IN VITRO AND *IN VIVO* ASSESSMENT OF RATE AND EXTENT OF STARCH DIGESTIBILITY IN WESTERN CANADIAN WHEAT MARKET CLASSES AND CULTIVARS IN BROILER CHICKENS

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Canada.

By

Namalika D. Karunaratne

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ABSTRACT

Two experiments were conducted to investigate the effect of Western Canadian wheat market classes and cultivars on rate and extent of starch digestibility in broiler chickens. The first experiment was an *in vitro* trial to determine starch digestibility rate and extent by mimicking chicken gastric and small intestinal (SI) phases. The study evaluated 18 spring wheat cultivars from eight Western Canadian wheat market classes. Each cultivar was replicated four times by growing them at four separate plots in a field nursery at Saskatoon, SK. Grain characteristics of these wheat cultivars were analyzed to determine the relationship with starch digestibility. The second experiment was designed to investigate genotypic variability of starch digestibility rate and extent, and also AME_n using broiler chickens. A total of 468 1-d-old male broiler chickens were randomly assigned to dietary treatments (6 cages/treatment, 6 birds/cage) from 0 to 21 day of age. The study evaluated two wheat cultivars from each of six Western Canadian wheat classes (selected according to the results of Experiment 1). Wheat cultivars were also subjected to *in vitro* starch digestion and grain characteristic analysis. Experiments 1 and 2 were completely randomized and randomized complete block designs, respectively. Wheat cultivars were nested within wheat market classes in both experiments. Differences were considered significant when $P \leq 0.05$. Pearson correlation was used to determine correlations.

In vitro starch digestibility was affected by wheat market class and cultivar nested within class according to results of Experiment 1. Starch digestibility ranges of wheat classes for the selected SI phase times are as follows: 15 min – 33.1 to 49.1%, 60 min – 80.2 to 93.3% and 120 min – 92.4 to 97.6%. Low to moderate positive correlations were found for starch digestibility

rate and extent with CP, ash, NSP and large granule size distribution, whereas negative correlations were found with total starch (TS), and small and medium granule proportions.

According to Experiment 2 results, the starch digestibility ranges were: proximal jejunum – 23.7 to 50.6%; distal jejunum – 63.5 to 76.4%; proximal ileum – 88.7 to 96.9%; distal ileum – 94.4 to 98.5%; excreta – 98.4 to 99.3%. Wheat class affected wheat AME_n with levels ranging from 3203 to 3411 kcal/kg at 90% DM. *In vivo* starch digestibility in all four segments of SI and total tract starch digestibility were affected by wheat market class. Moderate positive correlations were found for *in vitro* starch digestibility with CP and large granule size distribution, whereas it was negative with TS, and small and medium granule proportions. There were moderate positive correlations for *in vivo* starch digestibility with wheat hardness and ash content. Significant and moderate strong positive correlations were observed between *in vitro* and *in vivo* starch digestion rate, but no correlations were found between AME_n and starch digestion rate.

In conclusion, rate and extent of both *in vitro* and *in vivo* starch digestibility and AME_n were affected by Western Canadian wheat class, but starch digestibility did not predict wheat AME_n. The *in vitro* starch digestion model may have application in screening large numbers of samples for starch digestibility in poultry. Further, grain characteristics were related to differences in the rate and extent of starch digestion of Western Canadian wheat classes and cultivars, but to a limited degree.

Key words: wheat, AME, starch fermentation, nutrient retention, metabolism, poultry

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DEDICATION

The thesis is dedicated to my beloved parents who always supported and encouraged me to believe in myself

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LIST OF ABBREVIATIONS

AME	Apparent metabolizable energy
AME_n	Apparent metabolizable energy with nitrogen correction
ANF	Anti-nutritional factors
AX	Arabinoxylans
BW	Body weight
BWG	Body weight gain
CP	Crude protein
CPS	Canadian Prairie Spring
CWAD	Canadian Western Amber Durum
CWES	Canadian Western Extra Strong
CWGP	Canadian Western General Purpose
CWHWS	Canadian Western Hard White Spring
CWRS	Canadian Western Red Spring
CWRW	Canadian Western Red Winter
CWSWS	Canadian Western Soft White Spring
d	Day
DP	Degree of polymerization
DI	Distal ileum
DJ	Distal jejunum
DM	Dry matter
FFA	Free fatty acids
FI	Feed intake
GE	Gross energy

GI	Gastro Intestinal
HI	Hardness Index
IAX	Insoluble arabinoxylans
INSP	Insoluble non-starch polysaccharides
LPL	Lysophospholipids
Min	Minutes
N	Nitrogen
NSP	Non-starch polysaccharides
PI	Proximal ileum
PJ	Proximal jejunum
RDS	Rapidly digested starch
RS	Resistant starch
SAX	Soluble arabinoxylans
SCFA	Short chain fatty acids
SDI	Starch digestion index
SDS	Slowly digested starch
SI	Small intestine
SNSP	Soluble non-starch polysaccharides
TAX	Total arabinoxylans
TDF	Total dietary fibre
TiO_2	Titanium oxide
TNSP	Total non-starch polysaccharides
TS	Total starch

1. INTRODUCTION

Poultry production is an ever expanding industry in Western Canada due to increased consumer demand for meat and eggs. Feed is a major expense for poultry producers, and energy in feed alone accounts for 40% of the cost of producing poultry meat and eggs (Sibbald, 1982). Cereal grains are the major source of energy and make up the largest proportion of poultry diets. In Western Canada, wheat is the most common cereal grain used in poultry diets (Scott et al., 1998a).

A variety of wheat classes are grown in Western Canada to meet specific functional requirements, primarily for human food. The main wheat market classes that are grown in Western Canada are Canadian Prairie Spring (CPS), Canadian Western Amber Durum (CWAD), Canadian Western Extra Strong (CWES), Canadian Western General Purpose (CWGP), Canadian Western Hard White Spring (CWHWS), Canadian Western Red Spring (CWRS), Canadian Western Red Winter (CWRW) and Canadian Western Soft White Spring (CWSWS) (Canadian Grain Commission, 2015). Within wheat classes there can be large number of wheat cultivars, and most newly developed wheat cultivars have not been assessed for their nutrient profile, starch digestibility and energy values. The presence of many wheat classes and cultivars increases variability in these parameters and makes formulation of poultry diets less precise.

Starch digestibility has been shown in the past to vary considerably from sample to sample, and in turn to impact grain apparent metabolizable energy (AME) (Mollah et al., 1983; Rogel et al., 1987; Gutierrez del Alamo et al., 2008; Gutierrez del Alamo et al., 2009a,b; Yegani et al., 2013). In addition to overall starch digestibility (distal ileum or excreta), the rate of starch digestibility in the small intestine has also been suggested to impact poultry productivity

(Weurding et al., 2001b). The rate of starch digestibility in wheat has not been extensively investigated and only been examined using *in vitro* techniques in relationship to human nutrition (Ahuja et al., 2013) for Western Canadian wheat classes in terms of selected genotypes. However, establishment of a reliable *in vitro* starch digestibility technique is important for assessment of an adequate numbers of samples per treatment because of reduced cost and time in comparison to *in vivo* experiments (Weurding et al., 2001b). Englyst et al. (1992) established an *in vitro* technique that is widely used to determine starch digestibility in humans, but this may not be representative of the avian digestive tract. Ebsim (2013) modified the Englyst et al. (1992) technique to more closely approximate the digestive tract of chickens, but additional research is required to determine the relationship between *in vitro* and *in vivo* starch digestibility in order to validate *in vitro* starch digestibility results, and to confirm the reliability and accuracy of the *in vitro* model.

A feed ingredient is mainly evaluated for feed formulation based on its nutrient profile, digestibility of main nutrients and, AME_n. Therefore, it is relevant to determine the rate and extent of starch digestibility as well as the AME_n of different Western Canadian wheat classes/cultivars in chickens. Previous research on the AME_n of Western Canadian wheat has been inconsistent (Scott et al., 1998b; Yegani et al., 2013), that is in part due to a lack of adequate replication of wheat samples within a class or cultivar (Yegani et al., 2013).

The differences in digestible energy that ultimately contribute to AME_n are due to variation in grain content of total starch (TS), crude protein (CP), fat content and presence of anti-nutritional factors (ANF) in wheat cultivars (Scott et al., 1998a). Variation in the rate and extent of starch digestibility among wheat cultivars may be influenced by starch chemistry (e.g.

amylose content, amylopectin chain length distribution), crystallinity, starch granule size distribution, grain particle size, processing method and the association of starch with other compounds such as lipid, protein, fibre, minerals and ANF (Regmi et al., 2011). Different wheat cultivars may have distinct characteristics that lead to variable starch digestibility and eventually variable wheat AME_n values. Therefore, investigation of grain characteristics may help define the causes for variable starch digestibility in wheat cultivars.

In Experiment 1, it was hypothesized that wheat market class/cultivar will affect *in vitro* starch digestibility, and that grain characteristics would account for differences in the starch digestibility of wheat market classes/cultivars. Based on the results of Experiment 1, two cultivars within each of six wheat classes were chosen to examine *in vitro* and *in vivo* digestibility, AME_n as well as grain characteristics for Experiment 2. It was hypothesized that wheat market class/cultivar affects *in vitro* and *in vivo* starch digestibility in broiler chickens, and that these values are correlated and relate to grain AME_n and relevant grain characteristics. Overall, the objective of this thesis is to examine the impact of Western Canadian wheat class/cultivar on the nutritional value of wheat with specific emphasis on starch digestibility rate and extent, and also to investigate the grain characteristics that affect on starch digestibility.

2. LITERATURE REVIEW

2.1. Starch

Starch is the primarily digestible carbohydrate in plants and the main energy source for animals (Bednar et al., 2001). It is the main component in poultry diets, usually comprising ~40% of the diet and more than half of metabolizable energy intake (Svihus, 2011). Starch granules are embedded in a hydrophobic network and then covered by a cell wall to protect them from water. Pure starch is composed of α-glucan molecules and characterized as one of two types of molecules, amylose and amylopectin. It is distinguished from other glucans as it contains α-linked glucose whereas other glucans contain β-linked glucose (Bach Knudsen et al., 2006).

2.1.1. Chemical structure of starch

Starch is composed of α-D-glucopyranosyl units contained within amylose and amylopectin fractions. Amylose is a linear molecule with α-(1-4) links (0.99) and molecular weights around 1×10^5-1×10^6. Amylose is not branched, and has less surface area and more intra-molecular hydrogen bonds than amylopectin. Furthermore amylose forms complexes with surface components like fatty acids (Regmi et al., 2011). Amylopectin is larger than amylose, is heavily branched (0.95 α 1-4 linkages & 0.05 α 1-6 linkages) and molecular weights range from 1×10^7 to 1×10^9 (Buleon et al., 1998; Bach Knudsen et al., 2006).

Starch granules are 1-100 μm in diameter and are located within membrane bound organelles like chloroplasts in photosynthetic portions of plants and amyloplasts in storage organs (Stoddard et al., 2004). Starch is formed from D-glucose, which has a hexagonal pyranose ring

conformation (Figure 2.1). Highly reactive C1 of a D-glucose is attached to C4 of another D-glucose monomer to form polymers (Figure 2.2). The helical structure of starch is stabilized by hydrogen bonds located between glucose molecules (Figure 2.3). Starch normally contains 20 to 33% amylose, with the remainder amylopectin.

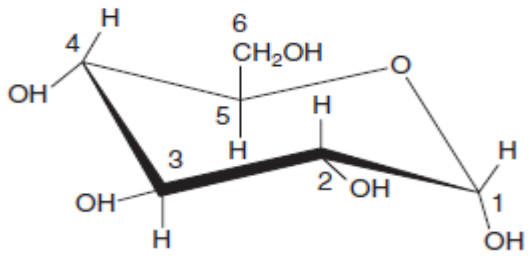

Figure 2.1. D-glucopyranose molecule (Stoddard, 2004).

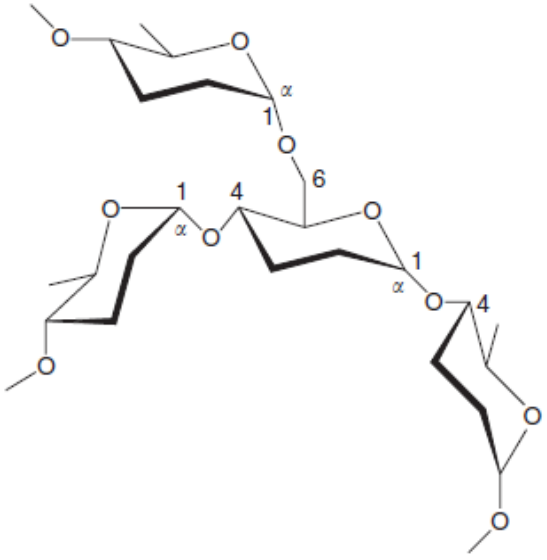

Figure 2.2. Glucose polymer with α-(1-4) and α-(1-6) linkages (Stoddard, 2004).

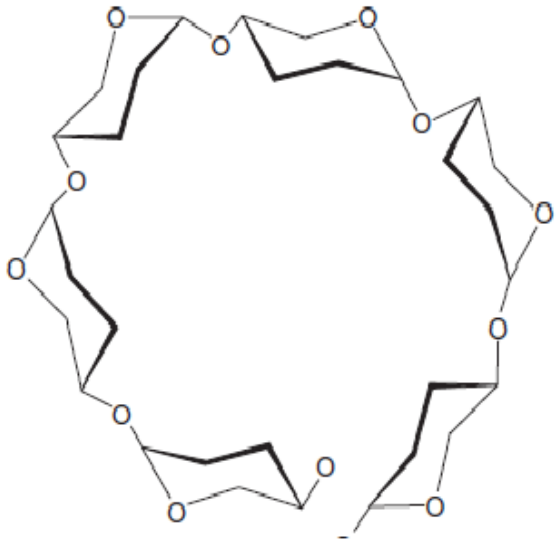

Figure 2.3. Helix of α-(1-4) glucose residues (Stoddard, 2004).

2.1.1.1. Amylose

A review by Tester et al. (2004) mentioned amylose is a linear polymer (Figure 2.4) and has a molecular weight of about 100 kDa. The degree of polymerization ranges from 324 to 4,920. The amylose molecule contains 9-20 branch points and 3-11 chains per molecule (Tester et al., 2004). The rate and extent of amylose digestion is low compared to amylopectin due to less access by α-amylase enzyme (Regmi et al., 2011).

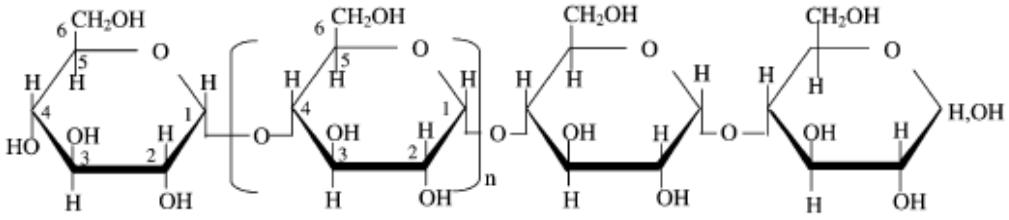

Figure 2.4. Structure of amylose (Tester et al., 2004).

2.1.1.2. Amylopectin

Amylopectin is a heavily branched polymer and has a molecular weight of about 10^4-10^6 kDa (Tester et al., 2004; Figure 2.5). The degree of polymerization of amylopectin is around 9,600-15,900. Amylopectin chains can be classified according to their chain lengths and position. Type A and B_1 amylopectin chains are located more externally and form double helices within starch granules. The chain lengths of A type and B_1 are 12-16 and 20-24, respectively. Further, chain lengths of B2, B3 and B4 are 42-48, 69-75 and 101-119, respectively. The A type chains of amylopectin link to B chains by α-(1-6) bonds and then this combination links to other B chains or single C chains in the backbone of the amylopectin molecule. Amylopectin molecules from high amylose starches contain a higher proportion of very long amylopectin chains (Tester et al., 2004).

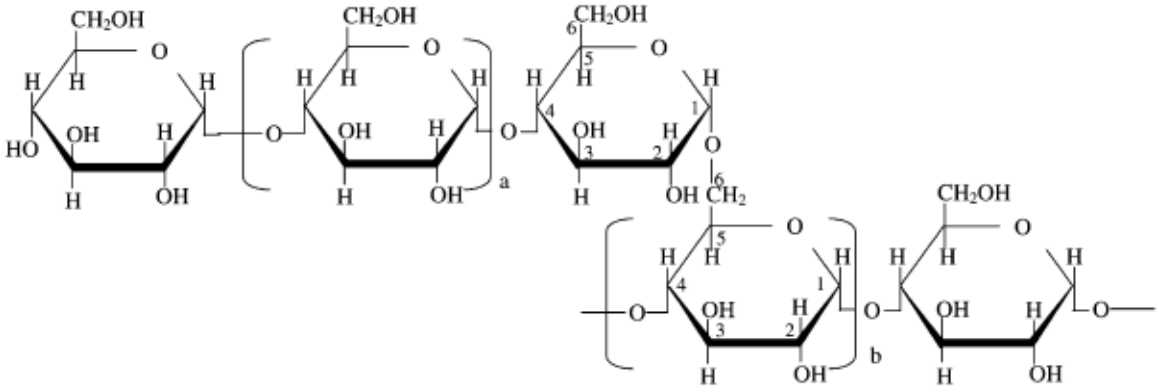

Figure 2.5. Structure of amylopectin (Tester et al., 2004).

2.1.1.3. Amylose to amylopectin ratio

Most cereal starches contain 72-82% amylopectin and 18-33% amylose (Buleon et al., 1998). Starch is divided into three types according to amylose to amylopectin ratio namely, normal, waxy and high amylose (amylo) starch. The amylose to amylopectin ratio of normal starch is 0.16-0.35 and for waxy starch the ratio is less than 0.15. The amylose: amylopectin ratio in amylo starch is greater than 0.36 (Bach Knudsen et al., 2006).

2.1.2. Physical structure of starch

Starch is a semi-crystalline material and is synthesized in plant tissues as spherical granules composed of both crystalline and amorphous material (Figure 2.6). Amylopectin forms the branched crystalline pattern consisting of double helices of α-(1-4) linear molecules. Amylose and amylopectin together form the amorphous lamellar consisting of α-(1-6) branched region (Bach Knudsen et al., 2006).

Starch granules have a layered organization with altering amorphous and semi-crystalline radial growth of 120-400 nm thickness rings. These semi-crystalline rings have a lamellar structure with alternating crystalline and amorphous regions, allowing 9-11 nm distance in between two rings (Figure 2.7). Amorphous regions contain both amylose and amylopectin in a disorganized conformation. Crystalline regions of lamellar are formed by double helices of amylopectin side chains, locating laterally to a crystalline lattice (Blazek et al., 2009).

Normally less than half of amylose is branched and one amylose molecule contains less than 20 branch points. However amylopectin contains one branch point per 20 glucose units (Stoddard, 2004).

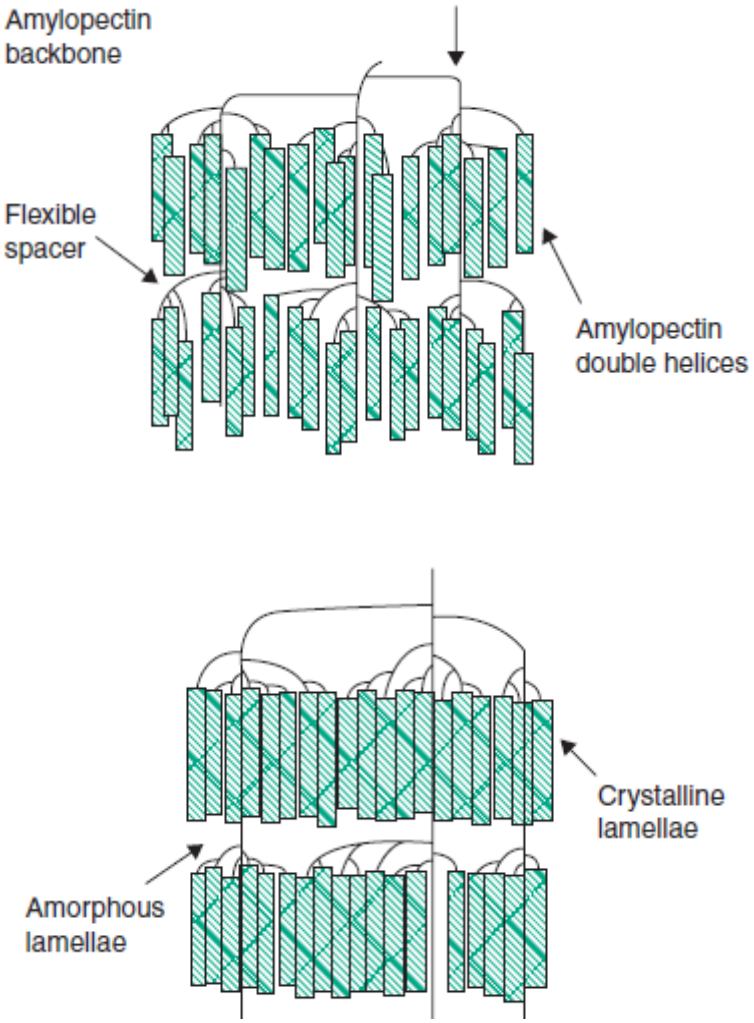

Figure 2.6. Amylopectin double helices with amorphous and crystalline regions (Stoddard, 2004).

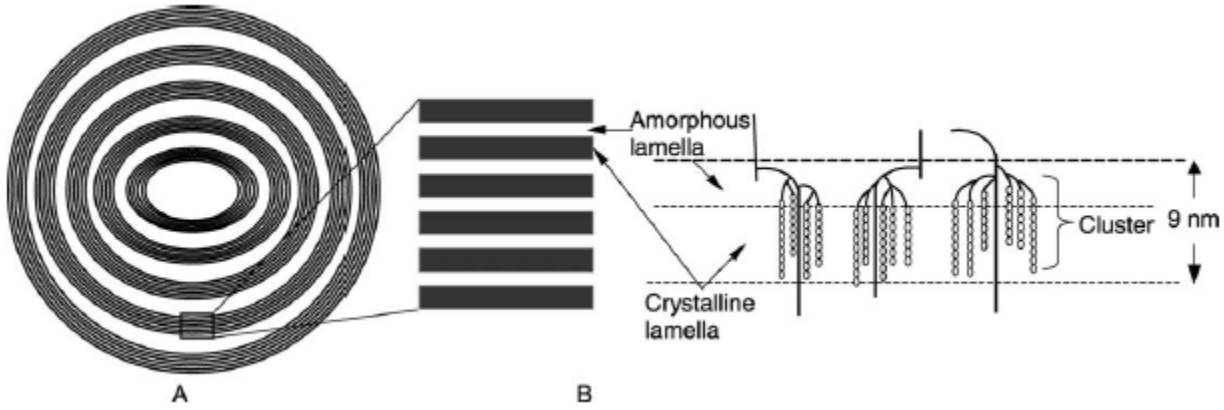

Figure 2.7. Lamellar structure of starch granule. (A) Microsatellite lamellae separated by amorphous rings. (B) Amorphous and crystalline regions- magnified (Tester et al., 2004).

