Increasing intake of dietary soluble nutrients affects digesta passage rate in the stomach of growing pigs

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Increasing intake of dietary soluble nutrients affects digesta passage rate in the stomach of growing pigs

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Abbreviations: BW, body weight; CP, crude protein; F, feed intake level; GIT, gastrointestinal tract; HF-HS, high feed intake – high nutrient solubility; HF-LS, high feed intake – low nutrient; solubility; HF-MS, high feed intake – medium nutrient solubility; *K*, consistency constant; LF-LS, low feed intake – low nutrient solubility; ME_m, metabolisable energy requirement for maintenance; MRT, mean retention time; n, power-law index; S, nutrient solubility; visco45, apparent viscosity at 45/s shear rate; WBC, water-binding capacity; η, viscosity (Pa×s)

ABSTRACT

The passage rate of solids and liquids through the gastrointestinal tract differs. Increased dietary nutrient solubility causes nutrients to shift from the solid to the liquid digesta fraction and potentially affect digesta passage kinetics. We quantified: 1) the effect of three levels of dietary nutrient solubility (8, 19, and 31 % of soluble protein and sucrose in the diet) at high feed intake level (S); and 2) the effect of low versus high feed intake level (F), on digesta passage kinetics in forty male growing pigs. The mean retention time (MRT) of solids and liquids in the stomach and small intestine was assessed using TiO_2 and Cr-EDTA, respectively. In addition, physicochemical properties of digesta were evaluated. Overall, solids were retained longer than liquids in the stomach (2·0 h, P<0·0001), and stomach + small intestine (1·6 h, P<0·001). When S increased, MRT in stomach decreased by 1·3 h for solids (P=0·01) and 0·7 h for liquids (P=0·002), but only at the highest level of S. When F increased using low soluble nutrients, MRT in stomach increased by 0·8 h for solids (P=0·041) and 0·7 h for liquids (P=0·0001). Dietary treatments did not affect water-binding capacity and viscosity of digesta. In the stomach of growing pigs, dietary nutrient solubility affects digesta MRT in a non-linear manner, while feed intake level increases digesta MRT depending on dietary nutrient solubility. Results can be used to improve predictions on the kinetics of nutrient passage and thereby of nutrient digestion and absorption in the gastro-intestinal tract.

INTRODUCTION

In humans and animals, the appearance kinetics of nutrients in portal blood depends on the kinetics of nutrient passage, hydrolysis, and absorption in the gastrointestinal tract (GIT). It has been shown that asynchronous appearance of metabolic complementary nutrients may affect the nutrient's metabolic fate. For example, pigs fed with a free lysine diet versus a protein-bound lysine diet[1], or pigs asynchronously fed amino acids and glucose within a day[2] showed an increased loss of amino acids as a result of oxidation. As the small intestine is the main site of nutrient absorption, the kinetics of nutrient passage prior to this site can influence the kinetics of portal blood appearance. Hence, the kinetics of nutrient passage through the stomach and small intestine is important to consider when one is interested in the metabolic fate of ingested nutrients.

The passage of nutrients through the stomach is a heterogeneous process[3]. Due to the morphology and motility of the stomach, solids pass slower than liquids[4, 5]. After ingestion, solids are first retained in the proximal stomach, whereas liquids rapidly distribute throughout, and empty from the stomach[4]. The passage of liquids from the stomach is driven by (fundic) pressure, and is related to stomach volume[6, 7]. Solids, however, first pass from the proximal to distal stomach, where they can be reduced in size before they are emptied into the small intestine[8, 9]. Moreover, several feedback mechanisms along the GIT are known to control the gastro-intestinal motility and inhibit digesta passage from the stomach and/or in the intestines. These feedback mechanisms can be triggered by receptors along the GIT by the presence of protein, carbohydrates, and fat degradation products[10, 11]. Increasing the nutrient load of a meal, for example, resulted in a decreased stomach emptying rate of both solids and liquids in both human and pigs[4, 12, 13]. Hence, the rate of passage of solids and liquids through the stomach is a net result of multiple factors that stimulate or inhibit the passage process.

The difference in passage rate of digesta phases (i.e. solids vs. liquids) and the influence of nutrient load on passage kinetics, indicates that dietary nutrient solubility can influence the passage rate of digesta from the stomach. An increase in dietary nutrient solubility causes nutrients to shift from the solid to the liquid digesta fraction. Nutrients in the latter fraction enter the small intestine quickly after ingestion, thereby potentially triggering nutrient feedback mechanisms that affect digesta passage kinetics in the proximal GIT. Moreover, relevant variation in nutrient solubility between feed ingredients exists. Protein solubility, for example, varies between 0 % in faba beans and 61 % in maize gluten meal at stomach pH[14], and close to 90 % in whey protein isolates at pH 4.6[15]. While previous studies observed an effect on stomach emptying rate by increasing the nutrient load of the liquid fraction of the diet[4, 13], the effect was confounded with the effect of increasing total nutrient intake[12]. In addition, although in humans and pigs the passage rate of solids and liquids in the stomach has been studied[4, 12, 13, 16, 17], only limited studies have quantified the passage rate of digesta

solids and liquids in other segments of the GIT[17]. Therefore, this study aimed to evaluate the effects of 1) dietary nutrient solubility (S), and 2) feed intake level (F), on the passage behaviour of solids and liquids in multiple GIT segments of growing pigs. It was hypothesized that an increase in S or F would result in an increase in mean retention time (MRT) of solids and liquids in the proximal GIT.

