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Temporal changes in postprandial intragastric pH: comparing measurement methods, food structure effects, and kinetic modelling

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Short Title: Postprandial intragastric pH

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Abstract (300 words max)

Intragastric pH greatly affects food disintegration and the release of nutrients in the gut. Here, the behaviour of two liquid meals (soymilk, pea emulsion) and two solid meals (tofu, seitan) was tested in miniature pigs fitted with gastric cannula. For 5 h, intragastric pH was recorded using one of three methods: *ex vivo* measurements of chyme samples, *in situ* measurements using pH catheters, or *in situ* measurements using wireless pH capsules, both inserted through a pig's cannula. The pH values obtained with the two *in situ* methods were highly correlated. The liquid and solid foods yielded distinct pH kinetics. For the solids, pH simply decreased exponentially. For the liquids, pH increased rapidly and then plateaued for 2 h before dropping Food macrostructure and, to a lesser extent, food buffering capacity clearly had an impact on intragastric pH. We modeled changes in intragastric pH over time with food-dependent nonlinear equations.

Keywords: pH; stomach;pH catheter;wireless pH capsule;food matrix

1 Introduction

Luminal pH plays a key role in the disintegration of food and tablets/capsules in the stomach because it impacts both the biochemical structure of ingested items and the activity of digestive enzymes. Changes in protein macrostructure occur in the gastric system. For example, casein clotting has been well described and is a consequence of acidification, which slows the gastric emptying of the resulting particles (compared to that of globulins that remain soluble) (Boirie et al., 1997). Thermodynamic laws act on protein structure (Dill, 1990); indeed, protein structure and protein behaviour in fluids are both affected by temperature, pH, and ionic forces (Carbonaro, Nardini, Maselli, & Nucara, 2015). It seems likely that plant proteins also undergo aggregation, coagulation, denaturation, solubilisation, or precipitation as a result of decreased pH in the gastric system, and all these processes have an impact on digestibility (Carbonaro, Cappelloni, Nicoli, Lucarini, & Carnovale, 1997). These phenomena can additionally affect gastric dynamics like antral grinding, which plays a major role in bolus deconstruction, notably that of solid foods (Marciani, Gowland, Fillery-travis, et al., 2001).

The acidification that occurs in the stomach results from the secretion of hydrochloric (HCl) acid, which is known to induce protein denaturation independently of pH (Goto, Calciano, & Fink, 1990). In addition to its impact on protein structure, pH greatly affects pepsin activity (Kazir et al., 2018) and, hence, the appearance of peptides (Kratzer & Porter, 1962). The effect of gastric pH on protein digestion has been well described for β-lactoglobulin gels (Dekkers, Kolodziejczyk, Acquistapace, Engmann, & Wooster, 2016) and egg-white lysozyme (Jiménez-Saiz et al., 2014), but few data are available concerning the effect of gastric pH on plant protein digestion. It is also clear that the food matrix influences the release of nutritional components of interest (Le Feunteun et al., 2014). It may likewise affect acid diffusion in food particles (Marcotte, Grabowski, Karimi, & Nijland, 2012) and broad temporal changes in intragastric pH. Indeed, postprandial intragastric pH kinetics appeared to be different between solid (Malagelada, Longstreth, Summerskill, & Go, 1976) and liquid foods (Kalantzi et al., 2006). However, data are relatively scarce and to our knowledge no direct comparison on postprandial intragastric pH from fully characterized liquid and solid foods exists. Some studies deal with gastric emptying but the kinetics of intragastric pH are still not well explored. Alterations in protein structure induced by pH conditions in the stomach could also affect pepsin-protein interactions and thus the degree of proteolysis. For dairy gels, it has been shown that pepsin mainly hydrolyses proteins that are

in contact with gastric juices (Nau et al., 2019); this result suggests that the structural changes in protein networks that occur during digestion may impact the overall degree of proteolysis and the generation of peptides.

In humans, dogs, and monkeys, pH has generally been measured via three techniques: 1) by chyme aspiration via a simple gastric tube (Malagelada et al., 1976); 2) by using single (Gardner, Ciociola, & Robinson, 2002) or multichannel (Simonian, Vo, Doma, & Fisher, 2005) transnasal catheters; or 3) by employing wireless capsules that were either allowed to move freely in the chyme (Cassilly et al., 2008) or that were fixed to gastric mucosa (Pandolfino et al., 2003). Solubilisation and absorption are pH dependent (Charman, Porter, Mithani, & Dressmann, 1997), and in most studies in which postprandial intragastric pH was recorded, the objective was to analyse drug performance. More recently, food scientists have become interested in the spatial and temporal changes in intragastric pH during digestion and their relationship with intrinsic food characteristics, such as pH or buffering capacity (Bornhorst et al., 2014; Nau et al., 2019). However, we still lack detailed knowledge on how changes in pH are affected by the food matrix, and this information is crucial if we wish to establish *in vitro* digestion protocol as accurately as possible (Kong & Singh, 2008). The study described here used a miniature pig model to characterise postprandial changes in intragastric pH following the ingestion of food-containing meals. Foods differed in macrostructure (liquid vs. solid) and plant protein type. We measured pH using the three techniques described above: the chyme sampling method, the pH catheter method, and the wireless pH capsule method.

2 Materials and methods

2.1 Animal Handling & Surgery

All procedures were carried out in accordance with European Union regulations (Directive 2010/63/EU) and were approved by the Auvergne Animal Experimentation Ethics Committee (CEMEAA) and the French government (APAFIS#11001-2017082312525562v2). We used four adult Yucatan miniature pigs (8 months old; bodyweight = 24.9 ± 1.2 kg). Three weeks before the experiment, each pig was surgically fitted with a cannula made of silicone rubber (internal diameter: 12 mm, external diameter: 17 mm). It was placed lateroventrally at the stomach's point of greatest curvature (Fig. 1). The animals were housed in separate pens in a ventilated room kept at a constant temperature (21°C). Between

112 experimental trials, the pigs received 500 g/d of a concentrate feed containing 16% protein,
113 1% fat, 4% cellulose, and 5% ash (Porcyprima; Sanders Centre Auvergne, France). This food
114 ration was distributed in two equal portions, given at 800 and 1600 hours, and the pigs had *ad*
115 *libitum* access to water.