Starch is present as granules in the endosperm of cereal grains (Buleon et al., 1998). Starch granule size distribution is affected by genetic (cultivars) (Peterson and Fulcher, 2001) and environmental factors including temperature at grain filling (Tester et al., 1995). Starch granule size in cereals generally ranges from 1 to 50 μm. There is a bimodal distribution of starch granule size particularly in wheat, rye and barley. In wheat, there are two types of starch granules. Large granule size ranges from 15 to 40 μm, whereas small granule size ranges from 1 to 10 μm (Salman et al., 2009). However, Raeker et al. (1998) reported a trimodal distribution of wheat starch granules, and the distribution ranges were < 2.8, 2.8-9.9 and > 9.9 μm for small, medium and large starch granules, respectively. The timing of large type A and small type B granule growth is different during grain-filling. Granule type A synthesis starts four days after anthesis and the further development occurs during the next 20 days. Synthesis of type B granules starts 10 days after anthesis and granule growth and development occurs during 20 days after anthesis (Salman et al., 2009).

2.1.3. Minor components of Starch

According to Buleon et al. (1998) review, minor components of starch can be divided into three parts called particulate material, surface components and internal components. Particulate material is mainly composed of cell wall fragments. Surface components are removable by extraction procedures. Protein, amino acids, nucleic acids and lipid are examples of surface components. Polypeptides, mainly puroindoline polypeptides, make up the protein part of surface components and, triglycerides, glycolipids and phospholipids are found in the lipid fraction of surface components. Internal components consist mainly of lipid, protein and minerals.

2.1.3.1. Lipid

Lipid is the most common minor component of starch. Approximately 1-14 g of lipid is present in 1 kg of cereal starch (Abdel-Aal et al., 2002). Lipid is mostly associated with amylose in starch granules and is present on the surface and inside of starch granules (Buleon et al., 1998). Amylose-lipid complexes reduce the availability of starch granules to digestive enzymes, and reduce starch digestibility (Vasanthan and Bhatty, 1996). The presence of internal lipid is a characteristic of cereals. Cereal starch is characterized by presence of monoacyl lipids like free fatty acids (FFA) and lysophospholipids (LPL), and these two components are positively related to amylose content. Wheat, barley, rye and triticale contain mostly LPL whereas other cereals contain mainly FFA and a low content of LPL. Lysophosphatidylcholine is the major lipid associated with wheat starch, with palmitic acid and linolenic acid as primary fatty acids (Buleon et al., 1998).

2.1.3.2. Protein

Starch granules generally contain 3 g or less protein/kg (Abdel-Aal et al., 2002). In general, proteins are associated with starch, but sometimes are associated with lipids on the granule surface. The size of surface proteins ranges from 5 to 60 kDa, whereas proteins present inside the starch granule are larger in size and range from 60 to 150 kDa (Baldwin, 2001).

2.1.3.3. Minerals

Starch contains less than 0.4% minerals, consisting mainly of calcium, magnesium, phosphorous, potassium and sodium. Phosphate monoesters, phospholipids and inorganic phosphate are the main sources of phosphorous in starch. Phosphate monoesters are bound to amylopectin regions of starch (Tester et al., 2004).

2.1.4. Classification of starch

In the literature, various classifications of starch are depicted. Englyst et al. (1999) described two types of dietary starch according to starch digestibility and rate of glucose release for absorption into the blood stream in humans. They are namely, rapidly available glucose and slowly available glucose. Rapidly available glucose increases blood glucose level within the first 20 minutes of digestion whereas slowly available glucose increase blood glucose level from 20 minutes to 2 hours of digestion. Slowly available glucose is beneficial as it results in a moderate level of post-prandial glycemic and insulinemic responses (Englyst et al., 1999). Englyst et al. (1992) classified starch (considering human nutrition purposes) into three categories called rapidly digestible starch (RDS), slowly digestible starch (SDS) and resistant starch (RS). Rapidly digestible starch and SDS are digested in the small intestine, but RS is not and, therefore enters

the large intestine. Resistant starch can be classified further into three sub types called RS1, RS2 and RS3. Physically inaccessible starch that is trapped in plant cells and food is defined as RS1, while RS2 is defined as starch granules which are hydrolyzed slowly. Lastly, RS3 is defined as retrograded starch that is produced during starch processing. Sajilata et al. (2006) also classified starch into RDS, SDS and RS. Resistant starch was further divided into four subtypes namely, RS1, RS2, RS3 and RS4. The RS1 type refers to starch which are not digestible as they are not physically accessible to amylase enzyme. Partly milled grains and some very dense processed starch foods are examples of the RS1 type. The starch granules that are present as a compact granular form and resist amylase hydrolysis are called RS2, and this type includes mostly raw starch. Retrograded starch accounts for the highest proportion of RS, and falls into the RS3 category. Starch which contains a high level of amylose is more susceptible to retrogradation and, therefore can form a high amount of type RS3 starch after processing. Physically or chemically modified starch, including esterified and cross-linked starches, is categorized as the RS4 type.

2.2. Starch digestion

Bach Knudsen et al. (2006) described the starch digestion procedure in non-ruminants and, it is presented in Figure 2.8. Initially, pancreatic α-amylase digests α-(1-4) glycosidic linkages in starch. Chickens do not secrete salivary amylase, however some starch digestion occurs in the crop as a result of microbial activity (Bolton, 1965). Therefore, the majority of starch is degraded by α-amylase in the SI. Pancreatic secretion causes dilution of feed particles and facilitates α-amylase containing polar solution penetration into feed particles. Starch is digested into oligosaccharides such as maltose, maltotriose and α-limit dextrin by α-amylase.

These oligosaccharides are further degraded into glucose by oligosaccharidases which are present on the SI brush border surface such as maltase and α-dextrinase. Single α-(1-4) linked glucose residues are released by cleaving from the oligosaccharide's non-reducing end, but this reaction is blocked when it reaches the terminal end of the disaccharide. The non-reducing terminal α-(1-6) links are cleaved by α-dextrinase. Finally the digested glucose is transported by glucose carriers that are located on enterocytes. Energy for glucose transportation into enterocytes is provided by Na^+ K^+ ATPase. Glucose is transported from basolateral surface of enterocytes into capillaries of the villous core and, then it is absorbed into portal vein.

Pancreatic α-amylase is the main limiting factor in starch digestion because it causes hydrolysis of water insoluble starch granules. At first, the outer surface of starch granules is hydrolyzed by pancreatic enzymes, and then the enzyme hydrolysis is propagated towards the inside of starch granules. Amorphous regions of starch granules are enzymatically hydrolyzed more rapidly compared to crystalline regions. Relatively high levels of α-amylase are needed to digest starch completely. Incomplete starch digestion is mainly related to the structure of starch granules, ANF and the association of starch granules with coarse particles (Carre et al., 2004).

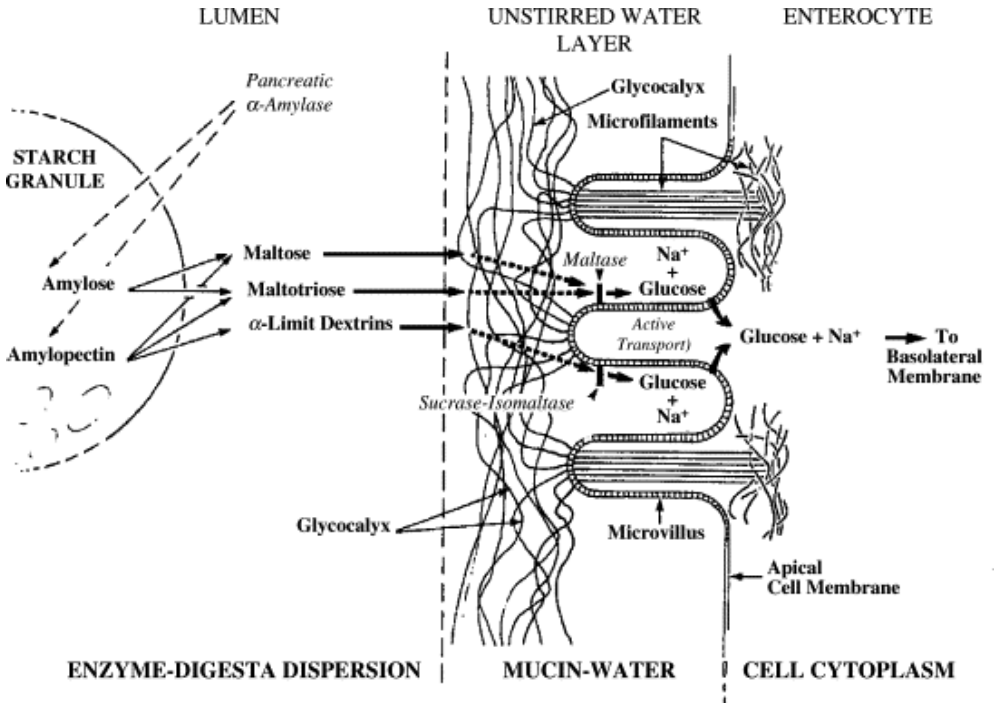

Figure 2.8. Starch granule hydrolysis by pancreatic α-amylase and brush border oligosaccharidases (Bach Knudsen et al., 2006).

2.2.1. Factors affecting starch digestibility

Rate and extent of starch digestion depends on starch chemistry, granule structure, particle size and surface area, amylose to amylopectin ratio, crystallinity and porosity of starch granules, digestion condition, processing method, association with other components such as lipids or protein, fibre, minerals and anti-nutritional factors (Al-Rabadi et al., 2009; Blazek and Copeland, 2010; Makhasukhonthachat et al., 2010; Regmi et al., 2011). Weurding et al. (2001a) stated that starch digestion rate is affected by animal related factors such as GI tract conditions, age, feed intake and passage rate, and glucose absorption capacity. Wiseman et al. (2000) summarized intrinsic and extrinsic factors that impact on starch granule degradation. Intrinsic factors include physical properties, chemical properties, presence of polysaccharides and protein,

and location of starch granules within the endosperm. Extrinsic factors include the amylase enzyme concentration in intestines and SI transit time.

2.2.1.1. Amylose to amylopectin ratio

Starch digestibility is influenced by amylose to amylopectin ratio. Normal starch contains around 20-35% amylose and, waxy starch contains less than 15% amylose. High amylose starch (Amylo) contains more than 40% amylose (Tester et al., 2004). Starch digestibility is reduced with higher amylose content as amylose is a more stable molecule due to the presence of large number of hydrogen bonds that interlink polymers of glucose (Ahuja et al., 2013). It might be also due to the interaction between amylose and fatty acids, which results in the formation of a less digestible complex on the surface of starch granules (Crowe et al., 2000).

2.2.1.2. Amylopectin chain length distribution

Starch digestibility is also influenced by amylopectin chain length distribution/degree of polymerization (DP). Higher amylose starch contains higher length amylopectin molecules (Tester et al., 2004). Starch digestibility is reduced with a higher proportion of long amylopectin chains because they form longer helices and, increase stabilization by hydrogen bonds (Ahuja et al., 2013).

2.2.1.3. Starch granule size distribution

Starch granule size distribution also affects starch digestibility rate in cereals. Starch is present as granules in the endosperm of cereal grains (Buleon et al., 1998). Starch granule size distribution is affected by genetic factors (cultivars) (Peterson and Fulcher, 2001) and environmental factors including temperature at grain filling (Tester et al., 1995). Theoretically, a

higher proportion of small starch granules increases starch digestibility as it increases surface area of starch granules and more free ends of glucan chains to contact with digestive enzymes (Ahuja et al., 2013).

2.2.1.4. Anti-nutritional factors

Starch digestibility is also influenced by anti-nutritional factors including α-amylase inhibitors, condensed tannins, dietary fibre and phytic acid (Carre et al., 2004). Alpha-amylase inhibitors are found in many cereals. Wheat endosperm contains heat-labile α-amylase inhibitors (Granum, 1979). Chickens can adapt to α-amylase inhibitors probably by increasing pancreatic α-amylase secretion (Macri et al., 1977). Condensed tannins are another ANF found in pea, sorghum and fava beans. Tannins have more of an effect on protein digestibility and their effect on starch digestibility is low in chickens (Lacassagne et al., 1988). Tannins are mostly found in hulls of field beans. They form complexes with amylase and thereby reduce the digestibility of starch (Longstaff and McNab, 1991). Dietary fibre also acts as an ANF. Soluble fibre increases digesta viscosity, slows down gastric emptying and absorption of digestive products, and ultimately reduces starch digestibility. The addition of enzyme additives, such as endo-xylanase, partially depolymerizes soluble NSP/fibre, reduces digesta viscosity and ultimately increase starch digestibility (Classen, 1996; Choct et al., 1999). Starch is encapsulated by cell wall NSP of starch granules, and it also reduces starch digestibility by minimizing enzyme access (Carre et al., 2007). Exogenous enzymes capable of depolymerizing NSP also act on these cell walls, and release the starch that has been entrapped inside cells. Phytic acid is another ANF, and is the most common phosphate reserve in many plants. It forms complexes with protein and minerals,

and reduces their biological availability. It affects starch digestibility by interacting with amylase or binding with salivary ions (Ca^{2+}) required for amylase activity (Singh et al., 2010).

2.2.1.5. Minor constituents of starch granules

Lipids are mostly found on surface of starch granules, but can be found inside of starch granules as well (Buleon et al., 1998). The concentration of lipid in starch granules is strongly correlated to amylose concentration in starch, as lipid forms complexes with long amylose molecules (Stoddard et al., 2004). Lipid and starch complexes reduce the contact of enzyme and substrate and reduce starch digestibility (Vasanthan and Bhatty, 1996). Protein can cause a reduction of starch digestibility by decreasing contact between starch and digestive enzymes due to the protein matrix formation surrounding the starch granules. Proteins such as albumins, globulins and glutenins form a matrix surrounding the starch granules and, act as a barrier for starch digestion. Furthermore, granule surface protein and lipids can reduce surface accessibility of enzymes by blocking the adsorption sites, and inhibiting digestive enzyme binding (Singh et al., 2010).

2.2.1.6. Hardness and starch damage

Grain hardness and starch damage also influence starch digestibility. Grain hardness can be defined as the relative resistance of grain to deformation when an external force is applied (Turnbull and Rahman, 2002). Hardness is related to the proportion of fine particles after milling. Hardness is increased when the proportion of fine particles decreases after grinding (Carre et al., 2005). In hard wheat, milling results in clean and well-defined particles since the fractures during milling pass along endosperm cell walls. However, it leads to a higher

proportion of damaged and broken starch granules as fracture occurs through the cell contents. Soft wheat releases starch granules more freely during milling as fractures occur around the granules but not through the granules, and also due to lower adhesion between starch and protein. Therefore, it results in lower starch damage in soft wheat. In addition, hardness is increased due to the strong interaction of starch granules and protein matrix (Barlow et al., 1973). Wheat having higher protein content is harder, and contains strong gluten compared to wheat with low protein content (Pasha et al., 2010).

Some starch granules undergo mechanical damage during wheat milling. The level of damage depends on severity of grinding and hardness of wheat. However, hard wheat results in a high proportion of damaged starch at milling (Pasha et al., 2010), and this damaged starch has a high water absorption capacity, which may lead to increased starch digestibility (Barrera et al., 2007). Starch damage affects starch granule surface, and modifies the interaction of particles with water vapor, which leads to increased hydrophilic bonds, and ultimately increased starch hydrolysis by enzymes (Saad et al., 2009). However, Carre et al. (2005) found low starch digestibility for hard wheat in comparison to soft wheat. The reason might be that the undigested starch of hard wheat is entrapped in a cell wall or protein matrix of starch granules.

2.2.2. Assessing starch digestion

2.2.2.1. *In vitro* starch digestion methods

A variety of *in vitro* starch digestion methods have been used in the scientific literature. All attempt to mimic starch digestion in the digestive tract of human or animals. Some methods mimic gastric and small intestinal phases of the chicken gastrointestinal (GI) tract by creating

temperature, pH and enzyme levels closer to chicken GI tract conditions. *In vitro* starch digestion techniques are less expensive, less time consuming, and feed or feed ingredient samples can be tested with an adequate number of replicates compared to *in vivo* starch digestion methods (Weurding et al., 2001b). These *in vitro* starch digestion techniques were able to predict *in vivo* starch digestion in broiler chickens (Wiseman et al., 2000; Weurding et al., 2001b).

2.2.2.2. *In vivo* starch digestion methods

In poultry species, starch digestibility can be determined in either excreta (often termed faecal) or ileal digesta. In excreta digestibility, after having been given dietary treatments to birds, excreta are collected to determine total tract starch digestibility. Starch digestion in the SI and starch fermentation in the terminal digestive tract or in excreta after defaecation (that releases SCFA) cannot be differentiated by measuring excreta digestibility. For ileal digestibility, digesta from the terminal ileum is collected after having been given dietary treatments and sacrificing birds. Therefore, starch digestibility in the SI, and starch fermentation in caeca can be differentiated by measuring both excreta digestibility and ileal digestibility (Weurding et al., 2001a). Ileal starch digestibility can vary depending on the exact site of digesta collection, as both the entire ileal content or content from the terminal (approximately 50%) section of the ileum have been used in starch digestibility research.

Bach Knudsen et al. (2006) compared three *in vivo* techniques which are qualitative and quantitative in determining starch digestibility in poultry. These are the slaughter, cannulation and catheterization techniques. In general, for the slaughtering technique, birds are sacrificed at a specific time after having being given experimental diets and then samples are collected from different segments of SI to determine the starch digestibility rate. An external indigestible marker

such as titanium oxide (TiO_2) or chromium oxide (Cr_2O_3) is required to analyze starch digestibility in the slaughtering technique. No surgery is required and also there is no risk of disturbing gastro-intestinal physiology in this technique. In addition, samples can be taken from various intestinal segments. There are some disadvantages of the technique as repeated samples cannot be taken from same animal. Therefore, the number of animals, or groups of animals in the case of pooled samples, is increased when using this technique.

2.3. Wheat

Wheat (*Triticum aestivum*) is a monocotyledonous grain that originated from polyploidization. High genetic variability of wheat leads to high adaption potential. Wheat is a major agricultural product and, several thousand wheat cultivars are used in the world. The annual global production of wheat is 729.1 million tonnes (FAO of the United Nations 2014/15).

Wheat consists of three main parts, the bran (13%), germ/embryo (2%) and endosperm (85%). The wheat bran is composed of NSP and aleurone layer. Protein, oil and minerals are the components of the aleurone layer. Wheat endosperm is comprised of starch granules that are surrounded by a protein matrix and cell wall (Amerah, 2015).

Wheat composition differences are mainly based on chemical and physical factors. Chemical characteristics including nutrient and ANF content are affected by genetic and environmental factors. Physical characteristics of wheat are mainly kernel weight and density. Wheat is a major feed ingredient in poultry rations due to its relatively high starch and CP content. When used, wheat usually accounts for the majority of poultry diets, providing 60-65% of AME and 35-40% of protein. (Gutierrez del Alamo et al., 2009a; Yegani et al., 2013). Wheat

carbohydrates include low molecular weight sugars (monosaccharides, sucrose, raffinose and stachyose), starch, and cell wall and storage NSP. Cell wall polysaccharides are mainly pentoses (arabinose and xylose) and hexoses (glucose, galactose and mannose) (Bach Knudsen, 1997).

2.3.1. Wheat classification

Wheat classification in Western Canada is based on seeding time (spring, winter), hardness (soft, hard) and colour (white, red). Winter wheat is sown in the fall and it grows to a certain extent before winter starts. Then growth ceases and the plant is dormant during winter; growth resumes in spring with harvest occurring in summer. Spring wheat is sown in early spring, and harvested in early fall. It is less tolerant to cold temperature (Acquaah, 2012). Hard wheat has a physically hard kernel that leads to flour with high protein content (gluten) during milling and, suitable to make bread and different types of noodles. Soft wheat contains less crude protein compared to hard wheat, and is primarily used to make cake and biscuits (Oleson, 1994). Hard wheat results in a high proportion of damaged starch at milling (Pasha et al., 2010), and this damaged starch has a high water absorption capacity which may leads to increase starch digestibility (Barrera et al., 2007). Wheat colour refers to the colour of aleurone or outer layer of wheat kernel (Oleson, 1994). Grain colour is associated with a red pigmentation that is caused by a plant metabolic product called flavonoid (Whan et al., 2014). It might be affected by the variations in grain protein content, hardness, vitreousness, and kernel size and shape. White wheat associates with less hardness and, it consists comparatively less crude protein content (Peterson et al., 2001). However, white wheat is more preferable in food industry due to high consumer demand for some end-products (Whan et al., 2014).

There are eight important wheat classes in Western Canada namely Canadian Prairie Spring (CPS), Canadian Western Amber Durum (CWAD), Canadian Western Extra Strong (CWES), Canadian Western General Purpose (CWGP), Canadian Western Hard White Spring (CWHWS), Canadian Western Red Spring (CWRS), Canadian Western Red Winter (CWRW) and Canadian Western Soft White Spring (CWSWS). Canadian Western Red Spring and CWAD are major wheat classes, whereas the other six wheat classes are relatively minor. The largest wheat class produced in Western Canada is CWRS with 11,438,627 annual insured commercial acres. It is mainly used to produce pan bread, hearth bread, flat bread and also use as a strong blending wheat. The second largest wheat class in Western Canada is CWAD with annual insured commercial acres of 4,135,990. This class is mainly used for pasta production. Wheat in the CPS class is mainly used to make hearth bread, flat bread and noodles, and the annual insured commercial acres are 838,622. The average annual insured commercial acres of CWSWS are 390,566, and this class of wheat is used to make cookies, cake and pastries. Wheat from the CWRW class is mainly used to make French bread, flat bread and noodles, and its annual insured commercial acres is 382,513. The average annual insured commercial acres of CWGP are 244,040, and it is mainly used as feed wheat and for ethanol production. Canadian Western hard white spring is basically used to produce bread and noodles, and the annual insured commercial acres is around 17,314. Canadian Western Extra Strong class has good gluten strength, and is used as blending wheat. Its annual insured commercial acres are around 465 (Canadian Grain Commission, 2015).