MATERIAL AND METHODS

The study was approved by the Dutch Animal Ethics Committee (2014.III.06.056) and carried out at the Swine Research Centre of Nutreco N.V. (Sint Anthonis, The Netherlands). This includes daily welfare assessments as required and guided by European legislation (European Commission: Directive 2010/63/EU). The study objective considers the pig as the main research subject.

Animals and housing. Forty male growing pigs (Hypor×Maxter; Hendrix Genetics, Boxmeer, The Netherlands) with an average initial body weight (BW) of 32·0 ± 1·4 kg were used. The experiment was performed in two sequential batches of twenty pigs each. Pigs were individually housed in pens (2·48×0·94 m) equipped with partial slatted floors and half-open walls between pens to allow visual and physical contact of adjacently housed pigs. Temperature was controlled at 23 ± 1°C and the facility was lid from 06.00 to 18.00 h.

Diets and feeding. In a randomized complete block design, the pigs were assigned to one of four treatments differing in S and F. Dietary treatments were a low, medium, and high S diet at high F (HF-LS, HF-MS, HF-HS, respectively), and a low S diet at low F (LF-LS). Low and high F represent feed intake levels of, respectively, 1·9 and 2·8 × metabolisable energy requirement for maintenance (ME_m: 419 kJ ME/kg $BW^{0·75}$) [18]. Low, medium and high S diets consisted of 8, 19, and 31 % of soluble protein and glucose-equivalents ($\frac{\boldsymbol{Starch}}{\mathbf{0.9}}$ + reducing sugars), respectively. Whereby dietary nutrient solubility was considered as the proportion of nutrients that are soluble when brought in a buffer solution (pH 3-3·5, stomach pH in pigs)[14, 15, 19, 20, 21].

The experimental diets were composed of two basal diets (**Table 1**): a basal low soluble diet and a basal high soluble diet, these diets were formulated using ingredients covering a low or high range of nutrient solubility, respectively. The basal diets were designed to be equal in crude protein (CP), glucose-equivalents, and crude fat content. These basal diets were produced as mash and were mixed in different ratios to obtain the four experimental diets (**Table 2**). Soybean meal, maize and wheat were hammer-milled to pass a 4-mm sieve, and sugar beet pulp and rapeseed meal to pass a 2·75 mm sieve.

Three days prior to the experiment, the pigs were gradually switched from a commercial diet to the experimental diets. The experiment lasted for 18 d **(Figure 1)**. Pigs were fed the experimental diets at a feeding level of 2·5 ME_m until d7, followed by the feeding level of the respective treatments until the end of the trial. The pigs were fed twice daily at 08.00 and 16.00 h until d15, followed by frequent feeding to induce steady state passage of digesta in the GIT. During the frequent feeding period, the daily feed allowance was divided in six equal portions. On d16 and d17 the pigs received portions once every 3 h from 05.30 until 20.30 h. On d18, the pigs received portions once every 2 h from 02.30 h until 2 h prior to euthanasia, with a minimum of three portions fed on this day. Feeding time on this day (d18) was scheduled according to the scheduled time of euthanasia of each pig, starting at 08.30 h with the first pig. The diets contained TiO_2 as the indigestible insoluble marker[22] from d8 onwards, and Cr-EDTA as the indigestible soluble marker[23] from d16 onwards. Diets were fed as mash and mixed with water (1:2·5, w: w) in the feed trough. In addition, the pigs received 0·5 L of water per day, 0·25 L in the morning and 0·25 L in the afternoon. During the frequent feeding period the pigs did not receive additional water. Twice weekly the pigs were weighed to adjust the amount of feed allowed based on the pigs' BW.

Sample collection and chemical analysis. At d18, the pigs (45·2 ± 3·2 kg BW) were euthanized for quantitative digesta collection from various segments of the GIT. Pigs were euthanized sequentially by sedating i.m. with Zoletil® 100 (0·06 ml/kg BW), followed by injecting Euthasol® (20 %; 24 mg/kg BW) in the ear vein, and exsanguinating via the carotid artery. The sequence of sacrificing pigs was balanced for treatment by block. Each block consisted of four adjacently housed pigs, each pig receiving a different dietary treatment. Immediately after exsanguination, the abdominal cavity was opened and the GIT was divided into segments by placing tie-wraps at the beginning and end of the stomach, small intestine, caecum, and colon + rectum (further mentioned as colon), and halfway the small intestine and colon. Digesta from the stomach, proximal and distal half of the small intestine, caecum, and proximal and distal half of the colon were collected by gentle stripping. After digesta collection, homogenous digesta subsamples were taken and stored at 4°C pending measurements of viscosity and water-binding capacity (WBC). The remaining digesta was stored at -80°C pending freeze-drying. After freeze drying, the samples were centrifugal-milled to pass a 1 mm sieve (Retsch ZM 200, Haan, North Rhine-Westphalia, Germany). The process from euthanasia until sample storage lasted 15 min per pig.

