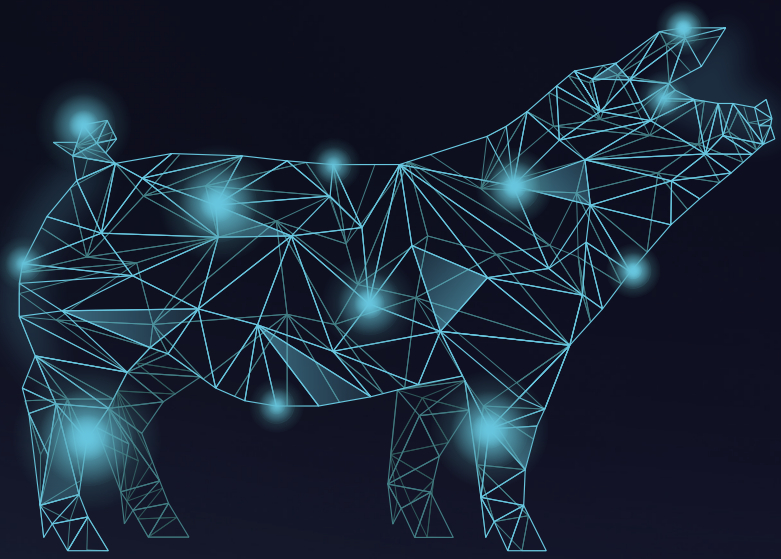
Modelling digestion kinetics in pigs

Predicting nutrient absorption based on diet and ingredient properties

Marijke Schop



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Thesis

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To the stars in my sky:

I lost you in life, but found you back in my heart.

Willeam Schop
(1983-2016)

PROPOSITIONS

1. Digesta viscosity is a consequence of, rather than a determinant for, digesta passage in the stomach.
   (this thesis)

2. *In vitro* kinetics of protein hydrolysis are not predictive for *in vivo* kinetics of amino acid absorption.
   (this thesis)

3. While time for peer review becomes scarce, its importance increases.

4. Quick and dirty methods are undervalued by researchers who take themselves too seriously.

5. People and planet are overburdened because profit is expressed in monetary-units.

6. Suffering of pet animals is avoided when welfare-assessments are a prerequisite for veterinary treatments.

Propositions belonging to the thesis, entitled

Modelling digestion kinetics in pigs
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Marijke Schop

Wageningen, 29 January 2020

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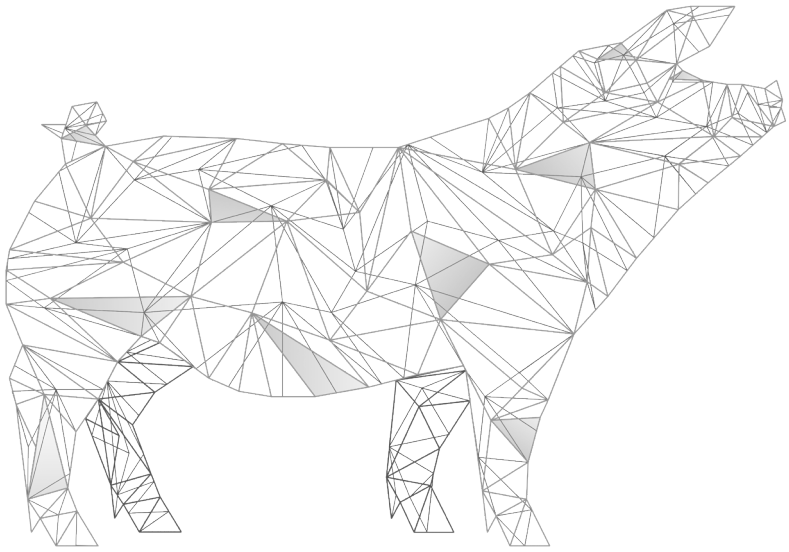
Chapter 1

General introduction



BACKGROUND

The current world population is expected to increase from 7.7 billion people in 2019 to 9.7 billion in 2050 [1]. Together with increasing wealth, the demand and competition for resources for food, feed, and fuel production increases [2]. In pig production, one of the strategies to cope with this competition is to increase the efficiency of production, i.e. by increasing the amount of output per unit of input. In pig nutrition, production efficiency is effectuated by formulating diets that meet the nutrient requirement for maintenance and production (i.e. growth, reproduction) of pigs. The amount of nutrients available to the pig are based on nutrient concentrations of constituting feed ingredients in the diet, and the ability of pigs to digest and absorb these nutrients from their gastrointestinal tract. Data on nutrient concentrations and nutrient digestibility per feed ingredient is provided by feed evaluation systems in the form of fixed tabulated data (e.g. CVB [3]; INRA [4]; NRC [5]). Feed formulation systems, hereby, use static nutrient digestibilities for feed ingredients. Nutrient digestibility, however, is not static as it is a result of digestive processes, and known to vary as a result of interactions among diet constituting feed ingredients. Current feed evaluation systems are indispensable to accurately predict the digestible nutrient supply to pigs, thereby optimising the production efficiency in pig production. Feed evaluation, however, can be improved by considering the effects of digestion kinetics, and interactions among feed ingredient and diets, on the nutritional value of feed ingredients fed to pigs.

DIGESTION KINETICS

When feed is ingested by the animal, it will pass through the gastrointestinal tract and simultaneously be hydrolysed by endogenous and microbial enzymes, eventually resulting in absorption or excretion of nutrients from the gastrointestinal tract. The location of hydrolysis and absorption of ingested nutrients affects the type of nutrients that becomes available to the animal for post-absorptive metabolism. Nutrients that are slowly hydrolysed or quickly pass along the gastrointestinal tract can reach the colon. In the colon, instead of being enzymatically hydrolysed, nutrients can be subjected to fermentation. Compared to enzymatic hydrolysis, fermentation yields nutrients of different nutritional value (e.g. amino-acids and glucose *v.* short-chain fatty acids) and my affect gut health [6]. Also, the availability of nutrients for post-absorptive metabolism is affected by the kinetics of nutrient digestion and can affect the metabolic use of absorbed nutrients. In pigs and humans, for example, post-absorptive protein metabolism and more specifically amino acid oxidation, protein deposition, and thereby net protein balance was affected when diets contained fast *v.* slow protein-sources [7; 8; 9; 10; 11], but protein metabolism was also affected when proteins were fed separately from carbohydrates [12]. In the latter case,

protein gain was 10% lower in pigs fed proteins and carbohydrates separately, compared to pigs fed proteins and carbohydrates combined. Hence, the nutritional value of feed ingredients is affected by the kinetics of digestion and absorption of nutrients from the gastrointestinal tract.

Interaction with animal and dietary factors

Nutrient digestion is the aggregated dynamic process comprising digesta passage, nutrient hydrolysis, endogenous secretions, and absorption. These processes do not occur independent of each other and can be affected by animal and dietary factors. Le Goff *et al.* [13], for example, showed that the passage of digesta in the gastrointestinal tract was affected by the age/BW of pigs (animal factor) and the source of dietary fibre fed (dietary factor). They observed that the mean retention time of digesta in the total gastrointestinal tract increased with age (growing pigs: 33 h, finishing pigs: 37 h, sows: 81 h). And, when the pigs were fed sugar beet pulp instead of maize bran or wheat bran, the total tract mean retention time increased by ~2 to 14 h. As a result of changes in the kinetics of digesta passage and nutrient hydrolysis, fibre digestibility was always higher for sows than for growing or finishing pigs. The digestibility of nutrients is a result of the underlying processes of digestion, and can be affected by animal and dietary factors, as well as, interactions among constituting feed ingredients in the diet. Owusu-Asiedu *et al.* [14], for example, showed that ileal digestibility of protein and energy decreased when maize starch was substituted for cellulose in the diet (protein: 72 *v.* 55 %; energy: 73 *v.* 51 %). This effect was stated to be caused by affecting passage, hydrolysis, and/or endogenous secretions of nutrients. The former shows that the digestibility of nutrients and subsequent nutritional value of feed ingredients depend on various underlying digestive processes which in turn are affected by interactions among animal and dietary factors (Figure 1.1).

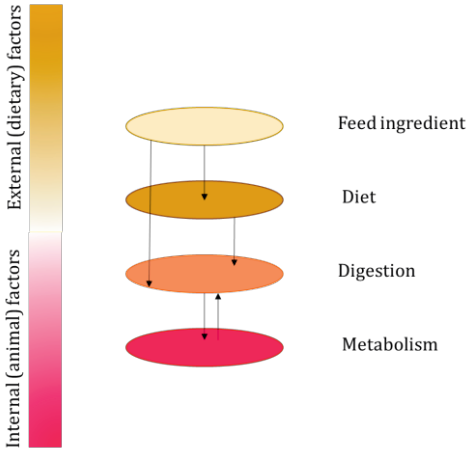


Figure 1.1. Schematic representation of the levels of interactions between feed ingredients, the diet, digestion, and metabolism.

Digesta passage and nutrient hydrolysis

It is assumed that digesta passage and nutrient hydrolysis, especially in the stomach and small intestine, are of influence on the nutritional value of feed ingredients. As the small intestine is the main site of nutrient absorption from the gut. Digesta passage and nutrient hydrolysis kinetics can be influenced by physicochemical properties of feed ingredients, the diet, and/or digesta as reviewed by Lentle and Janssen [15]; Lentle and Janssen [16]. Effects of dietary factors on the digestive process such as: particle size [17; 18; 19], viscosity [20; 21; 22], water-binding capacity [23], feed intake level/ bulkiness [24; 25; 26], either or not induced by dietary fibres [14; 23; 27; 28; 29], have been studied widely. However, this knowledge is currently not integrated to estimate the nutritional value of diets and feed ingredients in feed evaluation systems. Moreover, focussing on nutrient hydrolysis, variation exists among nutrients originating from different feed ingredients. For example, for protein, whey protein and casein [9] are classified as fast and slow digestible sources, while for starch these are wheat and pea starch [30]. Variation in the kinetics of nutrient hydrolysis can be caused by physicochemical properties of feed ingredients, such as the chemical structure of the nutrients themselves or those of the overall nutrient matrix [31; 32]. Hence, various dietary factors can affect the kinetics of digesta passage and nutrient hydrolysis, and subsequent quantitative nutrient digestibility.

Quantifying the kinetics of nutrient digestion

Since the relevance of the kinetics of nutrient digestion has been established, researchers have developed various methods to study it. The type of research conducted can be divided in three categories: *in vivo*, *in vitro*, and *in silico*. *In vivo* studies are conducted to directly assess nutrient digestion as occurring in pigs. Studies to assess the extent of nutrient digestibility in the gastrointestinal tract are routinely performed by collecting digesta or faeces from mainly intact pigs, but also from cannulated, or sacrificed pigs. In this case, pigs are generally fed indigestible markers to quantify nutrient digestibility, but these markers can also be used to study the kinetics of digesta passage[33]. The overall kinetics of digestion can be assessed by studying the appearance of nutrients in blood following a meal. At best, nutrient appearance is quantified in the portal vein of pigs, as it is the first location where absorbed nutrients become available after digestion for post-absorptive metabolism. These studies, however, are costly, invasive and subjected to the public debate regarding research on animals. Therefore, focus increased on development of *in vitro* and *in silico* models to study the kinetics of nutrient digestion in pigs. *In vitro* models are laboratory-based assays that simulate nutrient hydrolysis in the gastrointestinal tract, for example by mimicking the stomach, small intestine, and large intestinal environment. For an overview of *in vitro* assays see Wang and Zijlstra [34]. *In vitro* assays form excellent tools to

study the sole effect of feed ingredients on nutrient hydrolysis without interference of animal factors. These, together with their practical use and relative low experimental costs, are clear advantages of *in vitro* assays. One of the disadvantages, however, is that *in vitro* assays generally do not represent the overall kinetics of nutrient digestion, as passage and absorption are not taken into account. One of the exceptions is the TNO intestinal model (i.e. TIM) [35], in which digesta passage, nutrient hydrolysis, and absorption are combined. Results from *in vitro* assays are used as proxy for the kinetics of the overall digestive process occurring *in vivo*. For example, a good correlation can be found between the amount of *in vivo* absorbed glucose within 2 h after the meal, as indicated by the glycaemic index, and the *in vitro* rapidly degradable starch fraction[36]. This approach, however, does not provide information on the kinetics of glucose absorption during or after that 2 hour period. In order to simulate the complete process of digestion and its kinetics, *in silico* models can be developed. Provided sufficient knowledge and data is available, these models can simultaneously represent the underlying digestive processes of digesta passage, nutrient hydrolysis, endogenous secretions and absorption, as well as, interactions between these digestive processes and the ingested feed ingredients. Once validated, these models can run "experiments" using a fraction of time, budget, and utilities compared to *in vivo* and *in vitro* experiments. While *in silico* models can be used to simulate the kinetics of nutrient digestion, they can also be used to gain insight in the relative impact of underlying digestive processes, and hence identify knowledge gaps.

DEVELOPING A NUTRIENT-BASED DYNAMIC MECHANISTIC DIGESTION MODEL

Various approaches are available that need to be considered for the development of *in silico* models, see France and Kebreab [37]. Firstly, models can be empirical or mechanistic. Empirical models directly relate inputs and outputs in a mathematical manner, i.e. without considering underlying biological, physiological, or chemical mechanisms. In other words, empirical models represent a system as a 'black-box'. The accuracy of these models can be high, but predictions are generally considered to be poor outside the range of conditions under which their relationships are established. Mechanistic models, on the other hand, take into account the underlying mechanisms of a system, thereby aiming to increase the predictive behaviour of the model and understanding of the system as a whole. It has to be noted, however, that also mechanistic models rely on empirical relationships, as at lower levels of aggregation these relationships may be of empirical nature. Secondly, models can be static or dynamic. Static models do not represent changes occurring with time, whereas dynamic models, predict changes of a system with time. Thirdly, models can be deterministic or stochastic. Deterministic models present one output given a specific set of inputs, whereas stochastic models include a representation of variation on

model parameters, and can therefore also predict variation in addition to the output mean. The latter requires knowledge on the error-terms of variables or relationships. In order to increase our understanding of the complex processes and kinetics of digestion, a dynamic, mechanistic digestion model can be developed.

Current state of in silico digestion models

Feed evaluation systems such as CVB [3]; INRA [4]; NRC [5] are examples of empirical models considering their prediction of dietary net energy availability. These systems are based on nutrient digestibilities for each feed ingredient measured *in vivo*, as quantified at either the end of the small intestine (for amino acids) or total tract (for other nutrients). In turn, net energy available to the animal for growth, is estimated by assuming the maintenance energy and partial efficiencies for converting digested protein, fat, starch + sugars, and fermented fibre to energy gain. These partial efficiencies are based on empirical relationships and are derived from *in vivo* data [38]. Others have developed mechanistic models, that simulate digesta passage and/or the digestion of nutrients throughout (segments of) the digestive tract in pigs and humans [39; 40; 41; 42; 43; 44; 45; 46]. Models developed by Moxon *et al.* [40] and Taghipoor *et al.* [42] focussed on representing passage of digesta, as influenced by physicochemical properties of digesta in the stomach or small intestine. Models presented by Usry *et al.* [43], Rivest *et al.* [45], Bastianelli *et al.* [44], and Strathe *et al.* [46] simulate complete gastrointestinal tract digestion. Usry *et al.* [43] is one of the first representing a complete digestion model, they put much focus on the passage of digesta rather than hydrolysis of nutrients. Thereafter, Bastianelli *et al.* [44] presented their model with a less mechanistic approach for digesta passage compared to the model of Usry *et al.* [43], but with more focus on the hydrolysis and absorption of nutrients. The model presented by Rivest *et al.* [45] uses a similar approach for digesta passage as Usry *et al.* [43], but focussed more on protein digestion, i.e. hydrolysis, secretions and absorption. Finally, Strathe *et al.* [46] presented their model, showing much similarities to that of Bastianelli *et al.* [44]. They, however, also represented endogenous nitrogen secretions, and included the effects of fibre on digesta passage, nutrient hydrolysis and endogenous secretions in the distal part of the intestinal tract.

KNOWLEDGE GAPS

Current digestion models can be used simulate the kinetics of nutrient digestion in different gastrointestinal segments. However, these models focussed on the digestion of complete diets, leaving out variation in digestion kinetics as caused by diets varying in feed ingredients and physicochemical properties [43; 44; 45; 46]. These models can't be used to study the impact of physicochemical properties of feed ingredients, the diet, and digesta, on the kinetics of nutrient

digestion and absorption. To develop a model that can take the latter into account, focus must be put on variation in the kinetics of digesta passage and nutrient hydrolysis. Regarding the kinetics of digesta passage, there is some knowledge in pigs. Most studies, however, have assessed digesta passage at ileal (i.e. covering both stomach and small intestine) [14; 21; 47; 48] or total tract level [49; 50; 51]. Although useful, from these studies it is not able to derive the passage in individual segments of the gastrointestinal tract, focussing on stomach and small intestine separately [29]. In addition, most studies focussed on the passage of complete digesta or on the solid fraction by using insoluble markers. It has been established, however, that passage behaviour of solids and liquids differ in especially the stomach [52]. The latter might influence the absorption kinetics of soluble dietary nutrients. On top of it all, quantitative relations between diet or digesta physicochemical properties and the passage of digesta have only been studied to a limited extent [20; 24; 29; 53]. Hence, there is a lack of data on the passage of solids and liquids in different segments of the gastrointestinal tract in pigs, and on the relation between passage of these fractions and physicochemical properties of the diet and digesta. Regarding the kinetics of nutrient hydrolysis, it is known that the hydrolysis kinetics of protein and starch differ between feed ingredients [30; 32; 54; 55]. While for starch it has been studied more extensively, for protein only a limited set of feed ingredients have been studied.

THESIS OBJECTIVE AND OUTLINE

The objective of the research described in this thesis is to gain insight in the kinetics of nutrient digestion and absorption in the gastrointestinal tract of pigs, taking into account the effects of physicochemical properties of feed ingredients, diet, and digesta. To this end, we aimed to develop a nutrient-based dynamic mechanistic digestion model which can simulate the kinetics of nutrient digestion in pigs that are fed diets varying in feed ingredients and physicochemical properties. To parameterise the model, the passage of digesta solid and liquid fractions through the stomach and small intestine, and the physicochemical properties of digesta in growing pigs are studied (**Chapter 2 and 3**). In **Chapter 2**, the effects of increasing levels of diet viscosity, induced by oat β-glucans, is studied. Diet viscosity has been chosen as dietary treatment, as viscosity is often regarded a major influencer of the kinetics of digestion partly through its effect on digesta passage [15; 16; 18; 20; 21; 22; 47; 56]. Dose-response relationships, however, have not been studied and hence available information is biased towards highly viscous diets. In addition, differential effects of diet viscosity on digesta passage in different segments of the gastrointestinal tract are not available. In **Chapter 3**, the effects of dietary nutrient solubility in the diet (further mentioned as diet solubility), and feed intake level are studied. Effects of diet solubility are studied, as variation exists in the solubility of proteins [57; 58] and of glucose sources

(glucose-polymers in starch *v.* free glucose), and solids and liquids may leave the stomach at different rates. These differences in nutrient solubility are expected to be key in explaining variation in absorption kinetics of nutrient from different feed ingredients. Furthermore, the effect of feed intake level is studied, as individual variation exist between the feed intake of ad libitum-fed growing-finishing pigs [59]. In **Chapter 4,** the hydrolysis kinetics of protein in 19 feed ingredients is studied using an *in vitro* assay. Results from former studies and from literature are used to develop and evaluate the *in silico* dynamic mechanistic digestion model ('SNAPIG' - **S**imulating **N**utrient digestion and **A**bsorption kinetics in **PIG**s) and is presented in **Chapter 5**. The model simulates nutrient digestion kinetics as affect by diet and ingredient properties. Finally, **Chapter 6** contains the general discussion on the research presented in this thesis.

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Chapter 2

Increased diet viscosity by oat β-glucans decreases the passage rate of liquids in the stomach and affects digesta physicochemical properties in growing pigs

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ABSTRACT

Rheological properties of digesta play a role in digesta passage kinetics through the gastrointestinal tract, in turn affecting nutrient absorption kinetics. Therefore, we studied the effects of diet viscosity on digesta passage and physicochemical properties in pigs. Twenty male growing pigs (35 kg body weight at the start) were assigned to one of five diets with increasing dietary concentrations of β-glucans (BG; from 0% to 10 %), in exchange for maize starch. After a 17-day adaptation period, pigs were euthanised and the mean retention time (MRT) of digesta solids (TiO_2) and liquids (Cr-EDTA) in the stomach, and proximal and distal half of the small intestine was quantified. In the stomach, the MRT of liquids, but not of solids, increased when dietary BG level increased (6 min per % dietary BG, P = 0.008 and R^2 = 0.35). Concomitantly, stomach DM content (5 g/kg per % dietary BG, P < 0.001 and R^2 = 0.53) and apparent digesta viscosity (56 Pa × s at 1/s shear rate per % dietary BG, P = 0.003 and R^2 = 0.41) decreased. In the proximal half of the small intestine, no effects of dietary BG level were observed. In the distal half of the small intestine, water-binding capacity (WBC) of digesta increased (0.11 g/g digesta DM per % dietary BG, P = 0.028 and R^2 = 0.24) and starch digestibility decreased (0.3% per % dietary BG, P = 0.034 and R^2 = 0.23) when dietary BG level increased. In the colon, apparent digesta viscosity at 45/s shear rate increased (0.1 Pa × s per % dietary BG, P = 0.03 and R^2 = 0.24) in the proximal half of the colon, and digesta WBC increased (0.06 g/g digesta DM per % dietary BG, P = 0.024 and R^2 = 0.26) in the distal half of the colon when dietary BG level increased. To conclude, increasing dietary BG level caused the MRT of liquids, but not that of solids, to increase in the stomach, resulting in reduced separation of the solid and liquid digesta fractions. This caused dilution of the stomach content and reduction in digesta viscosity when dietary BG levels increased. Effects of dietary BG level on physicochemical properties in the proximal small intestine were absent and may have been due to a low DM content. The WBC of digesta in the distal small intestine and colon increased when dietary BG level increased, as did apparent digesta viscosity in the proximal colon. This likely reflects the concentration of BG in digesta when moving through the gastrointestinal tract.

Keywords: digesta mean retention time, gastrointestinal tract, solids, rheology, digestion kinetics

Implications

This study quantifies the relation between diet viscosity, induced by dietary β-glucans, digesta apparent viscosity and passage kinetics of liquid and solid digesta fractions in the gastrointestinal tract. The difference between passage of digesta solids and liquids decreased with increasing diet

viscosity. These results can be used to improve predictions of nutrient absorption kinetics, by using, for example, mechanistic digestion simulation models. Increased understanding of kinetics of the digestive process and absorption of nutrients will facilitate optimising diet formulation strategies to increase efficient metabolic use of nutrients, by taking into account variation in digestion kinetics among feed ingredients and diets.

INTRODUCTION

Currently, the nutritional value of feed ingredients for pigs is based on ileal or total tract nutrient disappearance. Feeding tables, containing (standardised) ileal digestibility values for amino acids per feed ingredient (e.g. CVB [1]; INRA [2]; NRC [3]), are of great importance to formulate diets that meet the pigs' requirement for essential amino acids. However, it was shown that the metabolic fate of absorbed nutrients can be influenced by differences in portal appearance kinetics between nutrients [4; 5]. Portal appearance kinetics of glucose and amino acids depend on the kinetics of feed intake, digesta passage and nutrient hydrolysis and absorption. As the small intestine is the major site of nutrient absorption, digesta passage in proximal segments of the gastrointestinal tract (GIT), especially the stomach, dominates portal nutrient appearance. In turn, dietary fibres can influence digesta passage kinetics [6; 7; 8], depending on, among others, their capacity to affect digesta viscosity [9; 10]. The latter can be dependent on dietary fibre concentration [6], fibre physical and chemical properties [11; 12] and location in the GIT [11; 13]. Hence, the current study aimed to evaluate the relation between diet viscosity, digesta passage and digesta physicochemical properties in various locations of the GIT in growing pigs. We hypothesised that an increase in diet viscosity would increase digesta viscosity in the stomach and small intestine, thereby increasing the mean retention time (MRT) of digesta in these segments.

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). Animals and housing Twenty male growing pigs (Hypor × Maxter; Hendrix Genetics, Boxmeer, the Netherlands) with an average initial BW of 34.6 ± 1.4 kg were used. 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°C ± 1°C, and facilities were lit from 0600 to 1800 h. Feeding schedule, sample collection and chemical analysis were executed as previously described by Schop *et al.* [14].

Diets and feeding

Pigs were assigned to one of five experimental dietary treatments. Dietary treatments consisted of five incremental levels of dietary β-glucans (BG): 0%, 2.5%, 5%, 7.5% and 10%, referred to as BG0, BG2.5, BG5, BG7.5 and BG10 (Table 2.1). The diets were obtained by mixing different ratios of the BG0 and BG10 diet. These two diets were formulated by exchanging maize starch in the BG0 diet, for a BG extract (PromOat, Tate & Lyle PLC, London, UK) in the BG10 diet, while maintaining equal levels of digestible nutrients and energy (Table 2.2). Diets were formulated to meet or exceed nutrient requirements for growing pigs according to CVB [1]. The feeds were produced as a mash. Soybean meal, maize and wheat were hammer-milled using a 4-mm sieve, and rapeseed meal and sugar beet pulp using a 2.75-mm sieve. Three days prior to the experiment, the pigs were gradually switched from the commercial diet to the experimental diets. The experiment lasted for 18 days. Pigs were fed the experimental diets at a daily feeding level of three times their metabolisable energy requirement for maintenance (419 kJ /kg $BW^{0.75}$; [15]). The pigs were fed twice daily at 0800 and 1600 h until day 15, followed by frequent feeding from day 16 onwards to induce steady-state passage of digesta in the GIT. During the frequent feeding period, daily feed allowance was divided in six equal portions. On days 16 and 17 pigs received portions once every 3 h from 0530 until 2030 h. On day 18 pigs received portions once every 2 h from 0230 h until 2 h prior to euthanasia, with a minimum of three portions fed on this day. Feeding time on day 18 was scheduled according to the pre-planned time of euthanasia of each pig, starting at 0830 h.