2.3.2. Wheat starch digestibility

World-wide, *in vivo* wheat starch digestion extent in broiler chickens has been studied fairly extensively, but not many studies have been conducted to compare starch digestibility among different wheat classes in Western Canada (Yegani et al., 2013; Ahuja et al., 2014). Generally the extent of wheat starch digestion is high according to the literature (Weurding et al., 2001a,b; Gutierrez del Alamo et al., 2008; Gutierrez del Alamo et al., 2009a,b). However there are some research findings that show a comparatively low extent of starch digestion for wheat (Mollah et al., 1983; Rogel et al., 1987; Yegani et al., 2013). Research has demonstrated that wheat genotype has an effect on starch digestion extent but the experiments were usually poorly replicated (Weurding et al., 2001a; Yegani et al., 2013), and therefore the results are less reliable. Little work has done regarding wheat genotype effects of Western Canada on either *in vivo* (Yegani et al., 2013) or *in vitro* (Ahuja et al., 2013; 2014) starch digestion extent.

Overall, there are very few studies that have been conducted regarding wheat starch digestion rate (Weurding et al., 2001a,b; Gutierrez del Alamo et al., 2009a,b). In Western Canada, very little research has been conducted to study genotypic effect of wheat on *in vitro* starch digestion rate (Ahuja et al., 2013; 2014). However no research has been found in literature regarding genotypic effect of Western Canadian wheat on *in vivo* starch digestion rate in broiler chickens. Therefore, further research is needed to establish starch digestion rate and extent values for different Western Canadian wheat classes and cultivars which will be supportive for feed ration formulation in broiler chickens.

2.3.3. Wheat AME

Energy is an important component of poultry feed, and it is mainly derived from cereal grains. Determination of digestible energy is important for accurate formulation of poultry rations as all the gross energy of an ingredient is not totally available to the bird (Scott et al., 1998a). Apparent metabolizable energy of a cereal is variable (Rogel et al., 1987; Scott et al., 1998b), and depends on the energy content of the cereal, its availability to the bird, and the presence of anti-nutrients such as soluble NSP (Scott et al., 1999).

Since starch is an important proportion of the AME of wheat, a positive relationship would be expected between starch digestibility or digestible starch content with AME. This has not always been the case as to obtain a good statistical correlation, there should be a wide range of difference for starch digestibility/digestible starch values. Significant positive correlations were observed for wheat AME_n with either starch digestibility (Mollah et al., 1983; Rogel et al., 1987; Wiseman et al., 2000) or digestible starch content (Wiseman et al., 2000) in broiler chickens in some studies, whereas no correlation was found in others (Gutierrez del Alamo et al., 2008 and Yegani et al., 2013). Therefore further research is required to investigate the relationship between starch digestibility and AME_n using wheat cultivars that belong to different Western Canadian wheat classes.

Scott et al. (1998b) provided evidence that the AME of Western Canadian wheat is affected by both cultivar (genotypic effect) and growing conditions (environmental effect). The study used nine wheat cultivars grown in replicate in three locations in each of two crop years, which indicate a very good replicated experiment. However evaluation of more Western

Canadian wheat market classes is still needed with the expansion of poultry industry which relies on accurate feed formulation using highly productive wheat varieties.

3. *IN VITRO* ASSESSMENT OF THE STARCH DIGESTIBILITY OF WESTERN CANADIAN WHEAT MARKET CLASSES AND CULTIVARS

3.1. Abstract

Starch provides the largest portion of energy in wheat used in poultry feeding, and both rate and extent of starch digestion may impact its nutritional value. Differences among wheat genetic backgrounds as represented by market class and cultivar may affect starch digestion characteristics. In addition, market class and cultivar variation is difficult to assess (with adequate replication) using *in vivo* techniques. The objective was to measure the effect of wheat market class and cultivar on starch digestibility using an *in vitro* model that mimics the chicken digestive tract and relate it to grain characteristics. The study evaluated 18 spring wheat cultivars from eight Western Canadian wheat market classes and each cultivar was replicated four times (different plots). Samples were subjected to 30 min gastric and 240 min small intestine (SI) digestion phases and each sample was assayed in triplicate; glucose release was measured starting at 15 min of the SI phase. Starch granule size distribution, amylose, total starch (TS), crude protein (CP), ash, and non-starch polysaccharides (NSP) were analyzed in all wheat samples. The experiment was a complete randomized design and wheat cultivars were nested within market classes. Significance level was set at $P \leq 0.05$. Pearson correlation was used to determine correlations. SI phase times of 15, 60 and 120 min were chosen to approximate digestion in terminal duodenum, jejunum and ileum. Starch digestibility ranges of wheat classes for these times are as follows: 15 min – 33.1 to 49.1%, 60 min – 80.2 to 93.3% and 120 min – 92.4 to 97.6%. Low to moderate positive correlations were found for starch digestibility with CP, ash, NSP and large granule size distribution whereas it was negative with TS, small and medium

granule proportions. In conclusion, market class and cultivar of Western Canadian wheat affects both rate and extent of *in vitro* starch digestibility and it is related to above grain characteristics.

Key words: chicken, slowly digested starch, rapidly digested starch

3.2. Introduction

Starch is the main energy source of poultry diets, and a major contributor to diet apparent metabolizable energy (AME). In Western Canada, wheat is the main cereal and starch source used in poultry diets because of its availability, and relatively high total starch (TS) and crude protein (CP) content. However, wheat can be variable as it is primarily grown to provide functional properties required by the food industry (Table 3.1) and not specifically for the feed industry. To meet the required properties, a variety of wheat market classes are grown in Western Canada, and within each market class are a number of cultivars. The predominant wheat market classes are Canadian Western Red Spring (CWRS), Canadian Western Amber Durum (CWAD), Canadian Prairie Spring Red (CPSR), Canadian Western Extra Strong (CWES), Canadian Western Red Winter (CWRW), Canadian Prairie Spring White (CPSW), Canadian Western Soft White Spring (CWSWS) and Canadian Western Hard White Spring (CWHWS). Canadian Western General Purpose does not meet the quality standards for milling due to its high starch and low protein content, and is not considered as a major wheat market class (Canadian Grain Commission, 2015). Feed formulation is based on the nutrient profile and digestibility of feed ingredients, and therefore the variability in wheat reduces the accuracy of feed manufacturing. However, limited data are available regarding the digestibility of wheat classes/cultivars because of the difficulty of testing the large number of samples. Therefore, the

determination of starch digestibility of wheat market classes/cultivars is important for accurate feed formulation and ultimately to improve poultry production.

Table 3.1. Western Canadian wheat market classes, their insured commercial acres in Western Canada and uses in food industry (Canadian Grain Commission, 2015)

Wheat class	Insured commercial acres in 2015	Use
CPS[1]	838,622	Hearth bread, flat bread, noodles
CWAD	4,135,990	Pasta
CWES	465	Blending wheat, gluten strength
CWGP	244,040	Ethanol production, animal feed
CWHWS	17,314	Bread, noodles
CWRS	11,438,627	Pan bread, hearth bread, flat bread, blending wheat
CWRW	382,513	French bread, flat bread, noodles
CWSWS	390,566	Cookies, cakes, pastries

[1] CPS – Canadian Prairie Spring; CWAD – Canadian Western Amber Durum; CWES – Canadian Western Extra Strong; CWGP – Canadian Western General Purpose; CWHWS – Canadian Western Hard White Spring; CWRS – Canadian Western Red Spring; CWRW – Canadian Western Red Winter; CWSWS – Canadian Western Soft White Spring.

In vivo digestibility trials in chickens are used to determine starch digestibility and energy utilization of grains, but they are expensive and time consuming. Because of these limitations, an adequate number of replications required to test variation among classes and cultivars is difficult to achieve, and often comparisons are limited to one sample of each class (cultivar) being tested (Gutierrez del Alamo et al., 2008; Gutierrez del Alamo et al., 2009a,b; Yegani et al., 2013). However, more than one sample should be tested per wheat market class/cultivar, as starch digestibility may differ due to the grain growing environment as well as genetic characteristics. Therefore, establishment of an *in vitro* starch digestibility technique is important to avoid these limitations.

Englyst et al. (1992) established an *in vitro* method to measure starch digestibility using a small intestine (SI) phase that mimics the human digestive tract. The method is used to estimate the rate and extent of starch digestion and separates starch into rapidly digestible starch (RDS), slowly digestible starch (SDS) and resistant starch (RS). It also permits estimation of the starch digestion index (SDI), which is a measure of the relative rate of starch digestion. This method, or modifications thereof, is widely used for assessing starch digestion in human nutrition. Ebsim (2013) modified this procedure to more accurately reflect digestive tract conditions in the chicken by using an incubation temperature of 41°C instead of 37°C, and a SI pH of 5.6 instead of 5.2. The digestive enzyme concentration in the SI phase was also increased so that the timing of starch digestion more closely approximated the time of digestion in the chicken. In addition, starch samples were not corrected for free glucose. This *in vitro* method permits the estimation of both the rate and extent of starch digestion in the chicken and has the potential to evaluate these characteristics in wheat classes and cultivars.

Most of the experiments assessing wheat starch digestibility in chickens, have determined the extent, but not the rate of starch digestibility (Rogel et al., 1987; Wiseman et al., 2000; Gutierrez del Alamo et al., 2008a; Yegani et al., 2013). However, rate of starch digestion is also important because it affects among other things, appearance of glucose in systemic blood and resulting metabolic effects, nutrient availability for enterocytes along the SI, and fermentation by gastrointestinal tract microbiota (Weurding et al., 2001a,b).

Rate and extent of starch digestion is affected by starch granule structure and composition, processing method, and association with other components including lipid content, the nature of protein matrix, fibre, minerals and anti-nutritional factors (ANF) (Al-Rabadi et al.,

2009; Blazek and Copeland, 2010; Mahasukhonthachat et al., 2010; Regmi et al., 2011). It has been proven that wheat starch digestion is affected by amylose concentration, amylopectin chain length distribution and starch granule size distribution (Ahuja et al., 2013). Starch digestibility is reduced with higher amylose content as amylose is a more stable molecule due to the presence of large numbers of hydrogen bonds. It might be also due to an interaction between amylose and fatty acids that results in complex formation on the surface of starch granules (Svihus et al., 2005). Starch digestibility is reduced with a higher proportion of long amylopectin chains because longer amylopectin chains form longer helices, and increase stabilization by hydrogen bonds. In wheat, there are two types of starch granules. Large lenticular-shaped granules are called A granules, and size is around 15-40 μm. Small spherical granules are called B granules, and size is around 1-10 μm (Salman et al., 2009). Higher proportion of small starch granules increase starch digestibility theoretically as it increases surface area of starch granules that contact with digestive enzymes (Svihus et al., 2005). All these relationships between grain characteristics and starch digestibility have been studied with *in vitro* digestion models. However, determination of relationships between grain characteristics with *in vivo* conditions is important to identify the reasons for variability of starch digestibility rate and extent in broiler chickens. Svihus et al. (2005) summarizes that feed processing methods may affect starch digestibility as it changes starch properties by interacting with its molecules. Severe processing conditions including extrusion result in complete gelatinisation, and increase starch availability due to high temperature and moisture conditions. In addition feed processing denatures α-amylase inhibitors, and results in higher starch digestibility. Starch digestibility is also affected by association with other non-starch compounds. Lipid-starch complexes on the surface of starch granules reduce starch digestibility by minimizing digestible enzyme access, and also by

reducing swelling due to hydrophobic nature of lipids (Vasanthan and Bhatty, 1996). Protein matrix that embeds the starch granules also reduce starch digestibility by minimizing exposure of enzymes (Rooney and Pflugfelder, 1986).

It was hypothesized that wheat market class and cultivar impact the rate and extent of *in vitro* starch digestibility because of differences in relevant grain characteristics. The objectives of this research were to determine the effect of wheat market class and cultivar on the rate and extent of *in vitro* starch digestibility, and to determine the effect of grain characteristics on *in vitro* starch digestibility.

3.3. Materials and methods

An experiment was conducted to determine the rate and extent of starch digestion using an *in vitro* model of the chicken digestive tract. The study used 18 wheat cultivars, consisting four independent samples for each cultivar, and they were obtained from the Crop Development Centre at the University of Saskatchewan. The cultivars were grown on fallow land in a Bradwell clay loam soil type at the University of Saskatchewan's North Seed Farm at Saskatoon, SK in 2012. The four samples of each cultivar were grown on different plots. The seeding rate was adjusted to 116 seeds per row. A standard fertilizer mix (11-51-0, N-P-K) was placed with the seed at approximately 50 kg ha^{-1}. When the plots reached maturity, the spikes were harvested and air-dried for 48 hours at 35°C. The spikes from each plot were then bulk-threshed using a rubber-belt deawner. The wheat cultivars tested and the market classes that they belong to are shown in Table 3.2.

Table 3.2. Wheat market classes and cultivars which were used for *in vitro* starch digestion assay

Wheat class	Wheat cultivars
CPS[1]	5702PR, SY985, Conquer
CWAD	Strongfield, CDC Verona, Transcend
CWES	CDC Rama
CWGP	NRG003, Minnedosa
CWHWS	Snowstar, Snowbird
CWRS	Glenn, CDC Stanley, CDC Utmost
CWSWS	AC Andrew, Sadash
Spelt	CDC Zorba, CDC Origin

[1] CPS – Canadian Prairie Spring; CWAD – Canadian Western Amber Durum; CWES – Canadian Western Extra Strong; CWHWS – Canadian Western Hard White Spring; CWRS – Canadian Western Red Spring; CWSWS – Canadian Western Soft White Spring; CWGP – Canadian Western General Purpose.

3.3.1. *In vitro* starch digestion

In vitro starch digestion was studied using a procedure that approximates the chicken gastric and small intestine (SI) digestion phases (Ebsim, 2013). The gastric phase contributes to sample mixing and moistening as well as exposure of samples to hydrochloric acid (HCl) and pepsin, which may increase digestive enzyme access to starch in the SI phase. In the SI phase, starch is hydrolyzed to glucose by the action of amylase (derived from pancreatin), amyloglucosidase and invertase enzyme activities. Protease and lipase activity derived from pancreatin may also benefit starch digestion by hydrolyzing lipid and protein blocking amylase access to starch. The released glucose is measured at different incubation times after the start of the SI phase using a glucose oxidase method. Digested starch is calculated based on released glucose, and starch digestibility is estimated based on the digested starch content in relationship to the total starch (TS) content of each wheat sample.

The *in vitro* starch digestibility method used in this research was primarily based on previously published *in vitro* methods (Englyst et al., 1992; Bedford and Classen, 1993) with modifications according to Ebsim (2013). Englyst et al. (1992) established an *in vitro* method to measure starch digestibility in the SI phase of humans. Bedford and Classen (1993) designed an *in vitro* digestion method to predict the intestinal viscosity of broiler chickens fed rye based diets with different dietary pentosanase levels. The gastric phase conditions of this experiment were used for the current *in vitro* starch digestibility assay. To more accurately reflect *in vivo* digestive tract conditions in the chicken, an incubation temperature of 41˚C was used instead of 37˚C, a SI buffer pH of 5.6 was used instead of 5.2 (Ebsim, 2013) and SI enzyme levels were increased to increase the rate of starch digestion. Total starch and digested starch values were also not corrected for free glucose which is contradictory to Englyst et al. (1992) technique (Ebsim, 2013).

Enzyme solution I was prepared by adding 1.818 g of pepsin (EC 3.4.23.1; Sigma ref. P-7125; St. Louis, MO-USA) into 60 ml of 0.1 M HCl. It provides 2000 U of pepsin per ml of solution. Enzyme solution II was prepared by weighing 3.0 g of pancreatin (Sigma ref. P-7545; Louis, MO. USA) to 9 centrifuge tubes followed by 20 ml of distilled water. The solution was stirred magnetically for 10 min, and centrifuged for 10 min at 1500 g (3000 rpm). Fourteen ml of supernatant from each tube was then added to a beaker (total 126 ml). The enzyme concentrations of pancreatin enzyme mixture were 228, 209 and 32.4 USP units/mg solid for amylase, protease and lipase respectively. Amyloglucosidase (22.5 ml; EC 3.2.1.3; Megazyme, Bray Business Park, Bray, Ireland) and invertase (9 ml; EC 3.2.1.26; Megazyme, Bray Business Park, Bray, Ireland) were added to make the solution contain 28.5 U/ml of amyloglucosidase and

60 U/ml of invertase. This amount was sufficient for 30 samples (Ebsim, 2013). Benzoic acid solution was prepared by dissolving 2.9 g of benzoic acid ($C_7H_6O_2$; Sigma ref. B-3250; St. Louis, MO, USA) in 1.0 l of distilled water. One molar calcium chloride ($CaCl_2$) solution was prepared by dissolving 11.1 g of $CaCl_2$ in 100 ml of distilled water. Sodium acetate buffer was prepared by dissolving 13.6 g of sodium acetate trihydrate ($CH_3COONa.3H_2O$; Sigma ref. S-6770; BDH ACS759; St. Louis, MO, USA) in 250 ml of saturated benzoic acid. Then pH was adjusted to 5.2 using acetic acid and adjusted to 1.0 l with distilled water. Finally 4 ml of 1 M $CaCl_2$ was added to 1.0 l of the buffer. Absolute (100%) ethanol was prepared by mixing 2.8 l of 95% ethanol into 4 l of distilled water. The glucose determination regent (GOPOD) from Megazyme (D-Glucose Assay Procedure- GOPOD format, K-GLUC 07/11, Megazyme International Ireland, Bray, Co. Wicklow, Ireland) was used for the glucose oxidase method. Distilled water was added into the glucose reagent buffer (50 ml) until it reaches 1.0 l, and then GOPOD reagent was dissolved in the buffer (Ebsim, 2013).

Samples were fine ground using a Retsch laboratory mill (Retsch ZM 200, Germany) using a screen-hole size of 0.5 mm; fine grinding was used to mimic the impact of the chicken's gizzard muscular function. Three replications of approximately 700 mg of each wheat sample were weighed, and added into 50 ml polypropylene centrifuge tubes, and 50 mg of guar gum powder was also added to each tube to standardize the viscosity. A blank tube containing 50 mg of guar gum powder was used to correct glucose content in the amyloglucosidase solution, and was used as the blank sample. A starch standard was prepared by adding regular maize starch and guar gum powder into a tube. *In vitro* starch digestion was completed on a set of 9 wheat samples at a time.

Initially, 1.5 ml of enzyme solution I (2000 U/ml pepsin-HCl solution) was added to each centrifuge tube. Then tubes were capped, mixed on a vortex mixer and placed horizontally in a water bath (41°C) for 30 min. The enzyme solution II was prepared during this time period. Tubes were taken out of the water bath after 30 min, and three glass balls (1.5 cm diameter) were added to each tube. Then 20 ml of sodium acetate buffer (41°C) was added to each sample, standard and blank tube, capped and vortexed. For the SI phase 5 ml of enzyme solution II was added to each tube, and then the tubes were capped, vortexed and immediately securely placed in a shaking water bath (41°C). The shaking water bath was set at a stroke length of 35 mm and 160 strokes per min. Timing was started immediately after adding enzyme solution to the first tube. In this phase, starch is digested into maltose, isomaltose and dextrin by α-amylase and further hydrolyzed into glucose by amyloglucosidase. Sucrose present in wheat is hydrolyzed into glucose and fructose by the action of invertase enzyme. Aliquots (0.5 ml) were taken from each tube at 15, 30, 45, 60, 90, 120, 180 and 240 min of the SI phase and added to 50 ml polypropylene centrifuge tubes containing 20 ml of absolute ethanol (stop the enzyme reaction). During aliquot removal, tubes were individually removed from the water bath, mixed before taking aliquots, and immediately returned to the water bath (30 sec for each tube to undergo this procedure).

Ethanol tubes which contained aliquots were centrifuged at 1500 rpm for 2 min to obtain a clear supernatant. The amount of released glucose was measured colourimetrically according to a glucose oxidase method of a Megazyme kit (D-Glucose Assay Procedure- GOPOD format, K-GLUC 07/11, Megazyme International Ireland, Bray, Co. Wicklow, Ireland). Aliquots of 100 µl were pipetted in duplicate into labelled round bottomed glass test tubes. Standards of four tubes

were prepared by adding 100 µl of glucose standard (1 mg/ml). Then 3 ml of prepared GOPOD reagent was added to each tube, and incubated in a 50°C water bath for 20 min. After 20 min, tube contents were transferred into cuvettes (4.5 ml PS Macro), and absorbance was read using a spectrophotometer (Genesys 20, Thermoscientific, USA) at 510 nm. Glucose was determined using the following formula for each sample.

$$\text{Glucose (\%)} = [A_t \times V_t \times C_s \times D / A_s \times W_t] \times 100$$

where A_t is the absorption of test solution, V_t is the total volume of test solution (26.5 ml + ml/g sample weight), C_s is the concentration of glucose of standard (1 mg/ml), A_s is the absorbance of standard, W_t is the weight of sample in mg and D represents the dilution factor of sub sample where the aliquot was taken from (Englyst et al., 1992).

The digested *in vitro* starch content of each sample was calculated using the following formula (Englyst et al., 1992).

$$\text{\% starch} = \text{\% Glucose} \times 0.9$$

Total starch was determined (method 996.11; AOAC, 1995) using a Megazyme kit (Amyloglucosidase /α-amylase method, K-TSTA 07/11, Megazyme International Ireland, Bray, Co. Wicklow, Ireland). Released glucose was measured colourimetrically using the glucose oxidase method. Total starch was calculated using the following formula:

$$\text{TS (\%)} = [A_s \times (0.1 \div A_g) \times 1000] \div W \times 0.9 \times 100$$

where A_s is the average absorbance of a sample, A_g is the absorbance of 0.1 mg glucose standard, 1000 is the volume correction (0.1 ml taken from 100 ml), W is the weight in mg of

analyzed sample, 0.9 is the correction factor from free glucose to anhydrous glucose (starch), 100 is the factor that allows to express starch as a % of sample weight.

Starch digestibility was calculated using the following formula.

Starch digestibility (%) = ($TS_{in\text{-}vitro} \div TS$) × 100

where $TS_{in\text{-}vitro}$ is the digested starch at a particular SI incubation time and TS is the total starch of the wheat sample.

3.3.2. Grain characteristics

All wheat samples were analyzed in duplicate for TS, CP, ash, soluble and insoluble NSP, soluble and total arabinoxylans (AX), amylose and starch granule size distribution. Amylose, TS, CP, ash, soluble and insoluble NSP, and soluble and total arabinoxylans were analyzed on dry matter (DM) basis. Moisture was determined using standard procedure of AOAC (1995).

Total starch was measured as described above. Crude protein was analyzed using a Leco protein analyzer (Model Leco–FP–528L, Leco Corporation, St. Joseph, MA, USA), and 6.25 was used as the N to CP correction factor. Samples were analyzed for ash content according to section 942.05 of AOAC (1995) method using a muffle oven (Model Lindberg/Blue BF51842C, Asheville, NC 28804, USA). Soluble and insoluble NSP, and soluble and total AX were analyzed using near-infrared (NIR) technique (Black et al., 2014). Amylose content of each sample was determined using the Megazyme amylose/amylopectin assay (Amylose/amylopectin method, K-TSTA 07/11, Megazyme International Ireland, Bray, Co. Wicklow, Ireland). Starch was extracted from wheat flour using cesium chloride density gradient centrifugation (Peng et

al., 1999) prior to analysis of starch granule size distribution. Starch granule size distribution (by volume) in purified starch of wheat samples was determined using a laser diffraction particle size analyzer (Hydro 2000S, Malvern Instruments, Malvern WR, UK). Malvern Mastersizer 2000 software was used to estimate starch granule size distribution by volume.

