Electronic Supplementary Information (ESI)

Supplementary Methods

A. Calculation of the amount of food used for digestion in the Human Gastric Simulator (HGS)

The amount of food used for digestion in the HGS was calculated using the amount of food eaten by pigs in previous *in vivo* study[1] that ate sufficient amount of food to obtain the presence of functional proximal/distal stomach regions. The size of the HGS stomach bag is closer to that of human stomach (~1 L) instead of a growing pig stomach (~3.5 L).[2] Therefore, a scaling factor was used (0.5 for semolina and 0.7 for other foods, based on the cut-off limits used in the respective *in vivo* study) to ensure the food and added simulated digestive fluids could fit within the capacity of the HGS stomach bag (Table A1). The averaged and median amount eaten were used in the calculation to anticipate potential variation in the distribution of the values. Both the averaged and median amount eaten after multiplication with the scaling factor gave similar values (~590 g) of cooked food. For practicality purposes, this value was rounded up to 600 g of food for each HGS digestion.

Table A.1 Calculation of the amount of food used for the in vitro digestion, based on data from Nadia *et al.*[1].

	Amount eaten by pigs that ate sufficient amount of food		**Scaling down to fit the HGS capacity**		
Food	Average amount eaten (g)	Median amount eaten (g)	Scaling factor (SF)	SF*Average amount eaten (g)	SF*Median amount eaten (g)
Couscous	915.6	856.0	0.7	640.9	599.2
Pasta	915.3	957.0	0.7	640.7	669.9
Semolina	1461.0	1410.9	0.5	730.5	705.5
Rice couscous	725.5	735.0	0.7	507.8	514.5
Rice noodle	712.8	722.0	0.7	499.0	505.4
Rice grain	748.2	774.9	0.7	523.7	542.4
Amount of food for HGS digestion, averaged across 6 foods (g)				**590.5**	**589.5**

B. Calculation of the contact time between food and SSF prior to digestion in the HGS

The amount of food used for digestion in the HGS was calculated using the amount of food eaten by pigs in an *in vivo* study using similar types of foods used in this study.[1] The loading rate of each food into the pig stomach was calculated by dividing the averaged amount of food eaten by the longest consumption time by the pigs in that study (Table B.1). The loading time for each food was calculated by multiplying the amount of food for digestion in the HGS obtained in section A (600 g) with its loading rate. The averaged loading time across the six foods was 14.7 min, which was rounded up to 15 min.

In the execution of the in vitro digestion, the calculated 15 min loading time comprised of: (1) the contact between SSF and the total amount of food and (2) loading time into the stomach. The contact between SSF and the total amount of food was determined to be 5 min, which was modified from the suggested oral phase duration (2 min) in the INFOGEST method,[3, 4] based on the consideration of the absence of simulated particle reduction and the large amount of the food used for the study. Following the 5 min of SSF-food contact time, the mixture was immediately introduced to the HGS stomach liner (~1 min time needed). The HGS motor was started as soon as the loading of the food-SSF mixture finished, to mimic the gastric wall contraction due to the presence of food in the stomach.

Table B.1 Rationale for the calculation of the pre-gastric digestion time (stomach loading time) in the *in vitro* experiments, based on *in vivo* data from Nadia *et al*.[1]

Food	Averaged food eaten (g)	Average consumption time by pigs (min)	Loading rate for averaged food eaten (g/min)	Loading time for 600 g (min) = 600*loading rate
Couscous	915.6	20	45.8	13.1
Pasta	915.3	20	45.8	13.1
Semolina	1461.0	30	48.7	12.3
Rice couscous	725.5	20	36.3	16.5
Rice noodle	712.8	20	35.6	16.8
Rice grain	748.2	20	37.4	16.0
Average stomach loading time for 600 g food, averaged across 6 foods (min)				**14.7**

C. Calculation of gastric secretion rate

Gastric secretion rate used in the HGS experiments was calculated by averaging the moisture addition rate to gastric digesta of six starch-rich foods during *in vivo* gastric digestion at 30, 60, 120, or 240 min digestion times.[1] The obtained value based on this calculation (Table C1) is 4.14 g/min, which was rounded to 4.1 g/min in the *in vitro* experiments. It is worth noting that the averaged moisture addition rate of larger-sized foods (pasta, rice noodle, rice grain) was lower compared to that of smaller-sized foods (couscous, semolina, rice couscous), possibly suggesting a potential relationship between food initial particle size with gastric secretion rate.