Diets and digesta were analysed for contents of DM[24], CP (nitrogen[25] × 6·25), starch[26], reducing sugars[27], titanium[28], and chromium (measured at 357·9 nm[29] after sample preparation according to Williams *et al.* [30]). Single analyses were carried out. In addition, 10 % randomly chosen samples

were analysed in duplicate to evaluate the precision of the analyses. Precision and thereby results from analyses were considered valid in case over 90 % of observed duplicate differences were below the set maximum allowable differences for the respective nutrients. In absolute terms, maximum differences were set for DM (2 g/kg), and for starch (2 g/kg, if starch concentration >100 g/kg; or 1 g/kg if starch concentration <100 g/kg). In relative terms, maximum differences were set for nitrogen (5 %), Ti (5 %), and Cr (10 %). Samples were reanalysed when values were outside the range of the mean value ± 2 × standard deviation (SD) within treatment and GIT segment.

Water-binding capacity of digesta was measured using centrifugational force. Fresh digesta samples were centrifuged at 4,000 × *g* for 10 min at 21°C after which the supernatant was decanted. The WBC, in g/g digesta DM, was calculated as the weighed amount of water retained after decanting. This analysis was performed in duplicate if the quantity of available sample allowed. In total, 25 samples were analysed single, 120 in duplicate, and for 95 samples insufficient material was available.

Dynamic viscosity of digesta was measured within 96 h after digesta collection by a MCR502 and MCR301 rheometer (Modular Compact Rheometer, Anton Paar GmbH, Graz, Styria, Austria). Measurements were carried out at 39°C with declining shear rates from 50/s to 1/s in 25 steps. Different geometries were used for digesta from the proximal and distal GIT segments due to differences in digesta consistencies within these segments. Stomach and small intestinal samples were measured in a titanium concentric cylinder (i.e. cup) system (CC17-SN2540, Anton Paar GmbH, Graz, Austria). Caecum and colon digesta samples were measured on a titanium parallel profiled plate-plate measuring system (PP25/P2-SN25463; PP25/P2-SN25491, Anton Paar GmbH, Graz, Austria) with a 1·5 mm gap width.

Calculations and statistics. Calculations and statistics were performed in Statistical Analysis Systems statistical software package version 9·3 (SAS Institute Inc., Cary, NC, USA). The mean retention time of digesta in each GIT segment was calculated (Eq. 1) based on the assumption that in a steady state, pool sizes of digestible marker in each segment reflects the MRT of digesta in that segment (discussed by de Vries and Gerrits [31]).

$$\text{MRT (h)} = \frac{Marker\ pool\ size\ in\ digesta\ (g)}{Marker\ intake\ (\frac{g}{h})} \quad \text{[Eq. 1]}$$

where, the marker is either Ti (as TiO_2) or Cr (as Cr-EDTA). Marker pool sizes in digesta in each GIT segment were calculated by multiplying the digesta marker concentration (g/kg DM) by the weight of digesta in the corresponding segment (g DM). Marker intake was calculated by multiplying marker concentration of the diet (g/kg DM) by the meal intake at d18 (kg DM/h).

The apparent digestibility of starch and protein in the proximal segments (i.e. stomach, proximal and distal half of the small intestine) of the GIT was calculated (Eq. 2) according to Kotb and Luckey[32]:

$$\text{Nutrient digestibility (\%)} = \left(1 - \frac{\left(\frac{[Nutrient]_{digesta}}{[Marker]_{digesta}}\right)}{\left(\frac{[Nutrient]_{diet}}{[Marker]_{diet}}\right)}\right) \times 100 \quad \text{[Eq. 2]}$$

where, $[\text{Nutrient}]_{\text{digesta}}$, $[\text{Nutrient}]_{\text{diet}}$, $[\text{Marker}]_{\text{digesta}}$, $[\text{Marker}]_{\text{diet}}$ are concentrations (g/kg DM) of nutrient (CP or starch) and marker (Ti or Cr) in the digesta or diet samples.

Dynamic digesta viscosity is described to have non-Newtonian shear-tinning flow behaviour[33]. Therefore, the non-Newtonian flow behaviour was fitted using a power-law model[34] (Eq. 3):

$$\eta = K\dot{\gamma}^{n-1} \quad \text{[Eq. 3]}$$

where, η = viscosity (Pa×s), K = consistency constant, $\dot{\gamma}$ = shear rate (/s) and n = power-law index. The power-law model parameters (K, n) were estimated per pig per GIT segment using non-linear least squares regression (PROC NLIN). The viscosity in the Newtonian region at 45/s was calculated from the power-law model and reported.