116 **2.2 Experimental foods and meals**

117 In this study, we focused on food types that differed in macrostructure (solid vs. liquid)
118 and plant protein type. The two solid foods were tofu (soy-based protein) and seitan (wheat-
119 based protein), and the two liquid foods were soymilk (soy-based protein) and a pea emulsion
120 (legume-based protein) The soymilk, tofu, and seitan were of commercial origin. The soymilk
121 had been ultra-pasteurised (UHT). The pea emulsion was made using a commercial pea
122 protein isolate (Pisane M9, Lot: N16231O04, Cosucra, Belgium) and commercial soy oil
123 (Emile Noël, France). Pre-emulsion was carried out using a disperser (T-50 Homogeniser,
124 Ultra-Turrax, IKA, Germany) and a 15 G dispersing element (IKA, Germany); the process
125 lasted 1 min and was run at 10,000 rpm. The solution was then homogenised twice using a
126 benchtop homogeniser (PandaPLUS 2000, GEA, USA) operated at 1,000 bar. We added
127 maltodextrine, sugar, and soy oil to the foods to equilibrate protein levels (30.0 g), fat levels
128 (23.1 g), and calorie contents (980 kcal). The portions of soymilk, pea emulsion, tofu, and
129 seitan weighed 1,162 g, 1,175 g, 435 g, and 290 g, respectively.

130 **2.3 Measuring intragastric pH**

131 Each pig was given each of the meal in a randomly determined order. Postprandial gastric
132 pH kinetics were characterised using the three different methods. Each meal was administered
133 on three different days, and a different measurement method was used each time. Pigs were
134 allowed to recover for at least one day between tests. All the meals were ingested in less than
135 15 min.

136 2.3.1 *Ex vivo* method

137 Samples of chyme were collected at the following time points: 5 min before food intake
138 and 20, 40, 60, 90, 120, 180, and 270 min after food intake. Each time, 10–20 ml of chyme
139 was obtained through the cannula via gravitational forces. The pH of the sample was then
140 measured *ex vivo* using an ISFET probe (1001-004 ISFET, Sentron, Holland) following
141 manual agitation.

142 2.3.2 *In situ* method 1: pH catheter system

143 A disposable dual-sensor catheter (Chongquing Jinshan Science & Technology Co. Ltd.,
144 China) was placed inside the stomach antrum via the cannula; the distance between the
145 cannula and the two sensors were 8.5 cm (**Cat1**) and 13.5 cm (**Cat2**), respectively (Figure 1).
146 The catheter was linked to an external pH meter (Ohmega Medical Measurement Systems,
147 Holland) placed on the animal's back, and pH was measured every 10 s. The pH meter was
148 calibrated before the experiment, and its continued functioning was verified after the
149 experiment. The measurements began at least 5 min before a meal was ingested and continued
150 for 5 h of the postprandial period.

151 2.3.3 *In situ* method 2: wireless pH capsule

152 The wireless pH capsule (length: 2.8 cm; model JSPC-1, Chongquing Jinshan Science &
153 Technology Co. Ltd., China) was attached to the cannula by a silk thread. The pH sensor was
154 located at the top of the capsule and was thus positioned 13.0 cm downstream from the
155 cannula, in the antrum (Figure 1). The calibration procedure was the same as for the pH
156 catheter. Measurements of pH were obtained every 3 s.

157 **2.4 Physicochemical analysis**

158 Just before the foods were given to the pigs, their pH was measured using the ISFET
159 probe (2.3.1) following manual agitation.

160 For the tofu and seitan, median particle sizes (i.e., the theoretical sieve through which
161 50% of the mass of particles could pass) were assessed as described by Peyron et al. (Peyron,
162 Mishellany, & Woda, 2004).

163 The buffering capacity (BC) of the meals was estimated under *in vitro* gastric conditions
164 for three pH ranges ([2.00–4.00]; [4.00–6.00]; [6.00–initial pH]). Foods were added to 50 ml
165 of simulated gastric fluid (SGF), which was made based on the method developed by
166 INFOGEST (Minekus et al., 2014); the objective was a final protein concentration of 50
167 $mg.ml_{SGF}^{-1}$. The mixtures were kept at 37°C using jacketed beakers equipped with magnetic
168 stirring bars, and then, using a 1 M HCl solution (Lot: 00718, Grosseron SAS, France), pH
169 was adjusted drop by drop to reach 2.00, 4.00, or 6.00. Glass electrodes (N61, SI Analytics
170 GmbH, Germany) with thermometers (W2180-KOAX, SI Analytics GmbH, Germany) and
171 pH meters (Titroline 7000, SI Analytics GmbH, Germany) were used. BC was calculated
172 using the equation described by Gaucheron (Gaucheron, Mollé, & Pannetier, 2001; Van
173 Slyke, 1922):

174 $$\mathrm{BC(pH)} = \frac{(volume\ of\ acid\ added)\times(normality\ of\ the\ acid)}{(volume\ of\ sample)\times(pH\ change\ produced)}$$

175 **2.5 Data and statistical analysis**

176 For the two *in situ* methods, pH measurements were averaged across 6-min intervals (pH
177 catheter method: 36 measurements; wireless pH capsule method: 129 measurements). The
178 kinetics of intragastric pH were analysed using the repeated option of the SAS PROC MIXED
179 procedure (SAS University Edition, v. 3.71; SAS Institute Inc., Cary, NC, USA); pig identity
180 was treated as a random effect, whereas time, measurement method, food-type, and their
181 interactions were independent factors. Significant interactions between time and method and
182 between time and meal were found, so the LSMEANS procedure was used to test differences
183 between methods and food types at specific time points. A similar procedure was used to
184 analyse the BC data. The alpha level for our statistical tests was 0.05. The data presented in
185 the results are the means ± standard error of the mean (SEM).

186 **2.6 Regression equations**

187 The regression analysis of the pH data obtained using the two *in vivo* methods was
188 performed using XLSTAT (XLSTAT Quality v. 18.07, Addinsoft, France). The values
189 employed were the means for the 6-min intervals. In the case of the pH catheter method, it
190 was also necessary to average across the two sensors.

191 An eight-parameter modified logistic regression was utilised:

192 $$pH(t) = pH_{MIN} + \left(\frac{pH_{MAX}-pH_{Infl}}{1+e^{(k_1(t_1-t))}+e^{(k_2(t-t_2))}}\right) + \left(\frac{(pH_{Infl}-pH_{MIN})\times \ln\left(1+e^{-t/t_3}\right)}{\ln(2)}\right) \quad (1)$$

193 where t was time since ingestion; pH_{MIN} was the basal pH; pH_{MAX} was the maximum pH;
194 pH_{Infl} was the inflection pH (the pH where a slow change of trend was observed, see
195 Supplementary data 1 for graphical explanation); t_1, t_2, and t_3 were the times to, respectively,
196 the initial rise in pH, global acidification, and acidification after inflection; and k_1 and k_2 were
197 the constants associated with the neutralisation and acidification rates, respectively.
198 Supplementary data 1 illustrate the contribution of parameters to the shape of some modelled
199 curves.

200 **3 Results**

201 **3.1 Meal characteristics and basal pH**

202 The pH values of the soymilk, pea emulsion, tofu, and seitan were 6.9 ± 0.2, 7.9 ± 0.1, 5.8
203 ± 0.1, and 5.6 ± 0.0, respectively. The final median particle sizes were 5.1 ± 0.1 mm for the
204 tofu and 3.5 ± 0.1 mm for the seitan (n = 4 trials).

