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ARTICLE

In vitro assessment of the starch digestibility of western Canadian wheat market classes and cultivars

Namalika D. Karunaratne, Dawn A. Abbott, Ravindra N. Chibbar, Pierre J. Hucl, Curtis J. Pozniak, and Henry L. Classen

Abstract: The objective of the study 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 wheat cultivars from eight western Canadian wheat classes and, each cultivar was replicated four times. Samples were subjected to gastric and small intestine (SI) digestion phases and each sample was assayed in triplicate; glucose release was measured in SI phase. Starch granule distribution, amylose, total starch, crude protein (CP), ash, and non-starch polysaccharides (NSP) were analyzed in all wheat samples. Small intestinal phase times of 15, 60, and 120 min were chosen to approximate digestion in the terminal duodenum, jejunum, and ileum. Starch digestibility of wheat classes ranged as follows: 15 min — 33.1% to 49.1%, 60 min — 80.2% to 93.3%, and 120 min — 92.4% to 97.6%. Starch digestibility positively correlated with CP, ash, NSP, and proportion of large granules, whereas it negatively correlated with total starch, and proportion of small and medium granules. In conclusion, market class and cultivar of western Canadian wheat affects both rate and extent of starch digestibility and it is related to various grain characteristics.

Key words: chicken, slowly digested starch, rapidly digested starch, fibre, starch granules.

Résumé : L'objectif de cette étude était de mesurer l'effet de la classe du marché et du cultivar de blé sur la digestibilité de l'amidon utilisant un modèle in vitro qui simule l'appareil digestif du poulet et le relier aux caractéristiques de grain. L'étude a évalué 18 cultivars de blé provenant de huit classes de blé de l'ouest du Canada. Chaque cultivar a été répliqué quatre fois. Les échantillons ont subi des phases de digestion gastrique et de l'intestin grêle (SI — « small intestine ») et chaque échantillon a été analysé en triplicata; la relâche de glucose a été mesurée dans la phase SI. La distribution des granules d'amidon, l'amylose, l'amidon total, les protéines brutes (CP — « crude protein »), les cendres, et les polysaccharides non-amidon (NSP — « non-starch polysaccharides ») ont été analysés dans tous les échantillons de blé. Des temps dans la phase de l'intestin grêle de 15, 60 et 120 minutes ont été choisis pour approximer la digestion dans le duodénum terminal, le jéjunum et l'iléon. Les digestibilités de l'amidon des classes de blé variaient comme suit : 15 min — 33,1 % à 49,1 %, 60 min — 80,2 % à 93,3 % et 120 min — 92,4 % à 97,6 %. La digestibilité de l'amidon avait une corrélation positive avec les CP, cendres, NSP et la proportion de grandes granules, tandis qu'il y avait une corrélation négative avec l'amidon total et la proportion de petites et moyennes granules. En conclusion, la classe du marché et le cultivar du blé de l'Ouest canadien a un effet autant sur le taux que l'étendu de la digestibilité de l'amidon et il y a relation avec diverses caractéristiques du grain. [Traduit par la Rédaction]

Mots-clés : poulet, amidon à digestion lente, amidon à digestion rapide, fibre, granules d'amidon.

Introduction

Starch is the main energy source of poultry diets, and a major contributor to diet apparent metabolizable energy. In western Canada, wheat is the main cereal and starch source used in poultry diets because of its availability, and relatively high total starch and crude protein (CP)

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content. However, wheat can be variable as it is primarily grown to provide functional properties required by the food industry 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, Canadian Western Extra Strong (CWES), Canadian Western Red Winter, Canadian Prairie Spring White, Canadian Western Soft White Spring (CWSWS), and Canadian Western Hard White Spring (CWHWS). Canadian Western General Purpose (CWGP) 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 (CGC) 2015]. In addition, an ancient subspecies of wheat called "Spelt" is commonly used in the food industry (Galkowska et al. 2014). Feed formulation is based on the nutrient profile and digestibility of feed ingredients, and therefore, 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. The determination of starch digestibility of wheat market classes/cultivars may improve poultry production by increasing the accuracy of nutrient delivery, and affecting other traits that influence post-prandial metabolism, enterocyte function, and gastrointestinal (GI) tract health.