Table 2.1 Dietary treatments consisting of five incremental levels of β-glucans (0, 2.5, 5. 7.5 and 10%) resulting from mixing of the control (BG0) and 10% β-glucans (BG10) diets, including apparent dynamic viscosity properties1 of the five diets, fed to growing pigs.

Dietary treatments[2]	BG0	BG10	K (SD) Pa×s	n (SD)	Visco45 (SD) Pa×s
BG0	100	0	39 (4.6)	-0.50 (0.517)	0.38 (4.4)
BG2.5	75	25	30 (9.8)	0.50 (0.0215)	4.4 (1.11)
BG5	50	50	117 (16.8)	0.29 (0.0437)	7.8 (0.26)
BG7.5	25	75	315 (46.5)	-0.13 (0.0170)	4.3 (0.36)
BG10	0	100	581 (97.6)	-0.27 (0.175)	5.1 (2.90)

[1] Derived from dynamic viscosity by using a power-law function: $\eta = K\dot{\gamma}^{n-1}$, where η = viscosity (Pa×s), $\dot{\gamma}$= shear rate (/s), n = power law index, K = consistency constant (Pa×s), and visco45= apparent viscosity at $\dot{\gamma}$ = 45/s (Pa×s).

[2] Number of observations was two per diet, except for BG5 and BG10 where the number of observations where three.

Table 2.2 Ingredient and chemical composition of the control (BG0) and 10% β-glucans (BG10) diet fed to growing pigs.

Ingredients, g/kg	BG0	BG10
Maize starch (Native)	232.3	0.0
PromOat Beta Glucan[1]	0.0	299.2
Sucrose	17.0	0.0
Oat hulls	48.7	0.0
Soy oil	20.8	10.4
Wheat gluten meal	18.1	9.1
Water	0.0	18.3
Wheat	200.0	
Soybean meal	139.9	
Maize	104.8	
Wheat middlings	100.0	
Rapeseed meal	80.0	
$CaCO_3$	11.3	
Monocalcium phosphate	7.0	
Premix[2]	5.0	
L-Lysine	3.5	
NaCl	2.5	
$Na(CO_3)_2$	1.3	
L-Threonine	0.9	
DL-Methionine	0.8	
L-Tryptophan	0.2	
TiO_2	4.0	
Cr-EDTA	1.9	
Analysed chemical composition (g/kg as-is) [3]		
DM	887	887
Crude ash	57	63
Crude protein	162	164
Crude fat	38	41
Starch	404	303
Reducing sugars	54	65
NSP[4]	173	254
ME[5], MJ/kg as-is	13.3	13.3

[1] PromOat Beta Glucan, Tate & Lyle PLC, London, United Kingdom. β-glucan content 35%. Analysed content, g/kg of product: 45 dry matter, 22 ash, 42 crude protein, 46 crude fat, 326 starch, 63 reducing sugars.

[2] Premix composition, /kg diet: 8 000 IU Vit. A, 1 600 IU Vit. D_3, 30 mg Vit. E, 1.5 mg Vit. K_3, 1.0 mg Vit. B_1, 4.0 mg Vit. B2, 1.5 mg Vit. B6, 20 µg Vit. B_{12}, 20 mg niacin, 12 mg D-pantothenic acid, 150 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.

[3] Chemical composition presented as g/kg as-is, unless stated otherwise.

[4] Non-starch polysaccharides as calculated from calculated diet composition: organic matter – CP – crude fat – starch – gluco-oligosaccharides – 0.9 × sugar (CVB, 2012).

[5] Metabolizable energy (MJ) = (20.0 × digestible CP + 39.1 × digestible ether extract + 17.5 × starch + 16.6 × sugars + 17.2 × digestible NSP)/1 000 [(18)].

The diets contained TiO_2 (4.0 g/kg diet) as the indigestible insoluble marker [16] from day 8 onwards, and Cr-EDTA (1.9 g/kg diet) as the indigestible soluble marker [17] from day 11 onwards. Diets were fed as mash and mixed with water (1:2.5, wt: wt) in the feed trough. In addition, pigs received 0.5 l of water per day, 0.25 l in the morning and 0.25 l in the afternoon. During frequent feeding, pigs did not receive additional water. Pigs were weighed twice weekly to adjust the feed allowance to the pigs' BW.

Sample collection and chemical analysis

At day 18 the pigs (48.9 ± 2.3 kg BW) were euthanised for quantitative digesta collection from the stomach, proximal and distal half of the small intestine based on length (further mentioned as proximal or distal small intestine, respectively), caecum, and proximal and distal half of the colon based on length (further mentioned as proximal or distal colon, respectively). After digesta collection, digesta samples were cooled and stored at 4°C pending analyses for dynamic viscosity (analysed within 96 h) and water-binding capacity (WBC; analysed within 24 h), while remaining digesta were stored at −80°C and freeze-dried before analyses for chemical content (DM [19], CP (N × 6.25, [20]), starch [21], reducing sugars [22], titanium [23] and chromium ([24], after sample preparation by Williams *et al.* [25]). Water-binding capacity of digesta was measured using centrifugal force. Fresh digesta samples were centrifuged at 4000 × *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 there were 12 missing observations: 9 in the proximal small intestine, 2 in caecum, 1 in the proximal colon. Dynamic viscosity of solutions can be quantified by measuring the force (i.e. stress) needed to make a sample flow at (various) rates. Considering the non-Newtonian, shear-thinning, behaviour of digesta and effects of particles on digesta flow behaviour [26], the apparent dynamic viscosity of digesta and diets was measured by applying a continuous shear rate sweep. Dynamic viscosity of digesta was measured within 96 h after digesta collection by an 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 after a 30 s pre-shear at 10/s. Due to variation in digesta consistency among GIT segments, different geometries were used. Stomach and small intestinal digesta samples were measured in a titanium concentric cylinder (i.e. cup) system (CC17-SN2540, Anton Paar GmbH, Graz, Austria). Caecal 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. The latter geometry was also used to measure dynamic diet viscosity of as-fed diet samples (diet to water ratio 1 : 2.5,

wt : wt). Measurements were carried out as for digesta samples, with the exception that temperature was 24°C.

Calculations and statistics

Calculations and statistical analyses were performed in SAS version 9.3 (SAS Institute Inc., Cary, NC, US). The retention time of digesta, being inversely related to the fractional passage rate, was studied in the stomach, and proximal and distal small intestine. The retention time was calculated (equation 1) and further defined as the MRT of digesta in each segment. Based on the assumption that in a steady state, pool sizes of indigestible marker in each segment reflect the MRT of digesta in that segment [27]:

$$\text{MRT (min)} = \frac{Marker\ pool\ size\ in\ digesta\ (g)}{Marker\ intake\ (\frac{g}{h})} \times 60 \qquad \text{[Eq. 1]}$$

where marker is either Ti (as TiO_2) or Cr (as Cr-EDTA), marker pool sizes in digesta were calculated for each GIT segment 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 diet marker concentration (g/kg DM) with hourly feed intake (g DM) during bi-hourly feeding. Apparent digestibility of starch and protein in the stomach, proximal and distal small intestine was calculated (equation 2) according to Kotb and Luckey [28]:

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

where $[Nutrient]_{digesta}$, $[Nutrient]_{diet}$, $[Marker]_{digesta}$, $[Marker]_{diet}$ are concentrations (g/kg DM) of nutrient (CP or starch) and marker (Ti) in the digesta or diet samples. Dynamic digesta viscosity is described to have non-Newtonian shear-thinning flow behaviour. Therefore, the non-Newtonian flow behaviour was fitted using a power-law model (equation 3; [26]):

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

where η = apparent shear 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). In addition, apparent viscosity at 45/s (Newtonian region) was calculated from the power-law model and reported. The effects of dietary BG level on digesta MRT, nutrient digestibility and digesta physicochemical properties were analysed per GIT segment using regression analysis (PROC REG) and dietary BG concentration as regressor. Pig was considered as the experimental unit. In addition, regression analysis was performed on dynamic diet viscosity parameters and dietary BG level (regressor).

Linear and quadratic regressions were performed. Model residuals were tested for normality using the Shapiro–Wilk Test, and visually evaluated to confirm heteroscedasticity. Results are presented as intercept, slope, pooled SEM, model established P-values and R^2 representing the goodness of fit. A Pearson's correlation matrix (PROC CORR) was established for digesta physicochemical properties per GIT segment, whereby observations of the proximal and distal halves of the intestines were combined for the small intestine and colon, respectively. Differences were considered significant at $P < 0.05$ and a trend at $P < 0.1$.

RESULTS

All pigs remained clinically healthy during the study. All meals' were finished within 15 min by the pigs. The results for the stomach segment of one pig were considered as outlier (MRT: 6.2 h, exceeded the overall mean + 2 × SD and was marked as outlier using Cook's D) and were excluded from further statistical analyses. An overview of mean and SD of all analysed parameters (i.e. MRT, nutrient digestibility, physicochemical properties) per dietary treatment is provided as supplementary tables (Supplementary Tables S2.1, S2.2 and S2.3, respectively). Dietary BG level appeared positively correlated with consistency constant *K* (36.9 – 20.8 × dietary BG (%) + 7.6 × dietary BG^2 (%), P quadratic term = 0.002, R^2 = 0.99, RMSE = 7.8) and apparent viscosity at 45/s shear rate (2.5 + 0.38 × dietary BG (%), P = 0.015 and R^2 = 0.31) of the diet (data not presented).

Mean retention time

On average (mean ± SD), over all dietary treatments, the MRT of solids and liquids was 122 (±38) and 69 (±34) min (stomach), 21(±9) and 21(±10) min (proximal small intestine) and 89(±25) and 100(±26) min (distal small intestine). Stomach MRT of liquids significantly increased when dietary BG level increased (6 min per % dietary BG, P = 0.008 and R^2 = 0.35; Table 2.3), thereby reducing the difference between stomach MRT of solids and liquids (6 min per % dietary BG, $P < 0.0001$ and R^2 = 0.63). No effects on the MRT of solids and liquids were observed in the proximal and distal small intestine.

Table 2.3 The effect of dietary β-glucan level (BG)[1] on the mean retention time (min) of digesta solids (TiO_2) and liquids (Cr-EDTA) in the stomach and small intestine of growing pigs estimated using linear regression[2].

Segment	Variable	Intercept (min)[2]	SE[2]	Slope (min per % diet BG)[2]	SE[2]	*P*[3]	R-square
Stomach	Solids	126	16.6	-1	2.6	0.796	0.00
	Liquids	39	11.8	6	1.9	0.008	0.35
	Difference	87	7.4	-6	1.2	<.0001	0.63
Proximal half small intestine[4]	Solids	21	3.8	0	0.6	0.928	0.00
	Liquids	21	4.1	0	0.7	0.989	0.00
	Difference	-0.18	2.1	-0.1	0.3	0.847	0.00
Distal half small intestine[4]	Solids	97	9.8	-2	1.6	0.335	0.05
	Liquids	111	9.9	-2	1.6	0.182	0.10
	Difference	-13	2.8	1	0.5	0.167	0.10
Stomach + small intestine	Solids	250	16.2	-3	2.6	0.255	0.08
	Liquids	177	12.8	3	2.0	0.204	0.09
	Difference[5]	73	8.3	-6	1.3	0.0004	0.53

[1] Dietary BG level ranged from 0 to 10% in five equidistant steps (i.e. 0, 2.5, 5, 7.5 and 10 % dietary BG level).

[2] Intercepts and slopes were estimated using linear regression: variable = intercept + slope x BG (% of diet), where the intercept represents estimated value of the dependent variable at 0% BG, and the slope represents the unit of change in the dependent variable per % of BG in the diet. SE = standard error of the estimated intercept and slope, respectively.

[3] P-value for H_0: slope=0.

[4] Division based on total length of small intestine.

[5] Quadratic relation: 99 – 15 × dietary BG level (%) + 0.8 × dietary BG level (%)[2] (P quadratic term= 0.034; R2=0.69; RMSE=16)

Digestibility

On average (mean ± SD), over all dietary treatments, apparent digestibility of starch and protein was 96% (±2%) and 56% (±13%) in the distal small intestine, respectively. Starch digestibility in the stomach and the distal small intestine decreased with increasing level of dietary BG (6% and 0.3% per % dietary BG, P = 0.006 and P = 0.034, R^2 = 0.36 and R^2 = 0.23, respectively; Table 2.4). Apparent protein digestibility in the stomach decreased when BG level increased (3% per % diet BG, P = 0.017 and R^2 = 0.29).

Table 2.4 The effect of diet β-glucan level (BG)[1] on the apparent digestibility of starch, and protein (%) in the stomach and small intestine of growing pigs estimated using linear regression[2].

Segment	Variable	Intercept (%)[2]	SE[2]	Slope (% per % diet BG)[2]	SE[2]	*P*[3]	R-square
Stomach	Starch	4	12.6	-6	2	0.006	0.36
	Protein	10	6.3	-3	1	0.017	0.29
Proximal half small intestine[4]	Starch	89	5.6	-0.4	0.9	0.638	0.01
	Protein	11	10.9	0.9	2	0.636	0.01
Distal half small intestine[4]	Starch	97	0.7	-0.3	0.1	0.034	0.23
	Protein	58	5.1	-0.5	0.8	0.580	0.02

[1] Dietary BG level ranged from 0 to 10% in five equidistant steps (i.e. 0, 2.5, 5, 7.5 and 10 % dietary BG level).
[2] Intercepts and slopes were estimated using linear regression: variable = intercept + slope x BG (% of diet), where the intercept represents estimated value of the dependent variable at 0% BG, and the slope represents the unit of change in the dependent variable per % of BG in the diet. SE = standard error of the estimated intercept and slope, respectively.
[3] P-value for H_0: slope=0.
[4] Division based on total length of small intestine.

Physicochemical properties

Dietary BG level affected specific digesta physicochemical properties in all GIT segments except for the proximal small intestine (Table 2.5). When dietary BG level increased, stomach digesta *K* (56 Pa × s per % diet BG, P = 0.003 and R^2 = 0.56), visco45 (2 Pa × s per % diet BG, P = 0.003 and R^2 = 0.38) and DM content (5 g/kg per % diet BG, P = 0.0004 and R^2 = 0.53) decreased, whereas *n* increased (0.02 per % diet BG, P < 0.0001 and R^2 = 0.61). Digesta WBC increased when dietary BG level increased in both the distal small intestine (0.1 g/g DM per % diet BG, P = 0.028 and R^2 = 0.24) and distal colon (0.06 g/g DM per % diet BG, P = 0.024 and R^2 = 0.26). In the proximal colon, visco45 increased when dietary BG level increased (0.1 Pa × s per % diet BG, P = 0.03 and

Table 2.5 Linear effect[1] of diet β-glucan level (BG)[2] on digesta viscosity[3] (K, n, visco45), dry matter content (DM), and water-binding capacity (WBC) of the digesta per segment of the gastrointestinal tract in growing pigs.

Variable	Unit	Intercept (unit)[1]	SE[1]	Slope (unit change per % diet BG)[1]	SE[1]	*P*[4]	R-square
Stomach							
K	Pa×s	512	102	-56	16	0.003	0.41
n		0.08	0.03	0.02	0.004	0.000	0.67
visco45	Pa×s	19	4	-2	0.7	0.008	0.35
DM	g/kg	251	7	-5	1	0.000	0.53
WBC	g/g DM	1.1	0.1	-0.01	0.02	0.818	0.00
Proximal small intestine[5]							
K	Pa×s	37	13	-2	2	0.450	0.03
n		0.3	0.08	-0.003	0.01	0.795	0.00
visco45	Pa×s	1.4	0.5	-0.01	0.09	0.902	0.00
DM	g/kg	135	9	-1	2	0.593	0.02
WBC	g/g DM	2.0	0.9	-0.02	0.2	0.893	0.00
Distal small intestine[5]							
K	Pa×s	123	40	-6	6	0.328	0.05
n		0.2	0.02	0.01	0.004	0.127	0.12
visco45	Pa×s	5.2	1.7	-0.2	0.3	0.426	0.04
DM	g/kg	115	8	1	1	0.448	0.03
WBC	g/g DM	1.9	0.3	0.1	0.05	0.028	0.24
Caecum							
K	Pa×s	28	6	0.4	1.0	0.683	0.01
n		0.2	0.03	0.01	0.005	0.061	0.18
visco45	Pa×s	1.0	0.3	0.1	0.04	0.090	0.15
DM	g/kg	119	9	1	1	0.519	0.02
WBC	g/g DM	3.3	0.3	-0.02	0.05	0.723	0.01
Proximal colon[5]							
K	Pa×s	35	5	2	0.9	0.056	0.19
n		0.2	0.03	0.002	0.004	0.668	0.01
visco45	Pa×s	1.8	0.3	0.1	0.05	0.030	0.24
DM	g/kg	193	12	-1	2	0.458	0.03
WBC	g/g DM	2.8	0.3	0.004	0.04	0.933	0.00
Distal colon[5]							
K	Pa×s	34	16	4	3	0.148	0.11
n		0.3	0.06	-0.002	0.01	0.858	0.00
visco45	Pa×s	2.3	1.0	0.3	0.2	0.111	0.14
DM	g/g	252	9	-3	2	0.108	0.14
WBC	g/g DM	2.7	0.1	0.06	0.02	0.024	0.26

[1] Intercepts and slopes were estimated using linear regression: variable = intercept + slope x dietary BG level (% of diet), where the intercept represents estimated value of the dependent variable at 0% BG, and

the slope represents the unit of change in the dependent variable per % of BG in the diet. SE = standard error of the estimated intercept and slope, respectively. Significant quadratic model (variable = intercept + slope x (dietary BG level x dietary BG level; % of diet) fits were observed for: stomach, n= 673– 169 × dietary BG level + 11 × dietary BG level[2] (P quadratic term= 0.04; R^2=0.55; RMSE=219); stomach, n= 0.13 – 0.015 × dietary BG level + 0.0038 × dietary BG level[2] (P quadratic term= 0.0016; R^2=0.83; RMSE=0.045); stomach, visco45= 26.1 – 6.89 × dietary BG level + 0.47 × dietary BG level[2] (P quadratic term= 0.026; R^2=0.52; RMSE=8.66).

[2] Dietary BG level ranged from 0 to 10% in five equidistant steps (i.e. 0, 2.5, 5, 7.5 and 10 % dietary BG level).

[3] Derived from dynamic viscosity by using a power-law function: $\eta = K\dot{\gamma}^{n-1}$, where η = viscosity (Pa×s), $\dot{\gamma}$= shear rate (/s), n = power law index, K = consistency constant (Pa×s), and visco45= apparent viscosity at $\dot{\gamma}$ = 45/s (Pa×s).

[4] P-value for H_0: slope=0.

[5] Small intestine and colon were, divided in proximal and distal halves based on length.

R^2 = 0.24). Digesta DM content tended to be positively correlated with digesta *K* in the stomach (R = 0.42, P = 0.07; Table 2.6) and small intestine (R = 0.31, P = 0.055), while significantly positive in the caecum (R = 0.77, P < 0.0001). In addition, digesta DM content was negatively correlated with digesta *n* in the stomach (R = −0.66, P = 0.002), but positively with digesta *n* in the colon (R = 0.44, P = 0.005). Digesta *K* tended to negatively correlate with digesta *n* in the stomach (R = −0.42, P = 0.07) and colon (R = −0.27, P = 0.09), and positively with digesta WBC in the colon (R = 0.29, P = 0.07). Finally, digesta WBC and *n* correlated negatively in the small intestine (R = −0.31, P = 0.09).

DISCUSSION

This study aimed to quantify the relation between diet viscosity, passage kinetics and physicochemical properties of digesta in segments along the GIT. Diet viscosity was induced by the inclusion of isolated oat BG in the diet, ranging from 0% (i.e. BG0) to 10% (i.e. BG10). When mixed with water prior to feeding, the BG0 diet formed an easily pourable suspension from which the solids directly sank to the bottom of the trough if left unstirred, whereas the BG10 diet formed a non-pourable dense dough-like mass. Diet viscosity parameters confirmed that apparent viscosity at 1 and 45/s shear rate (respectively indicated by *K* and visco45) increased when dietary BG level increased. Although apparent diet viscosity increased when dietary BG level increased, apparent digesta viscosity in the stomach decreased. In addition, liquids remained longer in the stomach when dietary BG level increased (6 min per %BG in the diet). This together with potentially increasing gastric secretions due to meal viscosity [7; 10] resulted in the dilution of stomach digesta in pigs fed diets with increasing BG levels. Based on the high correlation between stomach digesta DM and *K* (this study), and the relation between dynamic viscosity and the volume fraction of particles in suspensions [29] we speculate that the dilution of the stomach

Table 2.6 Pearson's correlation matrix of the physicochemical properties of digesta[1] in consecutive gastrointestinal tract segments of growing pigs, considering digesta viscosity[2] (K, n), dry matter content (DM), and water-binding capacity (WBC).

Segment		K	n	DM	WBC
Stomach	K	1			
	n	-0.42†	1		
	DM	0.42†	-0.66***	1	
	WBC	0.23	-0.01	-0.15	1
Small intestine[3]	K	1			
	n	-0.23	1		
	DM	0.31†	-0.15	1	
	WBC	0.11	-0.31†	-0.15	1
Caecum	K	1			
	n	-0.25	1		
	DM	0.77***	0.01	1	
	WBC	-0.26	-0.04	-0.37	1
Colon[3]	K	1			
	n	-0.27†	1		
	DM	0.08	0.44**	1	
	WBC	0.29†	-0.17	-0.22	1

[1] number of observations per variable: 19 in stomach, 20 in caecum (except for WBC:18), 40 in small intestine and colon (except for WBC: 31 and 39 for small intestine and colon).

[2] Derived from dynamic viscosity by using a power-law function: $\eta = K\dot{\gamma}^{n-1}$, where η = viscosity (Pa×s), $\dot{\gamma}$ = shear rate (/s), n = power law index, K = consistency constant (Pa×s).

[3] Combined proximal and distal small intestine or colon segments.

† P-value < 0.1, ** P < 0.01, *** P < 0.001.

digesta explains the decrease in digesta viscosity in pigs fed diets with increasing BG levels. In addition to dilution, depolymerisation of BG in the proximal GIT [30] in high BG diets, and maize starch [31] and wheat gluten [32] in low BG diets might have altered their subsequent viscosity-inducing properties. While increasing dietary BG level caused MRT of liquids to increase, the MRT of solids was not affected, in agreement with amongst others Rainbird and Low [7]. This resulted in a dramatic decrease in the separation of solids and liquids in the stomach when dietary BG level increased. Apparent digestibility of protein and starch in the stomach decreased when dietary BG level increased. In the case of protein, gastric secretions due to diet viscosity [10] may have increased the contribution of endogenous nitrogen, thereby reducing apparent protein digestibility when dietary BG level increased. In the proximal half of the small intestine no effects of dietary BG level on protein or starch digestibility were observed, while in the distal half of the

small intestine, starch, but not protein, digestibility reduced when dietary BG level increased. As the reduction in starch digestibility was not accompanied by increased apparent digesta viscosity or increased protein digestibility, we consider it unlikely that this reduction in digestibility can be ascribed to viscosity-inducing properties of BG. Differences in dietary starch source (maize starch v. oat starch) and level in the BG0 and BG10 diets might have contributed to the reduction in starch digestibility. Towards the end of the small intestine, most (enzymatic) digestible nutrients are absorbed. This caused concentration of BG contents in digesta to increase, bringing forth increased WBC of digesta in the distal small intestine when dietary BG level increased. The lack of effect of dietary BG level on apparent digesta viscosity in the distal small intestine might be related to the low DM content of digesta in this segment, as described earlier. Despite BG degradation towards the colon [33; 34], concentration of BG in colon digesta likely caused apparent viscosity at 45/s (proximal colon) and WBC (distal colon) of digesta to increase when dietary BG level increased. In addition, other variations in digesta composition in the colon together with the presence and activity of the microbial biomass might have caused variation in observed physicochemical properties of digesta when dietary BG level increased. In conclusion, the current study showed that when dietary BG level increased, the MRT of liquids, but not that of solids, in the stomach increased. This resulted in a strong reduction in separation of digesta liquids and solids in the stomach, causing dilution of the stomach content. This was illustrated by the decrease in stomach DM content and in turn caused the apparent digesta viscosity to decrease when dietary BG level increased. Effects of dietary BG level on physicochemical properties of digesta in the small intestine were absent and may be related to the low DM content. The water-binding capacity of digesta in the distal small intestine and colon increased with dietary BG level, as did apparent viscosity in the proximal, but not in the distal, colon. These findings likely reflect the concentration of BG in digesta, increasing along the small intestine and decreasing upon their fermentation towards the colon.

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Declaration of interest

The authors declare that there are no conflicts of interest.

Ethics statement

The study was approved by the Dutch Animal Ethics Committee (2014.III.06.056).

Software and data repository resources

None of the data were deposited in an official repository.

Supplementary material

To view supplementary material for this article, please visit https://doi.org/10.1017/S1751731119001824

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Supplementary table S2.1 The mean retention time (min) of digesta solids (TiO_2) and liquids (Cr-EDTA) in the stomach and small intestine of growing pigs fed dietary treatments consisting of five incremental levels of β-glucans (BG: 0, 2.5, 5. 7.5 and 10%).