Table C1. Moisture addition rate to six starch-based foods during gastric digestion in growing pigs reported in Supplemental Table 2 of the study of Nadia *et al*.[1] used for the calculation of SGF flow rate in this study

Food	**Moisture addition rate (g /min)**				**Averaged moisture addition rate (g/min)**
	30 min digestion	**60 min digestion**	**120 min digestion**	**240 min digestion**	
Couscous	15.18	5.52	1.72	1.40	5.95
Pasta	7.17	2.54	1.46	1.76	3.23
Semolina	6.48	2.18	8.67	3.41	5.18
Rice couscous	8.92	5.71	0.68	2.14	4.36
Rice noodle	5.02	2.36	1.12	1.44	2.49
Rice grain	11.61	0.21	1.40	1.21	3.61
Moisture addition rate, averaged across 6 foods (g/min)					**4.14**

D. Gastric emptying rate calculation

Gastric emptying rate of the digesta was calculated by utilizing gastric emptying of dry matter (DM) data and the moisture content of six starch-based foods (Table D1) from an *in vivo* study (growing pig model) that used similar food products to that of the current study [1]. Averaged DM emptied (Table D1) was calculated by averaging the dry matter emptied in each pig for each food and digestion time. DM emptied in each pig was calculated as follows:

$$DM\ emptied\ in\ each\ pig = DM\ of\ food\ eaten - DM\ remaining\ in\ the\ stomach$$

DM emptying rate was calculated by dividing the calculated averaged DM emptied by time difference between the current time point with the previous known digestion time point.

For example, at 120 min digestion:

$$DM_{emptying\ rate,120\mathrm{min}} = \frac{Averaged\ DM\ emptied_{120\ min} - Averaged\ DM\ emptied_{60\ \mathrm{min}}}{(120\ \mathrm{min} - 60\ \mathrm{min})}$$

The moisture content of digesta *in vivo* at different digestion time varied between foods, as a result of dynamic gastric emptying rate and gastric secretions over time [1]. This resulted in varying mass of emptied wet digesta over time. However, varied emptying of wet digesta at each sampling time is not practical for *in vitro* digestion experiments, thus a constant gastric emptying rate was estimated. To estimate the constant wet digesta emptying rate, the averaged initial DM of the foods was selected as the denominator of averaged DM emptying rate, based on the consideration that the dry matter emptied during digestion comprise of mainly the food itself. The division of the averaged DM emptying rate (1.71 g DM/min) by the averaged initial DM of the foods (1 g/g food - 0.696 g H_2O/g food = 0.304 g DM/g food) is 5.62 g wet digesta/min, which was used in the current study.

Table D.1 Calculation of digesta emptying rate using dry matter emptying data and moisture content of 6 starch-based foods from Nadia *et al.*[1].

Food	Digestion time (min)	Averaged DM emptied (g)	DM emptying rate (g/min)
Semolina	30	68.0 ± 14.3	2.26 ± 0.48
	60	101.0 ± 6.18	3.37 ± 0.21
	120	156.6 ± 14.5	2.61 ± 0.24
	240	202.2 ± 20.3	1.68 ± 0.17
Pasta	30	50.7 ± 4.37	1.69 ± 0.15
	60	68.8 ± 5.33	2.29 ± 0.18
	120	82.2 ± 7.25	1.37 ± 0.12
	240	147. 8 ± 13.3	1.23 ± 0.11
Couscous	30	34.1 ± 2.94	1.14 ± 0.10
	60	82.4 ± 11.0	2.75 ± 0.37
	120	120.7 ± 7.57	2.01 ± 0.13
	240	274.5 ± 83.3	2.29 ± 0.69
Rice grain	30	17.5 ± 14.4	0.58 ± 0.48
	60	21.07 ± 9.63	0.70 ± 0.32
	120	82.3 ± 6.44	1.37 ± 0.11
	240	141.4 ± 12.7	1.18 ± 0.11
Rice noodle	30	36.6 ± 16.5	1.22 ± 0.55
	60	37.0 ± 7.56	1.23 ± 0.25
	120	69.8 ± 6.30	1.16 ± 0.11
	240	155.8 ± 14.8	1.30 ± 0.12
Rice couscous	30	41.5 ± 13.4	1.38 ± 0.45
	60	84.4 ± 9.91	2.81 ± 0.33
	120	109.2 ± 7.69	1.82 ± 0.13
	240	184.4 ± 12.1	1.54 ± 0.1
		Averaged dry matter emptying rate (g DM/min)	1.71
		Averaged moisture content of 6 foods (g H_2O/g food)	0.696
		Averaged dry matter content of 6 foods (g DM/g food)	0.304
		Digesta emptying rate (g wet digesta/min)	**5.62**