205 The BCs of the different food types are presented in Table 1. The soymilk, pea emulsion,
206 and tofu exhibited the same profile: BC increased while pH decreased. Only the seitan
207 displayed a higher BC at a pH of 6 than at a pH of 4. Differences between meals were
208 observed for the three pH ranges. In generally, the BCs of food types with soy-based protein
209 were higher. When the foods were ordered based on BC, the result was as follows: tofu >
210 soymilk > pea emulsion ~ seitan.

211 **3.2 Postprandial kinetics of intragastric pH**

212 3.2.1 Comparison of the results for the two pH catheter sensors

213 The intragastric pH levels recorded with the two sensors (**Cat1** and **Cat2**) during the
214 postprandial period are illustrated in Figure 2. The overall statistical analysis (the final model
215 included time, meal, sensor identity, meal*sensor identity) found that there was a significant
216 difference between the pH levels recorded by **Cat1** versus **Cat2** ($P < 0.001$); there was also
217 an interaction between sensor identity and the nature of the meal ($P < 0.001$). The basal (pre-
218 meal) pH recorded by **Cat2** was consistently higher than that recorded by **Cat1** ($P < 0.05$).
219 When all the measurement data (across experimental days) were grouped, basal pH was 2.6 ±
220 0.4 with **Cat1** and 4.9 ± 0.6 with **Cat2**. When food-type-specific analyses were performed,
221 there was a difference between the pH levels recorded by **Cat1** and **Cat2** for the solid meals
222 (tofu and seitan) ($P < 0.0001$) but not for the liquid meals (soymilk and pea emulsion). For the
223 solid meals, the pH from **Cat2** was higher than the pH from **Cat1** for the entire postprandial
224 period. No significant interaction between time and sensor identity was observed, which
225 showed that the two sensors both picked up on the general patterns of temporal changes in
226 postprandial pH.

227 3.2.2 Comparison of the results obtained with the pH catheter method and the wireless
228 pH capsule method

229 First, the results from the pH catheter's two sensors were averaged. The postprandial
230 intragastric pH levels associated with the four meals, as measured via pH catheter or wireless

231 pH capsule, are shown in Figure 3. The overall statistical analysis (the final model included
232 time, food type, measurement method, and food type*measurement method) found that there
233 was a significant difference between the two measurement methods ($P < 0.001$) as well as a
234 significant interaction between food type and measurement method ($P < 0.001$). The methods
235 differed in their estimates of pH only in the case of the solid meals. Once again, no significant
236 interaction between time and measurement method was observed across the different food
237 types. Also, after solid meal intake, the gastric pH observed an inflection point, i.e. a given
238 pH reached at a considered time, after which the pH variation was slower than if the pH
239 kinetics was conventional sigmoid. Whereas pH kinetics of liquids had a sigmoid shape, pH
240 kinetics of solids decreased more rapidly.

241 3.2.3 Comparison of the results from the *ex vivo* and *in situ* measurement methods

242 To compare the pH levels obtained using the different methods, measurements made at
243 the time points corresponding to chyme sampling (n = 8) were employed (Figure 3). For the
244 pH catheter data, the mean of the two sensors was used. When measuring basal pH, the *ex*
245 *vivo* method yielded similar results to the *in situ* methods ($P = 0.6289$). The only interaction
246 between time and measurement method was observed for the soymilk ($P = 0.0430$): there was
247 a quicker drop in pH seen with the *ex vivo* method. For the other food types, changes in
248 intragastric pH were similar over time for the *ex vivo* and *in situ* methods. That said, the *ex*
249 *vivo* method yielded consistently lower pH values during the plateau associated with the
250 liquid meals (Figure 3). For the soymilk, pea emulsion, and tofu, the mean relative standard
251 error (SEM*100/mean; which expressed the degree of variability among the pigs) associated
252 with the pH catheter values was lower than that associated with other methods (Figure 3).

253 3.2.4 Effects of food type

254 The Meal comparisons depicted in Figure 4A are based on the pH catheter data (averaged
255 across **Cat1** and **Cat2**), but similar conclusions were reached when data from the wireless pH
256 capsules were employed (not shown). Time, food type, and the time*meal interaction were all
257 significant ($P < 0.001$). When comparisons were made at each time point, there were no
258 significant differences in intragastric pH between the soymilk and the pea emulsion. Tofu and
259 seitan exhibited a single significant difference, which occurred at 6 min ($P = 0.0154$). The
260 analysis revealed that, from 18 to 168 min after food ingestion, pH was significantly higher
261 for liquid meals (soymilk and pea emulsion) than for solid meals (tofu and seitan) ($P < 0.05$).
262 While pH rapidly decreased exponentially in the case of the solid meals, the liquid meals

263 reached a plateau where pH was 7. This plateau lasted about 2 h before pH levels declined.
264 After 192 min, pH was approximately 2 for all four food types.

265 **3.3 Modelling the kinetics of postprandial intragastric pH**

266 Table 2 summarises the parameter values obtained using the pH catheter method and the
267 wireless pH capsule method. The curves obtained by modelling the pH catheter measurements
268 are presented in Figure 4B. It was assumed that there was no inflection period when the
269 regression analysis of the intragastric pH kinetics associated with the liquid meals was
270 performed. Because the basal pH, pH_{MIN}, was set to equal pH_{Infl}, no t_3 was required. After the
271 initial rounds of regression, k_1 was no longer sensitive to changes in conditions. To allow
272 comparisons of t_1 across models, k_1 was fixed at 0.40.

273 The value of pH_{MIN} was estimated from the regression analysis and was situated between
274 1.0 and 1.8, which is slightly lower than the basal pH as measured via *in situ* methods (about
275 3.5). Regardless of nature of the food type, pH_{MAX} was quickly reached (t_1 was always < 5
276 min). Estimates of pH_{MAX} were similar for the different measurement methods (7.1–7.3 for
277 the soymilk and pea emulsion and 6.5–6.8 for the seitan), except in the case of the tofu, for
278 which the wireless pH capsule method yielded lower values than pH catheter method (4.5 vs
279 7.7), matching what was seen in the experimental data.

280 Based on the first derivative (data not shown), t_2 seemed to be the time point at which the
281 decrease in pH was at its fastest. This parameter was good at discriminating between liquid
282 and solid meals. Indeed, for the tofu and seitan, t_2 was less than 28 min. In contrast, for the
283 soymilk and pea emulsion, t_2 lay between 147 and 183 min.

284 **4 Discussion**

285 The translation to human of these results obtained in a pig model must be done
286 cautiously. Indeed, although the size and the geometry of the minipig stomach is similar to
287 those of the human stomach (1 – 1,6 l), some physiological differences remain: proportion of
288 the cardiac mucosa in the stomach is higher in pigs than in humans (Kararli, 1995) and the
289 gastric emptying time is considered as slower in pig (2 h – 24 h) than in humans (10 min – 2
290 h) (Henze et al., 2018). Moreover, the position and the orientation of the pig stomach is
291 different from those of the human stomach, because of the difference of the whole-body
292 position during the digestion. In fact, the lengthwise axis (fundus – antrum axis) of the human
293 stomach is vertical, whereas the lengthwise axis of the pig stomach is horizontal.