In vivo digestibility trials with 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. 2009*a*, 2009*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. Ebsim (2013) modified this procedure to more accurately reflect digestive tract conditions in the chickens. 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. 2008; 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 GI tract microbiota (Weurding et al. 2001*a*, 2001*b*; Regmi et al. 2011*a*, 2011*b*; Seal et al. 2003).

Rate and extent of starch digestion is affected by starch granule structure and composition, processing method, and association with other components including lipids, protein, fibre, minerals, and antinutritional factors (Al-Rabadi et al. 2009; Blazek and Copeland 2010; Mahasukhonthachat et al. 2010; Regmi et al. 2011*b*). Based on in vitro research, wheat starch digestion has been related to 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 also be 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, starch granule diameter ranges from around 1 to 50 μm and there are different classifications of starch granules according to their size (Raeker et al. 1998; Ahuja et al. 2013). A higher proportion of small starch granules theoretically increases starch digestibility due to the increased starch granule surface area accessible by digestive enzymes (Svihus et al. 2005).

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 examine the relationship of these values to grain characteristics.

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 spring wheat cultivars, consisting of four independent samples for each cultivar 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, Canada, in 2012. The four samples of each cultivar were grown on different plots. The wheat cultivars tested and the market classes that they belong to are shown in Table 1.

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Table 1. Wheat market classes and cultivars which were used for in vitro starch digestion assay.

Wheat class	Wheat cultivars
CPS	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

Note: 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.

In vitro starch digestion was studied using a procedure that approximates the chicken gastric and 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 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, whereas 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 from the latter research 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 to more closely match in vivo digestion in chickens. Total starch and digested starch values were not corrected for free glucose, in contrast to the Englyst et al. (1992) technique.

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 mol L^{-1} HCl. It provides 2000 U of pepsin per millilitre of solution. Enzyme solution II was prepared by weighing 3.0 g of pancreatin (Sigma ref. P-7545; Louis, MO, USA) to nine centrifuge tubes followed by 20 mL of distilled water. The solution was stirred magnetically for 10 min, and centrifuged at 6834*g* for 10 min at 3000 rev min^{-1}. Fourteen millilitres of supernatant from each tube were then added to a beaker (total 126 mL). The enzyme concentrations of pancreatin enzyme mixture were 228, 209, and 32.4 USP units mg^{-1} 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^{-1} of amyloglucosidase and 60 U mL^{-1} of invertase. 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. Sodium acetate buffer was prepared by dissolving 13.6 g of sodium acetate trihydrate ($CH_3COONa{\cdot}3H_2O$; Sigma ref. S-6770; BDH ACS759; St. Louis, MO, USA) in 250 mL of saturated benzoic acid. Then pH was adjusted to 5.6 using acetic acid and the volume adjusted to 1.0 L with distilled water. Finally, 4 mL of 1 mol L^{-1} calcium chloride was added to 1.0 L of the buffer. The glucose oxidase peroxidase determination (GOPOD) reagent 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 reached 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. 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 nine wheat samples at a time.

Initially, 1.5 mL of enzyme solution I (2000 U mL^{-1} of 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

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(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 s for each tube to undergo this procedure).

Ethanol tubes which contained aliquots were centrifuged at 513*g* and 1500 rev min^{-1} 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).

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; Association of Official Analytical Chemists (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.

Starch digestibility was calculated using the following formula.

$$\text{Starch digestibility}\ (\%) = (\text{TS}_{\text{in vitro}}/\text{TS}) \times 100$$

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

All wheat samples were analyzed in duplicate for total starch, CP, ash, soluble and insoluble non-starch polysaccharides (NSP), soluble and total arabinoxylans (AX), amylose and starch granule size distribution. Amylose, total starch, CP, ash, soluble and insoluble NSP, and soluble and total AX were analyzed on dry matter (DM) basis. Moisture was determined using method 930.15 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 nitrogen to CP conversion 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, USA). Soluble and insoluble NSP, and soluble and total AX were analyzed using near-infrared 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.