		Dietary β-glucan level (% of diet)									
		0		2.5		5		7.5		10	
Segment	Parameter	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Stomach	Obs[1]	3	3	4	4	4	4	4	4	4	4
	Solids	153	31	105	22	116	39	108	42	136	50
	Liquids	52	12	43	26	66	16	75	36	104	41
Proximal small intestine[2]	Obs[1]	4	4	4	4	4	4	4	4	4	4
	Solids	24	8	19	7	17	10	20	10	23	14
	Liquids	24	12	22	8	19	8	17	7	26	16
Distal small intestine[2]	Obs[1]	4	4	4	4	4	4	4	4	4	4
	Solids	81	5	111	37	104	17	69	20	82	15
	Liquids	91	6	128	38	112	18	79	17	88	11

[1] *number of observations.*

[2] *Division based on total length of small intestine.*

Supplementary table S2.2 The digestibility of starch and crude protein in the stomach and small intestine of growing pigs fed dietary treatments consisting of five incremental levels of β-glucans (BG: 0, 2.5, 5. 7.5 and 10%).

		Dietary β-glucan level (% of diet)									
		0		2.5		5		7.5		10	
Segment	Parameter	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Stomach	Obs[1]	3	3	4	4	4	4	4	4	4	4
	Solids	0.30	0.22	-0.34	0.25	-0.25	0.24	-0.63	0.24	-0.39	0.24
	Liquids	0.14	0.09	0.09	0.19	-0.10	0.11	-0.22	0.10	-0.04	0.11
Proximal small intestine[2]	Obs[1]	4	4	4	4	4	4	4	4	4	4
	Solids	0.85	0.11	0.91	0.07	0.88	0.05	0.90	0.11	0.79	0.29
	Liquids	0.14	0.28	0.12	0.18	0.09	0.22	0.26	0.40	0.18	0.37
Distal small intestine[2]	Obs[1]	4	4	4	4	4	4	4	4	4	4
	Solids	0.97	0.02	0.97	0.01	0.97	0.01	0.96	0.02	0.94	0.03
	Liquids	0.55	0.19	0.60	0.10	0.60	0.06	0.50	0.19	0.55	0.08

[1] *number of observations.*

[2] *Division based on total length of small intestine.*

Supplementary table S2.3 Physicochemical properties of digesta (viscosity: K, n, visco45[1], water-binding capacity: WBC) in various segments of the gastrointestinal tract of growing pigs fed dietary treatments consisting of five incremental levels of β-glucans (BG: 0, 2.5, 5. 7.5 and 10%).

		Dietary β-glucan level (% of diet)									
		0		2.5		5		7.5		10	
Segment	Parameter	MEAN	SD	MEAN	SD	MEAN	SD	MEAN	SD	MEAN	SD
Stomach	Obs[2]	3	3	4	4	4	4	4	4	4	4
	K	752	519	173	139	185	88	54	53	45	23
	n	0.12	0.04	0.14	0.03	0.14	0.03	0.23	0.08	0.37	0.04
	visco45	29.1	20.8	6.3	4.9	7.0	3.5	2.5	1.9	3.8	1.6
	WBC	1.2	0.2	1.0	0.5	1.2	0.5	1.0	0.2	1.1	0.2
Proximal small intestine[2]	Obs[2]	4	4	4	4	4	4	4	4	4	4
	K	47	62	24	21	24	26	19	24	29	21
	n	0.41	0.38	0.12	0.05	0.19	0.13	0.27	0.08	0.30	0.08
	visco45	1.9	2.2	0.9	0.8	1.3	1.5	1.0	1.0	1.8	1.2
	WBC[2]	1.8	1.2	1.6	0.8	2.7	2.4	1.3	.	1.0	.
Distal small intestine[2]	Obs[2]	4	4	4	4	4	4	4	4	4	4
	K	177	184	58	41	59	38	63	95	94	78
	n	0.16	0.03	0.20	0.03	0.20	0.04	0.27	0.09	0.20	0.07
	visco45	7.7	8.2	2.6	1.5	2.7	1.4	2.6	3.2	4.8	4.0
	WBC	2.1	0.4	2.2	0.3	2.0	0.3	3.2	1.4	2.9	0.4
Caecum	Obs[2]	4	4	4	4	4	4	4	4	4	4
	K	34	18	25	5	26	10	24	17	40	25
	n	0.13	0.06	0.19	0.06	0.23	0.05	0.20	0.11	0.25	0.11
	visco45	1.2	0.6	1.1	0.3	1.4	0.5	1.2	0.8	2.2	1.0
	WBC	3.4	0.6	3.0	0.5	3.7	1.2	3.0	0.5	3.1	1.0

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Proximal colon[3]	Obs[2]	4	4	4	4	4	4	4	4	4	4
	K	30	15	39	9	56	5	50	22	47	9
	n	0.22	0.04	0.26	0.04	0.18	0.07	0.21	0.08	0.27	0.07
	visco45	1.5	0.8	2.3	0.8	2.6	0.7	2.3	0.9	2.9	0.6
	WBC	2.9	0.5	2.5	0.6	3.0	0.5	2.9	0.7	2.7	1.2
Distal colon[3]	Obs[2]	4	4	4	4	4	4	4	4	4	4
	K	30	30	25	20	81	57	81	50	51	23
	n	0.33	0.26	0.36	0.16	0.23	0.13	0.33	0.13	0.32	0.09
	visco45	1.9	1.3	2.7	3.5	4.0	2.9	6.1	3.4	3.8	1.9

[1] Derived from dynamic viscosity by using a power-law function: $\eta = K\dot{\gamma}^{n-1}$, where η = viscosity (Pa×s), $\dot{\gamma}$ = shear rate (/s), n = power law index, K = consistency constant (Pa×s).

[2] number of observations, except for WBC in proximal small intestine (obs= 2 for BG0, 3 for BG2.5, 4 for BG5, 1 for BG7.5 and 1 for BG10).

[3] Division based on total length of small intestine, and colon, respectively.

Chapter 3

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

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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 *v.* 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 gastrointestinal 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 *v.* 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 before 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 gastrointestinal 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 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 *v.* liquids) and the influence of nutrient load on passage kinetics indicate 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 [6; 13], the effect was confounded with the effect of increasing total nutrient intake [12]. Although in humans and pigs the passage rate of solids and liquids in the stomach has been studied [6; 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 hypothesised 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.

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

A total of forty male growing pigs (Hypor × Maxter; Hendrix Genetics) with an average initial body weight (BW) of 32.0 (SD 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 lit from 06.00 to 18.00 hours.

Diets and feeding

In a randomised 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 and 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 ($\frac{Starch}{0.9}$ + reducing sugars), respectively. Whereby dietary nutrient solubility was considered as the proportion of nutrients that are soluble when brought in 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 3.1): 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 3.2). Soyabean 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. All pigs were gradually switched from a commercial diet to the experimental diets in 3 d before the experiment. The experiment lasted for 18 d (Fig. 3.1). Pigs were fed the experimental diets at a feeding level of 2.5 ME_m until day 7, 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 hours until day 15, 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 days 16 and 17, the pigs received

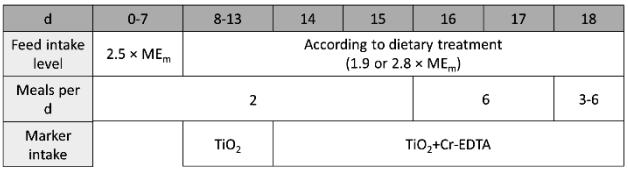

Figure 3.1. Timeline of the study

Table 3.1. 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. D3, 30 mg Vit. E, 1.5 mg Vit. K3, 1.0 mg Vit. B1, 4.0 mg Vit. B2, 1.5 mg Vit. B6, 20 µg Vit. B12, 20 mg niacin, 12 mg D-pantothenic acid, 150 mg choline chloride, 0.2 mg folic acid, 100 mg Fe (as FeSO4. H2O), 20 mg Cu (as CuSO4.5H2O), 30 mg Mn (as MnO), 70 mg Zn (as ZnSO4.H2O), 0.68 mg I (as KI), 0.20 mg Se (as Na2SeO3). Carrier: maize meal.

portions once every 3 h from 05.30 until 20.30 hours. On day 18, the pigs received portions once every 2 h from 02.30 hours until 2 h before euthanasia, with a minimum of three portions fed on this day. Feeding time on this day (day 18) was scheduled according to the scheduled time of euthanasia of each pig, starting at 08.30 hours with the first pig. The diets contained TiO_2 as the indigestible insoluble marker [22] from day 8 onwards, and Cr- EDTA [23] as the indigestible soluble marker from day 16 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 litre of water/d, 0.25 litre in the morning and 0.25 litre 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.

Table 3.2. 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 [25], 0.1 M at pH 3.5 and 39°C.*

[§] *Glucose-equivalents: (starch/0.9) + reducing sugars*

[‖] *NSP as calculated [24] 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 [26].*

[¶] *Metabolisable energy [27] (MJ) = (20.0 × digestible crude protein + 39.1 × digestible ether extract + 17.5 × starch + 16.6 × sugars + 17.2 × digestible NSP)/1,000.*

Sample collection and chemical analysis

At day 18, the pigs (45.2 (SD 3.2) kg BW) were euthanised for quantitative digesta collection from various segments of the GIT. Pigs were euthanised 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). The process from euthanasia until sample storage lasted 15 min/pig. Diets and digesta were analysed for contents of (DM [28], CP (N × 6.25, [29]), starch [30], reducing sugars [31], titanium [32] and chromium ([33], after sample preparation by Williams *et al.* [34]). 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 N (5 %), Ti (5 %) and Cr (10 %). Samples were reanalysed when values were outside the range of the mean value±2 × SD within treatment and GIT segment. WBC of digesta was measured using centrifugational force. Fresh digesta samples were centrifuged at 4000 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, twenty-five samples were analysed single, 120 in duplicate and for ninety-five samples insufficient materials were available. Dynamic viscosity of digesta was measured within 96 h after digesta collection by an MCR502 and MCR301 rheometre (Modular Compact Rheometer; Anton Paar GmbH). Measurements were carried out at 39°C with declining shear rates from 50/s to 1/s in twenty-five steps. Different geometries were used for digesta from the proximal and distal GIT segments due to the differences in digesta consistencies within these segments. Stomach and small intestinal samples were measured in a Ti concentric cylinder (i.e. cup) system (CC17- SN2540; Anton Paar GmbH). Caecum and colon digesta samples were measured on a Ti parallel profiled plate–plate measuring system (PP25/P2-SN25463, PP25/P2-SN25491; Anton Paar GmbH) with a 1.5mm gap width.

Calculations and statistics

Calculations and statistics were performed in Statistical Analysis Systems statistical software package version 9.3 (SAS Institute Inc.). The MRT 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 [35]).

$$\text{MRT (min)} = \frac{Marker\ pool\ size\ in\ digesta\ (g)}{Marker\ intake\ (\frac{g}{h})} \times 60 \quad (1)$$

where the marker is either Ti (as TiO_2) or Cr (as Cr-EDTA). Marker pool sizes in digesta of 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 the marker concentration of the diet (g/kg DM) by the meal intake at day 18 (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 [36]:

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

where $[Nutrient]_{digesta}$, $[Nutrient]_{diet}$, $[Marker]_{digesta}$, $[Marker]_{diet}$ 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 [37]. Therefore, the non-Newtonian flow behaviour was fitted using a power-law model [38] (Eq. (3)):

$$\eta = K\dot{\gamma}^{n-1} \quad (3)$$

where η = apparent shear 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. Studentised residuals were tested for normality using the Shapiro–Wilk test. Data distribution was visually evaluated to confirm heteroscedasticity. Non-normal distributed variables were transformed (i.e. logarithmic, exponential, reciprocal or 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 means and standard deviations of digesta physicochemical properties for WBC and viscosity were reported. Differences in digesta physicochemical properties between GIT segments were analysed using the previously mentioned general linear model including the fixed effect of GIT segment. Results are presented as back-transformed least square means, and pooled standard deviation (SDpooled), 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 a 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 seven consecutive days before the pigs' dissection.

Digesta passage

On average, the MRT of solids was longer than that of liquids in the stomach (3.2 *v.* 1.2h, P< 0.0001; Table 3.3) and in the stomach + small intestine (5.3 *v.* 3.7h, P< 0.0001) but shorter in the distal half of the small intestine (1.8 *v.* 2.3h, P< 0.0001). The HF-HS pigs had a shorter MRT of solids (2.9 *v.* 4.1h, P= 0.01) and liquids (0.8 *v.* 1.5h, 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 *v.* HF-MS *v.* 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 *v.* HF-LS), MRT in the stomach increased for both solids (2.5 *v.* 3.3h, P= 0.041) and liquids (0.6 *v.* 1.3h, P=0.0001). When F increased with additional intake of high-soluble nutrients (LF-LS *v.* 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 *v.* HF-LS: 2.1 *v.* 1.7h, P=0.006) as well as high-soluble nutrients (LF-LS *v.* HFHS: 2.1 *v.* 1.7h, 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 3.4). 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 *v.* HF-LS: –6 *v.* 25 %, P=0.013).

*Table 3.3. 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 +	TiO_2	5.0	5.1	6.0	5.0	0.92	0.071	0.748
SI	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 liquid phase MRT.

Table 3.4. 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.

** 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.*

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 (online Supplementary material). Therefore, results are presented as descriptive statistics (Table 3.5). 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, were on average higher in the distal half of the small intestine than in other GIT segments (visco 45: 8.4> 2.2–3.3Pa × 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 *v.* HF-MS *v.* HF-HS), (2) F (LF-LS *v.* 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.e. LF-LS *v.* 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) 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 BW per meal was shifted from insoluble to soluble nutrients, exceeding the nutrient load (approximately 33 kJ gross energy/kg metabolic BW per meal) that induced an effect on gastric emptying rate in previous studies in humans [6; 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 [6; 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

Table 3.5. Hydration and dynamic viscosity properties of digesta per GIT segment. (mean and standard deviations)

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* [38]*:* $\eta = K\dot{\gamma}^{n-1}$*, where* η *= viscosity in Pa×s,* K *= consistency constant,* $\dot{\gamma}$ *= shear rate (/s) and* n *= power-law index.*

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 (1230 *v.* 1967 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; 39]. 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; 12; 40]. Potentially the stimulus duration was too short, as high-soluble nutrients are generally absorbed rapidly after entering the small intestine [41; 42]. Unfortunately, the study design does not allow to speculate the dietary or animal factors that particularly caused the non-linear effect of S the passage kinetics of digesta.

The effect of F was dependent on S, as additional intake of high-soluble nutrients did not affect the digesta passage from the stomach, while additional intake of low-soluble nutrients caused the MRT of digesta in the stomach to increase. This is in agreement with the previous findings, where an increase in feed intake level caused stomach MRT to increase [12; 39]. 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, the passage of additional low-soluble nutrients depends on the gradual trituration process in the stomach [41] 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 *v.* 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 [43]. 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 *v.* 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 results from shifts of nutrients, and possibly of markers, between the solid and liquid digesta fractions during transit through the GIT [34]. However, as digestatransits 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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Supplementary material

For supplementary material/s referred to in this article, please visit https://doi.org/10.1017/S0007114518003756

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Supplementary Material

		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 water-binding capacity	Stomach	4	9	9	5					
	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 viscosity parameters	Stomach	10	9	10	10					
	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

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	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

Chapter 4

In vitro enzymatic protein hydrolysis kinetics of feed ingredients

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ABSTRACT

Kinetics of protein hydrolysis in the gastrointestinal tract is believed to influence the balance between enzymatic protein digestion in the small intestine and protein fermentation in the hindgut, and the metabolic fate of absorbed amino acids from the digestive tract. In order to parameterise a dynamic *in silico* digestion model, we quantified the kinetics of protein hydrolysis of 19 feed ingredients for pigs using an *in vitro* enzymatic method. We focussed on the appearance of soluble protein in a stomach simulation, and low molecular weight (MW) peptides and amino acids (<500 Da) in a small intestinal simulation. In the stomach phase, the fraction of protein which was instantly soluble (i.e. t=0 min) ranged from 8% (potato protein; wt/wt) to 100% (whey powder, whey protein isolate; wt/wt). The fractional rate of protein solubilisation in the stomach ranged from 0.031/h (fish meal) to 0.43/h (wheat). The potentially degradable protein fraction (%) was quantified as 100% minus the undegraded protein fraction (%) remaining in the residue at the end of the small intestinal simulation. The potentially degradable protein fraction ranged from 55% (soy hulls; wt/wt) to 100% (whey powder, whey protein isolate; wt/wt). At the onset of the small intestinal simulation, part of the potentially degradable protein fraction was already present as low MW peptides, ranging from 8% (oats; wt/wt) to 96% (extracted linseed; wt/wt). Estimates for the maximum extent of protein hydrolysed into low MW peptides in the small intestinal simulation ranged from 60% (soybean meal; wt/wt) to 123% (extracted linseed; wt/wt), for which the fractional hydrolysis rate ranged from 0.3/h (potato protein) to 15/h (whey powder). Our results showed that the instantly soluble protein fraction in the stomach simulation can vary substantial among feed ingredients and might influence *in vivo* the timing of protein appearance in the small intestine. In the small intestinal simulation, a substantial fraction of the potentially degradable protein fraction in feed ingredients appeared instantly as low MW peptides, which are assumed to be readily absorbed in the small intestine *in vivo*. Upon termination of the small intestinal simulations, however, for most ingredients the potentially degradable protein fraction was not completely present as low MW peptides, but rather as larger soluble proteins and peptides. The present data on variation in protein hydrolysis kinetics among feed ingredients are useful for the development of computer models that can simulate protein digestion kinetics of various feed ingredients in pigs.

INTRODUCTION

In pig feed formulation, the ileal protein digestibility value of feed ingredients is used as a proxy for the extent of protein that can be digested and absorbed as peptides and amino acids (AA) in the small intestine and become available for post-absorptive metabolism. The residual protein

fraction is assumed to escape enzymatic hydrolysis and can be fermented in the caecum and colon, yielding fermentation products that are of lesser metabolic use and even associate with impaired gut health [3]. Although the extent of enzymatic protein hydrolysis in the gut is important in relation to its nutritional value, the rate of protein hydrolysis and AA absorption is important as well. In pigs and humans, for example, feeding slow *v.* fast digestible protein sources, was shown to affect the metabolic use of AA for protein deposition and extent of amino acid oxidation [4; 5; 6; 7]. Hence, protein hydrolysis in the stomach and small intestine is considered of major importance for the rate of absorption of AA by the gut and their subsequent metabolic use. Mathematical models can be used to account for variation in the extent and rate (i.e. kinetics) of protein hydrolysis in future feed evaluation systems. Such models can simulate digestive processes considering ingestion, passage, hydrolysis, endogenous secretions, and absorption. Existing digestion models for growing pigs [8; 9], however, currently do not account for variation in protein hydrolysis kinetics [10; 11] among feed ingredients. It is known that protein hydrolysis kinetics are affected by the proteins' chemical and structural conformation [12], solubility [13], and their interactions with other nutrients [14] in feed ingredients or diets.

The kinetics of protein hydrolysis can be studied using *in vitro* hydrolysis methods which simulate stomach, small intestinal, and large intestinal digestion (see Wang and Zijlstra [15] for an overview). Such methods generally encompass the hydrolysis of substrate (e.g. feed ingredients) by enzymes (e.g. pepsin, trypsin, pancreatin and peptidases) in a buffered system (set pH) over a period of time. Depending on the aim of the study, the 'settings' for the substrate, enzymes, pH, and duration of the simulation can be adjusted to reflect the digestive process in the target animal species or to maximize hydrolysis of the substrate. Focussing on protein, the degraded protein fraction is generally quantified by difference considering the protein fraction that remains insolubilized at the end of a small intestinal simulation as undegraded protein, using filtration methods. Chen *et al.* [16], however, showed that the *in vitro* degradable protein fraction at the end of small intestinal simulation still consist for 20 (whey powder) to 62% (soybean meal) of high MW peptides (>500 Da), that *in vivo* cannot be absorbed prior to further hydrolysis. These results indicate that classifying the degradable protein fraction as absorbable amino acids/peptides without considering their degree of hydrolysis, may lead to considerable overestimation of the latter. We therefore propose an adapted *in vitro* method here based on the procedure of Boisen and Fernández [17]. Based on previous work by Chen *et al.* [16], we focussed on the solubilisation of protein in the stomach, as soluble proteins are emptied faster from the stomach than insoluble proteins [2] and become faster available for hydrolysis and absorption [5] in the small intestine after ingestion. For the small intestine, we focussed on the hydrolysis of protein into presumed absorbable peptides (reviewed by Silk *et al.* [18]) by the appearance of low MW peptides and free amino acids, further referred to as LMW-AA. The settings of the *in vitro* hydrolysis method were

chosen to reach maximum potential hydrolysis of protein in feed ingredients within a physiological relevant range for digestion considering the pig as target species. The adapted method was used to quantify protein hydrolysis kinetics in 19 feed ingredients commonly used in pig nutrition. The data on kinetics of protein hydrolysis of feed ingredients were meant to be used in a predictive mechanistic model for nutrient hydrolysis in pigs [1].

MATERIAL AND METHODS

Ingredients

To study the hydrolysis kinetics of protein, 19 feed ingredients were obtained from single batches of commercially available feed ingredients. The feed ingredients were selected based on their relevance for practical swine and poultry nutrition. The ingredients were ground to pass a 1 mm sieve (Retsch ZM 200, Haan, North Rhine-Westphalia, Germany) prior to their use in the *in vitro* protein hydrolysis assay.

In vitro protein hydrolysis

An adapted and up-scaled *in vitro* method based on Boisen and Fernández [17] was used to simulate stomach and small intestinal enzymatic hydrolysis (i.e. two steps). Adjustments were made to the stomach (pH 3.5 instead of 2.0) and small intestine (addition of amyloglucosidase) simulations in order to maximize enzymatic hydrolysis of protein and starch under physiologically relevant conditions for pigs. Aliquot samples of the incubation solutions were taken during the simulations, and total residue was collected at the end of the simulations. Each ingredient was incubated in duplicate per simulation, i.e. two duplicates for the stomach and two duplicates for the small intestinal simulations. Per simulation 5 ± 0.002 g of the ingredient was weighted into a 600 mL beaker with a magnetic rod (1 cm).

For the stomach simulation, disodium phosphate buffer (250 mL, 0.1 M, pH 6.0) and HCl (100 mL, 0.1 M) were added into the beakers. Successively, the pH was adjusted to 3.5 using HCl (1 M), and freshly prepared pepsin solution (10 mL, 0.025 mg/mL; 2000 FIP U/g, Merck, Darmstadt, Germany) was added to start the enzymatic hydrolysis. Beakers were placed into a water bath (39±1 °C) where the incubation solutions were gently stirred using a magnetic stirrer (210-240 rpm; Multipoint HP 15, Variomag). At t 5, 10, 20, 30, 60, 90, and 120 min after the start of the stomach simulation, a 15 mL aliquot sample of the incubation mixture was taken using a pipette. To ensure free entrance of particles, the pipette opening was increased by clipping off 2 mm of the tip. At the end of the simulation (i.e. t=120 min), the beaker was removed from the water bath for total residue collection after rinsing with water using a vacuum filtration unit with a nylon

filter (40 µm mesh). In addition, initial protein solubility (i.e. t=0 min) at the onset of the stomach simulations was quantified by separate single incubations per ingredient. For this aliquot mixture and residue samples were collected immediately after the pH was adjusted to 3.5 (i.e. before enzymatic hydrolysis by pepsin).

Small intestine simulations ran separately from the sampled stomach simulations. Hence, the small intestine simulations followed stomach simulations (120 min) without intermediate sampling and beakers were covered with aluminium foil to avoid evaporation of incubation solutions. Small intestine simulations were initiated by adding NaOH (50 mL, 0.6 M) and sodium phosphate buffer (100 mL, 0.2 M, pH 6.8) to the incubation mixture. Successively, the pH was adjusted to 6.8 using NaOH (10 M), and freshly prepared pancreatin solution (10 mL, 0.1 g/mL; porcine pancreas grade VI, Sigma-Aldrich, St-Louis, USA) and amyloglucosidase (27.5 mg; Aspergillus Niger, 120 U/g, Sigma-Aldrich, St-Louis, USA) were added to start the enzymatic hydrolysis. The beaker was placed back into the water-bath under constant gentle stirring. At t 5, 10, 20, 30, 60, 90, 120, 150, 180, and 240 min after the start of the small intestine simulation, 15 mL aliquot samples of the incubation mixture were taken using a pipette. At the end of the small intestine simulation (i.e. t=240 min), the beaker was removed from the water bath for total residue collection, after rinsing with water, using a vacuum filtration unit with a nylon filter (40 µm mesh). Finally, stomach and small intestine simulations were repeated in duplicate without substrate (i.e. blank simulations) and aliquot supernatant samples were analysed for the contribution of nitrogen originating from enzymes added during the stomach and small intestine simulations. All incubation solutions, except enzyme solutions, were preheated (39±1 °C) before addition. Aliquot samples of incubation mixtures were stored at -20 °C pending chemical analysis. Prior to chemical analysis, the aliquot samples of incubation mixtures were thawed to 4 °C, centrifuged (4000 g × 15 min), followed by supernatant collection using a pipette. The residue was air dried overnight at 24 °C and ground by hand using a mortar and pestle prior to chemical analysis.

Chemical analyses

Ingredients were analysed for contents of dry matter (DM [19]) and crude protein (CP: nitrogen [20] × 6.25). Aliquot supernatant samples from the small intestine simulations were analysed for concentrations of low molecular-weight peptides (<500 Da) [21] by nitrogen analysis [22] after precipitation (1:1 v/v) of proteins and high MW peptides (>500 Da) with sulfosalicylic acid solution (16% w/v). Per ingredient, the aliquot supernatant samples were alternately analysed per duplicate incubation and time point (i.e. aliquot ($t_{1\text{-}8}$)= $Aliquot_{t,y}$, $Aliquot_{t+1,y+1}$, $Aliquot_{t+2,y,}$ $Aliquot_{t+3,y+1}$..., $Aliquot_{t+n,y+1}$, where t=1,2, ..., 8 representing 5, 10, 20, 30, 60, 120, 180,

240 min; and y= 1 or 2 representing duplicate 1 or 2 per ingredient). Residues collected at the end of stomach and small intestine simulations were analysed for nitrogen [20].