294 **4.1 Comparison of the pH measurement methods**

295 In this study, three methods for measuring postprandial intragastric pH were compared:
296 the *ex vivo* sampling of chyme, the *in vivo* use of pH catheters, and the *in vivo* use of wireless
297 pH capsules. Compared to the *ex vivo* method, the *in vivo* pH catheter and wireless pH
298 capsule methods both allowed pH to be recorded continuously. For the solid meals (tofu and
299 seitan), no significant differences were seen between the *ex vivo* and *in vivo* measurements.
300 However, for the liquid meals, differences were observed during the pH plateau. After the
301 pigs consumed liquid foods, their stomachs were pretty much filled and contained
302 heterogenous contents. As a result, when the cannula was opened, the gastric contents leaked
303 out in large quantities. This fact may have led to the lower buffering capacities that were
304 observed and could explain the lower pH values obtained with the *ex vivo* method. Because
305 the *in situ* methods did not disturb the pigs or their stomach contents and because they also
306 allowed the continuous measurement of pH, they appear to be better suited to monitoring
307 postprandial intragastric pH. Moreover, because they displayed less variability, the pH
308 catheter data were more useful for evaluating the effect of meal on temporal changes in
309 postprandial intragastric pH. With the seitan, the *ex vivo* method provided more repeatable
310 results than did the *in situ* methods, probably because the chyme is more heterogeneous in the
311 core of the antrum than near the mucosa. Lastly, in the wireless pH capsule method, no
312 coupled external devices are needed, which is less constraining for the animal.

313 Overall, the pH catheter method and the wireless pH capsule method revealed similar
314 temporal patterns in postprandial intragastric pH. However, in the case of the solid meals, the
315 capsule consistently found lower pH values than did the catheter. This result is consistent with
316 the results of a previous study that also compared the two methods (Caparello et al., 2012). In
317 that study, the capsules were attached to the stomach wall, and the authors interpreted the
318 differences as resulting from the proximity of the pH sensor to the stomach parietal cells,
319 where HCl is secreted (Schubert & Peura, 2008). The lower pH values recorded with the
320 capsule were thus probably due to the lower BC of food occurring near the mucosa, in
321 comparison to food found in the middle of the gastric compartment. This same result could
322 arise for very heterogeneous chyme (Bornhorst et al., 2014). Thus, we could hypothesise that,
323 in this study, the wireless pH capsule remained close to the stomach wall while the pH
324 catheter, because of its rigidity, occupied a more central position within the stomach.

4.2 pH spatial distribution and chyme heterogeneity

It is noteworthy that the pH catheter's two sensors obtained significantly different measurements. Both before and after food ingestion, the sensor at the tip of the catheter yielded higher pH values. These differences between the two sensors, and the differences between the catheter and the capsule, clarify the spatial distribution of pH values both in the empty stomach and the stomach after it is filled with a solid meal; similar findings were seen in a previous study for soft and rigid foods (Bornhorst et al., 2014; Nau et al., 2019). Unfortunately, in this study, we were unable to precisely determine the positions of either the catheter sensors or the capsule. Thus, although we tried to direct the catheter towards the pylorus, we do not know if it was propelled backwards towards the fundus, which might explain the higher pH values found by the sensor at the catheter's tip in the case of the solid foods. Indeed, when a similar catheter (albeit equipped with four sensors) was used in humans, a gradient in intragastric pH was observed following complete food ingestion: the pH in the proximal part of the stomach was higher than the pH in the antrum (mid/distal region) (Simonian et al., 2005). In our study, no such difference was seen for liquid meals because of the large amount of liquid ingested, which rendered the gastric contents more homogeneous. Because the ultimate goal of the study was to characterise mean changes in intragastric pH over the postprandial period, it seemed important to use the mean of the values recorded by the two sensors when analysing the effects of food type on gastric pH kinetics.

4.3 Effects of food type on gastric pH kinetics

This study found clear differences in the postprandial pH kinetics elicited by liquid versus solid meals. While pH rapidly decreased exponentially in the case of the solid meals, there was an S-shaped drop in pH associated with the liquid meals, where pH plateaued for more than 2 hours. These results were similar to those obtained in previous studies in humans (Dressman, 1986; Gardner et al., 2002; Malagelada et al., 1976). An initial plateau has also been observed for liquid meals (Kalantzi et al., 2006). This plateau is probably caused by three phenomena: i) Meal volume and viscosity affect stomach distention and, as a consequence, the acid secretion rate (Marciani, Gowland, Spiller, et al., 2001); ii) proteins have higher buffering capacities when they occur in solutions than when they occur as solids, even if this pattern was not seen in our study; iii) pH results from the relative activity of H_3O^+ and OH^- ions in solution (aqueous phase) and, thus, ions inside the food network are not measured.

357 4.3.1 Meal volume

358 The volumes of the solid and liquid meals were different. The volume of the liquid meals
359 was 1.1 L, and their stomach filling rate was therefore 80% (total stomach volume = 1.31 ±
360 0.09 L; measured in six Yucatan miniature pigs weighing 25.4 ± 0.6 kg). In contrast, the
361 filling rate for the solid foods was less than 40%. Meal volume has been shown to affect
362 gastric emptying for liquid, but not solid meals.

363 4.3.2 Buffering capacity of meals

364 Buffering capacity is linked to the ability of acidic amino acids (*i.e.*, those with side
365 chains containing carboxylic groups) to trap the hydronium ion (Mat, Cattenoz, Souchon,
366 Michon, & Le Feunteun, 2018). Thus, for similar levels of protein, BC could be higher if
367 quantities of glutamic acid and aspartic acid are greater. However, in this study, although
368 wheat proteins contain more acidic amino acids (about 40%) than do soy proteins (about
369 30%), the seitan had a lower BC than did tofu. This finding underscores that, in addition to
370 primary structure, protein conformation matters; it is important to consider whether or not
371 acidic side chains are exposed when in the liquid phase. Furthermore, common methods for
372 identifying amino acids cannot distinguish between glutamine and glutamic acid or between
373 asparagine and aspartic acid.

