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Starch Characteristics and Their Influences on *In Vitro* and Pig Prececal Starch Digestion

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ABSTRACT: The main objective of this research was to study the characteristics of starch granules and their influences on *in vitro* and pig prececal starch digestion of corn, dehulled barley, wheat, and potato. Scanning electron microscopy was used to study the starch endosperm structure in the parent material as well as *in vitro* starch digestion. The results showed that corn starch granules were polyhedral, with a diameter ranging from 2 to 10 μm, whereas those of dehulled barley and wheat were spherical, with a diameter ranging from 5 to 20 μm. Potato had the largest starch granules among starch sources reported herein, with oval spheres of 10−50 μm in diameter. *In vitro* starch hydrolysis showed that starch granules of corn degraded faster than the starch of dehulled barley and wheat, with the potato starch being degraded the slowest. The *in vivo* digestibility trial using ileal-cannulated pigs confirmed the starch degradation of grains. The *in vitro* (x, %) and *in vivo* (y, %) digestibility were highly correlated [$y = 6.5304x - 538.48$ ($R^2 = 0.9924$)]. On the basis of the results, *in vitro* starch hydrolysis might be useful in predicting *in vivo* prececal starch digestibility. The digestion kinetic characteristics of different starch sources might be employed to evaluate the starch digestive rate at the pig ileum.

KEYWORDS: Cereal starch granules, digestion, pig, kinetic characteristics

INTRODUCTION

Starch derived from cereal grains is an important energy source for pigs. Different cereal starches differ in granular size, amylose content, and the length of amylopectin side chains.[1,2] As such, they also produce different digestive characteristics. The rate of starch digestion is important in pig nutrition because it may have an impact on the plasma insulin level and efficiency of dietary protein use.[3,4]

Starch hydrolysis in the digestive tract is affected by intrinsic and external factors. Intrinsic factors are its amylose/amylopectin ratio, the starch granular structure, and the matrix structure with protein or cell-wall components and dietary soluble non-starch polysaccharide (NSP) content.[5−7] Therefore, external factors include the method of industrial processing of cereal grains, time of mastication, and exogenous enzyme activity.[6,8]

Starch granules are stored as crystalline form in the plant intracellular bodies. They differ in shape and crystalline structure and are digested at different rates by pancreatic enzymes.[8,9] Types of grain (corn versus barley) and amylopectin content of barley grain (normal versus waxy) affected ruminal fermentation, digestibility, and use of ruminal ammonia nitrogen for milk protein syntheses.[10,11] Englyst et al.[12] fractionated the starch into rapidly degradable starch (RDS), slow-degradable starch (SDS), and resistant starch (RS) by chemical analysis. Slow digestion properties of the starch lead to slower glucose release and lower glycemic response.[13] In a healthy individual, most starch inclusive of SDS is degraded in the small intestine. RS is defined as the product of starch degradation not absorbed in the small intestine.[4,14] Measuring the contents of RDS and SDS in 12 different starch sources, Weurding et al.[15] used the test tubes containing diets and feedstuffs milled to pass a 1 mm screen, thus simulating the grinding action in the gizzard, and indicated that the starch digestive rate is well-predicted by the ratio of RDS/SDS measured using the *in vitro* method, while the RS reflected the nonhydrolyzed starch in the gastrointestinal tract of broilers. It also showed that a 4 h *in vitro* incubation well-represented the prececal starch digestion. Measuring digestibility is rather straightforward, but determining the rate of starch digestion in pigs is challenging. Recently, Doucet et al.[16] purposed a model to predict ($R^2 = 0.71$) *in vivo* average starch digestibility coefficients in the small intestinal region of weaned piglets fed cereal-based diets using seven laboratory variables describing starch properties and described that they were fundamentally associated with the quality of feed materials, i.e., structure, hydration, and amylolytic digestion.

The aim of the study was to examine the starch characteristics by electron microscope observation and their influences on *in vitro* and pig prececal starch digestion. The correlation of *in vitro* and *in vivo* results was determined, and a linear equation was generated for predicting the rate of digestion in pigs.

MATERIALS AND METHODS

Starch Sources. Four different cereal grains inclusive of corn (*Zea mays*), dehulled barley (*Hordeum vulgaris*), wheat (*Triticum aestivum*), and potato (*Solanum tuberosum*) were purchased from a local commercial market. Potatoes were then air-dried at 55−60 °C to a moisture content around 10%. All grains were grounded through a 1 mm mesh to prepare for different assays.

