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Nutrient Physiology, Metabolism, and Nutrient-Nutrient Interactions

Cooked Rice–Based and Wheat-Based Food Structure Influenced Digestion Kinetics and Glycemic Response in Growing Pigs

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A B S T R A C T

Background: How starch-based food structure can affect the rate and extent of digestion in the small intestine and resulting glycemic response is not properly understood. One possible explanation is that food structure influences gastric digestion, which subsequently determines digestion kinetics in the small intestine and glucose absorption. However, this possibility has not been investigated in detail.
Objectives: Using growing pigs as a digestion model for adult humans, this study aimed to investigate how physical structure of starch-rich foods affects small intestinal digestion and glycemic response.
Methods: Male growing pigs (21.7 ± 1.8 kg, Large White × Landrace) were fed one of the 6 cooked diets (250-g starch equivalent) with varying initial structures (rice grain, semolina porridge, wheat or rice couscous, or wheat or rice noodle). The glycemic response, small intestinal content particle size and hydrolyzed starch content, ileal starch digestibility, and portal vein plasma glucose were measured. Glycemic response was measured as plasma glucose concentration collected from an in-dwelling jugular vein catheter for up to 390 min postprandial. Portal vein blood samples and small intestinal content were measured after sedation and euthanasia of the pigs at 30, 60, 120, or 240 min postprandial. Data were analyzed with a mixed-model ANOVA.
Results: The plasma glucose $\Delta max_{overall}$ and $iAUC_{overall}$ for couscous and porridge diets (smaller-sized diets) were higher than that of intact grain and noodle diets (larger-sized diets): 29.0 ± 3.2 compared with 21.7 ± 2.6 mg/dL and 5659 ± 727 compared with 2704 ± 521 mg/dL·min, for the smaller-sized and larger-sized diets, respectively ($P < 0.05$). Ileal starch digestibility was not significantly different between the diets ($P \geq 0.05$). The $iAUC_{overall}$ was inversely related to the starch gastric emptying half-time of the diets ($r = -0.90$, $P = 0.015$).
Conclusions: Starch-based food structure affected the glycemic response and starch digestion kinetics in the small intestine of growing pigs.

Keywords: food structure, digestion kinetics, gastric emptying rate, glycemic response, growing pigs, small intestinal digestion, starch-based foods

Introduction

Consumption of starch-based foods largely affects glucose metabolism, which, if not well managed, may increase risk of type 2 diabetes and obesity [1]. The modification of starch conversion to glucose during digestion in the small intestine through changes in food structure can modulate glycemic response. In particular, the arrangement of starch and other components in the microstructural space of the food material (food microstructure) is important in regulating the rate and extent of starch digestion in the small intestine. In addition, many other factors have been studied in linking food structure to glycemic response management, such as resistant starch type and content [2,3], initial viscosity [4], initial particle size [5], fiber

Abbreviations: Δmax, maximum change in plasma glucose relative to the baseline concentration; D, Duodenum; D[3,2], volume mean diameter; D[4,3], surface area mean diameter; d_{10}, the diameter below which 10% of all particles are found; d_{50}, the diameter below which 50% of all particles are found; d_{90}, the diameter below which 90% of all particles are found; DJ, distal jejunum; DM, dry matter; I, ileum; iAUC, incremental area under the glycemic response curve; K_m, half-time to reach maximum accumulation; PJ, proximal jejunum; SSA, specific surface area; $t_{1/2,softening}$, gastric softening half-time; $t_{1/2,starch\ GE}$, starch gastric emptying half-time; TI, terminal ileum; V_{max}, maximum amount of accumulation.

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content [6], starch interactions with other components in the food matrix [7], and processing methods [8].

Despite the prevalence of studies on digestibility of starch-based foods, the mechanisms of how food structure affects gastric digestion, and subsequently the rate and extent of digestion in the small intestine, have not been investigated in detail. In previous studies where macrostructural differences in the foods were clear (e.g., contrasting particle size, viscosity, or the presence of bran), foods with slower gastric emptying rate were reported to have a lower glycemic response [9–11]. However, in other studies using porridge or hydrated flaked meals, glycemic response was not found to be dependent on gastric emptying rate [4,5,12] but was attributed to different starch microstructure and/or size of particles in the porridge. These contradictory findings may be related to the gastric emptying mechanism that typically allows particles smaller than 1–2 mm to enter the small intestine after a meal and how the food structure breaks down in the stomach. However, gastric digestion was not considered in detail in any of these studies. Meanwhile, understanding the link between gastric digestion, small intestinal digestion, and glycemic response should aid in developing targeted food structures for glycemic response management.

In this work, it was hypothesized that the glycemic response of starch-based foods of varying structures is affected by their small intestinal digestion kinetics, which is caused by differences in gastric breakdown and gastric emptying rates. Foods with a faster gastric breakdown rate and emptying were hypothesized to have a higher rate of digestion and absorption in the small intestine owing to a higher flow of nutrients from the stomach and, subsequently, a higher glycemic response. It was previously shown that macrostructural and microstructural differences of cooked rice and wheat-based foods led to different breakdown rates in the stomach, and subsequently different gastric emptying rates [13]. The breakdown rate in that study was measured as softening half-time, which was obtained from textural measurement of gastric digesta to reflect the overall effect of mixing with gastric secretions, particle size reduction by gastric contractions, and gastric emptying of solid. In the same work, foods with initial size of <1–2 mm were found to be emptied faster than foods with initial size of >2 mm [13]. The investigation on the chemical and enzymatic aspects of gastric digestion that occurred in this study were reported elsewhere [14]. It was reported that foods with smaller particle size and easier mixing with gastric secretions had not only faster starch emptying but also higher starch hydrolysis in the beginning of gastric digestion, which consequently were expected to cause faster starch digestion in the small intestine and glycemic response [14]. However, the further consequences of gastric digestion on small intestinal digestion and glycemic response of these same foods were not examined in the previous studies. Therefore, this work investigated how the glycemic response of varying structures of cooked rice–based and wheat-based foods was related to their digestion in the upper gastrointestinal tract, by combining glycemic response measurement and gut content property characterization. A growing pig model was used because of the similar metabolic responses between pigs and humans [15], which allowed for flexibility in acquiring small intestinal content and blood samples from different vein locations to achieve the objectives of the study.

Methods

Study diet preparation

Six study diets from 2 different starch sources (durum wheat and high amylose white rice) were used in the study. Durum wheat porridge (semolina); durum wheat couscous (couscous); durum wheat fettuccine (pasta); high amylose white rice grain (rice grain); high amylose white rice couscous (rice couscous); and high amylose white rice noodle (rice noodle) were sourced, cooked, and characterized as previously described [13,14]. Based on their initial particle size after cooking, these study diets were classified as smaller-sized diets (diameter of ≤2 mm: semolina, couscous, and rice couscous) and larger-sized diets (diameter of >2 mm: pasta, rice grain, and rice noodle). White bread was selected as a reference diet and was purchased from a local supermarket (Palmerston North, New Zealand). The crust was removed, and the crumb was cut to 20 × 20 mm on days before blood sampling (acclimatization period meal) or 15 × 15 mm (blood sampling day meal) before feeding.

Study design and sampling protocol

All protocols in this study were approved by the Animal Ethics Committee, Massey University, NZ (Protocol 18/128) and were performed according to the Guidelines for Care and Use of Laboratory Animals of Massey University. The study consisted of a glycemic response study and gut content collection study, which were conducted separately (Figure 1).

Glycemic response study

Thirty-three Large White × Landrace male pigs (21.3 ± 1.6 kg), distributed in 3 batches, were housed in individual pens (1.5 × 1.5 m) at the Massey University Animal Research Unit, Palmerston North, New Zealand. The pens were located in a temperature-controlled room (21°C ± 2°C) with 12:12-h light/dark cycles.

Each pig was randomly assigned to one of the 6 study diets or the reference diet on the day of arrival as their first diet/D1. Pigs were acclimatized to the first diet (D1) over 5 d (Supplemental Figure 1A). The acclimatization meals were supplemented with 10% casein, 10% soya oil, and 0.25% vitamin/mineral mix to meet the nutritional requirements of the pig [16]. During acclimatization, pigs were adapted to having their ears manipulated (to prepare for blood sampling through an ear vein catheter). On day 5, 19 pigs that adapted well to ear manipulation underwent in-dwelling ear catheter insertion (Supplemental Method A); the remaining pigs were re-acclimatized to randomly assigned test diets for gut content collection study. Each catheter was flushed with sterile heparinized saline at least twice daily. Catheterized pigs had ad libitum access to water except 2 h before and until the second hour of blood sampling. One catheterized pig was excluded from the study before the first blood sampling period owing to the signs of infection (Figure 1A).