3.3.3. Statistical analysis

The experiment was a Complete Randomized Design (CRD) with wheat cultivars nested within wheat market class. The experimental model used is as follows:

$$Y_{ijr} = \mu + \alpha_i + \beta_{ij} + \varepsilon_{ijr}$$

(Y_{ijr} is the value of the dependent variable observed at the r^{th} replication with the first factor (α) at its i^{th} level and the second factor (β) at its j^{th} level, ε is the error, μ is the overall (fixed) mean of the sampling population and $\alpha_i + \beta_{ij} + \varepsilon_{ijr}$ are mutually uncorrelated random effects).

All data were analyzed using Proc Mixed in SAS (SAS 9.4, Carey, N.C. 2008) and Tukey's studentized range test was used for mean separation of treatments when there was a significant difference. All data were checked for normal distribution using Shapiro-Wilk test. Differences were considered significant when $P \leq 0.05$. Correlations of *in vitro* starch digestibility with each grain characteristic and correlations among grain characteristics were determined using Proc Corr in SAS (SAS 9.4, Carey, N.C. 2008). Further, stepwise regression analysis was done using Proc Reg to determine the factors most affecting *in vitro* starch digestibility for each SI incubation time.

3.4. Results

3.4.1. *In vitro* starch digestibility

The nature of starch digestion pattern for wheat samples in the *in vitro* assay was as expected in the time frame of data collection. On average, 38.2% of starch digested at 15 min of the SI phase, and from this point digestion rose until reaching a plateau (average value of 96.9%) at 180 min. For each of the time points assessed, market class affected the degree of starch digestion (Table 3.3). The range in digestibility for each time period tended to decrease with increasing digestion time with a maximum range of 21% at 30 min and 3.8% at 240 min. Based on incubation time in the SI phase and Ebsim (2013) unpublished data, 15, 60 and 120 min were assumed to be representative of *in vivo* starch digestibility in the terminal duodenum, jejunum and ileum, respectively. These values were considered important in assessing rate of starch digestibility, and will be described in more detail. Starch digestibility of wheat classes at 15 min ranged from 33.1 (Spelt) to 49.1% (CWAD) with an overall difference between the minimum and maximum values of 16%. At 60 min, a 13.1% difference was found between the minimum value of 80.2% (CWRS) and the maximum value of 93.3% (CWAD). At 120 min, the range was from 92.4 (CWRS) to 97.6% (CWES), and the difference was 5.2%. At 15 min, the CWAD class resulted in the highest digestibility, followed by CWES and CWGP, and the remainder of the classes being lowest and statistically equal. Canadian Western Amber Durum maintained the highest digestibility at 60 min, followed by CWGP, which was not higher than CWES, but was higher than the remaining classes. Starting with CWES the digestibility ranking for the remaining cultivars was CWES, CWSWS, CPS, CWHWS, Spelt and CWRS (see Table 3.3 for statistical separation of means). At 120 min, CWES and CWAD demonstrated the highest

digestibility followed by, but not different than, CWSWS and CPS. The numerical ranking from high to low digestibility for the remaining cultivars was CWGP, CWHWS, Spelt and CWRS (again see Table 3.3 for statistical interpretation).

Table 3.3. Effect of wheat class on starch digestibility (%) at different incubation times of small intestine phase of *in vitro* starch digestion assay.

Wheat class	Small intestine phase incubation time (min)							
	15	30	45	60	90	120	180	240
CPS[1]	35.8^{c}	56.8^{c}	72.9^{cd}	84.5^{cd}	93.8^{bc}	95.5^{abc}	97.3^{abc}	97.8^{ab}
CWAD	49.1^{a}	74.6^{a}	88.8^{a}	93.3^{a}	97.7^{a}	97.5^{a}	97.1^{abc}	98.5^{a}
CWES	40.1^{b}	58.7^{c}	77.5^{bc}	87.9^{bc}	96.8^{ab}	97.6^{a}	99.4^{a}	97.4^{abc}
CWGP	42.2^{b}	65.1^{b}	81.6^{b}	89.7^{b}	95.3^{ab}	94.3^{bcd}	98.0^{ab}	97.1^{abc}
CWHWS	35.0^{c}	53.6^{d}	72.1^{d}	84.0^{d}	90.6^{c}	93.4^{cd}	95.0^{c}	95.1^{c}
CWRS	35.9^{c}	55.8^{cd}	71.2^{d}	80.2^{e}	92.3^{bc}	92.4^{d}	95.8^{bc}	95.9^{bc}
CWSWS	34.5^{c}	55.0^{cd}	73.9^{cd}	85.0^{cd}	93.0^{bc}	96.2^{abc}	97.0^{abc}	96.6^{abc}
Spelt	33.1^{c}	56.8^{cd}	71.3^{d}	83.9^{d}	91.0^{c}	92.7^{cd}	95.5^{bc}	94.7^{c}
SEM[2]	0.71	0.93	0.87	0.58	0.46	0.38	0.29	0.31

$^{a-e}$ Means within a column not sharing a common superscript are significantly different ($P \leq 0.05$).
[1] CPS – Canadian Prairie Spring (3 cultivars); CWAD – Canadian Western Amber Durum (2); CWES – Canadian Western Extra Strong (1); CWGP – Canadian Western General Purpose (2); CWHWS – Canadian Western Hard White Spring (2); CWRS – Canadian Western Red Spring (3); CWSWS – Canadian Western Soft White Spring (2).
[2] SEM- Pooled standard error of mean. Each mean represents 2 or 3 cultivars (except CWES – 1) and 4 replications per each cultivar.

Examination of variation in *in vitro* starch digestibility among cultivars is shown in Table 3.4. Similarly to class, cultivar affected starch digestibility at all time periods. *In vitro* starch digestibility (%) of wheat cultivars at 15 min ranged from 32.6 (CDC Zorba) to 51.6% (Transcend) with a maximum difference of 19.0%. At 60 min, digestibility ranged from 77.3 (Glenn) to 94.8% (CDC Verona) resulting in a maximum difference of 17.5%. At 120 min, the range was from 91.0 (CDC Origin) to 101.3% (Transcend) with a difference between these means of 10.3%. Similarity of cultivars within a class can be estimated based on separation of

cultivar means. When this is done, differences among cultivars within a class were found for CPS (120 min), CWAD (15, 30, 90, 120, 180 and 240 min), CWHWS (90 min), CWRS (30, 45, 60 min) and CWGP (45 min). Despite the importance of class in affecting starch digestion, there is still some variation within classes according to the statistical separation of means.

Table 3.4. Effect of wheat cultivar on starch digestibility (%) at different incubation times of small intestine phase of *in vitro* starch digestion assay.

Wheat class	Wheat cultivar	Small intestine phase incubation time (min)							
		15	30	45	60	90	120	180	240
CPS[1]	5702PR	36.2^{def}	57.4^{efg}	72.6^{bcde}	84.7^{def}	90.9^{bc}	92.7^{cde}	96.7^{abc}	95.8^{bc}
	SY985	35.5^{def}	54.5^{efgh}	70.8^{cde}	83.1^{def}	94.7^{b}	98.2^{ab}	97.3^{ab}	98.6^{ab}
	Conquer	35.8^{def}	58.4^{efg}	75.3^{bcd}	85.7^{de}	95.8^{ab}	95.6^{bcde}	97.9^{ab}	98.8^{ab}
CWAD[1]	Strongfield	49.5^{ab}	73.3^{ab}	87.9^{a}	92.2^{abc}	94.8^{b}	94.2^{bcde}	93.9^{bc}	95.0^{bc}
	CDC Verona	46.3^{b}	72.4^{b}	90.0^{a}	94.8^{a}	96.5^{ab}	96.8^{abcd}	98.2^{ab}	98.7^{ab}
	Transcend	51.6^{a}	78.1^{a}	88.5^{a}	92.9^{ab}	101.9^{a}	101.3^{a}	99.2^{a}	101.6^{a}
CWES	CDC Rama	40.1^{cd}	58.7^{def}	77.5^{bc}	87.9^{bcd}	96.8^{ab}	97.6^{abc}	99.4^{a}	97.4^{abc}
CWGP	NRG003	39.9^{cd}	64.2^{cd}	78.1^{b}	87.7^{cd}	93.6^{bc}	94.3^{bcde}	97.9^{ab}	96.8^{bc}
	Minnedosa	44.5^{bc}	66.0^{c}	85.1^{a}	91.7^{abc}	96.9^{ab}	94.2^{bcde}	98.1^{ab}	97.4^{abc}
CWHWS	Snowstar	34.3^{ef}	54.4^{efgh}	74.2^{bcd}	84.9^{def}	87.3^{c}	91.7^{de}	92.8^{c}	92.7^{c}
	Snowbird	35.8^{def}	52.9^{gh}	70.0^{de}	83.1^{def}	94.0^{b}	95.0^{bcde}	97.2^{abc}	97.4^{abc}
CWRS	Glenn	34.0^{ef}	51.3^{h}	66.9^{e}	77.3^{g}	91.4^{bc}	91.1^{e}	96.0^{abc}	95.2^{bc}
	CDC Stanley	39.3^{cde}	59.9^{de}	77.2^{bc}	83.4^{def}	95.0^{b}	94.4^{bcde}	97.6^{ab}	97.6^{ab}
	CDC Utmost	34.3^{ef}	56.1^{efgh}	69.5^{de}	79.9^{fg}	90.6^{bc}	91.6^{e}	94.0^{bc}	94.9^{bc}
CWSWS	AC Andrew	33.4^{f}	53.1^{fgh}	72.1^{bcde}	82.4^{ef}	91.8^{bc}	95.6^{bcde}	97.2^{abc}	96.4^{bc}
	Sadash	35.7^{def}	56.9^{efgh}	75.8^{bcd}	87.6^{bcde}	94.2^{bc}	96.7^{abcde}	96.9^{abc}	96.8^{bc}
Spelt	CDC Zorba	32.6^{f}	54.5^{efgh}	70.0^{de}	84.4^{def}	91.4^{bc}	94.5^{bcde}	97.2^{abc}	95.0^{bc}
	CDC Origin	33.5^{f}	59.0^{de}	72.6^{bcde}	83.4^{def}	90.6^{bc}	91.0^{e}	93.9^{bc}	94.3^{bc}
SEM[2]		1.03	1.09	1.33	0.99	1.25	1.01	0.86	0.93

[a – h] Means within a column not sharing a common superscript are significantly different ($P \leq 0.05$).
[1] CPS – Canadian Prairie Spring; CWAD – Canadian Western Amber Durum; CWES – Canadian Western Extra Strong; CWGP – Canadian Western General Purpose; CWHWS – Canadian Western Hard White Spring; CWRS – Canadian Western Red Spring; CWSWS – Canadian Western Soft White Spring.
[2] SEM- Pooled standard error of mean (n=4).

3.4.2. Grain characteristics

All grain characteristics were affected by wheat market class, and the results (DM basis) and statistical separation of means are presented in Table 3.5. The TS of wheat market classes varied from 53.4 (CWAD) to 58.7% (CWSWS) while CP varied from 15.6 (CWSWS) to 22.3% (CWAD). Ash content ranged from 2.0 (CWRS) to 2.3% (CWAD). Total, insoluble and soluble NSP levels ranged from 10.0 (CPS) to 12.4% (CWAD), 8.8 (CPS) to 10.8% (CWAD) and 1.0 (Spelt) to 1.6% (CWAD), respectively. The AX component of the NSP ranged from 5.1 (CPS) to 6.1% (CWAD), 4.4 (CPS) to 5.5% (CWAD) and 0.5 (Spelt) to 0.6% (CWES, CWSWS and CWGP) for the total, insoluble and soluble fractions, respectively. Starch characteristics including amylose content and starch granule size distribution were affected by wheat market class, and the data are presented in Tables 3.5 and 3.6, respectively. For amylose content, class means varied from 20.0 (CWRS) to 26.7% (Spelt). Starch granule size distribution varied from 4.5 (CWSWS) to 10.6% (CWRS), 25.9 (CWSWS) to 38.2% (Spelt) and 52.1 (Spelt) to 69.6% (CWSWS) for small, medium and large starch granules, respectively.

Table 3.5. Grain characteristics (% of DM) of wheat market classes

Wheat class	TS[1]	CP	Ash	TNSP	INSP	SNSP	TAX	IAX	SAX	Amylose
CPS[2]	56.4^{bc}	20.4^{c}	2.19^{ab}	9.96^{d}	8.78^{d}	1.18^{cd}	5.05^{c}	4.44^{e}	0.61^{b}	22.7^{bcd}
CWAD	53.4^{d}	22.3^{a}	2.32^{a}	12.39^{a}	10.84^{a}	1.55^{a}	6.08^{a}	5.48^{a}	0.60^{b}	23.3^{bc}
CWES	54.6^{cd}	22.2^{a}	2.22^{abc}	11.05^{b}	9.73^{b}	1.32^{bc}	5.61^{b}	4.97^{bc}	0.64^{a}	20.3^{cd}
CWGP	56.8^{b}	18.6^{d}	2.07^{bc}	10.88^{b}	9.53^{bc}	1.34^{b}	5.59^{b}	4.95^{bc}	0.64^{a}	22.9^{bcd}
CWHWS	56.0^{bc}	21.0^{b}	2.08^{bc}	10.27^{cd}	9.09^{cd}	1.18^{cd}	5.17^{c}	4.58^{e}	0.59^{b}	24.6^{ab}
CWRS	55.7^{bc}	20.9^{b}	1.99^{c}	10.23^{cd}	9.10^{cd}	1.12^{de}	5.22^{c}	4.62^{de}	0.60^{b}	20.0^{d}
CWSWS	58.7^{a}	15.6^{e}	2.08^{bc}	10.52^{bc}	9.29^{bc}	1.23^{bcd}	5.65^{b}	5.01^{b}	0.64^{a}	25.82^{ab}
Spelt	55.8^{bc}	22.0^{a}	2.22^{ab}	10.47^{bcd}	9.43^{bc}	1.04^{e}	5.19^{c}	4.65^{cde}	0.54^{c}	26.71^{a}
SEM[3]	0.223	0.241	0.019	0.110	0.092	0.022	0.051	0.050	0.004	0.524

[a – e] Means within a column not sharing a common superscript are significantly different ($P \leq 0.05$)

[1] TS – Total starch; CP – Crude protein; TNSP – Total NSP; INSP – Insoluble NSP; SNSP – Soluble NSP; TAX – Total arabinoxylans; IAX – Insoluble arabinoxylans; SAX – Soluble arabinoxylans.

[2] CPS – Canadian Prairie Spring (3 cultivars); CWAD – Canadian Western Amber Durum (2); CWES – Canadian Western Extra Strong (1); CWGP – Canadian Western General Purpose (2); CWHWS – Canadian Western Hard White Spring (2); CWRS – Canadian Western Red Spring (3); CWSWS – Canadian Western Soft White Spring (2).

[3] SEM- Pooled standard error of mean. Each mean represents 2 or 3 cultivars (except CWES – 1) and 4 replications per each cultivar.

Table 3.6. Starch granule size distribution (volume %) of wheat market classes

Wheat class	Starch granule size distribution (volume %)		
	Small (<5 μm)	Medium (5–15 μm)	Large (>15 μm)
CPS[1]	9.8[a]	30.4[c]	59.6[bc]
CWAD	5.9[bc]	33.4[b]	60.7[b]
CWES	8.3[ab]	28.2[cd]	63.5[ab]
CWGP	10.0[a]	29.3[c]	60.7[b]
CWHWS	9.2[a]	35.7[ab]	55.2[cd]
CWRS	10.6[a]	37.2[a]	52.2[d]
CWSWS	4.5[c]	25.9[d]	69.6[a]
Spelt	9.8[a]	38.2[a]	52.1[d]
SEM[2]	0.30	0.55	0.75

[a – d] Means within a column not sharing a common superscript are significantly different ($P \leq 0.05$).
[1] CPS – Canadian Prairie Spring (3 cultivars); CWAD – Canadian Western Amber Durum (2); CWES – Canadian Western Extra Strong (2); CWGP – Canadian Western General Purpose (2); CWHWS – Canadian Western Hard White Spring (2); CWRS – Canadian Western Red Spring (3); CWSWS – Canadian Western Soft White Spring (2).
[2] SEM- Pooled standard error of mean. Each mean represents 2 or 3 cultivars (except CWES 1) and 4 replications per each cultivar.

The impact of cultivar on grain characteristics are shown in Tables 3.7 and 3.8. With the exception of the proportion of small and large starch granules, cultivar affected all grain characteristics. In the interest of brevity, statistical interpretation is shown in Tables 3.7 and 3.8. As expected, the range in levels among cultivars is larger than seen for wheat classes. Variation among cultivars within a class was found for CP (CPS, CWHWS and CWSWS), total NSP (CWRS), insoluble NSP (CWRS), soluble NSP (CWAD and Spelt), total AX (CWRS), insoluble AX (CWRS), soluble AX (CPS and Spelt) and amylose (CPS, CWHWS, CWSWS and Spelt).

Table 3.7. Grain characteristics (% of DM) of wheat cultivars

Wheat class	Wheat cultivar	TS[1]	CP	Ash	TNSP	INSP	SNSP	TAX	IAX	SAX	Amylose
CPS[2]	5702PR	56.3^{abcde}	21.3^{bc}	2.22^{abcd}	9.73^{gh}	8.55^{g}	1.19^{defgh}	4.95^{f}	4.32^{h}	0.63^{abcd}	27.0^{abc}
	SY985	55.3^{cde}	20.6^{cd}	2.21^{abcd}	10.48^{bcdefgh}	9.22^{cdefg}	1.26^{cdefg}	5.23^{ef}	4.66^{defgh}	0.57^{f}	19.6^{ef}
	Conquer	57.7^{abc}	19.2^{ef}	2.13^{abcd}	9.67^{h}	8.57^{g}	1.10^{efghi}	4.98^{f}	4.35^{h}	0.63^{abcde}	21.3^{cdef}
CWAD	Strongfield	54.2^{ef}	22.0^{ab}	2.35^{ab}	12.22^{a}	10.61^{ab}	1.61^{ab}	5.93^{abc}	5.33^{abc}	0.60^{bcdef}	24.9^{bcd}
	CDC Verona	53.8^{ef}	21.9^{ab}	2.36^{a}	12.22^{a}	10.81^{a}	1.42^{bc}	6.08^{ab}	5.48^{ab}	0.60^{bcdef}	24.1^{cde}
	Transcend	52.2^{f}	22.9^{a}	2.26^{abc}	12.73^{a}	11.10^{a}	1.63^{a}	6.25^{a}	5.65^{a}	0.60^{cdef}	20.9^{def}
CWES	CDC Rama	54.6^{def}	22.2^{ab}	2.22^{abc}	11.05^{bc}	9.73^{cd}	1.32^{cd}	5.61^{bcde}	4.97^{bcde}	0.64^{abc}	20.3^{def}
CWGP	NRG003	56.4^{abcde}	18.9^{ef}	2.03^{bcd}	10.50^{bcdefgh}	9.20^{cdefg}	1.29^{cde}	5.43^{cdef}	4.79^{defgh}	0.64^{abc}	20.0^{cdef}
	Minnedosa	57.3^{abcd}	18.3^{f}	2.12^{abcd}	11.26^{b}	9.86^{bc}	1.39^{c}	5.76^{abcd}	5.12^{bcd}	0.64^{ab}	23.8^{cde}
CWHWS	Snowstar	57.3^{abcd}	20.0^{de}	2.09^{abcd}	9.89^{efgh}	8.79^{efg}	1.10^{fghi}	5.04^{f}	4.43^{fgh}	0.60^{bcdef}	28.9^{ab}
	Snowbird	54.6^{def}	22.1^{ab}	2.06^{abcd}	10.64^{bcdefg}	9.38^{cdefg}	1.26^{cdefg}	5.30^{def}	4.73^{defgh}	0.57^{f}	19.3^{ef}
CWRS	Glenn	56.0^{bcde}	20.5^{cd}	2.15^{abcd}	9.82^{fgh}	8.77^{fg}	1.06^{hi}	5.00^{f}	4.41^{gh}	0.59^{def}	18.4^{f}
	CDC Stanley	55.5^{cde}	21.6^{bc}	1.95^{cd}	10.02^{defgh}	8.94^{defg}	1.08^{ghi}	5.05^{f}	4.47^{efgh}	0.59^{ef}	20.1^{def}
	CDC Utmost	55.6^{cde}	20.7^{cd}	1.89^{d}	10.84^{bcd}	9.61^{cde}	1.23^{cdefgh}	5.62^{bcde}	5.00b^{cd}	0.62^{abcde}	22.0^{cdef}
CWSWS	AC Andrew	58.8^{a}	16.2^{g}	2.05^{abcd}	10.80^{bcde}	9.52^{cdef}	1.28^{cdef}	5.74^{abcd}	5.10^{bcd}	0.65^{a}	29.8^{ab}
	Sadash	58.6^{ab}	15.0^{h}	2.12^{abcd}	10.24^{cdefgh}	9.07^{cdefg}	1.18^{defgh}	5.56^{cde}	4.92^{cdef}	0.64^{abc}	20.4^{def}
Spelt	CDC Zorba	55.5^{cde}	21.9^{ab}	2.10^{abcd}	10.69^{bcdef}	9.76^{cd}	0.94^{i}	5.44^{cdef}	4.88^{cdefg}	0.57^{f}	32.0^{a}
	CDC Origin	56.1^{abcde}	22.2^{ab}	2.34^{a}	10.25^{cdefgh}	9.11^{cdefg}	1.14^{defgh}	4.95^{f}	4.43^{fgh}	0.52^{g}	21.5^{cdef}
SEM[3]		0.528	0.210	0.059	0.110	0.162	0.037	0.098	0.050	0.008	1.042

[a - i] Means within a column not sharing a common superscript are significantly different ($P \leq 0.05$). [1] TS – Total starch; CP – Crude protein; TNSP – Total NSP; INSP – Insoluble NSP; SNSP – Soluble NSP; TAX – Total arabinoxylans; IAX – Insoluble arabinoxylans; SAX – Soluble arabinoxylans. [2] CPS – Canadian Prairie Spring; CWAD – Canadian Western Amber Durum; CWES – Canadian Western Extra Strong; CWGP – Canadian Western General Purpose; CWHWS – Canadian Western Hard White Spring; CWRS – Canadian Western Red Spring; CWSWS – Canadian Western Soft White Spring.[3] SEM- Pooled standard error of mean (n=4).

