed per gain (F/G; g feed/g gain) of broiler starters[1] (d1-21) fed diets based on wheat and normal-starch hulled barley.

Inclusion level of barley	Enzyme	WG	FI	F/G
0	-	1102	1524	1.396
	+	1128	1530	1.358
141	-	1128	1522	1.354
	+	1140	1484	1.308
283	-	1142	1525	1.345
	+	1152	1473	1.287
424	-	1074	1456	1.357
	+	1119	1446	1.293
565	-	1102	1435	1.308
	+	1116	1439	1.290
SEM[2]		14.5	18.8	0.0150
Main effects				
Inclusion level of barley				
0		1115bc	1527a	1.377a
141		1134ab	1503a	1.331b
283		1147a	1499a	1.316bc
424		1097c	1451b	1.325bc
565		1109bc	1437b	1.299c
Enzyme				
	-	1110b	1492	1.352a
	+	1131a	1474	1.307b
Probabilities, $P \leq$				
Inclusion level of barley		0.010	0.001	0.001
Enzyme		0.025	0.141	0.001
Inclusion level of barley × Enzyme		0.726	0.448	0.575
Orthogonal polynomial contrast				
L[3]		0.148	0.001	0.037
Q[4]		0.056	0.623	0.011

Means in a column not sharing common letters (a,b,c) are different ($P < 0.05$).
[1]Each value represents the mean of five replicates (eight birds per replicate).
[2]Pooled standard error of mean.
[3]L = Linear effect of inclusion level of barley.
[4]Q = Quadratic effect of inclusion level of barley.

Table 4. 3. Influence of barley inclusion (g/kg) and enzyme supplementation on coefficient of apparent ileal digestibility (CAID)[1] of dry matter (DM), starch, nitrogen (N) and fat and N-corrected apparent metabolisable energy (AMEn; MJ/kg DM)[2] in broiler starters fed diets based on wheat and normal-starch hulled barley.

Inclusion level of barley	Enzyme	DM	Starch	N	Fat	AMEn
0	-	0.522	0.836	0.714	0.762	11.95
	+	0.531	0.861	0.733	0.771	12.02
141	-	0.520	0.864	0.731	0.741	12.12
	+	0.566	0.904	0.752	0.810	12.24
283	-	0.530	0.901	0.740	0.780	12.38
	+	0.589	0.937	0.761	0.849	12.49
424	-	0.536	0.888	0.730	0.797	12.40
	+	0.603	0.923	0.767	0.835	12.70
565	-	0.579	0.918	0.751	0.827	12.64
	+	0.642	0.948	0.796	0.911	13.03
SEM[3]		0.0142	0.0127	0.0145	0.0240	0.107
Main effects						
Inclusion level of barley						
0		0.526c	0.849d	0.723b	0.766c	11.98c
141		0.543bc	0.884c	0.741b	0.775bc	12.18c
283		0.559b	0.919ab	0.751ab	0.815b	12.44b
424		0.569b	0.905bc	0.749ab	0.816b	12.55b
565		0.610a	0.933a	0.773a	0.869a	12.83a
Enzyme						
	-	0.537b	0.881b	0.733b	0.782b	12.30b
	+	0.586a	0.914a	0.762a	0.835a	12.50a
Probabilities, $P \leq$						
Inclusion level of barley		0.001	0.001	0.026	0.001	0.001
Enzyme		0.001	0.002	0.003	0.001	0.006
Inclusion level of barley × Enzyme		0.270	0.981	0.849	0.539	0.479
Orthogonal polynomial contrast						
L[4]		0.001	0.001	0.003	0.001	0.001
Q[5]		0.383	0.099	0.962	0.481	0.930

Means in a column not sharing common letters (a,b,c,d) are different ($P < 0.05$).
[1]Each value represents the mean of five replicates (six birds per replicate).
[2]Each value represents the mean of five replicates (eight birds per replicate), measured from d-17 to 20.
[3]Pooled standard error of mean.
[4]L = Linear effect of inclusion level of barley.
[5]Q = Quadratic effect of inclusion level of barley.

4.4.5. Energy utilisation

Influence of inclusion level of barley and enzyme supplementation on AMEn in broiler starters is summarised in Table 4.3. The AMEn was not subject to an interaction ($P > 0.05$), showing a consistent and positive effect ($P < 0.01$) of enzyme at each level of barley inclusion. Inclusion level of barley had a significant ($P < 0.001$) effect on AMEn. A gradual improvement (linear effect, $P < 0.001$) was observed with increasing level of barley in the diet, while the highest ($P < 0.05$) value for AMEn was observed for the diet with complete replacement of wheat with barley.

4.4.6. Digestible nutrient intake

The influence of barley inclusion and supplementation of enzyme on digestible intake of nutrients (starch, protein and fat) and intake of AMEn is shown in Table 4.4. No interaction between barley inclusion and enzyme addition was present ($P > 0.05$). The main effect of inclusion level of barley was significant ($P < 0.01$ to 0.001) on digestible intake of each analysed nutrient. Digestible starch and protein (linear effects, $P < 0.01$ and 0.001, respectively) intakes were unaffected up to 283g/kg of barley inclusion, and then decreased with further inclusion. Digestible fat intake increased (linear effect, $P < 0.001$) with increasing inclusion of barley in the diet. Supplementation of enzyme increased ($P < 0.05$) digestible intake of starch and fat, but had no effect on N ($P > 0.05$). Neither inclusion level of barley nor enzyme supplementation affected AMEn intake ($P > 0.05$).

4.4.7. Relative weights of proventriculus and gizzard, gizzard pH and jejunal digesta viscosity

Table 4.5 shows the effect of barley inclusion level and enzyme supplementation on relative weight of proventriculus and gizzard, gizzard pH, and jejunal digesta viscosity. The relative weight of gizzard increased (linear effect, $P < 0.001$) with increasing inclusion of barley in the diet. The gizzard pH remained unchanged up to 283g/kg of barley and then reduced with further inclusion ($P < 0.01$), however, supplemental enzyme had no effect ($P > 0.05$).

No interaction ($P > 0.05$) between inclusion level of barley and enzyme supplementation was observed for digesta viscosity, while inclusion level of barley and enzyme supplementation had significant ($P < 0.001$) effects. Regardless of enzyme supplementation, the jejunal digesta viscosity decreased in a decreasing rate (quadratic effect, $P < 0.05$) with increasing barley inclusion in the diet. The addition of enzyme decreased the jejunal digesta viscosity at each level of barley inclusion.

4.4.8. Intestinal morphology

The influence of barley inclusion and enzyme supplementation on the morphometry of the duodenum and jejunum is shown in Table 4.6. A significant barley inclusion × enzyme interaction ($P < 0.05$) was observed only for duodenal crypt depth. Added enzyme increased the duodenal crypt depth only in the 424 g/kg barley inclusion, while it had no effect at other inclusion levels. Inclusion level of barley tended to have a significant effect on duodenal goblet cell number ($P = 0.07$). Supplemental enzyme increased the epithelial thickness in the duodenum ($P < 0.05$). In the jejunum, no interaction between barley inclusion and enzyme supplementation was observed for any morphometric parameter ($P > 0.05$). However, the inclusion level of barley had a significant effect on jejunal villus height ($P < 0.05$). All barley inclusion levels, except 424 g/kg, resulted in higher jejunal villi compared to 0 g/kg of barley. The inconsistent responses of jejunal villus height to increasing inclusion of barley tended to result in a quadratic effect ($P = 0.06$). Moreover, barley inclusion level tended to have a significant effect on jejunal epithelial thickness ($P = 0.08$). Jejunal epithelial thickness increased with increasing inclusion of barley (linear effect, $P < 0.01$). Enzyme supplementation had no effect on the morphometric parameters in the jejunum ($P > 0.05$).

Table 4. 4. Influence of barley inclusion (g/kg) and enzyme supplementation on digestible nutrient (starch, protein and fat) intake[1] (g/bird) and nitrogen-corrected apparent metabolisable energy (AMEn)[2] intake (MJ/bird) of broiler starters from 1 to 21 d, fed diets based on wheat and normal-starch hulled barley.

Inclusion level of barley	Enzyme	Starch	Protein	Fat	AMEn
0	-	438	272	22.2	16.07
	+	452	280	22.5	16.23
141	-	442	274	27.1	16.22
	+	452	274	28.8	15.98
283	-	454	277	33.8	16.68
	+	456	275	35.6	16.25
424	-	418	250	40.8	15.87
	+	432	262	42.4	16.15
565	-	417	249	44.5	16.01
	+	432	265	49.1	16.56
SEM[3]		8.4	6.7	1.19	0.176
Main effects					
Inclusion level of barley					
0		445a	276a	22.4e	16.15
141		447a	274a	28.0d	16.10
283		455a	276a	34.7c	16.46
424		425b	256b	41.6b	16.01
565		425b	257b	46.8a	16.29
Enzyme					
	-	434b	264	33.7b	16.17
	+	445a	271	35.7a	16.23
Probabilities, $P \leq$					
Inclusion level of barley		0.002	0.002	0.001	0.108
Enzyme		0.048	0.110	0.011	0.578
Inclusion level of barley × Enzyme		0.928	0.659	0.476	0.055
Orthogonal polynomial contrast					
L[4]		0.002	0.001	0.001	0.664
Q[5]		0.068	0.357	0.847	0.759

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

[1]Digestible nutrient intake (g/bird) = Feed intake (kg) × nutrient content of the feed (g/kg) × coefficient of apparent ileal digestibility of nutrient. Each value represents the mean of five replicates (eight birds per replicate).

[2]AMEn intake (MJ/bird) = Feed intake (kg, DM) × AMEn of the feed (MJ/kg, DM). Each value represents the mean of five replicates (eight birds per replicate), measured from d-17 to 20.

[3]Pooled standard error of mean.

[4]L = Linear effect of inclusion level of barley.

[5]Q = Quadratic effect of inclusion level of barley.

Table 4. 5. Influence of barley inclusion (g/kg) and enzyme supplementation on relative weight of proventriculus and gizzard (g/kg of body weight), gizzard pH, and viscosity (cP) in jejunal digesta of 21-d old broilers fed diets based on wheat and normal-starch hulled barley[1]

Inclusion level of barley	Enzyme	Relative weight		Gizzard pH	Jejunal digesta Viscosity
		Prov.	Gizzard		
0	-	3.87	7.59	3.68	5.32
	+	3.48	7.30	3.47	4.65
141	-	3.76	8.50	2.97	4.07
	+	3.04	7.33	3.79	3.68
283	-	3.68	9.11	3.63	3.89
	+	3.25	8.32	3.94	2.96
424	-	3.80	8.92	2.96	3.39
	+	3.68	9.28	2.80	2.76
565	-	3.70	10.23	3.07	3.09
	+	4.00	10.30	3.18	2.53
SEM[2]		0.291	0.520	0.245	0.274
Main effects					
Inclusion level of barley					
0		3.68	7.45d	3.58ab	4.99a
141		3.40	7.91cd	3.38ab	3.87b
283		3.47	8.71bc	3.79a	3.43bc
424		3.74	9.10b	2.88c	3.07cd
565		3.85	10.27a	3.13bc	2.81d
Enzyme					
	-	3.76	8.87	3.26	3.95a
	+	3.49	8.51	3.44	3.32b
Probabilities, $P \leq$					
Inclusion level of barley		0.515	0.001	0.006	0.001
Enzyme		0.149	0.273	0.271	0.001
Inclusion level of barley × Enzyme		0.492	0.589	0.238	0.905
Orthogonal polynomial contrast					
L[3]		0.306	0.001	0.016	0.001
Q[4]		0.220	0.472	0.519	0.030

Means in a column not sharing common letters (a,b,c,d) are different ($P < 0.05$).
[1]Each value represents the mean of five replicates (two birds per replicate).
[2]Pooled standard error of mean.
[3]L = Linear effect of inclusion level of barley.
[4]Q = Quadratic effect of inclusion level of barley.

Table 4. 6. Influence of barley inclusion (g/kg) and enzyme supplementation on villus height (µm), goblet cell number (per 100 µm villus height), epithelial thickness (µm) and crypt depth (µm) of the duodenum and jejunum of 21-d old broilers fed diets based on wheat and normal-starch hulled barley[1].

Inclusion level of barley	Enzyme	Duodenum				Jejunum			
		Villus height	Goblet cell number	Epithelial thickness	Crypt depth	Villus height	Goblet cell number	Epithelial thickness	Crypt depth
0	-	1081	13.5	20.1	94.3a	605	13.5	17.7	78.5
	+	1097	15.9	22.4	90.1ab	579	15.6	18.0	80.7
141	-	984	14.7	19.9	81.9de	636	15.1	18.9	79.7
	+	991	15.0	21.8	91.8ab	667	16.0	18.0	79.0
283	-	1014	17.1	21.1	87.5bcd	727	16.2	19.3	76.6
	+	988	16.2	20.9	86.7bcd	651	16.3	19.0	78.0
424	-	910	13.4	19.6	81.6e	618	14.7	18.9	73.0
	+	1040	14.1	21.5	82.6cde	626	13.8	20.0	81.7
565	-	1049	14.4	20.7	88.3bc	643	16.9	19.7	80.1
	+	1038	13.7	20.9	86.4bcd	673	13.9	19.9	78.8
SEM[2]		46.9	1.07	0.81	2.11	29.5	1.01	0.75	2.41
Main effects									
Inclusion level of barley									
0		1089	14.7	21.2	92.2	592c	14.5	17.8	79.6
141		987	14.9	20.9	86.9	651ab	15.6	18.4	79.4
283		1001	16.7	21.0	87.1	689a	16.3	19.2	77.3
424		975	13.8	20.5	82.1	622bc	14.2	19.4	77.4
565		1044	14.1	20.8	87.4	658ab	15.4	19.8	79.4
Enzyme									
	-	1008	14.6	20.3b	86.7	646	15.3	18.9	77.6
	+	1031	15.0	21.5a	87.5	639	15.1	19.0	79.7
Probabilities, $P \leq$									
Inclusion level of barley		0.101	0.066	0.940	0.001	0.019	0.258	0.077	0.766
Enzyme		0.438	0.576	0.019	0.554	0.729	0.812	0.884	0.182
Inclusion level of barley × Enzyme		0.480	0.568	0.419	0.014	0.332	0.133	0.737	0.253
Orthogonal polynomial contrast									
L[3]		0.323	0.328	0.514	0.004	0.123	0.878	0.004	0.673
Q[4]		0.017	0.116	0.778	0.007	0.055	0.343	0.618	0.306

Means in a column not sharing common letters (a-e) are different ($P < 0.05$).
[1]Each value represents the mean of five replicates (two birds per replicate, 10 readings per bird).
[2]Pooled standard error of mean.
[3]L = Linear effect of inclusion level of barley.
[4]Q = Quadratic effect of inclusion level of barley.

4.5. Discussion

In contrast to the study by Ankrah (1994) who reported no difference in the hardness of pellets made from normal starch hull-less barley and wheat, increasing inclusion of barley in the diet worsened the PDI in the present study. A high content of fat was used in diets with greater inclusion of barley to maintain similar energy levels and explains the results observed. Fat lubricates feed particles and reduces the friction generated in the die holes, which results in lower pellet durability. Dietary fat can also 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*., 2013a). Buchanan and Moritz (2009) evaluated the influence of small amounts of fibre, in the form of oat hulls, on pellet quality and observed that pellets tended to break at oat hull contact points. Ground NSH barley contained a considerable amount of hulls and this may have also contributed to the reduced PDI in diets with greater barley inclusions.

The lack of significant interaction between barley inclusion and enzyme addition for WG and F/G indicated that the efficacy of enzyme was similar at each barley inclusion level, and was strong enough to make significant improvements in WG of 21 g/bird and F/G of 4.5 points. Regardless of the enzyme supplementation, WG increased gradually up to 283 g/kg barley inclusion and reduced at inclusions above this point. Dietary inclusion of barley beyond 283 g/kg decreased the FI irrespective of enzyme supplementation. The reduced FI at 424 and 565 g/kg barley inclusions can be partly attributed to the deteriorated PDI at these inclusion levels (Abdollahi *et al*., 2018). The impaired WG at barley inclusion levels of 424 and 565 g/kg corresponded with lowered FI, and consequent reduction in digestible starch and protein intake at these inclusion levels. This observation confirms the importance of FI on the growth performance of broiler starters.

Slower feed passage rate associated with greater digesta viscosity (Salih *et al*., 1991; Almirall and Esteve-Garcia, 1995; Almirall *et al*., 1995) can affect FI in young broiler chickens (McNab and Smithard, 1992). However, this is not applicable to the findings of the current study, as the highest FI was observed in birds fed the diet with no barley, which had the highest jejunal digesta viscosity. The lower palatability of barley compared to wheat (Hughes, 1984) presumably played a role in determining FI in the

current study. Despite different AMEn values of diets, similar AMEn intakes were observed across all dietary treatments. Therefore, the lower FI associated with highest inclusions of barley (424 and 565 g/kg) can be considered as birds' response to maintain a constant energy intake (Classen, 2017).

Friesen *et al*. (1992) evaluated different inclusion levels of hulled barley (0, 350 and 700 g/kg diet) and supplementation of a cellulase enzyme and reported a reduction in FI with increasing dietary levels of barley. In agreement with the present study, the depression of FI reported by these researchers was most severe at the total replacement of wheat with hulled barley (700 g/kg diet), and feed efficiency of chicks fed the non-supplemented hulled barley diets (350 and 700 g/kg diet) was better compared to those fed the control wheat diet. The highest inclusion of hulled barley (700 g/kg) resulted in the lowest WG, while the WG in birds fed with 0 and 350 g/kg hulled barley were similar.

Moss *et al*. (1983) increased NSH barley inclusion in a wheat-based broiler diet from zero to 272, 408 and 544 g/kg with no enzyme supplementation and reported that increasing levels of barley consistently decreased WG and increased F/G by 14.0 points at barley inclusion of 544 g/kg. In their study, however, barley replaced wheat on a weight-to-weight basis, resulting in dietary treatments being different in respect to energy and protein contents. Therefore, the poor performance observed with the increasing levels of barley was most likely due to the lower AME content and digestible AA of barley-based diets compared to those based on wheat.

Yu *et al*. (1998) studied five isoenergetic and isonitrogenous diets with varying levels of barley (0, 70, 140, 278 and 557 g/kg in diet) substituted for maize and reported lower WG and FI with increasing inclusion of barley. However, when the diets were supplemented with β-glucanase, WG and FI increased up to 140 g barley/kg diet (25% replacement of maize), but depressed with barley inclusion beyond this point. It is noteworthy that even though these diets were formulated to be equivalent in energy and protein density, diets were formulated based on nutrient and total AA composition obtained from chemical analysis. Accordingly, decreased performance observed in the study by Yu *et al*. (1998) with increasing inclusions of barley may be partly due to the lower digestibility of nutrients, especially AA, in barley grain compared to maize.

Moreover, the inconsistencies in the literature suggest the importance of formulating diets based on digestible nutrient contents.

Bergh *et al*. (1999) added a carbohydrase enzyme complex consisting of xylanase and β-glucanase to a barley-based (696 g/kg) diet and reported greater BW and FI and improved feed conversion ratio. The increase in FI due to enzyme supplementation reported by these researchers was not observed in the current study. Mathlouthi *et al*. (2002) reported that addition of an NSP degrading enzyme to wheat- and barley-based diets resulted in a growth performance similar to a non-supplemented maize-based diet.

Contrary to the inconsistent responses of WG to the increasing levels of barley in the present study, F/G gradually improved with increasing barley inclusion regardless of enzyme supplementation. Observations on F/G in the current study are consistent with effects on jejunal digesta viscosity, indicating that changes in the digestive tract due to dietary NSP sources affect the feed efficiency of birds. Almirall *et al*. (1995), who evaluated the growth performance in broilers fed maize and two barley types, attributed the improved WG and feed efficiency in barley-based diets to the reduction in intestinal viscosity due to the action of supplemental enzyme. In agreement with Almirall *et al*. (1995), depressions in F/G in birds with greater digesta viscosity was observed in the current study. Consequently, the improvements in the WG and F/G with supplemental enzyme observed in the present study can be attributed to the reduction in digesta viscosity due to the action of enzymes (Bedford *et al*., 1991; Steenfeldt *et al*., 1998; Shakouri *et al*., 2009).

Increasing inclusions of barley in wheat-based diets improved the CAID of all analysed nutrients. Improvements of nutrient digestibility due to complete replacement of wheat with barley for DM, starch, N and fat were 16, 9.9, 6.9 and 13.5%, respectively. The improvement of DM digestibility with increasing barley in the diet was indicative of improved digestibility of all nutrients. Svihus (2001) compared the ileal starch digestibility of four varieties of wheat and barley substituted on a weight basis at 770 g/kg diet. Barley diets had greater CAID of starch (0.96) than all four wheat diets without supplemental enzyme (average of 0.78), and tended to have a greater starch digestibility than an enzyme-supplemented wheat-based diet (0.93). This observation implies the

presence of factors other than soluble NSP that interfere with starch digestion. Svihus and Hetland (2001) hypothesised that an overload of wheat starch in the digestive tract can lower the starch digestion in broiler chickens. According to the analysed starch contents of the experimental diets in the present study, the highest starch content (343 g/kg, as fed basis) was determined for the diet with 0 g/kg barley, and dietary starch values reduced with increasing barley inclusion in the diet.

Svihus and Hetland (2001) identified the gizzard as the key site for preventing starch overload in the digestive tract by regulating digesta passage rate (Hetland *et al*., 2004). The development of the gizzard is facilitated by the presence of insoluble NSP in the diet (Svihus, 2011a). According to the nutrient composition of the wheat and barley used in the current study analysed in Chapter 3, barley contained more insoluble NSP than wheat (142 vs. 119 g/kg). In consequence, greater concentrations of insoluble NSP are anticipated in diets with greater inclusion of barley. Increased weights of the gizzard and greater CAID of starch in birds fed diets with greater inclusion of barley in the current study lend support to the hypothesis of Svihus and Hetland (2001) that a well-developed gizzard can prevent the starch overload in the digestive tract and will facilitate better digestion and absorption. Moreover, a greater starch digestibility in wheat- and raw potato starch-based diets supplemented with oat hulls was attributed to actions of oat hulls in gizzard enlargement and mechanical abrasion resulting in disruption of starch granules and modification in gut microflora (Rogel *et al*., 1987a,b). Similarly, the increased occurrence of barley hulls with increasing inclusions of NSH barley in the diet might have contributed to improved starch digestion.

The proventriculus and gizzard (ventriculus) are the true stomach compartments in birds. Hydrochloric acid (HCl) and pepsinogen are secreted by the proventriculus and mixed with digesta in the gizzard. The proventriculus is the initial site of protein digestion in chickens where proteins are exposed to HCl, which denatures the protein and then exposes peptide bonds for enzyme hydrolysis. Adequate acid secretion is necessary for conversion of pepsinogen to pepsin, the enzyme initiating protein digestion. The amount of time that feed is retained in the proventriculus is insufficient for adequate exposure to secretions. Extended retention and mixing in the gizzard is necessary to allow for increased contact between feed, gastric juices and pepsin, thus, facilitating the

denaturation and digestion of proteins (Rynsburger, 2009). Accordingly, the larger gizzards in birds fed greater inclusion levels of barley might have aided in initial protein hydrolysis and, subsequently, resulted in greater CAID of N.

Due to the lower AMEn of barley (13.63 MJ/kg) compared to wheat (14.40 MJ/kg; Chapter 3), more fat was added to diets with greater inclusion levels of barley to equalise the energy content across diets in the current study. Therefore, the greater magnitude of response (13.5%) in CAID of fat in the diet with complete replacement of wheat with barley is mainly attributed to greater concentration of soybean oil.

Friesen *et al*. (1992) reported similar apparent excreta digestibility of protein and decreasing lipid digestibility in broilers as the hulled barley increased from 0 to 700 g/kg in a wheat-based diet with no enzyme supplementation. Svihus (2001) compared the CAID of protein and fat of four wheat types with barley (at 770 g grain/kg diet) and reported a greater average digestibility for wheat (0.79 vs. 0.68 and 0.73 vs. 0.66 for protein and fat, respectively). However, the nutrient composition of the grain samples was not provided in the paper. Bolarinwa and Adeola (2012) used wheat and barley to partly replace maize, soybean meal, maize starch, and soy oil, on a weight basis, in a reference diet at 100 or 200 g/kg. Inclusion of wheat did not cause any change in the CAID of any nutrient, while the CAID of DM and N decreased with increasing levels of barley in the diet. According to the nutrient composition, both barley and wheat were similar except for fibre fractions, with barley containing more crude fibre (55.4 vs. 23.8 g/kg).

Regardless of the inclusion level of barley, the magnitude of response to enzyme supplementation on ileal digestibility of DM, starch, N and fat were 9.1, 3.8, 4.0 and 6.8%, respectively. Starch and N digestibility might be facilitated from the enzyme action on cell wall integrity, which subsequently released the encapsulated starch and protein. Moreover, reduced digesta viscosity due to the added enzyme allows better interactions of digestive enzymes with respective substrates. Increased intestinal digesta viscosity is believed to be more detrimental on fat digestion (Edney *et al*., 1989; Almirall *et al*., 1995), making fat digestion the most affected by the presence of soluble NSP (Choct and Annison, 1992a). This observation was confirmed in the current work. High digesta

viscosity reduces the diffusion and passage of droplets of emulsion, fatty acids, mixed micelles, bile salts and lipase within the gastrointestinal tract, leading to reduced transport of micelles to the mucosal surface (Smulikowska, 1998, 2002). Three major modes of action of NSP-degrading enzymes have been proposed in the literature (i) reduction of digesta viscosity (Almirall *et al*., 1995), (ii) release of encapsulated nutrients via cell wall degradation (Hesselman and Åman, 1986; Bedford, 1996), and (iii) modification of gut microbiota through supply of prebiotic oligosaccharides (González-Ortiz *et al*., 2017; Bedford, 2018). The production of fermentable substrates for favourable microbial groups is proved to have beneficial effect on gut health (Józefiak *et al*., 2010) and villus growth (González-Ortiz *et al*., 2017), and to improve nutrient utilisation. Mathlouthi *et al*. (2002) attributed the improved protein and fat digestibility with supplementation of NSP-degrading enzymes in wheat- and barley-based diets to the reduction of total anaerobic bacterial load in the caeca. In addition, the presence of *Lactobacillius* and *Bifidobacter spp*. in the ileum induced by supplemental enzymes in barley-based diets (Rodriguez *et al*., 2012) might have indirectly enhanced the nutrient digestibility in broilers.

Linear improvements in AMEn were observed with increasing levels of barley in the diet, and AMEn was improved with enzyme addition at each inclusion level. Friesen *et al*. (1992) reported that increasing levels of hulled barley in a wheat-based diet (0, 350 and 700 g/kg) resulted in linear reductions in AMEn in diets without or with supplementation of a cellulase enzyme. The improvement of 7.1% (0.85 MJ/kg) in AMEn in the present study due to complete substitution of wheat with barley was contrary to reduction of AMEn (5.2%) reported by Friesen *et al*. (1992) with complete replacement of wheat with hulled barley. However, the increase of 0.2 MJ/kg in AMEn due to added NSP-degrading enzyme in the present study is lower than the improvement of AMEn (0.75 MJ/kg) due to enzyme supplementation reported by these researchers. Fuente *et al*. (1995), who evaluated increasing levels of barley in a maize-based diet, reported decreasing AMEn with increasing inclusion of barley. However, these researchers reported an increase in AMEn of enzyme-supplemented diet by 0.26 MJ/kg.

Despite that the diets in the present study were formulated to contain the same amount of energy by using AMEn values of the grains obtained in Chapter 3, AMEn values observed in the present study varied from 11.98 to 12.83 MJ/kg. Fuente *et al*.

(1995) suggested that digesta viscosity accounts for 97% of the variation in AMEn among barley-based diets and reported 59 kJ decline in AMEn per unit (cP) increase in digesta viscosity. A significant ($P < 0.001$) negative correlation (r = -0.488) between AMEn and jejunal digesta viscosity observed in the present study supports previous findings on the influence of digesta viscosity on energy utilisation by birds (Choct and Annison, 1992b; Smulikowska *et al*., 2002). Moreover, similar trends in treatment effects on nutrient digestibility and AME demonstrate a link between nutrient digestibility and AMEn.

Changes in gastrointestinal morphology associated with variation of dietary fibre concentrations were previously observed with special reference to the gizzard (Hetland *et al*., 2003; Amerah *et al*., 2009). A more developed musculature in the gizzard, as an adaptive response to increased dietary fibre in the diet, can lead to increased gizzard weight. In the present experiment, the complete replacement of wheat with barley resulted in 37.9% increase in the gizzard weight, from 7.45 to 10.27 g/kg body weight. More extensive grinding by larger gizzards might have facilitated the improvements in F/G, AMEn and nutrient utilisation at greater levels of barley inclusion. An increase in gizzard size can improve digestive function through increased retention time, lower pH, and better grinding and mixing with digestive enzymes (Svihus, 2011a, 2014).

Although the pH of gastric secretions is around 2.0 (Denbow *et al*., 1988), the amount, retention time and chemical characteristics of the digesta in the proventriculus/gizzard can result in a more variable and usually higher pH (Svihus, 2011a). When birds have a greater FI, the neutral pH in feed (Ravindran, 2013) can lead to a higher gizzard pH unless HCl secretion is able to increase in conjunction with intake (Svihus, 2014). Moreover, increased grinding in the gizzard and a longer retention time allows for more HCl secretion, resulting reduced pH. In accordance with these observations, the reduction of FI beyond 283 g/kg barley inclusion in the current study was associated with a reduction in gizzard pH. Besides lower FI, the increased size of the gizzard in birds fed greater inclusion levels of barley in the diet might have facilitated more HCl secretion resulting a lower pH.

Yu *et al*. (1998) measured the viscosity in duodenal digesta at different replacement levels of maize with barley, without and with a β-glucanase, and reported

increased intestinal viscosity as the inclusion of barley increased. Supplemental β-glucanase tended to decrease the viscosity at complete replacement of maize with barley, in which the concentration of soluble NSP was at maximum. Yaghobfar and Kalantar (2017) reported similar digesta viscosity for non-supplemented wheat and barley diets (150 g/kg diet), where the supplementation of a mixture of phytase and NSP degrading enzyme reduced the digesta viscosity in both diets. Fuente *et al*. (1995) reported a digesta viscosity increase of 3.5 cP per every 100 g/kg of barley inclusion. The lack of significant interaction for digesta viscosity in the current study is suggestive of a consistent enzyme efficacy at each level of barley inclusion. Contrary to previous observations, the highest digesta viscosity (4.99 cP) was observed, at 0 g/kg barley inclusion, and decreased with the increasing inclusion of barley. In agreement with present findings, Shakouri *et al*. (2009), who compared intestinal viscosity of broilers fed barley, maize, sorghum and wheat, reported greater digesta viscosity in the birds fed wheat-based diets (5.74 cP) compared to barley-based diets (2.92 cP). This surprising observation on decreasing digesta viscosity with increasing inclusion of barley confirms that digesta viscosity is dependent not only on the concentration of NSP, but also on its molecular weight. Therefore, a grain with a low content of soluble NSP might result in high viscosity if the NSP is of a high molecular weight (Saulnier *et al*., 1995; Dusel *et al*., 1997; Cowieson *et al*., 2005). Moreover, it was suggested that wheat gluten and its endosperm proteins (gliadins and glutenins) have an effect on the viscosity of aqueous extract of wheat flour. Glutenin acts as a cohesive elastic solid when hydrated while gliadin together with water behaves as a viscous liquid and, therefore, wheat varieties with a high level of crude protein can contain more gliadin and glutenin, resulting in greater viscosity in aqueous extracts (Dusel *et al*., 1997). Accordingly, the greater digesta viscosity observed in the current study from diets with low barley inclusion (i.e., great content of wheat) suggests that the wheat cultivar used had high molecular weight NSP, which consequently increased the digesta viscosity irrespective of NSP concentration.

Contrary to the current finding of increased jejunal villus height in barley fed birds, shorter jejunal villi in birds fed barley- compared to maize-based diets was reported previously (Viveros *et al*., 1994; Onderci *et al*., 2008; Kalantar *et al*., 2016). Shakouri *et al*. (2009) also reported decreased jejunal villus height in birds fed diets with 600 g barley/kg compared to three diets containing maize, wheat and sorghum (623 g/kg in each

diet). The observations on increased jejunal villus height and subsequent greater villus absorptive area correspond with the positive effect of barley inclusion on feed efficiency and CAID of nutrients in the current study.

The lack of response from gut morphology parameters (except epithelial thickness) to enzyme supplementation was in agreement with Iji *et al*. (2001) and Wu *et al*. (2004b). However, Wu *et al*. (2004b) reported that xylanase supplementation tended to increase the number of goblet cells in the duodenum and decreased jejunal crypt depth. Viveros *et al*. (1994) reported a relative reduction in the goblet cell number of jejunal mucosa in birds fed barley supplemented with β-glucanase. Enzyme supplementation increased the duodenal crypt depth only at 141 g/kg inclusion of barley in the current study, resulting a significant interaction between barley inclusion and enzyme supplementation. However, this observation is difficult to explain as enzyme supplementation in previous studies reduced the crypt depth (Wu *et al*., 2004b; Rebolé *et al*., 2010).

4.6. Conclusions

Despite impaired pellet quality, increasing inclusion of barley in wheat-based diets improved feed efficiency, nutrient digestibility and energy utilisation, due likely to lowered digesta viscosity and better functionality of gizzard. The corresponding improvements in feed efficiency, nutrient digestibility and energy utilisation with lowered digesta viscosity in birds fed enzyme-supplemented diets confirmed the benefits from the viscosity reducing mechanism of supplemental carbohydrases in barley-based diets. With respect to growth performance, the optimum inclusion level of barley in a wheat-based broiler starter diet is 283 g/kg of diet. Future studies *inter alia* on the influence of feed processing parameters such as grain particle size and conditioning temperature, in combination with enzyme supplementation, are warranted to explore the barley inclusion beyond this level.

CHAPTER FIVE

The effect of graded inclusions of waxy starch hull-less barley and a multi-component exogenous carbohydrase on the growth performance, nutrient digestibility and intestinal morphometry of broiler chickens[3]

5.1. Abstract

A 21-d experiment was conducted to investigate the effect of graded inclusions of waxy starch hull-less (WSHL) barley and a multi-component exogenous carbohydrase on the growth performance, nutrient digestibility and intestinal morphometry of broiler chickens. Five levels of WSHL barley inclusion (0, 65, 130, 195 and 260 g/kg) in a wheat-based diet and two levels of enzyme supplementation (0 and 150 g/tonne of feed) were evaluated in a 5 × 2 factorial arrangement of 10 dietary treatments. All diets were equivalent in metabolisable energy and digestible amino acid contents. A total of 400, one-d old male broilers (five cages/treatment; eight birds/cage) were used in the experiment. Regardless of enzyme supplementation, feed intake declined ($P < 0.001$) with increasing inclusion of WSHL barley. Increasing levels of WSHL barley ($P < 0.001$) and supplemental enzyme ($P < 0.05$) improved feed per gain. Birds fed diets with 0 g/kg WSHL barley showed the lowest ($P < 0.001$ to 0.01) digestibility for all nutrients except starch. Only the starch digestibility was improved ($P < 0.05$) by enzyme supplementation. The nitrogen-corrected apparent metabolisable energy improved with increasing inclusion of WSHL barley ($P < 0.001$) and supplemental enzyme ($P < 0.001$). Increasing inclusion of WSHL barley increased the relative weight of gizzard ($P < 0.001$) and reduced jejunal digesta viscosity ($P < 0.01$). Supplemental enzyme ($P < 0.001$) reduced the digesta viscosity. All levels of WSHL barley inclusion improved digestibility of dry matter, nitrogen and fat, whilst energy utilisation improved at inclusions of 130 g/kg WSHL and above, due likely to lowered digesta viscosity and better development of the gizzard. Feed per gain, starch digestibility, energy utilisation and jejunal digesta viscosity can benefit from carbohydrase supplementation in wheat-based diets, regardless of barley inclusion level.