After each blood sampling period, pigs received a new diet (D2/D3/D4) and were given 1 d to adjust to the new diet before the next blood sampling day (Supplemental Figure 1). The diets selected as D2/D3/D4 were randomly determined using the RAND() function in Excel, such that each pig would receive the reference diet and 3 study diets from the 6 possible study diets with different sequences. Five pigs were excluded from the study

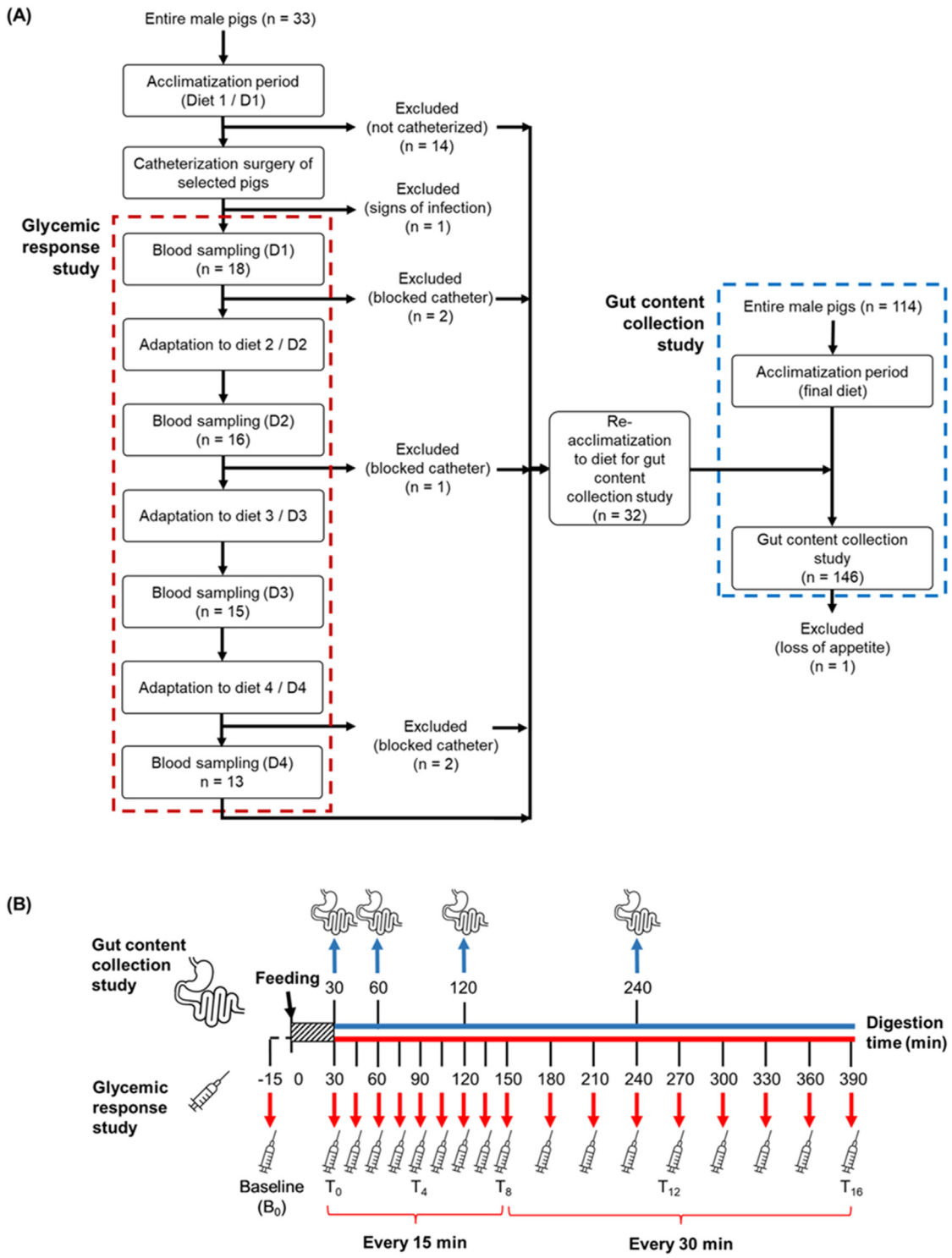

FIGURE 1. (A) Diagram of the design of the glycemic response and gut content collection studies. Pigs from the glycemic response study were re-acclimatized for the gut collection content study after their catheters were removed. (B) Timing of sampling for either the gut content collection or glycemic response study. The 2 studies were conducted in separate periods. In both studies, feeding started at 0 min and ended at 30 min digestion time.

at different times owing to blocked catheters, and they were re-acclimatized for the gut content collection study (Figure 1A). Two of the 5 pigs had not received the reference diet. Because of this exclusion, sequence randomization in the last batch of the study was adjusted to obtain at least 5 replicates for each study diet. At the end of the glycemic response study, catheters were removed from all pigs, and they proceeded with their D4 diet for the gut content collection study.

On blood sampling day, pigs were fed their assigned diet [250-g starch equivalent, dry basis (db)] as their morning meal. This quantity was selected because the suggested amount of carbohydrates for glycemic response measurement in humans is

50 g of starch (db), and pigs typically require 3–5 times this amount [17]. To minimize interference from the previous meal, the penultimate meal (≥16 h before blood sampling) consisted of 70% of the sampling day portion. Both the penultimate and sampling day meals were fed without supplemental ingredients, except for pasta that was mixed with a small amount of soya oil (~15 g) and salt (~4 g) to enhance its palatability. Pigs were given 30 min to consume their meals; then, their feeder was removed; the 30-minute time was determined based on the maximum duration the pigs could finish their meals observed in the acclimatization period of the first batch of the study. Most pigs were able to consume the required amount of meal (50% of the given amount for semolina, 70% of the given amount for the other 5 diets) without difficulties. No consistent trends were observed for the diet refusal when the pigs transitioned between the diets.

Blood samples were collected through the in-dwelling catheters (Figure 1B): ~15 min before the meal (B_0), immediately after the meal consumption (T_0/30 min of digestion time), every 15 min until 2 h later (T_1–T_8), then every 30 min until 6 h after the meal consumption (T_9–T_{16}). At each sampling time, the first 2 mL of blood that was drawn was discarded owing to potential dilution with heparinized saline. Subsequently, 2 mL of blood was collected into a BD Vacutainer tube containing NaF and Na_2EDTA and stored on ice until centrifugation. Then, the catheter was flushed with 4 mL of sterile heparinized saline. Blood samples were centrifuged for 10 min at 1200 × *g* (Tabletop Centrifuge DSC-200A-2; Digisystem Laboratory). Plasma was separated and stored on ice until the analysis (within 3 h).

Gut content collection study

A total of 146 Large White × Landrace male pigs (21.7 ± 1.8 kg body weight) were involved, comprising 33 pigs from the glycemic response study and 114 additional pigs that were specifically used for the gut content collection study (Figure 1A). Each pig was randomly assigned to one of the 6 study diets and 1 digestion time (30/60/120/240 min) (Figure 1B). Animal treatment and details for the gut content collection study were described elsewhere [13]. On sampling day, pigs were fed a standardized meal amount (250 g of starch equivalent, db) that was mixed with an indigestible marker [titanium dioxide (TiO_2); 0.5% meal dry matter (DM)] to trace the flow of the meal and nutrients in the gastrointestinal tract of the pigs [18–20]. Preliminary tests on TiO_2 mixing with the meal indicated a minimum separation between TiO_2 and the meal (~5% difference between the theoretical 0.5% TiO_2 added with the measured TiO_2), suggesting TiO_2 was a suitable indigestible marker for this study.

The pigs were anesthetized after their assigned digestion time (Figure 1B) using the same anesthetic as for the catheter insertion (Supplemental Method A). Blood samples (~2 mL) were collected from the jugular vein, vena cava, portal vein, hepatic vein, and left ventricle under anesthesia into BD Vacutainer tubes containing NaF and Na_2EDTA. Blood samples were stored on ice until plasma separation, then the plasma was immediately frozen on dry ice. Plasma samples were stored at −20°C until the analysis. Pigs were killed by an intracardiac injection of a lethal dose (0.3 mL/kg BW) of pentobarbitone (Pentobarb 300).

Clamps were placed at each end of the stomach and small intestine; then, the entire upper gastrointestinal tract was removed. Collection and characterization of the stomach content was reported elsewhere [13,14]. The small intestine was removed and divided into the following: duodenum (first ~25 cm of intestine from the pyloric sphincter), terminal ileum (TI) (final ~10 cm intestine from the ileal–cecal junction), and proximal jejunum (PJ), distal jejunum (DJ), ileum (3 equal-length regions of the remaining portion). Digesta was removed from the small intestinal lumen by gradual flushing with distilled water. Subsamples were taken for particle size measurement and were kept at 4°C for the same-day analysis. Another subsample was collected and frozen (−20°C) for the reducing sugar and glucose analysis; the rest were immediately frozen (−20°C), freeze dried, ground, and sieved with a 1-mm sieve before the analysis.

Sample analyses

Blood plasma glucose analysis

The glucose concentration in plasma samples from glycemic response and gut content collection studies was measured in duplicate using a glucose oxidase/peroxidase assay kit (GOPOD; Megazyme).

Intestinal content particle size analysis

Particle size distribution of intestinal digesta was determined in triplicate using the Mastersizer-2000 (Malvern Instruments), with a refractive index of 1.530 for starch-based samples [14]. From each measurement, volume mean diameter (D[4,3]), surface area mean diameter D[3,2]), specific surface area (SSA), and the diameter below which 10%, 50%, or 90% of all particles are found (d_{10}, d_{50}, and d_{90}, respectively) were quantified to identify any changes in the particles between 0.1 and 1000 μm in the digesta.

Intestinal content chemical analysis

Frozen samples were kept at 37°C for 15 min to transition the sample tubes between frozen and boiling conditions, followed by heating (≥90°C, 20 min) to denature digestive enzymes, cooling on ice for 10 min, and centrifugation (4300 × *g*, 10 min). Reducing sugar content in the supernatant was quantified as maltose equivalent using the dinitrosalicylic acid method [21] with modifications [14], whereas free glucose was determined as described for plasma glucose. Total glucose and maltose present in intestinal digesta DM were estimated as described in Supplemental Method B.

Freeze-dried intestinal contents were analyzed for TiO_2 following the study by Short et al. [20]. Total starch was quantified using Megazyme Total Starch Kit (Megazyme), following the procedure for samples not containing free glucose or maltodextrins. Moisture content was determined gravimetrically following the study by Nadia et al. [13].

Data and statistical analysis

Glycemic response

The incremental area under the curve (iAUC) and maximum change in plasma glucose from the baseline (Δmax) was determined for each pig and diet. The iAUC at 90, 150, 270, and 390 min was quantified using the trapezoid rule using the plasma glucose concentration at B_0 as the baseline [22]. Negative areas were not included in the calculation [23]. The Δmax was expressed as the maximum difference between the plasma glucose concentration at time *t* and the baseline concentration.