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Table 1. Composition of the Experimental Diet

	diets (as fed basis)			
ingredients	corn (g/kg)	dehulled barley (g/kg)	wheat (g/kg)	potato (g/kg)
corn	659			
dehulled barley		704		
wheat			746	
potato (dry)				641
soybean meal, 44%	252	188	110	291
wheat bran	31	40	60	0
soybean oil	19	32	45	31
salt	5	5	5	5
limestone	18	21	19	18
dicalcium phosphate	14	9	13	13
vitamin premix[a]	0.5	0.5	0.5	0.5
mineral premix[b]	1.5	1.5	1.5	1.5
total	1000	1000	1000	1000
analyzed value (%)				
dry matter	89.0	90.0	91.0	92.0
crude protein	16.3	15.5	17.2	16.8
ether extract	4.6	4.7	5.6	4.1
total starch	46.0	41.0	46.0	44.0
calculated value				
ME (MJ/kg)	13.8	13.8	13.6	12.6

[a] Vitamin premix (content per kilogram of diet): vitamin A, 15 000 IU; vitamin D_3, 3000 IU; vitamin E, 30 mg; vitamin K_3, 4 mg; thiamine, 3 mg; riboflavin, 8 mg; pyridoxine, 5 mg; vitamin B_{12}, 25 mg; Ca−pantothenate, 19 mg; niacin, 50 mg; folic acid, 1.5 mg; and biotin, 60 μg. [b] Mineral premix (content per kilogram of diet): Co($CoCO_3$), 0.255 mg; Cu($CuSO_4 \cdot 5H_2O$), 10.8 mg; Fe($FeSO_4 \cdot H_2O$), 90 mg; Mn($MnSO_4 \cdot H_2O$), 90 mg; Zn(ZnO), 68.4 mg; and Se(Na_2SeO_3), 0.18 mg.

Scanning Electron Microscopy Observation. A total of 1 g of each grain/feedstuff sample and 1 mL of enzyme cocktail, containing 4 g/L pancreatin (Sigma P-1625, 3× USP, Sigma Chemical, St. Louis, MO), 2 g/L amylase [Sigma P-3176, 30 international units (IU) of porcine pancreatic α-amylase type VI-B, Sigma Chemical, St. Louis, MO], 40 mg/L pancreatic lipase (Sigma L-3126, 100−400 units/mg of pancreatic lipase type II, Sigma Chemical, St. Louis, MO), and 80 mg/L bile salts (Sigma B-3883, oxgall powder), were placed in a shaking water bath (37 °C) for 0, 1, 3, and 6 h.[3] After each incubation time, the mixture was centrifuged (1500*g*) for 10 min and the supernatant was removed. The residue was washed with distilled water and ethanol (100%) and then dried at 50 °C. The dried samples were subjected to scanning electron microscopy (Bausch and Lomb, Ltd., Nonolab 2100).[17]

Transmision Electron Microscopy Observation. Dried samples derived from enzymatic hydrolysis were fixed overnight in 50 mM sodium phosphate buffer (pH 7.5) containing 2.5% glutaraldehyde. After several rinses with the sodium phosphate buffer, the samples were postfixed in sodium phosphate buffer containing 2% OsO_4 for 2 h. Dehydration was carried out in a graded ethanol series from 50 to 100%, and the samples were embedded in LR-White resin. Specimen sections of 75 nm were mounted on Formvar-coated nickel grids. After mounting, the sections were stained with uranyl acetate and lead citrate and observed under transmission electron microscopy (model 1200 EXII; JEOL, Tokyo, Japan) at 100 kV.[18]

Total Starch (TS) and RS Contents. Megazyme Resistant Starch Kit (Megazyme International Ireland, Ltd.) was employed to determine the TS and RS content of the tested starch sources. A total of 100 mg of each starch source were incubated with pancreatic α-amylase (3 units/mL) and amyloglucosidase (3 units/mL) at 37 °C for 16 h. The hydrolyzed sugars, nonresistant starch (non-RS), were removed by ethanol. The residue left was hydrolyzed with 2 M KOH, followed by amyloglucosidase. The RS content was determined colorimetrically at 510 nm. The TS content was determined by summing non-RS and RS. Starch hydrolysis was calculated as follows:

$$\text{starch hydrolysis (\%)} = 100 - \left(100 \times \frac{\text{RS (\%)}}{\text{TS(non-RS + RS) (\%)}}\right)$$

whereas TS was the sum of non-RS and RS.