[3]*British Poultry Science, 61*(4), 442-453.

5.2. Introduction

Inclusion of barley (*Hordeum vulgare* L.) in poultry diets is limited due mainly to its high fibre content, low energy and high content of non-starch polysaccharides (NSP; Jacob and Pescatore, 2012). Hull-less barley was developed to counter the perception of anti-nutritive influences from the fibrous hull and increase acceptance of barley as a poultry feed ingredient (Bhatty, 1999; Jacob and Pescatore, 2012). This development has resulted in a cereal that is more compatible with nutrient dense feeds preferred by the poultry industry (Campbell *et al*., 1993). Other advantages of using hull-less over hulled barley in poultry feed include elimination of cost and labour associated with dehulling and concentration of nutrients created from the removal of hull. Hull-less barley types have been reported to have greater concentrations of energy, fat, protein and starch compared to hulled barley types (Edney *et al*., 1992; Xue *et al*., 1997; Svihus and Gullord, 2002). Nevertheless, some studies report of hulled barley types with greater content of starch compared to hull-less barley types (Andersson *et al*., 1999; Asare *et al*., 2011).

Removal of the hull through the development of hull-less barley types in conjunction with incorporation of the waxy starch trait was expected to further improve the feeding value of barley for poultry. However, in contrary to the expectation that waxy grain starch with more amylopectin (970-1000 g/kg of starch, Ullrich *et al*., 1986) is more digestible (Björck *et al*., 1990), poor starch digestibility has been observed in birds fed waxy barley-based diets (Bergh *et al*., 1999; Ravindran *et al*., 2007). The poor growth performance observed in birds fed hull-less, waxy starch barley can be attributed to soluble β-glucan with high molecular weights, which occur in greater amounts in waxy starch barley types (Storsley *et al*., 2003).

Supplementation of barley-based broiler diets with NSP- degrading enzymes has been reported to increase feed intake (FI), weight gain (WG), flock uniformity, improve feed efficiency, and enhance nutrient utilisation (Hesselman and Åman, 1986; Marquardt *et al*., 1994; Almirall *et al*., 1995; Bergh *et al*., 1999). Exogenous NSP-degrading enzymes are thought to act on barley-based diets by: (i) reduction of digesta viscosity (Almirall *et al*., 1995; Józefiak *et al*., 2006), (ii) release of encapsulated nutrients via cell wall degradation (Hesselman and Åman, 1986; Bedford, 1996), and (iii) modification of gut microbiota through the supply of prebiotic oligosaccharides (González-Ortiz *et al*., 2017; Bedford, 2018). Because of the higher contents of soluble β-glucans, waxy starch

barley in comparison to normal and high amylose starch barley, and hull-less barley in comparison to hulled barley, have shown greater responses to supplemental enzymes (Rotter *et al*., 1990; Ravindran *et al*., 2007).

The benefits of waxy starch barley associated with lower starch gelatinisation temperature, such as higher physical pellet quality and reduced energy input in pellet production (Ankrah *et al*., 1999) and the efficacy of exogenous enzymes to mitigate the anti-nutritive effects of soluble NSP make waxy starch barley an attractive feed ingredient for poultry. Recommendations on the optimum inclusion of hull-less barley have been contradictory due to confounding factors such as starch type and cultivar differences, and most of the studies have overlooked the influence of starch type and cultivar. Three approaches have been used in previous research to replace other cereals with barley, namely: (a) weight to weight basis (Arscott *et al*., 1955; Petersen, 1969; Moss *et al*., 1983; Yu *et al*., 1998); (b) using nutrient composition data for barley and the substituted grain from established sources such as National Research Council (Moharrery, 2006) and Spanish Foundation for the Development of Animal Nutrition (FEDNA; Lázaro *et al*., 2003; de Blas *et al*., 2010); and (c) using nutrient composition data obtained from chemical analysis (Brake *et al*., 1997).

The fact that anti-nutritive components in barley play a key role in determining the availability of dietary components to poultry emphasises the importance of using nutrient profiles for the specific barley cultivar based on measured contents of apparent metabolisable energy (AME) and digestible amino acids (AA) to formulate barley-based diets. Starch characteristics of the barley grain can, to some extent, explain the variability in responses to some evaluated factors (e.g. enzyme addition) in previous reports, suggesting that observed variations could not be attributed to one factor alone. Therefore, the present study was aimed to evaluate the influence of graded levels of a waxy starch hull-less (WSHL) barley cultivar previously evaluated for nitrogen-corrected AME (AMEn) and digestible AA content (Chapter 3), and supplementation of a multi-component carbohydrase on the performance, nutrient and energy utilisation and intestinal morphometry in broiler starters.

5.3. Materials and methods

5.3.1. Enzymes

A multi-component NSP-degrading enzyme, Ronozyme® Multigrain, (produced by *Trichoderma reesei*, also known as *Trichoderma longiabrachiatum*) and Ronozyme® HiPhos were obtained from DSM Nutritional Products, Australia. 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 2700 XU/g, respectively. 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 min at pH 5.0 at incubation temperature of 40 °C for 20 min. One unit of xylanase (XU) is defined as the quantity of enzyme that releases 1µmol of reducing moieties from 1.5% arabinoxylan per min at pH 5.0 and incubation temperature of 40 °C for 20 min. Ronozyme® HiPhos was a granular 6-phytase preparation expressed by submerged fermentation of *Aspergillus oryzae* and contained > 10,000 phytase units (FYT)/g. One FYT is defined as the activity of enzyme that releases 1.0 µmole of inorganic phosphorus/min from 5.0 mM sodium phytate at pH 5.5 at 37 ºC (DSM Nutritional Products Ltd., 2013). The activities of 6-phytase, endo-1,3 (4)-β-glucanase and endo-1,4-β-xylanase in diet samples 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.

5.3.2. Diets

Waxy starch hull-less barley (cultivar, Streaker) was obtained from a seed multiplication company (Luisetti Seeds Ltd., Rangiora, New Zealand) and ground in a hammer mill to pass through the screen size of 3.0 mm. Wheat (undetermined cultivar) was obtained from a commercial supplier and ground through the same screen size. Nutrient composition, AMEn and standardised digestible AA contents of same batches of non-supplemented barley and wheat, determined in Chapter 3, were used in formulating the experimental diets. Considering the low AMEn value of WSHL, the maximum inclusion of WSHL in the wheat-based diet was set at 260 g/kg to avoid poor pellet quality and confounding effects on nutrient and energy utilisation associated with higher dietary fat inclusion.

Five levels of WSHL barley inclusion (0, 65, 130, 195 and 260 g/kg) in a wheat-based diet and two levels of enzyme supplementation (0 and 150 g/tonne of feed) were evaluated in a 5 × 2 factorial arrangement of 10 dietary treatments. Five basal diets, with different inclusion levels of WSHL barley, were formulated to meet the Ross 308 strain recommendations for major nutrients for broiler starters (Ross, 2014; Table 5.1), and to be equivalent in respect of AMEn and digestible AA contents. Ronozyme® HiPhos was added (1000 FYT/kg diet) across all basal diets. Each mixed diet was then divided into two equal batches, with one of the batches supplemented with Ronozyme® Multigrain (150 g/tonne of feed), resulting in 10 dietary treatments. The diets contained 5.0 g/kg of titanium dioxide (TiO_2, Merck KGaA, Darmstadt, Germany) as an indigestible marker to determine ileal nutrient digestibility. A pellet binder (KEMBIND®, Kemin Industries [Asia] Pte Ltd, Singapore), at an inclusion rate of 2.0 g/kg, was added on top of all diets. Diets were mixed in a single-screw paddle mixer. Following mixing, all diets were steam-conditioned to 70 °C for 30 seconds and pelleted using a pellet mill (Model Orbit 15; Richard Sizer, Kingston-upon-Hull, UK) capable of manufacturing 180 kg of feed/h and equipped with a die ring with 3.0 mm holes and 35 mm thickness. Representative samples of all diets were collected after pelleting for chemical analysis.

5.3.3. Pellet durability

Pellet durability was determined in a Holmen Pellet Tester (New Holmen NHP100 Portable Pellet Durability Tester, TekPro Ltd., Willow Park, North Walsham, Norfolk, UK) using the method described by Abdollahi *et al*. (2013b).

Table 5. 1. Composition, calculated analysis, analysed values (g/kg, as fed) and pellet durability index (PDI; %) of the basal experimental diets based on wheat and waxy-starch hull-less barley.

Item	Barley inclusion level (g/kg)				
	0	65	130	195	260
Wheat	629	550	471	393	314
Waxy starch hull-less barley	0.0	65.0	130	195	260
Soybean meal	278	282	286	289	293
Maize gluten meal	50.0	50.0	50.0	50.0	50.0
Soybean oil	3.4	13.3	23.2	33.1	43.0
Di-calcium phosphate	10.4	10.5	10.6	10.7	10.8
Limestone	9.3	9.1	9.2	9.1	9.2
L-Lysine HCl	3.8	3.8	3.7	3.7	3.6
DL-Methionine	2.0	2.1	2.2	2.2	2.3
L-Threonine	1.3	1.3	1.3	1.3	1.3
Sodium chloride	2.3	2.3	2.2	2.2	2.1
Sodium bicarbonate	3.4	3.5	3.5	3.6	3.6
Titanium dioxide[1]	5.0	5.0	5.0	5.0	5.0
Pellet binder[2]	2.0	2.0	2.0	2.0	2.0
Vitamin premix[3]	1.0	1.0	1.0	1.0	1.0
Mineral premix[3]	1.0	1.0	1.0	1.0	1.0
Phytase[4]	0.1	0.1	0.1	0.1	0.1
Calculated analysis					
Apparent metabolisable energy, MJ/kg	11.9	11.9	11.9	11.9	11.9
Digestible methionine	5.5	5.6	5.6	5.6	5.7
Digestible methionine + cysteine	9.0	9.0	9.0	9.0	9.0
Digestible lysine	12.2	12.2	12.2	12.2	12.2
Digestible threonine	8.2	8.2	8.2	8.2	8.2
Crude fat	18.9	28.2	37.5	46.8	56.1
Crude fibre	31.0	32.3	33.7	35.0	36.4
Calcium	9.6	9.6	9.6	9.6	9.6
Non-phytate phosphorus	4.8	4.8	4.8	4.8	4.8
Sodium	2.0	2.0	2.0	2.0	2.0
Chloride	2.0	2.0	2.0	2.0	2.0
Potassium	8.3	8.4	8.4	8.5	8.5
Analysed values					
Dry matter	883	885	888	886	894
Gross energy, MJ/kg	16.4	16.6	16.9	17.1	17.4
Crude protein (Nitrogen × 6.25)	250	253	247	246	251
Starch	343	333	322	312	301
Fat	19.1	29.5	39.0	49.8	58.8
PDI[5]	87.9a	86.1b	85.8b	86.2b	84.2c

[1]Merck KGaA, Darmstadt, Germany.
[2]KEMBIND® (Kemin Industries [Asia] Pte Ltd) pellet binder, which contained modified lignosulphonate, guar gum, edible fatty acids and mineral oil, was added on top of each diet.
[3]Supplied per kg of diet: antioxidant, 125 mg; biotin, 0.2 mg; calcium pantothenate, 20 mg; cholecalciferol, 5000 IU; cyanocobalamin, 0.02 mg; folic acid, 2.0 mg; menadione, 4 mg; niacin, 80 mg; pyridoxine, 5.0 mg; trans-retinol, 15000 IU; riboflavin, 9.0 mg; thiamine, 4.0 mg; dl-α-tocopheryl acetate, 80 IU; choline, 0.45 mg; ascorbic acid, 100 mg; Co, 1.0 mg; Cu, 20 mg; Fe, 40 mg; I, 2.0 mg; Mn, 100 mg; Mo, 1.0 mg; Se, 0.15 mg; Zn, 100 mg.
[3]Image Holdings Ltd., Auckland, New Zealand.
[4]Ronozyme® HiPhos (1000 phytase units (FYT)/kg diet). One FYT is defined as the activity of enzyme that releases 1.0 µmole of inorganic phosphorus/min from 5.0 mM sodium phytate at pH 5.5 at 37 ºC. Nutrient matrix values (1.5 g/kg non-phytate P and 1.8 g/kg Ca) were used in basal diet formulation.
[5]Each value represents the mean of ten replicate samples. Means not sharing common letters (a,b,c) are different ($P < 0.05$).

5.3.4. Birds and housing

The experimental procedures were approved by the Massey University Animal Ethics Committee (MUAEC protocol 17/13) and complied with the New Zealand Code of Practice for the Care and Use of Animals for Scientific Purposes. A total of 400, one-d old male broilers (Ross 308), obtained from a commercial hatchery, were individually weighed and allocated to 50 cages in electrically heated battery brooders so that the average bird weight per cage was similar. Each of the 10 dietary treatments was randomly assigned to five cages, each housing eight birds. The birds were transferred to grower cages on d 12 and continued on the same starter diets until the end of the trial (d 21). The battery brooders and grower cages were housed in an environmentally controlled room with 20 h of fluorescent illumination per d. The temperature was maintained at 31 ˚C on d 1 and was gradually reduced to 22 ˚C by 21 d of age. The diets, in pellet form, were offered *ad libitum* and water was available at all times.

5.3.5. Performance data

Body weights (BW) and FI were recorded on a cage basis at weekly intervals. Mortality was recorded daily. Feed per gain (F/G) values were corrected for the BW of any bird that died during the course of the experiment.

5.3.6. Energy and nutrient utilisation

5.3.6.1. Nitrogen-corrected apparent metabolisable energy

The AME_n was determined using the classical total excreta collection method. Feed intake and total excreta output of each cage were quantitatively measured from d 17 to 20 post-hatch. Daily collections from each cage were pooled, mixed in a blender and sub-sampled. Sub-samples were lyophilised (Model 0610, Cuddon Engineering, Blenheim, New Zealand), ground to pass through a 0.5 mm sieve and stored in airtight plastic containers at 4 ˚C pending analysis. The diets and excreta samples were analysed for dry matter (DM), gross energy (GE) and nitrogen (N).

5.3.6.2. Coefficient of apparent ileal digestibility (CAID) of nutrients

On d 21, six broilers per cage were euthanised by intravenous injection (0.5 mL per kg live weight) of sodium pentobarbitone (Provet NZ Pty Ltd., Auckland, New Zealand),

and digesta were collected from the lower half of the ileum by gently flushing with distilled water, as described by Ravindran *et al*. (2005). The ileum was defined as that portion of the small intestine extending from the Meckel's diverticulum to a point ~40 mm proximal to the ileo-caecal junction. The ileum was then divided into two halves and the digesta was collected from the lower half towards the ileo-caecal junction.

Digesta from birds within a cage were pooled, frozen immediately after collection and subsequently lyophilised. Diet and lyophilised digesta samples were ground to pass through a 0.5 mm sieve and stored at 4 ˚C until laboratory analysis. The diets and digesta samples were analysed for DM, titanium (Ti), N, starch and fat.

5.3.7. Intestinal morphology

Two birds from each replicate cage (euthanised for ileal digesta collection) were used for intestinal morphological examinations of villus height (the distance from the apex of the villus to the junction of the villus and crypt); crypt depth (the distance from the junction to the basement membrane of the epithelial cell at the bottom of the crypt); epithelial thickness (the distance from the epithelial surface to the basement membrane of the epithelial cell); and goblet cell numbers (per 100 µm villus height), using the methods described by Naderinejad *et al*. (2016). Measurements of villus height and crypt depth were made on 10 villi at 4× magnification while epithelium thickness and goblet cell number were made at 40× magnification using microscopy imaging software (cellSens Standard [Ver.1.18] Olympus, Tokyo, Japan).

5.3.8. Relative weight of the proventriculus and gizzard and jejunal digesta viscosity

On d 22, two additional birds per cage with body weights closest to the mean weight of the cage were weighed and euthanised by intravenous injection (0.5 mL per kg live weight) of sodium pentobarbitone. The proventriculus and gizzard were carefully excised and adherent fat was removed. The empty weight of these organs in individual birds were determined and reported as g/kg of BW.

Viscosity of jejunal digesta from two birds per cage (euthanised for the determination of relative weights of proventriculus and gizzard) was also measured. Digesta obtained from the lower jejunum was centrifuged at 3000 × g at 20 ˚C for 15 min. A 0.5 mL aliquot of the supernatant was used in a viscometer (Brookfield digital

viscometer, Model DV2TLV, Brookfield Engineering Laboratories Inc., Stoughton, MA) fitted with CP-40 cone spindle with shear rates of 5 to 500/s to measure the viscosity.

5.3.9. Chemical analysis

Dry matter was determined using standard procedures (Method 930.15; AOAC, 2016). Nitrogen was determined by combustion (Method 968.06; AOAC, 2016) using a 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). Fat was determined using Soxtec extraction procedure for animal feed, forage and cereal grains (Method 2003.06; AOAC, 2016). Samples were assayed for Ti on a UV spectrophotometer following the method of Short *et al*. (1996).

5.3.10. Calculations

The AMEn of diets was calculated using the following formula:

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

Correction for zero N retention was made using a factor of 36.54 kJ per gram N (g N/kg DM intake) retained in the body (Hill and Anderson, 1958).

$$AMEn_{diet}\ (MJ/kg) = AME_{diet} - (36.54 \times N\ retention)/1000$$

Apparent digestibility coefficients of nutrients were calculated from the dietary ratio of nutrients to Ti relative to the corresponding ratio in the ileal digesta.

$$CAID\ of\ nutrient = [(Nutrient / Ti)_d - (Nutrient / Ti)_i] / (Nutrient / Ti)_d$$

where, $(Nutrient / Ti)_d$ = ratio of nutrient to Ti in diet and $(Nutrient / Ti)_i$ = ratio of nutrient to Ti in ileal digesta.

5.3.11. Statistical analysis

The data were analysed as a 5 × 2 factorial arrangement of treatments using the general linear model procedure of SAS (version 9.4; SAS Institute Inc., Cary, NC.). Cage served

as the experimental unit. Significant differences between means were separated by Least Significant Difference test. In addition, data on inclusion level of barley in the diet (as an average for without and with enzyme supplementation) were subjected to orthogonal polynomial contrasts using the general linear model procedure of SAS to determine whether responses to increasing levels of barley had any linear or quadratic nature. Significance of effects was declared at $P < 0.05$.

5.4. Results

5.4.1. Pellet durability and enzyme recovery

The pellet durability deteriorated with increasing inclusion of barley in the diet (Table 5.1; $P < 0.05$). The average recovery of phytase, endo-1,3(4)-β-glucanase and endo-1,4-β-xylanase from enzyme-supplemented diets were 130, 101 and 93.4%, respectively.

5.4.2. Growth performance

Mortality during the experiment was negligible. Only 11 out of the 400 birds died and the deaths were not related to any dietary treatment. The interaction between the inclusion level of barley and enzyme supplementation was not significant ($P > 0.05$) for any of the performance parameters (Table 5.2). Inclusion of WSHL barley had a significant ($P < 0.001$) effect on FI and F/G. Increasing dietary inclusion of WSHL barley to 130 g/kg and above decreased FI, with the highest inclusion of WSHL barley (260 g/kg) showing the lowest FI. There was a gradual improvement in the F/G with increasing inclusions of barley.

Addition of enzyme improved ($P < 0.05$) the F/G of birds at all barley inclusions. According to orthogonal polynomial contrasts, regardless of enzyme supplementation, both WG ($P < 0.05$) and FI ($P < 0.001$) reduced linearly with increasing barley inclusion in the diet. Increasing barley inclusion from 0 to 65 g/kg improved F/G, resulting in a quadratic effect ($P < 0.05$) of barley inclusion level.

Table 5. 2. Influence of barley inclusion (g/kg) and enzyme supplementation on weight gain (WG; g/bird), feed intake (FI; g/bird) and feed per gain (F/G; g feed/g gain) of broiler starters (d1-21) fed diets based on wheat and waxy starch hull-less barley[1].

Inclusion level of barley	Enzyme	WG	FI	F/G
0	-	1102	1524	1.396
	+	1128	1530	1.358
65	-	1129	1489	1.326
	+	1137	1492	1.322
130	-	1135	1484	1.313
	+	1101	1427	1.300
195	-	1079	1419	1.317
	+	1120	1429	1.283
260	-	1073	1405	1.310
	+	1100	1403	1.272
SEM[2]		18.93	20.62	0.0154
Main effects				
Inclusion level of barley				
0		1115	1527a	1.377a
65		1133	1490ab	1.324b
130		1118	1456bc	1.307bc
195		1100	1424cd	1.300bc
260		1086	1404d	1.291c
Enzyme				
	-	1104	1464	1.333a
	+	1117	1456	1.307b
Probabilities, P ≤				
Inclusion level of barley		0.154	0.001	0.001
Enzyme		0.272	0.545	0.012
Inclusion level of barley × Enzyme		0.330	0.473	0.725
Orthogonal polynomial contrast				
L[3]		0.041	0.001	0.001
Q[4]		0.198	0.505	0.025

Means in a column not sharing common letters (a,b,c,d) are different ($P < 0.05$).
[1]Each value represents the mean of five replicates (eight birds per replicate).
[2]Pooled standard error of mean.
[3]L= Linear effect of inclusion level of barley.
[4]Q= Quadratic effect of inclusion level of barley.

5.4.3. Nutrient digestibility and energy utilisation

The results indicate no interaction between barley inclusion and enzyme supplementation for CAID of any analysed nutrient or AMEn (Table 5.3). Inclusion of WSHL barley increased CAID of DM, N and fat ($P < 0.01$ to 0.001), however, no differences were observed with level of inclusion. All barley diets showed a greater digestibility of DM, N and fat than the diet with no barley resulting in a significant quadratic effect ($P < 0.05$ to 0.01) of barley inclusion level. Supplemental enzyme improved CAID of starch ($P < 0.05$) only.

Because of consistent and significant ($P < 0.001$) effects of enzyme supplementation at each level of barley inclusion, no interaction ($P > 0.05$) between barley inclusion level and enzyme supplementation on AMEn was observed. Inclusion level of WSHL barley had a significant effect and elicited a progressive improvement of AMEn (linear effect, $P < 0.001$) with increasing level of barley in the diet. Regardless of barley inclusion level, carbohydrase supplementation improved ($P < 0.05$) the AMEn of the diet.

5.4.4. Digestible nutrient and energy intake

There was no interaction ($P > 0.05$) between the inclusion level of barley and enzyme supplementation for intake of digestible starch, protein and fat, and AMEn intake (Table 5.4). Despite the lack of significant differences in CAID of starch across different inclusion levels, the digestible starch intake was affected ($P < 0.001$) by barley inclusion level. The highest and lowest digestible starch intakes were observed for 0 and 260 g/kg barley inclusion levels, respectively. Linear ($P < 0.001$) decline and increase was observed for digestible starch and fat intakes, respectively, with increasing dietary inclusion of WSHL barley. The digestible protein intake tended ($P = 0.057$) to decline linearly with increasing barley inclusion from 0 to 260 g/kg of the diet. There was no influence ($P > 0.05$) from enzyme supplementation on the digestible intake of any analysed nutrient. Inclusion level of barley had no significant effect ($P > 0.05$) on AMEn intake, but orthogonal polynomial contrasts revealed a linear drop ($P < 0.05$) in AMEn intake with increasing WSHL barley inclusion in the diet. The addition of enzyme increased ($P < 0.05$) the intake of AMEn.

Table 5. 3. Influence of barley inclusion (g/kg) and enzyme supplementation on coefficient of apparent ileal digestibility (CAID)[1] of dry matter (DM), starch, nitrogen (N) and fat and N-corrected apparent metabolisable energy (AMEn; MJ/kg DM)[2] in broiler starters fed diets based on wheat and waxy starch hull-less barley.

Inclusion level of barley	Enzym	DM	Starch	N	Fat	AMEn
0	-	0.522	0.836	0.714	0.762	11.95
	+	0.531	0.861	0.733	0.771	12.02
65	-	0.548	0.853	0.755	0.831	12.04
	+	0.568	0.878	0.768	0.813	12.19
130	-	0.583	0.844	0.789	0.853	12.17
	+	0.569	0.849	0.766	0.818	12.56
195	-	0.562	0.809	0.777	0.846	12.17
	+	0.586	0.855	0.773	0.846	12.67
260	-	0.551	0.836	0.755	0.832	12.39
	+	0.587	0.838	0.773	0.863	12.74
SEM[3]		0.014	0.014	0.015	0.018	0.099
Main effects						
Inclusion level of barley						
0		0.526b	0.849	0.723b	0.766b	11.98c
65		0.558a	0.866	0.761a	0.822a	12.12c
130		0.576a	0.847	0.778a	0.835a	12.36b
195		0.574a	0.832	0.775a	0.846a	12.42a
260		0.569a	0.837	0.764a	0.847a	12.57a
Enzyme						
	-	0.553	0.836b	0.758	0.825	12.14b
	+	0.568	0.856a	0.762	0.822	12.43a
Probabilities, $P \leq$						
Inclusion level of barley		0.008	0.170	0.006	0.001	0.001
Enzyme		0.098	0.026	0.641	0.802	0.001
Inclusion level of barley × Enzyme		0.477	0.532	0.580	0.430	0.193
Orthogonal polynomial contrast						
L[4]		0.003	0.091	0.006	0.001	0.001
Q[5]		0.020	0.628	0.004	0.024	0.619

Means in a column not sharing common letters (a,b,c) are different ($P < 0.05$).
[1]Each value represents the mean of five replicates (six birds per replicate).
[2]Each value represents the mean of five replicates (eight birds per replicate), measured from d-17 to 20.
[3]Pooled standard error of mean.
[4]L= Linear effect of inclusion level of barley.
[5]Q= Quadratic effect of inclusion level of barley.

5.4.5. Relative weights of proventriculus and gizzard, and jejunal digesta viscosity

Neither the inclusion level of barley nor enzyme supplementation affected ($P > 0.05$) proventriculus weight (Table 5.5). However, inclusion level of barley significantly ($P < 0.001$) affected the gizzard weight and a linear ($P < 0.001$) gain was observed with increasing inclusion of barley in the diet.

No interaction between the inclusion level of WSHL barley and enzyme supplementation was evident for jejunal digesta viscosity (Table 5.5; $P > 0.05$). Both inclusion level of barley ($P < 0.01$) and enzyme supplementation ($P < 0.001$) reduced the jejunal digesta viscosity. Irrespective of enzyme supplementation, jejunal digesta viscosity dropped linearly ($P < 0.001$) with increasing barley inclusion in the diet.

5.4.6. Intestinal morphology

Neither barley inclusion level nor supplemental enzyme influenced ($P > 0.05$) duodenal villus height and epithelial thickness (Table 5.6). A significant ($P < 0.05$) barley inclusion × enzyme interaction was observed for duodenal goblet cell number. Enzyme addition increased the duodenal goblet cell number at 0 g/kg barley inclusion, but reduced goblet cell number at 260 g/kg barley inclusion with no effect at other barley inclusion levels. Showing a significant effect ($P < 0.05$), barley inclusion level influenced duodenal crypt depth in a quadratic manner ($P < 0.01$) with a reduction at 65, 130 and 195 g/kg barley inclusion levels, compared to 0 g/kg barley in the diet.

The inclusion level of barley influenced the villus height, epithelial thickness and crypt depth in the jejunum ($P < 0.011$ to 0.001). Inclusion of barley at all levels resulted in greater jejunal villus heights compared to the diet with no barley. Barley inclusion at 130 g/kg increased the jejunal epithelial thickness compared to other inclusion levels. The jejunal crypt depth increased beyond 65 g/kg of barley inclusion. Villus height, goblet cell number and epithelial thickness in the jejunum increased up to 130 g/kg barley inclusion, and declined afterwards, resulting in significant quadratic effects ($P < 0.05$ to 0.001). A significant barley inclusion × enzyme interaction was observed for jejunal goblet cell number ($P < 0.01$). Supplemental enzymes reduced the jejunal goblet cell number at inclusion levels of 65, 130 and 260 g/kg.

Table 5. 4. Influence of barley inclusion (g/kg) and enzyme supplementation on digestible nutrient (starch, protein and fat) intake[1] (g/bird) and nitrogen-corrected apparent metabolisable energy (AMEn)[2] intake (MJ/bird) of broiler starters from 1 to 21 d, fed diets based on wheat and waxy-starch hull-less barley.

Inclusion level of barley	Enzyme	Starch	Protein	Fat	AMEn
0	-	438	272	22.2	16.07
	+	452	280	22.5	16.23
65	-	423	284	36.5	15.85
	+	436	289	35.7	16.10
130	-	404	289	49.4	16.04
	+	390	270	45.5	15.91
195	-	357	271	59.8	15.30
	+	380	271	60.2	16.04
260	-	353	266	68.7	15.55
	+	354	272	71.1	15.96
SEM[3]		7.8	6.9	1.11	0.193
Main effects					
Inclusion level of barley					
0		445a	276	22.4e	16.15
65		429a	287	36.1d	15.97
130		397b	280	47.4c	15.97
195		369c	271	60.0b	15.67
260		353c	269	69.9a	15.76
Enzyme					
	-	395	276	47.3	15.76b
	+	402	276	47.0	16.05a
Probabilities, $P \leq$					
Inclusion level of barley		0.001	0.098	0.001	0.119
Enzyme		0.133	0.982	0.678	0.025
Inclusion level of barley × Enzyme		0.177	0.278	0.095	0.266
Orthogonal polynomial contrast					
L[4]		0.001	0.057	0.001	0.022
Q[5]		0.814	0.149	0.039	0.688

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

[1]Digestible nutrient intake (g/bird) = Feed intake (kg) × nutrient content of the feed (g/kg) × coefficient of apparent ileal digestibility of nutrient. Each value represents the mean of five replicates (six birds per replicate).

[2]AMEn intake (MJ/bird) = Feed intake (kg, DM) × AMEn of the feed (MJ/kg, DM). Each value represents the mean of five replicates (eight birds per replicate), measured from d-17 to 20.

[3]Pooled standard error of mean.

[4]L= Linear effect of inclusion level of barley.

[5]Q= Quadratic effect of inclusion level of barley.

Table 5. 5. Influence of barley inclusion (g/kg) and enzyme supplementation on relative weight and pH of proventriculus (prov.) and gizzard (g/kg of body weight), and viscosity in jejunal digesta (cP) of 21-d old broilers fed diets based on wheat and waxy-starch hull-less barley[1].

Inclusion level of barley	Enzyme	Relative weight		Jejunal digesta viscosity
		Prov.	Gizzard	
0	-	3.87	7.59	5.32
	+	3.48	7.30	4.65
65	-	3.89	8.38	4.98
	+	3.93	8.41	3.61
130	-	3.48	8.55	4.19
	+	3.47	8.85	3.62
195	-	3.87	9.99	4.27
	+	3.49	9.63	3.31
260	-	3.62	10.3	4.19
	+	3.54	10.2	2.82
SEM[2]		0.310	0.524	0.38
Main effects				
Inclusion level of barley				
0		3.68	7.45c	4.99a
65		3.91	8.39bc	4.29ab
130		3.47	8.70b	3.91bc
195		3.68	9.81a	3.79bc
260		3.58	10.3a	3.51c
Enzyme	-	3.75	8.97	4.59a
	+	3.58	8.89	3.60b
Probabilities, $P \leq$				
Inclusion level of barley		0.704	0.001	0.004
Enzyme		0.399	0.809	0.001
Inclusion level of barley × Enzyme		0.929	0.971	0.728
Orthogonal polynomial contrast				
L[3]		0.527	0.001	0.001
Q[4]		0.985	0.911	0.344

Means in a column not sharing common letters (a,b,c) are different (P < 0.05).
[1]Each value represents the mean of five replicates (two birds per replicate).
[2]Pooled standard error of mean.
[3]L = Linear effect of inclusion level of barley.
[4]Q = Quadratic effect of inclusion level of barley.

Table 5. 6. Influence of barley inclusion (g/kg) and enzyme supplementation on villus height (µm), goblet cell number (per 100 µm villus height), epithelial thickness (µm) and crypt depth (µm) of the duodenum and jejunum of 21-d old broilers fed diets based on wheat and waxy starch hull-less barley[1].

Inclusion level of barley	Enzyme	Duodenum				Jejunum			
		Villus height	Goblet cell number	Epithelial thickness	Crypt depth	Villus height	Goblet cell number	Epithelial thickness	Crypt depth
0	-	1081	13.5cd	20.1	94.3	605	13.5de	17.7	78.5
	+	1097	15.9ab	22.4	90.1	579	15.6bcd	18.0	80.7
65	-	1077	16.5a	20.6	86.9	657	17.3ab	19.2	75.5
	+	963	14.6abcd	21.6	84.5	632	14.9cde	18.3	76.8
130	-	1007	15.1abc	20.7	84.5	720	17.8a	21.1	80.6
	+	1051	14.1bcd	21.5	89.5	649	14.9cde	20.2	87.0
195	-	1011	14.2bcd	21.5	88.2	636	15.5bcd	18.7	81.6
	+	1074	14.7abcd	21.3	87.3	690	13.9de	18.8	81.8
260	-	1001	16.3a	20.7	89.4	688	16.3abc	18.7	82.2
	+	1017	12.8d	20.1	91.8	638	13.0e	17.8	80.4
SEM[2]		44.5	0.738	0.61	2.19	25.9	0.81	0.64	1.99
Main effects									
Inclusion level of barley									
0		1089	14.7	21.2	92.2a	592b	14.5	17.8b	79.6b
65		1020	15.5	21.1	85.7c	645a	16.1	18.8b	76.1c
130		1029	14.6	21.1	87.0b	685a	16.3	20.6a	83.8a
195		1043	14.4	21.4	87.7b	663a	14.7	18.8b	81.7a
260		1009	14.5	20.4	90.6a	663a	14.6	18.3b	81.3a
Enzyme									
	-	1035	15.1	20.7	88.7	661	16.1	19.1	79.7
	+	1041	14.4	21.4	88.6	638	14.5	18.6	81.3
Probabilities, $P \leq$									
Inclusion level of barley		0.415	0.568	0.575	0.022	0.008	0.058	0.001	0.004
Enzyme		0.854	0.126	0.085	0.981	0.154	0.002	0.248	0.198
Inclusion level of barley × Enzyme		0.308	0.002	0.156	0.231	0.168	0.009	0.752	0.336
Orthogonal polynomial contrast									
L[3]		0.167	0.422	0.338	0.814	0.008	0.567	0.557	0.050
Q[4]		0.523	0.765	0.382	0.002	0.019	0.031	0.001	0.498

Means in a column not sharing common letters (a,b,c,d,e) are different ($P < 0.05$).
[1]Each value represents the mean of five replicates (two birds per replicate, 10 readings per bird).
[2]Pooled standard error of mean.
[3]L = Linear effect of inclusion level of barley.
[4]Q = Quadratic effect of inclusion level of barley.