Table 3.8. Starch granule size distribution (volume %) of wheat cultivars

Wheat class	Wheat cultivar	Starch granule size distribution (volume %)		
		Small (<5 μm)	Medium (5–15 μm)	Large (>15 μm)
CPS[1]	5702PR	10.4	30.5^{cde}	59.1
	SY985	9.2	30.2^{cde}	60.5
	Conquer	9.9	30.6^{cde}	59.3
CWAD	Strongfield	5.1	35.8^{abc}	59.2
	CDC Verona	5.9	33.2^{abcde}	60.9
	Transcend	6.7	31.4^{bcde}	61.88
CWES	CDC Rama	8.3	28.2^{def}	63.5
CWGP	NRG003	9.2	28.4^{def}	62.5
	Minnedosa	10.9	30.2^{cde}	58.9
CWHWS	Snowstar	9.3	37.5^{a}	53.2
	Snowbird	9.0	33.9^{abcd}	57.2
CWRS	Glenn	12.0	36.4^{ab}	51.7
	CDC Stanley	8.8	36.9^{ab}	54.4
	CDC Utmost	11.0	38.5^{a}	50.6
CWSWS	AC Andrew	5.1	28.1^{ef}	66.9
	Sadash	4.0	23.7^{f}	72.4
Spelt	CDC Zorba	9.5	38.2^{a}	52.4
	CDC Origin	10.1	38.3^{a}	51.7
SEM[2]		0.71	1.10	1.66

[a - f] Means within a column not sharing a common superscript are significantly different ($P \leq .05$).
[1] CPS – Canadian Prairie Spring; CWAD – Canadian Western Amber Durum; CWES – Canadian Western Extra Strong; CWGP – Canadian Western General Purpose; CWHWS – Canadian Western Hard White Spring; CWRS – Canadian Western Red Spring; CWSWS – Canadian Western Soft White Spring.
[2] Pooled standard error of mean (n=4).

3.4.3. Correlations between grain characteristics and *in vitro* starch digestibility

Correlations of *in vitro* starch digestibility with grain characteristics are shown in Table 3.9. Total starch negatively correlated with starch digestibility at all time points examined. Crude protein was positively correlated with starch digestibility, but only during the early portions of the SI phases (15 and 30 min). Levels of NSP (total, insoluble, soluble) and AX (total, insoluble) positively correlated with starch digestibility at all time points (except at 180 min for total, insoluble and soluble NSP), with the size of the correlation decreasing with increasing digestion time. In contrast, soluble AX level was not correlated with starch digestibility. Amylose content negatively correlated with starch digestibility only at 240 min. No correlations were found between the proportions of large starch granules and starch digestibility at 15 and 30 min of the SI phase, but thereafter positive correlation coefficients were observed for the remainder of the times. For medium size starch granules, no correlations were found with starch digestibility at 15, 30 and 45 min, but thereafter a negative relationship was found. The proportion of small starch granules negatively correlated with starch digestibility for all time periods until 120 min. Stepwise regression analysis revealed the grain characteristics that explained the most variation in starch digestibility at different SI phase incubation times of the *in vitro* assay (Table 3.10). Regression coefficient values were cumulative for each of the time period.

Table 3.9. Correlations of starch digestibility at different small intestine phase incubation times of *in vitro* starch digestion assay with grain characteristics of wheat cultivars.

Time (min)	TS[1]	CP	Ash	TNSP	INSP	SNSP	TAX	IAX	SAX	Amylose	L. granules (>15 μm)	M. granules (5-15 μm)	S. granules (<5 μm)
15	-0.54[2]	0.29	0.37	0.76	0.71	0.79	0.66	0.66	NS	NS	NS	NS	-0.31
30	-0.53	0.29	0.39	0.75	0.71	0.74	0.64	0.65	NS	NS	NS	NS	-0.34
45	-0.42	NS	0.37	0.70	0.66	0.71	0.63	0.63	NS	NS	0.29	NS	-0.41
60	-0.37	NS	0.39	0.67	0.64	0.65	0.62	0.62	NS	NS	0.39	-0.28	-0.46
90	-0.48	NS	0.21	0.51	0.48	0.52	0.48	0.47	NS	NS	0.34	-0.31	-0.28
120	-0.39	NS	0.23	0.43	0.41	0.43	0.44	0.44	NS	NS	0.46	-0.40	-0.42
180	-0.30	NS	NS	NS	NS	NS	0.28	0.26	NS	NS	0.33	-0.39	NS
240	-0.40	NS	NS	0.34	0.32	0.36	0.35	0.34	NS	-0.28	0.33	-0.34	NS

[1] TS – Total starch; CP – Crude protein; TNSP – Total NSP; INSP – Insoluble NSP; SNSP – Soluble NSP; TAX – Total arabinoxylans; IAX – Insoluble arabinoxylans; SAX – Soluble arabinoxylans; L. granules – Large granules; M. granules – Medium granules; S. granules – Small granules.

[2] Correlation coefficient (r) is mentioned for all significant variables ($P \leq 0.05$).

n=72.

Table 3.10. Summary of stepwise regression selection of grain characteristics affecting *in vitro* starch digestibility

SI incubation time (min)	Grain characteristic	Regression coefficient (R^2)	*P*
15	SNSP[1]	0.63	<0.0001
	TNSP	0.66	0.0076
30	TNSP	0.59	<0.0010
	IAX	0.63	0.0044
	TAX	0.68	0.0035
45	TNSP	0.52	<0.0001
	SNSP	0.56	0.0096
60	TNSP	0.48	<0.0001
	Large granules	0.53	0.0112
	IAX	0.57	0.0171
	TAX	0.59	0.0414
90	Medium granules	0.45	0.0231
	CP	0.53	0.0357
	TS	0.55	0.0002
120	Large granules	0.30	0.0069
	TS	0.48	<0.0001
	CP	0.52	0.0169
180	Medium granules	0.15	0.0010
	TS	0.36	<0.0001
	SNSP	0.41	0.0205
	CP	0.48	0.0039
240	TS	0.17	0.0004
	Medium granules	0.43	<0.0001
	CP	0.47	0.0301

[1] SNSP – Soluble NSP; TNSP – Total NSP; IAX – Insoluble arabinoxylans; TAX – Total arabinoxylans; CP – Crude protein; TS – Total starch. n=72.

3.4.4. Correlations between grain characteristics

Correlation analysis among grain characteristics is presented in Table 3.11. Grain TS content negatively correlated with CP, ash, NSP (total, insoluble, soluble), AX (total, insoluble) and the percent medium sized starch granules. In contrast, TS was positively correlated with soluble AX and percent large starch granules. The level of CP positively correlated with ash, total and insoluble NSP, and level of medium and small size starch granules, CP negatively correlated with soluble AX and the percent large starch granules. Percent ash positively correlated with total, insoluble and soluble levels of NSP. Estimates of NSP (total, insoluble and soluble) highly correlated with each other as well as total and insoluble AX. All these fractions negatively correlated with the percent small starch granules. Positive correlations were found between soluble NSP, total AX, insoluble AX and soluble AX and the percent of large starch granules. Levels of total AX and soluble AX negatively correlated with the proportion of medium sized starch granules. The percent of large starch granules negatively correlated with medium and small size granules, and the percent of medium and small size starch granules were positively correlated.

Table 3.11. Correlation analysis among grain characteristics of wheat cultivars

	TS[1]	CP	Ash	TNSP	INSP	SNSP	TAX	IAX	SAX	Amylose	L. granules (>15 μm)	M. granules (5-15 μm)	S. granules (<5 μm)
TS	1	-0.78[2]	-0.38	-0.56	-0.55	-0.49	-0.35	-0.38	0.32	NS	0.25	-0.34	NS
CP		1	0.32	0.29	0.31	NS	NS	NS	-0.54	NS	-0.54	0.59	0.26
Ash			1	0.31	0.27	0.39	NS	NS	NS	NS	NS	NS	NS
TNSP[1]				1	0.99	0.82	0.93	0.95	NS	NS	NS	NS	-0.43
INSP					1	0.74	0.93	0.95	NS	NS	NS	NS	-0.42
SNSP						1	0.73	0.73	NS	NS	0.30	NS	-0.38
TAX							1	1	0.27	NS	0.38	-0.24	-0.51
IAX								1	NS	NS	0.35	NS	-0.50
SAX									1	NS	0.49	-0.56	NS
Amylose										1	NS	NS	NS
L. granules											1	-0.94	-0.78
M. granules												1	0.51
S. granules													1

[1] TS – Total starch; CP – Crude protein; TNSP – Total NSP; INSP – Insoluble NSP; SNSP – Soluble NSP; TAX – Total arabinoxylans; IAX – Insoluble arabinoxylans; SAX – Soluble arabinoxylans; L. granules – Large granules; M. granules – Medium granules; S. granules – Small granules.
[2] Correlation coefficient (r) is mentioned for all significant variables ($P \leq 0.05$).
n=72.

3.5. Discussion

Western Canadian wheat market classes and cultivars affect rate and extent of *in vitro* starch digestibility. In previous studies, it has been demonstrated that starch digestion extent is affected by Western Canadian wheat class (Yegani et al., 2013). However no research findings are available related to starch digestion rate in Western Canadian wheat classes, using chicken GI tract conditions. The extent of starch digestibility ranged from 91.0 to 101.3% (mean 96.2%), and is in accordance with the *in vitro* wheat starch digestibility results of Weurding et al. (2001b). The extent of starch digestibility in the current *in vitro* model was considered to be the value at 120 min of SI incubation time and equivalent to pre-fermentation starch digestibility at the terminal ileum of the digestive tract of broiler chickens (Weurding et al., 2001a). In Weurding et al. (2001b), *in vitro* starch digestion at 120 min in the SI phase was correlated with posterior jejunum starch digestibility, but not the terminal ileum as we hypothesized. However, that study used different starch sources having a broad range of starch digestibility including one wheat sample, and it is largely different from the current study that used only wheat samples that generally considered as rapidly digestible in chickens.

Starch digestibility rate values (starch digestibility % at different times of SI phase) are also in agreement with Weurding et al. (2001b). However, in the study of Ahuja et al. (2013) both the starch digestibility rate and extent are lower than these values, and it might be due to different conditions of two *in vitro* models, since Ahuja et al. (2013) study demonstrates human GI tract conditions, whereas our results were based on chicken GI tract environment that usually results in rapid starch digestion in comparison to humans. In addition, there was a starch digestion rate/extent variability of cultivars within a wheat class in our study, and there was no

such data available in literature. The significant differences of starch digestibility rates and extents of wheat classes/cultivars might be due to the following differences of grain characteristics.

Wheat can have a variable nutrient content (Yegani et al., 2013), based on both sample genotype and growing conditions. In agreement, levels of all nutrients analyzed in this research were affected by wheat market class and cultivar. Total starch content ranged from 52.2 to 58.8%, which is less than previously published values ranging from 68.6 to 69.8% (Hucl and Chibbar, 1996). In contrast, crude protein values of the wheat classes ranged from 15.0 to 22.9% and, were higher than values mentioned in Hucl and Chibbar (1996) (range from 12.8 to 17.0%). Appropriate standards and repeat analyses of samples confirmed the original analysis suggesting that analytical errors were not responsible for the variation in starch and protein levels from expected values. Grain growing conditions can have an important impact on nutrient content, and this may have been the case for these samples. Samples originating from research plots tend to have higher nitrogen fertilization rates than commercial production and, this may have been a reason for the increased protein levels (Gutierrez del Alamo et al., 2009b). Starch and protein are large components of grain composition and levels were negatively correlated in this work ($r = -0.78$). This value approximates correlations of -0.74 and -0.97 that found in recent studies (Ahuja et al., 2013, 2014).

Wheat amylose content was significantly different among wheat market classes and cultivars. The amylose content of wheat cultivars ranged from 18.4 to 32.0%, and indicates a wider range than previously analyzed values in the literature that range from 26.5 to 30.3% (Ahuja et al., 2014). The difference may relate to the method of analysis. Ahuja et al. (2013)

analyzed the amylose content of wheat using high-performance size exclusion chromatography (HPSEC), while the amylose content of wheat cultivars in this study was analysed using a Megazyme kit (Amylose/amylopectin method, K-TSTA 07/11, Megazyme International Ireland, Bray, Co. Wicklow, Ireland). The latter procedure uses Concanavalin A to precipitate amylopectin and leaves amylose to be measured in the resulting supernatant. The protocol mentions that Concanavalin A may also precipitate retrograded amylose, which would result in an under estimation of the amylose concentration. This is not in agreement with our results, and is unlikely because raw samples (without heat treatment) were analyzed, and therefore would not contain retrograde starch. Regardless, it is difficult to do a direct comparison of amylose values of these two studies.

Starch granule size distribution was significantly different among wheat market classes and cultivars. Small, medium and large starch granule size distributions ranged from 4.0 to 12.0, 23.7 to 38.5 and 50.6 to 72.4%, respectively. These values are in accordance with Ahuja et al. (2013). Similarly, non-starch polysaccharide and arabinoxylan values approximated previous values in the literature (Coles et al., 1997; Dornez et al., 2008; Gutierrez del Alamo et al., 2008), and were significantly different among wheat market classes and cultivars. Ash content range from 1.9 to 2.4%, and approximate the values of Canadian Grain Commission, (2015). Thus, these grain characteristics were within normal ranges according to previously analyzed values in literature.

Correlation analysis investigates the association of variables, but does not indicate a cause and effect relationship (Introductory statistics and analytics: a resampling perspective, 2015). Further, the ability of correlation analysis to establish relationships is also influenced by

the ranges in variable levels, with larger ranges more likely to result in a relationship. With these caveats, the current research used correlation analysis to investigate the association between grain characteristics and *in vitro* starch digestibility, as well as between grain characteristics. Total starch was negatively correlated with starch digestibility regardless of time in the SI phase. Although, increased starch content may be hypothesized to require longer digesting (Ahuja et al., 2013), relatively consistent correlations regardless of incubation time suggest that this is not the case. Since TS was negatively correlated with other nutrients including CP, ash, total NSP, soluble NSP, insoluble NSP, total and insoluble arabinoxylans, it is not possible to establish the factors which might impact starch digestibility.

Crude protein was positively correlated with starch digestion at the 15 and 30 min incubation times. Even at these times, the relationship was relatively weak and therefore of little predictive value. Wheat hardness is increased due to the strong interaction of starch granules and protein matrix (Barlow et al., 1973). Wheat having higher protein content is harder and contains strong gluten compared to wheat with low protein content. Hard wheat undergoes more starch damage compare to soft wheat (Pasha et al., 2010). Therefore higher protein content indirectly increases starch digestibility due to disruption of α-glycosidic linkages through starch damage, and it explains the positive correlation between starch digestion rate and CP. Ash was also positively correlated with starch digestion from 15 to 120 min of the SI phase. The range in ash values is quite small, suggesting that total ash *per se* is not the reason for the association. Specific components of ash or a chance association with starch digestion are more likely responsible for these correlations.

With the exception of soluble arabinoxylans, grain fibre content as estimated by measurement of NSP and arabinoxylans was positively correlated with starch digestibility with the relationships stronger earlier in the SI phase. Stepwise regression similarly showed a strong and positive association of total NSP, soluble NSP, total arabinoxylans and insoluble arabinoxylans with *in vitro* starch digestibility. Soluble arabinoxylans were not associated with starch digestion. Relatively strong correlations among fibre fractions preclude assigning responsibility to a specific fibre fraction.

In general, a positive relationship is opposite to a generally accepted negative association between soluble fibre and digestibility (Classen, 1996). Soluble NSP in wheat, mainly soluble arabinoxylans, increase viscosity of digesta, and decrease digesta passage rate (Choct et al., 1999). High digesta viscosity may also reduce the interaction between digestive enzymes and substrates like starch, and negatively affect the digestive tract microbiota (Choct et al., 1999). In addition, wheat starch can be entrapped in cell walls made up of NSP, and thereby reduce amylase access to this starch (Carre et al., 2007). Therefore, the positive correlations of starch digestibility with total NSP, insoluble NSP, soluble NSP, total arabinoxylans and insoluble arabinoxylans is unexpected based on the above mentioned theories.

Although there is no evidence from this work, it is possible that soluble and insoluble NSP together increase the time and energy required for grinding prior to *in vitro* testing. Samples were ground using a Retsch laboratory mill, and it is possible that increased grinding time affects starch damage, and as a result it increases starch digestibility. It is known in wheat processing for human consumption causes starch damage, and then increases starch digestibility (Saad et al., 2009). Little attention has been given to starch damage after particle size reduction in animal

feeding, and this possibility requires investigation. Another possible explanation for the lack of effect of soluble NSP may relate to the specific conditions of the *in vitro* assay. In addition, the above discussed digesta viscosity caused by soluble NSP in wheat is associated with chicken GI tract (*in vivo*), but the positive correlation of starch digestibility with soluble NSP was found in an *in vitro* experiment. The dry matter content of the *in vitro* model is much less than the digesta dry matter content in the middle to distal portion of the SI in chickens. The viscosity of a solution is strongly affected by its moisture content (Scott, 2002), and therefore viscosity is much lower inside the centrifuge tubes in the *in vitro* assay compared to digesta in the chicken GI tract. As a result, digesta viscosity is less likely play a negative role in *in vitro* starch digestibility.

Negative correlations of starch digestibility with small and medium starch granules, and a positive correlation of starch digestibility with large starch granules are in contrast to the results of Ahuja et al. (2013). The higher surface area of small starch granules is thought to increase enzyme-substrate interaction, which has the potential to increase starch digestibility (Ahuja et al., 2013). Nevertheless, our results were opposed to this theory. Direct comparison of starch digestibility between these studies is not possible because of major differences between the *in vitro* techniques used to assess starch digestibility. Differences included the use of a gastric phase prior to SI incubation for the current study, incubation temperature (41˚C vs. 37˚C) and grinding equipment (Retsch vs Udy). These factors all could affect the susceptibility of starch granules to hydrolysis. In addition, studying the relationship of starch digestibility with starch granule size distribution is problematic within one starch source (wheat) due to small ranges of starch granule size distribution, whereas it might be possible among different starch sources due to the increased variability of starch granule size distribution. In the current study, small and large starch granule size distributions among wheat cultivars were not statistically significant,

which further supports the above statement. Lindeboom et al. (2004) mentioned it is debatable whether starch granule size mainly affects digestive enzyme attack due to other proposed hypotheses, such as the presence of pores on starch granule surface impacting initial digestive enzyme attack. Starch hydrolysis is not only affected by starch granule size, but might also be affected by starch granule shape and complex formation of starch granules. Starch granule shape varies from spherical to polyhedral and the surface area to volume ratio is higher in polyhedral shaped granules compared to spherical granules, which increases digestive enzyme exposure. Some starch granules are packed together to make a granule complex, and it varied from single starch granules. These composite granules decrease digestive enzyme attack compare to separate granules. In addition, structural components including channels and blocklets present in starch granules also influence on starch hydrolysis (Tester et al., 2006). Therefore granule size, granule shape, granule complex and starch granule structural components together might impact starch hydrolysis rather than affecting this trait individually. In addition, amylopectin chain length distribution in starch granules may also affect *in vitro* starch digestibility (Ahuja et al., 2013).

The expected correlations were not observed between starch digestibility and some of the grain characteristics of wheat cultivars. There may be many factors that affecting starch digestibility other than the analyzed starch characteristics and nutrient constituents. Particle size, starch damage, crystallinity, amylopectin chain length distribution, associated compounds of starch granule surface including protein and lipid are some of the confounding factors that influence *in vitro* starch digestibility of wheat cultivars (Regmi et al., 2011; Ahuja et al., 2013).

Higher starch digestion extent refers to more complete SI starch digestion. Theoretically, it results in more energy retention in chicken, and therefore wheat samples having higher starch

digestion extent are obviously better than wheat samples with lower starch digestion extent. However starch digestion rate is not an indication of complete starch digestion, and it is related to time taken for starch digestion in the GI tract (Yegani et al., 2013). Impact of starch digestibility rate on bird performace parameters should be investigated to determine better starch digestion rates of wheat cultivars for broiler chickens, and it is important for accurate feed formulation in broiler chicken diets. In addition, specific grain characteristics which results in favorable starch digestion rates and extents can be used to develop better-quality wheat cultivars by plant breeding. The *in vitro* starch digestion model is a repeatable assay, and it was able to demonstrate genotypic differences in both estimated rate and extent of starch digestion. In addition, it requires less time and cost in comparison to *in vivo* broiler chicken experiments. However, the relevance and reliability of *in vitro* model need to confirm using *in vivo* comparison with the same wheat cultivars that used for the experiment.

4. STARCH DIGESTIBILITY AND APPARENT METABOLIZABLE ENERGY OF WESTERN CANADIAN WHEAT MARKET CLASSES IN BROILER CHICKENS

4.1. Abstract

Wheat is the primary grain fed to poultry in Western Canada, but its nutritional quality, including the nature of its starch digestibility, may be affected by wheat market class. The objectives of this study were to determine the rate and extent of starch digestibility of wheat market classes in broiler chickens, and to determine the relationship between starch digestibility and wheat AME_n. *In vitro* starch digestion was assessed using gastric and small intestinal phases mimicking the chicken digestive tract, while *in vivo* evaluation used 468 day-old male broiler chickens randomly assigned to dietary treatments (6 cages/treatment, 6 birds/cage) from 0 to 21 day of age. The study evaluated two wheat cultivars from each of six Western Canadian wheat classes: Canadian Prairie Spring (CPS), Canadian Western Amber Durum (CWAD), CW General Purpose (CWGP), CW Hard White Spring (CWHWS), CW Red Spring (CWRS) and CW Soft White Spring (CWSWS). All the samples were analyzed for grain characteristics. Data were analyzed as a randomized complete block design and cultivars were nested within market class. Pearson correlation was used to determine correlations. Significance level was $P \leq 0.05$. The starch digestibility range and wheat class rankings were: proximal jejunum – 23.7 to 50.6% ($CWHWS^{c}$, CPS^{bc}, $CWSWS^{bc}$, $CWRS^{ab}$, $CWGP^{a}$, $CWAD^{a}$); distal jejunum – 63.5 to 76.4% ($CWHWS^{c}$, CPS^{bc}, $CWSWS^{bc}$, $CWRS^{ab}$, $CWGP^{a}$, $CWAD^{a}$); proximal ileum – 88.7 to 96.9% ($CWSWS^{c}$, CPS^{bc}, $CWHWS^{bc}$, $CWRS^{b}$, $CWGP^{b}$, $CWAD^{a}$); distal ileum – 94.4 to 98.5% ($CWSWS^{b}$, $CWHWS^{b}$, CPS^{b}, $CWRS^{ab}$, $CWGP^{ab}$, $CWAD^{a}$); excreta – 98.4 to 99.3% (CPS^{b}, $CWRS^{b}$, $CWHWS^{b}$, $CWSWS^{ab}$, $CWGP^{ab}$, $CWAD^{a}$). Wheat class affected wheat AME_n with

levels ranging from 3203 to 3411 kcal/kg at 90% DM (CWRS[c], CWSWS[c], CPS[b], CWGP[b], CWAD[a], CWHWS[a]). Low to moderate positive correlations were observed for *in vivo* starch digestibility with kernel hardness index, ash content, and also with gain to feed ratio. Significant and moderately strong positive correlations were observed between *in vitro* and *in vivo* starch digestibility, but no correlations were found between AME_n and starch digestibility. In conclusion, rate and extent of starch digestibility and AME_n were affected by Western Canadian wheat class, but starch digestibility did not predict AME_n.