The effects of the dietary treatments on digesta MRT, nutrient digestibility, and viscosity parameters were analysed per GIT segment using a general linear model (PROC GLM). Dietary treatment, batch, treatment×batch, and block were considered as fixed effects, and the pig as experimental unit. Studentized residuals were tested for normality using the Shapiro-Wilk test. Data distribution was visually evaluated to confirm heteroscedasticity. Non-normal distributed variables where transformed (i.e. logarithmic, exponential, reciprocal, quadratic) before the statistical evaluation. Post-hoc separation of means was performed after Tukey-Kramer adjustment. Difference between the LF-LS and HF-LS treatment was considered as a pre-planned contrast and evaluated using a contrast statement. Due to unbalanced data and lack of fixed effects, only mean and SD of digesta physicochemical properties for water-binding capacity and viscosity were reported. Differences in digesta physicochemical properties between GIT segments were analysed using the previous mentioned general linear model including the fixed effect of GIT segment. Results are presented as back-transformed least square means, and pooled standard deviation (SD_{pooled}), unless indicated otherwise. Considering stomach MRT of solids and liquids as the most important parameters of this study, a power larger than 0·95 was reached on the main effect of treatment using retrospective power analysis (PROC GLMPOWER) with an two-sided α level of 0·05 and current study design and results. Differences among means with P-values <0·05 were considered significant and P-values between 0·05 and 0·10 were considered a trend.

RESULTS

All pigs remained clinically healthy during the study duration and no adverse events were observed in any of the experimental groups. Data of one pig from the HF-LS treatment were excluded from statistical analyses due to feed refusals that exceeded 10 % of the daily feed allowance for 7 consecutive days prior to the pigs' dissection.

Digesta passage. On average, the MRT of solids was longer than that of liquids in the stomach (3·2 vs 1·2 h, P<0·0001; **Table 3**) and in the stomach + small intestine (5·3 vs. 3·7 h, P<0·0001), but shorter in the distal half of the small intestine (1·8 vs. 2·3 h, P<0.0001). The HF-HS pigs had a shorter MRT of solids (2·9 vs. 4·1 h, P=0·01) and liquids (0·8 vs. 1·5 h, P=0·002) in the stomach than the HF-MS pigs, but no other differences were observed between treatments varying in the proportion of S (HF-LS vs. HF-MS vs. HF-HS). Nutrient solubility did not influence the MRT of solids or liquids in the small intestine. When F increased with the additional intake of low soluble nutrients (LF-LS vs. HF-LS), MRT in the stomach increased for both solids (2·5 vs. 3·3 h, P=0·041) and liquids (0·6 vs. 1·3 h, P=0·0001). When F increased with the additional intake of high soluble nutrients (LF-LS vs. HF-HS), no effects on MRT in the stomach were observed. In the distal half of the small intestine the MRT of solids decreased with additional intake of low soluble nutrients (LF-LS vs. HF-LS: 2·1 vs. 1·7 h, P=0·006), as well as, high soluble nutrients (LF-LS vs. HF-HS: 2·1 vs. 1·7 h, P=0·03).

Nutrient digestibility. Digestibility of starch was calculated using TiO_2 as marker, and apparent protein digestibility using both TiO_2 and Cr-EDTA as markers. Calculated digestibility values of starch (TiO_2), and protein (Cr-EDTA) in the stomach were negative, and therefore not presented. Dietary treatment did not affect starch digestibility **(Table 4)**. When F increased with additional intake of low soluble nutrients, only the apparent protein digestibility (based on Cr-EDTA) increased in the proximal half of the small intestine (LF-LS vs. HF-LS: -6 vs. 25 %, P=0·013).

Physicochemical properties. Dietary treatments did not affect the physicochemical properties of digesta in any GIT segment (P>0·12) as within treatment variation was greater than between treatment variation **(Supplementary Material)**. Therefore, results are presented as descriptive statistics (**Table 5**). Results on the WBC of digesta in the proximal half of the small intestine are not presented due to an insufficient number of samples. The average WBC of digesta was lowest in the stomach (1·9 g/g digesta DM), and highest in the caecum (5·7 g/g digesta DM) compared to the WBC of digesta in any other GIT segment (P<0·005). Dynamic viscosity properties of digesta, partly

represented by apparent viscosity at 45/s and *K*, was on average higher in the distal half of the small intestine than in other GIT segments (visco45: 8.4 > 2.2-3.3 Pa×s, P<0·0001; *K*: 177 > 35-54 Pa×s, P<0.0001).