5.5. Discussion

With a higher level of amylopectin and lower starch gelatinisation temperature compared to normal starch types, waxy cultivars of barley can benefit poultry feed in terms of starch hydrolysis (Li *et al*., 2004a) and physical pellet quality (Ankrah *et al*., 1999). According to Ankrah *et al*. (1999), equivalent pellet hardness in WSHL was achieved at a lower temperature (by 14.2 °C) than in normal starch hull-less barley. Even though better pellet quality was anticipated at greater dietary inclusions of WSHL, the higher levels of soybean oil used to equalise the AME contents across diets compromised the potential economic advantage offered by the inclusion of WSHL. High dietary fat lubricates feed particles and reduces the friction generated in the die holes, resulting in lower physical pellet quality. Dietary fat also partially covers feed particles and creates a barrier for penetration of steam to feed particles, reducing starch gelatinisation and development of binding adhesions (Löwe, 2005; Abdollahi *et al*., 2013a).

Despite the absence of effect of WSHL barley inclusion on WG and consistent with the previous studies (Moss *et al*., 1983; Friesen *et al*., 1992), orthogonal polynomial contrasts showed that WG reduced linearly with increasing WSHL inclusion in the diet. This observation can be attributed to the decreasing FI at dietary inclusions of WSHL beyond 65 g/kg. In the current study, FI linearly decreased with increasing inclusion of WSHL in the diet. In contrast, Yu *et al*. (2002) reported a linear increase in FI with increasing dehulled barley inclusion in a maize-based diet (0, 400 and 800 g/kg), which was partly attributed to improved palatability due to the removal of fibrous hull. The AMEn increased with increasing inclusions of barley in the diet, and the lower FI associated with higher WSHL inclusion (130, 195 and 260 g/kg) may be reflective of birds' attempt to maintain a constant energy intake (Classen, 2017), and is supported by similar AMEn intakes of the birds fed diets with different WSHL inclusions. The declining FI with increasing barley inclusion in the diet corresponded to lower PDI at these inclusion levels and support the positive relationship between pellet physical quality and FI suggested by Abdollahi *et al*. (2018).

Compared to the diet with no barley, WSHL inclusion at 65, 130, 195 and 260 g/kg improved the F/G by 5.3, 7.0, 7.7 and 8.6 points, respectively. Regardless of barley inclusion level, this improvement was 5.1% (0.729 vs. 0.766), compared to the diet with no barley. Consistent treatment effects on jejunal digesta viscosity, relative weight of

gizzard and F/G of birds explain the underlying reasons for improved feed efficiency in birds fed greater inclusion of barley and suggested the contribution of the changes in the digestive tract caused by dietary NSP sources on the feed efficiency of birds.

Moss *et al*. (1983), replaced wheat (w/w basis) with 0, 272, 408 and 544 g/kg of waxy starch hulled barley (cultivar, Wapana) in a broiler diet with no enzyme addition and reported that increasing levels of barley consistently decreased WG, but had no effect on F/G. Classen *et al*. (1988) substituted hull-less barley (cultivar, Scout; starch type, unidentified) on weight basis (0, 200, 400 and 600 g/kg) for wheat in a broiler starter diet and reported a linear decrease in BW with increasing levels of hull-less barley in the diet, while no depression was reported for F/G. Friesen *et al*. (1992) evaluated the influence of different inclusion levels of hull-less barley (0, 350 and 700 g/kg) in a wheat diet and supplementation of a cellulase enzyme on growth performance and, energy and nutrient utilisation in 14-d old broilers. Weight gain and F/G of birds fed the non-supplemented hull-less barley at 350 g/kg was similar to those fed the control wheat diet, wherein barley inclusion at 700 g/kg resulted in the lowest WG and highest F/G. The deterioration of growth performance associated with barley inclusion reported in previous studies may be partly explained by weight-to-weight substitution of barley for the major cereal in the diets (Moss *et al*., 1983; Friesen *et al*., 1992), resulting in lower metabolisable energy and digestible AA content than the corresponding cereal-based diet. Yu *et al*. (2002) evaluated the inclusion of de-hulled barley at levels of 0, 400 and 800 g/kg, and supplementation of β-glucanase in isonitrogenous and isocaloric maize-based diets. Contrary to the current results, these reserachers reported that increasing inclusion of barley increased FI and WG with no effect on the feed efficiency. The improvement in WG and FI reported by Yu *et al*. (2002) was attributed mainly to the greater amounts of fat added to the diets with higher inclusion of dehulled barley with a low energy value. Moreover, both the greater amount of fat and the removal of fibrous hull from barley in Yu *et al.* (2002) was believed to increase the palatability of the diets, improving the FI and WG.

As indicated by the absence of significant interaction between barley inclusion and enzyme addition, the efficacy of the enzyme on F/G was similar at each barley inclusion level and contributed to F/G by 2.6 points. In partial agreement with the current observation, Yu *et al*. (2002), who evaluated the inclusion of de-hulled barley and supplementation of β-glucanase in isonitrogenous and isocaloric maize-based diets, also

reported that added enzyme failed to improve WG, FI and F/G. Using a barley type similar to the current study, Ankrah *et al*. (1999) reported improved WG (429 vs. 650 g), FI (907 vs. 1083 g) and lowered F/G (2.12 vs. 1.67) in birds fed WSHL barley-based diets (610 g/kg) supplemented with β-glucanase. Bergh *et al*. (1999), in a study with a hulled waxy starch barley-based (696 g/kg) diet, reported increased BW (216 vs. 254 g) and FI (366 vs. 400 g), and lowered F/G (2.07 vs. 1.86) in 13-d old broilers due to carbohydrase enzyme supplementation. Although the enhanced BW and FI due to enzyme supplementation reported by Ankrah *et al*. (1999) and Bergh *et al*. (1999) was not evident in the current study, enhanced feed efficiency due to supplemental enzymes reported by these researchers was consistent with present findings.

Compared to the diet with no barley, WSHL inclusion supported higher CAID of DM by 8.2% (0.569 vs. 0.526), N by 6.4% (0.769 vs. 0.723) and fat by 9.3% (0.837 vs. 0.766), and this advantage was present regardless of barley inclusion level.

Interestingly, the inclusion of waxy barley, despite having higher amylopectin content than wheat (477 vs. 388 g/kg DM) which is thought to be highly digestible (Björck *et al*., 1990), did not cause any improvement in starch digestibility. Svihus and Hetland (2001) hypothesised that a well-developed gizzard can prevent starch overload in the digestive tract and facilitate better starch digestion. A close relationship between gizzard size and starch digestibility has also been reported in previous studies (Rogel *et al*., 1987a,b; Hetland *et al*., 2003; Amerah *et al*., 2009). Despite an increase of 38% in relative gizzard weight (10.3 vs. 7.45 g/kg BW) and 42% reduction in digesta viscosity (3.51 vs. 4.99 cP) of the broilers fed diet with 260 g/kg WSHL compared to the wheat-based diet in this study, CAID of starch was unaffected. The poor response of starch digestibility to variations in digesta viscosity has been previously reported (Carré *et al*., 2002; Zaefarian *et al*., 2015). It can be speculated that the extent of encapsulated starch might be increased with increasing inclusion of barley, owing to thicker endosperm cell walls in WSHL as observed in Chapter 3, and resulted in no effect from inclusion level on starch digestibility.

Regardless of barley inclusion level, enzyme supplementation enhanced CAID of starch by 2.4% (0.856 vs. 0.836). Ankrah *et al*. (1999) and Ravindran *et al*. (2007) found similar results investigating the effect of a carbohydrase in barley-based diets. Among different modes of action of NSP-degrading enzymes, starch digestibility seemed to

benefit from degradation of endosperm cell wall by added enzymes. This could consequently increase the release of encapsulated starch granules allowing better interaction with digestive enzymes (Hesselman and Åman, 1986; Bedford, 1996).

The prominent influence of enzyme supplementation to improve fat digestibility has been previously observed as fat digestion is the most affected by increased digesta viscosity caused by soluble NSP (Edney *et al*., 1989; Almirall *et al*., 1995; Choct and Annison, 1992a). In the present study, despite 42% reduction in jejunal digesta viscosity (4.59 vs. 3.60 cP) in enzyme-supplemented diets, no enzyme effect on CAID of fat was observed. This finding might indicate the presence of factors other than digesta viscosity, such as fat type (Dänicke *et al*., 1997) that can affect the efficacy of supplemental enzymes on digestibility of fat in birds fed grains rich in NSP.

The association between the changes in starch digestibility and AMEn of the diets reported in previous studies suggest that digestible starch is the major contributor to the AME in barley- (Wu *et al*., 2004a; Ravindran *et al*., 2007) and wheat- (Mollah *et al*., 1983) based diets. However, the lack of relationship between the CAID of starch and AMEn, and greater CAID of fat in barley containing diets in the current study suggests that improved AMEn with increasing inclusion of barley might be associated more with fat digestibility and digestible fat intake in WSHL diets. As discussed earlier, the amount of soybean oil was increased with increasing WSHL inclusion to balance the AME content across the experimental diets.

In the current study, regardless of the supplemental enzyme, all barley diets showed a similar level of N digestibility that was greater than that of the 0 g/kg barley diet. Compared to the diet with no barley, WSHL inclusion at 65, 130, 195 and 260 g/kg improved the CAID of N by 5.26, 7.61, 7.19 and 5.67% respectively. Rotter *et al*. (1990) replaced wheat with hull-less barley (cultivar, Scout) at 250 g/kg increments up to 750 g/kg in the diet and reported a reduction in apparent excreta protein digestibility from 89.5 to 76.1% and AMEn from 14.8 to 11.6 MJ/kg. In their study, a supplemental crude cellulase resulted in uniform response in both the apparent protein digestibility (89.9-90.8%) and AMEn (14.7-15.1 MJ/kg) regardless of hull-less barley inclusion level. In this study, however, barley replaced wheat on a weight-to-weight basis, resulting in dietary treatments being different in respect to energy (ranging from 12.54 to 13.22 MJ/kg) and protein (ranging from 218 to 232 g/kg) contents. Friesen *et al*. (1992) reported

quadratic reductions in the apparent excreta digestibility of protein with increasing inclusions of hull-less barley (0, 350 and 700 g/kg diet) in non-supplemented wheat-based diets. These researchers also observed linear reductions in the apparent excreta digestibility of lipids, with increasing inclusion of hull-less barley in non-supplemented diet. Yu *et al*. (2002) reported that increasing inclusions of processed de-hulled barley (or pearled barley) in maize-based diets at levels of 0, 400 and 800 g/kg, linearly reduced DM and digestibility coefficients from 0.726 to 0.698 and from 0.760 to 0.693, respectively, in three-week old broiler chicks. In six-week old birds, DM digestibility linearly decreased from 0.753 to 0.704, while digestibility of fat quadratically reduced from 0.803 to 0.757, with the lowest fat digestibility noted at 400 g barley/kg diet. The protein digestibility was unaffected by the inclusion level of barley at both ages.

Decreasing FI of birds and similar CAID of starch in diets with increasing inclusion of WSHL in the diet resulted in lower starch intakes in birds fed diets with barley inclusion beyond 65 g/kg. Compared to the diet with no barley, WSHL inclusion at 65, 130, 195 and 260 g/kg lowered the digestible starch intake by 16, 48, 76 and 92 g/bird, respectively. In contrast, the corresponding increases in digestible fat intake were 13.7, 25.0, 37.6 and 47.5 g/bird, respectively. The marked differences in digestible fat intake between dietary treatments were due to the greater incorporation of soybean oil into the diets with higher barley inclusions.

The effect of structural components such as insoluble fibre (hulls and wood shavings) on gizzard development is well documented (Rogel *et al*., 1987a; Hetland *et al*., 2003; Amerah *et al*., 2009; Svihus, 2011a; Abdollahi *et al*., 2019a). In the current study, the relative gizzard weight increased linearly by 38% (from 7.45 to 10.3 g/kg BW) with increasing inclusions of WSHL from 0 to 260 g/kg in the diet. However, the larger gizzards associated with the diet containing 260 g WSHL/kg could not have been caused solely by insoluble fibre as the insoluble NSP content in 260 g/kg barley diets (66.0 g/kg DM) was lower than the insoluble NSP content in the diet with no barley inclusion (74.9 g/kg DM). Moreover, due to the impaired FI in birds fed higher inclusions of WSHL in the diet, insoluble NSP intake of the birds fed the 260 g/kg barley diet was lower compared to the birds fed 0 g/kg barley diet (92.7 vs. 114 g/bird), suggesting a contribution of factors other than insoluble fibre content on gizzard development. Although the wheat and barley grains were not tested for grain hardness in the current

study, according to scanning electron microscopic images of the same grains obtained in Chapter 3, WSHL seemed to have thicker endosperm cell walls compared to wheat. Nair *et al*. (2011), who observed the microscopic images of endosperms of hard and soft-hulled spring barley lines, also reported thicker endosperm cell walls in hard barley lines. Moreover, Gamlath *et al*. (2008) reported that both β-glucan and arabinoxylan contents of the barley endosperm positively correlated with kernel hardness in barley. Therefore, it may be speculated that the WSHL barley in the current experiment had a greater grain hardness than wheat, which facilitated gizzard development to meet the increased requirement for grinding activity in the gizzard. More extensive grinding, increased retention time, regulation of digesta flow, lower pH and greater pancreatic enzyme secretion (Svihus, 2011a) by developed gizzards might have facilitated the improvements in F/G, AMEn, and digestibility of DM, N and fat at higher levels of barley inclusion. However, the reported advantage of having a well-developed gizzard on starch digestibility (Rogel *et al*., 1987a,b; Svihus and Hetland, 2001) was not observed in this study.

Surprisingly, regardless of the higher content of β-glucan in WSHL compared to wheat (68.6 vs. 7.74 g/kg DM; Chapter 3), the highest (4.99 cP) and lowest (3.51 cP) jejunal digesta viscosity values were observed for the 0 and 260 g/kg of WSHL, respectively. In agreement, Shakouri *et al*. (2009) reported higher digesta viscosity in broilers fed wheat-based diets (5.74 cP) compared to barley-based diets (2.92 cP). In contrast, Fuente *et al*. (1995) and Yu *et al*. (1998) reported increasing digesta viscosity in response to increasing inclusion of barley in the diet. This discrepancy in the literature suggests that digesta viscosity is reflective not only of the concentration of NSP, but also of its molecular weight. It has been suggested that a low content of soluble NSP can result in high intrinsic viscosity if the NSP is of a high molecular weight (Saulnier *et al*., 1995; Dusel *et al*., 1997; Cowieson *et al*., 2005). Moreover, Dusel *et al*. (1997) suggested the contribution of wheat gluten and its endosperm proteins (gliadins and glutenins) towards increased viscosity of aqueous extract of wheat flour. Due to the greater digesta viscosity observed in birds fed diets with low barley inclusion (i.e., greater content of wheat) in the current study, it is possible that the wheat cultivar contained NSP of high molecular weight, with a consequent increase in jejunal digesta viscosity regardless of NSP concentration.

In agreement with previous studies (Salih *et al*., 1991; Almirall *et al*., 1995; Józefiak *et al*., 2006), enzyme supplementation reduced the digesta viscosity by 0.99 cP, a reduction of 22%. The reduction in digesta viscosity due to the action of enzyme was associated with increases in starch digestibility, AMEn and F/G by 2.4%, 0.29 MJ/kg, and 2.6 points, respectively.

Jejunal villus heights were different only between 0 g/kg barley diet and all barley-included diets, while no differences were observed between different inclusions of barley. Interestingly, consistent treatment effects of jejunal villus height and DM, N and fat digestibility values in response to barley inclusion were observed, suggesting the contribution of increased absorptive surfaces on enhanced nutrient digestibility and consequently on AMEn and F/G. In contrast to the increased jejunal villus height caused by barley inclusion in wheat-based diets, inclusion of barley in maize-based diets, however, resulted in shorter jejunal villi (Viveros *et al*., 1994; Onderci *et al*., 2008; Kalantar *et al*., 2016). Shakouri *et al*. (2009) reported lower jejunal villus height in birds fed diets with 600 g/kg barley compared to diets containing maize, wheat or sorghum (623 g/kg).

The goblet cells in the chicken intestine secrete mucin glycoproteins, which are the main components of the mucus layer that protects epithelial cells and transports nutrients between the lumen and brush border membrane (Specian and Oliver, 1991; Wang and Peng, 2008). The lack of response from gut morphology parameters (except duodenal and jejunal goblet cell number) to enzyme supplementation was in agreement with Iji *et al*. (2001) and Wu *et al*. (2004b). The interactive influence of barley inclusion level and supplemental enzyme was observed as supplemental carbohydrase caused different responses in duodenal goblet cell numbers at 0 and 260 g/kg WSHL inclusions in the diet. In accordance with the present observation at 0 g/kg of barley inclusion (i.e., sole inclusion of wheat), Wu *et al*. (2004b) reported that xylanase supplementation tended to increase the duodenal goblet cell number in broilers fed wheat-based diets.

When alternative feed ingredients are included in commercial poultry diets, the current practice in the feed industry is to balance the energy and AA contents across the diets. It is, therefore, important that research data on the use of alternative ingredients should be generated using diets to resemble the feeding practice commonly used in the feed industry. To ensure the compatibility of the current research design to industry

context, the experimental diets were formulated to be isocaloric and isonitrogenous. Although the current design had some limitations, the results nevertheless have implications to the understanding of the effect of barley inclusion and supplemental enzyme on the growth performance and nutrient digestibility of broilers. First, considering the low AMEn value of WSHL, WSHL was included only up to 260 g/kg in the wheat-based diet to minimise the confounding effects associated with higher inclusions of dietary fat. Second, soybean oil added to equalise the energy content across the diets that might have resulted in a confounded fat effect especially on pellet durability and, fat and energy utilisation. However, a pellet binder was added to all diets to minimise the impact of higher fat inclusion on pellet quality.

5.6. Conclusions

The fact that maximum WSHL inclusion in the current study had no compromising effect on WG and even improved F/G efficiency suggests that WSHL could be safely included up to an inclusion level of 260 g/kg in a wheat-based broiler starter diet. Although one may question the applicability of this inclusion level for other barley cultivars, it is important to note that the broader objective of this study was to emphasise the importance of using nutrient profiles for the specific barley cultivar based on measured contents of AMEn and digestible AA to formulate barley-based diets and therefore, the optimum inclusion level obtained for the WSHL examined in this study may not be recommended to other barley types. The results of the present study confirmed the previously reported benefits of exogenous carbohydrases on starch digestibility, energy utilisation, digesta viscosity and feed efficiency when added to diets based on viscous grains.

CHAPTER SIX

The interactive influence of barley particle size and enzyme supplementation on growth performance, nutrient utilisation and intestinal morphometry of broiler starters[4]

6.1. Abstract

The influence of barley particle size and enzyme supplementation on performance, nutrient and energy utilisation, and intestinal morphometry of broiler starters (d 1-21) fed pelleted barley-based diets was evaluated. Two barley particle sizes (fine and coarse) and four enzyme treatments (non-supplemented [control], carbohydrase [0.15 g/kg of feed; Carb], phytase [0.10 g/kg; Phy] and combination of carbohydrase and phytase [0.15 and 0.10 g/kg, respectively; Carb + Phy]) were evaluated in a 2 × 4 factorial arrangement. Fine and coarse barley particles were achieved by grinding whole barley in a hammer mill to pass through 2.0 and 8.0 mm screens, respectively. A total of 384, one-d-old male broilers (eight birds/cage; six cages/treatment) were used. Supplemental enzymes tended ($P = 0.056$) to increase the weight gain of birds with a synergetic effect from Carb + Phy. The response of feed intake to supplemental enzymes interacted ($P < 0.05$) with barley particle size, as Phy increased feed intake only in fine barley diets. Both coarse particles and supplemental Carb, either individually or in combination with phytase, reduced feed per gain ($P < 0.001$). Digestibility of dry matter, nitrogen and fat was greater in birds fed coarse barley diets ($P < 0.05$). Dry matter, starch, fat and phosphorus digestibility values were improved by supplemental enzymes ($P < 0.05$). Coarse barley ($P < 0.05$) and Carb ($P < 0.001$), either individually or in combination, increased the nitrogen-corrected apparent metabolisable energy. Coarse barley reduced the gizzard pH ($P < 0.001$). Birds fed diet with supplemental enzymes had shorter jejunum ($P < 0.05$). Neither the barley particle size nor supplemental enzymes ($P > 0.05$) affected the jejunal digesta viscosity. In summary, feeding coarse barley particles and supplemental Carb improved the feed efficiency, and nutrient and energy utilisation. The effects of barley particle size on measured parameters suggest that the particle size effect was preserved even after pelleting. The combination of Carb and Phy tended to improve the weight gain but caused no further improvements in nutrient utilisation.

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6.2. Introduction

Due to possible impairment of pellet physical quality associated with coarser grain particles, fine grinding of ingredients followed by pelleting has become the standard practice in feed manufacture. However, the lack of structural components in highly processed poultry diets masks the benefits offered by superior pellet quality and, result in sub-optimal functionality of the foregut followed by feed overconsumption, poor nutrient digestibility, and increased consumption of litter leading to poor intestinal health (Hetland *et al*., 2004; Svihus, 2011a; Rodrigues and Choct, 2018). This concern has increased the interest on methods to restore the structure of the diet. Inclusion of insoluble fibre sources (Hetland *et al*., 2004), coarse cereal particles (Amerah *et al*., 2007a; Abdollahi *et al*., 2019a) or whole grains (Singh *et al*., 2014) in broiler diets has been practised to improve the physical microstructure of feed. However, insoluble fibre and whole grains can only be incorporated up to a certain level due to possible nutrient dilution, feed intake (FI) depression and increased segregation (Singh *et al*., 2014; Rodrigues and Choct, 2018). Manipulation of grain particle size therefore provides a promising solution due to easier adaptation into normal feed processing practice.

Cereal grains are ground to reduce the particle size with the aim of modifying their physical characteristics. Grinding facilitates handling, mixing and further processing (extrusion and pelleting) and increases the exposure of nutrients in the endosperm to digestive enzymes (Amerah *et al*., 2011). Fine grinding results in greater surface area, and consequently greater substrate availability for enzymatic digestion, and decreases segregation ensuring the homogeneity of mixed feed. Coarse grinding, on the other hand, stimulates gizzard development and functionality, facilitating digestion of nutrients through enhanced grinding activity and gut motility (Amerah *et al*., 2007a). A key benefit of feeding coarse particles is stronger reverse peristaltic contractions between the gizzard and proventriculus resulting in increased secretion of hydrochloric acid and proteolysis by pepsin (Svihus, 2011a). Accordingly, the use of coarse particles in pelleted diets may optimise intestinal development and function (Abdollahi *et al*., 2019a).

However, the pelleting process may further reduce the size of feed particles, especially of coarser particles, and equalise the differences in particle size distribution (Svihus *et al*., 2004; Amerah *et al*., 2007b; Abdollahi *et al*., 2013a), suggesting that the particle size impact is more pronounced in mash diets than in pelleted or crumbled diets

(Zaefarian *et al*., 2016). However, some reports indicate that the effects of feed particle size on bird performance exist even after pelleting (Nir *et al*., 1995; Naderinejad *et al*., 2016). Moreover, recommendations regarding the optimum particle size are contradictory due to the confounding effects from several factors including grain type, feed form, complexity of the diet, endosperm hardness, grinding method, particle size distribution and pellet quality (Amerah *et al*., 2007a; Abdollahi *et al*., 2018). The influence of grain particle size on growth performance and nutrient utilisation of broilers fed maize- (Amerah and Ravindran, 2009; Naderinejad *et al*., 2016) and wheat- (Lentle *et al*., 2006; Amerah *et al*., 2007b; Abdollahi *et al*., 2019a) based diets has been examined, but corresponding studies with barley are lacking.

In addition to carbohydrases (Carb) that target non-starch polysaccharides (NSP) present in viscous grains such as wheat and barley, phytases (Phy) are routinely added to cereal-based diets to facilitate the release of phytate-bound phosphorus (P) and reduce the P effluent from intensive poultry production (Ravindran *et al*., 1995). Several researchers have evaluated the individual and combined supplementation of Carb and Phy to maize- (Juanpere *et al*., 2005), wheat- (Ravindran *et al*., 1999; Wu *et al*., 2004a,b; Juanpere *et al*., 2005; Abdollahi *et al*., 2016) and barley- (Ravindran *et al*., 1999; Wu *et al*., 2004a; Juanpere *et al*., 2005) based diets. The combination of Carb and Phy is believed to facilitate each other's substrate access; however, the effects seem to be inconsistent (Selle *et al*., 2003b) and require further elucidation.

With the aim of maximising the benefit from supplemental enzymes, only a limited number of studies has focused on determining the optimum dietary conditions for enzyme action. Along with many other factors, particle size was recognised to cause variability in responses to supplemental enzymes (Ravindran, 2013) and their effectiveness could be improved by optimising the particle size in diet formulations (Amerah *et al*., 2008b). Consequently, there has been some interest in the interaction between particle size and supplemental enzymes (Amerah *et al*., 2011). Findings from limited studies that evaluated the interaction between particle size of maize (Kasim and Edwards, 2000; Amerah and Ravindran, 2009) and wheat (Amerah *et al*., 2008b) and supplemental enzymes are contradictory and, to the authors' knowledge, no corresponding studies are available with barley. Moreover, the interaction of particle size and supplemental enzymes can be influenced by the feed form due to pelleting-induced

particle size reduction. Accordingly, the present study was conducted to assess the potential interactive influence of barley particle size and Carb and Phy addition, individually or in combination, on growth performance, nutrient digestibility and intestinal morphometry of broiler starters fed pelleted diets.

6.3. Materials and methods

6.3.1. Enzymes

A multi-component NSP-degrading enzyme, Ronozyme® Multigrain (produced by *Trichoderma reesei*, also known as *Trichoderma longiabrachiatum*), and Ronozyme® HiPhos were obtained from DSM Nutritional Products, Australia. 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 2700 XU/g, respectively. One unit of β-glucanase activity (BGU) is defined as the quantity of enzyme that releases 1.0 µmol of reducing moieties from 1.5% β-glucan per minute at pH 5.0 at incubation temperature of 40 °C for 20 min. One unit of xylanase activity (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 and incubation temperature of 40 °C for 20 min. Ronozyme® HiPhos is a granular 6-phytase preparation expressed by submerged fermentation of *Aspergillus oryzae* and contains > 10,000 phytase units/g (FYT). One FYT is defined as the activity of enzyme that releases 1.0 µmole of inorganic P/min from 5.0 mM sodium phytate at pH 5.5 at 37 ºC (DSM Nutritional Products Ltd., 2013). The activities of phytase, endo-1,3 (4)-β-glucanase and endo-1,4-β-xylanase in samples of 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.

6.3.2. Diets

Normal-starch hulled barley (cultivar, Fortitude), obtained from a seed company (Luisetti Seeds Ltd, Rangiora, New Zealand), was ground in a hammer mill to pass through 2.0 and 8.0 mm screens, to achieve fine and coarse barley particles, respectively. Nutrient composition, nitrogen (N)-corrected apparent metabolisable energy (AMEn) and standardised digestible amino acid contents of barley, determined in Chapter 3, were used

to formulate a basal diet to meet the Ross 308 strain recommendations for major nutrients for broiler starters (Ross, 2019; Table 6.1). The basal diet contained 4.8 g/kg non-phytate phosphorus. Two diets, mixed using fine or coarse barley, were developed into eight dietary treatments using four methods of enzyme supplementation: non-supplemented (control), carbohydrase (0.15 g/kg of feed; Carb), phytase (0.10 g/kg; Phy) and combination of carbohydrase and phytase (0.15 and 0.10 g/kg, respectively; Carb + Phy). The diets contained 5.0 g/kg of titanium dioxide (TiO_2, Merck KGaA, Darmstadt, Germany) as an indigestible marker to determine ileal nutrient digestibility. Diets were mixed in a single-screw paddle mixer. Following mixing, all diets were steam-conditioned to 70 °C for 30 seconds and pelleted using a pellet mill (Model Orbit 15; Richard Sizer, Kingston-upon-Hull, UK) with capacity of manufacturing 180 kg of feed/h and equipped with a die ring with 3.0 mm holes and 35 mm thickness. Representative diet samples were collected after pelleting for chemical analysis, determination of particle size distribution and pellet durability.

6.3.3. Determination of particle size distribution

Particle size distribution of ground barley samples was determined using a dry sieving method as described by Baker and Herrman (2002). Briefly, ground barley samples (100 g; four replicates per particle size) were passed through a sieve stack with a set of six sieves (2.0, 1.0, 0.5, 0.25, 0.125 and 0.063 mm) on shakers for five min. The amount of sample retained on each sieve was determined and the geometric mean diameter (GMD) and geometric standard deviation (GSD) was calculated for each sample. These calculations assumed that weight distribution of the sample was logarithmically normal. The following equations were used to calculate the GMD and GSD.

$$di = (du \times do) \wedge 0.5$$

$$GMD = \log^{-1} \{\sum (Wi \log di) / \sum Wi\}$$

$$GSD = \log^{-1} \{\sum Wi (\log di - \log GMD)^2 / \sum Wi\}^{0.5}$$

Where,

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

du = diameter opening through which particles were passed (sieve preceding i^{th})

do = diameter opening through which particles were not passed (i^{th} sieve)

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

Particle size distribution of the two basal pelleted diets were determined by wet sieving using the method described by Lentle *et al*. (2006). Two weighed samples (100 g each; two replicates per particle size) of diets were used in the analysis. One sample was dried at 80 °C in a forced draft oven for 3 d for the determination of dry matter (DM). The second sample was soaked in 400 mL water and was left to stand for 2 h prior to sieving. The same sieve sizes used in the dry sieving method were used. The contents of each of the sieves were subsequently washed onto dried, pre-weighed filter papers, dried in a forced draft oven at 80 °C for 24 h and re-weighed. The dry weight of particles retained by each sieve was expressed as proportion of total DM recovered.

6.3.4. Pellet durability

Pellet durability was determined in a Holmen Pellet Tester (New Holmen NHP100 Portable Pellet Durability Tester, TekPro Ltd., Willow Park, North Walsham, Norfolk, UK) using the method described by Abdollahi *et al*. (2013b). Briefly, samples of whole pellets (100 g; five replicates per diet) with no fines, were rapidly circulated in an air stream around a perforated test chamber for 30 seconds. Resulting fines were removed continuously through the perforations during the test cycle. After the test cycle, pellets were ejected and weighed manually. The pellet durability index (PDI) was calculated as the percentage of weight of pellets not passing through the perforations at the end of the test to weight of whole pellets at the start.

Table 6. 1. Composition, calculated analysis and analysed values (g/kg, as fed) and pellet durability index (%) of the basal diet.

Item		*Calculated analysis*	
Normal starch hulled barley	550	Apparent metabolisable energy, MJ/kg	11.9
Soybean meal	318.4	Total protein	238
Maize gluten meal	50.0	Digestible protein	196
Soybean oil	33.8	Digestible methionine	5.8
Di-calcium phosphate	20.4	Digestible methionine+ cysteine	9.0
Limestone	6.0	Digestible lysine	12.2
L-Lysine HCl	3.1	Digestible threonine	8.2
DL-Methionine	2.4	Digestible arginine	13.1
L-Threonine	1.2	Digestible valine	9.5
Sodium chloride	1.9	Crude fat	46.0
Sodium bicarbonate	3.8	Crude fibre	43.9
Vitamin premix[1]	1.0	Calcium	9.6
Mineral premix[1]	1.0	Non-phytate phosphorus	4.8
Titanium dioxide[2]	5.0	Sodium	2.0
Pellet binder[3]	2.0	Chloride	2.0
		Potassium	8.4
		Analysed values	
		Dry matter	900
		Gross energy, MJ/kg	17.3
		Crude protein (Nitrogen × 6.25)	248
		Starch	315
		Fat	49.5
		Calcium	8.5
		Total phosphorus	7.6
		Pellet durability index (%)[4]	
		Finely ground diet	82.5[a]
		Coarsely ground diet	79.0[b]

[1]Supplied per kg of diet: antioxidant, 125 mg; biotin, 0.2 mg; calcium pantothenate, 20 mg; cholecalciferol, 5000 IU; cyanocobalamin, 0.02 mg; folic acid, 2.0 mg; menadione, 4 mg; niacin, 80 mg; pyridoxine, 5.0 mg; trans-retinol, 15000 IU; riboflavin, 9.0 mg; thiamine, 4.0 mg; dl-α-tocopheryl acetate, 80 IU; choline, 0.45 mg; ascorbic acid, 100 mg; Co, 1.0 mg; Cu, 20 mg; Fe, 40 mg; I, 2.0 mg; Mn, 100 mg; Mo, 1.0 mg; Se, 0.15 mg; Zn, 100 mg.
[1]Image Holdings Ltd., Auckland, New Zealand.
[2]Merck KGaA, Darmstadt, Germany.
[3]KEMBIND® (Kemin Industries [Asia] Pte Ltd) pellet binder, which contained modified lignosulphonate, guar gum, edible fatty acids and mineral oil.
[4]Each value represents the mean of five replicate samples. Means not sharing common letters (a,b) are different ($P < 0.05$).

6.3.5. Birds and housing

The experimental procedures were approved by the Massey University Animal Ethics Committee (MUAEC protocol 17/13) and complied with the New Zealand Code of Practice for the Care and Use of Animals for Scientific Purposes. A total of 384, one-d-old male broilers (Ross 308), obtained from a commercial hatchery, were individually weighed and allocated to 48 cages containing eight birds each of similar weight in electrically heated battery brooders so that the average bird weight per cage was similar. Each of the eight dietary treatments was randomly assigned to six cages. The birds were transferred to grower cages on d-12 and continued on the same starter diets until the end of the trial (d-21). The battery brooders and grower cages were housed in an environmentally controlled room with 20 h of fluorescent illumination per d. The temperature was maintained at 31 °C on d 1 and was gradually reduced to 22 °C by 21 d of age. The diets were offered *ad libitum* and water was available at all times.

6.3.6. Performance data

Body weights (BW) and FI were recorded on a cage basis at weekly intervals. Mortality was recorded daily. Feed per gain (F/G) values were corrected for the BW of any bird that died during the course of the experiment.

6.3.7. Energy and nutrient utilisation

6.3.7.1. Nitrogen-corrected apparent metabolisable energy

The AME_n was determined using the classical total excreta collection method. Feed intake and total excreta output of each cage were quantitatively measured from d 17 to 20 post-hatch. Daily collections from each cage were pooled, mixed in a blender and sub-sampled. Sub-samples were lyophilised (Model 0610, Cuddon Engineering, Blenheim, New Zealand), ground to pass through a 0.5 mm sieve and stored in airtight plastic containers at 4 ºC pending analysis. The diets and excreta samples were analysed for DM, gross energy (GE) and N.