To identify the overall effect of the diets on the progression of glycemic response, cumulative change of plasma glucose (Δglucose) and iAUC values of the diets over time from $t = 0$ min to $t = 360$ min were fit to the Gompertz model [56]:

$$Cumulative\ \Delta glucose\ or\ iAUC\ (t) = A_G \exp\left(-\exp(-k_G(t - T_i))\right) \quad (1)$$

where A_G, the plateau value of the growth curve [(mg/dL)·min]; k_G, growth rate coefficient (min^{-1}); and T_i, the time at inflection (min).

Ileal starch digestibility and nutrient flow

Ileal starch digestibility and glucose and maltose flow (as main starch hydrolysis products) in the ileum using measured TiO_2 concentration in the diet and digesta were calculated only after ensuring that the recovery of TiO_2 from the gastrointestinal tract of the pigs for all diets was ≥85%, indicating that risk of phase separation during digestion was limited and would have minimal interference on the calculation results. Ileal starch digestibility was calculated as follows [24]:

$$Ileal\ starch\ digestibility\ (\%) = \left[1 - \frac{(starch_{ileum}/Ti_{ileum})}{(starch_{diet}/Ti_{diet})}\right] \times 100 \quad (2)$$

where $starch_{ileum}$ or $starch_{diet}$, starch concentration in ileum content or diet, respectively (g/kg DM sample); Ti_{ileum} or Ti_{diet}, TiO_2 content in ileum content or diet, respectively (g/kg DM sample).

The flow of glucose or maltose in the ileum for each diet was calculated as follows [25]:

$$Component\ flow = Component_{ileum} \times \left(\frac{Ti_{diet}}{Ti_{ileum}}\right) \quad (3)$$

where $Component_{flow}$, flow (g/kg DM eaten) of maltose or glucose in the ileum; $Component_{ileum}$, concentration of maltose or glucose in ileal digesta (g/kg DM digesta); Ti_{ileum} or Ti_{diet}, TiO_2 concentration in ileal digesta or diet, respectively (g/kg DM sample).

Portal glucose accumulation, intestinal glucose, and maltose accumulation

To estimate the accumulation behavior of glucose in the portal vein and maltose or glucose in the small intestine of pigs in the gut content collection study, cumulative curves for these variables were established. Because each pig only represented 1 diet × digestion time, the mean portal glucose concentration, mean total intestinal glucose, or mean total intestinal maltose for each diet at each time point was summed over time (30–240 min) to generate a single cumulative curve for each diet. To empirically model the accumulation behavior, the cumulative value over time was fitted to the Michaelis–Menten nonlinear model in MATLAB [26,27]:

$$Accumulation\ (mg\ /\ dL\ or\ g) = \frac{V_{max} \times time}{K_m + time} \quad (4)$$

where V_{max}, maximum accumulation (milligrams per deciliter for portal glucose, grams of glucose or maltose for total intestinal glucose or maltose accumulation); K_m, half-time to reach the maximum accumulation (min).

It should be noted that the Michaelis–Menten model was used in this study as an empirical model that describes the overall accumulation behavior during digestion in the small intestine and absorption into the portal vein, such that the interpretation of the V_{max} (as the maximum accumulation mass or concentration) and K_m (as the rate of accumulation, expressed as half-time) in this study was different from when they are used to describe starch hydrolysis or glucose utilization kinetics.

Statistical analysis

The number of pigs in the glycemic response study and the gut content collection study was determined based on results from previous studies with a similar design and food types [28,29]. Four pigs per diet was necessary in the glycemic response study, to detect a minimum difference of 900 mg/dL·min in the iAUC of plasma glucose and SD of 1100 mg/dL·min, with a power of 80% and α of 0.05. For the gut content collection study, 6 pigs per treatment (diet × digestion time) was necessary to detect a 10% difference and SDs of 5.7% in the digesta properties (based on the previous measurements of marker concentration and rheological properties) with a power of 80% and α of 0.05. To match the number of replicates in the gut content collection study, a minimum of 6 pigs were determined for each study diet in the glycemic response study. To have 6 replicates per study diet in the glycemic response study, it was assumed that each catheterized pig could be fed 3 study diets and the reference diet (bread), which need at least 12 pigs. For each study diet, an additional 3 pigs were used to anticipate feed refusal and potential issues with the in-dwelling catheter, resulting in 18 pigs that were used in the glycemic response study.

Data from pigs that consumed <50% (semolina) or <70% (other diets) of the study diet were excluded from the analysis to ensure normal gastric emptying processes [13]. Exclusion of pigs by this criterion and blocked catheters resulted in an incomplete block design for the glycemic response study, with $5 < n \leq 7$ for each study diet and $n = 13$ for bread for each glycemic response parameter measured. Exclusion of pigs for the gut content collection study resulted in $5 \leq n \leq 6$ for each study diet and for each variable measured in the study.

The statistical analysis was performed using SAS Studio 3.8. One-way ANOVA was performed on glycemic response parameters (Δmax, iAUC, Gompertz model parameters for Δglucose, and iAUC) and ileal starch digestibility with the study diet as the main effect. Glycemic response parameters were screened for outliers using ±3 interquartile range before the statistical analysis. A square root transformation was applied to the iAUC data to achieve normality and homoscedasticity of residuals. Multifactor, repeated measures ANOVA (PROC MIXED) was performed on intestinal content properties (milligrams of glucose/DM digesta, milligrams of maltose/DM digesta, and particle size parameters). Pig was the subject, diet type and digestion time were the between-subject factors, and intestinal region (duodenum, proximal jejunum (PJ), distal jejunum (DJ), ileum, and terminal ileum (TI)) was the repeated factor within each pig. To achieve normality and homoscedasticity of residuals, glucose and maltose concentration were square-root-transformed, and all particle size parameters except d_{90} were log-transformed. Multifactor ANOVA was performed on portal vein plasma glucose, total intestinal glucose, total intestinal maltose, ileal glucose flow, and ileal maltose flow, where diet type and

digestion time were the main effects. A preliminary statistical analysis to remove outliers (using ±3 internally studentized residual criterion) was conducted for all data sets obtained from small intestinal digesta and portal vein, resulting in $4 \leq n \leq 6$ for the final data analysis.

In the analyses of both the glycemic response and gut content collection study data, batch of pigs ($n = 3$) was included in all statistical models to consider variability between the study periods. For all statistical analyses conducted, the Tukey–Kramer post hoc test was used to compare means between the treatments when main effects were significant. Statistical significance was determined at $P < 0.05$. All values are presented as mean ± SE.

Results

Glycemic response parameters

The baseline plasma glucose concentration of the catheterized pigs was not significantly different across diets and sampling days (mean 83.9 ± 1.2 mg/dL; $P = 0.169$). The glycemic response curve for each diet generally indicated a biphasic behavior (Figure 2A, B). The $\Delta max_{overall}$ was not significantly affected by the diet ($P = 0.420$). The iAUC was examined at 90, 150, 270, and 390 min ($iAUC_{overall}$) of the digestion time. Diet types significantly affected $iAUC_{270min}$ and $iAUC_{overall}$ ($P < 0.05$) (Table 1). A cumulative change in plasma glucose concenteration and iAUC over time also fit well to the Gompertz model (Table 1; examples of Gompertz model fit to the data sets are given in Supplemental Figure 3).

Smaller-sized (couscous, rice couscous, and semolina) and larger-sized (pasta, rice grain, rice noodle) diets exhibited different trends relative to bread (Figure 2A, B and Table 1). The $\Delta max_{overall}$ of reference, smaller-sized, and larger-sized diets were 27.8 ± 4.0, 29.0 ± 3.2, and 21.7 ± 2.6 mg/dL, respectively. The $iAUC_{overall}$ of reference, smaller-sized, and larger-sized diets were 3647 ± 762, 5659 ± 727, and 2704 ± 521 mg/dL·min, respectively (smaller-sized and larger-sized diets averaged together within their category).

Particle size distribution of intestinal digesta

In the measurement of particles between 1 and 1000 μm using the Mastersizer, a low concentration of particles in digesta samples (indicated by obscuration <2%) may produce unreliable results [30]. Because a considerable proportion (≥30%) of samples from the duodenum and terminal ileum (TI) had obscuration <2% (Supplemental Table 2), particle size data from these regions were excluded from the statistical analysis. The diet, small intestinal region, and diet × region interaction significantly affected D[4,3], D[3,2], d_{10}, d_{50}, d_{90}, and SSA of digesta from the proximal jejunum (PJ), distal jejunum (DJ), and ileum ($P < 0.001$) (Supplemental Tables 1 and 3). D[4,3] and D[3,2] were specifically measured to investigate any changes in the central point of the volume distribution and surface area distribution of the particles in the intestinal digesta because of changes in the distribution of larger and finer particles, respectively. However, limited trends were found. Rice couscous and rice noodle had smaller D[4,3] and D[3,2] than pasta and rice grain, which were all smaller than couscous and semolina in any small intestinal region. The trend was consistent in the SSA, where the SSA of rice couscous and rice noodle > pasta and rice grain > couscous and semolina ($P < 0.05$) (Supplemental Table 3).

Because limited trends could be observed using the particle size parameters (Supplemental Tables 3 and 4), the particle size distribution was visualized to monitor overall profile changes along the small intestine (Figure 3). All diets initially had at least 2 large peaks in the proximal and distal stomach; these trends have been reported elsewhere in another part of this study [14] but are shown in this study to allow for visualization of trends across the entire upper gastrointestinal tract. The large peaks visualized in the proximal and distal stomach were present in the small intestinal samples, but the percent volume of the peaks changed throughout the small intestine. As the digesta traveled from the proximal jejunum (PJ) toward the ileum, peaks for particles of <100 μm diminished in semolina, couscous, and rice grain; moreover, peaks for particles of >100 μm diminished in rice couscous and rice noodle (Figure 3).