Supplementary Figures

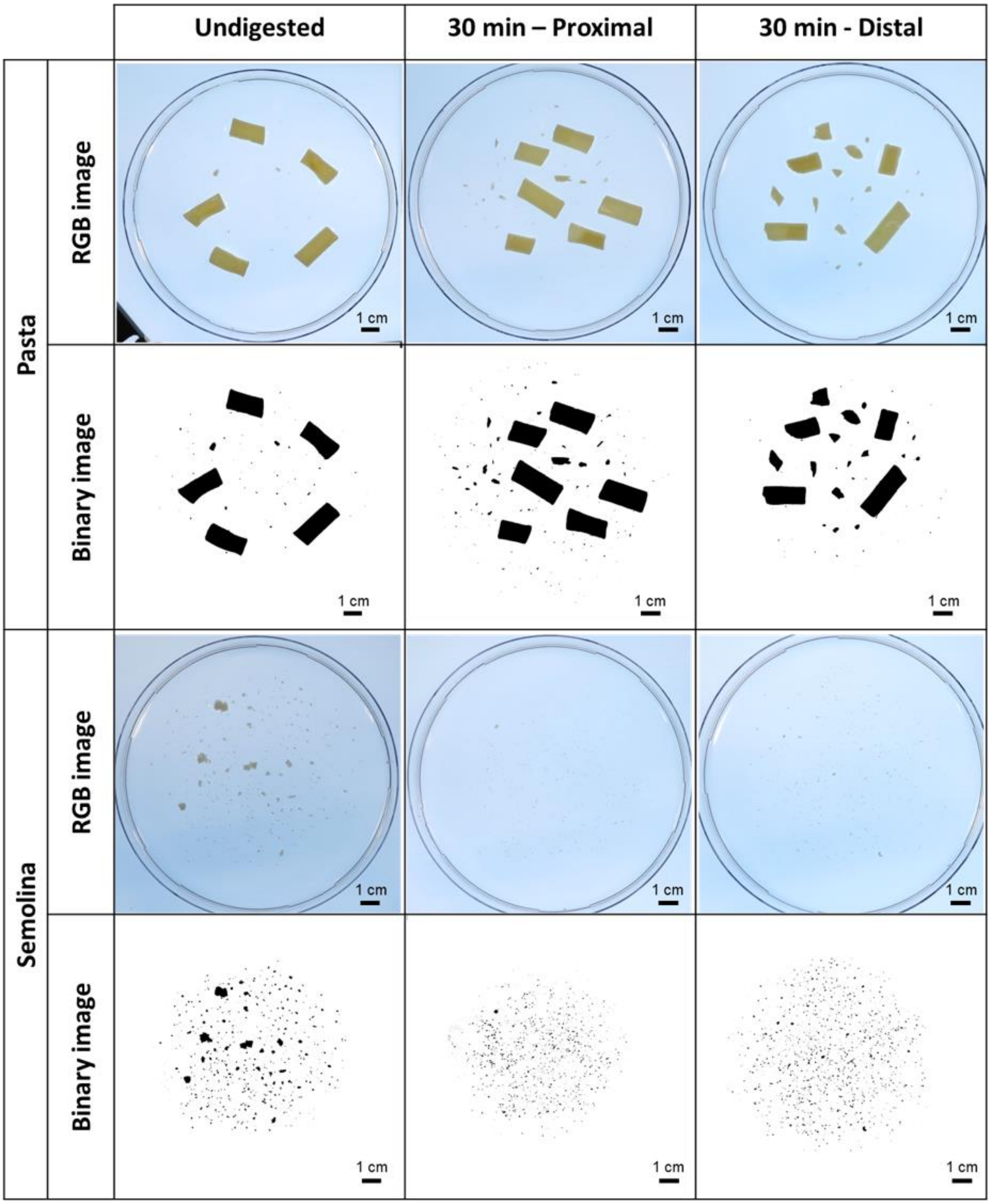

Figure S.1 Examples of binary images obtained from the image processing of RGB images (taken with a digital camera): undigested foods and their remaining digesta after 30 min gastric digestion. The resulting binary images were used to extract particle size parameters. The scale bar in each grid represents 1 cm.