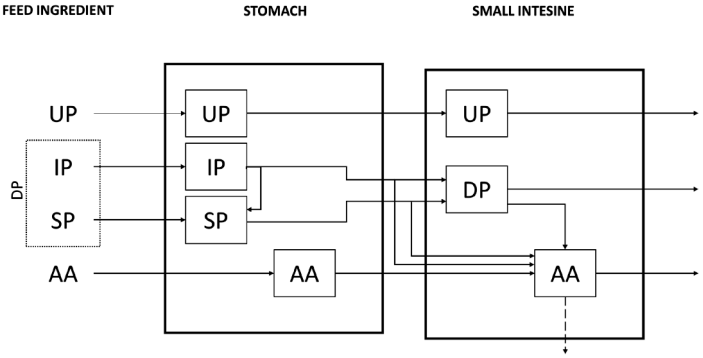

Figure 4.1. Conceptual framework of protein digestion in the stomach and small intestine of pigs, where the total amount of protein in the ingested feed ingredient is divided into an enzymatically undegradable (UP) and potentially degradable (DP) protein fraction, the latter encompassing a stomach insoluble (IP) and instantly soluble (SP) fraction. In the stomach, IP can be solubilised turning it into SP, the latter having a higher passage rate through the stomach [2]. Once entered the small intestine DP (i.e. IP+SP) hydrolysis yields absorbable amino acids, and di- and tripeptides (AA) which can be absorbed by the gut (dashed line) and be used in post-absorptive metabolism of the pig.

Calculations and statistical analysis

Calculations and statistics were performed in Statistical Analysis Systems statistical software package version 9·4 (SAS Institute Inc., Cary, NC, USA). A conceptual framework for the digestion of protein (Figure 4.1) was used to define protein hydrolysis parameters.

Protein solubility (SP) was quantified at the start of the stomach simulations (t=0 min; i.e. initial protein solubility), and at the end of the stomach (t=120 min) and small intestine (t=240 min) simulations:

$$\text{SP (\% of total protein)} = \frac{N_{substrate} - N_{residue}}{N_{substrate}} \times 100\% \qquad \text{eq. 1}$$

Where, $N_{substrate}$ = the amount of initial incubated nitrogen in the sample of the feed ingredient (g/mL); $N_{residue}$ = the amount of nitrogen left in the residue (g/mL) corrected for nitrogen removed by collection of aliquot samples during the simulations.

The SP fraction was expressed relative to the degradable protein fraction (DP) to quantify the fractional rate of solubilisation in the stomach (k_s) using first-order kinetics:

$$\frac{SP}{DP}(t) = \text{intercept} + 1 \times (1 - e^{-k_s \times t}) \quad \text{eq. 2}$$

$$DP = 1 - UP \quad \text{eq. 3}$$

$$UP = \frac{N_{residue}}{N_{substrate}} \quad \text{eq. 4}$$

where, intercept = the instantly soluble protein fraction (i.e. $\frac{SP}{DP}$ $(t = 0)$; g/g), k_s = the fractional solubilisation rate (/h), t = time in h, under the assumption that the potentially degradable protein fraction (DP, g/g) could be completely solubilised. The potentially degradable protein fraction was calculated by difference from the undegradable protein fraction (UP), where UP was quantified based on the protein fraction remaining in the residue at the end of small intestinal simulations, expressed as fraction of nitrogen in the original substrate (g/g).

In the small intestine, the appearance of low MW peptides and amino acids (i.e. LMW-AA), relative to DP was quantified as follows:

$$\frac{LMW-AA}{DP}(\%, t) = \frac{N_{supernatant} - N_{enzymes}}{N_{substrate} \times DP} \times 100\% \quad \text{eq. 5}$$

where, $N_{supernatant}$ = the amount of low MW peptide nitrogen in the supernatant, quantified as soluble N after precipitation with sulfosalicylic acid (g/mL), $N_{enzymes}$ = the amount of nitrogen originating from the enzymes added to the samples at the start of the incubation (g/mL).

The fractional rate of protein hydrolysis in the small intestine (k_d) was then estimated using the following first-order kinetics equation:

$$\frac{LMW-AA}{DP}(t) = \text{intercept} + D_{max} \times (1 - e^{-k_d \times t}) \quad \text{eq. 6}$$

where intercept = the degradable protein fraction appearing as low MW peptides and amino acids at onset of the small intestine simulation (i.e. $\frac{LMW-AA}{DP}$ $(t = 0)$, g/g), D_{max} = the fraction of potentially degradable protein hydrolysed into low MW peptides during the small intestine simulation (i.e. $\frac{LMW-AA}{DP}$ $(t = 0 - 4\ h)$, g/g), k_d = the fractional rate of protein hydrolysis (/h), and t = time in h. The maximum potential fraction of degradable protein ending up as low MW peptides is the sum of intercept and D_{max} (i.e. plateau). Parameters k_s (eq 2), intercept, D_{max}, and k_d (eq. 5) were fitted using ordinary least-squares parameter estimation (PROC NLIN) including

the bound of intercept ≥0. Outliers were marked by visual inspection of data plots per feed ingredient. Goodness of fit parameters i.e. root mean square error (RMSE) [23], R^2 (adjusted for number of model variables), and Lin's concordance correlation coefficient [24] (CCC) were calculated, as described by Ellis *et al.* [25].

RESULTS AND DISCUSSION

Differences in protein digestion kinetics of feed ingredients and diets can affect the efficiency of post-absorptive metabolic use of amino acids in pigs. Mathematical mechanistic models can be used to simulate the kinetics of protein digestion. Such models require data on the individual processes of digesta passage in the GIT and nutrient hydrolysis characteristics. As protein hydrolysis kinetics vary among feed ingredients [16; 26] we quantified here the hydrolysis kinetics of 19 feed ingredients (CP: 69 to 837 g/kg) commonly used in pig nutrition using an *in vitro* method.

We used an adapted two-step *in vitro* incubation method based on Boisen and Fernández [17]. Settings of the *in vitro* method were adjusted to reach maximum potential hydrolysis of protein and starch under conditions relevant for pigs. Firstly, instead of a single analysis of the residual protein fraction after the entire stomach and small intestinal simulation, as used in the original method, aliquot samples were taken during the course of stomach and small intestinal simulations to obtain data on the kinetics of nutrient hydrolysis. These aliquot samples were analysed for contents of low MW peptides and amino acids (<500 Da) [21] based on work by Chen *et al.* [16], as they were considered to better represent the fraction of protein hydrolysis products that can be absorbed in the small intestine of pigs compared to the potentially degradable soluble protein fraction (i.e. calculated as protein in the original sample (100%) minus the protein fraction (%) remaining in the residue). Secondly, the stomach pH was adjusted from pH 2.0 to 3.5. Although porcine pepsin activity is higher at pH 2.0 than pH 3.5 [27], a pH of 3.5 was considered closer to the observed average pH of stomach digesta in pigs ([27; 28; 29], unpublished data [2; 30]). Finally, amyloglucosidase [31] was added to the small intestinal simulation next to pancreatin, to maximize the hydrolysis of starch into glucose-units.

Protein hydrolysis in the stomach is initiated through effects of pepsin and low pH. We considered instant protein solubility and protein solubilisation the most important processes in protein digestion in the stomach, as soluble protein is emptied faster from the stomach than insoluble protein [2]. Soluble proteins can therefore be faster available for hydrolysis and absorption [5] in the small intestine after ingestion. Our results showed the protein fraction that was instantly soluble in the stomach, varied between feed ingredients (Table 4.1). Ranging from 8%

Table 4.1. Protein content[1], initial in vitro protein solubility[2], and protein solubilisation rate[4] (k_s) of feed ingredients during a 2 h stomach phase (i.e. pepsin) incubation.

Ingredient	Protein content (g/kg)	Instant protein solubility (%)	k_s (/h)
Barley	101	32	0.26
DDGS (maize)	262	26	0.09
Fishmeal	716	25	0.03
Linseed (extracted)	317	24	ND
Maize	69	12	0.32
Maize gluten meal	609	12	0.10
Oats	125	42	0.21
Peas	204	59	0.08
Potato protein	781	8	0.07
Rapeseed (full-fat)	169	25	0.23
Rapeseed meal	343	18	0.20
Rye	90	27	0.33
Soy hulls	126	31	0.03
Soybean meal	499	21	0.14
Sunflower meal	351	19	0.36
Wheat	108	28	0.43
Wheat middlings	144	18	0.38
Whey powder	250	100	NA
Whey protein isolate	837	100	NA

[1] Crude protein content (nitrogen[(20)] × 6.25)

[3] Protein solubility (SP) quantified as 100% - the protein fraction remaining in the residue at the start of the stomach simulations (t=0 min).

[4] The fractional solubilisation rate (k_s) was estimated (PROC NLIN, SAS 9.4) by fitting $\frac{SP}{DP}(0{,}120) = intercept + 1 \times (1 - e^{-k_s \times t})$*, where intercept = the instantly soluble degradable protein fraction (i.e.* $\frac{SP}{DP}(t = 0)$*; g/g), k_s = the fractional solubilisation rate (/h), t = time in h, and DP= degradable protein fraction calculated as 1 – the protein fraction (g/g) remaining in the residue at the end of the small intestinal simulation.*

ND = could not be determined, NA = not applicable, as instant protein solubility was 100%

(wt/wt) in potato protein to 100% (wt/wt) in whey powder and whey protein isolate. Protein solubility is a result of interactions between intrinsic factors of the feed ingredient, and the solution in which the protein is solubilised and therefore varies among feed ingredients [32; 33]. Comparing our results with those of studies applying similar *in vitro* hydrolysis methods, we observed greater fractions of instantly soluble protein in the stomach for rapeseed meal (18 *v.* 11%, wt/wt) and soybean meal (21 *v.* 7%, wt/wt) than observed by Chen *et al.* [16]. In contrast, we observed smaller values for barley (32 *v.* 54%, wt/wt), soybean meal (21 *v.* 32%, wt/wt), and wheat (28 *v.* 69%, wt/wt) than observed by Wilfart *et al.* [11]. These differences may be explained by a) variation among different batches of the same feed ingredients [16], b) differences in pH of the incubation mixture [32], and c) differences in filtration methods applied in the *in vitro* method, as the soluble protein fraction is calculated indirectly, via subtraction of the fraction of protein remaining in the residue after filtration from the original amount of protein in the sample.

Table 4.2. Enzymatic hydrolysis kinetics[1] of protein into low molecular weight peptides[2] of feed ingredients during a 4 h in vitro small intestinal simulation (i.e. pancreatin, amyloglucosidase) following a 2 h stomach simulation (i.e. pepsin).

Ingredient	DP (%)[1]	Intercept (%)[2]		D_{max} (%)[2]		k_d (/h)[2]		Plateau[2] (%)	N[2]	RMSPE[3] (%)	Adj. R-sq[3]	CCC[3]
			SE		SE		SE					
Barley	84	25	17	86	16	3.3	1.2	110	7	8.2	0.88	0.96
DDGS (maize)	78	45	7	29	9	0.7	0.8	73	8	11	0.58	0.83
Fishmeal	87	51	5	21	5	2.3	1.2	72	6	3.7	0.81	0.94
Linseed (extracted)	74	96	6	28	9	0.7	0.8	123	8	5.1	0.62	0.85
Maize	78	40	24	54	28	1.5	2.5	94	6	21	0.28	0.73
Maize gluten meal	73	22	1	52	3	0.5	0.1	74	8	2.6	0.99	1.00
Oats	87	8	8	82	7	2.4	0.4	91	6	3.1	0.98	1.00
Peas	89	0[4]	0	69	4	9.3	3.1	69	8	14	0.51	0.77
Potato protein	86	31	3	61	28	0.3	0.2	92	8	6.6	0.94	0.98
Rapeseed (full-fat)	64	72	25	31	25	2.3	4.1	104	7	14	0.01	0.51
Rapeseed meal	83	48	22	27	21	5.2	6.2	75	8	9.4	0.25	0.64
Rye	83	73	13	32	14	1.6	2.3	106	4	4.8	0.61	0.93
Soy hulls	55	69	11	29	12	2.0	2.2	98	5	6.3	0.50	0.86
Soybean meal	96	34	5	26	5	2.8	1.2	60	7	4.8	0.85	0.95
Sunflower meal	93	77	3	9	4	2.1	2.3	86	5	1.9	0.58	0.88
Wheat	88	59	4	40	15	0.4	0.2	99	5	6.6	0.75	0.93
Wheat middlings	70	71	2	37	3	0.8	0.2	108	5	1.3	0.98	1.00
Whey powder	100	0[4]	0[4]	91	6	15	4.2	91	4	7.0	0.42	0.88
Whey protein isolate	100	14	32	67	30	5.0	3.7	81	7	14	0.61	0.85

[1] The degradable protein (DP) fraction quantified as 100% - the protein fraction remaining in the residue at the end of small intestinal simulations, i.e. after 2 h stomach (i.e. pepsin) + 4 h small intestine (i.e. pancreatin and amyloglucosidase) simulations.

[2] The fractional rate of degradable protein hydrolysis into low molecular weight peptides and amino acids (LMW-AA) was fitted using non-linear parameter estimation (PROC NLIN, SAS 9.4): $\frac{LMW-AA}{DP}(t) = intercept + D_{max} \times (1 - e^{-k_d \times t})$, where intercept = the degradable protein fraction appearing as low molecular weight peptides and amino acids at onset of the small intestine simulation (i.e. $\frac{LMW-AA}{DP}(t = 0), g/g$), D_{max} = the maximum hydrolysis of the degradable protein fraction into low molecular

weight peptides during the small intestine simulation (i.e. $\frac{LMW-AA}{DP}$ *(* $t = 0 - 4$ *h), g/g),* k_d *= the fractional rate of protein hydrolysis (/h), and t = time in h. Plateau = intercept +* D_{max} *representing the maximum extent of hydrolysis. N= number of observations per ingredient (max. 8).*

[2] Low molecular weight peptides and amino acids defined as the fraction of peptides soluble after precipitation using sulfosalicylic acid (16%; 1:1 v/v).

[3] Root mean square prediction error (RMSPE, relative to observed mean), adjusted R-square for number of model variables (n=3), and concordance correlation coefficient (CCC [24]) are presented as goodness of fits.

[4] Intercept assumed to be 0, as fitted intercept met bound: intercept ≥0.

Compared to the study by Chen *et al.* [16], similar settings were applied and differences in instant protein solubility may be due to differences in composition and solubilisation behaviour between batches of the feed ingredients. In addition, compared to Wilfart *et al.* [11], we applied a different filtration technique (nylon filter *v.* celite filled glass crucible) and a higher pH (3.5 *v.* 2). The lower pH applied by Wilfart *et al.* [11] might have been more distant from the expected isoelectric point of proteins (i.e. pH where protein solubility is lowest) in feed ingredients (pH(I)≥4) than in our study, thereby likely explaining differences in obtained values for protein solubility.

The fractional rate of protein solubilisation observed in the stomach (i.e. k_s) ranged from 0.031 /h in fishmeal to 0.43 /h in wheat. These values were comparable for rapeseed meal (0.20 vs 0.17 /h) and soybean meal (0.14 *v.* 0.10 /h) based on recalculated data from Chen *et al.* [16], using our framework (Figure 4.1). However, we observed greater values for barley (0.15 *v.* 0.26 /h) and wheat (0.09 *v.* 0.43 /h) and a lower value for soybean meal (0.28 *v.* 0.14 /h) compared with recalculated values using data from Wilfart *et al.* [11]. Again differences among batches of feed ingredients and pH of the incubation mixture might have caused differences in solubilisation rate.

In the small intestine, soluble proteins and peptides are further hydrolysed into low MW peptides and amino acids. Our results showed that the potentially degradable protein fraction ranged from 55% (wt/wt) in soy hulls to 100% (wt/wt) in whey powder and whey protein isolate (Table 4.2). The values are in line with standardized ileal crude protein digestibility values of feed ingredients as presented in the Dutch feed evaluation system [34], although they were generally lower than values presented by Boisen and Fernández [35]. The latter is likely due to the longer incubation times (stomach: 6 h, small intestine 18 h) and lower stomach pH (2.0) applied in their study [35]. At onset of the small intestinal simulation a substantial part of the potentially degradable protein fraction was readily present as low MW peptides and amino acids, ranging from 8% (wt/wt) in oats to 96% (wt/wt) in extracted linseed. During the small intestinal simulation, the potentially degradable protein fraction continued to be hydrolysed yielding more low MW peptides and amino acids. The maximum potential fraction of degradable protein appearing as low MW peptides and amino acids was estimated at 60% in soybean meal and at 123% in extracted linseed. Values above 100% for this parameter relate to the inaccuracy of the independent predictions of the low MW peptide and amino acid fraction, and the degradable protein fractions. Relative to the total protein fraction in the original sample, the maximum low MW peptide and amino acids fraction represented 54% in soy hulls and maize gluten meal, and 93% in barley. These values show that part of the potentially degradable protein remained as high MW (>500 Da; e.g. 40% in soybean meal) at the end of the small intestinal simulation, in agreement with previous findings by Chen *et al.* [16]. As such, the degradable protein fraction is not completely

hydrolysed into absorbable peptides and amino acids [18]. *In vivo*, the final step in protein hydrolysis is dependent on the activity of brush-border peptidases[18]. As brush-border enzymes were not part of the set of enzymes added in our or other *in vitro* assays (e.g. [17; 36]), their absence likely explains the fraction of high MW peptides in the degradable protein fraction at the end of the small intestine simulation [37]. Overall these results indicate that, using traditional *in vitro* assays, estimations of the extent of protein digestion *in vivo* using the degradable protein fraction *in vitro* as proxy, may not adequately reflect the availability of absorbable peptide and amino acid to the animal.

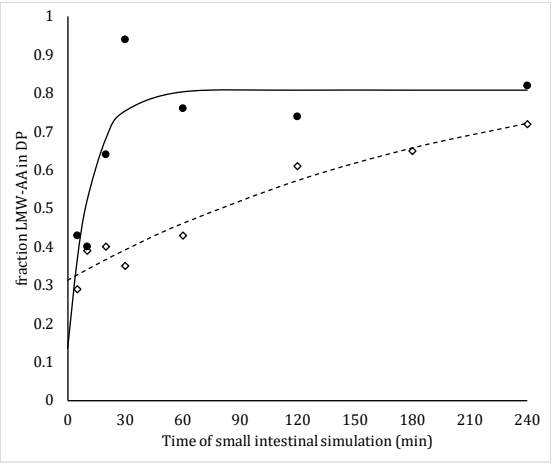

Figure 4.2. In vitro small intestinal hydrolysis kinetics of protein in potato protein (◇, dashed line) and whey protein isolate (●, solid line). Lines represent the first-order kinetic model fitted to the data points.

For the fractional rate of protein hydrolysis in the small intestine, the dataset contained 3 missing observations and 157 remaining observations of which 29 were considered outliers based on visual inspection of the data points and hydrolysis curve (e.g. Figure 4.2) of each ingredient. The fractional rate of protein hydrolysis in the small intestine (i.e. k_d) ranged from 0.3 /h in potato protein, to 5.2 /h in rapeseed meal, excluding results of peas and whey powder. For the latter a positive intercept (i.e. >0) could not be fitted by the model and was therefore assumed to be 0%, likely causing overestimated k_d values for these ingredients. Comparing observed k_d values with that of Chen *et al.* [16] shows deviations in absolute terms for rapeseed meal (5.2 *v.* 2.8 /h), soybean meal (2.8 *v.* 1.7 /h), and whey powder (15 *v.* 23.1 /h). The ranking of ingredients based on their fractional rate of hydrolysis, however, were similar in both studies.

The results from the present study were used as input for a computer model [1] that simulates passage and hydrolysis of ingested nutrients in the GIT of growing pigs (Figure 4.1). Applying pre-set passage rates of digesta in the stomach (i.e. solids < liquids) and small intestine (i.e. solids equal to liquids) and data on *in vitro* protein hydrolysis kinetics of the protein sources resulted in a difference in time of peak of protein digestion and amino acid absorption from the small intestine after a meal of 101 min (147 *v.* 46 min) and 37 min (44 *v.* 81 min) when simulating hydrolysis of potato protein and whey protein isolate (Figure 4.3). These ingredients differed

widely in *in vitro* protein solubilisation and hydrolysis in both the stomach and the small intestine. Also when simulating protein sources with similar instant protein solubility and potentially degradable protein fraction, e.g. potato protein *v.* maize, differences in time of peak of protein hydrolysis (147 *v.* 91 min) and amino acid absorption (81 *v.* 73 min) are simulated. Compared to *in vitro* methods which measure the extent rather than the kinetics of hydrolysis, results from the presented method yields insight in the variation in kinetics of protein hydrolysis among feed ingredients. These data can be used in computer models simulating the kinetics of nutrient digestion in pigs.

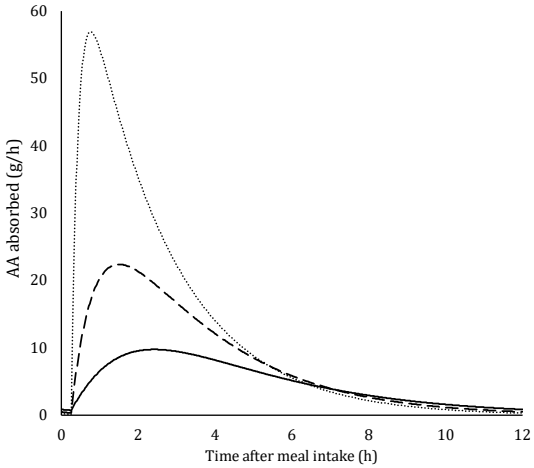

Figure 4.3. Simulated post-prandial absorption of amino acids from the gut after ingesting equal amounts of protein (195 g/ meal) from potato protein (solid line), maize (dashed line), and whey protein isolate (dotted line) in growing pigs using a computer simulation model [(1)].

In conclusion, our *in vitro* assay allowed to study the kinetics of protein hydrolysis of feed ingredients. A substantial but variable part of the protein fraction of feed ingredients is instantly soluble under stomach conditions. This fraction is relevant to consider when simulating protein digestion kinetics, as soluble proteins can be faster emptied from the stomach and become available for hydrolysis and absorption in the small intestine than the insoluble protein fraction. Under conditions of the small intestine, a variable fraction of the potentially degradable protein of feed ingredients is instantly present as low MW peptides and amino acids, which are supposed to be rapidly absorbed *in vivo*. At the end of the *in vitro* small intestinal simulations, potentially degradable protein fractions were not completely hydrolysed into low MW peptides and amino acids for some feed ingredients, likely due to lack of brush-border enzyme activity. The former, however, also indicates that the extent of protein digestion *in vivo*, as estimated based on the overall degradable protein fraction, may not adequately reflect the extent of absorbable peptide and amino acid availability *in vivo*. Data on the kinetics of protein hydrolysis per feed ingredient can be used in mathematical models to simulate the process of protein digestion in pigs.

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Chapter 5

Modelling digestion and absorption kinetics of nutrients in growing pigs

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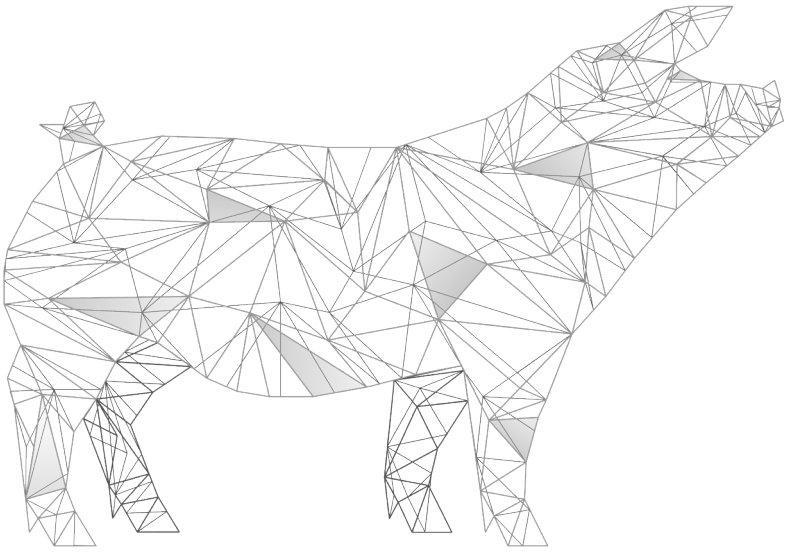

ABSTRACT

This paper describes a nutrient-based dynamic mechanistic digestion model for growing-finishing pigs. The model objective is to predict absorption kinetics of nutrients in pigs fed diets varying in feed ingredient and nutrient composition, as well as physicochemical properties. Digestion is represented by the passage, hydrolysis, absorption, and endogenous secretions of nutrients along the stomach, proximal small intestine, distal small intestine, and caecum + colon. The model comprises 48 state variables representing dietary protein, starch, fat, and non-starch polysaccharide pools, their hydrolysis products, endogenous protein and fat pools, and a microbial biomass pool. Driving variables are ingested nutrients. Dietary protein, starch, and fat are characterised by (enzymatically) degradable and undegradable fractions according to their feed ingredient origin. Rate and extent of starch and protein hydrolysis were derived from *in vitro* assays. Passage of digesta from the stomach is modelled as a function of nutrient solubility and by diet viscosity, diet solubility, and feed intake. Model output focusses on the prediction of glucose and amino acid absorption kinetics. Model evaluation includes testing against independent data from *in vivo* nutrient appearance studies in (portal) blood of growing pigs (studies = 12 and treatment means = 33 for glucose, studies = 8 and treatment means = 15 for amino acids). Evaluation of the model indicated adequate predictions of glucose absorption kinetics when simulating diets varying in physicochemical properties and starch sources. The extent of small intestinal protein digestion was adequately predicted. However, despite adequate mean predictions, variation in the kinetics of amino acid absorption between protein sources could not be predicted by the model. It was concluded that adequate data are missing for model calibration. The model can be used to gain insight in the quantitative impact of variation in the kinetics of nutrient digestion, induced by dietary feed ingredients and physicochemical properties, on absorption kinetics of nutrients.