374 The amount and output of acid secretions have been shown to directly correlate with a
375 food's initial BC (regardless of protein amount or source) (Williams, Forrest, & Campbell,
376 1968) and with postprandial gastric acidity during the first five hours of digestion (Gardner et
377 al., 2002). BC may thus help to shed light on changes in gastric pH over time. The longer
378 plateau observed for the soymilk compared to the pea emulsion could be explained by the
379 greater BC associated with the initial pH and the pH of 4 for soymilk compared to the values
380 for the pea emulsion. The same explanation could hold when interpreting the differences seen
381 between the seitan and the tofu at the beginning of digestion. While both food types had a
382 similar initial pH (~ 5.7), the tofu and the seitan reached maximal pH levels of 5.8 and 6.7,
383 respectively (pH catheter data). Tofu's higher BC could explain its resistance to neutralisation
384 in mouth and its subsequent acidification in the stomach. Furthermore, tofu had a higher BC
385 than did soymilk, although both food types are soy protein based; this dissimilarity could have
386 resulted from differences in macrostructure.

387 Lastly, BC could change during gastric digestion because of proteolysis and the addition
388 of endogenous components. Previous research found that gastric BC increased after meal

intake (Fordtran & Walsh, 1973), and, two hours later, its value was inversely correlated with the peak acid secretion rate. The authors of the study attributed this relationship to quicker gastric emptying, given that high acidity generally slows down the rate of gastric emptying (Fordtran & Walsh, 1973). Moreover, phase separation might have occurred, and thus buffer emptying would not have followed total gastric emptying.

4.3.3 Food particle size

Food particle size also plays a role in determining BC and thus gastric pH. Previous research explored the influence of particle size on pH distribution patterns in the pig stomach during digestion and showed that fine particles (D50: ~ 0.5 mm versus ~1.0 mm) promoted the diffusion of gastric acid within the stomach, which led to a more homogeneous pH (Hunt & Forrest, 1975; A. K. Mößeler, Wintermann, Beyerbach, & Kamphues, 2014; A. Mößeler, Köttendorf, Große Liesner, & Kamphues, 2010; Regina, Eisemann, Lang, & Argenzio, 1999). Another study examined acid diffusion patterns in an artificial stomach after the ingestion of solid meal and found similar results, with variation stemming mostly from particle size and meal temperature (Marcotte et al., 2012). In this study, the solid meals contained larger particles. Consequently, a more pronounced and persistent pH gradient within the stomach could be expected.

4.4 Regression analysis of gastric pH kinetics

Even if temporal changes in gastric pH were significantly different for liquid versus solid meals, we found that system kinetics could be described using the same equation, which made it possible to extract specific parameters. A recent modelling study (Sams, Paume, Giallo, & Carrière, 2016) that employed data from previous research showed that the kinetics of postprandial pH could be described with an exponential function or a polynomial equation. When we tested the fit between our pH data and a variety of functions, the coefficient of determination was higher for degree-four and degree-five polynomials and four-parameter logistic regressions (R^2 ~ 0.96–0.99) than for exponential functions (R^2 ~ 0.91–0.92). The most relevant regressions appeared to be those that included food type and/or physiological parameters, which is what Weinstein and colleagues found (Weinstein et al., 2013). They modelled postprandial gastric pH in healthy humans following the ingestion of liquid meal: like us, they observed a plateau in pH and determined that its duration depended on buffer concentration, gastric acid secretion rate, and gastric emptying time.

5 Conclusions

We compared the results yielded by three different methods for monitoring intragastric pH during the postprandial period in miniature pigs that had ingested liquid and solid meals. Across food types, *in situ* pH measurements obtained with pH catheters or wireless pH capsules were robust and consistent with each other. In contrast, the *ex vivo* method, in which chyme was sampled via the cannula, seemed to be poorly suited for following intragastric pH levels during the digestion of liquid meals. Both *in situ* methods captured the food-type-mediated differences in postprandial intragastric pH over time: for liquid meals, pH plateaued for approximately 2 h, but for solid meals, pH decreased quickly and immediately. Food macrostructure was thus clearly a determinant factor, as was food buffering capacity, which was assessed *in vitro*. Modelling temporal patterns of intragastric pH for liquid and solid meals will help clarify changes in pepsin activity in the stomach and the consequences for protein digestion. In addition, the equations that we defined could be used to differentially program an *in vitro* digester based on the food matrix to be studied.

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Disclosure

The authors have no competing interests.

Author Contributions

YR and DR designed the animal experiments. DD, IS, ML, YR, and DR defined the food types. YR, CB, JD, BC, MV, and DR performed the experiments. YR and DR analysed the data. YR drafted the manuscript, and DR, DD, and IS helped revise it.

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593 **Tables legends**

594

595 **Table 1**: Buffering capacity (BC) of the different food types for different pH ranges (n = 3,
596 expressed in terms of 10^3; mean ± SEM). The results were analysed using a repeated-
597 measures ANOVA where food type and pH*food type were fixed effects, and pH was a
598 repeated effect. As all the effects were significant ($P < 0.0001$), a post-hoc LSMEANS test
599 was performed. The letters (a through e) indicate when significant differences were present (P
600 < 0.05).

601 **Table 2:** Parameter values and coefficients of determination obtained from the logistic
602 regression analysis of intragastric pH kinetics for the different food types as assessed via the
603 two *in vivo* measurement methods (pH catheter = Cat, wireless pH capsule = WCap).

604 **Figure legends**

605 **Figure 1**

606 Approximate position of the pH monitoring equipment within the stomach. The dark red line
607 represents the pH catheter, and the two sensors are depicted using circles (Cat1 & Cat2). The
608 thin blue line is the silk thread that was attached to the wireless pH capsule, which is
609 represented by a rectangle (WCap). Cat1 and Cat2 were connected to a pH meter via the
610 catheter. In contrast, the pH capsule wirelessly transmitted data to a pH meter.

611 **Figure 2**

612 Temporal changes in postprandial intragastric pH (n = 4 animals) characterised with each of
613 the catheter sensors over a 300-min period after (A) seitan, (B) tofu, (C) pea emulsion, and
614 (D) soymilk ingestion. The measurements were averaged over 6-min intervals. The circles are
615 the means for each interval, and the bars represent the SEM. The filled dark circles represent
616 the data from the first pH catheter sensor (Cat1). The empty grey circles represent the data
617 from the second pH catheter sensor (Cat2).

618 **Figure 3**

619 Temporal changes in postprandial intragastric pH (n = 4 animals) characterised with the three
620 measurement methods over a 300-min period following meal ingestion. The effects of
621 different food types were tested: (A) seitan, (B) tofu, (C) pea emulsion, and (D) soymilk. For
622 the *in situ* methods, measurements were averaged over 6-min intervals. The circles are the
623 means for each interval, and the bars represent the SEM. The filled dark circles represent the
624 data from the pH catheter method (averaged across the two sensors; Cat). The empty grey
625 circles represent the data from the wireless pH capsule method (WCap). The filled grey
626 squares represent the data from the *ex vivo* chyme sampling method (ExV). The stars indicate
627 time points where there were significant differences. For each food type, relative standard
628 error (SEM*100/MEAN) was calculated for each 6-min interval for the Cat and WCap
629 methods and for the chyme sampling points for the ExV method. The results are represented
630 in the boxplots below. The values near the boxplots are the mean relative standard errors.