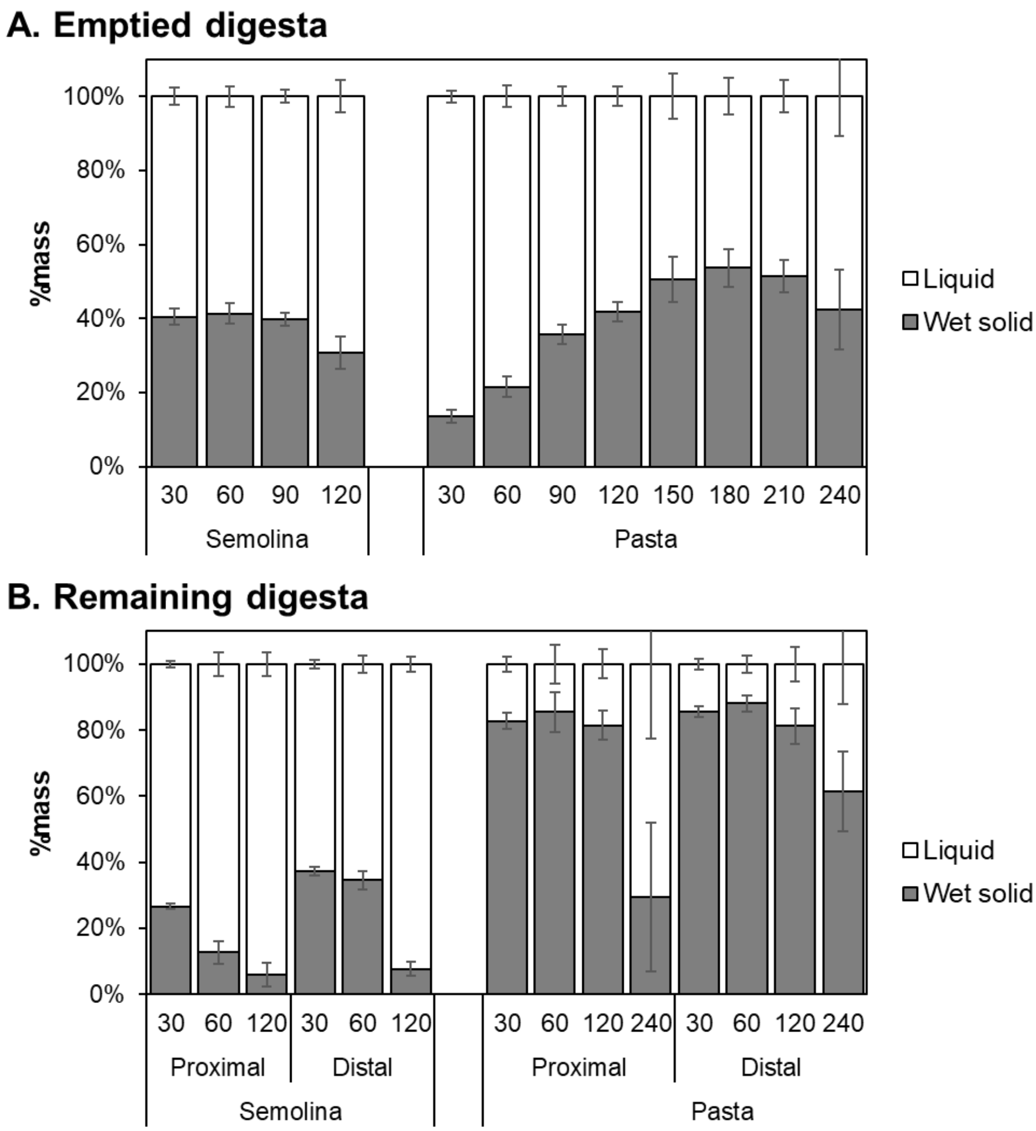

Figure S.2 Wet solid content of emptied (A) and remaining (B) digesta after digestion in the HGS, obtained by separating liquid from the solid and suspended solid contents of digesta samples after centrifugation. Numbers below the bar graph indicate the digestion times. Remaining digesta samples were separated to proximal and distal digesta samples, as indicated below the digestion times in (B). Values are mean ± SD (n = 3 for each bar graph, except n = 2 for pasta 240 min).