The experiment was a complete randomized design and the wheat cultivars were nested within wheat market class. Wheat class and cultivar were random effects. Four different samples (replications) were used from each cultivar. All data were analyzed using Proc Mixed in SAS version 9.4 (SAS Institute 2008) and Tukey's studentized range test was used for mean separation of treatments when there was a significant difference. 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 version 9.4 (SAS Institute 2008). Further, stepwise regression with forward selection was done using Proc Reg to determine the factors most affecting in vitro starch digestibility for each SI incubation time and, the prediction equations were developed for starch digestibility at each SI phase incubation time.

Results

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 2). 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) 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

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Table 2. Effect of wheat class on starch digestibility (%) at different incubation times of small intestine phase of in vitro starch digestion assay.

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

Note: Means within a column not sharing a lowercase letter differ significantly at the $P \leq 0.05$ level. 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).

[a]SEM, pooled standard error of mean. Each mean represents two or three cultivars (except CWES with one) and four replications per each cultivar.

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, Canadian Prairie Spring (CPS), CWHWS, Spelt, and CWRS (see Table 2 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 (see Table 2 for statistical interpretation).

Examination of variation in the in vitro starch digestibility among cultivars is shown in Table 3. 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 100.0% (Transcend) with a difference between these means of 9.0%. 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, and 60 min), and CWGP (45 min). Despite the importance of class in affecting starch digestion, there is still variation within classes according to the statistical separation of means.

All grain characteristics were affected by wheat market class, and the results (DM basis) and statistical separation of means are presented in Table 4. The total starch of wheat market classes varied from 53.4% (CWAD) to 58.7% (CWSWS), whereas 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 4 and 5, 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.

The impact of cultivar on grain characteristics are shown in Tables 6 and 7. 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 6 and 7.

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Table 3. Effect of wheat cultivar on starch digestibility (%) at different incubation times of small intestine phase of in vitro starch digestion assay.

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

Note: Means within a column not sharing a lowercase letter differ significantly at the $P \leq 0.05$ level. 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.

[a]SEM, pooled standard error of mean ($n = 4$).

Table 4. Grain characteristics of wheat market classes.

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

Note: Means within a column not sharing a lowercase letter differ significantly at the $P \leq 0.05$ level. TS, total starch; CP, crude protein; TNSP, total non-starch polysaccharides (NSP); INSP, insoluble NSP; SNSP, soluble NSP; TAX, total arabinoxylans; IAX, insoluble arabinoxylans; SAX, soluble arabinoxylans; 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).

[a]SEM, pooled standard error of mean. Each mean represents two or three cultivars (except CWES with one) and four replications per each cultivar.

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).

Correlations of in vitro starch digestibility with grain characteristics are shown in Table 8. Total starch negatively correlated with starch digestibility at all time

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Table 5. Starch granule size distribution of wheat market classes.

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

Note: Means within a column not sharing a lowercase letter differ significantly at the $P \leq 0.05$ level. 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).

[a]SEM, pooled standard error of mean. Each mean represents two or three cultivars (except CWES with one) and four replications per each cultivar.

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, and soluble) and AX (total and insoluble) were 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 9), and grain characteristics were used to develop the prediction equations for in vitro starch digestibility (Table 10). Regression coefficient values were cumulative for each of the time periods. Correlation analyses among grain characteristics are presented in Table 11.

Discussion

Western Canadian wheat market classes and cultivars affect rate and extent of in vitro starch digestibility. Previous studies have demonstrated that the extent of starch digestion is affected by western Canadian wheat class (Yegani et al. 2013). However, no published research has studied starch digestion rate in western Canadian wheat classes, using chicken GI tract conditions. The extent of starch digestibility ranged from 91.0% to 100.0% (mean 95.5%), and is in accordance with the in vitro wheat starch digestibility results of Weurding et al. (2001*b*). 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 starch digestibility at the terminal ileum of the digestive tract of broiler chickens (Weurding et al. 2001*a*). Starch digestibility at different SI phase incubation times of the in vitro assay are also in agreement with Weurding et al. (2001*b*). However, in the in vitro study of Ahuja et al. (2013), both the starch digestibility rate and extent are lower than values from the present study. This might be due to different conditions of the two in vitro models, since the Ahuja et al. (2013) study mimicked human GI tract conditions, whereas our results were based on chicken gastric and intestinal environments. In addition, there was variability in the rate and extent of starch digestion due to cultivars within a wheat class in our study, and there are no such data available in the literature.