Intestinal digesta chemical properties and ileal starch digestibility

Diet, small intestinal region, and their interaction significantly affected the intestinal reducing sugar (expressed as maltose equivalent) and glucose concentration ($P \leq 0.016$). Digestion time significantly influenced the maltose concentration ($P < 0.001$) (Supplemental Table 1). For all diets and times, maltose and glucose concentration was higher in the proximal and distal jejunum compared with those in the other 3 small intestinal regions, whereas the maltose and glucose concentration in the terminal ileum (TI) was lower than the rest of the small intestinal regions ($P < 0.05$) (Figures 4 and 5). Concentration of glucose and maltose in the proximal jejunum (PJ) did not significantly differ between diets ($P \geq 0.05$). Compared with that of the other 5 diets, maltose concentration in the ileum was significantly higher for rice couscous (344 ± 41.9 mg maltose/g DM digesta, averaged across digestion times; $P < 0.05$) but lower for pasta (173 ± 22.6 mg maltose/g DM digesta, averaged across digestion times; $P < 0.05$).

The total mass of glucose and maltose in the small intestine (Figure 6A, B) was significantly affected by diet and digestion time ($P < 0.001$). Diet × time interaction was significant only to total intestinal maltose ($P < 0.001$). For all diets, total glucose and maltose after 60 min (3.27 ± 0.23 g glucose and 8.36 ± 1.00 g maltose, respectively, averaged across diets) was higher than the other digestion time points ($P < 0.05$). The cumulative behavior of total intestinal maltose and glucose over time was well described by the Michaelis–Menten model ($R^2 \geq 0.87$) (Table 2 and Figure 6D, E). For both total intestinal maltose and glucose, the order of V_{max} was as follows: rice couscous > rice grain ≈ couscous > semolina > rice noodle > pasta, whereas the order of K_m was as follows: rice noodle > couscous > pasta > rice grain ≈ rice couscous > semolina.

Ileal starch digestibility was calculated using the 120-minute and 240-minute data for each diet to ensure constant digesta passage because, at earlier digestion times, minimum digesta and TiO_2 was found in the terminal ileum (TI). The ileal starch digestibility was not significantly different between the 120-minute and 240-minute digestion, thus the values for both digestion

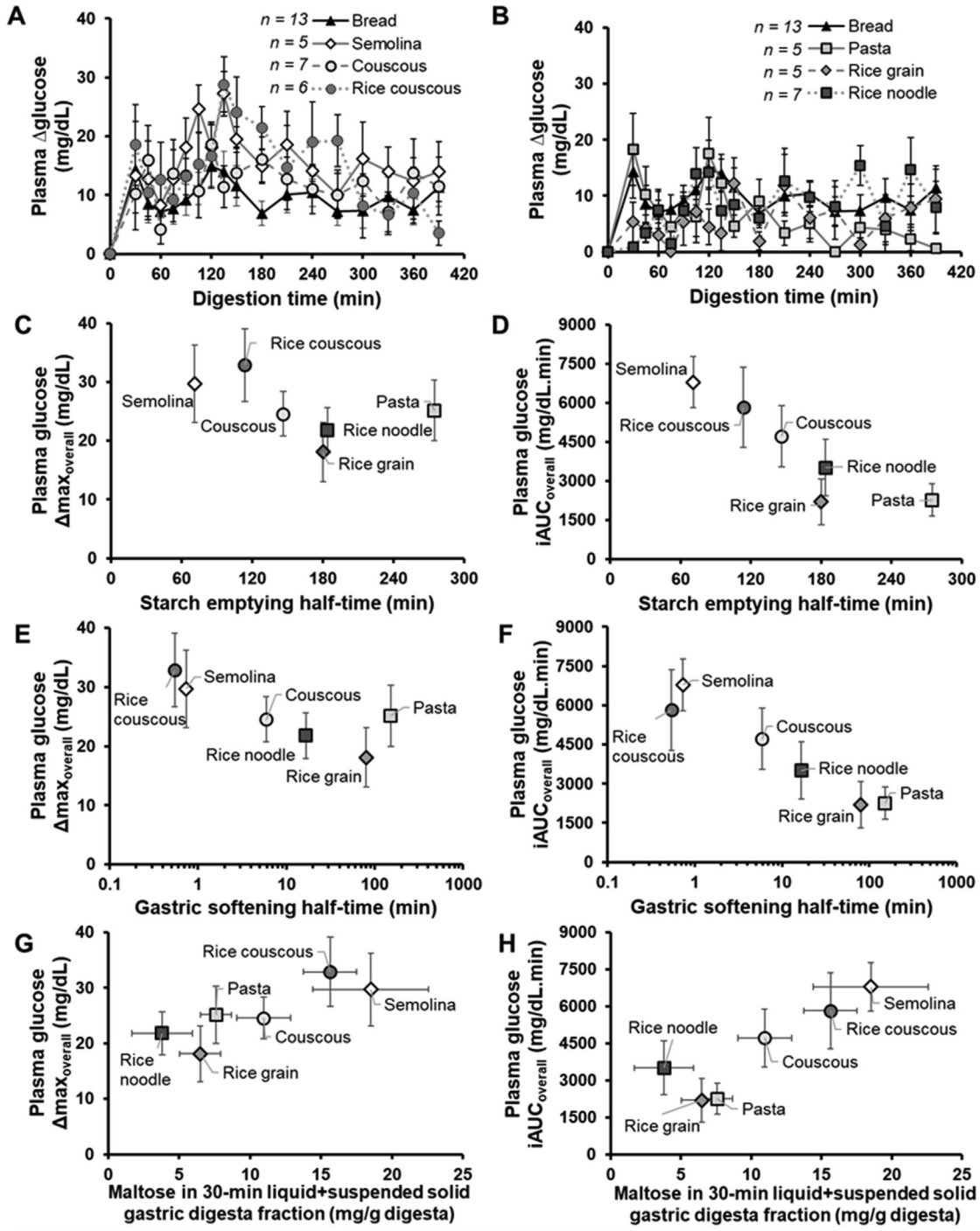

FIGURE 2. Glycemic response over time (mean ± SE; *n* indicates the number of pigs for each diet) for growing pigs fed with smaller-sized (A) and larger-sized (B) diets. Bread was included in both (A) and (B) as a reference. For variability in the glycemic response curves for each pig and diet, see Supplemental Figure 2. Relationship between: glycemic response parameters with starch-emptying half-time (C, D), glycemic response parameters with gastric softening half-time (E, F), and glycemic response parameters with maltose in the 30-min gastric digesta liquid and suspended solid fractions of the diets (G, H). Values are mean ± SE ($n = 4$–7 for each data point), except the half-times that are presented as estimated half-time obtained from model fitting. Starch-emptying half-time and maltose concentration in gastric digesta were reported in Nadia et al. [14]. Gastric softening half-time was reported in Nadia et al. [13]. Note that the gastric softening half-time is shown with a logarithmic scale owing to the wide range of the values across the 6 diets.

times were pooled. Diet did not significantly influence ileal starch digestibility ($P = 0.098$) (Table 3). Glucose and maltose flow in the ileum were significantly influenced by the diet, digestion time, and interaction of diet × time ($P < 0.001$). Both glucose and maltose flow in the ileum at 30 min of digestion was higher when pigs consumed semolina, couscous, or rice grain than that when they consumed rice couscous or the noodle diets ($P < 0.001$) (Table 3).

TABLE 1
Glycemic response parameters and Gompertz model (Equation 1) parameters for iAUC and change in plasma glucose relative to the baseline (Δglucose) of the glycemic response data in catheterized pigs fed one of the 6 study diets or bread as the reference diet[1]

	Diet[2]														*P*-diet[3]
	Bread	*n*	Semolina	*n*	Couscous	n	Pasta	*n*	Rice grain	*n*	Rice couscous	*n*	Rice noodle	*n*	
Glycemic response parameters															
$\Delta max_{overall}$ (mg/dL)	27.8 ± 4.0	13	29.7 ± 6.6	4	24.6 ± 3.8	7	25.1 ± 5.2	5	18.1 ± 5.0	5	32.9 ± 6.2	6	21.8 ± 3.9	7	0.420
$iAUC_{90min}$ (mg/dL·min)	776 ± 213	13	13,840 ± 305.8	4	625.4 ± 165	7	705 ± 251	5	240 ± 105	5	633 ± 165	6	189 ± 26.6	7	0.164
$iAUC_{150min}$ (mg/dL·min)	1658 ± 371	12	201,301 ± 389.5	5	1353.4 ± 338	7	1339 ± 375	5	593 ± 232	5	2203 ± 598	6	635 ± 60.7	7	0.088
$iAUC_{270min}$ (mg/dL·min)	2632 ± 529	13	3984 ± 517	5	3396.4 ± 867	7	1929 ± 519	5	1835 ± 541	4	4732 ± 1249	6	1522 ± 307	7	0.025
$iAUC_{overall}$ (mg/dL·min)	3647 ± 761^{ab}	13	6785 ± 988^{a}	5	4713 ± 1174^{ab}	7	22,567 ± 622^{ab}	5	2193 ± 878^{b}	5	5825 ± 1545^{ab}	6	3505 ± 1094^{ab}	7	0.018
Gompertz model parameters[4]															
iAUC A_G (mg/dL·min)[7]	4737 ± 930^{ab}	10	9197 ± 1423^{a}	5	4139 ± 886^{ab}	7	3127 ± 1140^{b}	3	1734 ± 724^{b}	4	4931 ± 1109^{ab}	6	5445 ± 1266^{ab}	6	0.010
iAUC k_G (min^{-1})[7]	0.71 ± 0.06	13	1.02 ± 0.29	5	0.96 ± 0.11	6	1.19 ± 0.09	5	1.23 ± 0.35	4	1.05 ± 0.07	5	0.65 ± 0.09	7	0.070
iAUC T_i (min)	161 ± 76^{ab}	12	194 ± 101^{ab}	5	162 ± 42^{ab}	7	88 ± 49^{b}	4	207 ± 114^{ab}	4	148 ± 36^{ab}	6	244 ± 120^{a}	7	0.039
iAUC R^2	0.98 ± 0.003	—	0.99 ± 0.002	—	0.99 ± 0.003	—	0.98 ± 0.013	—	0.99 ± 0.004	—	0.99 ± 0.001	—	0.99 ± 0.002	—	
Δglucose A_G (mg/dL)	164 ± 34.1	10	259 ± 19.2	4	160 ± 33.5	5	101 ± 31.2	3	96.7 ± 37.5	3	209 ± 45.5	5	137 ± 35.0	5	0.103
Δglucose k_G (min^{-1})	1.15 ± 0.07^{ab}	13	1.12 ± 0.19^{ab}	4	1.27 ± 0.2^{a}	6	1.31 ± 0.25^{a}	5	0.94 ± 0.1^{ab}	5	1.17 ± 0.09^{ab}	5	0.74 ± 0.08^{a}	6	0.015
Δglucose T_i (min)	98 ± 40^{ab}	13	104 ± 40^{ab}	5	103 ± 42^{ab}	7	71 ± 37^{b}	5	88 ± 67^{ab}	4	101 ± 35^{ab}	6	147 ± 63^{a}	7	0.119
Δglucose R^2	0.95 ± 0.022	—	0.98 ± 0.005	—	0.99 ± 0.003	—	0.96 ± 0.019	—	0.98 ± 0.014	—	0.99 ± 0.002	—	0.98 ± 0.009	—	