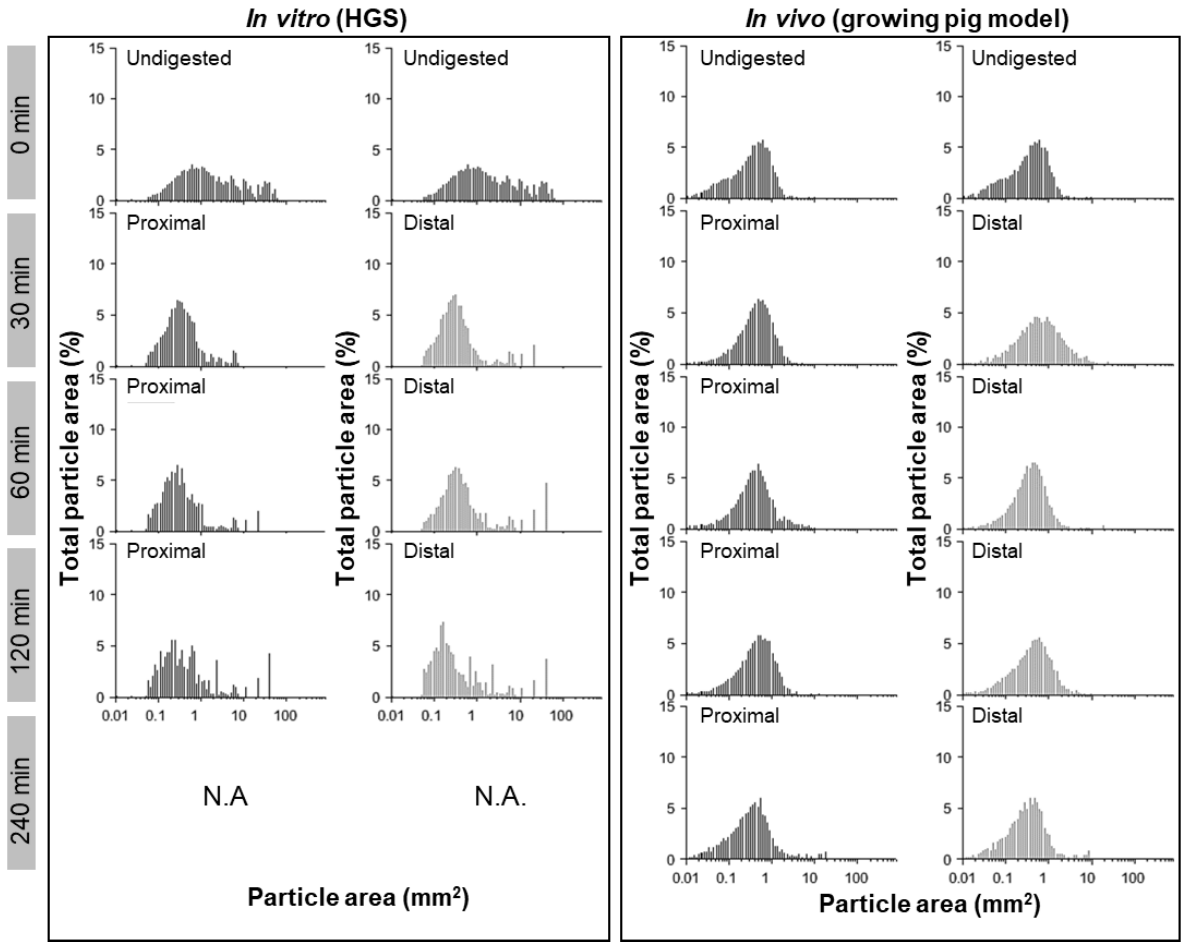

Figure S.3 Particle area distribution of remaining semolina digesta before digestion and after 30, 60, or 120 min in the HGS (2 columns on the left) or up to 240 min in the pig stomach (2 columns on the right). The distribution for each plot was established by combining the data from 3 (*in vitro*) or 5-6 experimental replicates (*in vivo*). *In vivo* plots were established using the data from Nadia *et al.*[1]. The x-axis is presented in a logarithmic scale to cover several magnitudes of area. For ease of comparison, the distribution of undigested food is given for each stomach region.