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, CP values of the wheat classes ranged from 15.0% to 22.9% and, were higher than the 12.8% to 17.0% range reported by Hucl and Chibbar (1996). Appropriate standards and

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Table 6. Grain characteristics of wheat cultivars.

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

Note: Means within a column not sharing a lowercase letter differ significantly at the $P \leq 0.05$ level. TS, total starch; CP, crude protein; TNSP, total non-starch polysaccharides (NSP); INSP, insoluble NSP; SNSP, soluble NSP; TAX, total arabinoxylans; IAX, insoluble arabinoxylans; SAX, soluble arabinoxylans; DM, dry matter; 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.

[a]SEM, pooled standard error of mean ($n = 4$).

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Table 7. Starch granule size distribution of wheat cultivars.

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

Note: Means within a column not sharing a lowercase letter differ significantly at the $P \leq 0.05$ level. 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.

[a]SEM, pooled standard error of mean ($n = 4$).

Table 8. 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	CP	Ash	TNSP	INSP	SNSP	TAX	IAX	SAX	Amylose	Large granules (>15 μm)	Medium granules (5–15 μm)	Small granules (<5 μm)
15	**−0.54**[a]	**0.29**	**0.37**	**0.76**	**0.71**	**0.79**	**0.66**	**0.66**	0.16	−0.11	0.19	−0.09	**−0.31**
30	**−0.53**	**0.29**	**0.39**	**0.75**	**0.71**	**0.74**	**0.64**	**0.65**	0.08	−0.05	0.17	−0.05	**−0.34**
45	**−0.42**	0.14	**0.37**	**0.70**	**0.66**	**0.71**	**0.63**	**0.63**	0.19	−0.01	**0.29**	−0.16	**−0.41**
60	**−0.37**	0.08	**0.39**	**0.67**	**0.64**	**0.65**	**0.62**	**0.62**	0.19	−0.07	**0.39**	**−0.28**	**−0.46**
90	**−0.48**	0.14	**0.21**	**0.51**	**0.48**	**0.52**	**0.48**	**0.47**	0.12	−0.03	**0.34**	**−0.31**	**−0.28**
120	**−0.39**	0.01	**0.23**	**0.43**	**0.41**	**0.43**	**0.44**	**0.44**	0.12	−0.08	**0.46**	**−0.40**	**−0.42**
180	**−0.30**	−0.01	0.08	0.19	0.21	0.19	**0.28**	**0.26**	0.25	−0.10	**0.33**	**−0.39**	−0.11
240	**−0.40**	0.06	0.12	**0.34**	**0.32**	**0.36**	**0.35**	**0.34**	0.14	**−0.28**	**0.33**	**−0.34**	−0.20

Note: TS, total starch; CP, crude protein; TNSP, total non-starch polysaccharides (NSP); INSP, insoluble NSP; SNSP, soluble NSP; TAX, total arabinoxylans; IAX, insoluble arabinoxylans; SAX, soluble arabinoxylans.

[a]Correlation coefficient (r) is bolded for all significant variables ($P \leq 0.05$). $n = 72$.

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. 2009*b*). 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 were found in recent studies (Ahuja et al. 2013, 2014).