631 **Figure 4**

632 (A) Temporal patterns in postprandial intragastric pH (n = 4 animals) characterised with the
633 pH catheter method (Cat; pH values averaged across the two sensors) over a 300-min period
634 following meal ingestion. Measurements were averaged over 6-min intervals. The circles are

635 the means for each interval, and the bars represent the SEM. The filled dark circles represent
636 the seitan data, the empty grey circles represent the tofu data, the filled dark squares represent
637 the pea emulsion data, and the empty grey squares represent the soymilk data. Significant
638 differences at a given time point are indicated by the presence of black lines above the plot.
639 (B) Results of the regression analysis of temporal patterns in postprandial intragastric pH as
640 characterised by the pH catheter method (Cat; pH values averaged across the two sensors;
641 solid lines) and by the wireless pH capsule method (WCap; dashed lines) over a 300-min
642 period following food ingestion. The different food types are indicated by different shades of
643 grey.

Figure 1

Approximate position of the pH monitoring equipment within the stomach. The dark red line represents the pH catheter, and the two sensors are depicted using circles (Cat1 & Cat2). The thin blue line is the silk thread that was attached to the wireless pH capsule, which is represented by a rectangle (WCap). Cat1 and Cat2 were connected to a pH meter via the catheter. In contrast, the pH capsule wirelessly transmitted data to a pH meter.

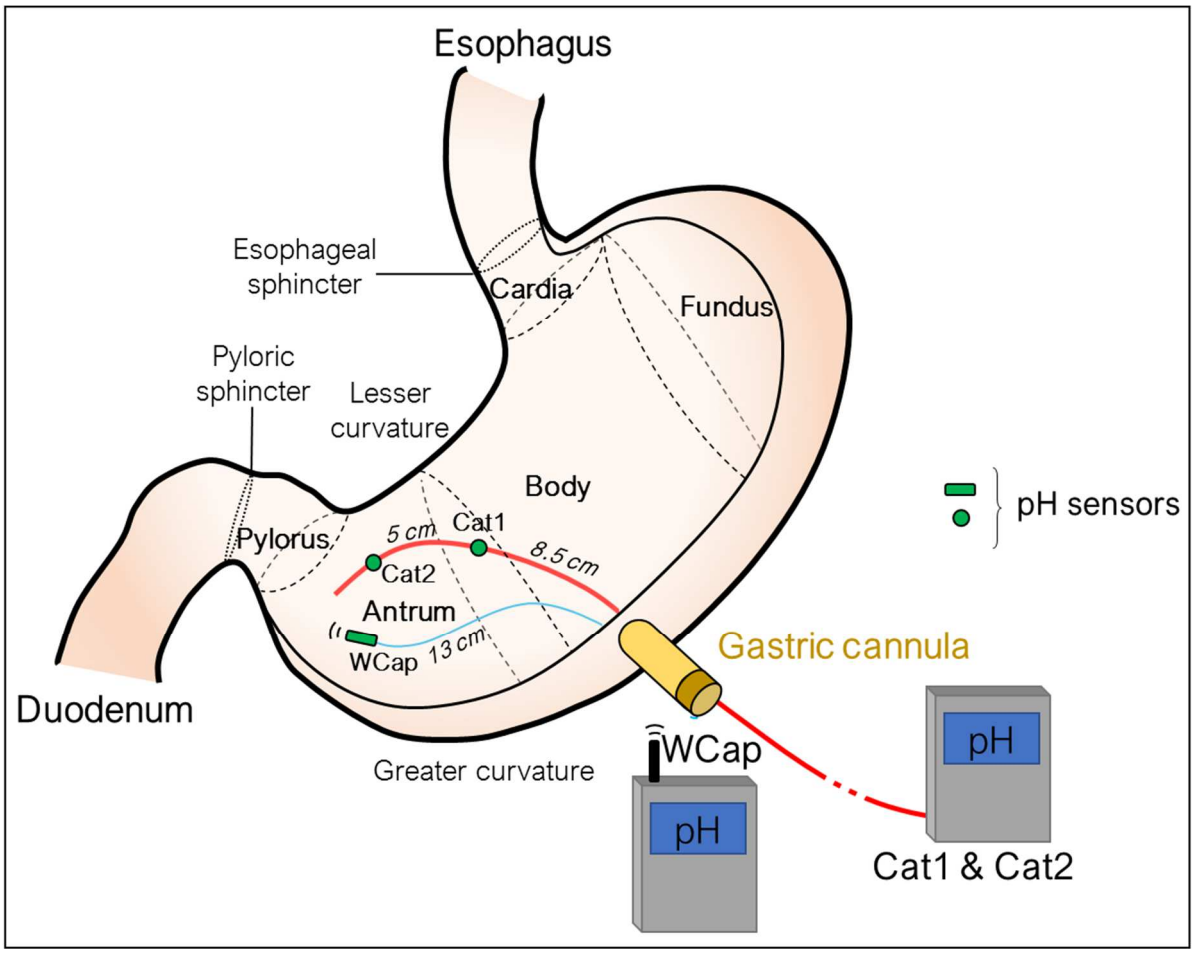


Figure 2

Temporal changes in postprandial intragastric pH (n = 4 animals) characterised with each of the catheter sensors over a 300-min period after (A) seitan, (B) tofu, (C) pea emulsion, and (D) soymilk ingestion. The measurements were averaged over 6-min intervals. The circles are the means for each interval, and the bars represent the SEM. The filled dark circles represent the data from the first pH catheter sensor (Cat1). The empty grey circles represent the data from the second pH catheter sensor (Cat2).