6.3.7.2. Coefficient of apparent ileal digestibility (CAID) of nutrients

On d 21, 6 broilers per cage were euthanised by intravenous injection (0.5 mL per kg BW) of sodium pentobarbitone (Provet NZ Pty Ltd., Auckland, New Zealand), and

digesta were collected from the lower half of the ileum by gently flushing with distilled water, as described by Ravindran *et al*. (2005). The ileum was defined as that portion of the small intestine extending from the Meckel's diverticulum to a point ~40 mm proximal to the ileo-cecal junction. The ileum was then divided into two halves and the digesta were collected from the lower half towards the ileo-cecal junction. Digesta from birds within a cage were pooled, frozen immediately after collection and subsequently lyophilised. Diet and lyophilised digesta samples were ground to pass through a 0.5 mm sieve and stored at 4 ˚C until laboratory analysis. The diets and digesta were analysed for DM, titanium (Ti), N, starch, fat, calcium (Ca) and P.

6.3.8. Gizzard pH and jejunal digesta viscosity

Gizzard pH was measured in two birds, from each replicate cage, euthanised for ileal collection using a pH meter (pH spear, Oakton Instruments, Vernon Hill, IL). The glass probe was inserted directly through an opening made in the gizzard and placed in the digesta. Three values were taken from the proximal, middle and distal sections of gizzard and the average value was considered as the final pH value.

The viscosity of jejunal digesta from two birds euthanised for ileal collection from each replicate cage was also measured. The jejunum is defined as portion of small intestine extending from pancreatic loop to the Meckel's diverticulum. The jejunum was divided into two halves and the digesta were collected from the lower half towards the Meckel's diverticulum. Digesta collected from each bird were centrifuged at 3000 × g at 20 °C for 15 min. A 0.5 mL aliquot of the supernatant was used in a viscometer (Brookfield digital viscometer, Model DV2TLV, Brookfield Engineering Laboratories Inc., Stoughton, MA) fitted with CP-40 cone spindle with shear rates of 5 to 500/s to measure the viscosity.

6.3.9. Digestive tract measurements

On d 22, two additional birds with body weights closest to the mean weight of the cage, were weighed and euthanised by cervical dislocation. The digestive tract from the proventriculus to ceca was carefully excised and adherent fat was removed. The length of duodenum (pancreatic loop), jejunum (from the pancreatic loop to Meckel's diverticulum), ileum (from Meckel's diverticulum to ileocecal junction) and ceca were recorded as described by Amerah *et al*. (2008b) and reported as cm/kg of BW. The empty

weights of proventriculus, gizzard, duodenum, jejunum, ileum and caeca were determined and reported as g/kg of BW.

6.3.10. Chemical analysis

Dry matter was determined using standard procedures (Method 930.15; AOAC, 2016). Nitrogen was determined by combustion (Method 968.06; AOAC, 2016) using a 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). Fat was determined using the Soxtec extraction procedure for animal feed, forage and cereal grains (Method 2003.06; AOAC, 2016). For mineral analysis, the samples were wet digested in a nitric and perchloric acid mixture, and concentrations of P and Ca were determined by inductively coupled plasma-optical emission spectroscopy (ICP-OES) using a Thermo Jarrell Ash IRIS instrument. Samples were assayed for Ti on a UV spectrophotometer following the method of Short *et al*. (1996).

6.3.11. Calculations

The AME of diets was calculated using the following formula:

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

Correction for zero N retention was made using a factor of 36.54 kJ per gram N retained in the body (Hill and Anderson, 1958).

$$AMEn_{diet}\ (MJ/kg) = AME_{diet} - (36.54 \times \text{N retention})/1000$$

Apparent ileal digestibility coefficients of nutrients were calculated from the dietary ratio of nutrients to Ti relative to the corresponding ratio in the ileal digesta.

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

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

6.3.12. Statistical analysis

The data were analysed as a 2 × 4 factorial arrangement of treatments using the general linear model procedure of SAS (version 9.4; SAS Institute Inc., Cary, NC.). Cage served as the experimental unit. Significant differences between means were separated by least significant difference test. Significance was declared at $P < 0.05$.

6.4. Results

6.4.1. Particle size distribution and pellet durability

As shown in Table 6.2, the GMD of barley ground through 2.0 and 8.0 mm screen sizes were 648 and 1249 µm, respectively, with corresponding GSD values of 2.0 and 1.9 µm. The GMD values of fine and coarse barley-based diets were 215 and 263 µm, respectively, with corresponding GSD values of 3.6 and 4.0 µm.

A significant effect of barley particle size ($P < 0.001$; Table 6.1) was observed for PDI, with poorer pellet durability in diets made of coarsely-ground barley (79.0%) compared to the diets made of finely-ground barley (82.5%).

Table 6. 2. Determined particle size distribution (percentage of retained particles on sieves), and geometric mean diameter ± geometric standard deviation (GMD ± GSD) of ground barley and diets.

Particle size	Sieve pore size (µm)							GMD ± GSD
	2,000	1,000	500	250	125	63	< 63	
Ground barley[1]								
Fine	0.04	28.9	43.1	17.5	7.93	1.83	0.70	648 ± 2.0
Coarse	31.8	44.1	15.0	5.74	2.25	0.94	0.17	1249 ± 1.9
Pelleted diets[2]								
Fine	0.66	13.1	20.0	18.1	7.55	4.82	35.8	215 ± 3.6
Coarse	5.96	16.9	16.8	16.3	7.60	3.39	33.0	263 ± 4.0

Fine and coarse grade were achieved using screen sizes of 2.0 and 8.0 mm, respectively.
[1]Each value represents the mean of four replicates.
[2]Each value represents the mean of two replicates. Fine and Coarse refers to particle size of barley used to make pellets.

6.4.2. Enzyme recovery

The average recovery of phytase, endo-1,3 (4)-β-glucanase and endo-1,4-β-xylanase from enzyme-supplemented diets were 78, 52 and 67%, respectively.

6.4.3. Growth performance

Mortality during the experiment was insignificant. Only seven out of the 384 birds died, and the deaths were not related to any dietary treatment.

As summarised in Table 6.3, supplemental enzymes tended ($P = 0.056$) to improve the weight gain (WG) of birds with a synergetic effect from the combined use of enzymes. Regardless of barley particle size and, in comparison to the control diet, the combination of enzymes increased the WG by 28 g/bird. The FI response to the supplemental enzymes interacted ($P < 0.05$) with barley particle size, as the individual supplementation of Phy resulted in greater FI only in fine barley diets. Coarse particle size and supplemental Carb, either individually or in combination with Phy, reduced ($P < 0.001$) the F/G.

6.4.4. Nutrient and energy utilisation

The effects of barley particle size and enzyme supplementation on nutrient and energy utilisation are summarised in Table 6.4. No significant ($P > 0.05$) interaction between particle size and enzyme supplementation was observed for the CAID of any nutrient or AMEn. Greater ($P < 0.05$) CAID of DM, N and fat were observed in birds fed coarse barley diets. Feeding coarse barley tended ($P = 0.071$) to increase the CAID of Ca. Regardless of barley particle size, all supplemental enzymes increased ($P < 0.05$) the DM digestibility. Carb addition (Carb and Carb + Phy) improved ($P < 0.05$) starch and fat digestibility. Phosphorus digestibility was positively influenced ($P < 0.01$) by enzyme supplementation, with greater P digestibility in diets with phytase (Phy and Carb + Phy; $P < 0.05$). Coarse grinding of barley ($P < 0.05$) and Carb enzyme improved ($P < 0.001$) the AMEn.

Table 6. 3. The influence of barley particle size and carbohydrase (Carb) and phytase (Phy) supplementation, individually or in combination (Carb + Phy) in pelleted diets on weight gain (WG; g/bird), feed intake (FI; g/bird) and feed per gain (F/G; g feed/g gain) of broiler starters[1] (d 1-21).

Particle size	Enzyme	WG	FI	F/G
Fine	Control	1185	1477bc	1.246
	Carb	1198	1442c	1.214
	Phy	1208	1519a	1.256
	Carb + Phy	1223	1501ab	1.235
Coarse	Control	1197	1474bc	1.235
	Carb	1204	1456c	1.209
	Phy	1199	1463c	1.220
	Carb + Phy	1215	1458c	1.203
SEM[2]		9.8	12.6	0.0074
Main effects				
Particle size				
Fine		1203	1485	1.238a
Coarse		1204	1463	1.217b
Enzyme				
	Control	1191	1475	1.240a
	Carb	1201	1449	1.211b
	Phy	1204	1491	1.238a
	Carb + Phy	1219	1479	1.219b
Probabilities, $P \leq$				
Particle size		0.962	0.018	0.001
Enzyme		0.056	0.014	0.001
Particle size × Enzyme		0.634	0.026	0.107

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

Table 6. 4. The influence of barley particle size and carbohydrase (Carb) and phytase (Phy) supplementation, individually or in combination (Carb + Phy) in pelleted diets on coefficient of apparent ileal digestibility (CAID)[1] of dry matter (DM), nitrogen (N), starch, fat, calcium (Ca), phosphorus (P) and N-corrected apparent metabolisable energy (AMEn; MJ/kg DM)[2] of 21-d old broiler starters.

Particle size	Enzyme	CAID						AMEn
		DM	N	Starch	Fat	Ca	P	
Fine	Control	0.582	0.725	0.930	0.811	0.311	0.455	12.57
	Carb	0.626	0.757	0.947	0.876	0.381	0.486	12.86
	Phy	0.607	0.751	0.936	0.810	0.343	0.557	12.46
	Carb + Phy	0.621	0.741	0.945	0.852	0.355	0.560	12.82
Coarse	Control	0.608	0.757	0.927	0.850	0.387	0.485	12.60
	Carb	0.631	0.768	0.940	0.864	0.382	0.508	12.89
	Phy	0.633	0.772	0.933	0.873	0.398	0.529	12.68
	Carb + Phy	0.639	0.775	0.948	0.905	0.374	0.554	12.94
SEM[3]		0.0112	0.0101	0.0067	0.0161	0.0287	0.0237	0.053
Main effects								
Particle size								
Fine		0.609b	0.744b	0.939	0.837b	0.347	0.514	12.68b
Coarse		0.628a	0.768a	0.937	0.873a	0.385	0.519	12.78a
Enzyme								
	Control	0.595b	0.741	0.929b	0.831c	0.349	0.470c	12.58b
	Carb	0.629a	0.763	0.943a	0.870ab	0.381	0.497bc	12.88a
	Phy	0.620a	0.761	0.935ab	0.842bc	0.370	0.543ab	12.57b
	Carb + Phy	0.630a	0.758	0.946a	0.878a	0.365	0.557a	12.88a
Probabilities, $P \leq$								
Particle size		0.022	0.002	0.600	0.003	0.071	0.773	0.013
Enzyme		0.012	0.129	0.044	0.014	0.722	0.002	0.001
Particle size × Enzyme		0.754	0.645	0.877	0.108	0.559	0.607	0.230

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

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

[2]Each value represents the mean of six replicates (eight birds per replicate) measured from d 17 to 20 post-hatch.

[3]Pooled standard error of mean.

6.4.5. Relative weight and length of intestinal segments, gizzard pH and jejunal digesta viscosity

Table 6.5 shows the influence of barley particle size and enzyme supplementation on the relative weight and length of intestinal segments, gizzard pH and jejunal digesta viscosity. A significant ($P < 0.01$) barley particle size × enzyme interaction was observed for the relative weight of gizzard, as supplemental phytase in fine and coarse barley diets resulted in the lowest and the highest relative gizzard weights, respectively. No significant ($P > 0.05$) differences in the weight of other digestive organs and segments were observed in response to either barley particle size or supplemental enzymes. Barley particle size had no effect ($P > 0.05$) on the relative length of intestinal segments. Carb and Carb + Phy tended ($P = 0.055$) to reduce the relative length of duodenum and significantly ($P < 0.01$) reduced the relative length of jejunum. Coarse grinding of barley reduced ($P < 0.001$) the gizzard pH. Neither barley particle size nor supplemental enzymes influenced ($P > 0.05$) jejunal digesta viscosity, but a tendency ($P = 0.071$) for an interaction between barley particle size and enzyme supplementation was observed.

Table 6. 5. The influence of barley particle size and carbohydrase (Carb) and phytase (Phy) supplementation, individually or in combination (Carb + Phy) in pelleted diets on relative weight (g/kg of body weight) of proventriculus (Prov.), gizzard (Giz.), duodenum (Duo.), jejunum (Jej.), ileum (Ile.) and caeca; relative lengths (cm/kg of body weight) of Duo., Jej., Ile. and caeca; pH of the gizzard; and jejunal digesta viscosity (cP) of 21-d old broilers[1].

Particle size	Enzyme	Relative empty weight						Relative length				Giz. pH	Jej. digesta viscosity
		Prov.	Giz.	Duo.	Jej.	Ile.	Caeca	Duo.	Jej.	Ile.	Caeca		
Fine	Control	3.80	9.13cd	4.09	7.53	5.71	2.11	22.2	64.0	64.8	13.8	3.66	2.83
	Carb	4.29	9.90bc	3.81	7.40	5.40	2.18	22.3	62.2	63.3	14.7	3.26	3.09
	Phy	3.59	8.33d	4.24	7.64	5.64	1.94	22.1	62.6	62.8	13.6	3.77	2.70
	Carb + Phy	4.17	9.69c	3.78	7.42	5.35	1.99	20.8	59.3	61.2	13.3	3.69	2.81
Coarse	Control	3.79	10.3bc	3.64	7.27	5.55	2.04	23.3	69.6	65.6	14.0	2.77	2.93
	Carb	3.70	11.2ab	3.82	7.93	5.46	2.17	20.9	61.0	60.8	13.8	2.81	2.50
	Phy	3.98	12.2a	3.63	7.46	5.30	1.97	23.3	61.5	64.6	13.6	2.67	2.91
	Carb + Phy	3.57	10.4bc	3.66	7.87	5.06	1.97	21.9	58.0	61.2	13.5	2.92	2.68
SEM[2]		0.286	0.459	0.331	0.557	0.319	0.134	0.62	2.27	2.31	0.55	0.228	0.159
Main effects													
Particle size													
Fine		3.96	9.26	3.98	7.50	5.52	2.06	21.85	62.0	63.0	13.84	3.60a	2.86
Coarse		3.76	11.0	3.69	7.63	5.34	2.04	22.34	62.5	63.0	13.70	2.79b	2.75
Enzyme													
	Control	3.79	9.70	3.86	7.40	5.63	2.08	22.74	66.8a	65.2	13.9	3.22	2.88
	Carb	4.00	10.53	3.82	7.67	5.43	2.18	21.61	61.6b	62.0	14.2	3.03	2.79
	Phy	3.79	10.26	3.93	7.55	5.47	1.95	22.70	62.1b	63.7	13.6	3.22	2.80
	Carb + Phy	3.87	10.06	3.72	7.64	5.20	1.98	21.33	58.6b	61.2	13.4	3.31	2.75
Probabilities, $P \leq$													
Particle size		0.327	0.001	0.218	0.732	0.423	0.841	0.273	0.756	0.999	0.712	0.001	0.357
Enzyme		0.874	0.338	0.933	0.963	0.605	0.346	0.055	0.010	0.319	0.438	0.674	0.872
Particle size × Enzyme		0.250	0.006	0.768	0.844	0.921	0.986	0.132	0.354	0.819	0.720	0.543	0.071

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

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

[2]Pooled standard error of mean.

6.5. Discussion

Particle size distribution results showed that the relative proportion of particles > 1,000 µm increased from 28.9% in the fine barley grind to 75.9% in coarse barley grind, showing the improvement in the diet structure by incorporating coarsely-ground barley. Proportion of particles > 1,000 µm in the diet were 13.8 and 22.9% for fine and coarse barley diets, respectively. Previous reports on the effect of grain particle size on pellet durability are contradictory. Some authors reported no effect of grain particle size on pellet durability (Reece *et al*., 1986a; Amerah *et al*., 2007b; Naderinejad *et al*., 2016), while Reece *et al*. (1986b) observed superior pellet durability of pellets made from coarsely ground maize particles compared to those made from fine particles. The current study showed a significant impact of barley particle size on pellet durability, which agrees with that of Angulo *et al*. (1996), supporting the suggestion that coarse grain particles result in more weak points in pellets, leading to pellet breakages and consequent poor pellet durability (Thomas *et al*., 1998). Although starch gelatinisation was not measured in the current study, it may be postulated that larger grain particles were more resistant to gelatinisation during processing than fine particles (Svihus *et al*., 2004) and, thus, resultant pellets were less durable.

Based on the lack of effect from grain particle size in pelleted diets, previous studies hypothesised that pelleting can mask the influence of particle size (Amerah *et al*., 2007b; Chewning *et al*., 2012). Amerah *et al*. (2007b) evaluated the effect of wheat particle size (3.0 vs. 7.0 mm) in mash and pelleted diets and, reported improvements in WG and F/G in broilers (d 1-21) fed 7.0 mm wheat in mash diets. In pelleted diets, however, wheat particle size had no influence on growth performance. Chewning *et al*. (2012) evaluated the effect of feed form (mash vs. pellets) and maize particle size (300 vs. 600 µm) on broiler performance and also reported the lack of particle size effect on performance of broilers (d 1-44) fed pelleted diets. In contrast, the present study showed that the effect of barley particle size on FI was preserved after pelleting and interacted with supplemental enzymes. The response of Phy on FI in the current study was influenced by barley particle size. Amerah and Ravindran (2009), in a study with broilers (d 1-21), evaluated medium and coarse grinds (3.0 and 7.0 mm, respectively) of maize in mash diets, without and with microbial Phy and reported increased FI by supplemental Phy regardless of particle size. Adding Phy to low P diets is expected to result in better

FI and WG; however, Phy inclusion in diets with adequate P levels do not necessarily generate greater responses in broilers (Selle and Ravindran, 2007). However, the FI response to phytase addition seems to be dependent on diet particle size, a finding that is not readily explainable.

Lentle *et al*. (2006) examined the performance of broiler starters fed pelleted diets based on wheat cultivars that were similar in nutrient composition and NSP content but differed in grain hardness. After grinding in a hammer mill to pass through a 4.0 mm screen, the wheat cultivars showed different particle size distributions owing to differences in grain hardness. The diet with a greater proportion of coarse particles resulted in improved feed efficiency. Amerah *et al*. (2007b) reported that wheat particle size did not influence the performance of birds fed pelleted diets, but in mash diets, F/G improved by 8.8 points in birds fed coarse compared to medium grind. Moreover, Deaton *et al*. (1995) and Naderinejad *et al*. (2016) reported that pelleting eliminated any possible effect of particle size on the F/G of birds fed pelleted maize-based diets. In the present study, however, the effects of particle size existed even after pelleting, with birds fed pellets made with coarsely ground barley having F/G improved by 2.1 points. This contradictory evidence from comparisons of pelleted diets with different grain particle sizes on growth performance can be explained, at least in part, by the changes in particle size distribution following pelleting process. It is evident that when particle size differences were preserved after pelleting, diets with coarser particles improved feed efficiency of broilers (Lentle *et al*., 2006). On the other hand, when pelleting evened out any differences in particle size distribution, no particle size effect on performance was observed (Naderinejad *et al*., 2016). It is, therefore, reasonable to speculate that grain hardness may have a substantial impact on the resistance of grain particles in the feed to the frictional force inside the pellet die and, hence, the particle size distribution after pelleting.

The presence of Carb in the diet (Carb and Carb + Phy) improved the F/G by an average of 2.5 points. Previous studies (Bedford *et al*., 1991; Shakouri *et al*., 2009) consistent with this finding, attributed the improvement in F/G to reduction in digesta viscosity by the action of Carb, but the viscosity effect was not observed in the current study. Amerah *et al*. (2008b) evaluated coarse and medium ground wheat (7.0 and 3.0 mm, respectively), without and with supplemental xylanases, on performance of broiler

starters. They observed a significant particle size × xylanase interaction for F/G as xylanase improved F/G only in the coarse wheat diet. In agreement with the current results with barley, these researchers did not observe any effect of wheat particle size or supplemental enzymes on digesta viscosity.

Surface area per unit volume of grain is increased with the extent of grinding, which can facilitate the in-situ gel formation by partial solubilisation of NSP in finely ground cereals, leading to poor efficacy of exogenous enzymes (Amerah *et al*., 2007a). In coarsely-ground grains, on the other hand, *in situ* gel formation happens to a lesser extent, causing only a minor impact on the efficacy of supplemental enzymes (Amerah *et al*., 2008b). Accordingly, the improvement in F/G observed only in birds fed coarse wheat diets by Amerah *et al*. (2008b) was attributed to enzyme action of hydrolysing the cell wall matrix (Bedford and Schulze, 1998), which can happen more effectively in coarse grain particles due to lower extent of *in situ* gel barriers. In the current study, however, as indicated by the absence of significant interaction, the action of supplemental enzymes on F/G was not influenced by the barley particle size. Furthermore, due to the lack of effect of barley particle size on jejunal digesta viscosity, it can be speculated that other mechanisms, as suggested by Amerah *et al*. (2008b), might have contributed to the 2.5 points improvement in feed efficiency by added Carb.

The improvements of 3.1, 3.2 and 4.3% in the CAID of DM, N and fat, respectively, in coarsely-ground barley diets is contrary to that of Naderinejad *et al*. (2016) and Abdollahi *et al*. (2019a), who reported no effect of maize and wheat particle size on the digestibility of nutrients. Improved DM, N and fat digestibility in birds fed coarse-barley diets can be attributed to a greater functionality of the gizzard (Svihus *et al*., 2011a) which results in greater mechanical breakdown of digesta (Svihus *et al*., 1997a; Hetland *et al*., 2002) and lower digesta pH, as illustrated by the lower gizzard pH of birds fed coarse barley in the current study. Moreover, coarse grain particles reduce the digesta passage rate through the gizzard (Nir *et al*., 1994b), and therefore, are retained longer than finer particles in the digestive tract (Amerah *et al*., 2007a), increasing the exposure time of nutrients to digestive enzymes.

With reference to protein digestion, extended retention and mixing in the gizzard is necessary for better contact between feed, gastric juices and pepsin, thus facilitating the denaturation and digestion of proteins. Accordingly, the larger gizzards in birds fed

coarse barley and the consequent increased gastric reflux between gizzard and proventriculus results in more time for gastric enzyme and protease activities in the foregut, aiding protein digestion. In addition, the lower gizzard pH increases the pepsin activity (Gabriel *et al*., 2003), which facilitates initial protein hydrolysis. All these modifications might have acted to enhance the CAID of N in birds fed coarsely ground barley in the current study. The improvement in CAID of N in birds fed coarse barley in the current study is, however, contrary to the findings by Naderinejad *et al*. (2016), who reported no effect of maize particle size on CAID of N in both mash and pelleted diets, despite well-developed gizzards and lower gizzard pH in birds fed coarser maize-based diets. Jacobs *et al*. (2010) also reported no effect of maize particle size on the apparent total tract digestibility of most amino acids in birds fed maize-based mash diets. According to Mtei *et al*. (2019), who evaluated the interaction between bird type (broilers and layers) and maize particle size, the CAID of N was not influenced by maize particle size, regardless of bird type. In the studies of Jacobs *et al*. (2010) and Mtei *et al*. (2019), despite well-developed gizzards, gizzard pH remained unaffected, suggesting that gizzard pH might be of more importance in enhancing the protein digestibility compared to other mechanisms facilitated by a functional gizzard.

The gizzard has been identified as a key site for regulating the digestibility of starch by preventing starch overload into the lower gut, and a positive correlation between gizzard weight and starch digestibility has been reported (Svihus, 2011b). Despite larger gizzards in birds fed coarse barley diets, no influence of barley particle size on the CAID of starch was observed in the current study. Fine feed structures do not facilitate gizzard development and can result in poor starch utilisation due to suboptimal regulation of feed flow (Svihus, 2011a). Naderinejad *et al*. (2016) reported a greater starch digestibility in pelleted coarse maize diets, while Péron *et al*. (2005) reported improved starch digestibility in birds fed pelleted fine wheat (hard cultivar) diets. The improved starch digestibility in coarse maize-based pelleted diets (Naderinejad *et al*., 2016) was attributed to higher gizzard weights and reduction in gizzard pH. On the other hand, the poor starch digestibility in coarse wheat-based pelleted diets (Péron *et al*., 2005) was attributed to a starch accessibility problem due to physical entrapment of starch granules in coarse particles of hard wheat (Carré, 2004) and, hence, the improved digestibility with fine grinding. The inconsistent response of starch digestibility with grain particle size is likely

related to a complex array of confounding factors such as grain type (Carré, 2004), hardness (Carré *et al*., 2002) and feed form (Naderinejad *et al*., 2016).

The CAID of Ca in birds fed coarse barley diets tended to be greater in the current study (0.347 vs. 0.385, $P = 0.071$), possibly due to a more acidic pH in the gizzard of birds fed coarse particles. Most phytate-mineral complexes are soluble at pH lower than 3.5 and become insoluble at pH values between 4.0 and 7.0 (Champagne, 1988; Selle *et al*., 2000). The low gizzard pH of birds fed coarse barley diets (2.79) fell within the soluble range of pH (< 3.5) and could explain the observed results. However, the lower pH failed to enhance the CAID of P in the current study. Naderinejad *et al*. (2016) evaluated the effect of different particle sizes of maize on the digestibility of minerals and reported an 18.3% improvement in Ca digestibility (0.429 vs. 0.508) and an improvement in CAID of P by 7.82% (0.467 vs. 0.504) for medium and coarse grinding compared to finely ground maize. Amerah and Ravindran (2009) also reported that coarse maize diets improved the total tract retention of Ca, by 16.4%, but had no effect on P retention.

Increasing coarseness of the barley grind in the current study caused a small, but significant, improvement of AMEn by 0.79% (from 12.68 to 12.78 MJ/kg DM). Naderinejad *et al*. (2016) observed greater AME in birds fed coarse maize-based pelleted diets (14.95 MJ/kg DM) compared to fine and medium maize-based pelleted diets (14.71 and 14.81 MJ/kg DM, respectively). Highlighting the inconsistent nature of particle size effect on energy utilisation, Svihus *et al*. (2004) and Amerah *et al*. (2007b) reported that different particle sizes in either mash or pelleted wheat-based diets had no effect on energy utilisation. These contradictory results may be explained by confounding factors such as grain type (Amerah *et al*., 2007b), hardness (Péron *et al*., 2005) and feed form (Kilburn and Edwards, 2001).

Regardless of the nature of response, previous studies (Péron *et al*., 2005; Svihus *et al*., 2011b) observed a strong correlation between starch digestibility and energy utilisation. The 4.66% improvement in AMEn (from 12.23 to 12.80 MJ/kg DM) reported by Péron *et al*. (2005) was attributed to a 6.18% enhancement in starch digestibility in response to increasing fineness. In contrast, the increase in AMEn (0.10 MJ/kg DM) with increasing coarseness of barley grind in the present study, was not reflected in starch digestibility response. Nevertheless, similar trends in AMEn with CAID of DM, N and

fat responses to barley particle size are reflective of a link between energy utilisation and nutrient digestibility.

Irrespective of the barley particle size, the magnitude of response to Carb, Phy and Carb + Phy on the ileal digestibility of DM were 5.7, 4.2 and 5.9%, respectively. Phytate in wheat and barley is largely located in the aleurone layer (Eeckhout and De Paepe, 1994). Therefore, improvement of CAID of DM in response to supplemental phytase, at least in part, was caused by the action of phytase in disrupting of cell wall and consequent release of encapsulated nutrients in a manner similar to that of carbohydrase (Ravindran *et al*., 1999).

Nutrients, such as starch and protein, encapsulated within intact endosperm cell walls in barley are released due to the action of supplemental carbohydrase on cell wall integrity (Bedford, 2018) and, as a consequence, digestibility increases. Similarly, supplemental phytase releases not only phytate P, but also phytate-bound protein and proteolytic enzymes, thus enhancing protein digestion (Ravindran *et al*., 2000; Selle and Ravindran, 2007). The benefits of individual and combined supplementation of carbohydrase and phytase in wheat- and barley-based diets in terms of protein and amino acid digestibility has been previously reported (Ravindran *et al*., 1999; Wu *et al*., 2004a). Wu *et al*. (2004a) reported that CAID of N enhanced by 13.8, 10.8 and 13.8% in broiler starters fed barley-based diets in response to glucanase, phytase and glucanase + phytase, respectively. The observations of the current study contrast from these findings by showing no effect from supplemental enzymes on the CAID of N, with only numerical improvements in the CAID of N (3.0, 2.7, 2.2% increments in response to Carb, Phy and Carb + Phy, respectively) being observed.

Regardless of barley particle size, starch digestibility was enhanced by carbohydrase in both individual and combined supplementation, with magnitude of response of 1.51 and 1.83%, respectively. The effect of supplemental carbohydrase on enhanced starch digestibility in barley is well documented (Bergh *et al*., 1999; Ravindran *et al*., 2007). Carbohydrase disrupts the endosperm cell wall and releases encapsulated starch granules, thus allowing them to interact unhindered with digestive enzymes. However, reports on improved starch digestibility in diets supplemented with phytase are limited (Camden *et al*., 2001). Improvements have been attributed to the release of starch

granules bound in phytate complexes (Thompson, 1988) and alleviation of the inhibitory action of phytate on α-amylases (Sharma *et al*., 1978).

Fat digestibility is detrimentally affected by greater digesta viscosity (Edney *et al*., 1989; Almirall *et al*., 1995). In this study, however, despite the lack of enzyme effect on digesta viscosity, CAID of fat was increased by 4.7 and 5.7% due to individual use of carbohydrases and combination with phytase, respectively. Carbohydrase is believed to enhance fat digestibility by the release of encapsulated nutrients, whilst phytase can partially prevent the formation of metallic soaps by prior hydrolysis of phytate in more proximal parts of the gut and thereby increasing fat digestibility (Selle and Ravindran, 2007). This beneficial impact of phytase on fat digestibility, however, was not evident in the current study.

Individual addition of phytase increased the CAID of P by 15.5%, from 0.470 to 0.543. The CAID of P was further improved by 18.5% when carbohydrases and phytase were added together, showing that activity of phytase was facilitated by NSP-degrading enzymes, perhaps by allowing greater access to substrates. Juanpere *et al*. (2005) evaluated the effect of carbohydrases and a phytase, individually and in combination, in maize-, wheat- and barley-based diets, and reported a synergistic effect of phytase + xylanase on P retention of wheat-based diets, and phytase + β-glucanase on P and Ca retention of barley-based diets. The improvement of Ca retention by supplemental enzymes, however, was not observed in the current study.

Regardless of barley particle size, 2.4% (0.30 MJ/kg DM) improvement in AMEn was reported in response to both individual and combined supplementation of carbohydrase. The beneficial effect from individual use of phytase (Selle *et al*., 2003b) on energy utilisation, reported in previous studies, was not observed in the current study. The improvement in AMEn in response to addition of carbohydrase was closely associated with enhanced digestibility of starch and fat, the main energy yielding nutrients. In agreement to the current findings, and despite the absence of effect on digesta viscosity, Amerah *et al*. (2008b) reported improved AMEn in response to added carbohydrase in both medium (1.6%) and coarse (5.6%) wheat diets. These improvements were attributed to the action of carbohydrase on the physical barriers of endosperm cell wall and gel barriers on digesta particles formed by partial solubilisation of NSP.

Barley particle size influenced the response of gizzard size to supplemental enzymes, as phytase in fine and coarse barley diets resulted in the lowest and the highest relative gizzard weights, respectively. This finding is hard to explain and highlights the need for evaluating the mechanisms of phytase interactions at different particle sizes.

Increased gizzard weights in birds fed coarse maize (Nir *et al*., 1994a,b; Parsons *et al*., 2006) and wheat (Amerah *et al*., 2007b; Abdollahi *et al*., 2019a) have been observed previously. Amerah *et al*. (2008a) reported higher gizzard weights in response to increasing grain particle size from 1.0 mm to 7.0 mm in maize-based (34% increase from 9.40 to 12.6 g/kg of BW) and wheat-based pelleted diets (10.7% increase from 9.03 to 10.0 g/kg of BW). Nir and Ptichi (2001) and Svihus *et al*. (2004) reported that coarse grinding increased gizzard size only when mash diets were fed, while this effect was not apparent in pelleted feeds. In the present study, however, the effects of particle size on gizzard size remained even after pelleting with 18.8% increase from 9.26 to 11.0 g/kg of BW.

In agreement with the present results, previous researchers reported no influence of maize (Naderinejad *et al*., 2016) and wheat (Péron *et al*., 2005) particle size on the relative weight and length of digestive tract components apart from the gizzard. However, Nir *et al*. (1994a) reported reduced duodenal weight in coarse wheat fed birds, but in a follow-up study, Nir *et al*. (1995) observed greater relative weights of jejunum, ileum and small intestine in birds fed coarse maize particles.

In a study by Wu *et al*. (2004b), supplementation of xylanase and phytase individually reduced the relative length (16.5 and 14.1%, respectively) and weight (15.5 and 11.4%, respectively) of the small intestine, while the combination of enzymes had no further effect. It was suggested that the heavier intestinal weight was caused by greater digesta viscosity (Wu *et al*., 2004b), reduced passage rate and subsequent rise in pathogenic microbial activity (Brenes *et al*., 2002) that stimulated intestinal tissue growth. In the current study, individual additions of carbohydrase and phytase significantly shortened the jejunum by 8.38 and 7.54%, respectively, while combining the two enzymes had a synergetic effect causing 13.9% reduction. As the reduction in the relative length of the jejunum paralleled the improvements in DM, starch and fat digestibility in response to supplemental enzymes, it is tempting to speculate that the reduced jejunal

length may be a consequence of the decreased need for digestive and absorptive capacity resulting from supplemental enzymes.

Birds fed coarse barley diets showed lower gizzard pH that tended (P = 0.058) to negatively correlate (r = -0.276) with the relative weight of gizzard. A significant negative correlation (r = -0.451) between the relative gizzard weight and gizzard pH reported by Liu *et al*. (2015) lends support to the present observation. Nir *et al*. (1994b) evaluated coarse, medium and fine particle sizes of maize, wheat and sorghum and observed that the pH of the gizzard contents decreased with increasing particle size, irrespective of the grain type. Naderinejad *et al*. (2016) reported a particle size × feed form interaction for gizzard pH of birds fed different maize particle sizes in mash and pellet diets, as in mash diets, gizzard pH was not influenced by particle size, whereas, in pelleted diets, medium and coarse grinding lowered gizzard pH compared to fine grinding. In addition, secretion of pepsinogen and hydrochloric acid from proventriculus is encouraged when digesta is refluxed into the proventriculus by contraction of a functional gizzard (Duke, 1992). On other hand, smaller gizzards may have resulted in fewer refluxes, which inhibited gastric secretions (Svihus, 2011a) and contributed to elevated pH in birds fed fine barley diets. Contrary to the current findings, Wu *et al*. (2004b) reported that addition of xylanase or the combination of xylanase plus phytase reduced the viscosity of digesta in all segments of the intestine.

The potential impact of grain hardness on particle size distribution, particularly after pelleting, justifies the need for further evaluation of the optimum particle size for different barley types that vary in grain hardness. Moreover, as a potential approach for restoring the structure in pelleted barley-based diets, whole barley inclusion should be evaluated concerning optimum inclusion and interactions with supplemental enzymes.