INTRODUCTION

Nutrient digestion kinetics is known to affect the nutritional value of feed ingredients. For example, nutrients that are more resistant to enzymatic hydrolysis will end up in the colon where they can be subjected to fermentation. As hydrolysis and fermentation yield different digestion products (e.g. amino acids and glucose, *v.* short-chain fatty acids), this partly explains the effect of digestion kinetics on the nutritional value of feed ingredients in pigs. In addition, the nutritional value is also affected by the rate at which ingested nutrients are absorbed. In pigs and humans, for example, the rate of protein digestion and absorption, e.g. fast *v.* slow protein, is shown to affect the oxidation of amino acids and the deposition of protein during post-absorptive

metabolism [6; 7; 8; 9; 10]. Moreover, the latter can also be affected by the availability of other nutrients, such as glucose [11]. Hence, nutrient digestion and absorption kinetics affect the nutritional value of feed ingredients in pigs. As current feed evaluation systems, presenting the nutritional value of feed ingredients, only take into account the extent of nutrient digestion [12; 13; 14], they can be improved by considering the kinetics of nutrient digestion.

The kinetics of nutrient digestion is mainly estimated using *in vitro* assays (e.g. [15; 16]). Although results of these assays are used to predict the kinetics of nutrient absorption kinetics *in vivo*, their capacity to do so is sometimes limited [17]. For example, although there is a good correlation between the extent of glucose absorption within 120 min after a meal. as indicated by the glycaemic index, and the rapid release of glucose measured *in vitro* [18], these results give no information on the rate of glucose absorption within, or on its kinetics after that timeframe. Results from another study [19], comparing four starch sources differing in rapid and slow degradable starch fractions, showed that the variation in time of peak (TOP) of glucose release *in vitro* did not match with that of glucose absorption *in vivo*: 14.5, 9.2, 0.03, 0.05 *v.* 78, 74, 76, 49 min. The authors [19] stated that, digesta passage in the stomach needs to be considered to better correlate the extent of glucose release *in vitro* with its absorption *in vivo*. These results indicate that the kinetics of nutrient absorption cannot simply be derived from *in vitro* assays that do not take into account other digestion processes than hydrolysis. Computer simulation models have been developed to account for the kinetics of both passage and hydrolysis on the kinetics of nutrient digestion. These models focus on digesta passage in the stomach [20; 21], small intestine [22; 23], or the complete digestion process [24; 25; 26; 27]. As latter models don't take into account variation in nutrient hydrolysis kinetics among feed ingredients, they do not, or to a limited extent, predict variation in absorption kinetics of nutrients originating from different feed ingredients. For example, the fractional rate of starch hydrolysis is considered equal among starch sources [24; 25; 26; 27], while results from *in vitro* assays show that potato starch is more resistant to hydrolysis than maize starch [28]. Similarly, current models only represent variation in the kinetics of digesta passage to a limited extent [27]. However, knowledge has been gained on the effects of dietary physicochemical properties [2; 5] and feed intake [2; 29] on the passage of digesta in the gastrointestinal tract (GIT) of pigs. Hence, to increase our understanding on the kinetics of nutrient digestion and its effect on the nutritional value of feed ingredients, we developed a nutrient-based dynamic mechanistic digestion model for growing-finishing pigs. As the kinetics of nutrient digestion is affected by feed ingredients and dietary physicochemical properties, we take into account effects in diet viscosity, diet and nutrient solubility, nutrient degradability, and level of feed intake. Hereby, we aimed to make the first step towards predicting

nutrient digestion and absorption kinetics from feed ingredients, varying in physicochemical properties and potential hydrolysis kinetics.

MODEL DESCRIPTION

The model simulates the process of digestion of nutrients in the GIT of a growing-finishing pig (35-110 kg bodyweight; Figure 5.1). Passage, hydrolysis, absorption, and endogenous secretions are the major processes simulated by the model. As these processes differ among GIT segments, the model represent the stomach (GS), the proximal small intestine (I1), the distal small intestine (I2), and the caecum + colon combined (CC) as anatomical compartments. Model abbreviations are presented in Table 5.1, parameter values in Table 5.2, and model notations in

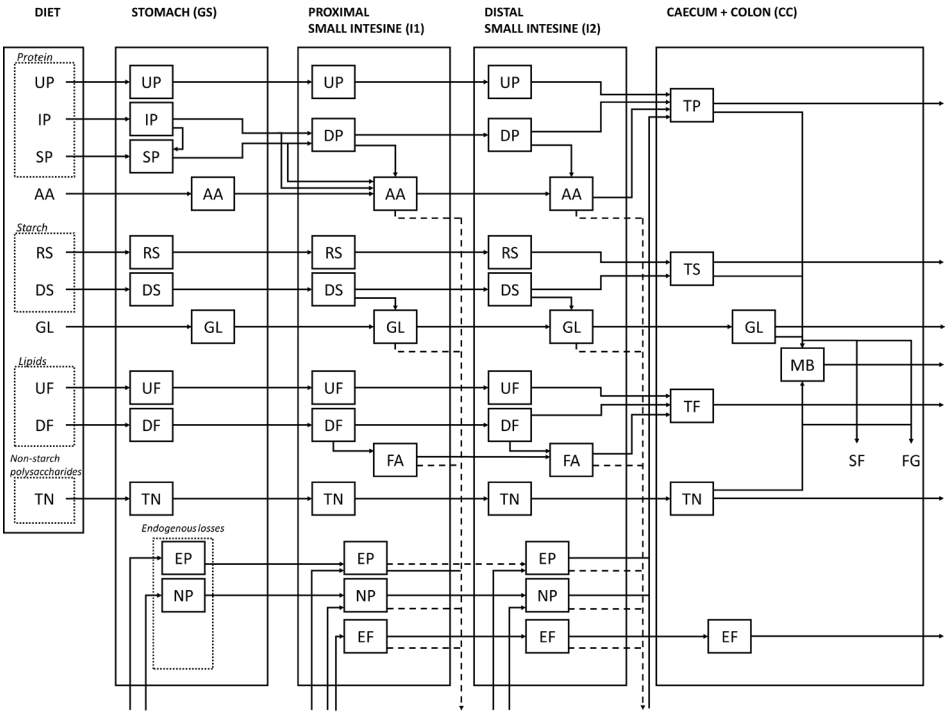

Figure 5.1. Schematic representation of the digestion model. Nutrients ingested during feed intake enter the stomach compartment (GS), where insoluble protein can be solubilised (IP→SP), and insoluble and soluble nutrients pass at diffierent rates into the proximal small intestine (I1). In the proximal and distal small intestine (I1, I2) degradable protein (DP), starch (DS), and fat (DF) can be hydrolysed into amino acids (AA), glucose (GL), and fatty acids (FA), respectively. These monomeric nutrients, together with endogenous secretions (ie. protein: EP, non-protein nitrogen: NP, fat: EF) can be (re-)absorbed in the small intestine (I1 and I2). Enzymatical undegradable protein (UP), starch (RS), and lipids (UF), together with undegraded counterparts forming total protein (TP), starch (TS), and fat (TF), and non-starch polysaccharides (TN) pass the small intestine and enter the colon (CC), where they can be fermented or excreted. Fermentation yields microbial biomass (MB) short-chain fatty acids (SF), and fermentation gasses (FG). Black lines indicate hydrolysis (within segments) or passage (between segments) or secretion, whereas dashed lines indicate absorption.

Supplementary Table S5.1. The kinetics of digesta passage, nutrient hydrolysis, and the consequential rate of change in nutrient pools are presented mainly by first-order kinetics and pools are presented on dry matter-basis (g). Differential equations are solved using Runga-Kutta fourth-order fixed numerical integration with a step size of 0.0167 h. Model outputs presented in this paper focus on the extent of digestion of protein, starch, and fat at the end of the small intestine (i.e. apparent or true ileal digestibility), and on the postprandial extent and kinetics of glucose and amino acids absorption from the small intestine (I1+I2). Model outputs is calculated when the model is in quasi-steady state, i.e. after running a 104 h simulation (i.e. 4 d). Time of peak absorption of glucose and amino acids are assessed in the last 12 h of a 104 h simulation run (i.e. representing the final meal). The model is driven by the ingestion of nutrients, originating from various ingredients, as described below.

Table 5.1. Abbreviations and general notation for model entities used to simulate digestion kinetics in growing pigs

Abbreviation/notation	Description	Unit
Diet (d)		
D_j	Feed intake level	× maintenance requirement for energy [30] (419 kJ /kg $BW^{0.75}$/d)
Ds	Diet solubility	g/g
Dr	Diet rheology	Pa×s
RAV	Real applied viscosity[1]	mL/g
Meal		
DMI	Dry matter intake	g/d
SFEED	Clock-time of initial meal	h
IFEED	Meal interval	h
TFEED	Duration of feed intake	h
FFEED	Number of meals per day	/d
Segments gastrointestinal tract		
gs	Stomach (i.e. gaster)	
i1	Proximal small intestine	
i2	Distal small intestine	
cc	Caecum + colon	
gb	Gallbladder	
bl	Portal blood	
Digesta phase		
sl	Solids	
lq	Liquids	
Nutrients		
cp	Total dietary crude protein (i.e. up+dp)	

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up	Enzymatically undegradable protein
dp	Enzymatically degradable protein (i.e. ip+sp)
ip	Stomach insoluble protein
sp	Stomach soluble protein
ep	Endogenous protein
np	Endogenous non-protein non-amino acid nitrogen
aa	Amino acids
tp	Total protein (cp+ep+np+aa)
ts	Total dietary starch (i.e. st+rs)
ds	Enzymatically degradable starch
rs	Ileal undegradable starch (i.e. resistant starch)
gl	Glucose
tf	Total dietary fat
uf	Undegradable fat
df	Degradable fat
fa	Fatty acids
ef	Endogenous fat
tn	Total non-starch polysaccharides
om	Organic matter
mb	Microbial biomass
sf	Short-chain fatty acids (e.g. acetate, propionate)
fg	Fermentation gasses (e.g. H_2, CO_2)

Notation format		
Q*xi*	Pool of nutrient *x* in segment *i*	g
Q*xi*0	Initial pool size of nutrient *x* in segment *i* (i.e. at t=0)	g
F*xi_yj*	Flux of nutrient *x* in segment *i*, to nutrient *y* in segment *j*	g/h
d*Qxi*	Auxilliary variable belonging to the pool of nutrient *x* in segment *i*	g/h
dQ*xi_yj*	Cumulative pool belonging to flux of nutrient *x* in segment *i,* to nutrient *y* in segment *j*	g
K*xi_yj*	Rate of change of nutrient *x* in segment *i* into nutrient *y* in segment *j*	/h
Kdyj	Rate of hydrolysis (kd) of nutrient *y* in segment *j*	/h
C*xi* or C*x_y*	Constant belonging to nutrient *x* in segment *i* or nutrient *x* in entity *y*	g/g

Model driving variables

Feed intake

Feed intake is based on a meal-fed pig and modelled as an episodic process (eq.[3], Supplementary Table S5.1) of a constant rate and interval. Meal size (eq. [2]) is calculated by

dividing the daily dry matter intake (DMI) over the number of meals per day (FFEED). Meals are ingested over a fixed period of time (TFEED). The ingestion rate depends on DMI, FFEED, and TFEED. Combined with daily timing of the first meal (SFEED) and meal interval (IFEED), they determine the overall daily feed intake pattern. Currently the daily feed intake pattern is as follows: the pig is fed a meal twice a day (FFEED=2) at 08.00 h (SFEED=8 h) in the morning and 20.00 h in the evening (IFEED=12 h), it is finishing a meal in 15 min (TFEED=0.25 h). Feed intake drives the input of nutrients to the pools in the stomach, calculated by multiplying the rate of feed intake with the concentration of the respective nutrients in the diet (eq. [10], [13], [17], [20], [25], [28], [31], [36], [39], [42]).

Dietary nutrient intake

Main dietary nutrients presented in the model are: protein (Crude protein: CP), starch (total starch: TS), fat (total fat: TF), and non-starch polysaccharides (NSP; total NSP: TN) which are calculated for feed ingredients and diets based on the Dutch feed evaluation system [12]. Moreover, dietary intake also includes amino acids (AA), and reducing sugars regarded as glucose (GL). Nutrients are further characterised by their degradability and solubility, which depends on the feed ingredient from which they originate (see Supplementary Table S5.4). For starch and protein data is used from *in vitro* studies [28; 31; 32; 33; 34], for fat based on work of [35; 36], and for NSP based on variation in extent of fermentation in pigs [12]. Nutrient fractions considered are as follows: for protein, enzymatically undegradable protein (UP) and enzymatically degradable protein (DP), of which DP encompasses: stomach insoluble (IP) and soluble protein (SP). The UP fraction is calculated by estimating the true ileal protein fraction using data from [12] regarding the apparent ileal protein digestibility values per feed ingredient (i), and assuming a level of basal and specific (i.e. arbitrarily set at 50% of basal) endogenous protein losses:

$$\mathrm{UP(i)} = 0.5 \times \left(1 - \frac{\mathrm{AIDCP} + 1.5 \times \mathrm{BEPL}}{\mathrm{CP}}\right)$$

where, AIDCP = the apparent ileal crude protein digestibility coefficient (g/g kg DM), BEPL = basal endogenous protein losses (i.e. 11.43 g/kg DM), and CP = crude protein content of the feed ingredient (g/kg DM), all based on the Dutch feed evaluation system [12]. The SP fraction is based on *in vitro* assays that consider protein hydrolysis kinetics of feed ingredients [4; 33].

For starch, ileal enzymatically undegradable starch (RS), and degradable starch (DS) are considered. The RS fraction, i.e. the fraction resistant to enzymatic hydrolysis in the small intestine, is derived from the starch fraction that is not hydrolysed after 6 h of *in vitro* small intestinal incubations:

$$RS(i, t_6) = Dmax \times (1 - e^{(-kds\_gl \times t_6)})$$

where, Dmax = the maximum degradable fraction of starch (g/g), Kds_gl = the rate of starch hydrolysis (/h), and t = 6 h. All parameters regarding starch hydrolysis are obtained from *in vitro* assays [28; 32; 34].

For fat, ileal undigestible (UF) and digestible fat (DF) are considered. Similar to the UP fraction, the UF fraction is calculated by estimating the true ileal digestibility of fat using data from Smink [35] and the Dutch feed evaluation system [12] on apparent ileal fat digestibility values per feed ingredient (i), and assuming a certain level of basal endogenous fat losses:

$$UF(i) = 0.5 \times \left(1 - \frac{(TF \times DC_{fat}) + BEFL}{TF}\right)$$

where, TF = fat content of the feed ingredient (g/kg DM) [12], BEFL = basal endogenous fat loss (i.e. 4.7 g/kg DM) [37], and DC_{fat} = digestibility coefficient of fat (g/g) based on work by Smink [35], who proposes to calculate fat digestibility based on chain length, degree of saturation, and positioning of fatty acids on the glycerol backbone. If fatty acid composition, i.e. chain length and saturation, was not presented by Smink [35] then DC_{fat} was based on the Dutch feed evaluation system [12].

Nutrient fractions per diet are calculated as weighted average of the diets' constituting feed ingredients (i) and macronutrient content. For example, dietary UP fraction is calculated as follows:

$$UP = \sum((\frac{CP_n}{\sum_{i=1}^{n} CP} \times UP_i) + \cdots + (\frac{CP_n}{\sum_{i=1}^{n} CP} \times UP_n))$$

where, i denotes a specific feed ingredient, n denotes the total number of feed ingredients in the diet.

The kinetics of nutrient hydrolysis vary among feed ingredients and therefore were considered as inherent feed ingredient properties. To compute fractional hydrolysis rates for protein and starch hydrolysis, data is taken from *in vitro* assays [4; 28; 32; 34; 38]. The kinetics of NSP and starch fermentation in the colon is modelled based on the fractional rates of fermentation required to reach the extent of faecal NSP, varying among feed ingredients, (i), and starch digestibility (~100%) as presented by the Dutch feed evaluation system[12]. Fractional rates of NSP and starch fermentation are calculated as follows:

$$\mathrm{Kdxcc_i} = (-\mathrm{DC_x} \times \mathrm{Kc\_o})/(\mathrm{DC_x} - 1)$$

where *x* = TN or TS, respectively, DC*x* = the faecal digestibility coefficient of *x* (g/g), which for TN is based on the Dutch feed evaluation system [12], and for TS is assumed to be 0.999 (g/g), Kc_o = the fractional passage rate of digesta in the colon (i.e. 0.0298 /h, see below).

Dietary physicochemical properties

The passage of digesta in the stomach is affected by diet solubility (D_s), feed intake level (D_j) and diet viscosity (D_r), as described below. Diet solubility, D_s, is calculated as the fraction of SP and GL in the diet (g/g), see [2]. Feed intake level, D_j, is calculated as dietary energy intake relative to the maintenance requirement for energy (ME_m=419 kJ metabolisable energy/kg $BW^{0.75}$ per d [30]; i.e. D_j = x ME_m). Diet viscosity, D_r, represents the apparent dynamic viscosity of the diet at 1/s shear rate. As data on the dynamic viscosity of diets and constituting feed ingredients is limited, D_r is deduced from rheological data (i.e. real applied viscosity: RAV, ml/g) by Carré *et al.* [39]. Diet viscosity is calculated as the weighted average of the RAV of each of the diets composing feed ingredients. The relationship between RAV and D_r is determined using the computed RAV and measured D_r of the viscous diets used to assess the effect of diet viscosity on digesta passage presented by Schop *et al.* [5]:

$$D_r = 30.33e^{(0.0693 \times \sum_{i=1}^{n}(w_i \times RAV_i + \cdots + w_n \times RAV_n))}$$

where, D_r = the apparent dynamic viscosity of the diet at 1/s shear rate (Pa·s), i = dietary feed ingredient (1 to *n*), w = weight factor according to ingredient content in the diet (g/g), and RAV = real applied viscosity (ml/g) [39].

Stomach

Upon ingestion, nutrients enter the stomach where they are mixed with endogenous secretions (i.e. HCl, pepsin). For protein, some of the proteins become instantly solubilised depending on intrinsic physicochemical properties of the ingested protein and the stomach environment [40](eq. [17]). Soluble proteins will leave the stomach with the liquid digesta fraction (eq. [18]) and will enter the small intestine quicker than the solid digesta fraction [2]. Insoluble proteins that are retained in the stomach will become solubilised as a result of protein hydrolysis (eq. [15]). The rate and extent of protein solubilisation differs among feed ingredients [4]. Parameters for initial protein solubility, and the rate of protein solubilisation are taken from *in vitro* assays [4; 33]. Dietary starch, fat, and NSP are assumed to leave the stomach unchanged with the solid fraction of digesta.

Passage of digesta through the stomach differs between the solid and liquid fractions of digesta [41]. The model considers a higher fractional passage rate for the liquid digesta fraction (eq. [4]) that contains soluble nutrients (SP, AA, GL, EP, NP; eq. [18], [21], [32], [48], [51]) than for the solid digesta fraction (eq. [5]) that contains insoluble nutrients (UP, IP, RS, DS, UF, DF, TN; eq. [11], [14], [26], [29], [37], [40], [43]). In addition, the fractional passage rate of digesta in the stomach is known to be affected by physicochemical properties of the diet and digesta (reviewed by Kong and Singh [42] and Lentle and Janssen [43]). As data lacks on interactions between effects of these physicochemical properties they are presented in the model according to the following basal equation which considers additivity:

$$kxgs\_xi1 = 1/(\text{intercept} + \text{a} + \text{b} + \text{c})$$

where, x = sl or lq, representing the solid or liquid fractions of digesta, intercept (±SD) = 3.2 (± 1.7) or 1.6 (± 0.7) h representing the baseline mean retention time (MRT; inversely related to the fractional passage rate) of solids and liquids, respectively, values are based on numerical means of study averages on digesta retention time in the stomach of growing pigs [2; 3; 5; 29; 44; 45; 46; 47; 48; 49; 50] (PROC MEANS, SAS version 9.4, SAS Institute Inc.), and a, b, and c are respectively the effects of feed intake level (D_j; a), diet solubility (D_s; b), and diet viscosity (D_r; c), further explained below. As D_j, D_s, and D_r affect the MRT of solids and liquids, their values at baseline need to be taken into account, i.e. D_j (± SD): 2.3 (± 0.7) × ME_m for solids and 2.4 (± 0.7) × ME_m for liquids, and D_s and D_r are assumed to be 0.1 g/g and 30 Pa×s, respectively.

Increasing feed intake causes the fractional passage rate of digesta to decrease [2; 29], presumably due to triggering of nutrient feedback mechanism in the GIT [51]. As the latter is considered to cause a generic effect, the effect of D_j is assumed to be equal for both solids and liquids. Data from Schop *et al.* [2]; Gregory *et al.* [29], used to quantify the effect of feed intake on digesta passage, showed that a one unit increase in D_j, increases the MRT of solids and liquids by 0.9(± 0.3; SE) h. To ensure sensible model behaviour across extreme feed intake levels, the effect of D_j is restrained to $1< D_j < 3$, and outside this range no effects of D_j on digesta passage rates were assumed. A Gompertz function was fitted to these data:

$$\text{a} = (1.9\ e^{(-[20.12e^{-1.7\times Dj}])} - \text{f})$$

where, a = the effect of feed intake on the passage of digesta solids or liquids in the stomach, D_j = feed intake relative to maintenance requirement for energy (ME_m=419 kJ metabolisable energy/kg $BW^{0.75}$ per d; [30]), and f = 1.2 h for solids and 1.3 h for liquids, respectively. Parameter

f is in place to adjust the effect of feed intake on ΔMRT for solids and liquids to be zero when Dj equals baseline feed intake ($kxgs\_xi1$, see above).

Diet solubility, represented as the fraction of soluble protein and sugar in the diet, affects digesta passage in a non-linear manner [2]. At increasing D_s (from 8 to 19 %) fractional passage rates of solids and liquids initially decreases (from 0.30 to 0.24 /h; from 0.77 to 0.67/h, respectively), whereas when D_s increases further (31 %), the fractional passage rates increases (to 0.34 and 1.25 /h, respectively). The effect of D_s on the passage of digesta is presumably caused by triggering of nutrient feedback mechanisms in the GIT [51]. As the latter is considered to cause a generic effect, the effect of D_s is assumed to be equal for both solids and liquids. Data from Schop *et al.* [2] is used to quantify the relative effect of D_s on the MRT of solids and liquids. This was done by taking the first derivative of quadratic functions that where fitted to quantify the relation between D_s and the MRT for solids and liquids, separately. In order to ensure sensible model behaviour at values of $0 < D_s < 0.4$, and outside this range no effects of D_s on digesta passage rates were assumed. A Gaussian function was fitted to these data:

$$\mathrm{b} = 0.87\, e^{-\left[\frac{(Ds-0.185)^2}{2\times 0.052^2}\right]}$$

where, b = the relative effect of diet solubility on the fractional passage rate of digesta solids and liquids in the stomach, and D_s = diet solubility (g/g) represented by the fraction of soluble protein and reducing sugars in the diet. The combined effect of feed intake level and diet solubility on the MRT of solids and liquids in the stomach is illustrated in Figure 5.2.

Diet viscosity is negatively related with the fractional passage rate of liquids in the stomach [5]. Increasing D_r resulted in an increase in MRT of liquids, but not of solids, thereby reducing the difference between the MRT of solids and liquids in the stomach. Data by Schop *et al.* [5] was used to quantify the relation between D_r and the difference in MRT of solids and liquids yielding, ΔMRT=1.2 (± 0.1; SE) − 0.00137 (± 0.0004; SE) × D_r. This relation was rescaled to apply to the average difference in MRT of solids and liquids predicted by the model, resulting in the following relation:

$$\mathrm{c} = \mathrm{g} \times 0.0017 \times \mathrm{D_r}$$

where, c = the effect of Dr on the fractional passage rate of liquids in the stomach, g = 1.5 h representing the average difference between the MRT of solids and liquids in the stomach of the model. The effect of D_r, as reflected by c, is applied in the model to the passage of liquids in order to reduce the difference in MRT of solids and liquids.