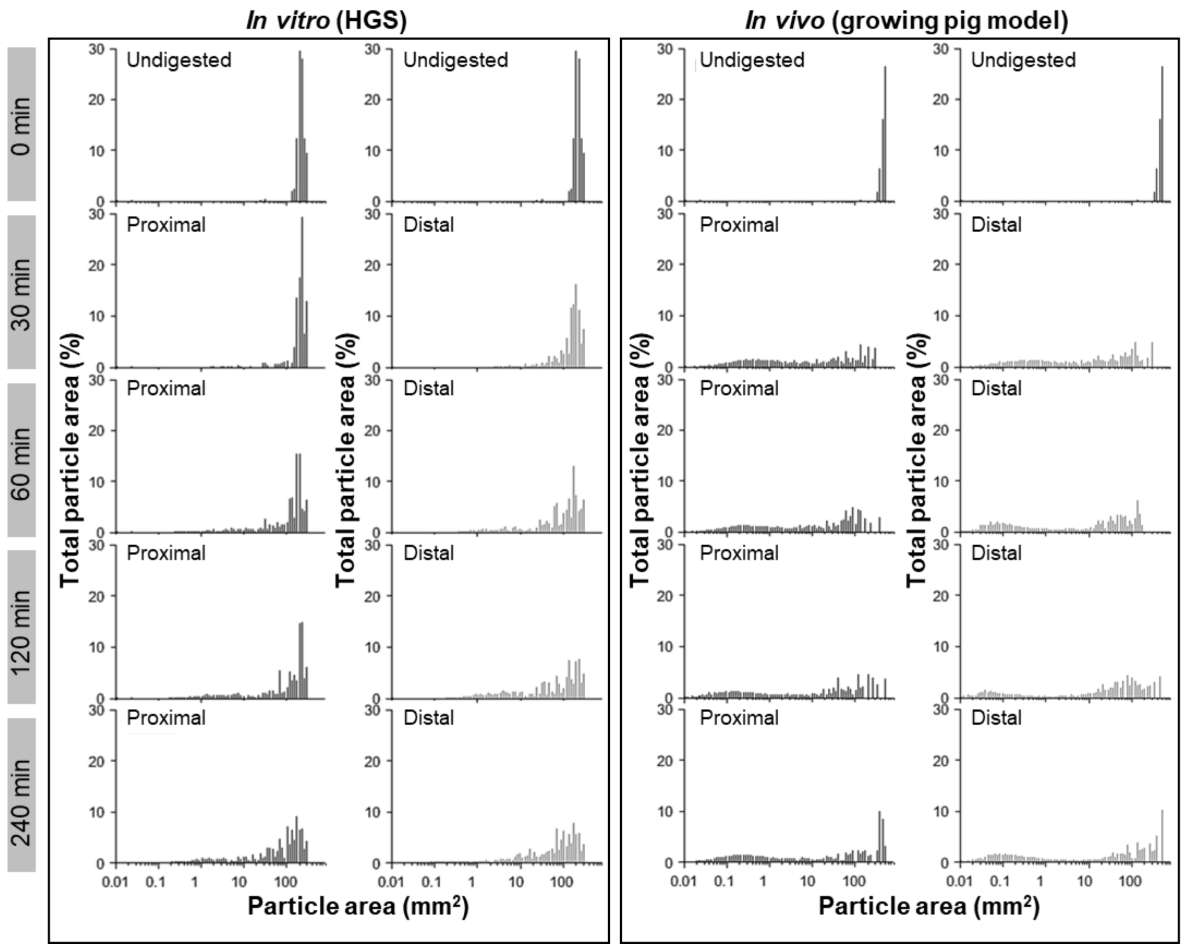

Figure S.4 Particle area distribution of remaining pasta digesta before digestion and after 30, 60, 120, or 240 min in the HGS (2 columns on the left) or in the pig stomach (2 columns on the right). The distribution for each plot was established by combining the data from 2-3 (*in vitro*) or 5-6 experimental replicates (*in vivo*). *In vivo* plots were established using the data from Nadia *et al*.[1]. The x-axis is presented in a logarithmic scale to cover several magnitudes of area. For ease of comparison, the distribution of undigested food is given for each stomach region.

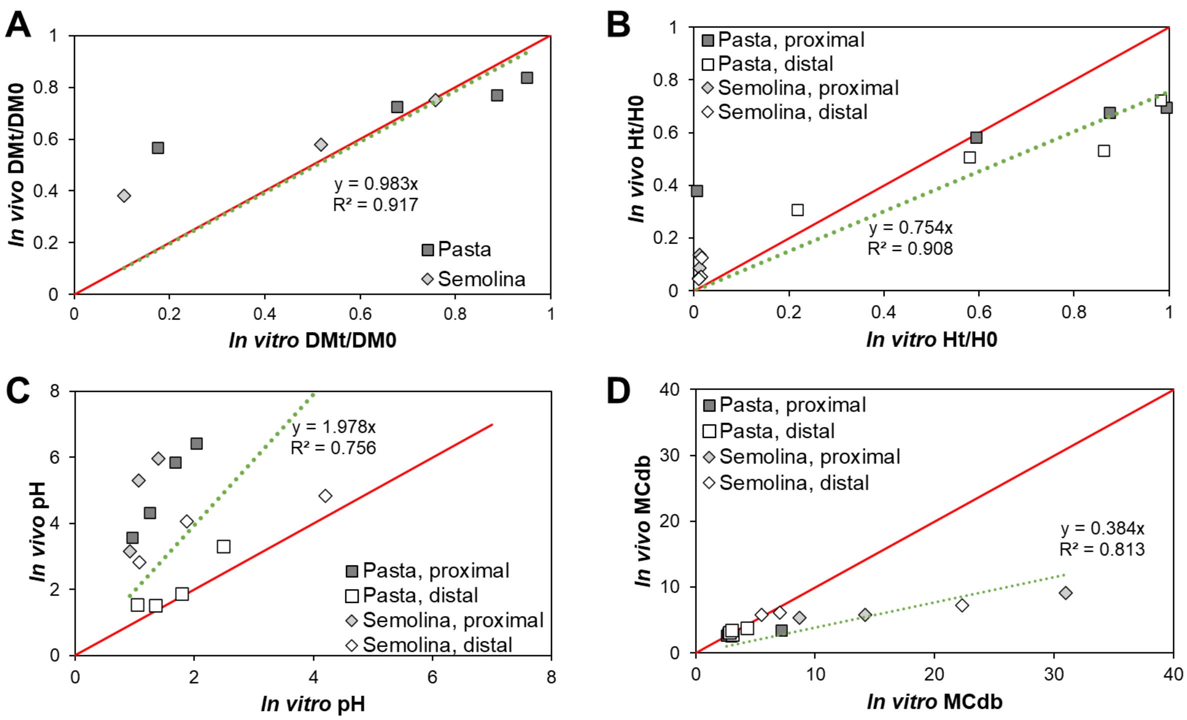

Figure S.5 Linear correlation with forced intercept at (0,0) between *in vitro*-*in vivo* dry matter retention (A), remaining digesta normalized hardness (B), remaining digesta pH (C), and remaining digesta dry basis moisture content (D). Linear regression with forced intercept at (0,0) was conducted to identify deviation from 1:1 line, which indicates agreement between *in vitro* and *in vivo* data points [5]. *In vivo* study data was obtained from Nadia *et al*.[1]. Solid red lines represent line of equality (1:1 correlation). Dotted green lines represent the linear regression conducted for each property.