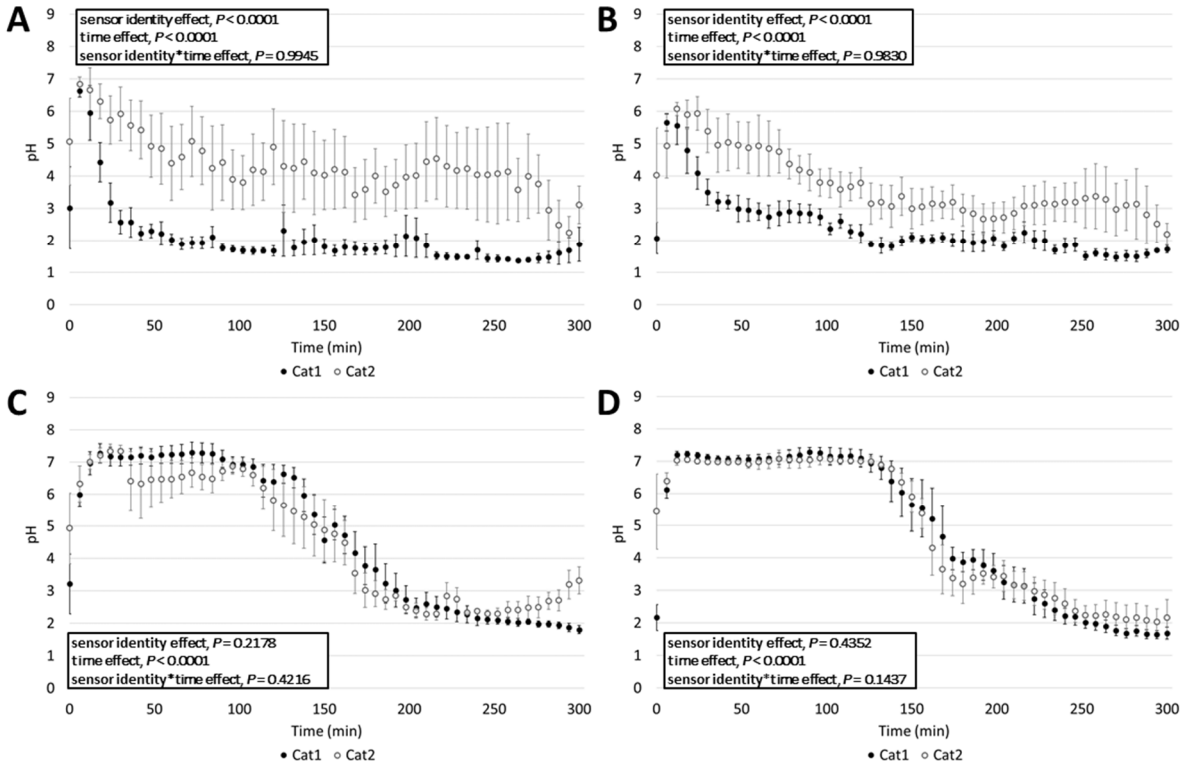


Figure 3

Temporal changes in postprandial intragastric pH (n = 4 animals) characterised with the three measurement methods over a 300-min period following meal ingestion. The effects of different food types were tested: (A) seitan, (B) tofu, (C) pea emulsion, and (D) soymilk. For the *in situ* methods, measurements were averaged over 6-min intervals. The circles are the means for each interval, and the bars represent the SEM. The filled dark circles represent the data from the pH catheter method (averaged across the two sensors; Cat). The empty grey circles represent the data from the wireless pH capsule method (WCap). The filled grey squares represent the data from the *ex vivo* chyme sampling method (ExV). The stars indicate time points where there were significant differences. For each food type, relative standard error (SEM*100/MEAN) was calculated for each 6-min interval for the Cat and WCap methods and for the chyme sampling points for the ExV method. The results are represented in the boxplots below. The values near the boxplots are the mean relative standard errors.

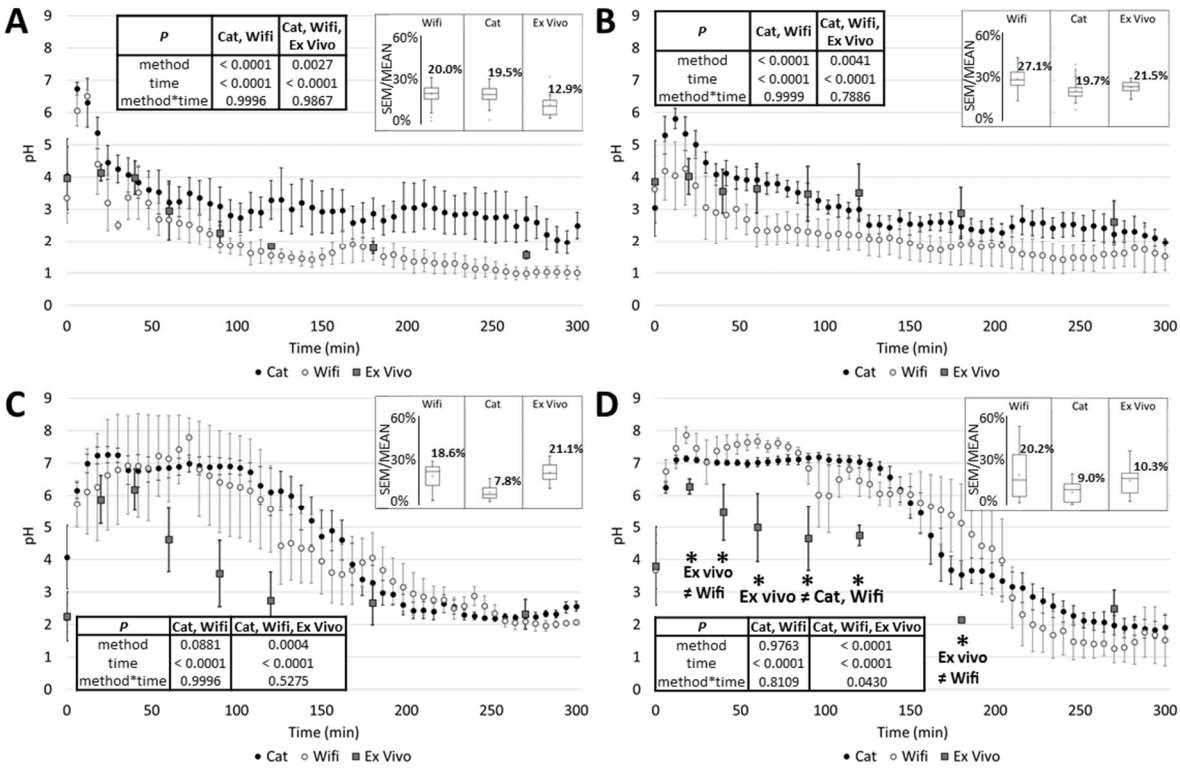
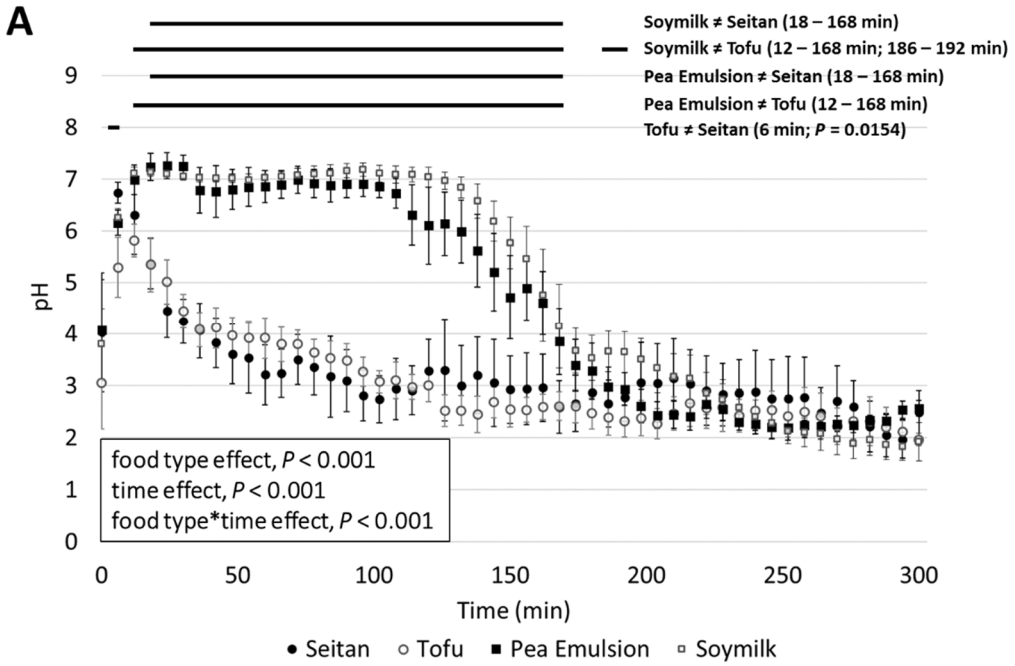
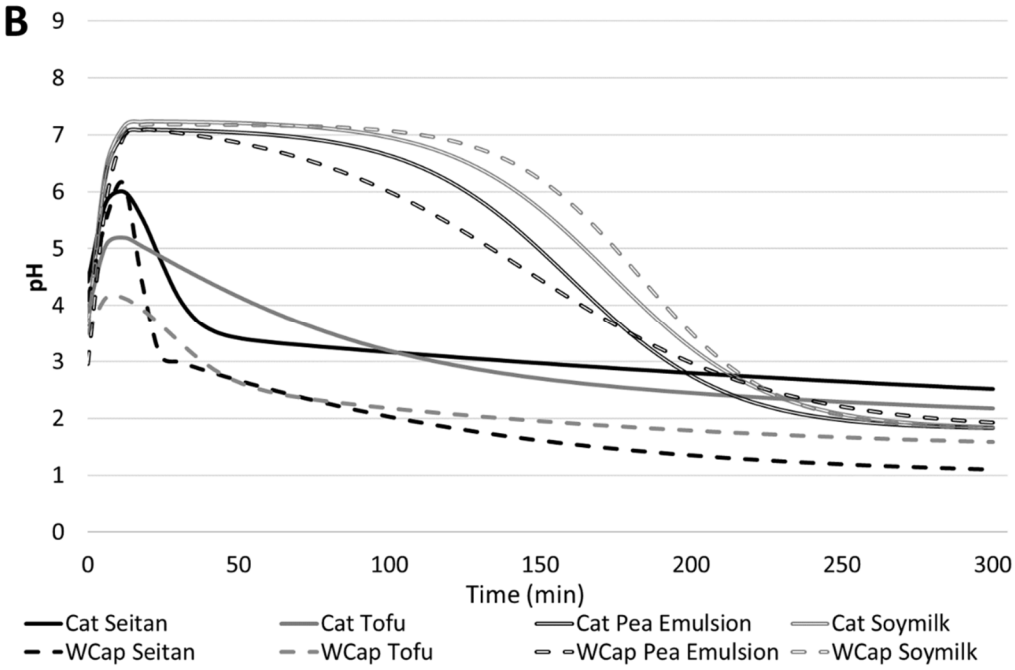