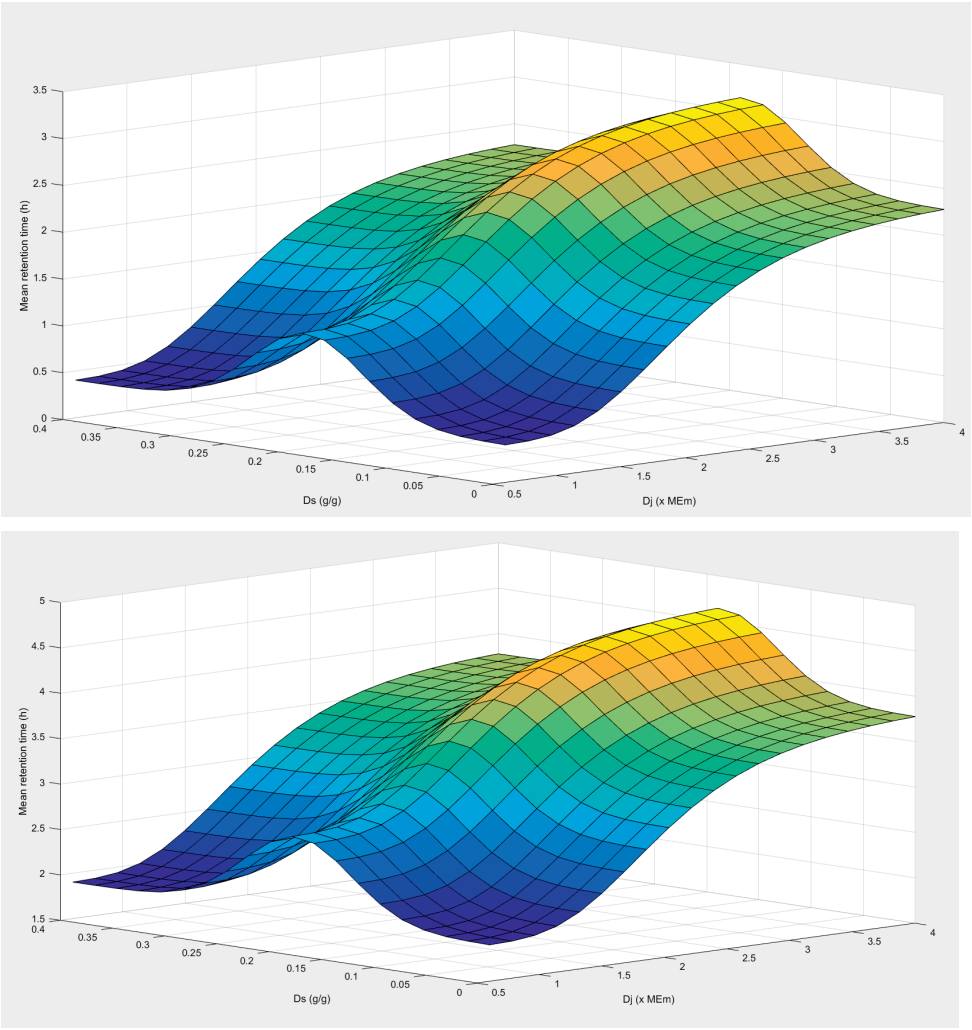

Figure 5.2. Effect of feed intake level (D_j: × maintenance requirement for energy [30]: 419 kJ /kg $BW^{0.75}$/d) and diet solubility (g/g) on the mean retention time of digesta liquids (above) and solids (below) in the stomach, as represented in the dynamic digestion model for growing pigs.

Small intestine

As digesta passes through the small intestine it becomes more homogenous, and no or limited differences between the retention time of digesta solids and liquids are reported [2; 5; 45; 52]. In the model, digesta passage is represented by a single fixed fractional rate for both solids and liquids. This is in contrast to literature stating that digesta passage rates can vary due to the physicochemical properties of diets or digesta [23; 43]. The effects of physicochemical properties of diets and/or digesta, however, were shown to be too small [2; 5], ambiguous [53; 54], or included

effects on gastric emptying (i.e. digesta passage until the ileum) [55; 56; 57; 58]. Hence, a fixed fractional passage rate for both insoluble and soluble nutrients. The rate is based on the numerical mean of study averages of digesta passage in the small intestine reported for growing pigs [2; 3; 5; 31; 45; 52] (PROC MEANS) (0.373 /h, i.e. MRT (±SD) of 2.7 (± 1) h; eq. [53], [55], [58], [61], [64], [67], [70], [73], [75], [77], [80], [83], [85], [87], [90], [93], [96], [99], [101], [110], [114], [118], [122], [127], [129]). The small intestine is divided into two segments (I1 and I2) to better model post-prandial nutrient appearance and to slow down transit of nutrients into the colon. The division between I1 and I2 is arbitrarily based on data used to parameterise fat hydrolysis kinetics [36]. In their study small intestine was divided based on length. To translate length to MRT, data by Martens *et al.* [1]; Schop *et al.* [2], and Van Erp [3] was used in which both were measured. Based on these data, I1 and I2 were set to 21 and 79 % of the total small intestinal MRT, respectively (Ci1_i2 = 0.21, eq. [6], [7]).

Upon arrival in the small intestine, protein, starch, and fat are subjected to enzymatic hydrolysis under influence of pancreatic and bile secretions (eq. [57], [60], [69], [72], [89], [92]). The fractional rates of hydrolysis and absorption per nutrient are not differentiated between the two small intestinal segments (I1 and I2). For protein, we consider no differences in the hydrolysis kinetics of insoluble and soluble protein, although there is little information that proves otherwise (e.g. [59]). Hence, they both enter the same degradable protein pool (eq. [59]). Data from *in vitro* assays show that at onset of small intestinal simulations part of degradable protein fraction is present as absorbable small peptides and amino acids [4; 33]. The latter is included in the model by representing part of the soluble and insoluble protein (i.e. Cdpgs_aai) to directly flow into the small intestinal amino acid pool, after they are emptied from the stomach (eq. [14], [18]). The remaining pool of degradable protein requires further hydrolysis in the small intestine before being present as absorbable small peptides and amino acids (eq. [57], [60]).

For starch, directly using the factional hydrolysis rates obtained *in vitro* in the model caused the extent of starch digestion by the end of the small intestine to be structurally lower than observed *in vivo* [45]. This might be due to underestimation of partial starch hydrolysis in the stomach, for which limited data exists [45]. The relationship between *in vitro* and *in vivo* fractional hydrolysis rates, therefore, are assessed based on experimental work by Martens *et al.* [1]; Schop *et al.* [2], and Van Erp [3] (see Figure 5.3: left panel). For fat, the fractional rate of hydrolysis varies among fat sources [60], however, available data are limited. Therefore, a generalised approach was adopted using a fixed fractional rate of fat hydrolysis across feed ingredients, this rate is set to meet the extent of fat digestibility in different segments of the small intestine as observed by Gunness *et al.* [36] (Kdfi_afi = 4.25 /h; eq. [89], [92]).

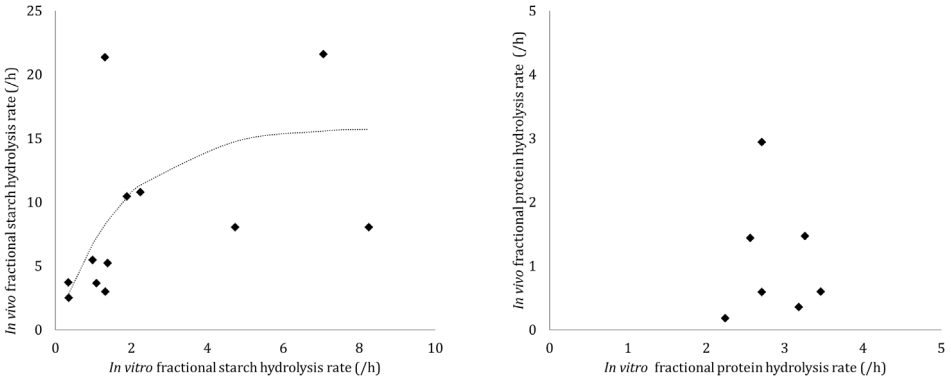

Figure 5.3. Relation between in vitro and in vivo fractional hydrolysis rates (/h) in the small intestine for starch (left-panel): y =12.87 × (1-e (-0.65×X)), RMSE=3.33; using data from Martens et al. (1); Schop et al. (2); Van Erp (3), and for protein (right-panel) no significant relation; using data from Chen et al. (4); Schop et al. (2); Van Erp (3); Schop et al. (5).

We assumed that the kinetics of portal appearance of absorbed nutrients from the gastrointestinal tract is dominated by the kinetics of passage and hydrolysis of protein and starch up to the end of the small intestine. Therefore, the absorption of amino acids, glucose, and fatty acids in the small intestine are assumed to occur at non-limiting fractional rates (eq. [63], [66], [79], [82], [95], [98]).

The hydrolysis of nutrients is facilitated by pancreatic and bile secretions, subsequently these lead to endogenous losses of protein (N × 6.25) and fat. Modelling of the endogenous secretions is based on previous work by Strathe *et al.* (27), where DMI and OM flows through the GIT affect gastric (eq. [47], [50]), pancreatic and bile secretions (eq. [109], [113], [125]), and gut wall abrasion (eq. [117], [121], [132],[133]). Parameters for the net secretion of endogenous losses were calibrated to the quantity of endogenous fat losses observed previously by Jørgensen *et al.* (37) and for protein reviewed by Jansman *et al.* (61). Contributions of the stomach, small intestine, and colon to total endogenous losses were assumed to be fixed based on data from Strathe *et al.* (27); Jansman *et al.* (61).

Colon

Enzymatically undigested nutrients in the small intestine entering the colon where they can be fermented by the residing microbiota. The fractional passage rate of digesta through the colon is based on the numerical mean of study averages reporting digesta retention times in the total tract of growing pigs (46; 53; 57; 62; 63; 64; 65; 66; 67)(39.6 ± 10.4 h, SD; PROC MEANS) minus the average retention time of digesta in the stomach and small intestine (Kc_o = 0.0298 /h; eq.[134], [137], [142], [147], [149], [152], [157], [160]). Fermentation of NSP and starch in the hindgut yields

microbial biomass (eq. [138], [143], [153]), short-chain fatty acids (eq. [139], [144], [154]), and fermentation gases (eq. [140], [145], [155]). Synthesis of microbial biomass is based on fermentation of carbohydrates (TN, TS, GL), which in turn is based on principles of NSP and starch fermentation in the rumen of dairy cows ([68] referenced in [69]). Synthesis of microbial biomass per unit TN or TS is calculated to be 0.35 g MB/g fermented, of which 62.5% is microbial protein (i.e. Ctn_mb= Cts_mb= 0.35 × (1 − 0.625) = 0.13 g MB/g TN or TS; Ccp_mb = 0.13/(0.35 × 0.625) =1.66). The synthesis of short-chain fatty acids is assumed to occur in a fixed ratio (65:25:10 for acetate: propionate: butyrate, on molar-basis). This ratio, however, can vary between substrate entering the colon, e.g. starch is known to increase the relative production of butyrate. The requirement for nitrogen associated with synthesis of microbial protein is delivered through dietary and endogenous protein entering the colon (eq. [135]), and whenever insufficient, from urea influx from blood (eq. [163]) which was assumed to be available in non-limiting quantities.

MODEL EVALUATION

Behaviour and sensitivity analysis

Sensitivity of model output parameters to changes in driving variables (i.e. behaviour analysis), and to changes in selected model parameters (i.e. sensitivity analysis) were evaluated. Selected model output parameters of interest are the absorption kinetics (i.e. TOP and AUC) of glucose and amino acids and the apparent ileal digestibility of protein, starch, and fat. Model driving variables are changed to lower and upper ranges of the respective parameters relevant in practical growing-pig diets (i.e. user defined variables; Δ ± values per variable representing a practical relevant range of physicochemical properties of feed ingredients and/or diets), whereas constant model parameters are changed up- and downwards by 25% compared to the initial setting (i.e. Δ ± 25%; Table 5.3). The sensitivity per in- and output parameter (i) is expressed as:

$$S_{y,x_i} = \frac{\frac{(y_{i\pm\Delta} - y_i)}{y_i} \times 100\ \%}{\frac{|x_{i\pm\Delta} - x_i|}{x_i} \times 100\ \%}$$

Where, y = the value of the selected model output parameter, and x = the value of the driving variable or constant model parameter, i = the value according to the initial settings without change, and i±Δ = the value according to the changed setting. The results can be interpreted as follows: 1) a negative S_{y,x_i} indicate that the output parameter decreases as a result of changing the input parameter, whereas a positive S_{y,x_i} indicate that the output parameters increases as a result of changing the input parameter, and 2) as $|S_{y,x_i}|$ is a relative value it ranges from 0 to 1,

Table 5.2. Parameter values of the model simulating digestion kinetics in growing pigs

Parameter	Description	value	unit
Diet			
SFEED	Clock-time of initial meal	08:00	h
IFEED	Meal interval	12	h
TFEED	Duration of feed intake	0.25	h
FFEED	Number of meals per day	2	/d
Passage			
Clqgs_lqi1	Intercept of equation for the fractional passage rate of liquids from the stomach	1.6	h
Cslgs_slqi1	Intercept of equation for the fractional passage rate of solids from the stomach	3.2	h
Ki_c	Fractional passage rate of digesta through the small intestine	0.373	/h
Ci1_i2	Proportion of the proximal small intestine relative to total small intestine based on mean retention time	0.21	
Kc_o	Fractional passage rate of digesta through the colon	0.0298	/h
Hydrolysis and fermentation			
kdfi_fai	Fractional rate of fat hydrolysis in the small intestine	4.25	/h
Kdtscc	Fractional rate of starch fermention in the colon	14.88	/h
Cxc_mbcc	Conversion of x (i.e. ts or tn) into microbial biomass	0.133	g/g
Cxc_sfcc	Conversion of x (i.e. ts or tn) into short-chain fatty acids	0.445	g/g
Cxc_fgcc	Conversion of x (i.e. ts or tn) into fermentation gasses	0.201	g/g
Ccp_mb	Unit of protein required per unit of microbial growth	1.66	g/g
Endogenous secretions			
Cepnp_gs	Endogenous protein (N * 6.25) secretion in the stomach	0.0024	g/g OM
Cepnp_i1	Endogenous protein (N * 6.25) secretion by the pancreas into the proximal small intestine	0.0047	g/g OM
Cepnp_gb	Endogenous protein (N * 6.25) secretion by bile into the proximal small intestine	0.0063	g/g DMI
Cepnp_i2	Endogenous protein (N * 6.25) loss due to cell abrasion in the distal small intestine	0.06	g/g OM
Cepnp_cc	Endogenous protein (N * 6.25) loss due to cell abrasion in the colon	0.059	g/g OM
Cefgb	Endogenous fat secretion by bile into the proximal small intestine	0.0237	g/g DMI
Cep_np_gs	Fraction of protein of total endogenous nitrogen (protein/6.25) secretion in the stomach	0.5	g/g
Cep_np_i1	Fraction of protein of total endogenous nitrogen (protein/6.25) secretion by the pancreas	0.7	g/g
Cep_np_gb	Fraction of protein of total endogenous nitrogen (protein/6.25) secretion by bile	0.65	g/g
Cep_np_i2	Fraction of protein of total endogenous nitrogen (protein/6.25) secretion in the distal small intestine	0.6	g/g
Cep_np_cc	Fraction of protein of total endogenous nitrogen (protein/6.25) secretion in the colon	0.5	g/g
Cepnpi_epnpbl	Fraction of total endogenous protein secretion reabsorbed in the small intestine	0.7	g/g
Cefi_efbl	Fraction of total endogenous fat secretion reabsorbed in the small intestine	0.8	g/g
Absorption			
Kaai_aabl	Fractional rate of amino acid absorption from the intestine	250	/h
Kgli_glbl	Fractional rate of glucose absorption from the intestine	500	/h
Kfai_fabl	Fractional rate of fatty acid absorption from the intestine	150	/h
Ksfc_sfbl	Fractional rate of short-chain fatty acid absorption from the intestine	150	/h

whereby a value of 0 indicates there is no response in the output parameter after changing the input parameter, while a value of 1 indicates that the relative change in the output parameter is

equal to the relative change in the input parameter. Hence, the higher the absolute value, the more sensitive the output parameter is to a change in the input parameter.

Model predictions

Model predictions of nutrient digestion kinetics are evaluated using independent *in vivo* data. Focus was on the prediction of starch and protein digestion kinetics, and thereby of glucose and amino acid absorption kinetics. While net portal appearance of nutrients can affected by first-pass metabolism [70], it is the only data available to evaluate the predicted absorption kinetics by the model. Hence, data were used from studies covering nutrient fluxes or changes in nutrient concentrations in portal and/or systemic blood in growing-finishing pigs. Model evaluation comprises predictions of the apparent ileal digestibility of protein [71] and fat ([12; 72; 73]; Figure 5.6). The kinetics of glucose and amino acid absorption are evaluated using predictions of the TOP and extent absorption (i.e. area-under-curve: AUC; [170][172]) of absorption. The following data from *in vivo* studies, used for model evaluation the absorption kinetics, were collected or calculated: 1) nutrient and feed ingredient composition of the diet, and feed intake level. These are used as model driving variables; 2) cumulative postprandial absorption of glucose and of amino acids; 3a) if presented: the TOP absorption of glucose and/or amino acid, preferably based on porto-arterial nutrient concentration differences (i.e. net portal appearance) or portal fluxes, otherwise on either portal or systemic blood nutrient concentrations. If TOP as mentioned under 3a was not presented: 3b) TOP of absorption was estimated by fitting the derivative of a generalised Michaelis-Menten equation[74] or a higher-order polynomial function (third, or fifth degree for data from Agyekum *et al.*[75]) using non-linear regression. Evaluation of model predictions were carried out based on root mean square prediction errors (RMSPE) [76] and Lin's concordance correlation coefficient [77], as explained in Ellis *et al.* [78].

RESULTS

Model behaviour and sensitivity

Table 5.3 presents the behaviour of model output parameters to changes in driving variables and sensitivity of outputs to model parameters. The TOP of nutrient absorption, especially of amino acids, is sensitive to the kinetics of digesta passage. Changes in the fractional passage rate in the stomach evoke a greater change than that in the small intestine (-0.28 and 0.34 % *v.* -0.07 and 0.07 % per % of change). The TOP of glucose absorption is not sensitive to changes in the fractional passage rate of digesta in the small intestine. Furthermore, the TOP of amino acids absorption is more sensitive than glucose for changes in feed intake (-0.44 and 0.21 % *v.* -0.9 and

0.09 % per % of change). The TOP of nutrient absorption is also sensitive to changes in nutrient hydrolysis kinetics. The TOP of glucose absorption is sensitive to changes in the fractional rate of statch hydrolysis, especially for downward changes (i.e. kds_gl: 1.31 [downward] to -0.03 [upward] % per % of change). For amino acids, the TOP and extent of absorption is sensitive to changes in protein hydrolysis kinetics in the stomach (0.03 to 0.1 % per % of change), and especially of that in the small intestine (-0.2 to 0.38 % per % of change). Similarly, the extent of ileal protein digestibility is sensitive to changes in the kinetics of digesta passage and protein hydrolysis in the stomach, though to a more limited extent (-0.02 to 0.01 % per % of change) compared to that in the small intestine (-0.15 to 0.09 % per % of change). In addition, apparent ileal protein digestibility is sensitive to changes in the size of the undegradable protein fraction of the diet (-0.08 to 0.08 % per % of change) and to changes in the net ileal endogenous protein excretions (-0.06-0.07 % per % of change). For fat, its apparent ileal digestibility is most sensitive to changes in endogenous fat absorption (-0.44 to 0.44 % per % of change), followed by endogenous fat secretion (-0.11 to 0.11 % per % of change), fat hydrolysis (0.04 to -0.07 % per % of change), digesta passage in the small intestine (-0.06 to 0.06 % per % of change), and finally the undegradable fat fraction (-0.01 to 0.02% per % of change).

Model predictions

For starch digestion kinetics, results of the evaluation of glucose absorption against independent (net) portal, arterial or systemic blood studies (studies = 12, dietary treatment means = 33; Supplementary Table S5.2) is provided in Figure 5.4 and Table 5.4. The simulated extent of glucose absorption ranges from 13% in high amylose maize starch to 99% in regular maize starch. The simulated TOP of glucose absorption ranged from 25 min for a soluble diet containing maltodextrin as "starch" source [70] to 98 min for a slowly degradable native tapioca starch source [79]. The extent of glucose absorption measured *in vivo,* are overestimated by the model (69±30 *v.* 63±20 %, n = 16, RMSPE = 39% relative to observed mean), whereby most error originated from deviation of the regression slope from unity (52%) followed by random error (42%). For the TOP of glucose absorption, model predictions underestimate that of *in vivo* (44±15 *v.* 56±20 min, RMSPE = 39% relative to observed mean). The prediction error is for 65% random and for 31% due to bias.

Table 5.3. Sensitivity[1] of output parameters of the digestion model for growing pigs, i.e. time of nutrient peak absorption (TOP) and area under the curve (AUC) of amino acids and glucose (AA, GL), and apparent ileal digestibility(AID) of crude protein, starch, and fat (CP, TS, TF), to changes in model driving variables (i.e. user input) and constants compared to the reference simulation[2].

Model parameters[3]	Set	Change	TOP	AUC	TOP	AUC	AID		
			AA		GL		CP	TS	TF
Unit			Min	%	min	%	%	%	%
Reference value			58	88	37	98	83	98	86
Driving variable									
DMI	975	669	0.00	0.00	0.00	0.00	0.00	0.00	0.00
		1545	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Feed intake level(D_i)	1.90	1.30	-0.44	0.00	-0.09	0.00	0.00	0.00	0.00
		3.00	0.21	0.00	0.09	0.00	0.00	0.00	0.00
Diet solubility (Ds)	0.09	0.05	-0.04	0.00	0.00	0.00	0.00	0.00	0.00
		1.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Diet viscosity (RAV)	0.95	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
		18.5	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Crs_ts	0.01	0.00	0.00	0.00	0.00	0.01	0.00	0.01	0.00
		0.50	0.00	0.00	0.00	-0.01	0.00	-0.01	0.00
Kds_gl	1.52	0.20	0.00	0.00	1.31	-0.13	-0.02	-0.12	0.00
		10.0	0.00	0.00	-0.03	0.00	0.00	0.00	0.00
Cup_cp	0.07	0.00	0.02	0.08	0.00	0.00	0.08	0.00	0.00
		0.14	0.00	-0.08	0.00	0.00	-0.08	0.00	0.00
Csp_cp	0.17	0.08	0.10	0.00	0.00	0.00	0.00	0.00	0.00
		1.00	-0.04	0.00	0.00	0.00	0.00	0.00	0.00
Kipgs_spgs	0.24	0.03	-0.06	0.00	0.00	0.00	0.00	0.00	0.00
		0.63	0.03	0.00	0.00	0.00	0.00	0.00	0.00
Cdpgs_aai	0.38	0.14	0.25	-0.04	0.00	0.00	-0.04	0.00	0.00
		0.49	-0.30	0.04	0.00	0.00	0.04	0.00	0.00
Kdpi_aai	2.18	1.10	0.38	-0.14	0.00	0.00	-0.15	0.00	0.00
		5.00	-0.20	0.03	0.00	0.00	0.03	0.00	0.00
Cuf_tf	0.013	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.02
		0.10	0.00	0.00	0.00	0.00	0.00	0.00	-0.01
Kslgs_sli1	0.34	0.25	0.34	0.00	0.22	0.00	-0.01	0.00	0.00
		0.42	-0.28	0.00	-0.11	0.00	0.01	0.00	0.00
klqgs_lqi1	0.65	0.49	0.07	0.00	0.00	0.00	0.00	0.00	0.00
		0.81	-0.07	0.00	0.00	0.00	0.00	0.00	0.00
Model constants									
Ki_c	0.37	0.28	0.07	0.05	0.00	0.00	0.09	0.01	0.06
		0.47	-0.07	-0.09	0.00	-0.04	-0.09	-0.02	-0.06
Kdfi_fai	4.25	3.19	0.00	0.00	0.00	0.00	0.00	0.00	-0.07
		5.31	0.00	0.00	0.00	0.00	0.00	0.00	0.04
Kdfi_fai	4.25	3.19	0.00	0.00	0.00	0.00	0.00	0.00	-0.07
		5.31	0.00	0.00	0.00	0.00	0.00	0.00	0.04

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Total endogenous CP secretion	0.13	0.10	0.00	0.00	0.00	0.00	0.07	0.00	0.00
		0.17	0.00	0.00	0.00	0.00	-0.06	0.00	0.00
Cepnpi_epnpbl	0.70	0.53	0.00	0.00	0.00	0.00	-0.15	0.00	0.00
		0.88	0.00	0.00	0.00	0.00	0.15	0.00	0.00
Cef_gb	0.024	0.018	0.00	0.00	0.00	0.00	0.00	0.00	0.11
		0.030	0.00	0.00	0.00	0.00	0.00	0.00	-0.11
Cefi_efbl	0.80	0.60	0.00	0.00	0.00	0.00	0.00	0.00	-0.44
		1.00	0.00	0.00	0.00	0.00	0.00	0.00	0.44

Red boxes indicate that input and output parameters change in the same direction (i.e. increase – increase, decrease – decrease), blue boxes indicate that input and output parameters change in the opposite direction (i.e. increase - decrease, decrease - increase). The more saturated the colour, the more sensitive the output value to a change in input value.

[1] presented as percentage point change in output (y)/ percentage point change in model parameter (x), calculated as sensitivity(y, x) = (Δy/y)/(Δx/x).

[2] See Supplementary TableS5.4 for reference diet

[3] See Table 5.1 for model abbreviations

For protein, the evaluation of amino acid absorption kinetics against independent (net) portal, arterial or systemic blood studies (Supplementary Table S5.3) is presented in Figure 5.4 and Table 5.4. The simulated extent of amino acid absorption ranges from 80% in a soybean meal based diet [7] to 87% in a mixed protein diet [80]. The simulated TOP of amino acid absorption ranges from 27 min for a diet containing black soldier fly larvae protein to 76 min for a diet containing potato protein as main protein source. Based on limited data (n= 6 dietary treatment means), the extent of amino acid absorption is overestimated by the model (83± *v.* 63± %, RMSPE = 40%; Figure 5.5). Model predictions regarding the TOP of amino acid absorption is evaluated at two levels: against the complete validation dataset (studies = 8, dietary treatment means = 15), and against a selection of the dataset that contains only studies regarding the net portal appearance of amino acids (studies = 6, dietary treatment means = 8). Evaluation against the complete dataset indicated that the model severely underestimates the observed mean of and variation in TOP of amino acid absorption (60±14 *v.* 115±79 min, RMSPE = 85% relative to observed mean). Evaluation against the selected dataset indicated that the model adequately estimates the observed mean of TOP of amino acids, but not the variation in TOP (61±11 *v.* 58±34 min, RMSPE = 60% relative to observed mean). For the latter, the prediction error originates almost completely from random error (96%).