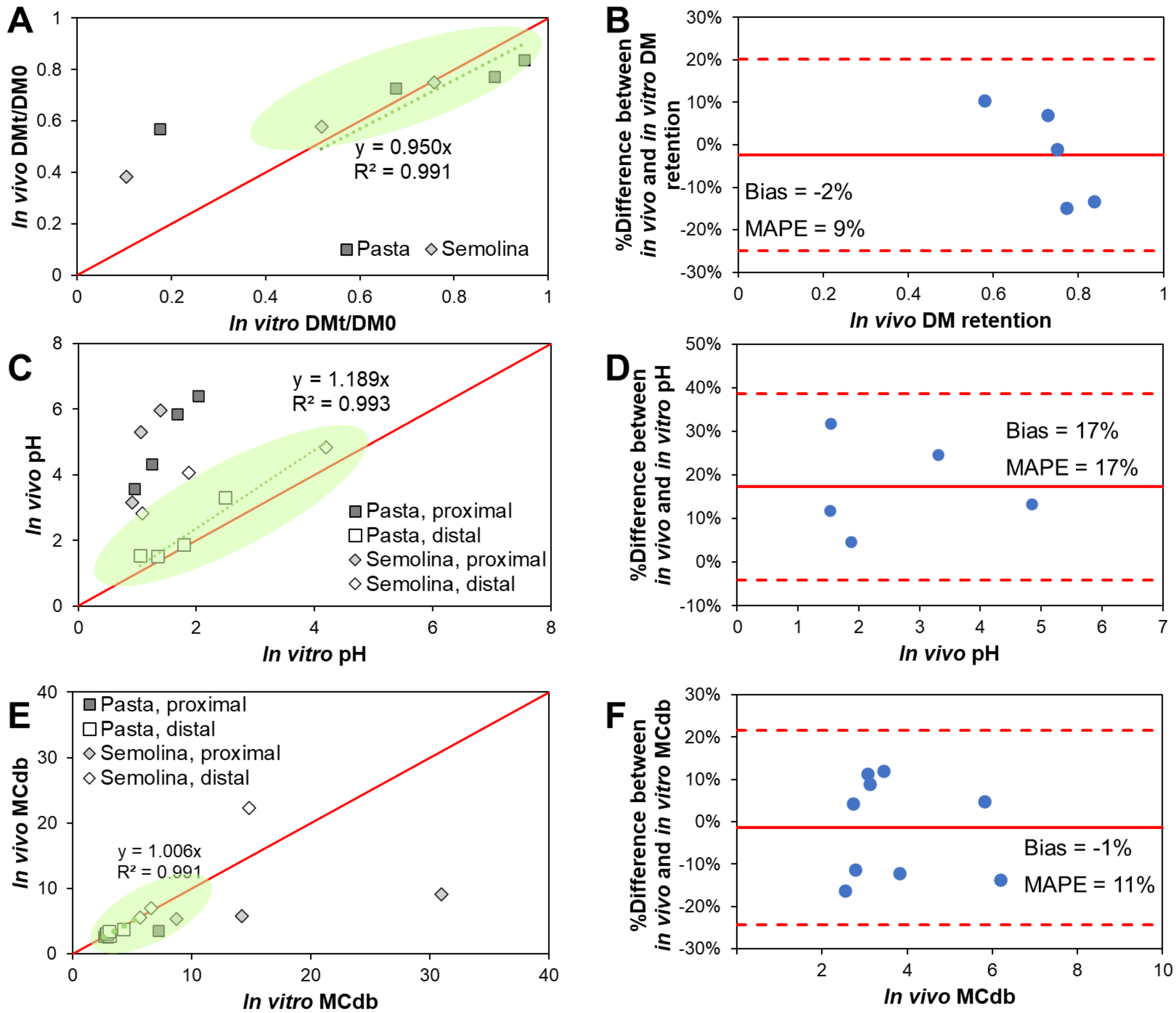

Figure S.6 *In vitro-in vivo* relationships between dry matter retention (A-B), remaining digesta pH (C-D), and remaining digesta dry basis moisture content (E-F) after excluding *in vitro* data with >50% difference from *in vivo* data (indicated by data points outside the green-shaded area in the plots on the left column). The relationships are presented as linear regression with forced intercept at (0,0) (left column) and the Bland-Altman plots (right column). *In vivo* study data was obtained from Nadia *et al*.[1]. For plots on the left column, solid red lines represent 1:1 correlation and dotted lines represent the linear regression conducted for each property. For the Bland-Altman plots, bias and limits of acceptance are shown by red solid line and red dashed lines, respectively. MAPE: mean absolute percentage error.

Supplementary Tables

Table S.1 Statistical significance of food, time, stomach region, or their two-way and three-way interaction effects on the properties of emptied digesta and remaining digesta.