DISCUSSION

This study aimed to evaluate the effects of 1) nutrient solubility and 2) feed intake level on the MRT of the solid and liquid digesta fraction in several GIT segments in growing pigs. The experimental design allowed to study the effects of 1) S, as the proportion of soluble nutrients within the diet (HF-LS vs. HF-MS vs. HF-HS) and 2) F (LF-LS vs. HF-LS) on the MRT of digesta solids and liquids in the stomach and small intestine, and 3) the dependency of F on S (*i.c.* LF-LS vs. HF-LS or HF-HS). Based on ingredient selection, nutrient solubility of the low soluble diet is considered representative for commercially fed dry diets to growing pigs. Dietary nutrient solubility was increased by exchanging low soluble ingredients for high soluble ingredients, thereby covering the range of variation in solubility between ingredients regarding protein (from 4 % in wheat to >80 % in whey protein isolate)[15, 19] and starch (i.e. glucose-equivalents; from 4 % in wheat to 100 % in sucrose)[19]. Concerning the treatments differing in S, the proportion of soluble nutrients in the diet increased from the HF-LS to the HF-HS treatment with a factor 2·3 for protein, and 4·6 for glucose-equivalents. Hereby, 45 kJ gross energy/kg metabolic body weight per meal was shifted from insoluble to soluble nutrients, exceeding the nutrient load (~33 kJ gross energy/kg metabolic body weight per meal) that induced an effect on gastric emptying rate in previous studies in humans[4,13].

Although it was expected that an increased intake of soluble nutrients could reduce gastric emptying through stimulation of nutrient feedback mechanisms in the small intestine[4, 13], the results in the present study do not support this hypothesis. Instead, increasing S, via the relative higher intake of soluble nutrients, resulted in a decreased MRT of digesta in the stomach. The latter indicates faster emptying of the stomach. This result, however, was only observed when S increased to the highest level applied (HF-MS to HF-HS), thereby indicating a non-linear effect of S on the MRT of digesta in the stomach. Previous studies showed an increase in MRT of digesta in the stomach with additional intake of soluble nutrients, the effect however being confounded with the effect of total nutrient and energy intake (1,230 vs. 1,967 kJ gross energy/ meal). Whereas it has also been shown that increasing feed intake level causes increased stomach MRT in both pigs and humans[12, 35]. By shifting nutrients from the solid to the liquid fraction of digesta in our study, we expected stimulation of nutrient feedback mechanisms in the small intestine by the rapid postprandial appearance of soluble nutrients in that segment. It seems that the intake of the high soluble nutrients in this study to increase S and F

were not able to trigger the feedback mechanisms. As the feedback mechanisms regulating digesta passage are complex in nature and their stimulation depends on many factors such as the type of stimuli, GIT location, and duration of stimulation[9 10, 11, 36]. Potentially the stimulus duration was too short, as high soluble nutrients are generally absorbed rapidly after entering the small intestine[37, 38]. Unfortunately, the study design doesn't allow to speculate which dietary or animal factors particularly caused the non-linear effect of S the passage kinetics of digesta.

The effect of F was dependent of S, as additional intake of high soluble nutrients did not affect digesta passage from the stomach, while additional intake of low soluble nutrients caused the MRT of digesta in the stomach to increase. This is agreement with previous findings, where an increase in feed intake level caused stomach MRT to increase[12, 35]. It seems that the low soluble nutrients were able to stimulate nutrient feedback mechanisms in the small intestine, in contrast to the high soluble nutrients. As with solids, passage of the additional low soluble nutrients depends on the gradual trituration process in the stomach[37] which might also have caused the observed increase in MRT.

In the small intestine no effects of S on the MRT of solids and liquids were observed. The dietary treatments with low, medium, or high S were designed to provide equal amounts of digestible nutrients. Exchange of ingredients from the low S to the high S diet, resulted in a slightly lower intake of NSP in pigs fed the (HF-LS vs. HF-MS and HF-HS). Differences in intake of NSP was not corrected by adding fibres, as (purified) fibres can affect physicochemical properties of digesta and subsequently affect gastric emptying rate[39]. As current dietary treatments were not designed to evoke effects on physicochemical properties of digesta, these properties were analysed for confirmation. The results confirmed that dietary treatment caused no differences between the physicochemical properties of digesta.

Regarding the digestibility of protein and starch in the small intestine, no treatment effects were observed, except in the proximal half of the small intestine. In the proximal half of the small intestine, using Cr-EDTA as marker, the apparent protein digestibility was lower for pigs fed low F compared to pigs fed high F (LF-LS vs. HF-LS). Negative digestibility values observed in particular GIT segments are likely related to endogenous protein secretions and/or discrepancies between the passage rates of nutrients and trace markers. The discrepancy in apparent protein digestibility values when using either TiO_2 or Cr-EDTA as marker, likely result from shifts of nutrients, and possibly of markers, between the solid and liquid digesta fractions during transit through the GIT[30]. However, as digesta transits along the GIT nutrients are hydrolysed and absorbed, and digesta becomes more homogenous. Therefore, differences between passage rates of solids and liquids become smaller, and artefacts in calculations of nutrient digestibility reduce.

In conclusion, the MRT of solids was greater than that of liquids in the stomach, and stomach + small intestine. Dietary nutrient solubility affected the stomach MRT of solids and liquids in a non-linear manner. When S increased the stomach MRT of solids and liquids decreased, but only at the highest level of S. Feed intake level increased stomach MRT of solids and liquids, only when F increased with additional low soluble nutrients. Furthermore, F decreased the MRT of solids and, to some extent, of liquids in the distal small intestine. Hence, dietary nutrient solubility and feed intake level affect the passage rate of digesta. These study results can be used to better predict the metabolic fate of nutrients taking into account the kinetics of nutrient passage and thereby the kinetics of nutrient absorption.