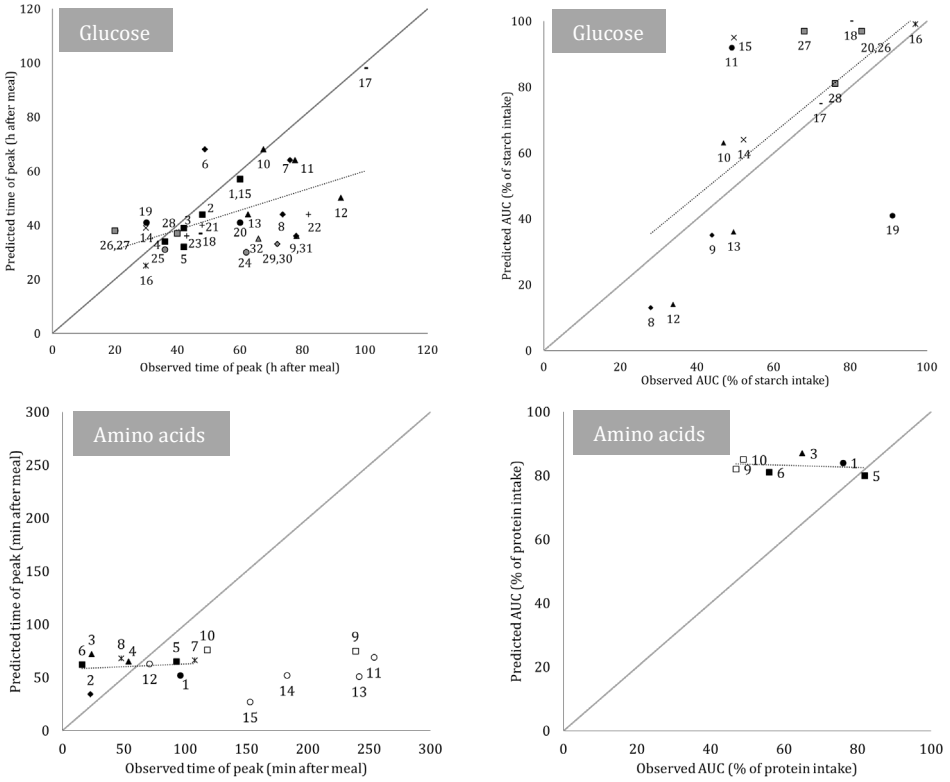

Figure 5.4. Predicted v. observed postprandial time of peak of absorption (left-panels), and area under curve (AUC) of postprandial appearance (right-panels) of glucose (top) and amino acids (bottom), using (portal) blood nutrient appearance studies. Symbols differ between studies, data labels represents treatment mean (see, for glucose: Supplementary Table S5.2, for amino acids: Supplementary Table S5.3), solid line represents y=x, dotted line represents regression line y=x.

The apparent ileal or faecal digestibility of protein (Figure 5.5) and fat (Figure 5.6) are, on average, overestimated by the model (protein: 70±5 *v.* 78±5 %, RMSPE=12%; fat: 82±5 *v.* 86±5 %, RMSPE=16%). In the case of protein, the prediction error is mainly due to bias (88%) followed by random error (12%), whereas for fat it is mainly due to random error (86%) followed by deviation of the regression slope from unity (8%).

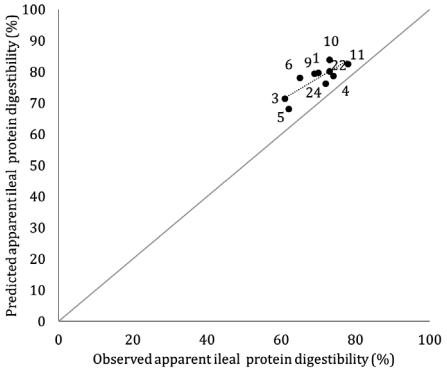

Figure 5.5. Comparing observed [71] and predicted values for apparent ileal crude protein digestibility (AID). Data labels refer to diet numbers as indicated by Just et al. [71]. Solid line represents y=x, dotted line represents regression line.

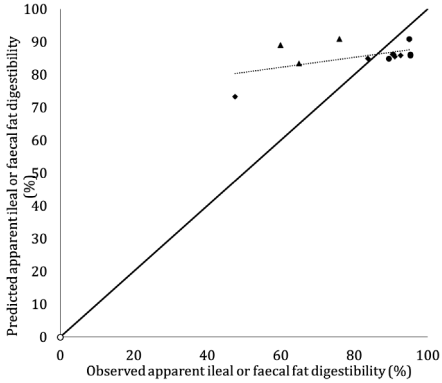

Figure 5.6. Comparing observed ileal (◆[72], ▲[73]) and faecal digestibility (●[12]) with predicted values for apparent ileal fat digestibility. Solid line represents y=x, dotted line represents regression line.

Table 5.4. Model validation parameters of the digestion model for growing pigs, presenting goodness of fit[1] of observed (obs) v. predicted (pred) postprandial time of peak (h) and area under the curve[2] (% of ingested) of glucose and amino acids absorption from the intestine, and apparent ileal protein digestibility (%).

Nutrient	Variable	Obs (SD)	Pred (SD)	R-sq[1]	RMSPE[1] (%)	ECT[1] (%)	ER[1] (%)	ED[1] (%)	CCC[1]	Cb[1]	v[1]	μ[1]
Glucose	Time of peak[3]	56 (20)	44 (15)	0.25	39	31	4	65	0.38	0.8	1.4	0.7
	Area under curve[4]	63 (20)	69 (30)	0.41	39	6	52	42	0.58	0.9	0.7	-0.2
Amino acids	Time of peak[5]	58 (34)	61 (11)	0.03	60	1	3	96	0.09	0.6	3.0	-0.1
	Time of peak[6]	115 (79)	60 (14)	0.00	85	32	2	67	0.00	0.2	5.8	1.7
	Area under curve[7]	63 (13)	83 (2)	0.03	40	69	0	27	-0.02	0.1	5.5	-3.7
Protein	Apparent ileal digestibility[8]	70 (5)	78 (5)	0.67	12	88	0	12	0.34	0.4	1.1	-1.7
Fat	Apparent ileal/faecal digetibility[9]	82 (15)	86 (4)	0.30	16	6	8	86	0.27	0.5	3.6	-0.4

[1] RMSPE = root mean square prediction error (as % of observed mean), ECT = error of overall bias, ER = error due to deviation of the regression slope from unity, ED = error due to disturbance (i.e. random error), where ECT, ER, and ED are expressed as % of total error, CCC = Lin's concordance correlation coefficient, Cb = bias correction faction, v = measure of scale shift, μ = measure of location shift (as presented by Ellis et al. [78]).

[2] Area under the curve calculated based on observed sampling time (varying from 5 to 12 h).

[3] Observed data(n=33) [19; 70; 75; 79; 80; 81; 82; 83; 84; 85; 86; 87; 88].

[4] Observed data (n=16)[19; 70; 79; 84; 85; 86; 87].

[5] Observed data based on net portal appearance of amino acids (n=8)[7; 75; 80; 89; 90]

[6] Observed data based under [5] plus studies considering arterial [79] or systemic venous [31] nutrient concentrations (n=15)

[7]Observed data (n=6) [7; 75; 79; 80; 90]

[8] Observed data (n=10)[71]

[9] Observed data (n=13)[12; 72; 73]

DISCUSSION

With the model described in this paper we aim to increase our understanding of the quantitative impact of variation in the kinetics of nutrient digestion on the kinetics of nutrient absorption. Focus was on variation in the kinetics of digesta passage and nutrient hydrolysis caused by physicochemical properties of the diet and constituting feed ingredients. Our ambition was to adequately predict variation in the kinetics of absorption of glucose and amino acids from the digestive tract of growing pigs that are fed diets composed of feed ingredients varying in physicochemical properties.

Digesta passage: from concept to model predictions

The absorption kinetics of nutrients is faster when nutrients pass the stomach at a higher fractional rate. In literature, this is observed when, for example, amino acid absorption kinetics is compared after ingestion of milk protein, and yoghurt and casein [91; 92]. In previous models, no variation in the passage rate of stomach digesta was considered [24; 25; 26; 27]. In contrast, in our current model, the fractional passage rates differs between solid and liquid phases of digesta and hence, insoluble and soluble nutrient fractions. By including other dietary factors that are known to affect gastric emptying (i.e. diet viscosity, diet solubility, and feed intake level) [2; 5; 29; 93; 94], the model is able to simulate variation in the kinetics of digesta passage in the stomach. For example, when simulating pigs fed diets varying in diet viscosity (0 – 18.5 RAV), diet solubility (2.6 to 100%) and at various feed intake levels (2 to 3.5 × ME_m), the model predicts variation the MRT of solids to range from 2.1 to 4.4 h, and of liquids from 0.7 to 3.2 h.

As a result of variation in the fractional passage rate of digesta in the stomach, some variation in the kinetics of glucose and amino acid absorption can be simulated. Sensitivity analysis of the model, however, revealed that a change in digesta passage in the stomach and small intestine only marginally affects the TOP of nutrient absorption. For example, increasing the MRT of solids in the stomach or digesta in the small intestine by roughly 1 h (see sensitivity analysis), the TOP of nutrient absorption is only delayed by 5 or 1-2 min, respectively. The latter is likely caused by representing passage of digesta using first-order kinetics. This causes a large fraction of ingested nutrients to enter the small intestine at onset of stomach emptying. These nutrients, in turn, can readily be hydrolysed and absorbed in the small intestine after a meal, thereby causing the general right skewed curve of the absorption of nutrients. This right skewed curve, causes 'stiffness' in the prediction of variation in TOP of nutrient absorption by the model. To better simulate the physiological nature of stomach emptying, representation of the different stomach emptying phases after a meal [42] might be considered (e.g. [24]). Moreover, instead of using first-

order kinetics it may be interesting to use higher-order kinetics such as a power-law model[95; 96]. The latter contains a shape parameter than can be used to adjust the stomach emptying curve, for example, to represent an initial period of delay or slower emptying. For the small intestine, representing digesta passage using first-order kinetics conflicts with the mechanism of plug flow in this segment [97]. While first-order kinetics has been applied in previous models [25; 27], others have simulated digesta passage in the small intestine according to the mechanism of peristaltic waves, which may be considered for model improvements [22; 24; 26].

Hydrolysis of macronutrients

The hydrolysis kinetics of nutrients affect the kinetics of nutrient absorption. As, for example, shown by Giuberti *et al.* [82] who observed that the absorption kinetics of glucose differs when pigs were fed starch sources varying in the rate and extent of *in vitro* hydrolysis. In the model, most of the variation in the absorption kinetics of glucose and amino acids is caused by variation in the hydrolysis kinetics of protein and starch. The hydrolysis kinetics of protein and starch are derived from *in vitro* assays [28; 31; 32; 33; 34], and parameters, as such, were used as model input variables. Directly using the *in vitro* hydrolysis kinetics of starch, however, resulted in model predictions whereby starch digestibility by the end of the mall intestine were structurally lower than observed *in vivo.* Hence, *in vitro* fractional starch hydrolysis rates seem to underestimate those occurring *in vivo*. Therefore, the relationship between the *in vitro* and *in vivo* fractional rate of hydrolysis was studied. The *in vivo* fractional rates of hydrolysis were estimated based on *in vivo* studies harvesting digesta from various small intestinal segments and analysing the MRT of digesta and starch digestion in each segment. The relation between *in vitro* and *in vivo* fractional hydrolysis rates resulted to be non-linear, i.e. the values *in vivo* increased with increasing values *in vitro* until a plateau was reached. An explanation for this could be that the extent of starch digestibility *in vivo* is limited by factors other than the potential hydrolysis of a single ingredient as measured *in vitro*, such as the kinetics of digesta passage and factors that induce nutrient-nutrient/matrix interactions [98].

For protein, there appeared no relationship between *in vitro* and *in vivo* fractional hydrolysis rates in the small intestine, although in contrast to starch, values observed *in vitro* seem higher than values *in vivo* (see Figure 5.3). The *in vitro* assays were designed to study the potential rate of protein hydrolysis as an inherent property of the feed ingredients, by grinding feed ingredients to fine particles (<1 mm), providing an overload of enzymes, and working in a diluted system [33]. As such, the *in vitro* assay might have provided more optimal conditions for the hydrolysis of protein compared to *in vivo*. Due to the lack of a relationship, *in vitro* fractional rates for protein were directly used as model input variables, in contrast to starch. Sensitivity analysis of the model

pointed out that the kinetics of amino acid absorption is more affected by the fractional rate of protein hydrolysis in the small intestine. In addition, also the direct transition of proteins into amino acids after entering the small intestine, as observed *in vitro* [33], affects the kinetics amino acid absorption. This is likely a result of partial protein digestion in the stomach, which can yield already some free amino acids and di- and tripeptides [4]. These observations indicate the importance of protein hydrolysis kinetics in the small intestine as elements to consider during evaluation of model predictions regarding the absorption kinetics of amino acids, as discussed below.

Fat hydrolysis and NSP fermentation were simulated by the model. The digestion of fat and NSP (Figure 5.7) yields (short-chain) fatty acids, thereby forming an undeniable energy source to the animal which can be used during post-absorptive metabolism. Variation and further validation of the kinetics of fat hydrolysis and NSP fermentation of diets varying in feed ingredients should be considered for model improvements.

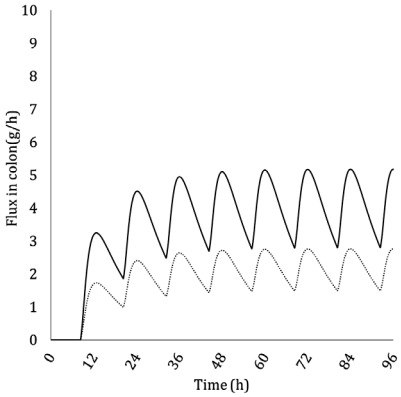

Figure 5.7. Simulated flux of short-chain fatty acid (solid line) and microbial biomass (dotted line) production in the colon of a pig (50 kg body weight) fed 975 g DM/d of a practical reference diet (i.e. LS diet [2]; see Supplementary TableS5.4) consisting of wheat (37%), maize (31%), rapeseed meal (14%), soybean meal (10%), sugar beet pulp (1.5%) and soybean oil (1.9%), with a nutrient content of (on dry matter basis): starch (54%), protein (22%), fat (5%), and NSP (19%).

Kinetics of starch and protein digestion

Net portal appearance of nutrients is commonly accepted as the ideal measure of absorption kinetics and was hence used to evaluate model predictions. Results of the evaluation indicated that most of the variation between observed and predicted TOP of nutrient absorption is random (65-67%). Random error is the error for which models inherently cannot account for, as models are parameterised based on relations found in observed data. Part of the random error, however, may be due to experimental error for which observed data in theory could be corrected for. In our case, however, the net portal appearance data used for model evaluation contained insufficient number of studies and treatments to perform a meta-analysis to account for this, between-study, variation.

Regarding glucose, the extent and TOP of absorption, as predicted by the model, fitted well with *in vivo* data on portal glucose appearance and systemic glucose concentrations when diets were

simulated varying in starch source and physicochemical properties. When evaluating the ability of the model to predict the extent of starch digestion, the model consistently predicted ileal starch digestion to be complete, whereas *in vivo* observations of the cumulative net portal glucose appearance were lower. Model predictions will predict close to 100% glucose absorption unless the starch source consists of a significant resistant fraction, whereas the appearance *in vivo* portal and system blood are less likely to reach 100%, as glucose can disappear in first-pass/ or whole body metabolism [99; 100]. This likely causes the regression slope in the observed-predicted plot to deviate from unity. For the TOP of glucose absorption, overall bias dominated the prediction error, with the model generally underpredicting the TOP. Based on the sensitivity analysis, priority should be given to reconsider passage of digesta in the stomach as presented in the model, followed by the relation between *in vitro* and *in vivo* fractional starch hydrolysis rates. For the latter it can be seen in Figure 5.3 (left panel) that the *in vivo* fractional rate of starch hydrolysis may be over predicted for *in vitro* slowly degradable starch sources (i.e. low fractional rates). To improve model predictions on the TOP of glucose absorption, it is worthwhile considering to use part of the validation dataset to estimate the *in vivo* fractional rates based on the kinetics of glucose appearance instead of on the kinetics of starch digestion.

Regarding protein, 'goodness of fit' of model predictions of the TOP of amino acid absorption depends on the dataset used for evaluation. An interesting range in protein sources was covered [31; 79] in the complete dataset, representing a wide range in TOP of amino acid absorption. Interestingly, all observation with late TOP of amino acid absorption (e.g. >120 min after the meal), which could not be adequately predicted by the model, were covered by two studies in which amino acid concentrations were measured in arterial [79] and systemic [31] blood. Such late peaks could only be predicted by the model by drastically reducing the fractional hydrolysis rates of protein, in turn, lowering the extent of protein digestion to unrealistic values (~10%). Further investigations showed that the estimated TOP of net portal amino acid appearance is generally earlier than that observed in either portal, arterial, or systemic blood (based on data from [7; 75; 80; 89; 90]). Differences range from 0 to ~100 min, depending on study and diet (data not shown), and are likely explained by first-pass and/or whole body metabolism [101]. These results indicate that, the TOP of amino acid appearance observed in arterial or systemic blood are not representative for that of net portal amino acid appearance, and they are therefore considered inadequate for model evaluation. Hence, despite the interesting range in protein sources that were studied [31; 79], the model should be evaluated only against studies that cover the net portal appearance kinetics of amino acids. Considering the latter, evaluation of the model showed that the mean TOP of amino acid appearance is adequately predicted, albeit based on a small number of observed data. The variation in TOP of amino acid absorption between protein sources is, however, poorly

predicted. This implies that the variation in protein hydrolysis kinetics observed *in vitro*, does not reflect the variation observed *in vivo.*

When simulating the digestion of a 'slow' or 'fast' degradable protein source, e.g. potato protein and whey powder [33], a difference in TOP of amino acids absorption of ~1 h is predicted (81 *v*. 23 min). Such variation, however, is not predicted when simulating diets of the validation dataset. In this dataset, the soybean meal based diets in the studies from [7; 75] induced part of the observed variation in TOP of amino acid absorption. The TOP in these studies were observed to be 91 and 103 min, whereas the model predicts the TOP after 66 and 65 min. The discrepancy between observed and predicted absorption kinetics of amino acids can be caused by inadequate representation of digesta passage kinetics (as explained earlier), overestimation of *in vivo* protein hydrolysis kinetics, and/or omitting the effects of gut metabolism. Based on data in Figure 5.3 (right panel) and the overestimation of apparent ileal protein digestibility compared to observed data, it seems that *in vivo* protein hydrolysis kinetics in the small intestine are overestimated by those measured *in vitro*. However, overestimation of apparent ileal protein digestibility may have also been caused by underestimation of the degradable protein fraction or endogenous protein losses. Reducing fractional rate of protein hydrolysis in the small intestine and omitting the direct appearance of amino acids after proteins enter the stomach, delayed the predicted TOP of amino acid absorption to 91 min (i.e. by adjusting Kdpi_aai from 2.1 to 1.2 /h, and Cdpgs_aai from 28 to 0 %). The resulting extent of digestion at the end of the small intestine dropped below that observed (~70% predicted *v.* 80-81% observed [12]). Hence, it is not likely for the discrepancy in observed and predicted absorption kinetics of amino acids to be only caused by overestimation of the kinetics of protein hydrolysis. When comparing nutrient absorption kinetics with the kinetics of net portal amino acid appearance, there is a differences caused by first-pass metabolism by gut tissue. It is known that the gut tissue is metabolic highly active, using and synthesizing amino acids and glucose [70] and it is postulated to hold a labile protein pool [102] in which amino acids and proteins can be temporarily stored. Hence, although net portal appearance is the closest estimation for amino acid absorption from the gut, the absorption kinetics of amino acids can be affected by gut metabolism which is not accounted for by the model. Kinetics on protein hydrolysis, digesta passage as well as gut metabolism may require modification to reduce the discrepancy between observed and predicted variation in TOP of amino acid absorption. Unfortunately, it appears that the availability of good data is limiting model development in this area. Studies in which the net portal appearance of amino acids is measured following a meal containing different protein sources are notorious for their large experimental error. A meta-analysis approach would allow to account for between study variation, but would also require the same protein sources to be tested in multiple studies. The data available is too limited to conclude which element contributes the most to the discrepancy

between the observed and predicted kinetics of amino acid absorption. Moreover, to gain insight in the relation between the kinetics of overall protein digestion and protein hydrolysis, as well as, the relation between *in vitro* and *in vivo* hydrolysis kinetics, a more extensive dataset is required. Such a dataset ideally covers data regarding the net portal appearance of amino acids in pigs fed diets varying in 'slow' and 'fast' *in vitro* degradable protein sources, whereby also passage kinetics of digesta and the extent of ileal protein digestibility are quantified.

In this paper we introduced a nutrient-based dynamic mechanistic digestion model for growing pigs. The model simulates the digestion of nutrients inside the gastrointestinal tract. As nutrient hydrolysis kinetics varies due to their feed ingredient origin, data from *in vitro* assays were used to estimate nutrient hydrolysis kinetics. Furthermore, variation in the kinetics of digesta passage due to dietary physicochemical properties were included. Based on these elements, the model predicts variation in absorption kinetics of nutrients, taking into account kinetics of nutrient hydrolysis and physicochemical properties of the diet and constituent feed ingredients. Model predictions of nutrient absorption kinetics and the extent of nutrient digestion were compared with independent data on the absorption kinetics of nutrients *in vivo.* Model predictions indicated that the data from arterial or systemic blood studies are unsuited for estimation of the net portal appearance of nutrients. Evaluation of the model indicated adequate predictions of glucose absorption kinetics when simulating diets varying in physicochemical properties and starch sources. The extent of small intestinal protein digestion was adequately predicted, but variation in the kinetics of amino acid absorption between protein sources could, despite adequate mean predictions, not be predicted by the model. It was concluded that adequate data are missing for model calibration. The model can be used to gain insight in the quantitative impact of variation in the kinetics of nutrient digestion, induced by dietary feed ingredients and physicochemical properties, on absorption kinetics of nutrients.

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Supplementary Table S5.1. Mathematical equations of the nutrient-based dynamic mechanistic digestion model for growing pigs.

Diet and feed intake		
Diet viscosity:		
Auxilliary equation	Dr=30.33*exp(0.0693*RAV)	[1]
Feed intake		
Auxilliary equation	mealsize=DMI/FFEED	[2]
Auxilliary equation[1]	meal= (mealsize/0.25)*PULSE(SFEED, IFEED, TFEED)	[3]
Digesta passage kinetics		
Stomach passage rate		
Auxilliary equation	if (meal.GT.0.0) then	
	Klqgs_lqi1=0.0	
	Kslgs_sli1=0.0	
	Else	
Auxilliary equation	Klqgs_lqi1=1/(Clqgs_lqi1+(-1.2+1.9158*exp(-20.12*exp(-1.7062*D_i)))+(0.87*exp(-(((0.185- Ds)^2)/(2*0.052^2))))+(1.5*0.00174*Dr))	[4]
	Kslgs_sli1=1/(Cslgs_sli1+(-1.3+1.9158*exp(-20.12*exp(-1.7062*D_i)))+(0.87*exp(-(((0.185- Ds)^2)/(2*0.052^2)))))	[5]
	Endif	
Small intestine passage rate		
Auxilliary equation	Ki1_i2=1/(Ci1_i2*(1/Ki_c))	[6]
	Ki2_cc=1/((1-Ci1_i2)*(1/Ki_c))	[7]
Stomach		
Total dietary protein		
Input	Fcpd_cpgs=meal*Ccp_d	[8]
Differential equation	dQcpd=Fcpd_cpgs	[9]
Undegradable dietary protein (Qupgs)		
Input	Fupd_upgs=meal*Ccp_d*Cup_cp	[10]
Output	Fupgs_upi1=Qupgs*Kslgs_sli1	[11]
Differential equation	dQupgs=Fupd_upgs-Fupgs_upi1	[12]
Insoluble dietary protein (Qipgs)		
Input	Fipd_ipgs=meal*Ccp_d*Cip_cp	[13]
Output	Fipgs_dpi1=Qipgs*Kslgs_sli1	[14]
	Fipgs_spgs=Qipgs*Kipgs_spgs	[15]
Differential equation	dQipgs=Fipd_ipgs-Fipgs_dpi1-Fipgs_spgs	[16]
Soluble dietary protein (Qspgs)		
Input	Fipgs_spgs=Qipgs*Kipgs_spgs	[15]
	Fspd_spgs=meal*Ccp_d*Csp_cp	[17]
Output	Fspgs_dpi1=Qspgs*Klqgs_lqi1	[18]
Differential equation	dQspgs=Fspd_spgs+Fipgs_spgs-Fspgs_dpi1	[19]

Amino acids (Qaags)		
Input	Faad_aags=meal*Caa_d	[20]
Output	Faags_aai1=Qaags*Klqgs_lqi1	[21]
Differential equation	dQaags=Faad_aags-Faags_aai1	[22]
Total starch		
Input	Ftsd_tsgs=meal*Cts_d	[23]
Differential equation	dQtsd=Ftsd_tsgs	[24]
Degradable starch (Qdsgs)		
Input	Fdsd_dsgs=meal*Cts_d*Cds_ts	[25]
Output	Fdsgs_dsi1=Qdsgs*Kslgs_sli1	[26]
Differential equation	dQdsgs=Fdsd_dsgs-Fdsgs_dsi1	[27]
Resistant starch (Qrsgs)		
Input	Frsd_rsgs=meal*Cts_d*Crs_ts	[28]
Output	Frsgs_rsi1=Qrsgs*Kslgs_sli1	[29]
Differential equation	dQrsgs=Frsd_rsgs-Frsgs_rsi1	[30]
Glucose (Qglgs)		
Input	Fgld_glgs=meal*Cgl_d	[31]
Output	Fglgs_gli1=Qglgs*Klqgs_lqi1	[32]
Differential equation	dQglgs=Fgld_glgs-Fglgs_gli1	[33]
Total fat		
Input	Ftfd_tfgs=meal*Ctf_d	[34]
Differential equation	dQtfd=Ftfd_tfgs	[35]
Undegradable fat (Qufgs)		
Input	Fufd_ufgs=meal*Ctf_d*Cuf_tf	[36]
Output	Fufgs_ufi1=Qufgs*Kslgs_sli1	[37]
Differential equation	dQufgs=Fufd_ufgs-Fufgs_ufi1	[38]
Degradable fat (Qdfgs)		
Input	Fdfd_dfgs=meal*Ctf_d*Cdf_tf	[39]
Output	Fdfgs_dfi1=Qdfgs*Kslgs_sli1	[40]
Differential equation	dQdfgs=Fdfd_dfgs-Fdfgs_dfi1	[41]
Non-starch polysaccharides (Qtngs)		
Input	Ftnd_tngs=meal*Ctn_d	[42]
Output	Ftngs_tni1=Qtngs*Kslgs_sli1	[43]
Differential equation	dQtngs=Ftnd_tngs-Ftngs_tni1	[44]
Organic matter		