$\Delta max_{overall}$, the maximum change in plasma glucose relative to the baseline (mg/dL) within 390 min of digestion; A_G, the plateau value of the glycemic response growth curve within 390 min of digestion; $iAUC_{overall}$, incremental area under the curve calculated throughout the examined digestion time (390 min); $iAUC_t$, incremental area under the curve calculated within *t* min of digestion; k_G, growth rate coefficient of the growth curve within 390 min of digestion; T_i, time at inflection of the growth curve within 390 min of digestion.

[1] Values are presented as mean ± SE. Labeled means in a row without a common letter differ, $P < 0.05$.

[2] The number of replicates for each diet and measured parameter is indicated by the column "*n*" on the right side of each presented mean. Different number of replicates between the diets were owing to different number of pigs that were able to consume the required amount of their assigned diet on the sampling day. Different numbers of replicates for parameters measured within the same diet were owing to outlier removal.

[3] Only the effect of diet is presented because the batch of pigs was not significant to the glycemic response and Gompertz model parameters ($P \geq 0.05$).

[4] Parameters were obtained through fitting to Equation *1*. R^2 indicates the goodness of fit of the Gompertz model to the cumulative values of iAUC or change in the plasma glucose (Δglucose) concenteration for each diet.

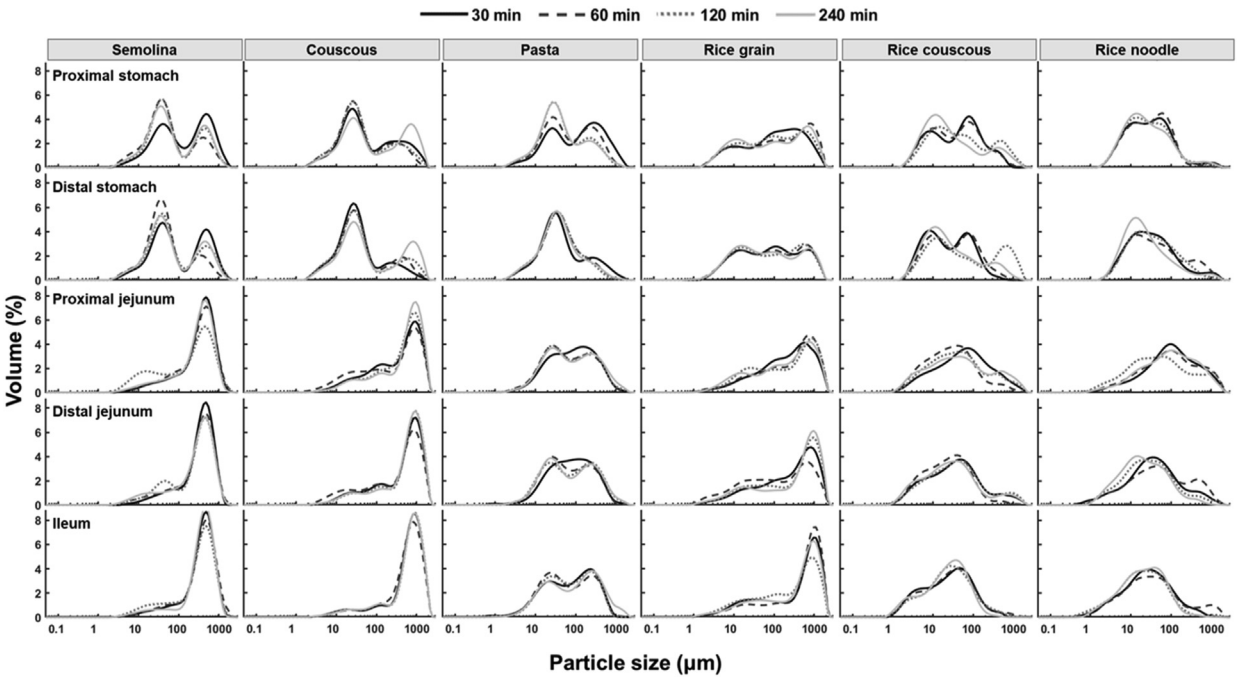

FIGURE 3. Particle size distribution over time of the suspended solid fraction of proximal stomach and distal stomach digesta (reported in Nadia et al. [14]) and small intestinal digesta obtained from the proximal jejunum, distal jejunum, and ileum regions of growing pigs fed one of the 6 study diets. Each column indicates particle size distribution data from one type of the diet. Each digestion time is indicated with different line style, as indicated on the top of the figure. Each curve represents the mean from 2 to 6 pigs. Individual curves with error shades indicate the range of the distribution, and the number of pigs used to establish each curve is given in Supplemental Figures 6–11.

Portal vein plasma glucose concentration

Among the vein locations measured for plasma glucose (Supplemental Table 5), the portal vein was the only location where plasma glucose concentration was significantly influenced by diet ($P = 0.010$). Portal plasma glucose concentration was also significantly influenced by digestion time ($P < 0.001$). Similar patterns in portal plasma glucose concentration were observed between semolina and rice couscous and between rice grain and rice noodle. The cumulative behavior of the portal plasma glucose concentration over time was well described by the Michaelis–Menten model ($R^2 \geq 0.97$) (Table 2 and Figure 6F). The V_{max} for portal plasma glucose was the lowest in semolina, followed by pasta, rice grain, rice noodle, couscous, and rice couscous. The K_m was the lowest in rice couscous and semolina, followed by pasta, rice grain and rice noodle, and couscous.

Discussion

This study aimed to identify the relationships between food structure, gastrointestinal digestion, and glycemic response of commonly consumed starch-based foods using growing pigs as a digestive model for adult humans. This aim was achieved by combining the findings from glycemic response data from the glycemic response study with small intestinal property data and gastric digestion data (published elsewhere [13,14]) from the gut content collection study. It was hypothesized that the trends observed in the glycemic response and small intestinal digesta in this study could be attributed to the differences in gastric breakdown and gastric emptying rate.

The glycemic effect of the diets ($\Delta max_{overall}$ and $iAUC_{overall}$) (Table 1) measured in the glycemic response study was in the order of smaller-sized diets > bread > larger-sized diets. This difference was also reflected in the mean glycemic response curves established for the 2 groups of diets (Figure 2A, B), which highlights the difference in the glycemic effects of the smaller-sized and larger-sized diets. The generally biphasic profile of the mean postprandial glycemic response curve (Figure 2A, B) can be associated with good glucose tolerance, as previously reported in studies using jugular-catheterized pigs [31–33] and in humans [34,35]. Separation in the trend between smaller-sized and larger-sized diets might be associated with their gastric emptying, as reported previously [13,14]. The $\Delta max_{overall}$ and $iAUC_{overall}$ were inversely related to the starch-emptying half-time ($t_{1/2,starch\ GE}$) (Figure 2C, D); similar relationship was observed between the asymptote of Δglucose or plasma glucose iAUC growth curve (A_G) and the starch-emptying half-time (Supplemental Figure 4). More specifically, a high inverse correlation was identified between $iAUC_{overall}$ and $t_{1/2,starch\ GE}$ ($r = -0.90$, $P = 0.015$), indicating that diets with slower gastric emptying generally had a lower glycemic response. The correlations between the glycemic response and starch gastric emptying rate agreed with previous studies that found an inverse relationship between the gastric emptying half-time and maximum rise in plasma glucose on consumption of an oral glucose load or starch-rich meal in humans [9,36,37].