6.6. Conclusions

In summary, improving the structure of the diet by increasing coarseness of barley grind enhanced the feed efficiency, and nutrient and energy utilisation in broiler starters fed pelleted diets. The fine barley diet was superior only from a pellet quality perspective. Pelleting did not mask the effect of barley particle size. Supplementation of carbohydrase individually or in combination with phytase enhanced the feed efficiency, and starch, fat and energy utilisation, while addition of phytase individually or in combination enhanced

P utilisation. Supplementation of either enzyme improved DM digestibility, and the combination of carbohydrase plus phytase tended to improve WG.

CHAPTER SEVEN

Influence of carbohydrase supplementation and conditioning temperature on performance, nutrient utilisation and gastrointestinal tract development of broiler starters fed barley-based diets

7.1. Abstract

The influence of supplemental carbohydrase (Carb) and conditioning temperature (CT) on growth performance, nutrient utilisation and intestinal morphometry of broilers (d 1-21) fed barley-based diets was examined in a 2 × 3 factorial arrangement, evaluating two levels of Carb (0 and 150 g/tonne of feed) and three CT (60, 74 and 88 °C). The activities of endo-1,4-β- glucanase, endo-1,3 (4)-β-glucanase and endo-1,4-β-xylanase in the tested Carb were 800 BGU/g, 700 BGU/g and 2700 XU/g, respectively. A total of 288, one-d-old male broilers (eight birds/cage; six cages/treatment) were used. On d 21, ileal digesta was collected for the determination of nutrient digestibility. There was no significant ($P > 0.05$) interaction between Carb and CT for any tested parameter. The pellet durability of diets conditioned at 88 °C was superior ($P < 0.05$) to those diets conditioned at 60 °C. Addition of Carb increased weight gain (WG; $P < 0.05$) and reduced feed per gain (F/G; $P < 0.001$) by 30 g/bird and 6.5 points, respectively. Birds fed diets conditioned at 60 and 74 °C had a similar ($P > 0.05$) WG but higher ($P < 0.05$) than those fed diets conditioned at 88 °C. Birds fed diets conditioned at 88 °C tended ($P = 0.054$) to have a lower feed intake than birds fed diets conditioned at 60 °C. Conditioning the diets at 88 °C increased ($P < 0.05$) F/G compared to the diets conditioned at 60 and 74 °C. Regardless of CT, Carb enhanced the CAID of starch ($P < 0.01$), and nitrogen-corrected apparent metabolisable energy (AMEn; $P < 0.05$) by 1.15% and 0.13 MJ/kg, respectively. Birds offered diets conditioned at 88 °C showed lower digestibility of dry matter, nitrogen, phosphorus, gross energy ($P < 0.001$), and AMEn ($P < 0.01$) compared to those fed diets conditioned at 60 and 74 °C. Diets conditioned at 88 °C resulted in poor ($P < 0.05$) starch digestibility compared to diets conditioned at 60 °C. Conditioning at 88 °C increased ($P < 0.05$) jejunal digesta viscosity by 10.2% compared to diets conditioned at 60 and 74 °C. In conclusion, supplementation of barley-based diets with Carb improved WG, F/G and, starch and energy utilisation in broilers. Conditioning barley-based diets at 88 °C negatively influenced WG, F/G and utilisation of dry matter, nitrogen, starch, phosphorus and energy. The lack of significant interactions between Carb and CT indicated that negative

impacts caused by high CT on bird performance and nutrient utilisation were regardless of supplemental Carb. Supplemental Carb *per se* failed to restore these deteriorated parameters.

7.2. Introduction

The use of barley in poultry diets is limited due mainly to its high contents of soluble non-starch polysaccharides (NSP) that results in increased intestinal digesta viscosity, leading to impaired nutrient utilisation and performance of birds fed barley-based diets. Different heat processing methods such as steam-cooking (Gracia *et al*., 2003), expansion, micronisation (Zheng *et al*., 1998; García *et al*., 2008) and extrusion (Vranjes and Wenk, 1995) have been evaluated as potential methods to enhance the feeding value of barley in poultry diets. Expansion, extrusion and micronisation are short-time high-temperature processes that involve temperature > 100 °C. Heat processing is believed to disrupt the cell structures and to release the encapsulated nutrients (Gracia *et al*., 2003; García *et al*., 2008) facilitating the nutrient utilisation. However, thermal processing can increase solubilisation of NSP (Silversides and Bedford, 1999), leading to greater viscosity in both feed and intestinal contents particularly in diets based on viscous grains such as barley (Svihus *et al*., 2000; Cowieson *et al*., 2005; García *et al*., 2008). Accordingly, to achieve the desired outcome of the thermal processing, the application of optimum conditions during feed manufacture is vital.

High conditioning temperatures (CT; > 80 °C) are commonly employed by poultry feed manufacturers to obtain high-quality pellets (Cutlip *et al*., 2008; Abdollahi *et al*., 2013a) and to maintain feed hygiene by controlling foodborne pathogens, such as *Salmonella* and *Campylobacter* (Amerah *et al*., 2011; Abdollahi *et al*., 2013a). High CT, however, can result in the formation of resistant starch (RS; Abdollahi *et al*., 2010b, 2011), degradation of heat-labile amino acids (AA; Papadopoulos, 1989), inactivation of synthetic vitamins (Jensen, 2000) and supplemental enzymes (Inborr and Bedford, 1994), reduced nutrient utilisation (Abdollahi *et al*., 2010a,b) and compromise growth performance (Cutlip *et al*., 2008; Abdollahi *et al*., 2011). Impaired nutrient utilisation of birds fed diets conditioned at higher CT can be attributed to losses in nutritional value of feed ingredients (Papadopoulos, 1989) and viscosity-induced interferences to nutrient absorption (Smulikowska *et al*., 2002) in the gastro-intestinal tract.

On the other hand, lower CT and consequent under-processing of diets can hinder the inactivation of anti-nutritive factors and result in insufficient starch gelatinisation and protein denaturation, while failing to assure satisfactory feed hygiene. These phenomena emphasise the importance of determining the optimum CT of feed and, the fact that impact of CT varies depending on the grain type (Abdollahi *et al*., 2010a,b) necessitates determination of optimum CT for each grain type. The influence of CT on growth performance and nutrient utilisation of broilers fed maize- (Cutlip *et al*., 2008; Abdollahi *et al*., 2010a,b; Loar II *et al*., 2014), wheat- (Abdollahi *et al*., 2010a, 2011) and sorghum- (Abdollahi *et al*., 2010b) based diets have been understood to a better extent. However, studies evaluating the influence of CT on barley-based diets are limited (Inborr and Bedford, 1994; Samarasinghe *et al*., 2000).

The NSP-degrading enzymes are routinely added to barley-based diets to overcome the adverse effects of anti-nutritional factors, mainly the higher digesta viscosity in birds fed barley-based diets. Improved performance and nutrient utilisation in birds fed barley-based diets by supplemental enzymes have been mostly attributed to the reduction of digesta viscosity (Almirall *et al*., 1995; Józefiak *et al*., 2006). As high CT may exacerbate the adverse effects of viscosity in diets based on viscous grains such as barley, use of exogenous enzymes becomes even more critical (Cowieson *et al*., 2005). A better understanding of possible interactions between enzyme and CT, particularly on intestinal digesta viscosity, whether enzymes are more effective in diets conditioned at higher CT, would allow poultry nutritionists to increase the barley inclusion in poultry diets, by strategically minimising the viscosity related negative consequences. Accordingly, the objectives of this study were set to evaluate whether interactive effects between Carb and CT exist on the performance, energy and nutrient utilisation, and gut morphometry in broiler starters fed barley-based diets.

7.3. Materials and methods

7.3.1. Enzymes

A multi-component NSP-degrading enzyme, Ronozyme® Multigrain, (produced by *Trichoderma reesei*, also known as *Trichoderma longiabrachiatum*) and Ronozyme® HiPhos were obtained from DSM Nutritional Products, Australia. 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 2700 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% β-glucan per minute at pH 5.0 at incubation temperature of 40 °C for 20 min. 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 and incubation temperature of 40 °C for 20 min. Ronozyme® HiPhos was a granular 6-phytase preparation expressed by submerged fermentation of *Aspergillus oryzae* and contained > 10,000 phytase units (FYT)/g. One FYT is defined as the activity of enzyme that releases 1.0 µmole of inorganic phosphorus/min from 5.0 mM sodium phytate at pH 5.5 at 37 ºC (DSM Nutritional Products Ltd., 2013). The activities of phytase, endo-1,4-β-xylanase, endo-1,3 (4)-β-glucanase and endo-1,4-β-glucanase in the diet samples obtained after pelleting 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.

7.3.2. Diets

Normal-starch hulled barley (cultivar, Fortitude) was obtained from a seed multiplication company (Luisetti Seeds Ltd., Rangiora, New Zealand) and ground in a hammer mill to pass through the screen size of 8.0 mm. Nutrient composition, nitrogen-corrected apparent metabolisable energy (AMEn) and standardised digestible AA contents of barley determined in Chapter 3, were used in formulating a basal diet to meet the Ross 308 strain recommendations for major nutrients for broiler starters (Ross, 2014; Table 7.1). Ronozyme® HiPhos (DSM Nutritional Products, Australia) was used in the basal diet and phytase matrix values (1.5 g/kg non-phytate phosphorus and 1.8 g/kg calcium) were used in basal diet formulation. The basal diet was then used to develop two feed batches, without and with an NSP-degrading enzyme (Ronozyme® Multigrain; DSM Nutritional Products, Australia). Each diet, without and with Carb, was divided into three equal batches and, conditioned at three different temperatures (60, 74 and 88 °C) by adjusting the steam flow rate. Mash diets were steam-conditioned for 30 s and the CT was measured at the outlet (close to the exit point) of the conditioner before the mash feed entered the pellet die. The CT of the mash was measured continuously, as a single-point measure during the conditioning time. Following conditioning, all diets were pelleted using a pellet

mill (Model Orbit 15; Richard Sizer, Kingston-upon-Hull, UK) capable of manufacturing 180 kg of feed/h and equipped with a die ring with 3.0 mm holes and 35 mm thickness. Representative samples were collected after pelleting for the determination of gelatinised starch (GS) content and pellet durability.

7.3.3. Pellet durability

Pellet durability of diets was determined in a Holmen Pellet Tester (New Holmen NHP100 Portable Pellet Durability Tester, TekPro Ltd., Willow Park, North Walsham, Norfolk, UK) using the method described by Abdollahi *et al*. (2013b). Briefly, clean pellet samples (100 g; five replicates per diet), with no fines, were rapidly circulated in an air stream around a perforated test chamber for 30 seconds. Resulting fines were removed continuously through perforations 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 percentage of weight of pellets not passing through the perforations at the end of the test to weight of whole pellets at the start.

Table 7. 1. Composition, calculated analysis and analysed values (g/kg, as fed) of the basal diet.

Item		*Calculated analysis*	
Normal starch hulled barley	565.4	Apparent metabolisable energy, MJ/kg	11.9
Soybean meal	316.1	Crude protein	238
Maize gluten meal	50.0	Digestible protein	196
Soybean oil	29.4	Digestible methionine	5.8
Di-calcium phosphate	11.5	Digestible methionine + cysteine	9.0
Limestone	8.2	Digestible lysine	12.2
L-Lysine HCl	3.1	Digestible threonine	8.2
DL-Methionine	2.4	Digestible arginine	13.1
L-Threonine	1.2	Digestible valine	9.5
Sodium chloride	1.8	Crude fat	42.1
Sodium bicarbonate	3.8	Crude fibre	44.7
Vitamin premix[1]	1.0	Calcium	9.6
Mineral premix[1]	1.0	Non-phytate phosphorus	4.8
Titanium dioxide[2]	5.0	Sodium	2.0
Pellet binder[3]	2.0	Chloride	2.0
Phytase[4]	0.1	Potassium	8.4
		Analysed values	
		Dry matter	916
		Gross energy, MJ/kg	17.1
		Crude protein (Nitrogen × 6.25)	250
		Starch	315
		Fat	53.0
		Insoluble NSP[5]	136
		Soluble NSP	30.0
		Total NSP[6]	166

[1]Supplied per kg of diet: antioxidant, 125 mg; biotin, 0.2 mg; calcium pantothenate, 20 mg; cholecalciferol, 5000 IU; cyanocobalamin, 0.02 mg; folic acid, 2.0 mg; menadione, 4 mg; niacin, 80 mg; pyridoxine, 5.0 mg; trans-retinol, 15000 IU; riboflavin, 9.0 mg; thiamine, 4.0 mg; dl-α-tocopheryl acetate, 80 IU; choline, 0.45 mg; ascorbic acid, 100 mg; Co, 1.0 mg; Cu, 20 mg; Fe, 40 mg; I, 2.0 mg; Mn, 100 mg; Mo, 1.0 mg; Se, 0.15 mg; Zn, 100 mg.
[1]Image Holdings Ltd., Auckland, New Zealand.
[2]Merck KGaA, Darmstadt, Germany.
[3]KEMBIND® (Kemin Industries [Asia] Pte Ltd) pellet binder, which contained modified lignosulphonate, guar gum, edible fatty acids and mineral oil, was added on top of each diet.
[4]Ronozyme® HiPhos (1000 phytase units (FYT)/kg diet). One FYT is defined as the activity of enzyme that releases 1.0 μmole of inorganic phosphorus/min from 5.0 mM sodium phytate at pH 5.5 at 37 ºC. Nutrient matrix values (0.15% non-Phytate P and 0.18% Ca) were used in basal diet formulation.
[5]NSP, non-starch polysaccharides.
[6]Total NSP= Insoluble NSP + Soluble NSP.

7.3.4. Birds and housing

The experimental procedures were approved by the Massey University Animal Ethics Committee (MUAEC protocol 17/13) and complied with the New Zealand Code of Practice for the Care and Use of Animals for Scientific Purposes. A total of 288, one-d-old male broilers (Ross 308), obtained from a commercial hatchery, were individually weighed and allocated to 36 cages in electrically heated battery brooders so that the

average bird weight per cage was similar. Each of the six dietary treatments was randomly assigned to six cages, each housing eight birds. The birds were transferred to grower cages on d 12 and continued on the same starter diets until the end of the trial (d 21). The battery brooders and grower cages were housed in an environmentally controlled room with 20 h of fluorescent illumination per d. The temperature was maintained at 31 °C on d 1 and was gradually reduced to 22 °C by 21 d of age. The diets were offered *ad libitum* and water was available at all times.

7.3.5. Performance data

Body weights and feed intake (FI) were recorded on a cage basis at weekly intervals. Mortality was recorded daily. Feed per gain (F/G) values were corrected for the body weight (BW) of any bird that died during the course of the experiment.

7.3.6. Energy and nutrient utilisation

7.3.6.1. Nitrogen-corrected apparent metabolisable energy (AME_n)

The AME_n was determined using the classical total excreta collection method. Feed intake and total excreta output of each cage were quantitatively measured from d 17 to 20 post-hatch. Daily collections from each cage were pooled, mixed in a blender and sub-sampled. Sub-samples were lyophilised (Model 0610, Cuddon Engineering, Blenheim, New Zealand), ground to pass through a 0.5 mm sieve and stored in airtight plastic containers at 4 ºC pending analysis. The diets and excreta samples were analysed for dry matter (DM), gross energy (GE) and nitrogen (N).

7.3.6.2. Coefficient of apparent ileal digestibility (CAID) of nutrients

On d 21, six broilers per cage were euthanised by intravenous injection (0.5 mL per kg live weight) of sodium pentobarbitone (Provet NZ Pty Ltd., Auckland, New Zealand), and digesta were collected from the lower half of the ileum by gently flushing with distilled water, as described by Ravindran *et al*. (2005). The ileum was defined as that portion of the small intestine extending from the Meckel's diverticulum to a point ~40 mm proximal to the ileo-caecal junction. The ileum was then divided into two halves and the digesta was collected from the lower half towards the ileo-caecal junction. Digesta from birds were pooled within a cage, frozen immediately after collection and subsequently lyophilised. The diets and lyophilised digesta samples were ground to pass

through a 0.5 mm sieve and stored at 4 ºC until laboratory analysis. The diets and digesta samples were analysed for DM, titanium (Ti), N, starch, fat, calcium, phosphorus and GE.

7.3.7. Gizzard pH

In two birds from each replicate cage euthanised for ileal collection, gizzard pH was measured using a pH meter (pH spear, Oakton Instruments, Vernon Hill, IL). The glass probe was inserted directly through an opening made in the gizzard and placed in the digesta. Three values were taken from the proximal, middle and distal sections of gizzard and the average value was considered as the final pH value.

7.3.8. Jejunal digesta viscosity

The viscosity of jejunal digesta from two birds euthanised for ileal collection from each replicate cage was also measured. Digesta obtained from the lower jejunum was centrifuged at 3000 × g at 20 ºC for 15 min. A 0.5 mL aliquot of the supernatant was used in a viscometer (Brookfield digital viscometer, Model DV2TLV; Brookfield Engineering Laboratories Inc., Stoughton, MA) fitted with CP-40 cone spindle with shear rates of 5 to 500/s to measure the viscosity.

7.3.9. Relative length and weight of digestive tract segments

Two additional birds, with body weights closest to the mean weight of the cage, were weighed and euthanised by cervical dislocation. The digestive tract, from the crop to caeca was carefully excised and adherent fat was removed. The lengths of duodenum (pancreatic loop), jejunum (from the pancreatic loop to Meckel's diverticulum), ileum (from Meckel's diverticulum to ileo-caecal junction) and caeca were recorded as described by Amerah *et al*. (2008b). The empty weights of crop, proventriculus, gizzard, duodenum, jejunum, ileum and caeca in individual birds were determined and reported as g/kg of body weight.

7.3.10. Chemical analysis

Dry matter was determined using standard procedures (Method 930.15; AOAC, 2016). Nitrogen was determined by combustion (Method 968.06; AOAC, 2016) using a 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). Fat was determined using Soxtec extraction procedure for animal feed, forage and cereal grains (Method 2003.06; AOAC, 2016). For mineral analysis, the samples were wet digested in a nitric and perchloric acid mixture, and concentrations of phosphorus and calcium were determined by inductively coupled plasma-optical emission spectroscopy (ICP-OES) using a Thermo Jarrell Ash IRIS instrument. Total, soluble and insoluble NSP were determined using an assay kit (Megazyme International Ireland Ltd., Wicklow, Ireland) based on thermostable α-amylase, protease and amyloglucosidase (Englyst *et al*., 1994). Gelatinised starch content of diet samples was determined using an assay kit (Megazyme International Ireland Ltd., Wicklow, Ireland). Samples were assayed for Ti on a UV spectrophotometer following the method of Short *et al*. (1996).

7.3.11. Calculations

The AME of diets was calculated using the following formula:

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

Correction for zero N retention was made using a factor of 36.54 kJ per gram N retained in the body (Hill and Anderson, 1958).

$$AMEn_{diet}\ (MJ/kg) = AME_{diet} - (36.54 \times \text{N retention})/1000$$

The CAID of nutrients were calculated from the dietary ratio of nutrients to Ti relative to the corresponding ratio in the ileal digesta.

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

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

Ileal digestible energy (IDE) was calculated using the following formula.

$$IDE\ (MJ/kg) = GE_{diet} \times \text{CAID of GE}$$

7.3.12. Statistical analysis

The data for GS contents were analysed as a 2 × 3 factorial arrangement evaluating two stages of feed processing (conditioned-only and conditioned-pelleted) and three CT. All other data were analysed as a 2 × 3 factorial arrangement of treatments evaluating two levels of Carb supplementation and three CT. Cage served as the experimental unit. The general linear model procedure of SAS (version 9.4; SAS Institute Inc., Cary, NC.) was used. Significant differences between means were separated by Least Significant Difference test. Significance of effects was declared at $P < 0.05$.

7.4. Results

7.4.1. Gelatinised starch content, pellet durability and enzyme recovery

There was a significant ($P < 0.05$) processing stage × CT interaction for GS content of the diets (Table 7.2). In conditioned-only diets, increasing CT to 88 °C resulted in higher ($P < 0.05$) GS content compared to the diets conditioned at 60 and 74 °C. In conditioned-pelleted diets, GS content was not influenced by CT of the diet. At each CT, pelleting increased ($P < 0.05$) the GS content compared to the respective conditioned-only diets. As shown in Table 7.3, PDI improved ($P < 0.05$) with increasing CT, with a greater PDI for the diet conditioned at 88 °C than the diet conditioned at 60 °C (66.4 vs. 62.2%).

The recovery of phytase at 60, 74 and 88 °C was 153, 128 and 48.5% respectively. The recovery of endo-1,4-β-xylanase was 81, 55 and 16% at 60, 74 and 88 °C, respectively. The endo-1,4-β-glucanase recovery at 60, 74 and 88 °C was 70, 50 and 0%, respectively. Moreover, endo-1,3 (4)-β-glucanase recovery at 60, 74 and 88 °C was 62, 46 and 0%, respectively.

7.4.2. Growth Performance

Mortality during the experiment was negligible. Only three out of the 288 birds died, and the deaths were not related to any specific treatment. The effects of dietary treatments on growth performance are shown in Table 7.3. There was no interaction between Carb and CT for any of growth performance parameters. Addition of Carb increased weight gain (WG; $P < 0.05$) and reduced F/G ($P < 0.001$) by 30 g/bird and 6.5 points, respectively. Regardless of the Carb addition, WG ($P < 0.001$) and F/G ($P < 0.01$) was deteriorated by increasing CT. Birds fed diets conditioned at 60 and 74 °C had a similar ($P > 0.05$) WG

but higher ($P < 0.05$) than those fed the diets conditioned at 88 °C. Birds fed diets conditioned at 88 °C tended ($P = 0.054$) to have a lower FI than birds fed diets conditioned at 60 °C. Conditioning at 88 °C increased ($P < 0.05$) F/G compared to the diets conditioned at 60 and 74 °C.

7.4.4. Nutrient and energy utilisation

As shown in the Table 7.4, no interaction between supplemental Carb and CT was observed for CAID of any analysed nutrient. Supplemental Carb enhanced ($P < 0.01$) the starch digestibility. Digestibility of phosphorus tended ($P = 0.079$) to be lowered by the supplemental Carb. Birds offered diets conditioned at 88 °C had lower ($P < 0.05$) digestibility of DM, N, phosphorus and GE compared to the birds fed diets conditioned at 60 and 74 °C. Diets conditioned at 88 °C resulted in lower ($P < 0.05$) starch digestibility than diets conditioned at 60 °C.

Regardless of CT, supplemental Carb increased AMEn by 0.13 MJ/kg. Steam-conditioning at 88 °C reduced ($P < 0.05$) IDE and AMEn compared to the diets conditioned 60 and 74 °C.

Table 7. 2. Influence of processing stage and conditioning temperature on gelatinised starch content (g per 100 g total starch) of the diets[1]

Processing stage	Conditioning temperature, (°C)	Gelatinised starch[2]
Conditioned-only	60	11.4c
Conditioned-only	74	10.8c
Conditioned-only	88	13.2b
Conditioned-pelleted	60	16.0a
Conditioned-pelleted	74	15.4a
Conditioned-pelleted	88	16.3a
SEM[3]		0.31
Main effects		
Processing stage		
Conditioned-only		11.8
Conditioned-pelleted		15.9
Conditioning temperature, (°C)		
	60	13.7
	74	13.1
	88	14.8
Probabilities, $P \leq$		
Processing stage		0.001
Conditioning temperature		0.001
Processing stage × Conditioning temperature		0.044

Means not sharing common letters (a,b,c) are different.
[1]Each value represents mean of four replicate samples.
[2]Non-supplemented diets (0 g/kg of Ronozyme® Multigrain) were used in the analysis. Unconditioned diet contained 9.93 g gelatinised starch per 100 g total starch.
[3]Pooled standard error of mean.

Table 7. 3. Influence of carbohydrase enzyme addition and conditioning temperature on weight gain (WG; g/bird), feed intake (FI; g/bird) and feed per gain (F/G; g feed/g gain) of broiler starters[1] (d1-21), and pellet durability index (PDI; %).

Enzyme addition	Conditioning temperature, (°C)	WG	FI	F/G	PDI[2]
-	60	1040	1405	1.365	-
-	74	1026	1376	1.355	-
-	88	938	1360	1.452	-
+	60	1064	1369	1.288	-
+	74	1033	1371	1.327	-
+	88	996	1357	1.363	-
SEM[3]		13.6	11.2	0.0217	-
Main effects					
Enzyme addition					
-		1001b	1380	1.391a	-
+		1031a	1366	1.326b	-
Conditioning temperature, (°C)					
	60	1052a	1387	1.327b	62.2b
	74	1029a	1373	1.341b	64.8ab
	88	967b	1358	1.408a	66.4a
Probabilities, $P \leq$					
Enzyme addition		0.011	0.122	0.001	-
Conditioning temperature		0.001	0.054	0.002	0.021
Enzyme addition × Conditioning temperature		0.175	0.272	0.355	-

Means in a column not sharing common letters (a,b) are different ($P < 0.05$).
[1]Each value represents the mean of six replicates (eight birds per replicate).
[2]Each value represents the mean of five replicate samples.
[3]Pooled standard error of mean.

Table 7. 4. Influence of carbohydrase enzyme addition and conditioning temperature on coefficient of apparent ileal digestibility (CAID)[1] of dry matter (DM), nitrogen (N), fat, starch, calcium (Ca), phosphorus (P), gross energy (GE), ileal digestible energy (IDE; MJ/kg DM)[1] and N-corrected apparent metabolisable energy (AMEn; MJ/kg DM)[2] of 21-d old broilers.

Enzyme addition	Conditioning temperature, (°C)	CAID							IDE	AMEn
		DM	N	Fat	Starch	Ca	P	GE		
-	60	0.648	0.792	0.936	0.963	0.475	0.618	0.660	12.31	12.47
-	74	0.672	0.821	0.951	0.956	0.466	0.606	0.687	12.82	12.46
-	88	0.617	0.752	0.921	0.951	0.458	0.512	0.633	11.80	12.28
+	60	0.654	0.818	0.925	0.973	0.453	0.606	0.670	12.50	12.67
+	74	0.656	0.809	0.931	0.971	0.440	0.574	0.673	12.56	12.56
+	88	0.597	0.772	0.891	0.958	0.423	0.479	0.614	11.45	12.39
SEM[3]		0.0139	0.0136	0.0193	0.0046	0.0272	0.0175	0.0136	0.254	0.006
Main effects										
Enzyme addition										
-		0.646	0.788	0.936	0.956b	0.466	0.579	0.660	12.31	12.41b
+		0.636	0.800	0.916	0.967a	0.439	0.553	0.652	12.17	12.54a
Conditioning temperature, (°C)										
	60	0.651a	0.805a	0.931	0.968a	0.464	0.612a	0.665a	12.41a	12.57a
	74	0.664a	0.815a	0.941	0.963ab	0.453	0.590a	0.680a	12.69a	12.51a
	88	0.607b	0.762b	0.906	0.954b	0.440	0.495b	0.623b	11.63b	12.33b
Probabilities, $P \leq$										
Enzyme addition		0.381	0.310	0.211	0.007	0.226	0.079	0.496	0.502	0.021
Conditioning temperature		0.001	0.001	0.192	0.021	0.688	0.001	0.001	0.001	0.003
Enzyme × Conditioning temperature		0.591	0.347	0.884	0.705	0.974	0.802	0.536	0.541	0.718

Means in a column not sharing common letters (a,b) are different ($P < 0.05$).
[1]Each value represents the mean of six replicates (six birds per replicate).
[2]Each value represents the mean of six replicates (eight birds per replicate) measured from d 17 to 20.
[3]Pooled standard error of mean.

7.4.5. Relative length and weight of digestive tract segments, gizzard pH and jejunal digesta viscosity

No interaction was observed between supplemental Carb and CT on the relative empty weight or length of any measured intestinal segment (Table 7.5). Supplemental Carb reduced *(P* < 0.05) the relative length of the ileum and small intestine and, tended to reduce the relative length of the duodenum (*P* = 0.076) and jejunum (*P* = 0.087). Conditioning the diets at 74 °C tended (*P* = 0.082) to reduce the relative weight of small intestine compared to those conditioned at 60 and 88 °C. Increasing CT to 88 °C tended (*P* = 0.093) to increase the relative weight of the gizzard. Birds offered diets conditioned at 88 °C had lighter (*P* = 0.050) caeca compared to those fed diets conditioned at 60 °C, and longer (*P* < 0.05) duodenum and jejunum compared to those fed diets conditioned at 60 and 74 °C.

Supplemental Carb and CT did not interact (*P* > 0.05) to influence the gizzard pH or jejunal digesta viscosity. However, the gizzard pH tended (*P* = 0.065) to increase with the increasing CT. Jejunal digesta viscosity was significantly (*P* < 0.05) influenced by the CT, as the diet conditioned at 88 °C resulted in 10.1% (0.32 cP) higher digesta viscosity compared to the diets conditioned at 60 and 74 °C.

Table 7. 5. Influence of carbohydrase enzyme addition and conditioning temperature on relative weight (g/kg of body weight) of crop, proventriculus (Prov.), gizzard (Giz.), duodenum (Duo.), jejunum (Jej.), ileum (Ile.) and caeca, and relative lengths (cm/kg of body weight) of Duo., Jej., Ile. and caeca, pH of the gizzard and jejunal digesta viscosity (cP) of 21-d old broilers.

Enzyme addition	Conditioning temperature, (°C)	Relative empty weight								Relative length					Giz. pH	Jej. digesta viscosity
		Crop	Prov.	Giz.	Duo.	Jej.	Ile.	Caeca	SI[3]	Duo.	Jej.	Ile.	Caeca	SI[3]		
-	60	2.38	3.55	11.7	4.93	11.2	7.09	2.46	23.2	20.6	55.2	61.3	24.8	137	2.35	3.06
-	74	2.20	3.75	11.8	4.49	9.2	6.30	2.44	20.0	19.7	52.2	57.7	24.0	130	2.56	3.20
-	88	2.65	3.65	12.1	5.07	10.5	6.64	2.11	22.2	22.1	57.3	61.0	24.8	140	2.91	3.55
+	60	2.31	3.40	11.5	4.92	10.2	6.62	2.60	21.7	19.3	49.5	54.6	24.3	123	2.60	3.20
+	74	2.43	3.79	11.8	4.98	10.3	6.25	2.39	21.5	19.9	53.6	56.5	25.6	130	2.59	3.15
+	88	2.43	3.84	12.8	4.88	10.9	6.75	2.42	22.5	20.7	54.8	58.0	25.5	134	2.71	3.38
SEM[2]		0.128	0.159	0.40	0.215	0.52	0.307	0.105	0.84	0.57	1.53	1.76	0.75	3.5	0.14	0.131
Main effects																
Enzyme addition																
-		2.41	3.65	11.9	4.83	10.3	6.67	2.34	21.8	20.8	54.9	60.0a	24.5	136a	2.60	3.27
+		2.39	3.68	12.0	4.93	10.5	6.54	2.47	22.0	19.9	52.7	56.4b	25.2	129b	2.63	3.24
Conditioning temperature, (°C)																
	60	2.34	3.47	11.6	4.92	10.7	6.86	2.53a	22.5	19.9b	52.3b	58.0	24.5	130	2.47	3.13b
	74	2.31	3.77	11.8	4.73	9.75	6.27	2.42a	20.8	19.8b	52.9b	57.1	24.8	130	2.58	3.17b
	88	2.54	3.74	12.5	4.97	10.7	6.69	2.26b	22.4	21.4a	56.1a	59.5	25.2	137	2.81	3.47a
Probabilities, $P \leq$																
Enzyme addition		0.836	0.824	0.727	0.575	0.697	0.590	0.127	0.852	0.076	0.087	0.018	0.302	0.028	0.814	0.806
Conditioning temperature		0.178	0.135	0.093	0.500	0.113	0.163	0.050	0.082	0.015	0.046	0.405	0.709	0.095	0.065	0.032
Enzyme × Conditioning temperature		0.221	0.572	0.502	0.285	0.176	0.622	0.219	0.244	0.325	0.082	0.294	0.359	0.152	0.293	0.494

Means in a column not sharing common letters (a,b) are different ($P < 0.05$).
[1]Each value represents the mean of six replicates (two birds per replicate).
[2]Pooled standard error of mean.
[3]Small intestine = duodenum + jejunum + ileum.

7.5. Discussion

In the current study, the effect of CT on the formation of GS interacted with the stage of processing. In conditioned-only diets, CT of 88 °C resulted in higher GS content compared to both 60 and 74 °C, while in conditioned-pelleted diets the GS content was not influenced by CT. The differences of GS contents in response to CT in conditioned-only diets were equalised in conditioned-pelleted diets. Pelleting increased the GS content of the conditioned-pelleted diets compared to the respective conditioned-only diets in mash form (15.9 vs. 11.8 g GS per 100 g total starch). The higher GS formation in conditioned-pelleted diets demonstrates that pelleting has a greater effect on starch gelatinisation than steam conditioning. In agreement, Abdollahi *et al*. (2010a) reported that GS content of maize- and sorghum-based diets increased in the conditioned-pelleted diets compared to the conditioned-only diets (9.9 vs. 15.5 g GS per 100 g total starch), attributable to the frictional heat and mechanical shear generated during the pelleting process. Accordingly, it has been hypothesised that only a portion of starch gelatinisation occurs during steam-conditioning, but most of the gelatinisation takes place during the actual pelleting process (Abdollahi *et al*., 2013a).

Increasing CT from 60 to 88 °C enhanced the pellet durability by 4.2 percentage points. This finding agrees with the literature (Cutlip *et al*., 2008; Abdollahi *et al*., 2010a, 2011) that has attributed the improved pellet quality to an increased GS content in response to increasing CT. In the current study, however, no difference in GS contents of pelleted diets conditioned at different temperatures was observed, suggesting the lack of GS effect on pellet durability. Svihus *et al*. (2005) suggested that an increase in diet viscosity, due partly to starch gelatinisation, may enhance the binding capacity of feed particles leading to improved pellet quality. Even though not assessed in the current study, the positive impact of Maillard reaction products generated at higher CT on pellet binding ability has also been acknowledged (Thomas *et al*., 1998; Abdollahi *et al*., 2013a). It can, therefore, be speculated that a combination of factors induced by high CT might have resulted in the higher pellet durability in diets conditioned at 88 °C.

Regardless of the CT, addition of Carb to barley-based diets in the present study increased the WG by 30 g/bird and improved F/G by 6.5 points. Inborr and Bedford (1994), evaluated the supplementation of β-glucanase (0.0, 1.0 and 10 g/kg) to a barley-based diet conditioned at 75, 85 or 95°C for either 30 s or 15 min and reported no

interaction between enzyme, temperature and time for growth performance. These researchers, however, reported a linear improvement in WG and F/G with increasing enzyme addition. Samarasinghe *et al*. (2000) reported that CT of 90 °C compared to 60 °C, in a non-supplemented barley-maize-soybean meal diet numerically impaired WG of broilers (d 7-21) by 2.6 g/bird, daily FI by 2.0 g/bird and F/G by 4.1 points. These researchers reported that despite the 82.2% reduction in exogenous enzyme activity in diets conditioned at 90 °C compared to 75 °C, the impaired WG at 90 °C was restored by the added enzyme. The 11.1% improvement in WG due to the enzyme addition at 90 °C in study by Samarasinghe *et al*. (2000) was not, however, observed at 60 and 75 °C, indicating a greater enzyme efficacy at higher CT. In contrast, as indicated by the lack of interaction between the Carb and CT in the current study for WG and F/G, the exogenous enzymes had similar efficacy at each CT, despite the low recovery at higher CT. Moreover, due to the lack of effect of Carb on jejunal digesta viscosity, it can be speculated that enzyme action of hydrolysing the cell wall matrix (Bedford and Schulze, 1998) and generation of prebiotic oligosaccharides (González-Ortiz *et al*., 2017) might have contributed to the improvements in WG and F/G by supplemental Carb.