***In Vitro* Enzyme Hydrolysis Characteristics.** A total of 100 mg of each and individual cereal grain or its respective diet formulated for prececal-cannulated pig study was incubated at 37 °C for 0, 1, 2, 3, 4, 5, 6, 10, and 16 h with the enzyme cocktail described above. The remainder of undigested RS content was determined at the end of each incubation time. The data of starch hydrolysis at each incubation time were fitted to the following equation described by Ørskov and McDonald[19] to estimate the starch digestibility characteristics:

$$DC_t = D(1 - e^{-k(d)t})$$

where DC_t (%) is the proportion of starch digested at time t (h), fraction D is the potential starch digestibility (%) that will digest at a rate of k_d, and k_d is the digestion rate (h^{-1}). The Marquardt method of the SAS PROC NLIN procedure was used.

***In Vivo* Starch Digestion Trial.** The experiment was conducted on the National Chung-Hsing University experimental farm, with the experimental protocol for animal use approved by the Animal Care and Use Committee. Three pigs (Landrace, Yorkshire, and Duroc) averaging 40 kg of body weight at approximately 90 days of age were surgically fitted with cannula at the end of ileum. They were housed individually in metabolic cages situated in a temperature-controlled room. The average temperature during the experimental period was 26.3 °C. The daily feed supply was sufficient to simulate *ad libitum* intake. The prececal-cannulated pigs were allotted to a 3 × 3 Latin Square design. Three experimental diets (Table 1) were formulated according to the National

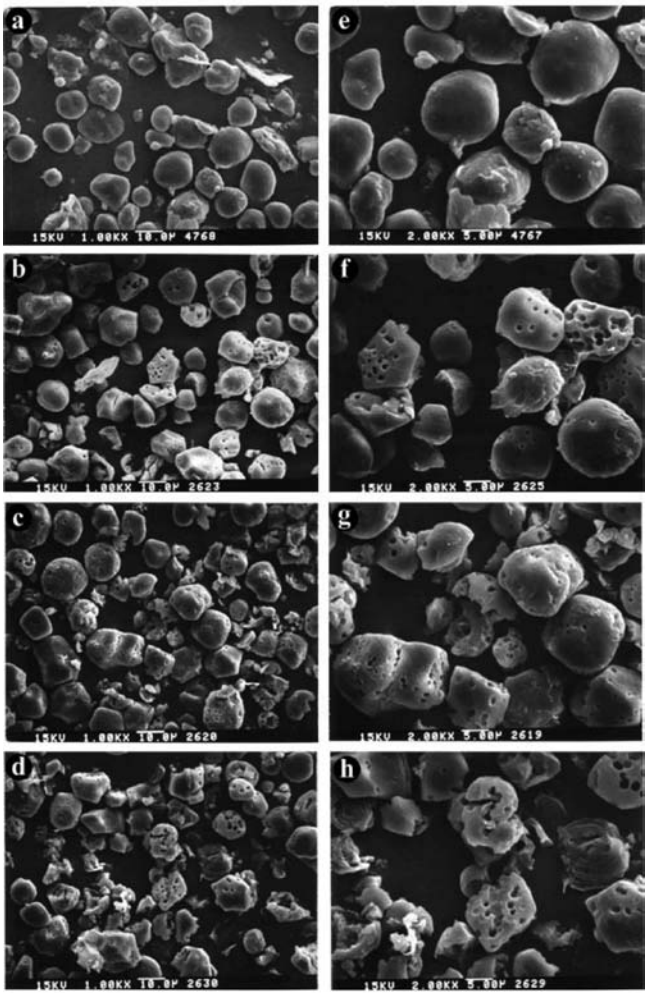

Figure 1. Scanning electron photomicrographs of corn starch granules following incubation in a mixture of pancreatic amylase and amyloglucosidase at different times: a and e, control; b and f, 1 h; c and g, 3 h; and d and h, 6 h. a−d, 1000×; e−h, 2000×.