To investigate the link between the glycemic response observed in this work and gastric digestion, the relationship between the rate of food breakdown in the stomach and the

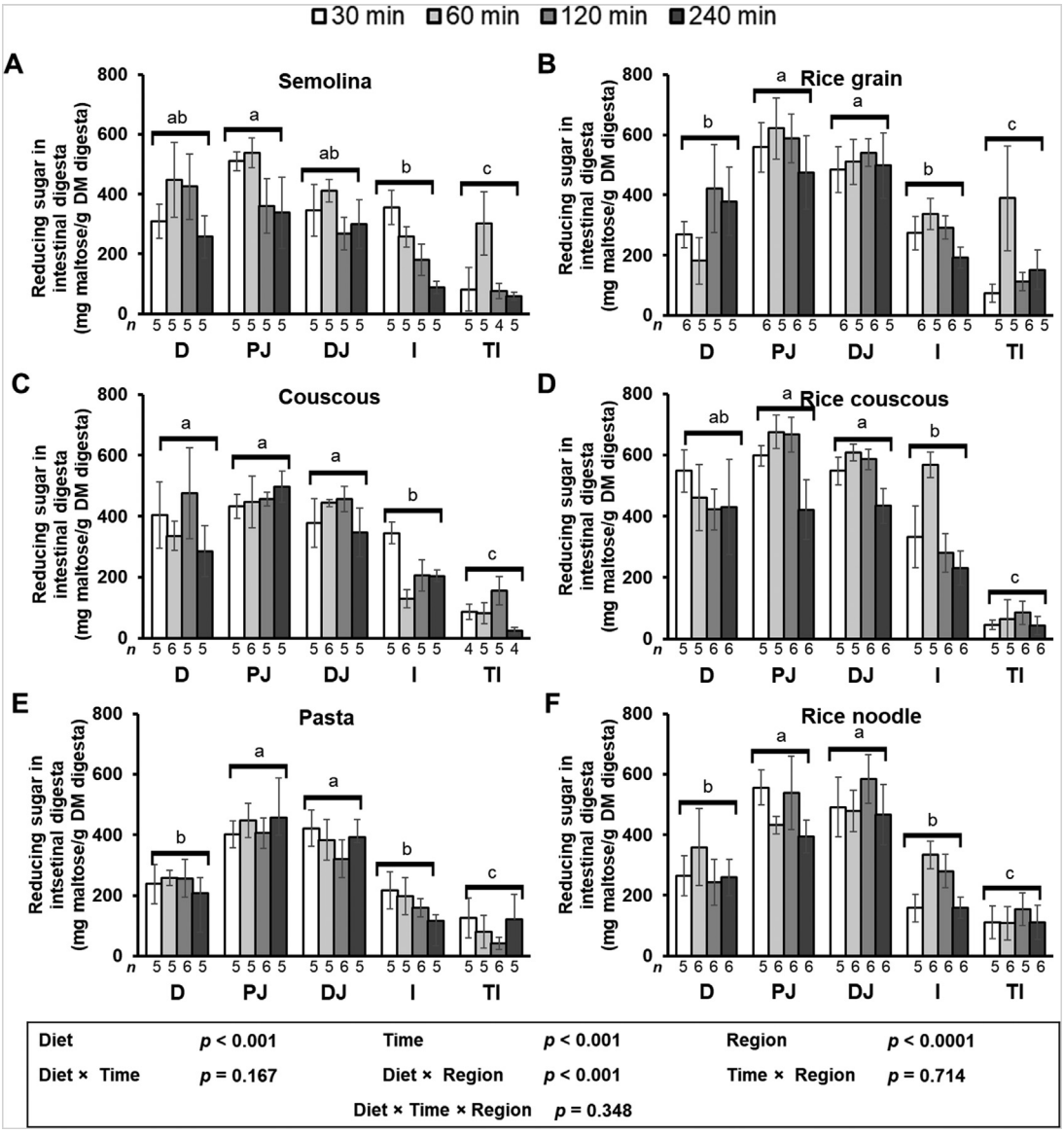

FIGURE 4. Reducing sugar concentration (measured as maltose equivalent) in the small intestinal digesta of pigs fed with semolina (A), rice grain (B), couscous (C), rice couscous (D), pasta (E), and rice noodle (F) obtained from 5 small intestinal regions at different digestion times. Values are mean ± SE; *n* indicates number of replicates for each diet × time × small intestinal region. In each panel, small intestinal regions (means of data across digestion times for each small intestinal region; diet × region interaction) labeled without a common letter differ, $P < 0.05$. D, duodenum; DJ, distal jejunum; DM, dry matter; I, ileum; PJ, proximal jejunum; TI, terminal ileum.

glycemic response parameters was explored further. The gastric emptying rate in the pigs that consumed the same diets used in this study was found to be limited by the diet breakdown rate during gastric digestion (quantified as gastric softening half-time, $t_{1/2,softening}$) [13]. The $t_{1/2,softening}$ was inversely related to $\Delta max_{overall}$ and $iAUC_{overall}$ (Figure 2E, F), suggesting that food with a slower breakdown during gastric digestion resulted in a lower glycemic effect. This finding aligns with an in vitro gastric digestion study using various foods that identified a negative correlation between $t_{1/2,softening}$ and glycemic indices of carbohydrate-based foods [38]. The rate of breakdown during gastric digestion might be related to the rate of mixing between the food bolus and gastric secretions in the stomach [14], which was also observed in the glycemic response profile of the reference diet (white bread) that was between the 2 groups of study diets (Figure 2A, B). Although the $t_{1/2,softening}$ of white bread was not measured in this study, a bread-based meal was reported to form a homogeneous food bolus that underwent slow mixing with gastric secretions [39], indicating that food bolus properties after mastication, in addition to its initial structure, may affect the glycemic response of the food. The link between $t_{1/2,softening}$, $t_{1/2,starch\ GE}$, and glycemic response parameters implies that the gastric breakdown rate of the diets in this study limited their gastric emptying rate and, subsequently, limited their glycemic responses. The negative-exponential relationship between $t_{1/2,softening}$ and the glycemic response parameters (Figure 2E, F) (logarithmic regression $R^2 = 0.65$ and 0.97 for $\Delta max_{overall}$ and $iAUC_{overall}$, respectively) possibly indicates a certain maximum limit of gastric breakdown rate to cause low glycemic response, which merits further investigation.

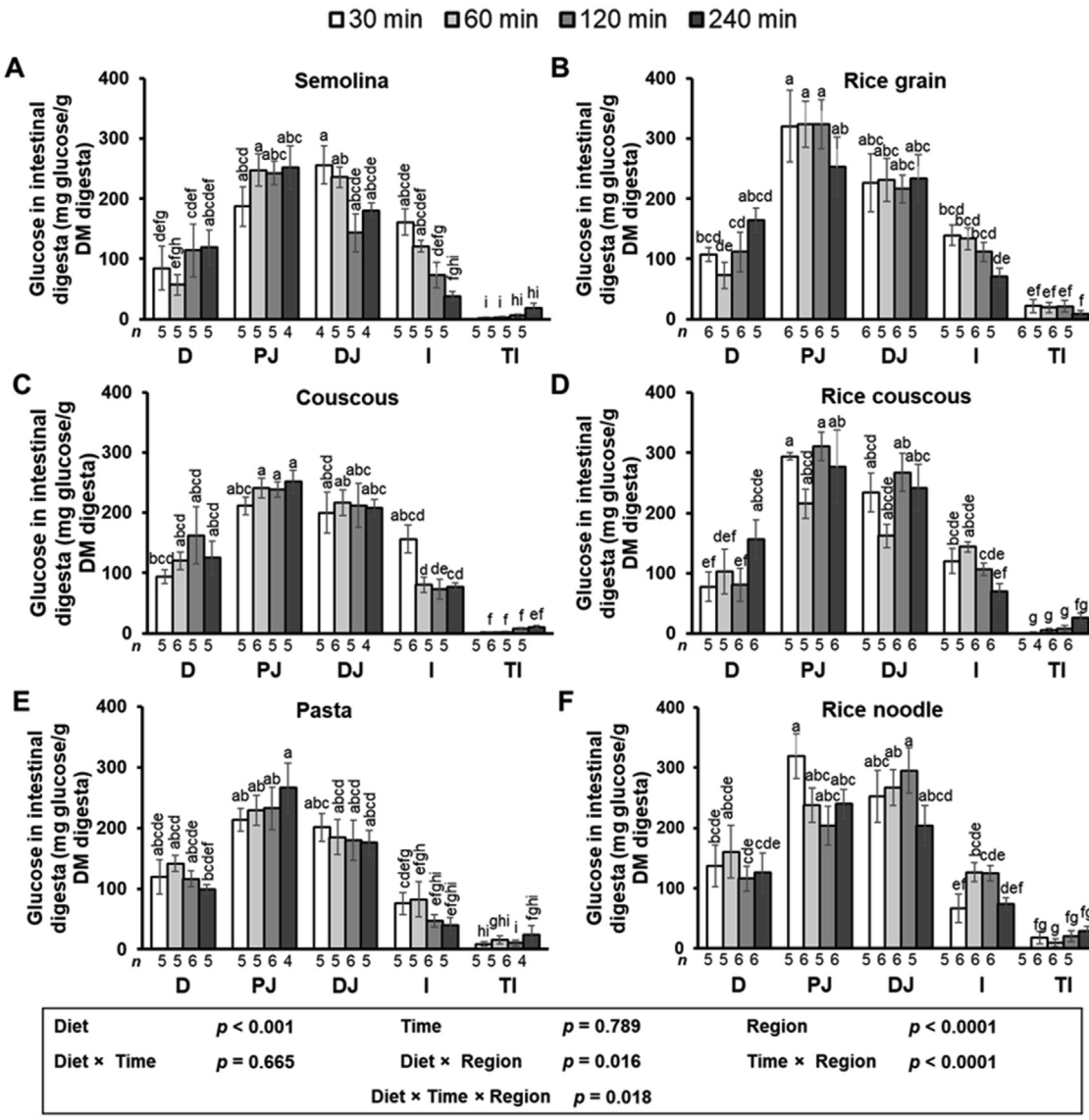

FIGURE 5. Glucose concentration in the small intestinal digesta of pigs fed with semolina (A), rice grain (B), couscous (C), rice couscous (D), pasta (E), and rice noodle (F) obtained from 5 small intestinal regions at different digestion times. Values are mean ± SE; *n* indicates number of replicates for each diet × time × small intestinal region. In each panel, labeled means without a common letter differ, $P < 0.05$. D, duodenum; DJ, distal jejunum; DM, dry matter; I, ileum; PJ, proximal jejunum; TI, terminal ileum.