Feeding pelleted diets enhances economics of meat chicken production mainly through facilitating ingestion, increased FI (Abdollahi *et al*., 2018) and, subsequent improvements in growth rate and feed efficiency. However, the benefits of pellet feeding on bird performance partly depends on the CT applied during the pelleting process (Abdollahi *et al*., 2010a,b, 2011). In the current study, compared to diets conditioned at 60 °C, birds offered diets conditioned at 88 °C tended to consume 29 g less feed, gained 85 g less weight and showed deterioration of F/G by 8.1 points during the 21-d experimental period, while no differences were observed when the CT increased from 60 to 74 °C. These observations are in agreement with previous studies (Samarasinghe *et al*., 2000; Creswell and Bedford, 2006) reporting deteriorated growth performance in broilers fed diets conditioned at temperatures above 80 °C. Consistent with the present findings, Inborr and Bedford (1994) reported no effect from increasing CT of a barley-based diet from 75 to 85 °C. However, when CT increased from 85 to 95 °C, both WG and F/G were poorer. Loar II *et al*. (2014) reported that increasing the CT from 74 to 85 and 96 °C in a maize-soybean meal diet deteriorated F/G by 3.0 (1.96 vs. 1.99) and 8.0 (1.96 vs. 2.04) points, respectively. Raastad and Skrede (2003) reported similar BW and F/G in 21-d old broilers fed maize-wheat-oat-based diets conditioned at 69 and 78 °C, but lower BW by

5.4% and impaired feed efficiency by 11.5 points in birds fed diets conditioned at 86 °C. Cowieson *et al*. (2005) showed that increasing CT from 80 to 90 °C reduced the WG by 154 g per bird and increased F/G by 9.0 points (1.94 vs. 2.03) in broilers (1-42 d) fed non-supplemented wheat-based diets. Supplemental xylanase restored WG and F/G in the birds fed diets conditioned at 85 or 90 °C but not in those fed the diet conditioned at 80 °C.

The negative effect of high CT on digesta viscosity is believed to be primarily responsible for the poorer performance of birds fed high-temperature conditioned diets (Cowieson *et al*., 2005; Abdollahi *et al*., 2019b). Lending support to this thesis, conditioning the diets at 88 °C tended to lower FI by 29 g/bird compared to the conditioning at 60 °C, due possibly to the slower feed passage associated with greater digesta viscosity (McNab and Smithard, 1992; Almirall *et al*., 1995) in birds fed the diets conditioned at 88 °C. Moreover, F/G of the birds was impaired by 2.4 points per 0.1 cp increase in jejunal digesta viscosity in response to the increasing CT from 60 °C to 88 °C. In contrast, Abdollahi *et al*. (2010a) reported lack of CT effect on F/G of birds fed maize- and sorghum-based diets conditioned at 60, 75 and 90 °C, showing that the feed efficiency deterioration due to the application of high CT is more severe in diets based on viscous grains than those made of non-viscous grains.

Evaluating the influence of increasing CT in maize- and wheat-based diets, Abdollahi *et al*. (2010b) reported that reduction in WG and FI in response to increasing CT from 60 to 75 °C in maize-based diets was restored in the birds fed diets conditioned at 90 °C. This effect was not, however, reported for wheat-based diets, with WG of birds fed diets conditioned at 75 and 90 °C were lower than those fed diets conditioned at 60 °C. In another study, Abdollahi *et al*. (2010a) reported that increasing CT from 60 to 75 °C in both maize- and sorghum-based diets reduced the WG, but the gain was restored in birds fed diets conditioned at 90 °C. These observations led to the hypothesis that WG and FI responses of broilers fed diets conditioned at different temperatures represent a balance between the negative effect of high CT on nutrient availability and the positive effect of high CT on pellet quality. Accordingly, the positive effect of conditioning at 90 °C on pellet quality in non-viscous maize- and sorghum-based diets reported by Abdollahi *et al*. (2010a,b), might have been greater than the negative effect on nutrient utilisation. On the other hand, the improvements in pellet quality gained by applying higher CT to

diets based on viscous grains such as wheat and barley seemed to be insufficient to overcome the adverse effects of high CT on nutrient utilisation (Abdollahi *et al*., 2010b), due, most probably, to the greater magnitude of damage to nutrient utilisation caused by the increased digesta viscosity. In agreement with above explanation, the higher pellet quality achieved at 88 °C in the current study was probably incapable of ameliorating the negative impacts of high CT and restoring the impaired WG and F/G.

Regardless of the CT, supplemental Carb enhanced starch digestibility by 1.15%. The positive effect of the supplemental Carb on starch digestibility in broilers fed barley-based diets has been reported previously (Bergh *et al*., 1999; Ravindran *et al*., 2007). The enhanced starch digestibility, and the lack of Carb effect on digesta viscosity, implies the action of Carb on hydrolysing the cell wall matrix (Hesselman and Åman, 1986; Bedford, 1996) that releases encapsulated starch granules leading to better interactions with digestive enzymes.

Enzyme addition increased the AMEn by 0.13 MJ/kg in the current study, which is paralleled to the enhanced digestibility of starch as the main energy yielding nutrient. Both the improvement of AMEn in response to exogenous enzymes and the correlation with starch digestibility is recognised in the literature (Ravindran *et al*., 2007; Svihus *et al*., 2011b).

It has been suggested that heat treatment of diets containing viscous grains at high temperatures may impair the ability of birds to utilise the nutrients through both increased digesta viscosity and reduced activity of the enzymes (Amerah *et al*., 2011; Abdollahi *et al*., 2013a). In the current study, when the CT increased to 88 °C, digestibility of all nutrients except fat and calcium reduced. Despite the recognised sensitivity of fat digestibility to the higher digesta viscosity (Edney *et al*., 1989; Almirall *et al*., 1995), the CAID of fat was only numerically reduced (by 2.69%) in response to increasing CT from 60 to 88 °C.

Digestibility of N in the current study was influenced by the CT with diets conditioned at 88 °C had lower N digestibility by 5.3% compared to the diets conditioned at 60 °C. Increasing the CT to a certain level can benefit the protein digestibility through inactivation of enzyme inhibitors and protein denaturation that exposes new sites for enzyme attack (Camire *et al*., 1990; Abdollahi *et al*., 2013a). However, extreme CT can

potentially reduce the N digestibility by degradation of heat-labile AA with a marked impact on cysteine, the most heat-labile AA, followed by lysine, arginine, threonine and serine (Papadopoulos, 1989). Loar II *et al*. (2014) reported that methionine, isoleucine and proline digestibility was reduced by 3-5% in response to increasing CT from 74 to 85 and 96 °C. Even though not measured in the current study, inactivation of proteolytic enzymes at higher CT may also have impaired the protein digestibility (Abdollahi et al., 2013a).

Starch gelatinisation increases the susceptibility for amylolytic degradation due to loss of crystalline structure (Svihus *et al*., 2005). Upon gelatinisation, the starch granules are opened allowing the entrance of enzymes into the granule structure (Abdollahi *et al*., 2013a). Starch gelatinisation can occur in temperature ranged from 45-90 °C depending on the starch source and moisture content (Eliasson and Gudmundsson, 1996; Abdollahi *et al*., 2013a). Song and Jane (2000) evaluated the starch extracted from different barley types (normal, waxy, high amylose) and reported the gelatinisation of starch extracted from normal starch barley to onset at 55 °C and reach peak gelatinisation at 59 °C. It can, therefore, be speculated that conditioning the normal starch barley-based diets at 60 °C might have generated a substantial amount of GS to result in starch digestibility high as 0.968. Moreover, as a linear relationship between extent of gelatinisation due to processing and starch digestibility is not evident, higher GS contents does not necessarily mean a higher starch digestibility (Svihus *et al*., 2005). Accordingly, despite the similar GS contents in conditioned-pelleted diets, CAID of starch in birds offered the diet conditioned at 88 °C was 1.45% lower than those fed diets conditioned at 60 °C. In partial agreement, Abdollahi *et al*. (2010b) reported that conditioning wheat-based diets at 90 °C lowered the starch digestibility compared to that of 60 and 75 °C, while starch digestibility in maize-based diets was not affected by increasing CT. Abdollahi *et al*. (2011) reported that CAID of starch in pelleted wheat-based diets decreased from 0.977 in diets conditioned at 60 °C to 0.940 and 0.913 in diets conditioned at 75 and 90 °C, respectively. Crystallisation of the GS upon cooling to the room temperature, known as retrogradation, re-associates starch molecules separated during gelatinisation. As the opposite of gelatinisation, retrogradation can decrease the digestibility of starch (Abdollahi *et al*., 2013a) by forming RS that is resistant to enzymatic hydrolysis. Higher RS content in response to increasing CT to 90 °C has been reported in maize-, sorghum- (Abdollahi *et al*., 2010a) and wheat- (Abdollahi *et al*., 2011) based broiler diets. Even

though RS content was not measured in the current study, it can be speculated that conditioning barley-based diets at higher temperature would have encouraged the formation of RS, negatively influencing the starch digestibility.

Studies on the effect of CT on mineral digestibility are scant. However, it might be reasonable to speculate that the higher digesta viscosity in birds fed diets conditioned at 88 °C is partly responsible for the 17.6% reduction in CAID of phosphorus compared to those fed the diets conditioned at 60 °C. The reductions in the CAID of GE, IDE and AMEn in response to increasing CT from 60 to 88 °C were 6.32, 6.29 and 1.91%, respectively. In comparison to diets conditioned at 60 °C, conditioning diets at 88 °C reduced the digestible protein and phosphorus contents of diets by 10.75 and 0.79 g/kg, respectively. As protein and phosphorus play critical roles in driving broiler growth, the deficit in the digestible contents of these nutrients due to the extreme heat treatment can cause a substantial negative impact on performance parameters, as evidenced by impaired WG and F/G at 88 °C in the present study. Moreover, poor digestibility in birds fed high CT diets can result in greater amounts of substrate available for bacterial growth in the hindgut (Creswell and Bedford, 2006). In consequence, the efforts to sterilise the feed by application of higher CT may unwittingly increase the risk of other microbial infections such as necrotic enteritis (Creswell and Bedford, 2006; Amerah *et al*., 2011; Abdollahi *et al*., 2019b).

Compared to diets conditioned at 60 °C, conditioning at 88 °C reduced the IDE and AMEn by 0.78 and 0.24 MJ/kg, respectively. The reports on the effect of CT on energy utilisation in broilers are not consistent and seemed to be confounded by the grain type. Abdollahi *et al*. (2010a) reported grain type × CT interaction for energy utilisation, with increasing CT from 60 to 90 °C decreased the AME of sorghum-based diets but had no effect on the AME of maize-based diets. Abdollahi *et al*. (2010b), in a study with maize- and wheat-diets, reported no effect of CT on AME of the diets conditioned at either 60, 75 or 90 °C. However, in a follow up study (Abdollahi *et al*., 2011), increasing CT of pelleted wheat-based diets from 60 °C to 90 °C reduced the AME by 0.31 MJ/kg. In agreement with these studies, the negative impact of high CT on IDE and AMEn in the present study showed a direct link to CAID of starch and can be attributed to possible formation of RS, which is refractory to enzymatic hydrolysis.

Birds offered diets with supplemental enzymes had 6.0 and 5.1% shorter ileums and small intestines, respectively, compared to those fed non-supplemented diets. Reduction in the length of jejunum in response to the enzyme supplementation has been observed previously (Wu *et al*., 2004b) and was attributed to an enzyme-induced improvement in nutrient digestibility that might have decreased the need for digestive and absorptive capacity.

Compared to the diet conditioned at 60 °C, conditioning diets at 88 °C resulted in 10.7% reduction in caecal weight. It was hypothesised that high viscosity of digesta impedes the passage of material into the caeca, allowing only small, non-viscous polysaccharides, but not large, highly viscous materials (Svihus *et al*., 2013). Caeca enlarge as a consequence of an increased amount of fermentable material in the diet (Svihus, 2014) and it can be therefore speculated that the impeded passage of fermentable material into the caeca, by greater digesta viscosity in birds offered diets conditioned at 88 °C, has resulted in a significant reduction in the relative weight of caeca.

Feeding diets conditioned at 88 °C increased the relative length of duodenum and jejunum by 7.5 and 7.3%, respectively, compared to the diets conditioned at 60 °C. In agreement, Abdollahi *et al*. (2010b) reported 6.3% longer small intestine in birds fed diets conditioned at 75 °C and 90 °C than at 60 °C. This can be considered as the natural response of the small intestine to the reduced availability of nutrients in diets exposed to higher CT.

The proven impact of NSP-degrading enzymes in alleviating the higher digesta viscosity caused by extreme heat treatments of the wheat- (Silversides and Bedford, 1999; Cowieson *et al*., 2005) and barley- (Samarasinghe *et al*., 2000; Gracia *et al*., 2003; García *et al*., 2008) based diets was not observed in the current study. Gracia *et al*. (2003) evaluated steam-cooked barley grains in mash diets, without or with a supplemental multi-component enzyme, for broiler-starters (d 1-21). An interaction between steam cooking and enzyme addition was reported for intestinal digesta viscosity due to the marked reduction of digesta viscosity in response to the supplemental enzyme in steam-cooked barley diets. Samarasinghe *et al*. (2000) reported greater dietary viscosity in a barley-maize-soy diet due to conditioning at 75 and 90 °C compared to 60 °C. Enzyme addition reduced the viscosity by 11, 14 and 17% in diets conditioned at 60, 75 and 90 °C, respectively, showing greater magnitudes of response at high CT diets. Despite the

lack of enzyme effect on digesta viscosity in the current study, WG, F/G, AMEn and CAID of starch improved by supplemental Carb, suggesting the involvement of mechanisms other than reduction of digesta viscosity.

Application of higher temperatures during conditioning process can increase the viscosity of feed and intestinal digesta by increasing starch gelatinisation (Svihus *et al*., 2005), greater release of encapsulated NSP (Cowieson *et al*., 2005), increased solubilisation of NSP (García *et al*., 2008), increased molecular weights due to less depolymerisation of carbohydrates (Abdollahi *et al*., 2013a) or destruction of enzymes (Inborr and Bedford, 1994; Silversides and Bedford, 1999; Samarasinghe *et al*., 2000). It has been hypothesised that digesta viscosity is dependent not only on NSP concentration but also on its molecular weight. A diet with a low content of soluble NSP might result in high viscosity if the NSP is of a high molecular weight (Cowieson *et al*., 2005). Impaired enzyme activity due to high CT has been reported to result in less depolymerisation of NSP contributing to an increase in molecular weight of NSP and consequent greater digesta viscosity (Silversides and Bedford, 1999; Cowieson *et al*., 2005).

The thermostable enzyme product with maximum CT tolerance of 90 °C (DSM, 2020) used in this experiment has been used in previous studies in this thesis (Chapters 3, 4, 5 and 6) and were found to have high enzyme recoveries under high-temperature thermal processing. In contrast, extremely low enzyme recoveries were determined in diets conditioned at 88 °C in the present study. It is difficult to provide a reason for this unexpected finding. In this study, CT was continuously measured and maintained at desired temperatures of 60, 74 and 88 °C by adjusting the steam flow rate. During the conditioning process, the temperature of diets increases from ambient temperature in mash diets to higher temperatures in the conditioning chamber. The amount of heat required to achieve a particular CT depends on the difference between the pre-conditioning diet temperature (which is almost identical to ambient temperature) and the desired temperature in the conditioning chamber. Accordingly, lower the gap between ambient temperature and conditioner temperature, the requirement of heat to achieve that CT will be low. When the gap is greater, extra heat is needed to achieve the target CT. The current experiment was conducted during early spring and the diets were processed during a day with an average ambient temperature of < 10 °C. To achieve the 88 °C,

therefore, excess heat, and consequently excess moisture, was added to the diets, which may explain, at least in part, the low enzyme recoveries that are atypical for this enzyme product. Moreover, it can be hypothesised that the amount of heat and moisture applied to the diet seems to be more important than final CT and that, if the same diets were conditioned at 88 °C under higher ambient temperature, the recoveries of enzyme activity would have been much higher. Moreover, the pellet die hole frictional heat generated during the pelleting process (Abdollahi *et al*., 2010b) may also have exacerbated the loss of enzyme activity in high CT diets.

7.6. Conclusions

In conclusion, the efficacy of the test enzyme was similar at each CT as indicated by the lack of significant interactions between supplemental Carb and CT. Supplementation of Carb in barley-based diets improved WG, F/G, starch digestibility and AMEn in broiler starters. Steam-conditioning diets at 88 °C negatively influenced the WG, F/G, ileal digestibility of N, starch, phosphorus and GE, IDE and AMEn. Even though conditioning barley-based diets at 88 °C delivered more durable pellets, nutrient utilisation was seriously compromised, most likely due to the increased digesta viscosity, causing a substantial negative impact on growth rate and feed efficiency of the birds. Taken together with previous published data, it is evident that the response of viscous grains to increasing CT differ from those of non-viscous grains highlighting the need of determining grain-specific optimum CT.

CHAPTER EIGHT

GENERAL DISCUSSION

8.1. Introduction

With the ever-increasing demand for poultry products, the supply of adequate and sustainable feed resources become critical justifying continuous exploration for alternative poultry feed ingredients. Despite potential as a poultry feed ingredient, barley remains a comparatively underutilised grain because of the anti-nutritive impact of non-starch polysaccharides (NSP) and, the variability in nutrient composition and quality. Different measures that have been evaluated to ameliorate the anti-nutritive factors in barley have failed to prompt widespread utilisation because of variable responses in birds fed barley-based diets.

Inconsistent research methodology used in published studies has exacerbated the variability and prevented a clearer understanding of the feed value of barley. The lack of characterisation of tested barley types in publications questions the validity of previous recommendations for barley inclusion in poultry diets. Moreover, most research aimed to optimise processing conditions for barley-based diets have not used barley as the sole cereal in the diet, which makes it difficult to reach clear conclusions on the effect of barley *per se*. Accordingly, the broader objective of this thesis was to establish the correct scientific approach for evaluating barley in poultry diets based on grain specific metabolisable energy and digestible amino acid (AA) contents. In order to achieve the optimal performance of broilers fed pelleted barley-based diets, the effect of supplemental enzymes and the optimum feed processing parameters were also investigated. It was hoped that recommendations based on this thesis research will facilitate greater inclusions of barley in commercial broiler diets while maintaining bird performance.

8.2. Development of the study

The first experiment in this project was conducted to characterise the nutrient composition, and to determine grain specific nitrogen (N)-corrected apparent metabolisable energy (AMEn), and coefficient of standardised ileal digestibility of AA in two barley types (NSH [normal starch hulled barley] and WSHL[waxy starch hull-less barley]) compared with wheat (Chapter 3). The two barley types were New Zealand origin

and available in adequate quantities for a series of experiments. Wheat, the most commonly used cereal grain for poultry diets in New Zealand, was evaluated in parallel as the control grain. Nutrient composition, AMEn and standardised digestible AA contents of non-supplemented barley and wheat, determined in Chapter 3, were used to formulate the dietary treatments in subsequent experiments.

The next two experiments were conducted to determine the optimum inclusion levels of NSH (Chapter 4) and WSHL (Chapter 5) in wheat-based diets. When alternative feed ingredients are included in commercial poultry diets, the current industry practice is to balance the energy and AA contents across the diets. To ensure the current research designs are compatible to industry context, experimental diets (Chapters 4 and 5) were formulated to be isocaloric and isonitrogenous.

After determining the optimum inclusion rate for barley in wheat-based diets, the optimum barley particle size (Chapter 6) and conditioning temperature (CT; Chapter 7) were evaluated in diets containing barley as the sole grain source. These evaluations were limited to NSH barley (Chapters 6 and 7). In addition, effects of carbohydrase and phytase (that are routine additions in cereal-based commercial diets) and their possible interactions with grain type (Chapter 3), barley inclusion rate (Chapter 4 and 5), barley particle size (Chapter 6), and CT (Chapter 7) were evaluated.

8.3. The effect of grain type on nutritional quality

The results from Chapter 3 suggested that the β-glucan content, rather than starch composition and presence or absence of hulls, plays an important role in determining the utilisation of nutrients in barley for broilers. Moreover, digestible AA and AMEn contents of NSH were superior to WSHL, despite the higher concentrations of nutrients in WSHL. This finding questions the appropriateness of table values or chemical composition data in formulating barley-based diets in commercial poultry production. The main conclusion from this study was that cultivar-specific values for metabolisable energy and digestible nutrients, AA in particular, should be used when formulating broiler diets to account for barley variation and ensure that birds' nutrient requirements are met.

8.4. Influence of barley inclusion rate and feed processing on pellet quality of barley-based diets

Apart from the first study (Chapter 3), barley-based diets were offered in pellet form, and pellet durability index (PDI) of the diets was determined in each study. Increasing inclusion levels of NSH (Chapter 4) and WSHL (Chapter 5) in wheat-based diets resulted in decreased pellet durability. Soybean oil added to barley diets to maintain isocaloric conditions is the likely cause of the decreased PDI at higher barley inclusions (Chapters 4 and 5). This decreased pellet quality in diets with high oil and barley was observed despite the precautionary use of pellet binder in all diets. The confounding effect of oil on pellet quality was more prominent in WSHL-based diets (Chapter 5) due to the greater amount of added oil compared to NSH diets (Chapter 4). Moreover, the presence of hulls in NSH barley may have generated weak points in pellets, thus contributing to reduced PDI in these diets (Chapter 4) with increasing barley inclusions.

Based on the assumption that large grain particle size results in poor pellet quality, the grains were finely ground for the manufacture of pellets. This assumption was confirmed by the impairment of PDI in coarsely ground barley diets (Chapter 6), due probably to more weak points leading to pellet breakages. Data reported in Chapter 7 demonstrated that increasing the CT for barley-based diets improved the pellet quality, as shown by greater PDI with conditioning at 88 vs. 60 °C. However, greater PDI at higher CT was not due to starch gelatinisation as no difference in gelatinised starch contents of pelleted diets was observed.

8.5. Influence of barley inclusion rate on growth performance of broilers

The optimum inclusion levels of NSH and WSHL in wheat-based diets were determined in Chapters 4 and 5, respectively. In Chapter 4, weight gain of birds increased up to 283 g/kg of NSH inclusion and then decreased with further inclusion. The feed per gain, however, was improved with increasing NSH inclusions in diets. Accordingly, the optimum inclusion level of NSH in a pelleted wheat-based broiler diet was determined as 283 g/kg of diet. In Chapter 5, because maximum WSHL inclusion had no negative effect on weight gain and even improved feed efficiency, it was concluded that WSHL could be safely included up to an inclusion level of 260 g/kg in a balanced, pelleted wheat-based broiler diet.

8.6. Influence of feed processing on growth performance of broilers fed barley-based diets

According to the results from Chapter 6, the effects of barley particle size existed even after pelleting, with birds fed pellets made with coarsely ground barley having improved feed per gain by 2.1 points. Moreover, the effect of barley particle size on feed intake was preserved after pelleting and interacted with supplemental enzymes. These findings contradict the previous hypothesis that pelleting can mask the influence of particle size on growth performance (Amerah *et al*., 2007b; Chewning *et al*., 2012).

Abdollahi *et al*. (2010a,b) hypothesised that growth responses of broilers fed diets conditioned at different temperatures represent a balance between the negative effect of high CT on nutrient availability and the positive effect of high CT on pellet quality. In diets based on viscous grains (e.g., barley and wheat), improvements in pellet quality gained by applying higher CT seemed insufficient to overcome the adverse effects of high CT on nutrient utilisation (Abdollahi *et al*., 2010b). Results reported in Chapter 7 support this contention, with better pellet quality achieved at 88 °C failing to ameliorate the negative impact of high CT on nutrient utilisation and consequently causing substantial losses of growth and feed efficiency.

8.7. Influence of barley type and inclusion rate on nutrient utilisation of broilers

The findings from Chapter 3 showed that, despite the higher contents of N and AA in WSHL compared to NSH, coefficient of standardised ileal digestibility values were lower for WSHL, emphasising the importance of using grain specific digestible AA contents for formulation of barley-based diets. The enhanced coefficient of apparent ileal digestibility (CAID) of N reported in response to increasing NSH inclusion (Chapter 4) can be attributed to a higher functionality of the gizzard that resulted in greater mechanical breakdown of feed particles, longer retention time and lower digesta pH.

The higher CAID of starch for NSH compared to WSHL reported in Chapter 3 contradicted the expectation that WSHL, with greater amounts of amylopectin, would be highly digestible compared to NSH. Enhanced starch digestibility with increasing NSH inclusion reported in Chapter 4 was primarily attributed to gizzard development induced by insoluble NSP, which can prevent starch overload in the digestive tract. In both Chapters 4 and 5, the highest dietary starch content (343 g/kg, as fed basis) was associated

with the diet containing 0 g/kg barley, and dietary starch content reduced with increasing barley inclusion in the diet. The starch digestibility was enhanced with increasing NSH inclusion (Chapter 4), but remained unchanged across WSHL inclusion levels (Chapter 5) despite the significant impact of WSHL inclusion on relative gizzard weight. This observation suggests that dietary starch content does not always influence starch digestibility.

Similar trends in AMEn and CAID of starch reported in Chapter 3 suggested that digestible starch content is the major contributor to metabolisable energy in barley. Despite the experimental diets in Chapters 4 and 5 being formulated to be isoenergetic, AMEn was improved linearly with increasing barley inclusion. The AMEn intake, however, was not influenced by barley inclusion level, suggesting that the lower feed intake associated with higher barley inclusion may be reflective of birds' attempt to maintain a constant energy intake (Classen, 2017).

8.8. Influence of feed processing on nutrient utilisation of broilers fed barley-based diets

The enhanced CAID of N reported in response to increasing coarseness of the barley grind (Chapter 6) can be attributed to a higher functionality of the gizzard that results in greater mechanical breakdown of feed particles *in situ*, longer digesta retention time and lower digesta pH. Despite larger gizzards in birds fed coarse barley diets, no influence of barley particle size on the CAID of starch was observed in Chapter 6. Coarse grinding of barley in Chapter 6 caused a small, but significant, improvement of AMEn by 0.10 MJ/kg (from 12.68 to 12.78 MJ/kg DM).

According to Chapter 7, N digestibility of birds fed diets conditioned at 88 °C were lower than those fed diets conditioned at 60 and 74 °C. It has been suggested that even though increasing CT to a certain level can benefit the protein digestibility through inactivation of enzyme inhibitors and protein denaturation that exposes sites for enzyme action (Camire *et al*., 1990; Abdollahi *et al*., 2013a), extreme CT can potentially reduce N digestibility by degrading heat-labile AA (Papadopoulos, 1989). Results from Chapter 7 showed that birds fed diets conditioned at 88 °C had a poor starch digestibility compared to those fed diets conditioned at 60 °C due probably to increased intestinal digesta viscosity in birds fed diets conditioned at 88 °C. It was also found that the birds offered

diets conditioned at 88 °C had lower AMEn compared to those fed diets conditioned at 60 and 74 °C. Based on these findings, coarse particle size (8.0 mm) and conditioning the diets up to 74 °C is recommended for the tested NSH barley type.

8.9. Influence of enzyme supplementation on feeding value of barley for broilers

Previously reported benefits of exogenous carbohydrase on nutrient and energy utilisation, digesta viscosity and feed efficiency when added to barley-based diets were confirmed in this thesis research. Chapter 3 showed that supplemental carbohydrase improved starch and energy utilisation, with a more pronounced effect in the barley (WSHL) that contained the highest content of β-glucan. In Chapters 4 and 5, improvements in feed efficiency, nutrient digestibility and energy utilisation corresponded with reduced digesta viscosity in birds fed enzyme-supplemented diets. In Chapter 5, however, regardless of the recognised fact that fat digestion is highly sensitive to digesta viscosity, fat digestibility of WSHL-based diets remained unaffected despite 42% reduction in jejunal digesta viscosity in enzyme-supplemented diets. This finding implies that factors other than digesta viscosity, such as fat type (Dänicke *et al*., 1997), affects the efficacy of supplemental enzymes on fat digestibility in birds fed diets based on viscous grains.

Despite the lack of carbohydrase effect on digesta viscosity in Chapters 6 and 7, supplemental carbohydrase enhanced nutrient utilisation and growth performance which implies the involvement of other mechanisms, such as hydrolysis of cell wall matrix (Bedford and Schulze, 1998) and generation of prebiotic oligosaccharides (González-Ortiz *et al*., 2017). The study reported in Chapter 7 showed that the addition of carbohydrase resulted in increased weight gain and reduced feed per gain by 30 g/bird and 6.5 points, respectively. The lack of interaction between the carbohydrase and CT reported in Chapter 7 indicated that the exogenous carbohydrase used in this study had similar efficacy at each CT.

8.10. The role of intestinal digesta viscosity in broilers fed barley-based diets

The negative impact of high intestinal digesta viscosity on growth performance and nutrient utilisation in poultry fed barley-based diets is well recognised. In Chapters 4 and 5, however, increasing inclusion of barley in wheat-based diets reduced the intestinal digesta viscosity despite the higher content of β-glucan in barley compared to wheat. This

observation contrasted with most of the previous literature and implies the contribution of factors other than β-glucan concentration that can influence the intestinal digesta viscosity of birds fed barley-based diets.

The barley particle sizes used in this thesis research did not impact the intestinal digesta viscosity. Application of extreme heat during the conditioning process could exacerbate the adverse effects of intestinal digesta viscosity on nutrient utilisation and bird performance. Supplemental carbohydrase reduced the intestinal digesta viscosity in Chapters 4 and 5, while no effects were observed in Chapters 6 and 7. The variable response of digesta viscosity to supplemental carbohydrase in different experiments of this thesis emphasises the need for strategic determination of enzyme dosage in barley-based diets, with close attention to feed processing conditions. The observation that the positive effects of supplemental carbohydrase in barley-based diets were not necessarily mediated through a reduction in digesta viscosity suggested the involvement of other mechanisms of action by added enzyme.

8.11. The role of gizzard in broilers fed barley-based diets

The studies reported in Chapters 4 and 5 demonstrated greater relative gizzard weight in response to increasing barley inclusions, regardless of the barley type. The greater gizzard weight observed in Chapter 4 was attributed to increased insoluble NSP in the diets with greater NSH inclusions. However, as neither dietary insoluble NSP content nor insoluble NSP intake was greater in WSHL-based diets compared to the control wheat diet, this postulation was not applicable to WSHL (Chapter 5). Consequently, the greater gizzard weight in response to increasing WSHL inclusion reported in Chapter 5 led to speculation that high level of β-glucan in WSHL (68.6 g/kg; Chapter 3) would have positively contributed to the barley hardness (Gamlath *et al*., 2008) and subsequently to the gizzard development. This speculation was supported by the microscopic images with thicker endosperm cell walls for WSHL (Chapter 3). Feeding coarsely ground barley benefited the gizzard development (Chapter 6).

Recently, attempts have been made to understand the sub-optimal starch digestibility in pellet-fed broilers with relation to gizzard development. Consequently, it was hypothesised that a well-developed gizzard could regulate feed consumption and prevent starch overload in the digestive tract, facilitating better starch digestion (Svihus,

2011a,b). The positive influence of gizzard development on starch digestibility reported in Chapter 4 supports this hypothesis. Nevertheless, the CAID of starch remained unaffected despite an increase in relative gizzard weight in response to increasing WSHL inclusion (Chapter 5) or coarse barley particles (Chapter 6). This shows that the relationship between gizzard development and starch digestibility can be confounded by barley characteristics such as hardness and particle size, which can affect the access to starch granules regardless of mechanical grinding by a functional gizzard.

In addition to starch utilisation, the beneficial impact of a functional gizzard may also extend to a favourable influence on feed efficiency, protein and energy utilisation. A well-developed gizzard can improve digestive function through increased retention time, lower pH, and better grinding and mixing with digestive enzymes (Svihus 2011a, 2014). This hypothesis is supported by the findings from Chapters 4, 5 and 6, and suggests the potential of manipulating feed processing practices to enhance gizzard development and, thereby improve the feeding value of barley in poultry diets. Matching physical characteristics of barley, such as hull type and grain hardness, with the appropriate feed processing method, with particular attention to grain particle size, will enhance use of barley in poultry diets.

8.12. Suggestions for future studies

Future studies on optimum inclusion level of barley for broiler finisher diets are justified. The optimum inclusion level of barley for starter and finisher growth phases can then be used to build a complete understanding of the economic feasibility of feeding barley to broilers up to market age. Further evaluation of the optimum particle size for different barley types that vary in grain hardness and hull type is warranted. Whole barley feeding is an unexplored area and inclusion of whole barley either pre-or post-pelleting should be evaluated as a potential approach for restoring the structure in pelleted barley-based diets. Hard barley types with a continuous protein matrix show greater starch-protein adhesion than soft barley types, suggesting that starch-protein binding may be one of the factors influencing barley hardness (Nair *et al*., 2011). Accordingly, evaluation of protease enzyme in combination with carbohydrase and phytase in diets based on barley types that differ in hardness is also suggested to explore effect of the protein matrix and cell wall on nutrient accessibility in barley-based diets.

8.13. Conclusions

The primary objective of this thesis research was to establish a scientific approach for the evaluation and application of barley in broiler diets. Moreover, to build a complete picture on barley use in broiler diets, the influence of feed processing and supplemental enzymes were also evaluated. The comprehensive discussion in this thesis on the effect of feed processing and supplemental enzymes in barley-based diets enables a nutritionist to manipulate conditions to minimise the inherent variability of barley grains and, consequently, increase inclusion of barley in broiler diets. This thesis showed the importance of using nutrient profiles for the specific barley cultivar based on measured contents of AMEn and digestible AA to formulate barley-based diets. Moreover, considering the variability of barley grain, processing conditions should be tailored to the specific barley in use and, hence, the optimum processing parameters reported herein may not be recommended to other barley types. Apart from its direct value in providing information on the optimum barley use in poultry diets, this thesis research can also be used as a model for evaluating other alternative feedstuffs.

REFERENCES

Abdel-Aal, E. S., Hucl, P., Chibbar, R. N., Han, H. L., & Demeke, T. (2002). Physicochemical and structural characteristics of flours and starches from waxy and nonwaxy wheats. *Cereal Chemistry, 79*(3), 458-464.

Abdollahi, M. R., Ravindran, V., & Amerah, A. M. (2016). Influence of partial replacement of ground wheat with whole wheat and exogenous enzyme supplementation on growth performance, nutrient digestibility and energy utilization in young broilers. *Journal of Animal Physiology and Animal Nutrition, 100*(5), 929-937.

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

Abdollahi, M. R., Ravindran, V., Wester, T. J., Ravindran, G., & Thomas, D. V. (2013b). 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*(2), 114-120.

Abdollahi, M. R., Ravindran, V., Wester, T. J., Ravindran, G., & Thomas, D. V. (2010a). Influence of conditioning temperature on performance, apparent metabolisable energy, ileal digestibility of starch and nitrogen and the quality of pellets, in broiler starters fed maize-and sorghum-based diets. *Animal Feed Science and Technology, 162*(3-4), 106-115.