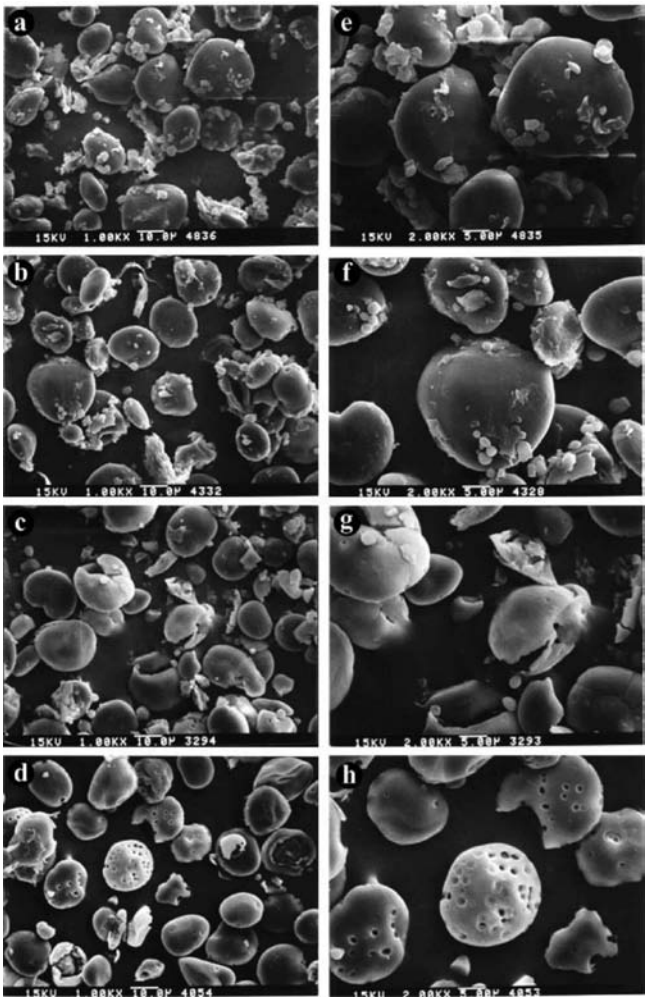

Figure 2. Scanning electron photomicrographs of dehulled barley starch granules following incubation in a mixture of pancreatic amylase and amyloglucosidase at different times: a and e, control; b and f, 1 h; c and g, 3 h; and d and h, 6 h. a−d, 1000×; e−h, 2000×.

Research Council (NRC).[20] All diets contained Cr_2O_3 added at 3 g/kg as an indicator for the determination of starch digestibility. After the 3 day adaptation period, pigs then followed a 7 day experiment period. Pigs were fed at 8:00, 16:00, and 24:00, with water available at all times. The collection of prececal digesta was carried out in the last 3 days of the experimental period. Prececal digesta were collected for 4 h following 1 h after meal. They were weighed and stored at −20 °C until processing. Samples were freeze-dried and ground passing through a 1 mm screen. Samples were analyzed for TS (Megazyme International Ireland, Ltd.). Cr_2O_3 was determined colorimetrically with a spectrophotometer (U-2001, Hitachi, Japan) according to the methods described by Williams et al.[21] Chemical analysis of feeds was determined by the method of the Association of Official Analytical Chemists (AOAC).[22] Prececal starch digestibility was calculated by the following equation:

$$\text{starch digestibility } (\%) = 100 - \left(100 \times \frac{Cr_2O_{3\ \text{feed}}\ (\%)}{Cr_2O_{3\ \text{digesta}}\ (\%)} \times \frac{\text{starch}_{\text{digesta}}\ (\%)}{\text{starch}_{\text{feed}}\ (\%)}\right)$$

Statistical Analysis. The means of starch content, hydrolysis, and digestibility were analyzed by analysis of variation (ANOVA) using the general linear model (GLM) procedure, and all statistical analyses were performed with the Statistical Analysis Systems Institute, Inc.[23] Duncan's new multiple-range test was used to determine the difference of means, and $p < 0.05$ was considered to be statistically significant.[24]

RESULTS

Starch Granule Morphology. The scanning electron micrographs of partial hydrolyzed starch granules of corn, dehulled barley, wheat, and potato following different incubation times are shown in Figures 1−4, respectively. The starch granules of corn (Figure 1) are smaller than those of dehulled barley (Figure 2), wheat (Figure 3), and potato (Figure 4). Corn starch granules showed a polygonal sphere of 2−10 μm in diameter, whereas those of dehulled barley and wheat were spherical, with a diameter ranging from 5 to 20 μm. Potato had the largest starch granules among starch sources reported herein, with oval spheres of 10−50 μm in diameter.