The trends observed in the glycemic response study were expected to be because of starch digestion in the small intestine and glucose absorption into the portal vein, which was investigated in the gut content collection study. Portal vein plasma glucose profiles (Figure 6C) could affect glycemic response and were hypothesized to be related to varying starch delivery rates into the small intestine (quantified as $t_{1/2,starch\ GE}$). Previous studies reported that the glycemic response of healthy and type 2 diabetic human subjects after the intraduodenal infusion of glucose at 1 kcal/min was lower than at 2 and 4 kcal/min, but little difference was found between the glycemic response at 2 and 4 kcal/min [40,41]. This suggests there was a maximum nutrient delivery rate that could affect glucose loading in the small intestine and glucose absorption into the portal vein, which in turn affected the glycemic response [40,41]. Similar patterns between intestinal maltose and glucose and portal vein plasma glucose profile over time (Figure 6A-C) implied that portal plasma glucose was related to starch hydrolysis and absorption along the small intestinal regions. The accumulation behavior of glucose in the portal plasma glucose with intestinal glucose (estimated as K_m and V_{max}) was proportionally correlated (Supplemental Figure 12A, B), suggesting that with a faster accumulation and higher amount of glucose in the small intestinal lumen, a higher rate and concentration of glucose accumulation into the portal vein would be expected. However, the V_{max} and K_m parameters in this study were estimated using a single data set established from mean values (Figure 6D–F), such that the statistical difference between diets and in the identified correlations could not be determined. Future investigation with a different study design, that is, continuous portal and gut luminal glucose measurement in individual pigs, is needed to provide replicates to establish clearer correlations between starch intestinal hydrolysis and glucose absorption.

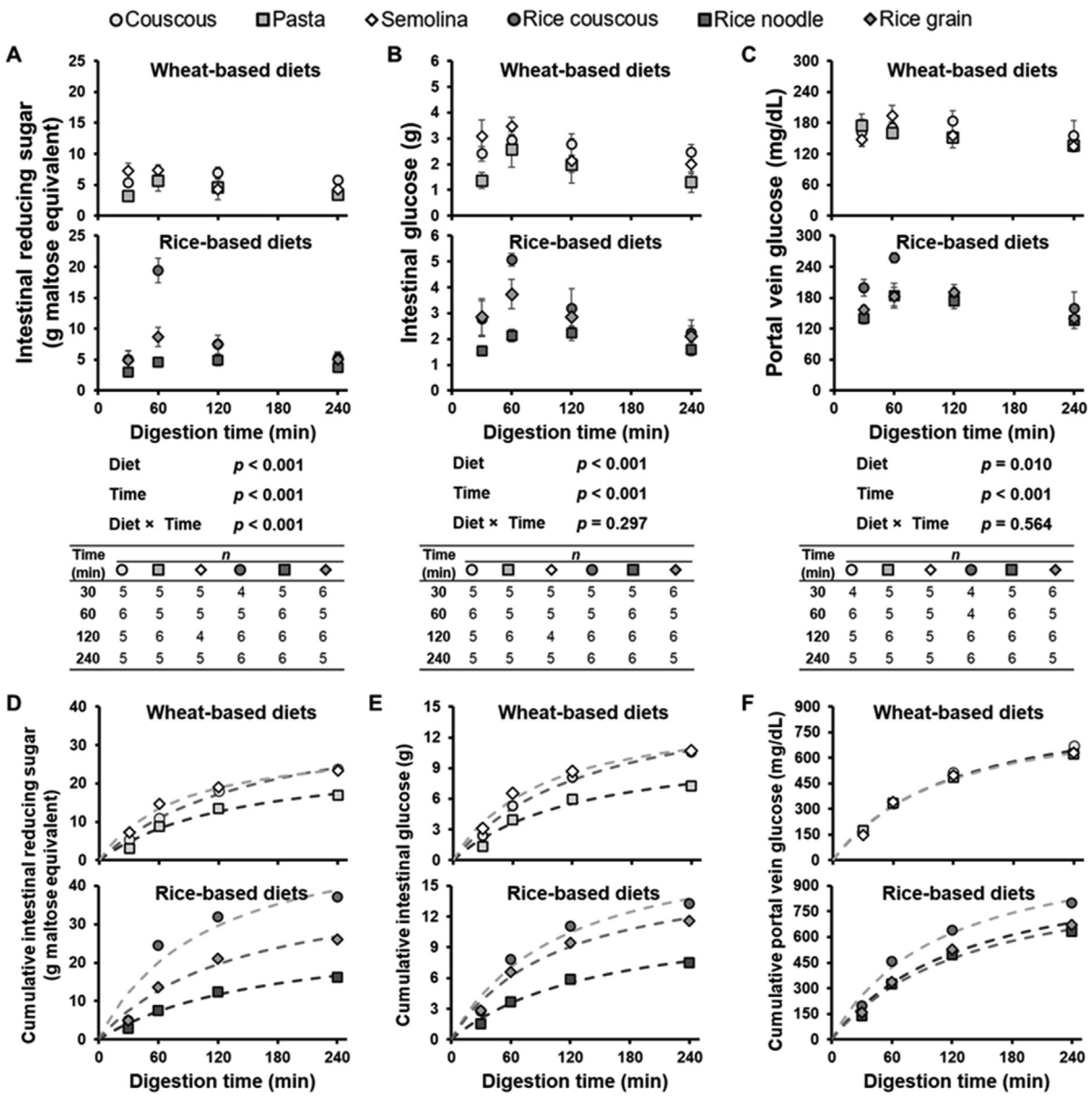

FIGURE 6. Total intestinal reducing sugar expressed as total intestinal maltose (A), total intestinal glucose (B), and portal vein plasma glucose concentration (C) in growing pigs fed with one of the 6 study diets. Values are mean ± SE; *n* indicates number of pigs for each data point. SE is presented but not always visible owing to the small error bars. Plots for cumulative intestinal reducing sugar (D), cumulative intestinal glucose (E), and cumulative portal vein plasma glucose (F) over time were established by summing mean values from panels A, B, or C over time. Dashed lines for panels D–F indicate Michaelis–Menten model (Equation 4) fit to the cumulative data. Parameters and confidence bounds for each model fit can be found in Table 2 and Supplemental Figure 5, respectively.

Because continuous feeding was not applied in this study, differences between small intestinal regions in their starch hydrolysis and glucose production could be observed. Starch hydrolysis products were quantified as glucose and reducing sugars (expressed as maltose equivalent), which were proportional to one another in their accumulation behavior (V_{max} and K_m) (Supplemental Figure 12C, D). More maltose equivalent was present than that of glucose owing to the presence of various reducing sugars from the starch intestinal hydrolysis process (Supplemental Figure 12E) [42]. At any digestion time, maltose and glucose concentration in the duodenal and terminal ileal digesta was the lowest (Figures 4, 5). This may be attributed to the shorter length of the duodenum and terminal ileum (TI) (≤25 cm) than that of the 3 other intestinal regions (>2 m each), which led to shorter digesta retention time in these regions. This difference in the maltose and glucose concentration in the different regions of the small intestine also provided experimental evidence to support that rapid hydrolysis and absorption typically occurs in the duodenum and that the majority of hydrolysis and glucose absorption typically occurs in the jejunum, leaving minimum material retention in the duodenum and terminal ileum (TI) [24,43].

Changes in the particle size distribution along the jejunum and ileum also indicated increasing starch hydrolysis and absorption along the small intestine (Figure 3). Consistent trends were observed for all diets when the particle size distribution of

TABLE 2
Michaelis–Menten parameters (Equation 4) for the total intestinal glucose, total intestinal maltose, and portal vein plasma glucose concentration shown in Figure 6D–F [1]

Parameter	Diet					
	Semolina	Couscous	Pasta	Rice grain	Rice couscous	Rice noodle
Total intestinal glucose (g)						
V_{max} (g glucose)	14.9 ± 7.49	17.0 ± 9.18	11.6 ± 11.7	17.2 ± 11.0	20.1 ± 19.0	12.5 ± 9.43
K_m (min)	88 ± 106	141 ± 153	132 ± 274	108 ± 153	111 ± 231	151 ± 223
R^2	0.98	0.99	0.95	0.97	0.95	0.98
Total intestinal maltose (g)						
V_{max} (g maltose)	31.4 ± 13.9	40.5 ± 19.8	27.6 ± 26.5	42.7 ± 41.0	56.8 ± 90. 3	28.7 ± 24.9
K_m (min)	79 ± 87	162 ± 152	142 ± 273	141 ± 272	110 ± 385	176 ± 283
R^2	0.98	0.99	0.96	0.96	0.87	0.98
Portal vein plasma glucose concentration (mg/dL)						
V_{max} (mg/dL)	918 ± 273	1077 ± 471	972 ± 592	1018 ± 662	1184 ± 717	1066 ± 607
K_m (min)	110 ± 72	140 ± 123	123 ± 158	137 ± 181	109 ± 145	134 ± 156
R^2	0.98	0.99	0.98	0.98	0.97	0.98

[1] Values are expressed as predicted parameters ± 95% CI. No statistical comparison was conducted and the parameters have large confidence intervals because only 1 data point was present for each digestion time (owing to the use of mean values to build the cumulative curve). The goodness of fit of the model to the data for each variable is indicated with the R^2.

small intestinal digesta was compared with that of the liquid and suspended solid fractions of gastric digesta measured in another study [14]. Peaks between 1 and 30 μm diminished over time as the digesta traveled distally toward the ileum (Figure 3). This might reflect increasing digestion of starch granules (ranging from 3 to 15 μm for rice, or 20 to 25 μm for durum wheat [44, 45]) by digestive enzymes along the small intestine, which produced <1-μm hydrolyzed molecules that were not detectable by the Mastersizer. For each diet, the digestion time did not affect particle size parameters ($P < 0.05$), as also observed in the similar profiles between digestion times within 1 small intestinal region, possibly suggesting process homeostasis in each region. The lack of correlation between the particle size parameters with glycemic response may indicate that glycemic response did not only depend on the size characteristics of starch emptied from the stomach.