Key words: wheat, AME, energy retention, slowly digestible starch, rapidly digestible starch

4.2. Introduction

Wheat is primarily grown as human food, and the nature of wheat classes (cultivars) grown in Western Canada reflects the functional properties required by various segments of the human food industry. Wheat is also used in animal feeding, and is the main cereal grain used in poultry diets in Western Canada. Wheat is fed because of its relatively high energy content as well as to provide protein and other nutrients. Starch is the primary source of energy in wheat, and because of the relatively high inclusion of wheat in poultry diets, is an important contributor to diet AME. Wheat classes have been suggested to contribute to variability in wheat feeding value (Scott et al., 1998b; Yegani et al., 2013), and cultivars within a class may further increase this variability. Variability in the rate and extent of starch digestion in poultry as affected by wheat class and cultivar is poorly understood and warrants further investigation because of the potential effects of these characteristics on diet AME and poultry productivity.

The extent of starch digestion is relatively well studied in poultry nutrition, but less knowledge is available on rate of starch digestion among starch containing ingredients. Rate of starch digestion has been considered important in broiler nutrition as it may affect among other things, plasma insulin response, enterocyte health and beneficial carbohydrate fermentation (Weurding et al., 2003). A slow starch digestion results in a gradual but longer lasting insulin response, and it leads to a higher glucose supply to enterocytes in lower part of SI, and it minimizes protein degradation into amino acids since glucose is available as nutrients for enterocytes. Therefore muscle protein catabolism is minimized, and it facilitates feed efficiency in broiler chickens. In addition, slowly digestible starch results in gradual glucose absorption which results in less conversion of glucose into lactate in the gut wall compared to rapidly digestible starch. The converted lactate is absorbed into portal blood, and it is converted back into glucose inside the liver, and it requires energy. Therefore slowly digestible starch save energy that is required by this metabolic process, and therefore that energy is available for growth and production of birds (Weurding et al., 2003).

Starch that reaches the distal SI, caeca and colon may undergo microbial fermentation, resulting in the production of short chain fatty acids (SCFA). In turn, SCFA may reduce lumen pH, affect the nature of the microbial community and minimize the occurrence of enteric disease and digestive tract colonization by zoonotic organisms (Jozefiak et al., 2004). However, starch fermentation products (SCFA) result in less efficient energy utilization than enzymatic starch digestion in the SI (Weurding et al., 2001a). It means the energy derived from starch fermentation is less than the energy derived from starch digestion that occurs in SI in chickens. Starch fermentation does not represent the total starch digestibility of resistant starch, since the

majority of undigested starch bypass caeca, and only small, water soluble molecules and free granules can enter caeca (Carré, 2004). The rest of the undigested starch bypass the caeca, and may be fermented in the colon or lost in excreta.

The starch digestion characteristics of wheat have been demonstrated on several occasions. Wiseman et al. (2000) evaluated starch digestion in sixteen varieties of wheat (one sample per variety) and found higher starch digestibility rates were associated with higher AME. Weurding et al. (2001a) examined twelve starch sources including one wheat sample and found wheat starch was relatively rapidly digested compared to other feed ingredients in broiler chickens. Gutierrez del Alamo et al. (2009a) studied starch digestion rate in three cultivars, and each from two origins, and observed the rate of starch digestion varies among wheat cultivars and that environmental factors can also affect this trait. Cultivar affected rate of digestion, but digestibility values were only affected by cultivar in the proximal jejunum. Gutierrez del Alamo et al. (2009b) studied starch digestibility in three wheat cultivars grown under two nitrogen fertilizer rates (one sample per nitrogen fertilization rate) and found wheat starch was rapidly digested and digestion rate varied among cultivars. Overall, previous research including Chapter 3 results suggests that starch digestion rate and extent can be affected by grain genotype, and although there are exceptions, that wheat starch is rapidly digested.

Worldwide, the wheat AME results are variable. In addition, it is affected by wheat genotype and geographic conditions. A study which used twenty two wheat samples in broiler chickens resulted in a wide variation of AME value of 2627.3 - 3797.6 kcal/kg of DM (Mollah et al., 1983), which proves the variability of AME. Scott et al. (1998b) observed wheat AME variation among nine cultivars grown in replicate in three locations in each of two crop years in

Western Canada, and it is a descriptive study demonstrating genotypic and environmental effect on energy value of wheat. However, more research is required for further understanding of effect of Western Canadian wheat classes and cultivars on AME in broiler chickens as it is important for accurate feed formulation.

It was hypothesized that wheat market class/cultivar impacts on rate and extent of starch digestibility in broiler chickens, and that *in vitro* and *in vivo* starch digestibilities are positively correlated, and relate to grain AME_n. The objectives of this research were to determine the impact of wheat market class/cultivar on rate and extent of starch digestibility in broiler chickens, and to study the relationship between *in vitro* and *in vivo* starch digestibility assessments. A further objective was to relate *in vitro* and *in vivo* starch digestibility to wheat AME_n.

4.3. Materials and methods

The experimental procedure was approved by the Animal care committee of University of Saskatchewan and it was carried in accordance with the Guide to the Care and Use of Experimental Animals, Canadian Council on Animal Care (1993).

4.3.1. Birds and housing

A total of 468 one day old male (Ross × Ross 308) broiler chickens were obtained from a commercial hatchery, and housed six birds per cage in battery cages (51 cm length, 51 cm width, 46 cm height) with wire mesh floor. The wire mesh floor grid was 2.54 × 2.54 cm, but was covered by a removable 1.27 × 1.27 cm mesh from 0 to 7 d of age. Cages were located in two rows with back to back cages and each row had two levels. Room temperature was 32°C at day 0

and was gradually decreased by 2.8°C per week. Day length was 23 h from day 0 to 7 and 18 h from day 8 to 21. Light intensity was a minimum of 25 lux through-out the trial. Birds were provided with ad-libitum feed and water through-out the experiment. Each battery cage was equipped with a front mounted feed trough (51 cm length) and two height adjustable nipple drinkers. Extra feed and water were supplied by supplementary feeders (d 0 to 4 of age) and ice cube trays (d 0 to 5), respectively. Birds and feed intake were measured on a cage basis and treatments were randomly assigned to dietary treatments (13) and there were 6 replications per treatment.

4.3.2. Experimental diets

There were 12 wheat containing experimental diets and one basal diet used for wheat AME_n calculation. All 12 experimental diets were formulated using wheat as the only source of starch, and the wheat quantity was constant at 63.08%. The ingredients and calculated nutrient levels are presented in Table 4.1. Titanium oxide (TiO_2) was used as an indigestible marker to determine starch digestibility and AME. The basal diet contained the same ingredients as the experimental diets except wheat. All experimental diets were made by mixing basal diet with particular wheat cultivar. The basal diet was fed from d 14 to 21, and until d 14 a non-medicated commercial starter diet (crumbles) was given to these birds. The other experimental diets were fed from d 0 to 21. All diets were fed in mash form.

Table 4.1. Ingredients and calculated nutrient levels of experimental diets

Nutrient	Quantity (%)
Wheat	63.08
Soybean meal	25.17
Porcine meal	5.00
Canola oil	3.13
Mono-dicalcium phosphate	0.50
Limestone	1.35
Sodium chloride	0.32
Vitamin-mineral premix[1]	0.50
Choline chloride	0.10
Enzyme[2]	0.10
TiO_2	0.30
Nutrient, calculated	
AME (kcal/kg)	3050
Crude protein	22.54
Calcium	1.00
Non-phytate phosphorous	0.48
Sodium	0.18
Digestible arginine	1.32
Digestible lysine	1.14
Digestible methionine	0.59
Digestible methionine and cysteine	0.85
Digestible threonine	0.75

[1] Vitamin-mineral premix provided the following per kilogram of complete diet: vitamin A, 2,200,000 IU; vitamin D, 440,000 IU; vitamin E, 6000 IU; menadione, 400 mg; thiamine, 300 mg; riboflavin, 1200 mg; pyridoxine, 800 mg; vitamin B_{12}, 4 mg; niacin, 12,000 mg; pantothenic acid, 2000 mg; folic acid, 120 mg; biotin 30 mg; copper, 2000 mg; iron, 16,000 mg; manganese 16,000 mg; iodine, 160 mg; zinc, 16,000 mg; selenium, 60 mg; calcium carbonate 100,000 mg; Ethoxyquin 125 mg; wheat middlings 754,546 mg.

[2] Econase XT (ABVista, Wiltshire, UK), β 1-4 endo-xylanase enzyme, xylanase activity – 160,000 BXU/g.

Two wheat cultivars from each of six classes were selected on the basis of *in vitro* assessment of starch digestibility (Chapter 3), including cultivars having high as well as low starch digestibility. The classes and cultivars (in brackets) were Canadian Prairie Spring (CPS – 5702PR, Conquer), Canadian Western Amber Durum (CWAD – Transcend, CDC Verona), Canadian Western General Purpose (CWGP – Minnedosa, NRG003), Canadian Western Hard White Spring (CWHWS – Snowstar, Snowbird), Canadian Western Red Spring (CWRS – CDC Stanley, Glenn), and Canadian Western Soft White Spring (CWSWS Sadash, AC Andrew). All 12 spring wheat cultivars were grown in Saskatoon in the summer of 2014. Wheat cultivars were ground using a hammer mill (Model 160–D, Jacobson Machine Works, Minneapolis, MN 55427, USA) with a 3.97 mm screen-hole size. Particle size distribution of wheat samples post-grinding was analyzed in triplicate using dry sieving by a digital particle size analyzer (Hoskins scientific, Burlington, ON). The basal diet was mixed with each wheat cultivar to make treatment diets using a Hobart mixer (Hobart mixer, Model L–800, Hobart Canada, Don Mills, ON M3B 1B1).

4.3.3. Data collection

Feed intake (FI) and body weight (BW) were measured on cage basis weekly (d 7, 14 and 21) and, body weight gain (BWG) and gain to feed ratio were calculated based on these values. Mortality was recorded daily and, body weights of dead birds were used to correct the gain to feed ratio calculation.

4.3.4. Excreta collection

Clean aluminum trays were placed under each battery cage and excreta was collected on a cage basis at 12 h intervals for 36 h on d 20 (morning and evening) and 21 (morning). Feed and

feather contaminants were removed, and excreta were collected into polythene bags, and immediately frozen at -20°C. Excreta samples were later dried using a forced air oven (55°C) and pooled by replication.

4.3.5. Digesta collection

All birds were euthanized at day 21 by giving intravenous administration of T-61 (Embutramide, mebezonium iodide and tetracaine hydrochloride injectable euthanasia solution) into the brachial vein. After opening the bird carcass, the gastro-intestinal tract was removed and the small intestine (SI) divided into four sections, proximal jejunum (PJ), distal jejunum (DJ), proximal ileum (PI) and distal ileum (DI). The jejunum and ileum were separated at Meckel's diverticulum. The ileum was separated from the lower digestive tract by cutting 2 cm anterior to the ileo-caecal junction. The jejunum and ileum were separated into proximal and distal parts by dividing at the middle of each section. The digesta content from each SI section was gently squeezed out into a 50 ml plastic snap-cap vial. Digesta samples were pooled by replicate, and then stored at -20°C. Then the digesta samples were freeze-dried, and the samples were finely ground using a mortar and pestle.

4.3.6. Chemical analysis

Experimental diets and excreta were ground using a Retsch laboratory mill (Retsch ZM 200, Germany) using 1 mm (for GE, N and TiO_2 analysis) and 0.5 mm (for starch analysis) screen-hole sizes. Diets, excreta and SI digesta were analyzed for moisture, TiO_2 and total starch (TS). Diets and excreta were analyzed for gross energy (GE) and nitrogen (N). Wheat samples were analyzed for TS, crude protein (CP), ash, total dietary fibre (TDF), amylose content, kernel

hardness index (HI) and starch granule size distribution. Moisture was determined using standard procedure of AOAC (1995), and TiO_2 was determined using the procedure described by Myers et al. (2014). Gross energy was determined using an oxygen bomb calorimeter (Model A1435DDEB, Parr Instruments, Moline, IL, USA). Total starch was analyzed (method 996.11; AOAC, 1995) using a Megazyme analysis kit (Amyloglucosidase/α-amylase method, K-TSTA 07/11, Megazyme International Ireland Ltd., Bray Business Park, Bray, Co. Wicklow, Ireland). Nitrogen was analyzed using a Leco protein analyzer (Model Leco–FP–528L, Leco Corporation, St. Joseph, MA, USA), and 6.25 was used as the N to CP correction factor. Ash content was analyzed according to AOAC (1995) method 942.05 using a muffle oven (Model Lindberg/Blue BF51842C, Asheville, NC 28804, USA). Total dietary fibre content was determined (method 991.43; AOAC, 1990) using a Megazyme kit (Total dietary fibre assay procedure, K-TDFR 06/14, Megazyme International Ireland Ltd., Bray Business Park, Bray, Co. Wicklow, Ireland). Amylose content was determined using a Megazyme kit (Amylose/amylopectin assay procedure K-AMYL 07/11, Megazyme International Ireland Ltd., Bray Business Park, Bray, Co. Wicklow, Ireland). Starch granule size distribution (by volume) in purified starch of wheat cultivars was determined using a laser diffraction particle size analyzer (Hydro 2000S, Malvern Instruments, Malvern WR, UK). Kernel HI was determined by analyzing 300 individual kernels per wheat cultivar using a Perten Model SKCS 4100 Single Kernel Characterization System (Perten Instruments North America Inc., Springfield, IL). All samples were analyzed in duplicate for each chemical analysis. Wheat cultivars were analyzed for *in vitro* starch digestibility using the procedure described in Chapter 3.

4.3.7 Starch digestibility and AME_n calculation

Starch digestibility in each SI section was calculated using TS and TiO_2 values of diet and digesta using the following equation (Weurding et al., 2001a):

Starch digestibility (%) = $1 - [(\%\ TiO_{2\ diet} \div \%\ TiO_{2\ digesta}) \times (\%\ starch_{\ digesta} \div \%\ starch_{\ diet})] \times 100$

The same equation was used for total tract starch digestibility except % $starch_{\ digesta}$ was replaced by % $starch_{\ excreta}$.

Starch digestibility that occurs in the caeca and colon was calculated by subtracting distal ileum starch digestibility from total tract starch digestibility. The digestible starch content of diets was calculated by multiplying the level of starch in the wheat by the starch digestibility at various locations in the SI and in excreta.

Nitrogen corrected AME was determined using GE, N and TiO_2 values of diet and excreta. Diet AME_n values were calculated according to Hill and Anderson (1958). The following equations were used for calculations.

$AME_{n\ (Cal/g.diet)} = AME_{\ Cal/g.diet} - (8220 \times ANR_{\ g/g.diet})$

$AME_{\ Cal/g.diet} = GE_{\ Cal/g.diet} - [GE_{\ Cal/g.excreta} \times (\%\ TiO_{2\ diet} \div \%\ TiO_{2\ excreta})]$

$ANR_{\ g/g.diet} = N_{\ g/g.diet} - [N_{\ g/g.excreta} \times (\%\ TiO_{2\ diet} \div \%\ TiO_{2\ excreta})]$

Where:

$ANR_{g/g.diet}$ = Apparent N Retained (g/g of diet)

8220 = Correction factor (Cal per g N retained in the body)

Wheat AME_n was calculated according to the following formula (Scott et al., 1998a).

Wheat AME_n = (treatment diet AME_n – basal diet AME_n) × 100/63.08 (63.08% is the quantity of starch that includes in experimental diets)

4.3.8. Statistical analysis

The experimental design was a Randomized Complete Block Design (RCBD) as treatments were blocked by battery cage level to account for potential differences in light intensity and air flow pattern between levels. Wheat cultivars were nested within each wheat market class. Each experimental diet (treatment) had 6 replications (battery cages) with 6 birds per replication, and replications of each treatment were equally distributed in battery cage levels. Cage was the experimental unit. The experimental model is as follows:

$$Y_{ijr} = \mu + \alpha_i + \beta_{ij} + \varepsilon_{ijr}$$

(Y_{ijr} is the value of the dependent variable observed at the r^{th} replication with the first factor (α) at its i^{th} level and the second factor (β) at its j^{th} level, ε is the error, μ is the overall (fixed) mean of the sampling population and $\alpha_i + \beta_{ij} + \varepsilon_{ijr}$ are mutually uncorrelated random effects)

All data were analyzed using Proc Mixed model of SAS software (SAS 9.4, Carey, N.C. 2008). Data were checked for normality using Shapiro-Wilk test prior to other analysis. Fisher's Least Significant Difference (LSD) test was used to perform mean separation of treatments of wheat market classes and cultivars when a significant difference was found. Differences were

considered significant when $P \leq 0.05$. Correlation analyses were completed to examine the relationships between *in vivo* starch digestibility, *in vitro* starch digestibility and AME_n using Proc Corr of SAS (SAS 9.4, Carey, N.C. 2008). Both *in vivo* and *in vitro* starch digestibility of wheat cultivars were further correlated with grain characteristics.

4.4. Results

4.4.1. AME_n

Apparent metabolizable energy (kcal/kg, 90% DM basis) of wheat was affected by wheat market class, and cultivars nested within classes (Table 4.2). Wheat AME_n ranged from 3203 kcal/kg (CWRS) to 3411 kcal/kg (CWHWS). The consistency of AME_n for wheat cultivars within a class was assessed based on mean separation. Conquer (3338 kcal/kg) and 5702PR (3274 kcal/kg) within CPS, Transcend (3386 kcal/kg) and CDC Verona (3415 kcal/kg) within CWAD, and Snowstar (3446 kcal/kg) and Snowbird (3377 kcal/kg) within CWHWH were examples of AME_n consistency. However, other wheat classes failed to demonstrate AME_n consistency based on significant differences between cultivars; Minnedosa (3249 kcal/kg) and NRG003 (3415 kcal/kg) within CWGP, CDC Stanley (3291 kcal/kg) and Glenn (3116 kcal/kg) within CWRS, and Sadash (3348 kcal/kg) and AC Andrew (3135 kcal/kg) within CWSWS.

Table 4.2. Effect of class and cultivar on wheat AME_n in broiler chickens (d 21)

Wheat class	AME_n (kcal/kg, 90% DM)	Wheat cultivar	AME_n (kcal/kg, 90% DM)
CPS[1]	3306[b]	5702PR	3274[cd]
		Conquer	3338[bc]
CWAD	3400[a]	CDC Verona	3415[ab]
		Transcend	3386[ab]
CWGP	3332[b]	NRG003	3415[ab]
		Minnedosa	3249[d]
CWHWS	3411[a]	Snowstar	3446[a]
		Snowbird	3377[ab]
CWRS	3203[c]	Glenn	3116[e]
		CDC Stanley	3291[cd]
CWSWS	3241[c]	AC Andrew	3135[e]
		Sadash	3348[bc]
SEM[2]	14.48	SEM[2]	14.48

[a - e] Means within a column not sharing a common superscript are significantly different ($P \leq 0.05$).
[1] CPS – Canadian Prairie Spring; CWAD – Canadian Western Amber Durum; CWGP – Canadian Western General Purpose; CWHWS – Canadian Western Hard White Spring; CWRS – Canadian Western Red Spring; CWSWS – Canadian Western Soft White Spring.
[2] SEM – pooled standard error of mean (n=6).

4.4.2. *In vivo* starch digestibility

Starch digestibility at four SI locations was affected by wheat market class (Table 4.3). Starch digestibility at the PJ ranged from 23.7 (CWHWS) to 50.6% (CWAD) yielding a maximum difference of 26.9%. Starch digestibility at the DJ, PI and DI ranged from 63.5 (CWHWS) to 76.4% (CWAD), 88.7 (CWSWS) to 96.9% (CWAD), and 94.4 (CWSWS) to

98.5% (CWAD) with maximum differences of 12.8, 8.2 and 4.1%, respectively. Only 0.9% difference in total tract starch digestibility was seen among wheat classes with values ranging from 98.4 (CPS) to 99.3% (CWAD). Digestible starch content in different locations of the GI tract was also affected by wheat market class. Digestible starch in PJ ranged from 14.4 (CWHWS) to 31.4% (CWAD). Digestible starch of DJ, PI and DI ranged from 38.8 (CWHWS) to 47.5% (CWAD), 54.6 (CWSWS) to 60.3% (CWAD) and, 57.9 (CWHWS) to 62.4% (CPS) respectively. Total tract digestible starch range was from 60.3 (CWHWS) to 64.3% (CPS). Digestible starch content range was also gradually reduced when it reached the distal part of GI tract.

Starch digestibility values and digestible starch content in different wheat cultivars at 4 SI locations are presented in Table 4.4. Starch digestibility at PI and DI was affected by wheat cultivars nested within classes whereas, PJ, DJ and total tract starch digestibility values were not affected by wheat cultivar. Starch digestibility ranges of cultivars in all SI locations were similar but larger than the wheat market class digestibility values. Starch digestibility values for wheat cultivars within the CWHWS class were different (not consistent) for the PI and DI areas. Wheat cultivars within the CWSWS class were also not consistent for DI starch digestibility. As with the wheat class data, the range in starch digestibility decreased from the proximal to distal portions of the SI, and even further for total tract starch digestibility values. Slowly digestible starch (SDS) of wheat cultivars can be calculated from the difference between starch digestibility of distal ileum and proximal ileum according to the nature of digestibility values in 4 different SI locations. It ranged from 1.5 (Transcend) to 7.2% (AC Andrew), demonstrating an average of 3.7% (Table 4.5).

Table 4.3. Effect of wheat market class on starch digestibility and digestible starch content in small intestine segments in broiler chickens (d 21)

	Starch digestibility (%)					Digestible starch (%)				
Wheat class	Proximal jejunum	Distal jejunum	Proximal ileum	Distal ileum	Total tract	Proximal jejunum	Distal jejunum	Proximal ileum	Distal ileum	Total tract
CPS[1]	31.2^{bc}	65.2^{bc}	90.6^{bc}	95.5^{b}	98.4^{b}	20.4^{bc}	42.6^{bcd}	59.2^{ab}	62.4^{a}	64.3^{a}
CWAD	50.6^{a}	76.4^{a}	96.9^{a}	98.5^{a}	99.3^{a}	31.4^{a}	47.5^{a}	60.3^{a}	61.3^{ab}	61.7^{c}
CWGP	44.7^{a}	74.1^{a}	93.5^{b}	96.8^{ab}	98.9^{ab}	27.7^{a}	45.8^{ab}	57.9^{b}	59.9^{b}	61.2^{d}
CWHWS	23.7^{c}	63.5^{c}	91.7^{bc}	94.9^{b}	98.7^{b}	14.4^{c}	38.8^{d}	56.0^{c}	57.9^{c}	60.3^{f}
CWRS	41.7^{ab}	70.6^{ab}	92.9^{b}	96.6^{ab}	98.5^{b}	26.7^{ab}	45.1^{abc}	59.4^{ab}	61.8^{a}	63.0^{b}
CWSWS	31.9^{bc}	67.4^{bc}	88.7^{c}	94.4^{b}	98.8^{ab}	19.7^{c}	41.6^{cd}	54.6^{c}	58.0^{c}	60.8^{e}
SEM[2]	3.90	2.21	1.06	0.97	0.19	2.45	1.39	0.66	0.61	0.12

[a - f] Means within a column not sharing a common superscript are significantly different ($P \leq 0.05$).