Morphological changes in starch granules of corn, barley, and wheat were observed after 1 h of incubation with pancreatic amylase and amylogucosidase (Figures 1f, 2f, and 3f). All starch granules showed a gradually increased degree of destruction as the incubation time increased. Corn starch granules were more susceptible to enzymatic hydrolysis than wheat starch granules. They appeared with multiple pinholes and became almost graveled without a smooth surface (Figure 1h) following 6 h of incubation. In contrast, barley and wheat starch granules started to break down after 6 h of hydrolysis (Figures 2h and 3h). Potato

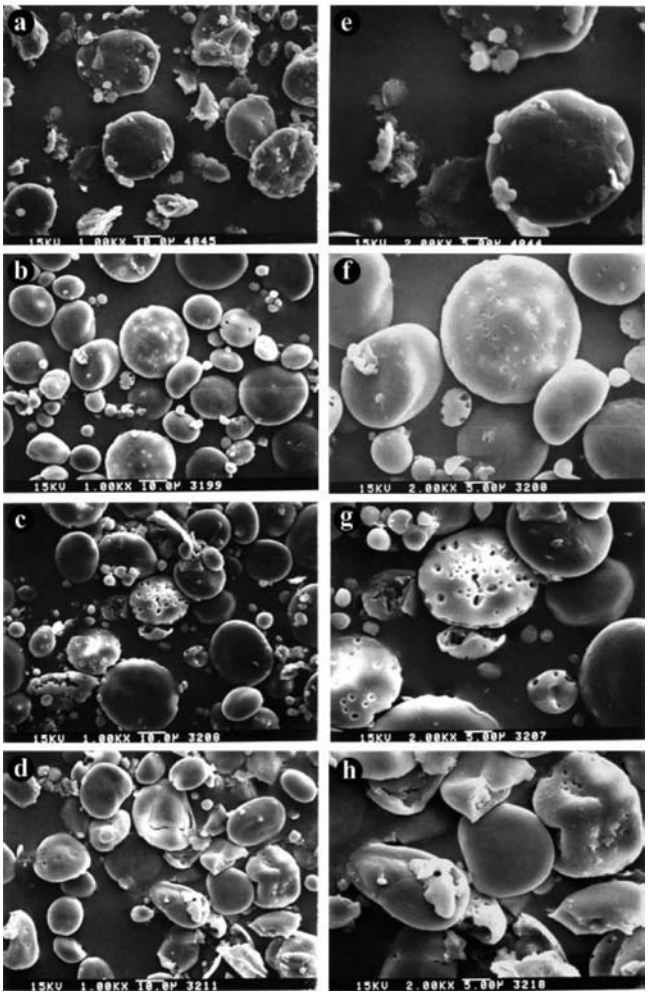

Figure 3. Scanning electron photomicrographs of wheat starch granules following incubation in a mixture of pancreatic amylase and amyloglucosidase at different times: a and e, control; b and f, 1 h; c and g, 3 h; and d and h, 6 h. a−d, 1000×; e−h, 2000×.