Because the study diets exhibited different glycemic response owing to conversion of the starch to glucose in the small intestine, it was expected that the diets would have different starch digestibility in the ileum. However, ileal starch digestibility, which reflects the end point of starch hydrolysis of the diets, ranged between 96% and 98%, and was not significantly different between diets and between the 120 minute and 240 minutes of digestion (Table 3). This range is similar to values reported in the literature (92.9%–99.4%) for white rice–based and wheat-based diets in pigs obtained from continuous feeding [46–48]. It is noteworthy that diets in those previous studies were fed in milled form and did not require further breakdown during gastric digestion before gastric emptying. The similar ileal digestibility of the diets in this study to those of previous studies, although they were in different physical forms, suggests that starch digestion kinetics in the small intestine was crucial in determining the observed differences in the glycemic response, portal glucose absorption, and intestinal glucose and maltose, although these differences in kinetics do not ffect the overall starch digestibility.

Starch digestion kinetics in the small intestine, which affected portal glucose absorption, were affected by gastric emptying, as highlighted by the correlation between $t_{1/2,\text{starch GE}}$ and glycemic response parameters found in this study (Figure 2C, D). Similar to this finding, previous research reported that portal glucose appearance in growing pigs after the consumption of various starch sources can be accurately predicted with the in vitro Englyst assay [49] whether the in vitro results are corrected for in vivo gastric emptying data [50]. An association between the kinetics of in vitro starch digestibility and in vivo portal glucose absorption in growing pigs of breads with varying dietary fiber content and composition has also been reported [51]. This implication of the influence of gastric emptying (e.g., the rate and the extent of starch hydrolysis of the emptied materials) on small intestinal digestion is important, particularly when starch digestibility is used as a measure to predict the glycemic response using in vitro approach.

The maltose-equivalent content of the suspended solid and liquid fraction of gastric digesta, which provides an estimation of the extent of starch hydrolysis of the emptied materials [14], was proportional to the $\Delta max_{overall}$ and $iAUC_{overall}$ of the diets only at the 30-minute digestion (Figure 2G, H and Supplemental Figure 13). This implies the presence of physiological responses owing to the output of gastric digestion at early digestion times. These relationships aligned with findings from previous oral glucose tolerance and gastric emptying studies in human subjects, where the glucose emptying rate was highly correlated with the glycemic response only up to 60 minutes of digestion [36,37].

Because the 30-minute emptied gastric digesta and glycemic response parameters were correlated, it was hypothesized that glucose and maltose flow to the ileum at 30 min of digestion would align with the trend in glycemic response because of ileal triggering mechanisms [52–54], especially for the larger-sized, slow-emptying diets owing to different gastric breakdown and emptying rates of the diets. However, the maltose and glucose flow to the ileum was greater for rice grain than that for the noodle diets (Table 3). Meanwhile, rice grain had the lowest $iAUC_{overall}$ among the 3 diets. Similarly, in the smaller-sized, fast-emptying diets, the order of their glucose and maltose flow (semolina ≥ couscous > rice couscous) did not follow the order of their glycemic response parameters (semolina ≥ rice couscous > couscous). This disagreement between the trend in ileal glucose and maltose flow with the trend in glycemic response might indicate that the presence of nutrients (starch and/or its

TABLE 3
Ileal starch digestibility (Equation 2) and glucose and maltose flow in the ileum at different digestion time points (Equation 3) for growing pigs fed one of the 6 study diets[1]

Time (min)	Diet[2]											P		
	Semolina	*n*	Couscous	*n*	Pasta	*n*	Rice grain	*n*	Rice couscous	*n*	Rice noodle	*n*	Diet	Time	Diet × time
Ileal starch digestibility (%)[3]	96.7 ± 0.51	9	97.4 ± 0.33	10	98.1 ± 0.27	11	96.8 ± 0.49	10	97.8 ± 0.24	11	97.7 ± 0.24	12	0.098		
Glucose flow (g/kg DM eaten)												—	<0.001	<0.001	<0.001
30	101 ± 8.88^{a,z}	4	84.7 ± 13.9^{ab,z}	3	14.2 ± 3.85^{d}	5	69.4 ± 11.42^{bc,z}	5	47.4 ± 8.04^{c,z}	5	9.06 ± 3.48^{d}	5			
60	35.7 ± 5.05^{ab,y}	5	20.0 ± 6.70^{bc,y}	6	9.30 ± 3.34^{c}	5	30.8 ± 2.24^{abc,y}	4	41.2 ± 2.86^{a,z}	5	20.2 ± 6.56^{bc}	6			
120	17.4 ± 5.46^{yx}	5	13.1 ± 3.38^{y}	5	6.33 ± 2.01	6	16.8 ± 2.70^{yx}	6	17.0 ± 3.64^{y}	6	13.0 ± 2.22	6			
240	7.89 ± 1.86^{x}	5	12.8 ± 2.17^{y}	5	6.17 ± 2.31	5	10.7 ± 4.52^{x}	5	7.49 ± 1.46^{y}	6	9.17 ± 1.52	6			
Maltose flow (g/kg DM eaten)												—	<0.001	<0.001	<0.001
30	226 ± 27.1^{a,z}	4	206 ± 17.7^{ab,z}	4	39.6 ± 11.2^{cd}	5	161 ± 25.7^{b,z}	5	92.9 ± 23.3^{c,y}	4	19.6 ± 5.11^{d}	5			
60	76.5 ± 14.1^{b,y}	5	26.1 ± 4.67^{c,y}	6	23.1 ± 6.88^{c}	5	79.6 ± 8.41^{bc,y}	4	167 ± 21.7^{a,z}	5	52.4 ± 16.5^{bc}	6			
120	43.8 ± 14.5^{yx}	5	36.5 ± 9.62^{y}	5	21.7 ± 6.10	6	44.5 ± 7.81^{yx}	6	39.0 ± 8.23^{x}	6	29.9 ± 7.04	6			
240	18.0 ± 3.99^{x}	5	34.5 ± 6.55^{y}	5	16.9 ± 4.00	5	28.5 ± 11.3^{x}	5	24.7 ± 5.90^{x}	6	20.6 ± 5.24	6			

DM, dry matter.
[1] Values are presented as mean ± SE. For each parameter, labeled means in a row (abcd) or in a column (zyx) without a common letter differ, $P < 0.05$.
[2] The number of replicates for each diet and measured parameter is indicated by the column "*n*" on the right side of each presented mean. The different number of replicates between diets (for starch digestibility) or between diets × times (for glucose and maltose flow) were owing to the different number of pigs that were able to consume the required amount of their assigned diet on sampling day and owing to outlier removal.
[3] Starch digestibility was averaged across 120- and 240-min digestion time owing to insignificant effect of time ($P = 0.875$) to the digestibility values.

hydrolysis products) in the ileum is not the only factor determining the physiological response to regulate plasma glucose concentrations [52–54]. Future studies could include the measurement of hormonal responses and nutrient flow to other small intestinal regions at earlier and later digestion times to elucidate the exact mechanisms of small intestinal feedback as affected by gastric digestion.

This study was able to identify relationships between food structure, gastrointestinal digestion, and glycemic response, despite some limitations associated with the study design. The glycemic response study was limited by the number of replicates that varied between diets, which might have caused the lack of statistical significance and degree of power between the diets and variability in the observed glycemic response [55]. It was initially expected that all 18 pigs in the glycemic response study could be fed with 4 diets (reference diet and 3 study diets) until the end of study period, resulting in 9 replicates for each study diet and 18 replicates for the reference diet. However, the difficulties in keeping the catheters patent in several pigs and ensuring the pigs ate the minimum amount required for each diet resulted in fewer than the ideal number of replicates for each treatment.

The glycemic response study reported in this study also was coupled with a gut content collection study, of which the gastric digestion-related aspects have been reported elsewhere [13,14]. To enable comparison across the studies, the pigs in the glycemic response study were required to consume the same amount as in the gut content collection study to ensure that the stomach was filled to its working volume [13]. Some pigs could not consume the minimum required amount (23% of the total data), possibly owing to the total volume of the diets (because of their intact food form and high moisture) compared with conventional pig diets (milled grain form). For future studies with similar design to this study, the volume of the meal should be considered.

In addition to the identified links between food structure, gastrointestinal digestion, and glycemic response, this study also showed that the ileal digestibility across the diets and times was similar, emphasizing the importance of gastric digestion in influencing the kinetics of starch hydrolysis and glucose absorption. The initial size of cooked, starch-rich diets affected their breakdown and gastric emptying rates, which further influenced their digestion kinetics in the small intestine, glucose absorption into the portal vein, and ultimately their maximum change in plasma glucose concentrations and overall glycemic effects. Smaller-sized diets used in this study, which had looser starch arrangement in the microstructure than those of the larger-sized diets [14], had a shorter starch-emptying half-time than the larger-sized diets, resulting in their higher glycemic responses. The different emptying half-times between the diets might be associated with their breakdown rates during gastric digestion, as reported in a separate study [13]. These identified relationships should be explored further for improving food structuring strategies to modulate glycemic response.

Acknowledgments

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Author contribution

The authors' responsibilities were as follows—GMB, SMH, NS: designed the study; NS, SJH, JN, AGO, TGE, GMB: conducted the in vivo study; JN, AGO, PS, TGE: analyzed glycemic response study samples; JN, AGO, TGE, PS, NS: analyzed gut content collection study samples; JN: performed data analysis and wrote the manuscript; GMB: had primary responsibility for final content; AGO, PS, SMH, NS, TGE, RPS, HS, GMB: contributed to review, and/or revision of the manuscript; and all authors read and approved the final manuscript.

Data availability

The data described in the manuscript can be made available on request.

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Author disclosures

The authors report no conflicts of interest.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.tjnut.2023.03.009.

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