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CONFLICTS OF INTEREST

None

AUTHORSHIP

M.S., A.J.M., W.J.J.G. designed research; M.S. conducted research and handled data; M.S., A.J.M., S.D.V., W.J.J.G. interpreted data and wrote paper. All authors read and approved the final manuscript.

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d	0-7	8-13	14	15	16	17	18
Feed intake level	2.5 × Me_m	According to dietary treatment (1.9 or 2.8 × Me_m)					
Meals per d	2				6		3-6
Marker intake		TiO_2	TiO_2+Cr-EDTA				

Table 1 Ingredient composition of the basal low soluble, and high soluble diets used to compose the experimental diets

Ingredients, g/kg as-is	Low soluble	High soluble
Wheat	365·5	0·0
Maize	310·0	0·0
Soybean meal	140·0	0·0
Rapeseed meal	100·0	0·0
Sugar beet pulp	15·0	0·0
Soybean oil	18·9	41·0
Agglomerated whey*	0·0	238·3
Sucrose	0·0	660·0
Premix†	5·0	5·0
Monocalcium phosphate	10·0	18·0
Limestone	14·0	14·5
Sodium-bicarbonate	5·6	13·3
NaCl	4·0	4·0
L-Lysine	4·3	0·0
DL-Methionine	0·7	0·0
L-Threonine	0·8	0·0
L-Tryptophan	0·3	0·0
TiO_2	4·0	4·0
Cr-EDTA	1·9	1·9

* Volactive UltraWhey 90 instant = agglomerated, instantised whey protein isolate 90%, Volac International Ltd, Orwell, Cambridgeshire, UK.

† Composition of premix, /kg diet: 2·4 mg Vit. A, 40 µg Vit. D_3, 30 mg Vit. E, 1·5 mg Vit. K_3, 1·0 mg Vit. B_1, 4·0 mg Vit. B_2, 1·5 mg Vit. B_6, 20 µg Vit. B_{12}, 20 mg niacin, 12 mg D-pantothenic acid, 150 mg choline chloride, 0·2 mg folic acid, 100 mg Fe (as $FeSO_4.H_2O$), 20 mg Cu (as $CuSO_4.5H_2O$), 30 mg Mn (as MnO), 70 mg Zn (as $ZnSO_4.H_2O$), 0·68 mg I (as KI), 0·20 mg Se (as Na_2SeO_3). Carrier: maize meal.

Table 2 Experimental design: intake of basal diets and resulting intake of nutrients of pigs fed diets with a low (LS), medium (MS), or high (HS) nutrient solubility, and low (LF) or high feed intake (HF)*

	Experimental treatments			
	LF-LS	HF-LS	HF-MS	HF-HS
Diet intake (g DM/kg $BW^{0.75}$ per d)				
Basal low soluble diet	51	76	64	51
Basal high soluble diet	0	0	10	20
Nutrient intake (g/kg $BW^{0.75}$ per d) †				
Dry matter	51	76	74	71
Crude protein	9·3	14	14	13
Soluble protein‡	1·6	2·4	3·7	5·1
Starch	23	35	30	24
Reducing sugars	2·5	3·7	10	17
Glucose-equivalents§	28	43	43	43
NSP‖	10	16	13	11
Insoluble NSP‖	1	2	2	1
ME¶, MJ/kg $BW^{0.75}$/d	0·78	1·2	1·2	1·1

LF-LS, low feed intake – low nutrient solubility; HF-LS, high feed intake – low nutrient solubility; HF-MS, high feed intake – medium nutrient solubility; HF-HS, high feed intake – high nutrient solubility; BW, body weight; ME, metabolisable energy.

* Feed intake level at 1·9 (LF) or 2·8 (HF) × ME requirement for maintenance (419 kJ ME/ kg $BW^{0.75}$)[18].

† Unless stated otherwise.

‡ Protein solubility in phosphate buffer A[40], 0·1 M at pH 3·5 and 39°C.

§ Glucose-equivalents: (starch/0·9) + reducing sugars

‖ NSP as calculated[41] from calculated diet composition: organic matter – crude protein – crude fat – starch – gluco-oligosaccharides – 0·9 × sugar. Insoluble NSP calculated based on water insoluble cell wall content from calculated diet composition[42].

¶ Metabolisable energy[43] (MJ) = (20·0 × digestible crude protein + 39·1 × digestible ether extract + 17·5 × starch + 16·6 × sugars + 17·2 × digestible NSP)/1,000.