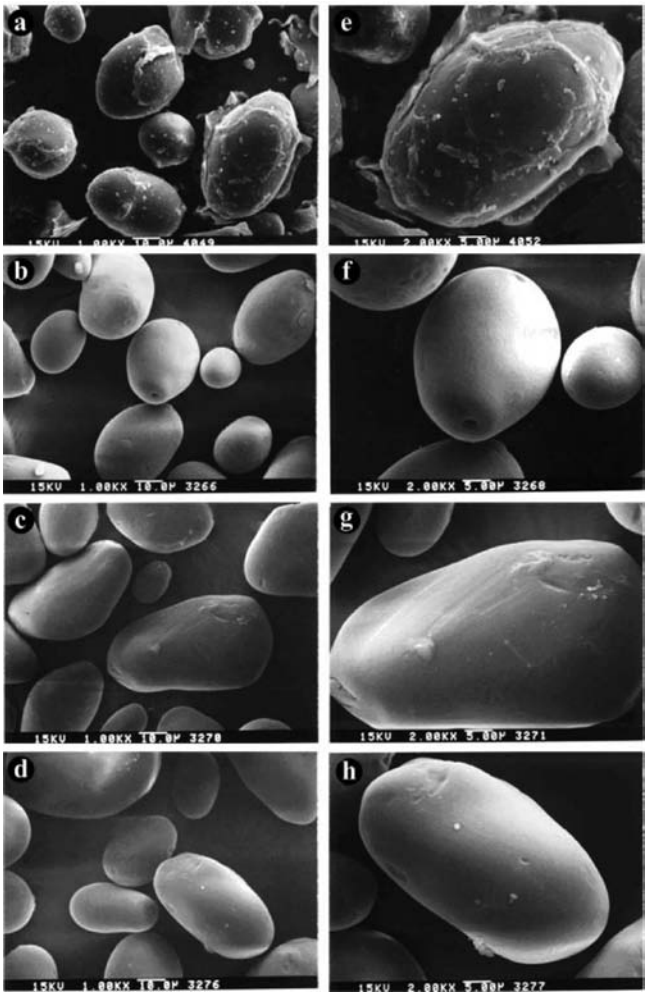

Figure 4. Scanning electron photomicrographs of potato starch granules following incubation in a mixture of pancreatic amylase and amyloglucosidase at different times: a and e, control; b and f, 1 h; c and g, 3 h; and d and h, 6 h. a−d, 1000×; e−h, 2000×.

starch granules adhered to various miscellaneous particles before processing (panels a and e of Figure 4) and appeared smoother, despite the presence of traces of enzymatic action. However, there was no indication of increased destruction of potato starch granules as the incubation time increased (panels b−d of Figure 4). Figure 5 showed the transmission electron micrographs of corn (Figure 5A) and potato (Figure 5B) starch granules digested by enzymes for 6 h. The channel/pinholes and boundary membrane were observed in both starch granules. Larger volume channels were seen in the corn starch granules. However, potato starch granules were digested with channel/pinholes in the inner substrate but not in the surface.

***In Vitro* Starch Hydrolysis.** The content of TS and RS of corn, dehulled barley, wheat, and potato is shown in Table 2. Among all cereal grains, potato contains the largest amount of RS (44.39%), whereas corn, dehulled barley, and wheat contain 2.15, 2.63, and 1.82%, respectively. In relation to the content of RS in each cereal tested herein, similar trends were observed in *in vitro* digestibility of starch in its diet, with corn (97.28%), dehulled barley (95.96%), and wheat (97.03%) being degraded to the greatest extend and potato (32.26%) being degraded the least (Table 3).

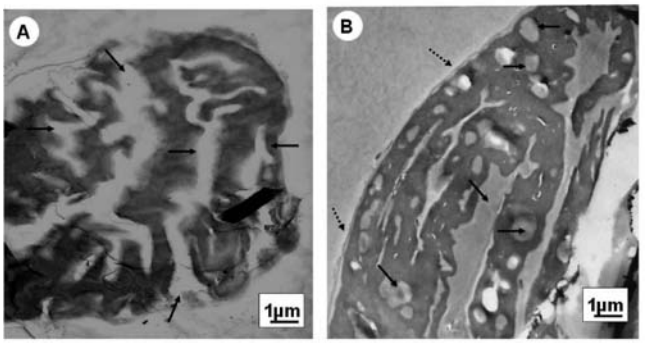

Figure 5. Electron microscopy of (A) corn and (B) potato starch granules digested by enzyme for 6 h. The locations of some channel/pinholes and boundary membranes are indicated by filled and dotted arrows, respectively.

Correlation of *In Vivo* and *In Vitro* Starch Digestibility. Table 3 shows the *in vivo* starch digestibility of diets formulated with the tested cereal grains. The potato diet was excluded from the pig prececal digestibility assay because of its high content of

Table 2. TS and RS Contents of Different Feedstuffs[a]

starch sources	TS (g/100 g of sample, dry weight)	RS (g/100 g of sample, dry weight)
corn	66.08 ± 0.53	2.15 ± 0.27
dehulled barley	60.62 ± 0.65	2.63 ± 0.05
wheat	58.09 ± 0.56	1.82 ± 0.36
potato	68.48 ± 1.02	44.39 ± 1.04

[a] Mean ± standard deviation (SD); $n = 5$.

Table 3. *In Vitro* Hydrolysis and *in Vivo* Digestibility of Starch in Different Diets[a]

item	starch digestibility	
	in vitro (%)	*in vivo* (%)
corn diet	97.28 ± 0.44 a	97.15 ± 0.75 a
dehulled barley diet	95.96 ± 0.90 b	88.19 ± 3.23 c
wheat diet	97.03 ± 0.86 ab	94.72 ± 2.35 b
potato diet	32.26 ± 1.12 c	nd[b]
SEM[c]	0.32	0.51

[a] Mean ± SD; $n = 3$. Means within the same column without the same letters are significantly different ($p < 0.05$). [b] nd = not detectable. [c] SEM = standard error of means.