Figure 4

(A) Temporal patterns in postprandial intragastric pH (n = 4 animals) characterised with the pH catheter method (Cat; pH values averaged across the two sensors) over a 300-min period following meal ingestion. Measurements were averaged over 6-min intervals. The circles are the means for each interval, and the bars represent the SEM. The filled dark circles represent the seitan data, the empty grey circles represent the tofu data, the filled dark squares represent the pea emulsion data, and the empty grey squares represent the soymilk data. Significant differences at a given time point are indicated by the presence of black lines above the plot.

(B) Results of the regression analysis of temporal patterns in postprandial intragastric pH as characterised by the pH catheter method (Cat; pH values averaged across the two sensors; solid lines) and by the wireless pH capsule method (WCap; dashed lines) over a 300-min period following food ingestion. The different food types are indicated by different shades of grey.

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pH
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50
100
150
200
250
300
Time (min)
Cat Seitan
Cat Tofu
Cat Pea Emulsion
Cat Soymilk
WCap Seitan
WCap Tofu
WCap Pea Emulsion
WCap Soymilk

Table 1

Buffering capacity (BC) of the different food types for different pH ranges (n = 3, expressed in terms of 10^3; mean ± SEM). The results were analysed using a repeated-measures ANOVA where food type and pH*food type were fixed effects, and pH was a repeated effect. As all the effects were significant ($P < 0.0001$), a post-hoc LSMEANS test was performed. The letters (a through e) indicate when significant differences were present ($P < 0.05$).

	pH 2 ← pH 4	pH 4 ← pH 6	pH 6 ← initial pH
Seitan	16.0 ± 0.8^{e}	4.4 ± 0.4^{d}	10.3 ± 0.2^{c}
Tofu	34.9 ± 1.3^{a}	21.3 ± 0.8^{b}	14.4 ± 0.3^{c}
Pea Emulsion	16.0 ± 0.5^{e}	7.0 ± 0.6^{d}	6.7 ± 0.3^{d}
Soymilk	21.8 ± 3.2^{b}	$13.5 \pm 0.2^{e,c}$	$12.0 \pm 0.4^{e,c}$

Table 2

Parameter values and coefficients of determination obtained from the logistic regression analysis of intragastric pH kinetics for the different food types as assessed via the two *in vivo* measurement methods (pH catheter = Cat, wireless pH capsule = WCap).

Food type	Method	R^2	pH_{MIN}	pH_{Infl}	pH_{MAX}	t_1	t_2	t_3	k_1	k_2
Seitan	Cat	0.906	1.8	3.6	6.5	2	23	256	0.40	0.17
	WCap	0.946	1.0	3.6	6.5	4	18	89	0.40	1.00
Tofu	Cat	0.955	1.5	3.0	7.7	3	9	300	0.40	0.02
	WCap	0.960	1.4	2.8	4.5	1	28	125	0.40	0.10
Pea emulsion	Cat	0.987	1.8	1.8	7.1	2	160	-	0.40	0.04
	WCap	0.956	1.8	1.8	7.3	3	147	-	0.40	0.02
Soymilk	Cat	0.983	1.8	1.8	7.3	2	174	-	0.40	0.04
	WCap	0.965	1.8	1.8	7.2	2	183	-	0.40	0.05

Foods tested:

Two Solids

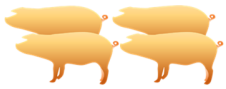
- Tofu (soy based)
- Seitan (wheat gluten based)

Two Liquids

- Soymilk
- Pea Emulsion

Methods to follow the postprandial intragastric pH:

4 adult Yucatan minipigs with a gastric cannula



- Wireless pH capsule
- pH catheters
- Sampling through the cannula