Table 3 Mean retention time (h) of digesta solids (TiO_2) and liquids (Cr-EDTA) in consecutive segments of the gastrointestinal tract of pigs subjected to dietary treatments varying in feed intake level (F) and nutrient solubility (S)*

		Experimental treatments†					P-value‡	
Segment	Marker	LF-LS	HF-LS	HF-MS	HF-HS	SD_{pooled}	Treatment	LF-LS vs. HF-LS
Stomach	TiO_2	2·5^{a}	3·3^{ab}	4·1^{b}	2·9^{a}	0·83	0·001	0·041
	Cr-EDTA	0·6^{a}	1·3^{bc}	1·5^{c}	0·8^{ab}	0·43	<0·001‖	<0·001
	Difference§	***	***	***	***			
Proximal SI	TiO_2	0·4	0·3	0·3	0·4	0·16	0·382	0·719
	Cr-EDTA	0·3	0·3	0·2	0·3	0·14	0·355	0·355
	Difference§	**						
Distal SI	TiO_2	2·1^{b}	1·7^{a}	1·6^{a}	1·7^{a}	0·32	0·003	0·006
	Cr-EDTA	2·5	2·3	2·0	2·2	0·43	0·155	0·371
	Difference§	***	***	***	***			
Stomach + SI	TiO_2	5·0	5·1	6·0	5·0	0·92	0·071	0·748
	Cr-EDTA	3·4	4·0	3·9	3·4	0·70	0·105	0·068
	Difference§	***	**	**	***			

LF-LS, low feed intake – low nutrient solubility; HF-LS, high feed intake – low nutrient solubility; HF-MS, high feed intake – medium nutrient solubility; HF-HS, high feed intake – high nutrient solubility; SD_{pooled}, pooled standard deviation; Proximal SI, proximal half small intestine; Distal SI, distal half small intestine.

[a,b,c] Means within a row without a common superscript differ (P<0·05).

[*] Feed intake level at 1·9 (LF) or 2·8 (HF) × ME requirement for maintenance (419 kJ ME/kg $BW^{0·75}$)[(18)]. Dietary nutrient solubility levels were 8 % (LF-LS and HF-LS), 19 % (HF-MS), and 31 % (HF-HS) regarding the amount of soluble protein and sucrose in the diet.

[†] Number of pigs per treatment: HF-LS=9; LF-LS, HF-MS, and HF-HS=10.

[‡] Model established P-values for fixed effects of treatment (overall dietary treatments), and the contrast between low or high feed intake level (LF-LS vs. HF-LS).

[§] Significant difference (*: P<0·05; **: P<0·001; ***: P<0·0001) between MRT of the solid and liquid phase of digesta per treatment within segment.

[‖] Significant treatment×batch effect (P=0·025) for solid phase MRT.

Table 4 Apparent digestibility of starch and nitrogen (%) in the proximal and distal half of the small intestine (SI), based on TiO_2 and Cr-EDTA as indigestible markers in pigs subjected to dietary treatments varying in feed intake level (F) and nutrient solubility (S)*, including the overall effects of dietary treatment (LF-LS vs. HF-LS vs. HF-HS vs. HF-MS) and feed intake level (LF-LS vs. HF-LS).

			Experimental treatments†					P-value‡	
Nutrient	Segment	Marker	LF-LS	HF-LS	HF-MS	HF-HS	SD_{pooled}	Treatment	LF-LS vs. HF-LS
Starch§	Proximal SI	TiO_2	73	72	69	63	15·0	0·484	0·889
	Distal SI	TiO_2	94	95	94	91	2·8	0·093	0·707
Protein	Proximal SI	TiO_2	27	31	9	35	21·7	0·068	0·659
		Cr-EDTA	-6	25	1	16	25·4	0·051	0·013
		Difference‖	**			*			
	Distal SI	TiO_2	69	67	60	64	7·9	0·085	0·555
		Cr-EDTA	74	74	71	73	5·5	0·532	0·808
		Difference‖	***	***	***	***			

LF-LS, low feed intake – low nutrient solubility; HF-LS, high feed intake – low nutrient solubility; HF-MS, high feed intake – medium nutrient solubility; HF-HS, high feed intake – high nutrient solubility; SD_{pooled}, pooled standard deviation; SI, small intestine.

[a,b,c] Means within a row without a common superscript differ ($P<0·05$).

* Feed intake level at 1·9 (LF) or 2·8 (HF) × ME requirement for maintenance (419 kJ ME/kg $BW^{0·75}$)[18]. Dietary nutrient solubility levels were 8 % (LF-LS and HF-LS), 19 % (HF-MS), and 31 % (HF-HS) regarding the amount of soluble protein and sucrose in the diet.

† Number of pigs per treatment: HF-LS=9; LF-LS, HF-MS, and HF-HS=10.

‡ Model established P-values for fixed effects of treatment (overall dietary treatments), and P-values representing the contrast between low or high feed intake level (LF-LS vs. HF-LS).

§ Significant batch effect in SI1 and SI2 (P=0·038 and P=0·003): starch digestibility of pigs in batch 1 smaller than pigs in batch 2.

‖ Significant difference (*: P<0·05; **: P<0·001; ***: P<0·0001) between protein digestibility based on TiO_2 and Cr-EDTA per treatment within segment.

Table 5 Mean and standard deviation (SD) of hydration and dynamic viscosity properties of digesta per GIT segment.