[1] CPS – Canadian Prairie Spring; CWAD – Canadian Western Amber Durum; CWGP – Canadian Western General Purpose; CWHWS – Canadian Western Hard White Spring; CWRS – Canadian Western Red Spring; CWSWS – Canadian Western Soft White Spring.

[2] SEM – pooled standard error of mean (n=12).

Table 4.4. Effect of wheat cultivar on starch digestibility and digestible starch content in small intestine segments in broiler chickens (d 21)

Wheat class	Wheat cultivar	Starch digestibility (%)					Digestible starch (%)				
		PJ[1]	DJ	PI	DI	Total tract	PJ	DJ	PI	DI	Total tract
CPS[2]	5702PR	33.6	65.7	90.4^{cde}	94.5^{bcd}	98.0	22.3	43.5^{abc}	59.9^{ab}	62.65	65.0^{a}
	Conquer	28.8	64.8	90.7^{cde}	96.5^{ab}	98.7	18.6	41.8^{bcd}	58.5^{abcd}	62.17	63.6^{b}
CWAD	CDC Verona	51.0	77.0	96.8^{a}	98.6^{a}	99.4	31.9	48.1^{a}	60.5^{a}	61.62	62.1^{d}
	Transcend	50.1	75.8	97.0^{a}	98.5^{a}	99.2	31.0	46.8^{ab}	60.0^{ab}	60.89	61.3^{e}
CWGP	NRG003	40.7	74.4	93.2^{abcd}	95.9^{abc}	98.9	24.8	45.4^{ab}	56.9^{cde}	58.52	60.3^{f}
	Minnedosa	48.8	73.7	93.8^{abc}	97.7^{ab}	98.9	30.6	46.2^{ab}	58.9^{abc}	61.28	62.1^{d}
CWHWS	Snowstar	16.7	58.8	88.0^{e}	92.0^{d}	98.7	10.3	36.3^{d}	54.3^{ef}	56.73	60.9^{e}
	Snowbird	30.7	68.3	95.4^{ab}	97.7^{ab}	98.7	18.6	41.3^{bcd}	57.7^{bcd}	59.11	59.7^{g}
CWRS	Glenn	35.8	65.0	91.6^{bcde}	95.6^{abcd}	98.7	22.9	41.6^{bcd}	58.5^{abcd}	61.12	63.1^{c}
	CDC Stanley	47.6	76.1	94.2^{abc}	97.6^{ab}	98.3	30.5	48.6^{a}	60.2^{ab}	62.41	62.8^{c}
CWSWS	AC Andrew	26.1	64.3	89.3^{de}	96.5^{ab}	98.7	15.6	38.4^{cd}	53.3^{f}	57.59	58.9^{h}
	Sadash	37.7	70.5	88.2^{e}	92.2^{cd}	99.0	23.9	44.7^{ab}	55.9^{def}	58.43	62.7^{c}
SEM[3]		5.52	3.13	1.49	1.37	0.27	0.90	0.68	0.37	0.32	0.20

[a - h] Means within a column not sharing a common superscript are significantly different ($P \leq 0.05$).
[1] PJ – proximal jejunum; DJ – distal jejunum; PI – proximal ileum; DI – distal ileum.
[2] CPS – Canadian Prairie Spring; CWAD – Canadian Western Amber Durum; CWGP – Canadian Western General Purpose; CWHWS – Canadian Western Hard White Spring; CWRS – Canadian Western Red Spring; CWSWS – Canadian Western Soft White Spring.
[3] SEM – pooled standard error of mean (n=6).

Starch disappearance between the DI and excreta may be the result of microbial fermentation. When calculated in this research, starch disappearance was not affected by wheat market class, but it was affected by wheat cultivars nested within classes, and varied from 0.8% (CDC Verona) to 5.5% (5702PR) (Table 4.5).

Table 4.5. Effect of wheat class and cultivar on slowly digestible starch and, starch disappearance between the terminal ileum and total tract in broiler chickens (d 21)

Wheat class	SDS (%)	Starch disappearance (%)	Wheat cultivar	SDS (%)	Starch disappearance (%)
CPS[1]	4.9^{ab}	3.9	5702PR	4.1^{abc}	5.5^{a}
			Conquer	5.8^{ab}	2.2^{bcd}
CWAD	1.6^{c}	0.9	CDC Verona	1.7^{c}	0.8^{d}
			Transcend	1.5^{c}	0.9^{d}
CWGP	3.3^{abc}	2.4	NRG003	2.8^{bc}	3.5^{abcd}
			Minnedosa	3.9^{abc}	1.3^{d}
CWHWS	3.2^{bc}	3.1	Snowstar	4.0^{abc}	4.8^{ab}
			Snowbird	2.3^{c}	1.3^{cd}
CWRS	3.8^{abc}	2.0	Glenn	4.1^{abc}	3.0^{abcd}
			CDC Stanley	3.4^{bc}	0.9^{d}
CWSWS	5.6^{a}	3.2	AC Andrew	7.2^{a}	2.2^{bcd}
			Sadash	4.0^{abc}	4.2^{abc}
SEM[2]	0.36	0.06	SEM[2]	0.36	0.06

[a - d] Means within a column not sharing a common superscript are significantly different ($P \leq 0.05$).
[1] CPS – Canadian Prairie Spring; CWAD – Canadian Western Amber Durum; CWGP – Canadian Western General Purpose; CWHWS – Canadian Western Hard White Spring; CWRS – Canadian Western Red Spring; CWSWS – Canadian Western Soft White Spring.
[2] SEM – pooled standard error of mean (class: n=12/cultivar: n=6).

4.4.3. *In vitro* starch digestibility

In vitro starch digestibility is affected by wheat market class at all the time points of SI phase (Table 4.6). *In vitro* starch digestibility of wheat market classes increased (by starting from a mean of 42.4% at 15 minutes) gradually until reaching a plateau at 120 minutes (a mean value of 98.0%) of the SI phase. Starch digestibility varied from 38.9 (CWHWS) to 53.5% (CWAD) at 15 min of the SI phase, with a difference of 14.6% between maximum and minimum values. A difference of 16.9% was found between the minimum value of 84.2% (CWRS) and a maximum value of 101.1% (CWSWS) for starch digestibility at 60 min of the SI phase. Starch digestibility at 120 min varied from 93.9 (CWRS) to 102.0% (CWSWS), resulting a difference of 8.1%. *In vitro* starch digestibility is affected by wheat cultivar nested within market class at all the SI phase incubation times except 90 min (Table 4.7). *In vitro* starch digestibility ranges of wheat cultivars at different incubation times of SI phase were more or less similar to the values of wheat market classes.

Table 4.6. Starch digestibility (%) of wheat market classes at different incubation times of the small intestine phase of the *in vitro* starch digestion assay

Wheat class	Small intestine phase incubation time (min)					
	15	30	45	60	90	120
CPS[1]	39.3^{cd}	60.8^{c}	76.4^{d}	86.3^{e}	95.0^{c}	95.3^{c}
CWAD	53.5^{a}	77.8^{a}	92.0^{a}	97.0^{b}	98.4^{b}	99.0^{b}
CWGP	42.3^{b}	66.6^{b}	83.9^{c}	93.9^{c}	99.3^{b}	99.5^{b}
CWHWS	38.9^{d}	59.4^{cd}	72.6^{e}	91.2^{d}	96.2^{c}	98.5^{b}
CWRS	39.5^{cd}	59.0^{d}	73.2^{e}	84.2^{f}	92.1^{d}	93.9^{c}
CWSWS	40.7^{c}	66.8^{b}	86.7^{b}	101.1^{a}	103.1^{a}	102^{a}
SEM[2]	0.92	1.21	1.38	1.07	0.65	0.54

[a - f] Means within a column not sharing a common superscript are significantly different ($P \leq 0.05$). [1] CPS – Canadian Prairie Spring; CWAD – Canadian Western Amber Durum; CWGP – Canadian Western General Purpose; CWHWS – Canadian Western Hard White Spring; CWRS – Canadian Western Red Spring; CWSWS – Canadian Western Soft White Spring. [2] SEM – pooled standard error of mean (n=6).

Table 4.7. Starch digestibility (%) of wheat cultivars at different incubation times of the small intestine phase of the *in vitro* starch digestion assay

Wheat class	Wheat cultivar	Small intestine phase incubation time (min)					
		15	30	45	60	90	120
CPS[1]	5702PR	39.0^{ef}	60.1^{de}	73.4^{fg}	84.0^{g}	94.0	92.6^{d}
	Conquer	39.5^{def}	61.5^{de}	79.4^{de}	88.7^{f}	96.0	98.0^{c}
CWAD	CDC Verona	54.2^{a}	78.0^{a}	89.3^{b}	95.9^{c}	98.1	97.9^{c}
	Transcend	52.6^{a}	77.5^{a}	94.7^{a}	98.2^{b}	98.6	100.1^{abc}
CWGP	NRG003	43.1^{b}	65.2^{c}	82.2^{d}	93.8^{cd}	98.8	100.2^{abc}
	Minnedosa	41.6^{bcd}	68.1^{b}	85.5^{c}	94.1^{cd}	99.7	98.8^{bc}
CWHWS	Snowstar	38.3^{f}	59.3^{e}	74.4^{f}	92.3^{de}	97.2	97.9^{c}
	Snowbird	39.4^{ef}	59.5d^{e}	70.8^{gh}	90.2^{ef}	95.3	99.2^{bc}
CWRS	Glenn	38.4^{f}	56.0^{f}	68.8^{h}	80.4^{h}	90.9	93.9^{d}
	CDC Stanley	40.6^{cde}	61.9^{d}	77.6^{e}	88.0^{f}	93.3	93.9^{d}
CWSWS	AC Andrew	42.8^{bc}	68.7^{b}	88.0^{bc}	103.1^{a}	103.5	103.1^{a}
	Sadash	38.5^{ef}	65.0^{c}	85.4^{c}	99.1^{b}	102.8	100.8^{ab}
SEM[2]		0.92	1.21	1.38	1.07	0.65	0.54

[a - h] Means within a column not sharing a common superscript are significantly different ($P \leq 0.05$).

[1] CPS – Canadian Prairie Spring; CWAD – Canadian Western Amber Durum; CWGP – Canadian Western General Purpose; CWHWS – Canadian Western Hard White Spring; CWRS – Canadian Western Red Spring; CWSWS – Canadian Western Soft White Spring.

[2] SEM – pooled standard error of mean (n=3).

4.4.4. Grain characteristics and correlations with starch digestibility

Grain analysis results are shown in Table 4.8. Total starch, CP, ash, TDF and amylose values on a DM basis ranged from 59.7 (AC Andrew) to 66.3% (5702PR), 12.5 (Sadash) to 17.4% (Snowbird), 1.4 (Sadash) to 2.0% (CDC Verona and Transcend), 10.9 (Sadash) to 14.3% (Minnedosa) and 17.0 (NRG003) to 35.3% (CDC Verona), respectively. Kernel hardness index ranged from 23 (AC Andrew) to 86 (CDC Verona). Starch granule size distribution in wheat cultivars is shown in Table 4.9. Small granule size distribution ranged from 3.4 (AC Andrew) to 11.0% (Snowbird) whereas the proportion of medium granule size starch ranged from 18.6 (CDC Verona) to 40.0% (Snowbird). Large granule size starch varied from 49.0 (Snowbird) to 77.8% (CDC Verona).

Table 4.8. Grain characteristics of wheat cultivars (DM basis)

Wheat class	Wheat cultivar	TS[1] (%)	CP (%)	Ash (%)	TDF (%)	Amylose (%)	Kernel hardness index
CPS[2]	5702PR	66.3	15.5	1.5	11.8	22.1	54
	Conquer	64.4	16.0	1.6	13.5	28.1	43
CWAD	CDC Verona	62.5	16.5	2.0	12.3	35.3	86
	Transcend	61.8	16.4	2.0	12.2	30.7	79
CWGP	NRG003	61.0	16.1	1.6	13.5	17.0	63
	Minnedosa	62.7	16.2	1.6	14.3	23.5	60
CWHWS	Snowstar	61.7	17.3	1.6	11.7	25.1	63
	Snowbird	60.5	17.4	1.5	13.1	21.9	65
CWRS	Glenn	63.9	16.7	1.6	12.8	22.0	70
	CDC Stanley	63.9	17.2	1.7	12.8	29.7	58
CWSWS	AC Andrew	59.7	13.1	1.5	11.6	20.9	23
	Sadash	63.4	12.5	1.4	10.9	21.5	27

[1] TS – total starch; CP – crude protein; TDF – total dietary fibre. All the analyses were completed in duplicate for one sample for each wheat cultivar.
[2] CPS – Canadian Prairie Spring; CWAD – Canadian Western Amber Durum; CWGP – Canadian Western General Purpose; CWHWS – Canadian Western Hard White Spring; CWRS – Canadian Western Red Spring; CWSWS – Canadian Western Soft White Spring.

Table 4.9. Starch granule size distribution (volume %) of wheat cultivars

Wheat class	Wheat cultivar	Starch granule size distribution (volume %)		
		Small (<5 μm)	Medium (5-15 μm)	Large (>15 μm)
CPS[1]	5702PR	6.9	26.0	67.1
	Conquer	8.2	29.2	62.7
CWAD	CDC Verona	3.6	18.6	77.8
	Transcend	5.1	22.9	72.0
CWGP	NRG003	8.4	31.5	60.1
	Minnedosa	7.9	28.5	63.7
CWHWS	Snowstar	9.3	38.6	52.1
	Snowbird	11.0	40.0	49.0
CWRS	Glenn	6.9	30.0	63.1
	CDC Stanley	10.4	38.8	50.7
CWSWS	AC Andrew	3.4	27.6	69.1
	Sadash	4.9	23.7	71.4

[1] CPS – Canadian Prairie Spring; CWAD – Canadian Western Amber Durum; CWGP – Canadian Western General Purpose; CWHWS – Canadian Western Hard White Spring; CWRS – Canadian Western Red Spring; CWSWS – Canadian Western Soft White Spring. All the analyses were completed in duplicate for one sample for each wheat cultivar.

No significant correlations were observed between *in vitro* starch digestibility and CP, ash, TDF, amylose and kernel hardness index. However significant negative correlations were found between small starch granule proportion and *in vitro* starch digestibility at 30 (r = -0.66) and 45 min (r = -0.67). In addition, *in vitro* starch digestibility at 15 (r = -0.62), 30 (r = -0.69) and 45 min (r = -0.67) negatively correlated with the proportion of medium starch granules. Further *in vitro* starch digestibility at 15 (r = 0.61), 30 (r = 0.70) and 45 min (r = 0.69) of the SI

phase positively correlated with proportion of large starch granules. Significant negative correlations were observed between TS and *in vitro* starch digestibility at 60 (r = -0.65) and 120 (r = -0.79) min.

Kernel hardness index and ash content were the only two grain characteristics that resulted in significant correlations with *in vivo* starch digestibility. Kernel hardness index positively correlated with starch digestibility in PJ (r = 0.69) and PI (r = 0.75). Ash content positively correlated with starch digestibility in PJ (r = 0.69), PI (r = 0.75), DI (r = 0.62) and total tract (r = 0.58).

4.4.5. Correlations between *in vivo* and *in vitro* starch digestibility

In vivo starch digestibility in PJ and DJ positively correlated with *in vitro* starch digestibility at 15 and 30 min whereas starch digestibility in PI and DI positively correlated with *in vitro* starch digestibility only at 15 min of SI phase. Total tract *in vivo* starch digestibility positively correlated with *in vitro* starch digestibility at all the SI phase incubation times until 120 min except 90 min (Table 4.10).

Table 4.10. Correlations between *in vitro* starch digestibility at different small intestine phase incubation times and *in vivo* starch digestibility at various sites in the broiler small intestine

SI incubation time (min)	Starch digestibility				
	PJ[1]	DJ	PI	DI	Total tract
15	0.63[2]	0.64	0.71	0.63	0.71
30	0.62	0.65	NS	NS	0.74
45	NS	NS	NS	NS	0.68
60	NS	NS	NS	NS	0.59
90	NS	NS	NS	NS	NS
120	NS	NS	NS	NS	0.59

[1] PJ – proximal jejunum; DJ – distal jejunum; PI – proximal ileum; DI – distal ileum.
[2] Correlation coefficient (r) presented for all significant variables ($P \leq 0.05$).
n=12.

4.4.6. Correlation of starch digestibility and AME_n

No correlations were found for AME_n with either *in vivo* or *in vitro* starch digestibility. Further, there were no correlations between AME_n and *in vivo* digestible starch content.

4.4.7. Wheat particle size distribution

Relative proportion of particle size distribution and mean particle size after hammer mill grinding were affected by wheat market class and cultivar, and is presented in Tables 4.11 and 4.12, respectively. No significant correlations were observed for relative proportion of particle size distribution of wheat cultivars with either i*n vivo* or *in vitro* starch digestibility.

Table 4.11. Relative proportion of particle size distribution (%) in wheat market classes

Wheat class	Particle size distribution by volume (%)								
	≤ 300 µm	> 300 ≤ 425 µm	> 425 ≤ 600 µm	> 600 ≤ 850 µm	> 850 ≤ 1180 µm	> 1180 ≤ 1700 µm	> 1700 ≤ 2360 µm	> 2360 µm	Mean particle size (µm)
CPS[1]	15.6[a]	6.6[ab]	8.2[ab]	13.2[ab]	17.4[a]	23.1[cd]	11.6[c]	2.8[c]	857.8[c]
CWAD	8.9[c]	6.4[ab]	8.5[a]	13.8[a]	17.5[a]	25.8[ab]	14.4[b]	4.0[b]	954.5[b]
CWGP	12.5[b]	5.8[b]	7.5[b]	11.7[c]	16.4[b]	25.6[ab]	16.0[b]	4.2[b]	945.5[b]
CWHWS	11.6[b]	4.6[c]	5.8[c]	9.7[d]	14.4[d]	26.9[a]	20.6[a]	5.8[a]	1030.5[a]
CWRS	13.4[b]	5.9[ab]	7.8[ab]	12.3[bc]	15.9[c]	24.6[bc]	14.9[b]	4.6[b]	923.0[b]
CWSWS	16.7[a]	7.0[a]	8.5[a]	13.3[a]	17.2[a]	21.4[d]	11.7[c]	3.3[c]	841.0[c]
SEM[2]	0.73	0.35	0.35	0.33	0.16	0.72	1.04	0.23	21.45

[a - d] Means within a column not sharing a common superscript are significantly different ($P \leq 0.05$).

[1] CPS – Canadian Prairie Spring; CWAD – Canadian Western Amber Durum; CWGP – Canadian Western General Purpose; CWHWS – Canadian Western Hard White Spring; CWRS – Canadian Western Red Spring; CWSWS – Canadian Western Soft White Spring.

[2] SEM- pooled standard error of mean (n=3).

Table 4.12. Relative proportion of particle size distribution (%) in wheat cultivars

Wheat class	Wheat cultivar	Particle size distribution by volume (%)									
		≤ 300 µm	> 300 ≤ 425 µm	> 425 ≤ 600 µm	> 600 ≤ 850 µm	> 850 ≤ 1180 µm	> 1180 ≤ 1700 µm	> 1700 ≤ 2360 µm	> 2360 µm	Mean (µm)	SD of Mean
CPS[1]	5702PR	14.2^{bc}	6.3^{abc}	7.8^{bc}	13.1^{b}	17.5^{bc}	24.0^{bcd}	12.5^{ef}	2.8^{d}	884.3^{def}	2.30
	Conquer	16.9^{ab}	7.0^{ab}	8.6^{abc}	13.4^{b}	17.4^{c}	22.3^{de}	10.7^{f}	2.8^{d}	831.3^{ef}	2.35
CWAD	CDC Verona	10.2^{de}	7.5^{a}	9.8^{a}	15.5^{a}	18.3^{a}	23.6^{bcd}	11.0^{f}	3.2^{cd}	883.0^{def}	2.22
	Transcend	7.6^{e}	5.2^{cd}	7.3^{cd}	12.1^{bcd}	16.6^{d}	28.1^{a}	17.8^{bc}	4.8^{b}	1026.0^{ab}	2.13
CWGP	NRG003	12.7^{cd}	5.6^{bcd}	7.2^{cd}	11.0^{de}	15.3^{e}	25.4^{abc}	17.8^{bc}	4.9^{b}	963.3^{bcd}	2.30
	Minnedosa	12.2^{cd}	6.0^{bc}	7.8^{bc}	12.5^{bc}	17.6^{bc}	25.9^{ab}	14.2^{de}	3.5^{cd}	927.7^{cd}	2.25
CWHWS	Snowstar	11.1^{d}	4.2^{d}	5.3^{e}	9.0^{f}	14.2^{g}	28.0^{a}	21.8^{a}	5.5^{ab}	1055.7^{a}	2.22
	Snowbird	12.2^{cd}	5.0^{cd}	6.3^{de}	10.4^{ef}	14.6^{fg}	25.9^{ab}	19.4^{ab}	6.1^{a}	1005.3^{abc}	2.27
CWRS	Glenn	12.6^{cd}	6.3^{abc}	8.3^{bc}	13.2^{b}	16.5^{d}	23.3^{bcd}	13.7^{def}	5.3^{ab}	912.3^{de}	2.28
	CDC Stanley	14.1^{bc}	5.5^{bcd}	7.3^{cd}	11.4^{cde}	15.3^{ef}	26.0^{ab}	16.0^{cd}	3.8^{c}	933.7^{cd}	2.31
CWSWS	AC Andrew	14.6^{bc}	6.3^{abc}	7.8^{bc}	13.2^{b}	18.1^{ab}	22.8^{cde}	12.8^{ef}	3.4^{cd}	880.3^{def}	2.30
	Sadash	18.9^{a}	7.7^{a}	9.2^{ab}	13.4^{b}	16.3^{d}	20.1^{e}	10.7^{f}	3.1^{cd}	801.7^{f}	2.39
SEM[2]		0.54	0.20	0.23	0.30	0.23	0.15	0.64	0.20	14.39	

[a - g] Means within a column not sharing a common superscript are significantly different ($P \leq 0.05$).