	Effect						
Parameter	**Food**	**Time**	**Region**	**Food × Time**	**Food × Region**	**Time × Region**	**Food × Time × Region**
Emptied digesta							
Moisture content, dry basis	**	***	-	***	-	-	-
pH	****	****	-	****	-	-	-
Dry matter (DM) retention	****	****	-	****	-	-	-
Hardness	NS	**	-	**	-	-	-
Reducing sugar content [g maltose/g starch]	*	***	-	****	-	-	-
Remaining digesta							
Moisture content, dry basis	NS	****	****	****	****	NS	NS
pH	NS	****	****	****	****	****	****
Hardness	****	****	*	*	**	*	NS
Normalized hardness	****	**	**	NS	**	**	NS
particles/g DM	****	**	NS	****	NS	NS	NS
x_{10}	****	****	NS	***	*	NS	NS
x_{50}	****	****	*	**	*	NS	NS
x_{90}	****	*	NS	NS	NS	NS	NS
Broadness of particle area distribution (*b*)	****	***	NS	****	NS	NS	*
Reducing sugar content [g maltose/g starch]	****	**	*	**	*	NS	NS

Asterisk (*) symbols indicate different levels of statistical significance. *: $p < 0.05$; **: $p < 0.01$, *** : $p < 0.001$, ****: $p < 0.0001$. NS: not significant. Irrelevant effect to the measured parameter is shown as “-“.

Table S.2 Additional particle area parameters (x_{10} and x_{90}) of remaining digesta (mean ± SD; n = 3 separate experimental replicates per data point, except n = 2 for 240-min pasta). N/A indicates no available data due to the termination of semolina digestion at 120 min. For each parameter, significantly different values within the same column are denoted with superscript abcd, and significantly different values within the same row are denoted with zywx ($p < 0.05$).

	Semolina		Pasta	
Time (min)	**Proximal**	**Distal**	**Proximal**	**Distal**
Particle area x_{10} (mm^2)				
30	0.04 ± 0.00^{y}	0.04 ± 0.00^{y}	68.24 ± 38.38^{a,z}	11.65 ± 9.75^{a,z}
60	0.04 ± 0.00^{x}	0.05 ± 0.00^{x}	0.28 ± 0.08^{b,z}	0.11 ± 0.05^{b,}y
120	0.04 ± 0.01^{y}	0.04 ± 0.01^{y}	0.33 ± 0.32^{b,z}	0.14 ± 0.03^{b,z}
240	N/A		6.25 ± 8.74	0.54 ± 0.19
Particle area x_{90} (mm^2)				
30	0.67 ± 0.08^{y}	0.56 ± 0.13^{y}	329.64 ± 121.14^{z}	403.36 ± 175.21^{z}
60	0.52 ± 0.16^{y}	0.69 ± 0.17^{y}	624.68 ± 106.98^{z}	400.52 ± 214.99^{z}
120	0.41 ± 0.26^{y}	0.27 ± 0.02^{y}	453.5 ± 238.22^{z}	196.24 ± 122.9^{z}
240	N/A		105.05 ± 8.88	218.15 ± 62.08

Table S.3 Hardness of emptied digesta (mean ± SD; n = 3 separate experimental replicates per data point, except n = 2 for 240-min pasta). N/A indicates no available data due to the termination of semolina digestion at 120 min. Significantly different values within the same column are denoted with superscript abcd, and significantly different values within the same row are denoted with zy ($p < 0.05$).

	Hardness (N)	
Time (min)	**Semolina**	**Pasta**
30	0.18 ± 0.04^{z}	0.10 ± 0.004^{ab,y}
60	0.14 ± 0.01^{z}	0.10 ± 0.002^{b,y}
90	0.14 ± 0.003	0.15 ± 0.04^{ab}
120	0.10 ± 0.01^{y}	0.28 ± 0.11^{a,z}
150	N/A	0.52 ± 0.58
180	N/A	0.67 ± 0.05
210	N/A	0.51 ± 0.38
240	N/A	0.30 ± 0.05

Table S.4 Average gastric emptying of dry matter obtained in vivo[1] and in vitro in the current study that were compared to obtain the bias and absolute percent error shown in Figure 7M (for all data) and Figure S.6B (for only those data points up to 120 min digestion in pasta and 60 min in semolina).

Gastric Emptying of Dry matter					
Food	**Time (min)**	**Average value**		**Bias (%)**	**Absolute Percent Error (%)**
		***In vivo* (A)**	***In vitro* (B)**	**(A-B)/A *100**	**|(A-B)|/A*100**
Pasta	30	0.84	0.95	-13%	13%
	60	0.77	0.89	-15%	15%
	120	0.73	0.68	7%	7%
	240	*0.57*	*0.18*	*69%*	*69%*
Semolina	30	0.75	0.76	-1%	1%
	60	0.58	0.52	10%	10%
	120	*0.38*	*0.10*	*73%*	*73%*
		Average of all values		**Bias = 19%**	**Mean absolute percent error = 27%**
Average of values from 30 - 120 min in pasta and 30-60 min in semolina				**Bias = -2%**	**Mean absolute percent error = 9%**

References

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2. H. N. Lærke and M. S. Hedemann, in *Nutritional physiology of pigs - Online publication*, eds. K. E. Bach Knudsen, N. J. Kjeldsen, H. D. Poulsen and B. B. Jensen, Videncenter for Svineproduktion, Foulum, 2012.
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