Physicochemical property	Unit	Segment	n*	Mean	SD
Hydration					
Water-binding capacity	g water/ g DM	Stomach	27	1·9	0·76
		Proximal SI	ND†	ND†	ND†
		Distal SI	36	3·8	1·30
		Caecum	7	5·7	0·86
		Proximal C	39	3·8	1·10
		Distal C	30	3·9	1·10
Viscosity‡					
Apparent viscosity at 45/s shear rate (visco45)	Pa×s	Stomach	39	3·1	1·92
		Proximal SI	36	2·7	4·05
		Distal SI	39	8·4	6·79
		Caecum	36	2·2	2·63
		Proximal C	39	2·5	1·22
		Distal C	39	3·3	1·98
Power-law index (n)		Stomach	39	0·38	0·417
		Proximal SI	36	0·32	0·167
		Distal SI	39	0·20	0·066
		Caecum	36	0·21	0·136
		Proximal C	39	0·23	0·080
		Distal C	39	0·29	0·111
Consistency constant (K)	Pa×s	Stomach	39	45	33·5
		Proximal SI	36	54	83·9
		Distal SI	39	177	140·9
		Caecum	36	35	27·0
		Proximal C	39	49	34·2
		Distal C	39	52	33·0

WBC, water-binding capacity; Proximal SI, proximal half small intestine; ND, not determined; Distal SI, distal half small intestine; Proximal C, proximal half colon; Distal C, distal half colon.

* n= number of pigs

† Not determined, due to insufficient observations (n=1).

‡ Viscosity parameters derived by using a power-law function[33]: $\eta = K\dot{\gamma}^{n-1}$, where η = viscosity in Pa×s, K = consistency constant, $\dot{\gamma}$ = shear rate (/s) and n = power-law index.

		Model estimates				SE				P-Value
Variable	Segment	LF-LS	HF-LS	HF-MS	HF-HS	LF-LS	HF-LS	HF-MS	HF-HS	Treatment
Hydration										
number of observations	Stomach	4	9	9	5					
water-binding capacity	Proximal SI	0	0	1	0					
	Distal SI	9	9	9	9					
	Caecum	2	2	3	0					
	Proximal C	10	9	10	10					
	Distal C	8	7	8	7					
Water-binding capacity in g/g DM	Stomach	1.9	1.9	2.1	1.3	0.37	0.25	0.25	0.33	0.335
	Proximal SI	.	.	0.5	.	.	.	.	.	.
	Distal SI	4.2	4.1	3.5	3.5	0.45	0.45	0.45	0.45	0.587
	Caecum	6.1	5.2	5.8	.	0.67	0.67	0.55	.	0.649
	Proximal C	3.8	3.9	3.6	3.9	0.37	0.39	0.37	0.37	0.948
	Distal C	4.0	3.7	4.1	3.9	0.42	0.45	0.42	0.45	0.930
Viscosity										
number of observations	Stomach	10	9	10	10					
viscosity parameters	Proximal SI	8	8	10	10					
	Distal SI	10	9	10	10					
	Caecum	8	9	9	10					
	Proximal C	10	9	10	10					
	Distal C	10	9	10	10					
Apparent viscosity at 45/s shear rate (visco45) in Pa×s	Stomach	3.0	3.0	3.3	3.0	0.68	0.73	0.68	0.68	0.988
	Proximal SI	4.2	2.4	1.4	2.7	1.32	1.30	1.12	1.12	0.470
	Distal SI	8.8	11.6	4.5	8.9	1.96	2.10	1.96	1.96	0.117
	Caecum	1.4	1.4	1.1	1.9	0.30	0.27	0.21	0.34	0.240
	Proximal C	2.5	2.6	2.2	2.5	0.38	0.41	0.38	0.38	0.856
	Distal C	3.0	3.2	2.9	3.7	0.61	0.65	0.61	0.61	0.801
Consistency constant (K) in Pa×s	Stomach	38.8	43.6	33.1	59.9	10.35	11.07	10.35	10.35	0.315
	Proximal SI	89.7	44.0	28.7	51.9	26.48	26.02	22.51	22.51	0.386
	Distal SI	164.9	245.2	106.0	190.4	40.12	42.89	40.12	40.12	0.146
	Caecum	29.4	24.4	21.8	35.1	6.41	4.83	4.32	6.50	0.331
	Proximal C	41.2	44.8	35.6	43.1	5.66	6.58	4.88	5.92	0.669
	Distal C	41.8	51.6	51.8	59.9	8.98	9.60	8.98	8.98	0.572
Power-law index (n)	Stomach	0.39	0.24	0.37	0.19	0.105	0.0690	0.101	0.0541	0.215
	Proximal SI	0.25	0.28	0.35	0.30	0.0478	0.0522	0.0569	0.0485	0.591
	Distal SI	0.22	0.20	0.17	0.21	0.0208	0.0222	0.0208	0.0208	0.296
	Caecum	0.18	0.24	0.22	0.22	0.0451	0.0410	0.0411	0.0384	0.837
	Proximal C	0.22	0.23	0.25	0.24	0.0273	0.0291	0.0273	0.0273	0.912
	Distal C	0.31	0.28	0.27	0.30	0.0366	0.0391	0.0366	0.0366	0.899