Abdollahi, M. R., Ravindran, V., Wester, T. J., Ravindran, G., & Thomas, D. V. (2010b). 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*(5), 648-657.

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

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

Abdollahi, M. R., Zaefarian, F., & Ravindran, V. (2019b). Maximising the benefits of pelleting diets for modern broilers. *Animal Production Science, 59*(11), 2023-2028.

Abdollahi, M. R., Zaefarian, F., Hunt, H., Anwar, M. N., Thomas, D. G., & Ravindran, V. (2019a). 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*(1), 146-161.

Aberle, T., Burchard, W., Vorwerg, W., & Radosta, S. (1994). Conformational contributions of amylose and amylopectin to the structural properties of starches from various sources. *Starch-Stärke, 46*(9), 329-335.

Adibmoradi, M., Navidshad, B., & Faseleh Jahromi, M. (2016). The effect of moderate levels of finely ground insoluble fibre on small intestine morphology, nutrient digestibility and performance of broiler chickens. *Italian Journal of Animal Science*, *15*(2), 310-317.

Adret-Hausberger, M., & Cumming, R. B. (1985). Behavioural aspects of food selection in young chickens. In: Farrell, D. J. (Eds.), *Recent Advances in Animal Nutrition in Australia*. University of New England, Armidale, Australia.

Afsharmanesh, M., Paghaleh, A. S., & Kheirandish, R. (2013). Effects of sprouted and nonsprouted wheat and barley with and without enzyme on intestinal morphometry of broiler chickens. *Comparative Clinical Pathology*, *22*(5), 993-998.

Al Bustany, Z. (1996). The effect of pelleting an enzyme-supplemented barley-based broiler diet. *Animal Feed Science and Technology, 58*(3-4), 283-288.

Al-Kaisey, M. T., Mohammed, M. A., Alwan, A. K. H., & Mohammed, M. H. (2002). The effect of gamma irradiation on the viscosity of two barley cultivars for broiler chicks. *Radiation Physics and Chemistry, 63*(3-6), 295-297.

Al-Marzooqi, W., & Leeson, S. (2000). Effect of dietary lipase enzyme on gut morphology, gastric motility, and long-term performance of broiler chicks. *Poultry Science*, *79*(7), 956-960.

Al-Marzooqi, W., Kadim, I. T., Mahgoub, O., Al-Busaidi, M., Al-Lawati, S. M., Al-Maqbaly, R., Al-Wheebi, S., & Al-Bakery, A. N. (2010). Apparent ileal amino acids digestibility of four varieties of barley for two strains of chickens. *International Journal of Poultry Science, 9*(6), 527-532.

Almirall, M., & Esteve-Garcia, E. (1994). Rate of passage of barley diets with chromium oxide: Influence of age and poultry strain and effect of β-glucanase supplementation. *Poultry Science, 73*(9), 1433-1440.

Almirall, M., & Esteve-Garcia., E. (1995). In vitro stability of a β-glucanase preparation from *trichoderma longibrachiatum* and its effect in a barley based diet fed to broiler chicks. *Animal Feed Science and Technology*, *54*(1-4), 149-158.

Almirall, M., Francesch, M., Perez-Vendrell, A. M., Brufau, J., & Esteve-Garcia, E. (1995). The differences in intestinal viscosity produced by barley and β-glucanase alter digesta enzyme activities and ileal nutrient digestibilities more in broiler chicks than in cocks. *The Journal of nutrition, 125*(4), 947-955.

Al-Rabadi, G. J. (2014). Microscopic analysis of in vitro digested milled barley grains: influence of particle size heterogeneity. *Jordan Journal of Biological Sciences, 7*(3), 199-203.

Al-Rabadi, G. J., Torley, P. J., Williams, B. A., Bryden, W. L., & Gidley, M. J. (2012). Particle size heterogeneity in milled barley and sorghum grains: Effects on physico-chemical properties and starch digestibility. *Journal of Cereal Science, 56*(2), 396-403.

Åman, P., & 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*(sup129), 42-51.

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

Amerah, A. M., & Ravindran, V. (2009). Influence of particle size and microbial phytase supplementation on the performance, nutrient utilisation and digestive tract parameters of broiler starters. *Animal Production Science, 49*(8), 704-710.

Amerah, A. M., Gilbert, C., Simmins, P. H., & Ravindran, V. (2011). Influence of feed processing on the efficacy of exogenous enzymes in broiler diets. *World's Poultry Science Journal, 67*(1), 29-46.

Amerah, A. M., Ravindran, V., & 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*(3), 366-375.

Amerah, A. M., Ravindran, V., Lentle, R. G., & 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*(12), 2615-2623.

Amerah, A. M., Ravindran, V., Lentle, R. G., & Thomas, D. G. (2008a). 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*(11), 2320-2328.

Amerah, A. M., Ravindran, V., Lentle, R. G., & Thomas, D. G. (2008b). Influence of particle size and xylanase supplementation on the performance, energy utilisation, digestive tract parameters and digesta viscosity of broiler starters. *British Poultry Science, 49*(4), 455-462.

Amerah, A. M., Ravindran, V., Lentle, R. G., & Thomas, D. G. (2007a). Feed particle size: Implications on the digestion and performance of poultry. *World's Poultry Science Journal, 63*(3), 439-455.

Andersson, A. A. M., Elfverson, C., Andersson, R., Regnér, S., & Åman, P. (1999). Chemical and physical characteristics of different barley samples. *Journal of the Science of Food and Agriculture, 79*(7), 979-986.

Andriotis, V. M., Rejzek, M., Barclay, E., Rugen, M. D., Field, R. A., & Smith, A. M. (2016). Cell wall degradation is required for normal starch mobilisation in barley endosperm. *Scientific reports*, *6*(33215), 1-15.

Angulo, E., Brufau, J., & Esteve-Garcia, E. (1996). Effect of a sepiolite product on pellet durability in pig diets differing in particle size and in broiler starter and finisher diets. *Animal Feed Science and Technology*, *63*(1-4), 25-34.

Ankrah, N. O. (1994). Heat and moisture effects on the nutritional and physical characteristics of starch in normal and waxy hull-less barley. M. Sc. Thesis. University of Saskatchewan, Saskatoon, Canada.

Ankrah, N. O., Campbell, G. L., Tyler, R. T., Rossnagel, B. G., & 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*(3-4), 205-219.

Ao, Z., & Jane, J. L. (2007). Characterization and modeling of the A-and B-granule starches of wheat, triticale, and barley. *Carbohydrate polymers*, *67*(1), 46-55.

AOAC. (2016). Official Methods of Analysis. 20th edition. *Association of Official Analytical Chemists*, Washington DC, USA.

Arscott, G. H., & Rose, R. J. (1960). Use of barley in high-efficiency broiler rations: 4. influence of amylolytic enzymes on efficiency of utilization, water consumption and litter condition. *Poultry Science*, *39*(1), 93-95.

Arscott, G. H., Hulit, V. L., & Pautz, R. K. (1957). The use of barley in high-efficiency broiler rations: 3. effect of pellets and reground pellets on growth and efficiency of feed utilization. *Poultry Science*, *36*(6), 1388-1389.

Arscott, G. H., Johnson, L. E., & Parker, J. E. (1955). The use of barley in high-efficiency broiler rations: 1. The influence of methionine, grit and stabilized animal fat on efficiency of utilization. *Poultry Science*, *34*(3), 655-662.

Asare, E. K., Jaiswal, S., Maley, J., Båga, M., Sammynaiken, R., Rossnagel, B. G., & Chibbar, R. N. (2011). Barley grain constituents, starch composition, and structure

affect starch in vitro enzymatic hydrolysis. *Journal of Agricultural and Food Chemistry*, *59*(9), 4743-4754.

Baidoo, S. K., & Liu, Y. G. (1998). Hull-less barley for swine: ileal and faecal digestibility of proximate nutrients, amino acids and non-starch polysaccharides. *Journal of the Science of Food and Agriculture, 76*(3), 397-403.

Baker, S., & Herrman, T. (2002). Evaluating particle size, MF-2051. *Feed manufacturing*. Department of Grain Science and Industry, Kansas State University. Manhattan. KS.

Bandegan, A., Golian, A., Kiarie, E., Payne, R. L., Crow, G. H., Guenter, W., & Nyachoti, C. M. (2011). Standardized ileal amino acid digestibility in wheat, barley, pea and flaxseed for broiler chickens. *Canadian Journal of Animal Science, 91*(1), 103-111.

Bao, Y. M., Romero, L. F., & Cowieson, A. J. (2013). Functional patterns of exogenous enzymes in different feed ingredients. *World's Poultry Science Journal, 69*(4), 759-774.

Bartnik, M., & Szafrańska, I. (1987). Changes in phytate content and phytase activity during the germination of some cereals. *Journal of Cereal Science, 5*(1), 23-28.

Barua, M., Abdollahi, M. R., Zaefarian, F., Wester, T. J., Channarayapatna, G., & Ravindran, V. (2019). Influence of feed form on the standardised ileal amino acid digestibility of ingredients for broiler chickens. *Proceedings of the 21st Massey University technical update conference* (pp. 72-77). Monogastric Research Centre, Massey University: Palmerston North, New Zealand.

Bdour, M. A., Al-Rabadi, G. J., Al-Ameiri, N. S., Mahadeen, A. Y., & Aaludatt, M. H. (2014). Microscopic analysis of extruded and pelleted barley and sorghum grains. *Jordan Journal of Biological Sciences, 7*(3), 227-231.

Beames, R. M., Helm, J. H., Eggum, B. O., Boisen, S., Knudsen, K. B., & Swift, M. L. (1996). A comparison of methods for measuring the nutritive value for pigs of a range of hulled and hulless barley cultivars. *Animal Feed Science and Technology, 62*(2-4), 189-201.

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

Bedford, M. R. (1996). The effect of enzymes on digestion. *Journal of Applied Poultry Research, 5*(4), 370-378.

Bedford, M. R. (2018). The evolution and application of enzymes in the animal feed industry: the role of data interpretation. *British Poultry Science, 59*(5), 486-493.

Bedford, M. R., & Schulze, H. (1998). Exogenous enzymes for pigs and poultry. *Nutrition Research Reviews*. 11(1), 91-114.

Bedford, M. R., Classen, H. L., & Campbell, G. L. (1991). The effect of pelleting, salt, and pentosanase on the viscosity of intestinal contents and the performance of broilers fed rye. *Poultry Science, 70*(7), 1571-1577.

Behall, K. M., Scholfield, D. J., & Hallfrisch, J. (2004). Lipids significantly reduced by diets containing barley in moderately hypercholesterolemic men. *Journal of the American College of Nutrition, 23*(1), 55-62.

Bengtsson, S., Åman, P., Graham, H., Newman, C. W., & Newman, R. K. (1990). Chemical studies on mixed-linked β-glucans in hull-less barley cultivars giving different hypocholesterolaemic responses in chickens. *Journal of the Science of Food and Agriculture, 52*(4), 435-445.

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

Bergh, M. O., Razdan, A., & Åman, P. (1999). Nutritional influence of broiler chicken diets based on covered normal, waxy and high amylose barleys with or without enzyme supplementation. *Animal Feed Science and Technology, 78*(3-4), 215-226.

Bewley, J. D., & Black, M. (1978). The structure of seeds and their food reserves. Chap. 2, In: *Physiology and Biochemistry of Seeds. Vol. 1*. (pp. 7-37). Springer-Verlag, New York.

Bhatty, R. S. (1999). The potential of hull-less barley. *Cereal Chemistry*, *76*(5), 589-599.

Bhatty, R. S., Berdahl, J. D., & Christison, G. I. (1975). Chemical composition and digestible energy of barley. *Canadian Journal of Animal Science, 55*(4), 759-764.

Bhatty, R. S., Christison, G. I., Sosulski, F. W., Harvey, B. L., Hughes, G. R., & Berdahl, J. D. (1974). Relationships of various physical and chemical characters to digestible energy in wheat and barley cultivars. *Canadian Journal of Animal Science, 54*(3), 419-427.

Biel, W., & Jacyno, E. (2013). Chemical composition and nutritive value of spring hulled barley varieties. *Bulgarian Journal of Agricultural Science, 19*(4), 721-727.

Biggs, P., & Parsons, C. M. (2009). The effects 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*(9), 1893-1905.

Björck, I., Eliasson, A. C., Drews, A., Gudmundsson, M., & Karlsson, R. (1990). Some nutritional properties of starch and dietary fiber in barley genotypes containing different levels of amylose. *Cereal Chemistry, 67*(4), 327-333.

Black, J. L., A. M. Tredrea, S. G. Nielsen, P. C. Flinn, A. G. Kaiser, and R. J. Van Barneveld 2005. "Feed Uses for Barley." *Proceedings of the 12th Australian Barley Technical Symposium*. Hobart, Tasmania, Australia.

Bolarinwa, O. A., & Adeola, O. (2012). Energy value of wheat, barley, and wheat dried distillers grains with solubles for broiler chickens determined using the regression method. *Poultry Science, 91*(8), 1928-1935.

Brake, J. D., Brann, D. E., & Griffey, C. A. (1997). Barley without enzyme supplementation in broiler grower and finisher diets. *Journal of Applied Poultry Researc*h, *6*(4), 422-431.

Brenes, A., Marquardt, R. R., Guenter, W., & Viveros, A. (2002). Effect of enzyme addition on the performance and gastrointestinal tract size of chicks fed lupin seed and their fractions. *Poultry Science, 81*(5), 670-678.

Brenes, A., Smith, M., Guenter, W., & 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*(9), 1731-1739.

Bryden, W. L., Li, X., Ravindran, G., Hew, L. I., & Ravindran, V. (2009). Ileal digestible amino acid values in feedstuffs for poultry. Rural Industries Research and Development Corporation, Canberra, Australia (RIRDC Publication No 09/071).

Buchanan, N. P., & Moritz, J. S. (2009). Main effects and interactions of varying formulation protein, fiber, and moisture on feed manufacture and pellet quality. *The Journal of Applied Poultry Research*, *18*(2), 274-283.

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*(1), 55-75.

Caldwell, R. A. (1992). Effect of calcium and phytic acid on the activation of trypsinogen and the stability of trypsin. *Journal of Agricultural and Food Chemistry*, *40*(1), 43-46.

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

Camire, M. E., Camire, A., & Krumhar, K. (1990). Chemical and nutritional changes in foods during extrusion. *Critical Reviews in Food Science and Nutrition*, *29*(1), 35-57.

Campbell, G. L., Classen, H. L., & Ballance, G. M. (1986). Gamma irradiation treatment of cereal grains for chick diets. *The Journal of Nutrition*, *116*(4), 560-569.

Campbell, G. L., Rossnagel, B. G., & Bhatty, R. (1993). Evaluation of hull-less barley genotypes varying in extract viscosity in broiler chick diets. *Animal Feed Science and Technology*, *41*(3), 191-197.

Campbell, G. L., Rossnagel, B. G., Classen, H. L., & Thacker, P. A. (1989). Genotypic and environmental differences in extract viscosity of barley and their relationship to its nutritive value for broiler chickens. *Animal Feed Science and Technology, 26*(3-4), 221-230.

Carré, B. (1990). Predicting the dietary energy value of poultry feeds. In: J. Wiseman & D. J. A. Cole (Eds.), *Feedstuff evaluation* (pp. 283-300), Butterworths, London.

Carré, B. (2004). Causes for variation in digestibility of starch among feedstuffs. *World's Poultry Science Journal, 60*(1), 76-89.

Carré, B., Idi, A., Maisonnier, S., Melcion, J. P., Oury, F. X., Gomez, J., & Pluchard, P. (2002). Relationships between digestibilities of food components and characteristics of wheats (*Triticum aestivum*) introduced as the only cereal source in a broiler chicken diet. *British Poultry Science, 43*(3), 404-415.

Cengiz, Ö., Köksal, B. H., Önol, A. G., Tatlı, O., Sevim, Ö., Avcı, H., Bilgili, S. F. (2012). Influence of dietary enzyme supplementation of barley-based diets on growth performance and footpad dermatitis in broiler chickens exposed to early high-moisture litter. *Journal of Applied Poultry Researc*h, *21*(1), 117-125.

Cengiz, Ö., Köksal, B. H., Tatlı, O., Sevim, Ö., Ahsan, U., Bilgili, S. F., Önol, A. G. (2017). Effect of dietary tannic acid supplementation in corn-or barley-based diets on growth performance, intestinal viscosity, litter quality, and incidence and severity of footpad dermatitis in broiler chickens. *Livestock Science, 202*, 52-57.

Champagne, E. T. (1988). Effects of pH on mineral-phytate, protein-mineral-phytate and mineral-fiber interactions. Possible consequences of atrophicgastritis on mineral bioavailability from high fiber foods. *Journal of the American College of Nutrition, 7*(6), 499-508.

Chesson, A. (1992). Effects of supplementary enzymes in barley diets. *Options Mediterraneennes, 20*, 55-62.

Chesson, A. (1993). Feed enzymes. *Animal Feed Science and Technology, 45*, 65-79.

Chewning, C. G., Stark, C. R., & Brake, J. (2012). Effects of particle size and feed form on broiler performance. *Journal of Applied Poultry Research, 21*(4), 830-837.

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

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

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

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

Choct, M., & Annison., G. (1992a). The inhibition of nutrient digestion by wheat pentosans. *British Journal of Nutrition, 67*(1), 123-132.

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

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

Classen, H. L. (1996). Cereal grain starch and exogenous enzymes in poultry diets. *Animal Feed Science and Technology, 62*(1), 21-27.

Classen, H. L. (2017). Diet energy and feed intake in chickens. *Animal Feed Science and Technology, 233*, 13-21.

Classen, H. L., Campbell, G. L., Rossnagel, B. G., & Bhatty, R. S. (1988). Evaluation of hulless barley as replacement for wheat or conventional barley in laying hen diets. *Canadian Journal of Animal Science, 68*(4), 1261-1266.

Classen, H. L., Campbell, G. L., Rossnagel, B. G., Bhatty, R., & Reichert, R. D. (1985). Studies on the use of hulless barley in chick diets: Deleterious effects and methods of alleviation. *Canadian Journal of Animal Science, 65*(3), 725-733.

Cowieson, A. J., Hruby, M., & Faurschou Isaksen, M. (2005). The effect of conditioning temperature and exogenous xylanase addition on the viscosity of wheat-based diets and the performance of broiler chickens. *British Poultry Science, 46*(6), 717-724.

Crampton, E. W. (1936). The comparative feeding values for poultry of barley, oats, wheat, rye, and corn: a review and analysis of published data, prepared for the associate committee on grain research of the national research council and the dominion dept. of agriculture (no. 96).

Creswell, D., & Bedford, M. (2006). High pelleting temperatures reduce broiler performance. *Proceedings of the Australian Poultry Science Symposium* (pp. 1-6). Sydney, Australia.

Cutlip, S. E., Hott, J. M., Buchanan, N. P., Rack, A. L., Latshaw, J. D., Moritz, J. S. (2008). The effect of steam-conditioning practices on pellet quality and growing broiler nutritional value. *Journal of Applied Poultry Research, 17*(2), 249-261.

Dänicke, S., Simon, O., Jeroch, H., & Bedford, M. (1997). Interactions between dietary fat type and xylanase supplementation when rye-based diets are fed to broiler chickens. 2. performance, nutrient digestibility and the fat-soluble vitamin status of livers. *British Poultry Science, 38*(5), 546-556.

Danisco Animal Nutrition. (2014). Feed enzyme innovation-past present and potential. International Poultry Production-September. Retrieved from: http://animalnutrition.dupont.com/fileadmin/user_upload/live/animal_nutrition/documents/op en/Feed-enzyme-innovation-International-Poultry-Production-September_2014.pdf

de Blas, C., Mateos, G. G., & Rebollar, P. G. (2010). *FEDNA Tables of Composition and Nutritive Value of Foods for the Manufacture of Compound Feed*. (3rd ed.). Spanish Foundation for the Development of Animal Nutrition. Madrid, Spain.

Deaton, J. W., Lott, B. D., & Branton, S. L. (1995). Corn grind size and broilers reared under two temperature conditions. *Journal of Applied Poultry Research, 4*(4), 402-406.

Dembicki Jr., H. (2017). Interpreting crude oil and natural gas data. Chap. 4, In: *Practical Petroleum Geochemistry for Exploration and Production.* (pp. 135-188). Elsevier, Amsterdam, Netherlands.

Denbow, D. M., Duke, G. E., & Chaplin, S. B. (1988). Food intake, gastric secretion, and motility as affected by avian pancreatic polypeptide administered centrally in chickens. *Peptides, 9*(3), 449-454.

Dersjant-Li, Y., Van De Belt, K., Van Der Klis, J. D., Kettunen, H., Rinttilä, T., & Awati, A. (2015). Effect of multi-enzymes in combination with a direct-fed microbial on performance and welfare parameters in broilers under commercial production settings. *Journal of Applied Poultry Research, 24*(1), 80-90.

DSM Nutritional Products Ltd. (2013). Ronozyme® Multigrain (GT)-Specification and Tests- Global Quality management OPS/QM. Basel, Switzerland.

DSM in Animal Nutrition & Health. (2020) Retrieved from https://www.dsm.com/markets/anh/en_US/products/products-feedenzymes/products-feed-carbohydrases/products-feed-carbohydrases-ronozyme-multigrain.html

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

Dusel, G., Kluge, H., Glaser, K., Simon, O., Hartmann, G., Lengerken, J. V., & Jeroch, H. (1997). An investigation into the variability of extract viscosity of wheat-relationship with the content of non-starch-polysaccharide fractions and metabolisable energy for broiler chickens. *Archives of Animal Nutrition, 50*(2), 121-135.

Edney, M. J., Campbell, G. L., & Classen, H. L. (1989). The effect of β-glucanase supplementation on nutrient digestibility and growth in broilers given diets

containing barley, oat groats or wheat. *Animal Feed Science and Technology, 25*(1-2), 193-200.

Edney, M. J., Tkachuk, R., & MacGregor, A. W. (1992). Nutrient composition of the hull-less barley cultivar, condor. *Journal of the Science of Food and Agriculture, 60*(4), 451-456.

Eeckhout, W., & De Paepe, M. (1994). Total phosphorus, phytate-phosphorus and phytase activity in plant feedstuffs. *Animal Feed Science and Technology, 47*(1-2), 19-29.

Ehsani, M., Baratian, V., & Torki, M. (2011). Performance of broilers fed barly-based diets supplemented by two sources of commercial probiotics. *World Applied Science Journal, 14*, 9-14.

Eliasson, A. C., & Gudmundsson, M. (1996). Starch: Physicochemical and functional aspects. In: A. C. Eliasson, (Eds.), *Carbohydrates in Food* (pp. 431-503). Marcel Dekker, Inc., New York, NY.

Englyst, H. (1989). Classification and measurement of plant polysaccharides. *Animal Feed Science and Technology*, 23, 27-42.

Englyst, H. N., Quigley, M. E., & Hudson, G. J. (1994). Determination of dietary fibre as non-starch polysaccharides with gas–liquid chromatographic, high-performance liquid chromatographic or spectrophotometric measurement of constituent sugars. *Analyst, 119*(7), 1497-1509.

Fairbairn, S. L., Patience, J. F., Classen, H. L., & Zijlstra, R. T. (1999). The energy content of barley fed to growing pigs: characterizing the nature of its variability and developing prediction equations for its estimation. *Journal of Animal Science, 77*(6), 1502-1512.

FAOSTAT. Food and Agriculture Organization of the United Nations. (2018). Retrieved from http://www.fao.org/faostat/en/#data/QC

Fayez, K. A., & 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 Sciences, 13*(1), 45-55.

Fedak, G., & Roche, I. D. L. (1977). Lipid and fatty acid composition of barley kernels. *Canadian Journal of Plant Science, 57*(1), 257-260.

Fengler, A. I., Aherne, F. X., & Robblee, A. R. (1990). Influence of germination of cereals on viscosity of their aqueous extracts and nutritive value. *Animal Feed Science and Technology, 28*(3-4), 243-253.

Fernandes, V. O., Costa, M., Ribeiro, T., Serrano, L., Cardoso, V., Santos, H., Lordelo, M., Ferreira L. M. A. and Fontes, C. M. G. A. (2016). 1,3-1,4-β-Glucanases and not 1,4-β-glucanases improve the nutritive value of barley-based diets for broilers. *Animal Feed Science and Technology, 211*, 153-163.

Flores, C., Williams, M., Pieniazek, J., Dersjant-Li, Y., Awati, A., & Lee, J. T. (2016). Direct-fed microbial and its combination with xylanase, amylase, and protease enzymes in comparison with AGPs on broiler growth performance and foot-pad lesion development. *Journal of Applied Poultry Research, 25*(3), 328-337.

Fox, G. P., Panozzo, J. F., Li, C. D., Lance, R. C. M., Inkerman, P. A., & Henry, R. J. (2003). Molecular basis of barley quality. *Australian Journal of Agricultural Research, 54*(12), 1081-1101.

Francesch, M., & Brufau, J. (2004). Nutritional factors affecting excreta/litter moisture and quality. *World's Poultry Science Journal, 60*(1), 64-75.

Friesen, O. D., Guenter, W., Marquardt, R. R., & Rotter, B. A. (1992). The effect of enzyme supplementation on the apparent metabolizable energy and nutrient digestibilities of wheat, barley, oats, and rye for the young broiler chick. P*oultry Science, 71*(10), 1710-1721.

Frølich, W. (1990). Chelating properties of dietary fiber and phytate. The role for mineral availability. In I. Furda & C. J. Brine (Eds.) *New developments in dietary fiber*. (pp. 83-93). Springer, Boston, MA.

Fry, R. E., Allred, J. B., Jensen, L. S., & McGinnis, J. (1958). Effect of pearling barley and of different supplements to diets containing barley on chick growth and feed efficiency. *Poultry Science, 37*(2), 281-288.

Fuente, J. M., De Ayala, P. P., Flores, A., & Villamide, M. J. (1998). Effect of storage time and dietary enzyme on the metabolizable energy and digesta viscosity of barley-based diets for poultry. *Poultry Science*, *77*(1), 90-97.

Fuente, J. M., Pérez, P., & Villamide, M. J. (1995). Effect of dietary enzyme on the metabolizable energy of diets with increasing levels of barley fed to broilers at different ages. *Animal Feed Science and Technology*, *56*(1-2), 45-53.

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

Gabriel, I., Mallet, S., & Leconte, M. (2003). Differences in the digestive tract characteristics of broiler chickens fed on complete pelleted diet or on whole wheat added to pelleted protein concentrate. *British Poultry Science, 44*(2), 283-290.

Gamlath, J., Aldred, G. P., & Panozzo. J. F. (2008). Barley (1→3; 1→4)-β-glucan and arabinoxylan content are related to kernel hardness and water uptake. *Journal of Cereal Science*, *47*(2), 365–371.

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

Gohl, B., Alden, S., Elwinger, K., & Thomke, S. (1978). Influence of β-glucanase on feeding value of barley for poultry and moisture content of excreta. *British Poultry Science, 19*(1), 41-47.

González-Ortiz, G., Kozłowski, K., Drażbo, A., & Bedford, M. R. (2017). Response of turkeys fed wheat-barley-rye based diets to xylanase supplementation. *Animal Feed Science and Technology*, *229*, 117-123.

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

Güler, M. (2003). Barley grain β-glucan content as affected by nitrogen and irrigation. *Field Crops Research*, 84(3), 335-340.

Helm, C. V., & Francisco, A. D. (2004). Chemical characterization of Brazilian hulless barley varieties, flour fractionation, and protein concentration. *Scientia Agricola, 61*(6), 593-597.

Hesselman, K., & Åman, P. (1986). The effect of β-glucanase on the utilization of starch and nitrogen by broiler chickens fed on barley of low-or high-viscosity. *Animal Feed Science and Technology, 15*(2), 83-93.

Hesselman, K., Elwinger, K., & Thomke, S. (1982). Influence of increasing levels of β-glucanase on the productive value of barley diets for broiler chickens. *Animal Feed Science and Technology, 7*(4), 351-358.

Hetland, H., Choct, M., & Svihus, B. (2004). Role of insoluble non-starch polysaccharides in poultry nutrition. *World's Poultry Science Journal, 60*(4), 415-422.

Hetland, H., Svihus, B., & 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*(2), 275-282.

Hetland, H., Svihus, B., & Olaisen, V. (2002). Effect of feeding whole cereals on performance, starch digestibility and duodenal particle size distribution in broiler chickens. *British Poultry Science, 43*(3), 416-423.

Hew, L. I., Ravindran, V., Mollah, Y., & Bryden, W. L. (1998). Influence of exogenous xylanase supplementation on apparent metabolisable energy and amino acid digestibility in wheat for broiler chickens. *Animal Feed Science and Technology, 75*(2), 83-92.

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

Holtekjølen, A. K., Uhlen, A. K., Bråthen, E., Sahlstrøm, S., & Knutsen, S. H. (2006). Contents of starch and non-starch polysaccharides in barley varieties of different origin. *Food Chemistry, 94*(3), 348-358.

Hughes, B. O. (1984). The principles underlying choice feeding behaviour in fowls-with special reference to production experiments. *Worlds Poultry Science Journal, 40*(20), 141-150.

Hughes, R. J., & 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*(5), 689-702.

Iji, P. A. (1999). The impact of cereal non-starch polysaccharides on intestinal development and function in broiler chickens. *World's Poultry Science Journal, 55*(4), 375-387.

Iji, P. A., Hughes, R. J., Choct, M., & Tivey, D. R. (2001). Intestinal structure and function of broiler chickens on wheat-based diets supplemented with a microbial enzyme. *Asian-Australasian Journal of Animal Sciences, 14*(1), 54-60.

Inborr, J., & Bedford, M. R. (1994). Stability of feed enzymes to steam pelleting during feed processing. *Animal Feed Science and Technology, 46*(3-4), 179-196.

Izydorczyk, M. S., & Dexter, J. E. (2008). Barley β-glucans and arabinoxylans: Molecular structure, physicochemical properties, and uses in food products–a Review. *Food Research International, 41*(9), 850-868.

Izydorczyk, M. S., Macri, L. J., & MacGregor, A. W. (1998). Structure and physicochemical properties of barley non-starch polysaccharides-I. Water-extractable β-glucans and arabinoxylans. *Carbohydrate Polymers, 35*(3-4), 249-258.

Izydorczyk, M. S., Storsley, J., Labossiere, D., MacGregor, A. W., & 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*(4), 982-989.

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

Jacob, J. P., & Pescatore, A. J. (2014). Barley β-glucan in poultry diets. *Annals of Translational Medicine*, *2*(2), 20.

Jacobs, C. M., Utterback, P. L., & Parsons, C. M. (2010). Effects of corn particle size on growth performance and nutrient utilization in young chicks. *Poultry Science*, *89*(3), 539-544.

Jamroz, D., Jakobsen, K., Knudsen, K. E. B., Wiliczkiewicz, A., & Orda, J. (2002). Digestibility and energy value of non-starch polysaccharides in young chickens, ducks and geese, fed diets containing high amounts of barley. *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiolo*gy, *131*(3), 657-668.

Jane, J. (2006). Current understanding on starch granule structures. *Journal of Applied Glycoscience*, *53*(3), 205-213.

Jang, D. A., Fadel, J. G., Klasing, K. C., Mireles Jr, A. J., Ernst, R. A., Young, K. A., Cook, A., & Raboy, V. (2003). Evaluation of low-phytate corn and barley on broiler chick performance. *Poultry Scienc*e, *82*(12), 1914-1924.

Jensen, L. S., Fry, R. E., Allred, J. B., & McGinnis, J. (1957). Improvement in the nutritional value of barley for chicks by enzyme supplementation. *Poultry Science*, *36*(4), 919-921.

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

Jensen, M. S., Knudsen, K. B., Inborr, J., & Jakobsen, K. (1998). Effect of β-glucanase supplementation on pancreatic enzyme activity and nutrient digestibility in piglets

fed diets based on hulled and hulless barley varieties. *Animal Feed Science and Technology, 72*(3-4), 329-345.

Jeroch, H., & Dänicke, S. (1995). Barley in poultry feeding: a review. *World's Poultry Science Journal, 51*(3), 271-291.

Józefiak, D., Rutkowski, A., Jensen, B. B., & Engberg, R. M. (2007). Effects of dietary inclusion of triticale, rye and wheat and xylanase supplementation on growth performance of broiler chickens and fermentation in the gastrointestinal tract. *Animal Feed Science and Technology*, *132*(1-2), 79-93.

Józefiak, D., Rutkowski, A., Jensen, B. B., & Engberg, R. M. (2006). The effect of β-glucanase supplementation of barley- and oat-based diets on growth performance and fermentation in broiler chicken gastrointestinal tract. *British Poultry Science, 47*(1), 57-64.

Józefiak, D., Rutkowski, A., Kaczmarek, S., Jensen, B. B., Engberg, R. M., & Højberg, O. (2010). Effect of β-glucanase and xylanase supplementation of barley-and rye-based diets on caecal microbiota of broiler chickens. *British Poultry Science, 51*(4), 546-557.

Juanpere, J., Perez-Vendrell, A. M., Angulo, E., & Brufau, J. (2005). Assessment of potential interactions between phytase and glycosidase enzyme supplementation on nutrient digestibility in broilers. *Poultry Science, 84*(4), 571-580.

Kalantar, M., Khajali, F., & Yaghobfar, A. (2016). Effect of cereal type and enzyme addition on performance, pancreatic enzyme activity, intestinal microflora and gut morphology of broilers. *Poultry Science Journal, 4*(1), 63-71.

Kaldhusdal, M., & Hofshagen, M. (1992). Barley inclusion and avoparcin supplementation in broiler diets. 2. Clinical, pathological, and bacteriological findings in a mild form of necrotic enteritis. *Poultry Science, 71*(7), 1145-1153.

Kasim, A. B., & Edwards Jr, H. M. (2000). Effect of sources of maize and maize particle sizes on the utilization of phytate phosphorus in broiler chicks. *Animal Feed Science and Technology, 86*(1-2), 15-26.

Khalil, M. M., Abdollahi, M. R., Zaefarian, F. and Ravindran, V. 2019. Influence of feed form on the metabolisable energy of ingredients for broilers. *Proceedings of the 21st Massey University technical update conference* (pp. 78-85). Monogastric Research Centre, Massey University: Palmerston North, New Zealand.

Kilburn, J., & Edwards Jr, 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*(4), 484-492.

Knudsen, K. E. B. (1997). Carbohydrate and lignin contents of plant materials used in animal feeding. *Animal Feed Science and Technology, 67*(4), 319-338.

Knudsen, K. E. B. (2014). Fiber and nonstarch polysaccharide content and variation in common crops used in broiler diets. *Poultry science, 93*(9), 2380-2393.

Kocher, A., Hughes, R. J., & Barr, A. R. (1997). β-Glucanase reduces but does not eliminate variation in AME of barley varieties. *Proceedings of the Australian Poultry Science Symposium* (pp. 142-145). Sydney, Australia.

Lamp, A. E., Evans, A. M., & Moritz, J. S. (2015). The effects of pelleting and glucanase supplementation in hulled barley based diets on feed manufacture, broiler performance, and digesta viscosity. *Journal of Applied Poultry Research, 24*(3), 295-303.

Lázaro, R., Garcia, M., Aranibar, M. J., & Mateos, G. G. (2003). Effect of enzyme addition to wheat-, barley- and rye-based diets on nutrient digestibility and performance of laying hens. *British Poultry Science, 44*(20), 256-265.