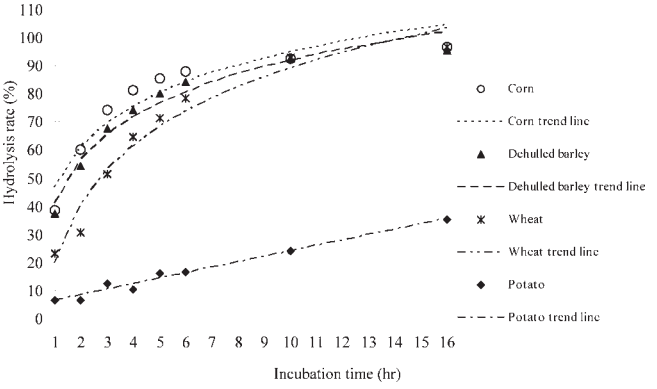

Figure 6. Kinetics of starch hydrolysis in corn (○), dehulled barley (▲), wheat (∗), and potato (◆). The predicted curves are based on observed *in vitro* starch hydrolysis curves for the feedstuffs; $n = 3$.

RS and potential digestion disturbance. The corn diet (97.15%) attained the highest starch digestibility determined at the terminal pig ileum. The prececal starch digestibilities of wheat diet and dehulled barley diet were 94.72 and 88.19%, respectively.

The *in vivo* starch digestibility (y, %), with the exception of the potato diet, was highly correlated with the *in vitro* digestibility (x, %), as evidenced by the linear regression equation, $y = 6.5304x - 538.48$ ($R^2 = 0.9924$). The results of the estimation reported herein indicated that the *in vitro* starch digestibility might accurately predict the *in vivo* starch digestibility determined at the terminal pig ileum.

***In Vitro* Enzyme Hydrolysis Curve and Characteristics of Starch.** Figure 6 illustrates the predicted and observed *in vitro* starch hydrolysis curves of the cereal grains containing corn, dehulled barley, wheat, and potato. The hydrolysis of corn, dehulled barley, and wheat starch produced an exponential curve, with the rate of hydrolysis increasing as the processing time increased and gradually stabilizing after 10 h. In contrast, the hydrolysis of potato starch increased linearly; albeit, the rate of hydrolysis was slower than the other three cereal grains.

Table 4. Kinetics of Starch Hydrolysis Characteristics in Different Starch Sources and Diets[a]

item	hydrolysis characteristics	
	D (%)	k_d (h^{-1})
starch source		
corn	94.03	0.510
dehulled barley	93.06	0.433
wheat	101.2	0.238
potato	50.53	0.071
diet		
corn diet	96.02	0.497
dehulled barley diet	93.57	0.504
wheat diet	95.37	0.365
potato diet	43.72	0.088

[a] Calculated using the exponential curve equation $DC_t = D(1 - e^{-k(d)t})$, where DC_t is the proportion of starch digested at time t and D is the potential starch digestibility.

Table 4 shows the kinetic characteristics of starch hydrolysis of different starch sources and diets using the exponential curve equation,[19] where D represents the potential starch digestibility (%) and k_d is the starch digestion rate. All cereal grains demonstrated similar potentials in digestibility and digestion rate of starch. All cereal grains and their diets exhibited more than 93% potential starch digestibility, whereas 50.53 and 43.72% were observed in potato and its diet, respectively. Corn and dehulled barley starch digested the fastest (0.510 versus 0.433 h^{-1}). This was followed by wheat (0.238 h^{-1}), with potato being the slowest (0.071 h^{-1}). Similar trends were also reflected in the diets formulated with the cereal grains. Although the wheat starch had a slower rate of hydrolysis than corn and dehulled barley, its potential starch digestibility was close to corn.

DISCUSSION

Starch digestion is a function of the surface area, structure, and degree of crystallinity of the starch granules.[1,25] Smaller granules possess greater surface area and are, therefore, more susceptible to enzymatic hydrolysis than larger granules.[26,27] Pores were observed and evenly distributed in the granular surface of corn starch, whereas pits were found in those of larger starch granules, such as dehulled barley and wheat. The pores found on corn starch granules are evidence of the penetration of amylase; however, some starch granules from wrinkled pea and potato are hardly digested because they produced a packed structure.[28]

Pores served as exits of the interconnected tunnel found inside the starch granules that allow enzymes to penetrate inwardly from pores through these channels.[29] Channels become greater as the degree of hydrolysis increased (Figure 5). The small raw-type starch granules of cereal grains, therefore, could be directly used as an energy source by the animal without further processing.