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Table 9. 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	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
	(>15 μm)	0.53	0.0112
	IAX	0.57	0.0171
	TAX	0.59	0.0414
90	(5–15 μm)	0.45	0.0231
	CP	0.53	0.0357
	TS	0.55	0.0002
120	(>15 μm)	0.30	0.0069
	TS	0.48	<0.0001
	CP	0.52	0.0169
180	(5–15 μm)	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
	(5–15 μm)	0.43	<0.0001
	CP	0.47	0.0301

Note: SI, small intestine; SNPS, soluble non-starch polysaccharides (NSP); TNSP, total NSP; IAX, insoluble arabinoxylans; TAX, total arabinoxylans; >15 μm, large granules; 5–15 μm, medium granules; CP, crude protein; TS, total starch. $n = 72$.

Table 10. Prediction equations for starch digestibility at different small intestine phase incubation times of in vitro starch digestion assay.

Prediction equation	R^2	P value	SEM
$D_{15} = -8.2232 + 18.7469 \times$ SNSP $+ 2.1687 \times$ TNSP	0.79	<0.0001	7.975
$D_{30} = -50.9019 + 17.4906 \times$ TNSP $+ 69.6373 \times$ TAX $- 94.1892 \times$ IAX	0.77	<0.0001	16.026
$D_{45} = 20.2171 + 16.8616 \times$ SNSP $+ 3.269 \times$ TNSP	0.66	0.0003	18.926
$D_{60} = 16.2384 + 9.5422 \times$ TNSP $+ 32.5894 \times$ TAX $- 47.1423 \times$ IAX $+ 0.3073 \times$ L. granules	0.72	0.0014	7.851
$D_{90} = 225.4020 - 1.9252 \times$ TS $- 0.5192 \times$ CP $- 0.4103 \times$ M. granules	0.68	0.0008	4.170
$D_{120} = 138.7477 - 1.0899 \times$ TS $- 0.1674 \times$ CP $+ 0.3474 \times$ L. granules	0.74	0.0002	2.256

Note: SEM, standard error of mean; SNSP, soluble non-starch polysaccharides (NSP); TNSP, total NSP; TAX, total arabinoxylans; IAX, insoluble arabinoxylans; TS, total starch; CP, crude protein; L. granules, large granules; M. granules, medium granules.

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, whereas the amylose content of wheat cultivars in this study was analysed using a Megazyme kit. 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 from the two studies.

Starch granule size distribution was significantly different among wheat market classes and cultivars. Small, medium, and large starch granule size distribution values are in accordance with Ahuja et al. (2013). Similarly, NSP and AX values approximated previous values in the literature (Coles et al. 1997; Dornez et al. 2008; Gutierrez del Alamo et al. 2008). In addition, ash content approximates the values of CGC (2015). Thus, these grain characteristics were within normal ranges according to previously analyzed values in the literature.

Correlation analysis investigates the association of variables, but does not indicate a cause and effect relationship (Bruce 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. Because total starch was negatively correlated with other nutrients including CP, ash, total NSP, soluble NSP, insoluble NSP, total and insoluble AX, it is not possible to establish the factors which might impact starch digestibility.

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Table 11. Correlation analysis among grain characteristics of wheat cultivars.

	TS	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**	**−0.38**	**−0.56**	**−0.55**	**−0.49**	**−0.35**	**−0.38**	**0.32**	0.10	**0.25**	**−0.34**	0.01
CP		**1**	**0.32**	**0.29**	**0.31**	0.17	−0.0003	0.04	**−0.54**	−0.13	**−0.54**	**0.59**	**0.26**
Ash			**1**	**0.31**	**0.27**	**0.39**	0.13	0.15	−0.21	0.01	0.02	0.05	−0.16
TNSP				**1**	**0.99**	**0.82**	**0.93**	**0.95**	0.01	−0.003	0.20	−0.04	**−0.43**
INSP					**1**	**0.74**	**0.93**	**0.95**	−0.02	0.02	0.17	−0.004	**−0.42**
SNSP						**1**	**0.73**	**0.73**	0.19	−0.12	**0.30**	−0.20	**−0.38**
TAX							**1**	**1**	**0.27**	0.06	**0.38**	**−0.24**	**−0.51**
IAX								**1**	0.18	0.05	**0.35**	−0.19	**−0.50**
SAX									**1**	0.16	**0.49**	**−0.56**	−0.18
Amylose										**1**	−0.03	0.13	−0.14
L. granules											**1**	**−0.94**	**−0.78**
M. granules												**1**	**0.51**
S. granules													1

Note: Correlation coefficients (r) are bolded for all significant variables ($P \leq 0.05$). $n = 72$. TS, total starch; CP, crude protein; TNSP, total non-starch polysaccharides (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.