Lee, C. J., Horsley, R. D., Manthey, F. A., & Schwarz, P. B. (1997). Comparisons of β-glucan content of barley and oat. *Cereal Chemistry, 74*(5), 571-575.

Lemme, A., Ravindran, V., & Bryden, W. L. (2004). Ileal digestibility of amino acids in feed ingredients for broilers. *World's Poultry Science Journal, 60*(4), 423-438.

Lentle, R. G., Ravindran, V., Ravindran, G., & 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*(2), 135-142.

Li, J. H., Vasanthan, T., Hoover, R., & Rossnagel, B. G. (2004a). Starch from hull-less barley: V. In-vitro susceptibility of waxy, normal, and high-amylose starches towards hydrolysis by alpha-amylases and amyloglucosidase. *Food Chemistry, 84*(4), 621-632.

Li, J. H., Vasanthan, T., Rossnagel, B., & Hoover, R. (2001). Starch from hull-less barley: I. Granule morphology, composition and amylopectin structure. *Food Chemistry, 74*(4), 395-405.

Li, M., Lopato, S., Kovalchuk, N., & Langridge, P. (2013). Functional genomics of seed development in cereals. In: P. K. Gupta & R. K. Varshney (Eds.), *Cereal Genomics II* (pp. 215-245). Springer, Dordrecht.

Li, S., Sauer, W. C., Huang, S. X., & Gabert, V. M. (1996). Effect of β-glucanase supplementation to hulless barley-or wheat-soybean meal diets on the digestibilities of energy, protein, β-glucans, and amino acids in young pigs. *Journal of Animal Science, 74*(7), 1649-1656.

Li, W. F., Sun, J. Y., & Xu, Z. R. (2004b). Effects of NSP degrading enzyme on in vitro digestion of barley. *Asian-Australasian Journal of Animal Sciences, 17*(1), 122-126.

Linares, L. B., Broomhead, J. N., Guaiume, E. A., Ledoux, D. R., Veum, T. L., & Raboy, V. (2007). Effects of low phytate barley (*Hordeum vulgare L.*) on zinc utilization in young broiler chicks. *Poultry Science, 86*(2), 299-308.

Liu, S. Y., Truong, H. H., & Selle, P. H. (2015). Whole-grain feeding for chicken-meat production: possible mechanisms driving enhanced energy utilisation and feed conversion. *Animal Production Science, 55*(5), 559-572.

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*(2), C334-C342.

Loar, R. E II., Wamsley, K. G. S., Evans, A., Moritz, J. S., Corzo, A. (2014). Effects of varying conditioning temperature and mixer-added fat on feed manufacturing

efficiency, 28-to 42-day broiler performance, early skeletal effect, and true amino acid digestibility. *Journal of Applied Poultry Research, 23*(3), 444-455.

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

Marquardt, R. R., Boros, D., Guenter, W., & Crow, G. (1994). The nutritive value of barley, rye, wheat and corn for young chicks as affected by use of a *Trichoderma reesei* enzyme preparation. *Animal Feed Science and Technology, 45*(3-4), 363-378.

Martinez, V. M., Newman, R. K., & Newman, C. W. (1992). Barley diets with different fat sources have hypocholesterolemic effects in chicks. *The Journal of Nutrition, 122*(5), 1070-1076.

Mateos, G. G., Jiménez-Moreno, E., Serrano, M. P., & Lázaro, R. P. (2012). Poultry response to high levels of dietary fiber sources varying in physical and chemical characteristics. *Journal of Applied Poultry Research, 21*(1), 156-174.

Mathlouthi, N., Mallet, S., Saulnier, L., Quemener, B., & Larbier, M. (2002). Effects of xylanase and β-glucanase addition on performance, nutrient digestibility, and physico-chemical conditions in the small intestine contents and caecal microflora of broiler chickens fed a wheat and barley-based diet. *Animal Research, 51*(05), 395-406.

Mayne, R. K., Else, R. W., & Hocking, P. M. (2007). High litter moisture alone is sufficient to cause footpad dermatitis in growing turkeys. *British Poultry Science, 48*(5), 538-545.

Mccleary, B. V., Gibson, T. S., & Mugford, D. C. (1997). Measurement of total starch in cereal products by amyloglucosidase-α-amylase method: Collaborative study. *Journal of AOAC International, 80*(3), 571-579.

McIntosh, J. I., Slinger, S. J., Sibbald, I. R., & Ashton, G. C. (1962). Factors affecting the metabolizable energy content of poultry feeds: 7. the effects of grinding, pelleting and grit feeding on the availability of the energy of wheat, corn, oats and barley; 8. a study on the effects of dietary balance. *Poultry Science*, *41*(2), 445-456.

McNab, J. M., & Smithard, R. R. (1992). Barley β-glucan: An anti-nutritional factor in poultry feeding. *Nutrition Research Reviews, 5*(1), 45-60.

Messia, M. C., Candigliota, T., De Arcangelis, E., & Marconi, E. (2017). Arabinoxylans and β-glucans assessment in cereals. *Italian Journal of Food Science, 29*(1), 112-122.

Moharrery, A. (2006). Comparison of performance and digestibility characteristics of broilers fed diets containing treated hulled barley or hulless barley. *Czech Journal of Animal Science, 51*(3), 122-131.

Mollah, Y., Bryden, W. L., Wallis, I. R., Balnave, D., & Annison, E. F. (1983). Studies on low metabolisable energy wheats for poultry using conventional and rapid assay procedures and the effects of processing. *British Poultry Science, 24*(1), 81-89.

Morel, P. C., & Cottam, Y. H. (2007). Effects of particle size of barley on intestinal morphology, growth performance and nutrient digestibility in pigs. *Asian-Australasian Journal of Animal Sciences, 20*(11), 1738-1745.

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

Moss, B. R., El-Negoumy, A. M., & Newman, C. W. (1983). Nutritional value of waxy barleys for broiler chickens. *Animal Feed Science and Technology, 8*(1), 25-34.

Moughan, P. J., & Marlies Leenaars, G. S. (1992). Endogenous amino acid flow in the stomach and small intestine of the young growing pig. *Journal of the Science of Food and Agriculture, 60*(4), 437-442.

Mtei, A. W., Abdollahi, M. R., Schreurs, N. M., & Ravindran, V. (2019). Impact of corn particle size on nutrient digestibility varies depending on bird type. *Poultry Science, 98*(11), 5504-5513.

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

Nahas, J., & 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*(2), 195-202.

Nair, S., Knoblauch, M., Ullrich, S., & Baik, B. K. (2011). Microstructure of hard and soft kernels of barley. *Journal of Cereal Science*, *54*(3), 354-362.

National Research Council. (1994). *Nutrient requirements of poultry*. (Ninth revised ed.). National Academy Press, Washington D.C.

Nikkhah, A. (2012). Barley grain for ruminants: A global treasure or tragedy. *Journal of Animal Science and Biotechnology, 3*(1), 22.

Nir, I., & I. Ptichi. (2001). Feed particle size and hardness: Influence on performance, nutritional, behavioral and metabolic aspects. *Proceedings of the 1st World Feed Conference*, (pp. 157-186.). Utrecht, The Netherlands, Wageningen Press, Wageningen, The Netherlands.

Nir, I., Hillel, R., Ptichi, I., & Shefet, G. (1995). Effect of particle size on performance.: 3. Grinding pelleting interactions. *Poultry Science, 74*(5), 771-783.

Nir, I., Hillel, R., Shefet, G., & Nitsan, Z. (1994a). Effect of grain particle size on performance.: 2. grain texture interactions. *Poultry Science, 73*(6), 781-791.

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

O'Neill, H. M., Smith, J. A., & Bedford, M. R. (2014). Multicarbohydrase enzymes for non-ruminants. *Asian-Australasian Journal of Animal Sciences, 27*(2), 290.

Onderci, M., Sahin, N., Cikim, G., Aydin, A., Ozercan, I., Ozkose, E., Ekinci, S., Hayirli, A., & Sahin, K. (2008). β-Glucanase-producing bacterial culture improves

performance and nutrient utilization and alters gut morphology of broilers fed a barley-based diet. *Animal Feed Science and Technology, 146*(1-2), 87-97.

Oscarsson, M., Andersson, R., Åman, P., Olofsson, S., & 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(3), 359-366.

Oscarsson, M., Andersson, R., Salomonsson, A. C., & Åman, P. (1996). Chemical composition of barley samples focusing on dietary fibre components. *Journal of Cereal Science, 24*(2), 161-170.

Oscarsson, M., Parkkonen, T., Autio, K., & Åman, P. (1997). Composition and microstructure of waxy, normal and high amylose barley samples. *Journal of Cereal Science, 26*(2), 259-264.

Papadopoulos, M. C. (1989). Effect of processing on high-protein feedstuffs: a review. *Biological Wastes, 29*(2), 123-138.

Parsons, A. S., Buchanan, N. P., Blemings, K. P., Wilson, M. E., & Moritz, J. S. (2006). Effect of corn particle size and pellet texture on broiler performance in the growing phase. *Journal of Applied Poultry Research, 15*(2), 245-255.

Péron, A., Bastianelli, D., Oury, F. X., Gomez, J., & 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*(2), 223-230.

Perttilä, S., Valaja, J., & Jalava, T. (2005). Apparent ileal digestibility of amino acids and metabolisable energy value in grains for broilers. *Agricultural and Food Science, 14*, 325-334.

Perttilä, S., Valaja, J., Partanen, K., Jalava, T., Kiiskinen, T., & Palander, S. (2001). Effects of preservation method and β-glucanase supplementation on ileal amino acid digestibility and feeding value of barley for poultry. *British Poultry Science 42*(2), 218-229.

Petersen, S. T., Wiseman, J., & Bedford, M. R. (1999). Effects of age and diet on the viscosity of intestinal contents in broiler chicks. *British Poultry Science*, *40*(3), 364-370.

Petersen, V. E. (1969). A comparison of the feeding value for broilers of corn, grain sorghum, barley, wheat, and oats, and the influence of the various grains on the composition and taste of broiler meat. *Poultry Science, 48*(6), 2006-2013.

Pettersson, Å., & Lindberg, J. E. (1997). Ileal and total tract digestibility in pigs of naked and hulled barley with different starch composition. *Animal Feed Science and Technology, 66*(1-4), 97-109.

Pirgozliev, V. R., Rose, S. P., & Bedford, M. R. (2010). The effect of amylose: amylopectin ratio in dietary starch on growth performance and gut morphology in broiler chickens. *Archiv für Geflügelkunde, 74*(1), 21-29.

Pirgozliev, V. R., Rose, S. P., & Graybosch, R. A. (2002). Energy and amino acid availability to chickens of waxy wheat. *Archiv für Geflügelkunde, 66*(3), 108-113.

Raastad, N., & Skrede, A. (2003). Feed pelleting temperature influences growth performance of broiler chickens. *Proceedings of European Symposium on Poultry Nutrition* (pp. 115-116). Lillehammer, Norway.

Ravindran, V. (1995). Phytases in poultry nutrition. An overview. *Proceedings of the Australian Poultry Science Symposium* (pp. 135-139). Sydney, Australia.

Ravindran, V. (2013). Feed enzymes: The science, practice, and metabolic realities. *Journal of Applied Poultry Research, 22*(3), 628-636.

Ravindran, V., & Blair, R. (1991). Feed resources for poultry production in Asia and the Pacific region. I. Energy sources. *World's Poultry Science Journal*, 47(3), 213-231.

Ravindran, V., Abdollahi, M. R., & Bootwalla, S. M. (2014). Nutrient analysis, metabolizable energy, and digestible amino acids of soybean meals of different origins for broilers. *Poultry Science, 93*(10), 2567-2577.

Ravindran, V., Bryden, W. L., & Kornegay, E. T. (1995). Phytates: occurrence, bioavailability and implications in poultry nutrition. *Poultry and Avian Biology Reviews, 6*(2), 125-143.

Ravindran, V., Cabahug, S., Ravindran, G., Selle, P. H., & Bryden, W. L. (2000). Response of broiler chickens to microbial phytase supplementation as influenced by dietary phytic acid and non-phytate phosphorous levels. II. Effects on apparent metabolisable energy, nutrient digestibility and nutrient retention. *British Poultry Science, 41*(2), 193-200.

Ravindran, V., Cowieson, A. J., & Selle, P. H. (2008). Influence of dietary electrolyte balance and microbial phytase on growth performance, nutrient utilization, and excreta quality of broiler chickens. *Poultry Science*, *87*(4), 677-688.

Ravindran, V., Hew, L. I., Ravindran, G., & Bryden, W. L. (2005). Apparent ileal digestibility of amino acids in dietary ingredients for broiler chickens. *Animal Science, 81*(1), 85-97.

Ravindran, V., Selle, P. H., & Bryden, W. L. (1999). Effects of phytase supplementation, individually and in combination, with glycanase, on the nutritive value of wheat and barley. *Poultry Science, 78*(11), 1588-1595.

Ravindran, V., Thomas, D. V., Camden, B. J., & Voon, S. H. (2001). Apparent metabolisable energy content of new zealand wheats. *Proceedings of the Australian Poultry Science Symposium* (pp. 240). Sydney, Australia.

Ravindran, V., Tilman, Z. V., Morel, P. C. H., Ravindran, G., & 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*(1-2), 45-55.

Ravn, J. L., Martens, H. J., Pettersson, D., & Pedersen, N. R. (2016). A commercial GH 11 xylanase mediates xylan solubilisation and degradation in wheat, rye and barley as demonstrated by microscopy techniques and wet chemistry methods. *Animal Feed Science and Technology, 219*, 216-225.

Rebolé, A., Ortiz, L. T., Rodríguez, M., Alzueta, C., Treviño, J., & Velasco, S. (2010). Effects of inulin and enzyme complex, individually or in combination, on growth performance, intestinal microflora, cecal fermentation characteristics, and jejunal histomorphology in broiler chickens fed a wheat-and barley-based diet. *Poultry Science*, *89*(2), 276-286.

Reece, F. N., Lott, B. D., & Deaton, J. W. (1986a). Effects of environmental temperature and corn particle size on response of broilers to pelleted feed. *Poultry Science*, *65*(4), 636-641.

Reece, F. N., Lott, B. D., & Deaton, J. W. (1986b). The effects of hammer mill screen size on ground corn particle size, pellet durability, and broiler performance. *Poultry Science*, *65*(7), 1257-1261.

Riddell, C., & Kong, X. M. (1992). The influence of diet on necrotic enteritis in broiler chickens. *Avian Diseases*, *36*, 499-503.

Roberts, J. R., Ball, W., & Leary, A. (1998). Effects of different cereal grains on egg and egg shell quality in laying hens. *Proceedings of Australian Poultry Science Symposium* (pp. 199). Sydeny, Australia.

Rodehutscord, M., Rückert, C., Maurer, H. P., Schenkel, H., Schipprack, W., Bach Knudsen, K. E., Schollenberger, M., Laux, M., Eklund, M., Siegert, W., & Mosenthin, R. (2016). Variation in chemical composition and physical characteristics of cereal grains from different genotypes. *Archives of Animal Nutrition*, *70*(2), 87-107.

Rodrigues, I., & Choct, M. (2018). The foregut and its manipulation via feeding practices in the chicken. *Poultry Science*, *97*(9), 3188-3206.

Rodriguez, M. L., Rebole, A., Velasco, S., Ortiz, L. T., Trevino, J., & Alzueta, C. (2012). Wheat- and barley-based diets with or without additives influence broiler chicken performance, nutrient digestibility and intestinal microflora. *Journal of the Science of Food and Agriculture*, *92*(1), 184-190.

Rogel, A. M., Annison, E. F., Bryden, W. L., & Balnave. D. (1987b). The digestion of wheat starch in broiler chickens. *Australian Journal of Agricultural Research, 38*(3), 639-649.

Rogel, A. M., Balnave, D., Bryden, W. L., and Annison. E. F. (1987a). Improvement of raw potato starch digestion in chickens by feeding oat hulls and other fibrous feedstuffs. *Australian Journal of Agricultural Research, 38*(3), 629-637

Rose, R. J., & Arscott, G. H. (1962). Use of barley in high-efficiency broiler rations: 5. further studies on the use of enzymes, soaking and pelleting barley for chicks. *Poultry Scien*ce, *41*(1), 124-130.

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

Rotter, B. A., Friesen, O. D., Guenter, W., & Marquardt, R. R. (1990). influence of enzyme supplementation on the bioavailable energy of barley. *Poultry Science, 69*(7), 1174-1181.

Rynsburger, J. M. (2009). Physiological and Nutritional Factors Affecting Protein Digestion in Broiler Chickens. M.Sc. Thesis. University of Saskatchewan, Saskatoon, Canada.

Sacranie, A., Svihus, B., Denstadli, V., Moen, B., Iji, P. A., Choct, M. (2012). The effect of insoluble fiber and intermittent feeding on gizzard development, gut motility, and performance of broiler chickens. *Poultry Science, 91*(3), 693-700.

Salarmoini, M., Campbell, G. L., Rossnagel, B. G., & Raboy, V. (2008). Nutrient retention and growth performance of chicks given low-phytate conventional or hull-less barleys. *British Poultry Science, 49*(3), 321-328.

Salih, M. E., Classen, H. L., & Campbell, G. L. (1991). Response of chickens fed on hull-less barley to dietary β-glucanase at different ages. *Animal Feed Science and Technology, 33*(1-2), 139-149.

Samarasinghe, K., Messikommer, R., & Wenk, C. (2000). Activity of supplemental enzymes and their effect on nutrient utilization and growth performance of

growing chickens as affected by pelleting temperature. *Archives of Animal Nutrition, 53*(1), 45-58.

Saulnier, L., Peneau, N., & Thibault, J. F. (1995). Variability in grain extract viscosity and water-soluble arabinoxylan content in wheat. *Journal of Cereal Science, 22*(3), 259-264.

Scott, T. A., Silversides, F. G., Classen, H. L., Swift, M. L., & Bedford, M. R. (1998). Effect of cultivar and environment on the feeding value of Western Canadian wheat and barley samples with and without enzyme supplementation. *Canadian Journal of Animal Science, 78*(4), 649-656.

Selle, P. H., & Ravindran, V. (2007). Microbial phytase in poultry nutrition. *Animal Feed Science and Technology, 135*(1-2), 1-41.

Selle, P. H., Ravindran, V., Caldwell, A., & Bryden, W. L. (2000). Phytate and phytase: consequences for protein utilisation. *Nutrition Research Reviews, 13*(2), 255-278.

Selle, P. H., Ravindran, V., Ravindran, G., Pittolo, P. H., & Bryden, W. L. (2003b). Influence of phytase and xylanase supplementation on growth performance and nutrient utilisation of broilers offered wheat-based diets. *Asian-Australasian Journal of Animal Sciences, 16*(3), 394-402.

Selle, P. H., Walker, A. R., & Bryden, W. L. (2003a). Total and phytate-phosphorus contents and phytase activity of australian-sourced feed ingredients for pigs and poultry. *Australian Journal of Experimental Agriculture, 43*(5), 475-479.

Shaik, S. S., Carciofi, M., Martens, H. J., Hebelstrup, K. H., & Blennow, A. (2014). Starch bioengineering affects cereal grain germination and seedling establishment. *Journal of Experimental Botany, 65*(9), 2257-2270.

Shakouri, M. D., Iji, P. A., Mikkelsen, L. L., & Cowieson, A. J. (2009). Intestinal function and gut microflora of broiler chickens as influenced by cereal grains and microbial enzyme supplementation. *Journal of Animal Physiology and Animal Nutrition, 93*(5), 647-658.

Sharma, C. B., Goel, M., & Irshad, M. (1978). Myoinositol hexaphosphate as a potential inhibitor of α-amylases. *Phytochemistry, 17*(2), 201-204.

Shepherd, E. M., & Fairchild, B. D. (2010). Footpad dermatitis in poultry. *Poultry Science, 89*(10), 2043-2051.

Sheppy, C. (2001). The current feed enzyme market and likely trends. In: M. R. Bedford, & G. G. Partridge (Eds.), *Enzymes in farm animal nutrition* (1st ed., pp. 1-10). CABI Publishing, Oxon, UK.

Short, F. J., Gorton, P., Wiseman, J., & Boorman, K. N. (1996). Determination of titanium dioxide added as an inert marker in chicken digestibility studies. *Animal Feed Science and Technology, 59*(4), 215-221.

Short, F. J., Wiseman, J., & Boorman, K. N. (1999). Application of a method to determine ileal digestibility in broilers of amino acids in wheat. *Animal Feed Science and Technology, 79*(3), 195-209.

Silversides, F. G., & Bedford, M. R. (1999). Effect of pelleting temperature on the recovery and efficacy of a xylanase enzyme in wheat-based diets. *Poultry Science, 78*(8), 1184-1190.

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

Smits, C. H., & Annison, G. (1996). Non-starch plant polysaccharides in broiler nutrition–towards a physiologically valid approach to their determination. *World's Poultry science Journal, 52*(2), 203-221.

Smits, C. H., Veldman, A., Verstegen, M. W., & Beynen, A. C. (1997). Dietary carboxymethylcellulose with high instead of low viscosity reduces macronutrient digestion in broiler chickens. *The Journal of Nutrition, 127*(3), 483-487.

Smulikowska, S. (1998). Relationship between the stage of digestive tract development in chicks and the effect of viscosity reducing enzymes on fat digestion. *Journal of Animal Feed Sciences, 7*, 125-134.

Smulikowska, S., Mieczkowska, A., Nguyen, C. V., & Babelewska, M. (2002). The influence of digesta viscosity on the development of the stomach, on in vitro small intestinal motility, and on digestion of nutrients in broiler chickens. *Journal of Animal and Feed Sciences, 11*(4), 683-694.

Song, Y., & Jane, J. L. (2000). Characterization of barley starches of waxy, normal, and high amylose varieties. *Carbohydrate Polymers, 41*(4), 365-377.

Specian, R. D., & Oliver, M. G. (1991). Functional biology of intestinal goblet cells. *American Journal of Physiology*, *260*(2), C183-C193.

Steenfeldt, S. (2001). The dietary effect of different wheat cultivars for broiler chickens. *British Poultry Science*, *42*(5), 595-609.

Steenfeldt, S., Müllertz, A., & Jensen, J. F. (1998). Enzyme supplementation of wheat-based diets for broilers: 1. Effect on growth performance and intestinal viscosity. *Animal Feed Science and Technology*, *75*(1), 27-43.

Stein, H. H., Pedersen, C., Wirt, A. R., & Bohlke, R. A. (2005). Additivity of values for apparent and standardized ileal digestibility of amino acids in mixed diets fed to growing pigs. *Journal of Animal Science*, *83*(10), 2387-2395.

Storsley, J. M., Izydorczyk, M. S., You, S., Biliaderis, C. G., & Rossnagel, B. (2003). Structure and physicochemical properties of β-glucans and arabinoxylans isolated from hull-less barley. *Food Hydrocolloids, 17*(6), 831-844.

Svihus, B. (2001). Research note: a consistent low starch digestibility observed in pelleted broiler chicken diets containing high levels of different wheat varieties. *Animal Feed Science and Technology, 92*(1-2), 45-49.

Svihus, B. (2011a). The gizzard: function, influence of diet structure and effects on nutrient availability. *World's Poultry Science Journal, 67*(2), 207-224.

Svihus, B. (2011b). Limitations to wheat starch digestion in growing broiler chickens: a brief review. *Animal Production Science*, *51*(7), 583-589.

Svihus, B. (2014). Function of the digestive system. *Journal of Applied Poultry Research*, *23*(2), 306-314.

Svihus, B., & 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*(1-4), 71-92.

Svihus, B., & Hetland, H. (2001). Ileal starch digestibility in growing broiler chickens fed on a wheat-based diet is improved by mash feeding, dilution with cellulose or whole wheat inclusion. *British Poultry Science, 42*(5), 633-637.

Svihus, B., Choct, M., & Classen, H. L. (2013). Function and nutritional roles of the avian caeca: a review. *World's Poultry Science Journal, 69*(2), 249-264.

Svihus, B., Edvardsen, D. H., Bedford, M. R., & Gullord, M. (2000). Effect of methods of analysis and heat treatment on viscosity of wheat, barley and oats. *Animal Feed Science and Technology, 88*(1-2), 1-12.

Svihus, B., Herstad, O., Newman, C. W., & Newman, R. K. (1997a). Comparison of performance and intestinal characteristics of broiler chickens fed on diets containing whole, rolled or ground barley. *British Poultry Science, 38*(5), 524-529.

Svihus, B., K. H. Kløvstad, V. Perez, O. Zimonja, S. Sahlström, R. B. Schüller, W. K. Jeksrud, & E. Prestløkken. (2004). Physical and nutritional effects of pelleting of broiler chicken diets made from wheat ground to different coarsenesses by the use of roller mill and hammer mill. *Animal Feed Science and Technology, 117*(3-4), 281-293.

Svihus, B., Newman, R. K., & Newman, C. W. (1997b). Effect of soaking, germination, and enzyme treatment of whole barley on nutritional value and digestive tract parameters of broiler chickens. *British Poultry Science, 38*(4), 390-396.

Svihus, B., Uhlen, A. K., & 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*(3-4), 303-320.

Szczurek, W. (2009). Standardized ileal digestibility of amino acids from several cereal grains and protein-rich feedstuffs in broiler chickens at the age of 30 days. *Journal of Animal and Feed Sciences, 18*(4), 662-676.

Szczurek, W., Szymczyk, B., Arczewska-Włosek, A., & Świątkiewicz, S. (2020). Apparent and standardised ileal digestibility of amino acids in wheat, triticale and barley for broiler chickens at two different ages. *British Poultry Science*, *61*(1), 63-69.

Talati, R., Baker, W. L., Pabilonia, M. S., White, C. M., & Coleman, C. I. (2009). The effects of barley-derived soluble fiber on serum lipids. *The Annals of Family Medicine, 7*(2), 157-163.

Tang, D. F., Liu, X. X., Shi, X. G., & Aftab, U. (2017). Effect of cereal type and Xylanase supplementation on nutrient retention and growth performance of broilers. *Journal of Applied Poultry Research, 26*(4), 529-535.

Taylor, R. D., & Jones, G. P. D. (2004). The incorporation of whole grain into pelleted broiler chicken diets. II. Gastrointestinal and digesta characteristics. *British Poultry Science, 45*(2), 237-246.

Terzi, V., Tumino, G., Pagani, D., Rizza, F., Ghizzoni, R., Morcia, C., & Stanca, A. M. (2017). Barley developmental mutants: The high road to understand the cereal spike morphology. *Diversity*, *9*(2), 21.

Tester, R. F., Karkalas, J., & Qi, X. (2004). Starch-composition, fine structure and architecture. *Journal of Cereal Science*, *39*(2), 151-165.

Tester, R. F., Qi, X., & Karkalas, J. (2006). Hydrolysis of native starches with amylases. *Animal Feed Science and Technology*, *130* (1), 39-54.

Tester, R. F., South, J. B., Morrison, W. R., & 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*(2), 113-127.

Thomas, M., Van Vliet, T., & Van der Poel, A. F. B. (1998). Physical quality of pelleted animal feed 3. Contribution of feedstuff components. *Animal Feed Science and Technology*, *70*(1-2), 59-78.

Thompson, L. U. 1988. Anti-nutrients and blood glucose. Food Technology, *36*, 123-131.

Tricase, C., Amicarelli, V., Lamonaca, E., & Rana, R. L. (2018). Economic analysis of the barley market and related uses. *Grasses as Food and Feed*. IntechOpen.

Ullrich, S. E., Clancy, J. A., Eslick, R. F., & Lance, R. C. M. (1986). β-Glucan content and viscosity of extracts from waxy barley. *Journal of Cereal Science*, *4*(3), 279-285.

Villamide, M. J., Fuente, J. M., de Ayala, P. P., & Flores, A. (1997). Energy evaluation of eight barley cultivars for poultry: effect of dietary enzyme addition. *Poultry Science*, *76*(6), 834-840.

Viveros, A. A., Brenes, A., Pizarro, M., & Castano, M. (1994). Effect of enzyme supplementation of a diet based on barley, and autoclave treatment, on apparent digestibility, growth performance and gut morphology of broilers. *Animal Feed Science and Technology*, *48*(3-4), 237-251.

Vranjes, M. V., & Wenk, C. (1995). The influence of extruded vs. untreated barley in the feed, with and without dietary enzyme supplement on broiler performance. *Animal Feed Science and Technology*, *54*(1-4), 21-32.

Waldron, L. A. (1997). The nutritive value of different wheat varieties for broiler chickens. PhD. thesis, Harper Adams Agricultural College, Newport, UK.

Wang J. X., & Peng K. M. (2008). Developmental morphology of the small intestine of african ostrich chicks. *Poultry Science*, *87*(12), 2629-2635.

Wang, L., Newman, R. K., Newman, C. W., & Hofer, P. J. (1992). Barley β-glucans alter intestinal viscosity and reduce plasma cholesterol concentrations in chicks. *The Journal of Nutrition*, *122*(11), 2292-2297.

Welch, R. W. (1978). Genotypic variation in oil and protein in barley grain. *Journal of the Science of Food and Agriculture*, *29*(11), 953-958.

Weurding, R. E., Veldman, A., Veen, W. A., van der Aar, P. J., & Verstegen, M. W. (2001). Starch digestion rate in the small intestine of broiler chickens differs among feedstuffs. *The Journal of Nutrition*, *131*(9), 2329-2335.

White, W. B., Bird, H. R., Sunde, M. L., Prentice, N., Burger, W. C., & Marlett, J. A. (1981). The viscosity interaction of barley beta-glucan with Trichoderma viride cellulase in the chick intestine. *Poultry Science*, *60*(5), 1043-1048.

Wu, Y. B., Ravindran, V., & Hendriks, W. H. (2004a). Influence of exogenous enzyme supplementation on energy utilisation and nutrient digestibility of cereals for broilers. *Journal of the Science of Food and Agriculture, 84*(14), 1817-1822.

Wu, Y. B., Ravindran, V., Thomas, D. G., Birtles, M. J., & Hendriks, W. H. (2004b). Influence of phytase and xylanase, individually or in combination, on performance, apparent metabolisable energy, digestive tract measurements and gut morphology in broilers fed wheat-based diets containing adequate level of phosphorus. *British Poultry Science, 45*(1), 76-84.

Xue, P. C., Ragland, D., & Adeola, O. (2014). Determination of additivity of apparent and standardized ileal digestibility of amino acids in diets containing multiple protein sources fed to growing pigs. *Journal of Animal Science*, *92*(9), 3937-3944.

Xue, Q., Wang, L., Newman, R. K., Newman, C. W., & Graham, H. (1997). Influence of the hulless, waxy starch and short-awn genes on the composition of barleys. *Journal of Cereal Science*, *26*(2), 251-257.

Yaghobfar, A., & Kalantar, M. (2017). Effect of non-starch polysaccharide (NSP) of wheat and barley supplemented with exogenous enzyme blend on growth performance, gut microbial, pancreatic enzyme activities, expression of glucose transporter (SGLT1) and mucin producer (MUC2) genes of broiler chickens. *Brazilian Journal of Poultry Science, 19*(4), 629-638.

Yasar, S., & Forbes, J. M. (1999). Performance and gastro-intestinal response of broiler chickens fed on cereal grain-based foods soaked in water. *British Poultry Science, 40*(1), 65-76.

Yegani, M., & Korver, D. R. (2008). Factors affecting intestinal health in poultry. *Poultry Science*, 87(10), 2052-2063.

Yin, Y. L., Baidoo, S. K., Jin, L. Z., Liu, Y. G., Schulze, H., Simmins, P. H. (2001). The effect of different carbohydrase and protease supplementation on apparent (ileal

and overall) digestibility of nutrients of five hull-less barley varieties in young pigs. *Livestock Production Science, 71*(2-3), 109-120.

Yu, B., Hsu, J. C., & Chiou, P. W. S. (1998). Effects of β-glucanase supplementation of barley diets on growth performance of broilers. *Animal Feed Science and Technology, 70*(4), 353-361.

Yu, B., Sun, Y. M., & Chiou, P. W. S. (2002). Effects of glucanase inclusion in a de-hulled barley diet on the growth performance and nutrient digestion of broiler chickens. *Animal Feed Science and Technology, 102*(1-4), 35-52.

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

Zaefarian, F., Abdollahi, M. R., & Ravindran, V. (2016). Particle size and feed form in broiler diets: impact on gastrointestinal tract development and gut health. *World's Poultry Science Journal, 72*(2), 277-290.

Zheng, G. H., Fasina, O., Sosulski, F. W., & Tyler, R. T. (1998). Nitrogen solubility of cereals and legumes subjected to micronization. *Journal of Agricultural and Food Chemistry, 46(*10), 4150-4157.

Zijlstra, R. T., Lange, C. D., & Patience, J. F. (1999). Nutritional value of wheat for growing pigs: chemical composition and digestible energy content. *Canadian Journal of Animal Science, 79*(2), 187-194.

APPENDIX

Statement of contributions to doctoral thesis containing publications

Statement of contributions to doctoral thesis containing publications from Chapters 3, 4, 5 and 6 are attached.

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STATEMENT OF CONTRIBUTION
DOCTORATE WITH PUBLICATIONS/MANUSCRIPTS

We, the candidate and the candidate's Primary Supervisor, certify that all co-authors have consented to their work being included in the thesis and they have accepted the candidate's contribution as indicated below in the *Statement of Originality*.

Name of candidate:	Wellawattage Nipuna Udayani Perera
Name/title of Primary Supervisor:	Dr. M. Reza Abdollahi
Name of Research Output and full reference:	
Perera, W.N.U., Abdollahi, M.R., Ravindran, V., Zaefarian, F., Weaver, T.J., Ravindran, G., 2019. Nutritional evaluation of two barley cultivars, without and with CT supplementation, for broilers: Metabolisable energy and standardised amino acid digestib	
In which Chapter is the Manuscript /Published work:	Chapter 3
Please indicate:	
• The percentage of the manuscript/Published Work that was contributed by the candidate:	65%
and	
• Describe the contribution that the candidate has made to the Manuscript/Published Work:	
Candidate contributed to experimental design, field work, data collection and analyses, lab analysis, and manuscript writing	
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This work was published in British Poultry Science, 2019	
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Name of Research Output and full reference:	
Perera, W.N.U., Abdollahi, M.R., Ravindran, V., Zaefarian, F., Wester, T.J., Ravindran, G. (2019). Influence of inclusion level of barley in wheat based diets and supplementation of xylanase on growth performance, nutrient utilisation and gut morph	
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and	
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Candidate contributed to experimental design, field work, data collection and analyses, lab analysis, and manuscript writing	
For manuscripts intended for publication please indicate target journal:	
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W. N. U. Perera, M. R. Abdollahi, F. Zaefarian, T. J. Wester & V. Ravindran (2020) The effect of graded inclusions of waxy starch, hull-less barley and a multi-component exogenous carbohydrase on the growth performance, nutrient digestibility and intes	
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• The percentage of the manuscript/Published Work that was contributed by the candidate:	70%
and	
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Perera, W.N.U., Abdollahi, M.R., Zaefarian, F., Wester, T.J., Ravindran, V. (2020). The interactive influence of barley particle size and enzyme supplementation on growth performance, nutrient utilisation and intestinal morphometry of broiler starters. Po	
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