Potato starch granules were covered by a boundary membrane (Figure 5b) of lipid and protein complexes.[30] Thompson[31] elucidated that potato starch granules being denser, more integrated, and structured with packed arrangement were not

susceptible to enzyme action. Furthermore, various sizes of blicklet pileups ranging from 200 to 500 nm found within the potato starch granules may be partially related to why potato starch is resistant to the enzyme hydrolysis.

Englyst et al.[12] proposed an *in vitro* method simulating starch digestion in the small intestine of humans. Results showed that the *in vitro* starch hydrolysis rate was significantly correlated to the *in vivo* digestibility. Weurding et al.[26] showed that the starch digestion rate and extent in broiler chickens was well-predicted by the *in vitro* method that mimics their digestive processes in the gastrointestinal tract. This is in contrast to the barley diet, from which *in vivo* starch digestibility was lower than *in vitro*. The observed differences between *in vivo* and *in vitro* potential digestion of dehulled barley diet may be attributed to the presence of antinutritional factors, such as β-glucan and NSPs, by which their unfavorable effects on digestive processes, such as passage rate and viscosity in the gastrointestinal tract, are not simulated in the *in vitro* method. In the present study, the comparison between *in vivo* starch digestibility (Table 3) and *in vitro* kinetic exponential curve methods (Table 4) showed that the *in vivo* starch digestibility was in agreement with the potential starch digestibility predicted in corn and wheat diets (D value in Table 4). This suggests that the starch is mostly digested in the small intestine of pigs. The starch digestibility of dehulled barley determined by the *in vivo* method and predicted by the *in vitro* method is 88.19 and 93.57%, which implies an incomplete digestion of starch in the small intestine of pigs. The potential starch digestion of feedstuffs calculated from the exponential curve also agreed well with the digestibility of feedstuffs determined by the *in vivo* method.[26]

The degree of starch granule hydrolysis by amylase agreed with their crystalline characteristics and the digestion by the artificial digestive solution in cereal grains and potato. Furthermore, cereal grains contain lesser minerals to have a negative impact on nutrient digestion, whereas high phosphate salts found in the amorphous zone of potato starch granules may potentially hinder starch digestion.[2] The reason why wheat is digested slower than corn may be attributed to its endosperm, which is surrounded by the aleuronic layer, germ cell, and pericarp or testa (seed coat), containing high contents of cellulose, lignin, and NSPs. The insoluble NSPs embracing the endosperm cell encapsulate the nutrients and their digestion by digestive enzymes.[32] The soluble NSPs are mainly composed of β-glucan and arabinoxylans and form gel during digestion, resulting in increased viscosity of digesta and hindering the contact of digestive enzyme and their substrates and, hence, the nutrient absorption via villi.[33]

The *in vivo* starch digestibility of dehulled barley diet was 6−9% lower than that of the wheat and corn diets. In addition to the NSPs found in wheat, dehulled barley also contains antinutrient factors, such as phytic acid and tannin. Incomplete dehulling may also contribute to the lower digestibility of the barley diet, as compared to the corn and wheat diets.

The rate of starch digestion varied considerably among cereal grains and potato. Wheat starch produced a digestion rate slower than corn and barley; albeit, the extent of its digestion was similar to corn, but it was superior to dehulled barley. A faster rate of starch digestion may lead to a more complete digestion of starch and better efficiency of energy use in broilers.[15,26] However, a slower rate of starch digestion allows for a continuous supply of glucose in the circulation, promoting cellular uptake of amino acids and, subsequently, protein synthesis. This reflects the importance of the starch digestion rate in pigs. Although a number of advantages of the *in vivo* methods were demonstrated, this study, however, illustrated the close relationship between the *in vitro* and *in vivo* methods for measuring starch digestion.

In conclusion, the results reported herein imply that the *in vitro* starch digestion method may provide a useful tool in predicting starch digestion at the terminal pig ileum.

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ABBREVIATIONS USED

NSP, non-starch polysaccharide; RDS, rapid-degradable starch; SDS, slow-degradable starch; RS, resistant starch; TS, total starch

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