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 a higher protein content is harder and contains strong gluten compared with wheat with low protein content. Hard wheat undergoes more starch damage compared with soft wheat during flour milling (Pasha et al. 2010). Therefore, a higher protein content may indirectly increase starch digestibility due to disruption of α-glycosidic linkages through starch damage, and thereby explain 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 AX, grain fibre content as estimated by measurement of NSP and AX was positively correlated with starch digestibility, with the relationships stronger earlier in the SI phase. Stepwise regression similarly showed a strong association of total NSP, soluble NSP, total AX, and insoluble AX with in vitro starch digestibility, mostly in the earlier SI phase incubation times. One or more of the above fibre fractions were able to predict in vitro starch digestibility in those earlier incubation times of the SI phase. However, soluble AX was 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 AX, increase viscosity of digesta, decrease digesta passage rate, 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 (Carré et al. 2007). Further, in vivo experiments have shown significant negative correlations between total NSP and ileal starch digestibility coefficient in broiler chickens (Ball et al. 2013). Therefore, the positive correlations of starch digestibility with fibre estimates are unexpected based on the above mentioned theories.

The reason for the opposing relationships between starch digestion and fibre fractions may relate to the nature of the in vitro assay. It is possible that NSP increases the time and energy required for grinding prior to in vitro testing as fibre is resistant to processing. Samples were ground using a Retsch laboratory mill (0.5 mm screen-hole size) and increased grinding time could affect starch damage, and as a result increase

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starch digestibility (Saad et al. 2009). Another possible explanation for the lack of effect of soluble NSP may relate to the specific conditions of the in vitro assay. The DM content of the in vitro model is much less than the digesta DM 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 with 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 are 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 vs. 37 °C) and grinding equipment (Retsch vs. Udy). These factors all could affect the susceptibility of starch granules to hydrolysis, but the impact of grinding on starch granule damage, larger granules more affected than smaller granules (Hossen et al. 2011), deserves consideration.

The expected correlations were not observed between starch digestibility and some of the grain characteristics including starch granule proportions and NSP. There are factors that affect 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*a*, 2011*b*; Ahuja et al. 2013). In some instances, in vitro starch digestibility at a specific SI phase incubation time is significantly predicted by particular grain characteristics by multiple stepwise regression analysis. However, all of these grain characteristics were not correlated with the in vitro starch digestibility at the same SI phase incubation time. It suggests that some of the analyzed grain characteristics interact each other to produce a combined effect on in vitro starch digestibility rather than having individual effects.

The ability of an in vitro model to predict the rate and extent of starch digestion has a number of practical implications. Extent of starch digestion estimates complete SI starch digestion, which theoretically results in more energy retention in the chicken. Therefore, wheat samples having higher starch digestion are better than wheat samples with lower starch digestion. Although starch digestion rate is not an indication of complete starch digestion, it provides valuable information on where digestion occurs and as a consequence effects related to that site. For instance, starch digestion rate may impact post-prandial metabolism, nutrient utilization and also GI tract health in animals (Regmi et al. 2011*a*, 2011*b*; Yin et al. 2011). Therefore, the effects of class and cultivar on the rate and extent of starch digestion both have value in selecting superior wheat cultivars for poultry feed to improve production and health in poultry.

The in vitro starch digestion model is a repeatable assay and 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 with in vivo broiler chicken experiments, and therefore, it can be used to test a large number of starch containing feed ingredients. Limitations of the in vitro assay are that it cannot exactly mimic the dynamic and adaptable nature of the chicken digestive tract. Therefore, it serves as a procedure to select promising effects and differences for later in vivo testing. The relevance and reliability of the in vitro model also needs to be confirmed using an in vivo comparison with the same wheat samples.

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