[1] CPS – Canadian Prairie Spring; CWAD – Canadian Western Amber Durum; CWGP – Canadian Western General Purpose; CWHWS – Canadian Western Hard White Spring; CWRS – Canadian Western Red Spring; CWSWS – Canadian Western Soft White Spring.

[2] SEM – pooled standard error of mean (n=3).

4.4.8. Performance variables

The experiment was not primarily designed to investigate the effect of wheat sample on bird performance, but FI, BW, BWG and gain to feed ratio were determined mainly to estimate the physiological status of birds in the experimental period. Performance variables of broiler chickens were within normal range at 0-21 d period of production cycle (Table 4.13) when comparing to Ross 308 broiler performance objectives (Aviagen 2014). Mortality was 1.5% for the entire period of the trial and there was no treatment effect on mortality. All the performance variables were affected by wheat market class. Gain: feed positively correlated with starch digestibility in PJ, DJ and PI. However, there were no correlations of starch digestibility with BW, BWG and FI (Table 4.14).

Table 4.13. Effect of wheat class and cultivar on performance variables of broiler chickens (0-21 d)

Wheat class	BW[1] (g/bird)	BWG (g/bird)	FI (g/bird)	Gain: feed (g/g)	Wheat cultivar	BW (g/bird)	BWG (g/bird)	FI (g/bird)	Gain: feed (g/g)
CPS[2]	898.0^{c}	854.7^{c}	1046.4^{c}	0.82^{c}	5702PR	895.0	848.8^{d}	1066.2	0.80^{b}
					Conquer	901.0	860.5^{d}	1026.5	0.84^{ab}
CWAD	1024.3^{a}	980.7^{a}	1131.6^{ab}	0.87^{a}	CDC Verona	1022.3	975.5^{abc}	1127.8	0.87^{a}
					Transcend	1026.3	985.8^{abc}	1135.4	0.87^{a}
CWGP	1023.8^{a}	982.8^{a}	1148.1^{ab}	0.86^{ab}	NRG003	1047.5	1012.2^{a}	1166.6	0.87^{a}
					Minnedosa	1000.0	953.3^{abc}	1129.7	0.84^{a}
CWHWS	997.1^{ab}	957.1^{ab}	1113.3^{abc}	0.86^{ab}	Snowstar	985.3	939.3^{abc}	1102.7	0.85^{a}
					Snowbird	1008.8	974.8^{abc}	1123.9	0.87^{a}
CWRS	973.4^{b}	925.8^{b}	1108.9^{bc}	0.83^{bc}	Glenn	982.5	935.0^{bc}	1122.3	0.84^{ab}
					CDC Stanley	964.3	916.5^{cd}	1095.6	0.84^{ab}
CWSWS	1029.7^{a}	994.4^{a}	1177.1^{a}	0.85^{abc}	AC Andrew	1030.8	1006.2^{ab}	1175.3	0.87^{a}
					Sadash	1028.5	982.7^{abc}	1178.9	0.84^{ab}
SEM[3]	8.658	9.167	10.345	0.004		8.658	9.167	10.345	0.004

$^{a - d}$ Means within a column not sharing a common superscript are significantly different ($P \leq 0.05$).

[1] BW – body weight; BWG – body weight gain; FI – feed intake.

[2] CPS – Canadian Prairie Spring; CWAD – Canadian Western Amber Durum; CWGP – Canadian Western General Purpose; CWHWS – Canadian Western Hard White Spring; CWRS – Canadian Western Red Spring; CWSWS – Canadian Western Soft White Spring.

[3] SEM- pooled standard error of mean (n=12).

Table 4.14. Correlations of *in vivo* starch digestibility at different small intestine segments with performance variables at 0-21 d period of broiler chickens.

Performance variable	Starch digestibility				
	PJ	DJ	PI	DI	Total tract
BW[1]	NS[2]	NS	NS	NS	NS
BWG	NS	NS	NS	NS	NS
FI	NS	NS	NS	NS	NS
Gain: feed	0.72	0.57	0.41	NS	NS

[1]PJ – proximal jejunum; DJ – distal jejunum; PI – proximal ileum; DI – distal ileum.
[2] BW – body weight; BWG – body weight gain; FI – feed intake.
[3] Correlation coefficient (r) presented for all significant variables ($P \leq 0.05$).
n=12.

4.5. Discussion

Establishing the impact of grain class or cultivar on grain nutritional value is difficult because of the cost and effort required to properly replicate each class or cultivar. Many studies on this topic have used one replication per cultivar, and as such grain nutritional value can be affected by both the genotype of the grain and also its growing conditions. Scientifically, this type of study should be considered a comparison of grain samples. A notable exception is research completed by Scott et al. (1998b) who studied the impact of wheat and barley cultivar on AME by using six replications (two replicate sources grown at three growing locations in each of two crop years) per cultivar. Although not compared statistically, grain class effects could be seen because the study used two or three cultivars per class. The current study used one sample of each of two cultivars per wheat class and does not reach the level of statistical comparison accomplished by Scott et al. (1998b). Based on this reservation in the current study, the AME_n (kcal/kg of 90% DM basis) of wheat was affected by wheat market class and wheat cultivars nested within classes. Further, the values for CPS, CWAD and CWRS were in close agreement with the AME_n results of Scott et al. (1998b). In contrast, AME_n values for CPS, CWAD, CWHWS, CWRS and CWSWS classes in the current study were higher than those found by Yegani et al. (2013). The latter study used one cultivar per wheat market class to determine energy retention, and this might account for the differences compared to the current study and Scott et al. (1998b). However, it is more probable that using test diets without a supplemental endo-xylanase, and completing the testing at a younger age (13 vs 21 d) contributed to the lower values of Yegani et al. (2013). Enzyme addition has been shown to have a positive impact on Western Canadian wheat AME_n (Scott et al., 1998b), and AME_n

determinations in young birds are lower than older birds (Scott et al., 1998a; Gutierrez del Alamo et al., 2008). The Canadian Western Amber Durum wheat class consistently ranked highest in terms of AME in all the above mentioned studies despite different conditions used, indicating high nutritional value of this Canadian wheat class for poultry.

In the current study, the AME_n of wheat cultivars within CPS, CWAD, and CWHWS classes were consistent. These results suggest that the functional properties used to select cultivars within these classes impact AME_n. However, the cultivars within CWGP, CWRS, and CWSWS classes were significantly different from one another for AME_n. Canadian Western Soft White Spring and CWGP are relatively minor wheat market classes in Western Canada and both of them have comparatively low protein content. Moreover, CWGP is mostly used for ethanol production and animal feed production. Therefore CWGP and CWSWS might not have been selected as extensively or specifically for specific functional grain properties. Wheat cultivars belong to CWRS were also not consistent in terms of energy retention. It is a major Western Canadian wheat market class, having the highest average annual production and, a high selection pressure for gluten quality and bread production. However, it is expected CWRS to have a selection for energy yield indirectly due to its' specific nutrient value including protein content. The use of only two, un-replicated cultivars for each class may also have impacted the ability to judge AME_n consistency within a class.

In vivo starch digestibility determined in all four segments of SI as well as in excreta (total tract digestibility) was affected by wheat market class. In turn, this demonstrates that both the rate and extent of starch digestion vary among Western Canadian market classes. Little research has examined rate of starch digestion in wheat cultivars, and none has studied the

impact of wheat market class. Gutierrez del Alamo et al. (2009a) studied rate of starch digestion in three wheat cultivars, each from two origins. Starch digestibility ranged from 23.7 to 50.6% in the PJ, 63.5 to 76.4% in the DJ, 88.7 to 96.9% in the PI and 94.4 to 98.5% in the DI in the current study, in contrast to 41.9 to 56.1, 77.4 to 80.0, 92.9 to 95.2 and 95.2 to 96.1% were reported by Gutierrez del Alamo et al. (2009a) for the same SI sections. Weurding et al. (2001a) determined starch digestibility in one wheat sample and reported values of 88.2, 92.9 and 94.4% for the DJ, PI and DI, respectively. In general, values from these studies were higher and less variable than the present study, possibly due to differences in genotypic and environmental effects for European wheat. In addition, more variability in the present study could have been affected by the larger number and more diverse classes examined. In conclusion, wheat starch has been shown to be rapidly digested and the research from the current study is in general agreement. The significance of differences in digestibility in the various sections of the SI will be discussed later in the manuscript.

Many factors affect starch digestibility, but as noted above, grain genotype plays a role. Starch digestibility comparisons of Western Canadian wheat classes have not studied extensively. In comparison to the relatively high DI digestibility (94.4 to 98.5%) in the current study, Yegani et al., (2013) reported values ranging 84.0 to 92.0%. The lower values found in this study likely relate to not using an endo-xylanase in test diets, bird age at testing and importantly the use of digesta from the entire ileum rather than the DI to obtain starch digestibility values.

The extent of starch digestion in chickens is often estimated by values derived in the terminal ileum, while comparisons of the terminal ileum and total tract digestibility are assumed

to be the result of starch fermentation in the caeca and colon. Starch, including from wheat, is generally well digested in chickens (Weurding et al., 2001a; Gutierrez del Alamo et al., 2008), but research has found that in some cases, the digestibility of wheat starch can be low and affect its feeding value (Mollah et al., 1983; Rogel et al., 1987; Yegani et al., 2013). Differences in starch digestibility between the DI and total tract estimates (fermentation) were not affected by wheat market class in this work, but differences ranged from 0.9 to 3.9%. The role of caecal and colonic fermentation in these differences remains undefined. Previous comparisons of DI and excreta starch digestibility values have not been consistent with some showing clear differences (Svihus and Hetland, 2001; Gutierrez del Alamo et al., 2009a; b) and others none (Yutste et al., 1991; Steenfeldt et al., 1998; Ankrah et al., 1999; Weurding et al., 2001a). The caeca are the primary site of fermentation, and entry is limited to soluble and small particle fractions (Svihus et al., 2013), and starch encapsulation by NSP, heat processing and retrograded starch could impact entry of undigested starch into caeca in chicken. In addition, it is possible that some fermentation can occur after defaecation, with factors such as frequency of excreta collection and excreta moisture level affecting this possibility. In addition, site of digesta collection in the ileum (entire ileum vs. distal ileum) also is a factor that affects estimation of starch fermentation. In the case of collecting digesta from the entire ileum, differences between ileal and total tract starch digestibility cannot be totally attributed to starch fermentation. This is demonstrated by differences of 4% or less for the current study and Gutierrez del Alamo et al. (2009a), which both used the distal ileum for measuring starch digestibility, in comparison to the 10% difference noted by Yegani et al. (2013) using content from the entire ileum.

Grain characteristics of wheat cultivars including TS, CP, ash, small, medium and large starch granule size distribution were in accordance with the results of another study on Canadian wheat classes (Ahuja et al., 2013). Kernel hardness index values for wheat cultivars were also in general agreement with previous research (Carré et al., 2002). No significant correlations were observed between *in vitro* starch digestibility and grain characteristics except for starch granule size distribution and TS. *In vitro* starch digestibility was positively correlated with large granule distribution, whereas it was negatively correlated with both small and medium granule proportions, and correlations were moderately strong. These results are similar to the correlations found in Chapter 3 (section 3.4.3), but are in contrast to the results of Ahuja et al. (2013). A direct comparison of the current study with Ahuja et al. (2013) is difficult due to differences in the *in vitro* models. Differences included incubation temperature (41.0 vs 37.5°C) and grinding equipment (Retsch laboratory mill vs. UDY mill). Total starch content negatively correlated with *in vitro* starch digestibility, and it is in accordance to Ahuja et al. (2013). Kernel hardness index positively correlated with *in vivo* starch digestibility at several SI segments. It may be explained by greater hardness results in a higher proportion of damaged starch (Pasha et al., 2010), and this damaged starch increases water absorption capacity and ultimately increases starch digestion (Barrera et al., 2007).

In vitro starch digestibility at initial incubation times of the SI phase positively correlated with *in vivo* starch digestibility in the small intestine (PJ and DJ – with 15 and 30 min; PI and DI – with 15 min) as well as for the total digestive tract (r range – from 0.62 to 0.74). However, later *in vitro* starch digestibility values were only correlated with total tract *in vivo* values (r range – 0.59 to 0.68; Table 4.10). Weurding et al. (2001b) compared the *in vitro* and *in vivo* rate and

extent of starch digestibility for a wide range of diets (twelve starch sources including wheat) and found a strong positive correlation (r = 0.87) between the two methods of assessing the rate of starch digestion. In Weurding et al. (2001b), experimental diets were used for both *in vitro* and *in vivo* assessment of starch digestion, which may be more precise than the comparison of *in vitro* assessment of wheat sources with *in vivo* assessment of diets in the current work. In the same experiment by Weurding et al. (2001b), starch digestibility values after 2 h and 4 h of SI incubation were highly correlated with *in vivo* starch digestibility at the distal jejunum (r = 0.94) and distal ileum (r = 0.96), respectively. The study by Weurding et al. (2001b) used diets that contained starch ranging from rapidly (wheat) to slowly digested (potato starch), and this broad range may increase the probability of a stronger relationship. The narrow range of starch digestion rate and the other mentioned limitations might be the cause for moderate strong correlations between *in vivo* and *in vitro* starch digestibility in the current study. Despite the presence of correlations only at early incubation times of SI phase (PJ to DI), the *in vitro* digestion assay still has the ability to predict *in vivo* starch digestion in chickens, and it is particularly useful in comparing large numbers of samples. The assay is repeatable and is more time and cost efficient than *in vivo* trials.

The current research failed to demonstrate a relationship between starch digestion (rate or extent) and grain digestible starch content with wheat AME_n. This is in contrast to previous research showing a strong relationship (Mollah et al., 1983; Rogel et al., 1987; Wiseman et al., 2000) between these traits, but in agreement with other work (Gutierrez del Alamo et al., 2008; Yegani et al., 2013). Gutierrez del Alamo et al. (2008) did not find a relationship of AME_n with excreta (total tract) starch digestibility or grain digestible starch content, while Yegani et al.

(2013) similarly found no relationship between AME_n of wheat and ileal starch digestibility. Many differences exist between previous research reports, but one factor that may account for the lack of agreement is the relatively narrow range in total starch content (59.7 – 66.3%), digestible starch and starch digestibility (Table 4.4) in the current research. This may reduce the predictive ability of these parameters, despite the importance of starch in the total energy availability from wheat. In contrast, studies by Mollah et al. (1983), Rogel et al. (1987) and Wiseman et al. (2000) had broader range of digestible starch content and starch digestibility that likely enhanced the potential for a stronger correlation.

There were no correlations between AME_n and performance variables. Historically, chickens have been thought to have an ability to control feed intake according to AME_n of feed, but this relationship has been questioned by many including Classen (2016). Based on this premise, differences in performance as a result of wheat energy levels would only occur if the diets were deficient in energy and, protein (amino acids) were redirected for use as energy instead of protein synthesis. The lack of effect seen in this work, and also in Scott et al. (1999) study supports the latter conclusion. Scott et al. (1999) observed significant positive correlations between AME_n in wheat and barley with BW and negative correlations between AME_n and feed to gain ratio, but the strength of correlations was not sufficient to predict the performance variables using AME_n values.

In the current research, strong positive correlations were found between *in vivo* starch digestibility (in PJ, DJ and PI) and gain to feed ratio. This finding suggests that rapid starch digestion (more anterior in the SI) results in a better feed efficiency in broiler chickens and is therefore a desirable trait in starch containing feed ingredients. This argues against the

suggestion that slowly digested starch beneficially affects feed efficiency (Weurding et al., 2003) and indicates the need for more extensive research in this area. However, an accurate assessment of broiler performance parameters related to digestibility values should be done cautiously, as the experimental diets are not well balanced in the experiment.

In conclusion, rate and extent of *in vivo* starch digestibility and AME_n were affected by Western Canadian wheat class, but starch digestibility did not predict AME_n. In addition, *in vivo* starch digestibility can be predicted using an *in vitro* digestion model and may serve a role in examining larger numbers of starch containing ingredients. Increased starch digestion in proximal sections of the SI may improve feed efficiency in broiler chickens.

5. OVERALL DISCUSSION

The extent of wheat starch digestibility has been studied in broiler chickens, but information regarding the rate of starch digestibility in chickens is minimal, especially for Western Canadian wheat. Moreover there are large numbers of wheat cultivars that belong to different wheat market classes available in Western Canada and not much research has been done to compare variation in starch digestibility and nutritive value among wheat market classes. In addition, *in vivo* experiments that have already been conducted, often did not use an adequate number of wheat samples per wheat market class to compare starch digestibility among wheat classes. It is important to have more than one wheat sample per market class in order to compare nutritive value of wheat classes, as the nutritive value is not only dependent on genotype, but also on growing conditions as well.

The current study demonstrated Western Canadian wheat is generally rapidly digestible. According to the results discussed in Chapter 3, *in vitro* starch digestibility is affected by wheat market class, and cultivars nested within wheat classes. Chapter 4 results similarly demonstrated that starch digestibility in PJ, DJ, PI, DI and total tract were affected by wheat market class. Starch digestibility values were generally in accordance to previous studies on broiler chicken starch digestibility (Gutierrez del Alamo et al., 2008a; Gutierrez del Alamo et al., 2009a; Yegani et al., 2013). Apparent metabolizable energy of wheat also affected by wheat market class, and cultivar nested within market class, and the AME_n values were in close agreement with results reported by Scott et al. (1998b). The above experiments emphasize that the nutritive value of wheat is variable, and therefore it is important to establish the digestible nutrient content of wheat market classes and cultivars for future use in accurate poultry diet formulation.

Weurding et al. (2001b) established a strong positive correlation ($r = 0.87$, $p \leq 0.05$) between *in vitro* and in *vivo* starch digestion rate. However we were only able to establish significant correlations for *in vivo* starch digestibility of PJ, DJ, PI, DI with *in vitro* starch digestibility at early incubation times; whereas total tract starch digestibility was positively correlated with all the SI phase incubation times except 90 min of the *in vitro* starch digestibility procedure. Moreover, the established correlations of our study were moderately strong. As mentioned in the Chapter 4 discussion (section 4.5), Weurding et al. (2001b) used a set of different starch sources with a wide range of starch digestion rates, in contrast to the narrow range of digestion rates for wheat cultivars in the current study. This could affect the nature of the correlation between *in vitro* and *in vivo* starch digestibility. In addition Weurding et al. (2001b) used experimental diets for *in vitro* starch digestion, whereas in the current research wheat cultivars were used for *in vitro* digestion. The other ingredients in the diet may affect starch digestion, and it might be a cause for moderate strong correlations. However, the purpose of establishing an *in vitro* starch digestion model is to use it as a reliable technique to determine starch digestibility as *in vivo* starch digestibility trials are comparatively expensive, time consuming and therefore may use inadequate numbers of replications per treatment. The current *in vitro* starch digestion model results were repeatable and the method was relatively less time consuming and cost effective, and can test many replications per sample. Despite moderate strong correlations (most of the occasions only with early SI phase incubation times), this assay has value for predicting *in vivo* starch digestion rate and extent in broiler chickens when there are large numbers of samples to analyse. Therefore the *in vitro* starch digestion model contributes to analyse large numbers of samples, and then the number of samples to analyse using *in vivo* trials can get narrowed down according to *in vitro* digestion results.

The previous studies regarding broiler chicken starch digestibility observed positive correlations for AME_n with *in vivo*/*in vitro* starch digestion extent and/or digestible starch (Mollah et al., 1983; Rogel et al., 1987; Wiseman et al., 2000), but some studies did not detect a significant correlation between AME_n and starch digestion extent/digestible starch (Gutierrez del Alamo et al., 2008a; Yegani et al., 2013). In the present study, a significant correlation was not observed for AME_n with *in vivo*/*in vitro* starch digestion rate/extent and digestible starch. The narrow range of TS might be attributed to the absence of correlation between AME_n and starch digestibility or digestible starch content (Yegani et al., 2013). Apparent metabolizable energy represents the portion of gross energy available to the bird that is not lost in the excreta. If there is a positive correlation between AME_n and the extent of starch digestibility, then it indicates higher starch digestibility is better for energy retention in chicken. The experiment failed to prove the hypothesis that AME_n and starch digestibility extent are positively correlated. Theoretically, higher starch digestion should lead to higher availability of energy to chickens.

On the other hand, starch digestion rate effects may be independent of complete starch digestion as starch digestion rate depends on mean retention time in GI tract and digestive capacity of birds (Yegani et al., 2013). Rapid starch digestion may result in the same extent of starch digestion as in slow starch digestion, but the starch digestion can occur in different locations of the SI (PJ, DJ, PI and DI). The different sites of starch digestion can result in different metabolic responses that affect feed utilization and efficiency (Weurding et al., 2003). Therefore, wheat cultivars having higher starch digestion rates are not necessarily the wheat cultivars with higher energy retention in birds. Weurding et al. (2003) found broiler chickens performed better when fed diets containing slowly in comparison to rapidly digested starch. They

hypothesized that this may be due to a longer lasting insulin response, more efficient protein deposition, and synchronization of energy and protein digestion in birds. However, the current study resulted in a positive correlation between starch digestion rate and gain to feed ratio, and it indicates rapidly digestible starch (RDS) is associated with better feed conversion efficiency in broiler chickens. A possible explanation is that lower levels of slowly digestible starch (SDS) result in less starch fermentation in the distal SI, which is considered as less energy efficient. Therefore, a higher percentage of RDS in the diet increases energy retention due to higher energy retention from starch digestion than starch fermentation.

Slowly digestible starch may have advantages over RDS in terms of broiler chicken health which eventually contributes broiler production. Slowly digestible starch may be beneficial to broiler chickens in disease challenging conditions, as it can act as a substrate for beneficial microbes in gut, and facilitates enhancement of beneficial bacteria while reducing pathogenic bacteria colonization. It is attributed to production of SCFA as a result of starch fermentation (Jozefiak et al., 2004) since lower pH dissipate the proton motive force across the bacteria (Russel, 1992) or might be due to bacteriostatic effect (Van der Wielen et al., 2000) of SCFA against pathogenic bacteria.

Differences in the rate and extent of starch digestion were related to a certain extent to grain characteristics as indicated by moderately strong correlations between starch digestibility and grain characteristics such as NSP (including arabinoxylans), starch granule size distribution and TS. However, additional research may benefit our understanding of factors affecting starch digestion. Other starch grain characteristics such as amylopectin chain length distribution, and starch damage due to processing are two areas that might be useful in understanding wheat starch

digestion in poultry. Furthermore, analysis of grain characteristics in undigested starch found in the terminal ileum might be helpful to predict starch digestibility in broiler chickens and it also can be directed for future research.

In conclusion, both *in vitro* and *in vivo* starch digestion rate and extent is affected by Western Canadian wheat market classes according to the results of two experiments, and it has a relationship with some grain characteristics. Further, *in vivo* starch digestibility in broiler chickens can be predicted using the *in vitro* starch digestion model, as the results were repeatable, and it is time and cost effective, which facilitate testing of large numbers of samples.

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