Auxilliary equation	Fomgs_omi1=Fupgs_upi1+Fipgs_dpi1+Fspgs_dpi1+Fdsgs_dsi1+Frsgs_rsi1+Fufgs_ufi1+Fdfgs_dfi1+Ftngs_tni1	[45]
	dQomgs=dQupgs+dQipgs+dQspgs+dQaags+dQdsgs+dQrsgs+dQglgs+dQufgs+dQdfgs+dQtngs	[46]

Endogenous protein (Qepgs)

Input	Fepgs=(Cepnp_gs*Cep_np_gs)*Qomgs	[47]
Output	Fepgs_epi1=Qepgs*Klqgs_lqi1	[48]
Differential equation	dQepgs=Fepgs-Fepgs_epi1	[49]

Endogenous non-protein nitrogen (Qnpgs)

Input	Fnpgs=(Cepnp_gs*(1-Cep_np_gs))*Qomgs	[50]
Output	Fnpgs_npi1=Qnpgs*Klqgs_lqi1	[51]
Differential equation	dQnpgs=Fnpgs-Fnpgs_npi1	[52]

Small intestine 1 and 2 (i1 and i2)

Undegradable dietary protein in i1 (Qupi1)

Input	Fupgs_upi1=Qupgs*Kslgs_sli1	[11]
Output	Fupi1_upi2=Qupi1*Ki1_i2	[53]
Differential equation	dQupi1=Fupgs_upi1-Fupi1_upi2	[54]

Undegradable dietary protein in i2 (Qupi2)

Input	Fupi1_upi2=Qupi1*Ki1_i2	[53]
Output	fupi2_tpcc=Qupi2*Ki2_cc	[55]
Differential equation	dQupi2=Fupi1_upi2-fupi2_tpcc	[56]

Degradable dietary protein in i1 (Qdpi1)

Input	Fipgs_dpi1=Qipgs*Kslgs_sli1	[14]
	Fspgs_dpi1=Qspgs*Klqgs_lqi1	[18]
Output	Fdpi1_aai1=Qdpi1*Kdpi1_aai1	[57]
	Fdpi1_dpi2=Qdpi1*ki1_i2	[58]
Differential equation	dQdpi1=((Fipgs_dpi1+Fspgs_dpi1)*(1-Cdpgs_aai))-Fdpi1_aai1-Fdpi1_dpi2	[59]

Degradable dietary protein in i2 (Qdpi2)

Input	Fdpi1_dpi2=Qdpi1*ki1_i2	[58]
Output	Fdpi2_aai2=Qdpi2*Kdpi2_aai2	[60]
	fdpi2_tpcc=Qdpi2*Ki2_cc	[61]
Differential equation	dQdpi2=Fdpi1_dpi2-Fdpi2_aai2-fdpi2_tpcc	[62]

Amino acids in i1 (Qaai1)

Input	Fipgs_dpi1=Qipgs*Kslgs_sli1	[14]
	Fspgs_dpi1=Qspgs*Klqgs_lqi1	[18]
	Faags_aai1=Qaags*Klqgs_lqi1	[21]
	Fdpi1_aai1=Qdpi1*Kdpi1_aai1	[57]
Output	Faai1_aabl=Qaai1*Kaai1_aabl	[63]
	Faai1_aai2=Qaai1*Ki1_i2	[64]
Differential equation	dQaai1=Faags_aai1+((Fipgs_dpi1+Fspgs_dpi1)*Cdpgs_aai)+Fdpi1_aai1-Faai1_aabl-Faai1_aai2	[65]

Amino acids in i2 (Qaai2)		
Input	Fdpi2_aai2=Qdpi2*Kdpi2_aai2	[60]
	Faai1_aai2=Qaai1*Ki1_i2	[64]
Output	Faai2_aabl=Qaai2*Kaai2_aabl	[66]
	faai2_tpcc=Qaai2*Ki2_cc	[67]
Differential equation	dQaai2=Faai1_aai2+Fdpi2_aai2-Faai2_aabl-faai2_tpcc	[68]
Degradable starch in i1 (Qdsi1)		
Input	Fdsgs_dsi1=Qdsgs*Kslgs_sli1	[26]
Output	Fdsi1_gli1=Qdsi1*Kdsi1_gli1	[69]
	Fdsi1_dsi2=Qdsi1*Ki1_i2	[70]
Differential equation	dQdsi1=Fdsgs_dsi1-Fdsi1_gli1-Fdsi1_dsi2	[71]
Degradable starch in i2 (Qdsi2)		
Input	Fdsi1_dsi2=Qdsi1*Ki1_i2	[70]
Output	Fdsi2_gli2=Qdsi2*Kdsi2_gli2	[72]
	Fdsi2_dscc=Qdsi2*Ki2_cc	[73]
Differential equation	dQdsi2=Fdsi1_dsi2-Fdsi2_gli2-Fdsi2_dscc	[74]
Resistant starch in i1 (Qrsi1)		
Input	Frsgs_rsi1=Qrsgs*Kslgs_sli1	[29]
Output	Frsi1_rsi2=Qrsi1*Ki1_i2	[75]
Differential equation	dQrsi1=Frsgs_rsi1-Frsi1_rsi2	[76]
Resistant starch in i2 (Qrsi2)		
Input	Frsi1_rsi2=Qrsi1*Ki1_i2	[75]
Output	Frsi2_rscc=Qrsi2*Ki2_cc	[77]
Differential equation	dQrsi2=Frsi1_rsi2-Frsi2_rscc	[78]
Glucose in i1 (qgli1)		
Input	Fglgs_gli1=Qglgs*Klqgs_lqi1	[32]
	Fdsi1_gli1=Qdsi1*Kdsi1_gli1	[69]
Output	Fgli1_glbl=Qgli1*Kgli1_glbl	[79]
	Fgli1_gli2=Qgli1*Ki1_i2	[80]
Differential equation	dQgli1=Fglgs_gli1+(Fdsi1_gli1/0.9)-Fgli1_glbl-Fgli1_gli2	[81]
Glucose in i2 (qgli2)		
Input	Fdsi2_gli2=Qdsi2*Kdsi2_gli2	[72]
	Fgli1_gli2=Qgli1*Ki1_i2	[80]
Output	Fgli2_glbl=Qgli2*Kgli2_glbl	[82]
	Fgli2_glcc=Qgli2*Ki2_cc	[83]
Differential equation	dQgli2=Fgli1_gli2+(Fdsi2_gli2/0.9)-Fgli2_glbl-Fgli2_glcc	[84]
Undegradable fat in i1 (Qufi1)		
Input	Fufgs_ufi1=Qufgs*Kslgs_sli1	[37]

Output	Fufi1_ufi2=Qufi1*Ki1_i2	[85]
Differential equation	dQufi1=Fufgs_ufi1-Fufi1_ufi2	[86]

Undegradable fat in i2 (Qdufi2)

Input	Fufi1_ufi2=Qufi1*Ki1_i2	[85]
Output	Fufi2_ufcc=Qufi2*Ki2_cc	[87]
Differential equation	dQufi2=Fufi1_ufi2-Fufi2_ufcc	[88]

Degradable fat in i1 (Qdfi1)

Input	Fdfgs_dfi1=Qdfgs*Kslgs_sli1	[40]
Output	Fdfi1_fai1=Qdfi1*Kdfi1_fai1	[89]
	Fdfi1_dfi2=Qdfi1*Ki1_i2	[90]
Differential equation	dQdfi1=Fdfgs_dfi1-Fdfi1_fai1-Fdfi1_dfi2	[91]

Degradable fat in i2 (Qdfi2)

Input	Fdfi1_dfi2=Qdfi1*Ki1_i2	[90]
Output	Fdfi2_fai2=Qdfi2*Kdfi2_fai2	[92]
	Fdfi2_dfcc=Qdfi2*Ki2_cc	[93]
Differential equation	dQdfi2=Fdfi1_dfi2-Fdfi2_fai2-Fdfi2_dfcc	[94]

Fatty acid in i1 (Qfai1)

Input	Fdfi1_fai1=Qdfi1*Kdfi1_fai1	[89]
Output	Ffai1_fabl=Qfai1*Kfai1_fabl	[95]
	Ffai1_ai2=Qfai1*Ki1_i2	[96]
Differential equation	dQfai1=Fdfi1_fai1-Ffai1_fabl-Ffai1_ai2	[97]

Fatty acid in i2 (Qfai2)

Input	Fdfi2_fai2=Qdfi2*Kdfi2_fai2	[92]
	Ffai1_ai2=Qfai1*Ki1_i2	[96]
Output	Ffai2_fabl=Qfai2*Kfai2_fabl	[98]
	Ffai2_facc=Qfai2*Ki2_cc	[99]
Differential equation	dQfai2=Fdfi2_fai2+Ffai1_ai2-Ffai2_fabl-Ffai2_facc	[100]

Non-starch polysaccharides in i1 (Qtni1)

Input	Ftngs_tni1=Qtngs*Kslgs_sli1	[43]
Output	Ftni1_tni2=Qtni1*Ki1_i2	[101]
Differential equation	dQtni1=Ftngs_tni1-Ftni1_tni2	[102]

Non-starch polysaccharides in i2 (Qtni2)

Input	Ftni1_tni2=Qtni1*Ki1_i2	[101]
Output	Ftni2_tncc=Qtni2*Ki2_cc	[103]
Differential equation	dQtni2=Ftni1_tni2-Ftni2_tncc	[104]

Organic matter in i1 (Qomi1)

Auxilliary equation	Fomi1_omi2=Fupi1_upi2+Fdpi1_dpi2+Faai1_aai2+Fdsi1_dsi2+Frsi1_rsi2+Fgli1_gli2+Fufi1_ufi2+Fdfi1_dfi2+Ftni1_tni2	[105]

Differential equation	dQomi1=dQupi1+dQdpi1+dQaai1+dQdsi1+dQrsi1+dQgli1+dQufi1+dQdfi1+dQtni1	[106]

Organic matter in i2 (Qomi2)

Auxilliary equation	Fomi2_omcc=fupi2_tpcc+fdpi2_tpcc+faai2_tpcc+Fdsi2_dscc+Frsi2_rscc+Fgli2_glcc+Fufi2_ufcc+Fdfi2_dfcc+Ftni2_tncc	[107]
Differential equation	dQomi2=dQupi2+dQdpi2+dQaai2+dQdsi2+dQrsi2+dQgli2+dQufi2+dQdfi2+dQtni2	[108]

Endogenous protein in i1 (Qepi1)

Input	Fepgs=(Cepnp_gs*Cep_np_gs)*Qomgs	[47]
	Fepi1=Fomgs_omi1*(Cepnp_i1*Cep_np_i1)+Fdmi*(Cepnp_gb*Cep_np_gb)	[109]
Output	Fepi1_epi2=Qepi1*Ki1_i2	[110]
	Fepi1_epbl=(Fepgs_epi1+Fepi1)*Cepnpi_epnpbl	[111]
Differential equation	dQepi1=Fepgs_epi1+Fepi1-Fepi1_epi2-Fepi1_epbl	[112]

Non-protein nitrogen in i1 (Qnpi1)

Input	Fnpgs=(Cepnp_gs*(1-Cep_np_gs))*Qomgs	[50]
	Fnpi1=Fomgs_omi1*(Cepnp_i1*(1-Cep_np_i1))+Fdmi*(Cepnp_gb*(1-Cep_np_gb))	[113]
Output	Fnpi1_npi2=Qnpi1*Ki1_i2	[114]
	Fnpi1_npbl=(Fnpgs_npi1+Fnpi1)*Cepnpi_epnpbl	[115]
Differential equation	dQnpi1=Fnpgs_npi1+Fnpi1-Fnpi1_npi2-Fnpi1_npbl	[116]

Endogenous protein in i2 (Qepi2)

Input	Fepi1_epi2=Qepi1*Ki1_i2	[110]
	Fepi2=Fomi1_omi2*(Cepnp_i2*Cep_np_i2)	[117]
Output	fepi2_tpcc=Qepi2*Ki2_cc	[118]
	Fepi2_epbl=Fepi2*Cepnpi_epnpbl	[119]
Differential equation	dQepi2=Fepi1_epi2+Fepi2-fepi2_tpcc-Fepi2_epbl	[120]

Non-protein nitrogen in i2 (Qnpi2)

Input	Fnpi1_npi2=Qnpi1*Ki1_i2	[114]
Input	Fnpi2=Fomi1_omi2*(Cepnp_i2*(1-Cep_np_i2))	[121]
Output	fnpi2_tpcc=Qnpi2*Ki2_cc	[122]
	Fnpi2_npbl=Fnpi2*Cepnpi_epnpbl	[123]
Differential equation	dQnpi2=Fnpi1_npi2+Fnpi2-fnpi2_tpcc-Fnpi2_npbl	[124]

Endogenous fat in i1 (Qefi1)

Input	Fefi1=Fdmi*Cefgb	[125]
Output	Fefi1_efbl=Fefi1*Cefi_efbl	[126]
	Fefi1_efi2=Qefi1*Ki1_i2	[127]
Differential equation	dQefi1=Fefi1-Fefi1_efbl-Fefi1_efi2	[128]

Endogenous fat in i2 (Qefi2)

Input	Fefi1_efi2=Qefi1*Ki1_i2	[127]
Output	Fefi2_efcc=Qefi2*Ki2_cc	[129]

Differential equation	dQefi2=Fefi1_efi2-Fefi2_efcc	[130]
Auxilliary equation	dQefi2_efcc=Fefi2_efcc	[131]

Colon

Total protein (Qtpcc)

Input	fupi2_tpcc=Qupi2*Ki2_cc	[55]
	Fdpi1_dpi2=Qdpi1*ki1_i2	[58]
	Faai1_aai2=Qaai1*Ki1_i2	[64]
	fepi2_tpcc=Qepi2*Ki2_cc	[118]
	fnpi2_tpcc=Qnpi2*Ki2_cc	[122]
	Fepcc=Fomi2_omcc*(Cepnp_c*Cep_np_c)	[132]
	Fnpcc=Fomi2_omcc*(Cepnp_c*(1-Cep_np_c))	[133]
Output	Ftpcc_cpo=Qtpcc*Kc_o	[134]
	Ftpcc_mbcc=Rtpcc_mbcc	[135]
Differential equation	dQtpcc=(fupi2_tpcc+fdpi2_tpcc+faai2_tpcc+fepi2_tpcc+fnpi2_tpcc+Fepcc+Fnpcc)-Ftpcc_cpo-Ftpcc_mbcc	[136]

Total starch (Qtscc)

Input	Fdsi2_dscc=Qdsi2*Ki2_cc	[73]
	Frsi2_rscc=Qrsi2*Ki2_cc	[77]
Output	Ftscc_tso=Qtscc*Kc_o	[137]
	Ftscc_mbcc=Qtscc*Kdtscc*Ctscc_mbcc	[138]
	Ftscc_sfcc=Qtscc*Kdtscc*Ctscc_sfcc	[139]
	Ftscc_fgcc=Qtscc*Kdtscc*Ctscc_fgcc	[140]
Differential equation	dQtscc=Fdsi2_dscc+Frsi2_rscc-Ftscc_tso-Ftscc_mbcc-Ftscc_sfcc-Ftscc_fgcc	[141]

Glucose (Qglcc)

Input	Fgli2_glcc=Qgli2*Ki2_cc	[83]
Output	Fglcc_glo=Qglcc*Kc_o	[142]
	Fglcc_mbcc=Qglcc*Ctscc_mbcc	[143]
	Fglcc_sfcc=Qglcc*Ctscc_sfcc	[144]
	Fglcc_fgcc=Qglcc*Ctscc_fgcc	[145]
Differential equation	dQglcc=Fgli2_glcc-Fglcc_mbcc-Fglcc_sfcc-Fglcc_fgcc-Fglcc_glo	[146]

Total fat (Qtfcc)

Input	Fufi2_ufcc=Qufi2*Ki2_cc	[87]
	Fdfi2_dfcc=Qdfi2*Ki2_cc	[93]
	Ffai1_ai2=Qfai1*Ki1_i2	[96]
Output	Ftfcc_tfo=Qtfcc*Kc_o	[147]
Differential equation	dQtfcc=Fufi2_ufcc+Fdfi2_dfcc+Ffai2_facc-Ftfcc_tfo	[148]

Endogenous fat (Qefcc)

Input	Fefi2_efcc=Qefi2*Ki2_cc	[129]
Output	Fefcc_efo=Qefcc*Kc_o	[149]
Differential equation	dQefcc=Fefi2_efcc-Fefcc_efo	[150]
Auxilliary equation	dQefcc_efo=Fefcc_efo	[151]

Non-starch polysaccharides (Qtncc)		
Input	Ftni2_tncc=Qtni2*Ki2_cc	[103]
Output	Ftncc_tno=Qtncc*Kc_o	[152]
	Ftncc_mbcc= Qtncc*kdtncc*Ctncc_mbcc	[153]
	Ftncc_sfcc= Qtncc*kdtncc* Ctncc_sfcc	[154]
	Ftncc_fgcc= Qtncc*kdtncc* Ctncc_fgcc	[155]
Differential equation	dQtncc=Ftni2_tncc-Ftncc_mbcc-Ftncc_sfcc-Ftncc_fgcc-Ftncc_tno	[156]
Organic matter (Qomcc)		
Input	Fomi2_omcc=fupi2_tpcc+fdpi2_tpcc+faai2_tpcc+Fdsi2_dscc+Frsi2_rscc+Fgli2_glcc+Fufi2_ufcc+Fdfi2_dfcc+Ftni2_tncc	[107]
Output	Fomcc_omo=Ftpcc_cpo+Ftscc_tso+Fglcc_glo+Ftncc_tno+Fmbcc_mbo	[157]
Differential equation	dQomcc=Fomi2_omcc-Fomcc_omo	[158]
Microbial biomass (Qmbcc)		
Input	Fmbcc=Ftncc_mbcc+Ftscc_mbcc+Fglcc_mbcc	[159]
Output	Fmbcc_mbo=Qmbcc*kc_o	[160]
Differential equation	dQmbcc=Fmbcc-fmbcc_mbo	[161]
Auxilliary equations	Rtpcc_mbcc=Fmbcc*Ccp_mb	[162]
	Furbl_mbcc=Rtpcc_mbcc-Ftpcc_mbcc	[163]
	Fsfcc=Ftncc_sfcc+Ftscc_sfcc+Fglcc_sfcc	[164]
	Ffgcc=Ffgcc=Ftncc_fgcc+Ftscc_fgcc+Fglcc+sfcc	[165]
Auxilliary equations	Last meal (t = 92-104):	
	Qauc_aabl= Faai1_aabl+Faai2_aabl	[166]
	Qauc_glbl= Fgli1_glbl+Fgli2_glbl	[167]
	Terminal equations	
	TID (CP)=((Qcpd+Qaad)-(Qupi2_upcc+Qdpi2_dpcc+Qaai2_aacc))/(Qcpd+Qaad)	[168]
	AID (CP)=((Qcpd+Qaad)-(Qupi2_upcc+Qdpi2_dpcc+Qaai2_aacc+Qepnpi2_epnpcc))/(Qcpd+Qaad)	[169]
	AUC (AA)=Qauc_aabl/(meal*(Ccp_d+Caa_d))	[170]
	TID (ST)=((Qtsd+Qgld)-(Qdsi2_dscc+Qrsi2_rscc+Qgli2_glcc))/(Qtsd+Qgld)	[171]
	AUC (GL)=(Qauc_glbl)/(meal*((Cst_d/0.9)+Cgl_d)	[172]
	TID (TF)=(Qtfd-(Qdfi2_dfcc+Qufi2_ufcc+Qfai2_facc))/Qtfd	[173]
	AID (TF)=(Qtfd-(Qdfi2_dfcc+Qufi2_ufcc+Qfai2_facc+Qefi2_efcc))/Qtfd	[174]

[1] PULSE is an acslX statement used to initiate and repeat feed intake at a certain time and interval.

Supplementary Table S5.2. Driving variables of the dataset obtained from literature for the independent evaluation of postprandial glucose absorption and model estimates of digesta passage kinetics in stomach and stomach + small intestine by the digestion model for growing pigs.

Ref[1]	Obs	Ingredients/diets	Driving variables[2]						Model estimates of mean retention time (h)[3]			
			D_j	RAV	Ds (%)	Cts_d (%)	Crs_ts (%)	Kds_gl (/h)	slgs	lqgs	sli12	lqi12
(82)	1	Corn starch	2.6	0	3	69	69	0.5	3.4	2.0	6.1	4.7
	2	Pea starch	2.6	0	3	69	26	1.0	3.4	2.0	6.1	4.7
	3	Rice starch slow	2.6	0	3	69	18	1.4	3.4	2.0	6.1	4.7
	4	Rice starch rapid	2.6	0	3	69	15	2.5	3.4	2.0	6.1	4.7
	5	White bread	2.6	0	3	69	4	3.7	3.4	2.0	6.1	4.7
(19)	6	Corn starch	1.7	0	3	70	85	0.3	2.5	1.1	5.2	3.8
	7	Pea starch	1.7	0	3	70	61	0.3	2.5	1.1	5.2	3.8
	8	Rice starch slow	1.7	0	3	70	34	0.8	2.5	1.1	5.2	3.8
	9	Rice starch rapid	1.7	0	3	70	2	1.5	2.5	1.1	5.2	3.8
(87)	10	Corn starch	1.7	0	3	70	85	0.3	2.5	1.1	5.2	3.8
	11	Pea starch	1.7	0	3	70	61	0.3	2.5	1.1	5.2	3.8
	12	Rice starch slow	1.7	0	3	70	34	0.6	2.5	1.1	5.2	3.8
	13	Rice starch rapid	1.7	0	3	70	3	0.8	2.5	1.1	5.2	3.8
(86)	14	Corn starch	1.9	0.3	5	63	0	1.2	2.8	1.4	5.5	4.0
	15	Pea starch	1.9	0.3	20	64	22	0.5	3.6	2.2	6.3	4.8
(70)	16	Maltodextrin	1.3	0	100	28	0	10	0.7	0.7	3.4	3.4
(79)	17	Native tapioca starch	2	0.3	5	64	48	0.1	2.9	1.5	5.6	4.2
	18	Gelatinised wheat starch	2	0.3	14	47	2	1.8	3.5	2.1	6.2	4.8
(75)	19	Control diet	2.3	0.6	9	41	0	1.2	3.3	1.9	6.0	4.6
	20	High fibre diet	2.3	1.1	7	31	0	1.2	3.3	1.9	6.0	4.5
(83)	21	Western-style Diet	2.4	0	29	42	2	1.8	3.4	2.0	6.1	4.6
	22	Resistant-starch diet	2.4	0	17	47	44	1.1	4.1	2.7	6.8	5.4
	23	Arabinoxylan-enriched diet	2.4	17.8	17	47	9	2.3	4.1	2.9	6.8	5.6
(81)	24	Dextrose	2.7	5.8	51	30	0.1	1.6	3.5	2.1	6.2	4.8
	25	Fat+cellulose	2.7	5.8	39	30	0.1	1.6	3.5	2.1	6.2	4.8

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[85]	26	Low fibre	3.5	0.2	22	57	2	1.8	4.4	3.0	7.1	5.7
	27	high insoluble fibre	3.5	0.7	22	50	2	1.8	4.4	3.0	7.1	5.7
	28	high soluble fibre	3.5	18.5	22	46	18	12.0	4.4	3.2	7.1	5.9
[80]	29	Control diet - high CP	2.8	2.8	12	47	0.03	1.8	3.9	2.5	6.6	5.2
	30	Control diet - low CP	2.8	1.6	10	50	0.2	2.2	3.8	2.3	6.4	5.0
[88]	31	Wheat flour	2.6	0.7	36	47	0.03	0	3.4	2.0	6.1	4.7
	32	Rye flour	2.5	9.9	27	51	0.04	4.2	3.6	2.3	6.3	4.9

[1] References: Giuberti et al. [82]; van Kempen et al. [19]; Regmi et al. [87]; Meulen et al. [86]; Deutz et al. [70]; Bakker et al. [79]; Agyekum et al. [75]; Ingerslev et al. [83]; Fledderus et al. [81]; Knudsen et al. [85]; Bakker et al. [80]; Theil et al. [88]

[2] Driving variables estimated based on study information. D_j = feed intake relative to maintenance requirement for metabolisable energy (419 kJ ME/kg $BW^{0.75}$/d; [30]), RAV = real applied viscosity (mL/g; [39]), Ds = diet solubility (as % of soluble protein and glucose in the diet), C_ts = total starch content diet (%), Crs_ts = resistant starch (% of total starch, based on in vitro starch hydrolysis kinetics = plateau × $(1-e^{(-k_{st\_gl} \times t)})$, where t = 6 hours), K_{st_gl} = starch hydrolysis rate (fraction/h)

[3] Mean retention times estimated by the model based on dietary input parameters and modelled digesta passage kinetics: slgs = solids in the stomach, lqgs = liquids in the stomach, sli12 = solids stomach + total small intestine, lqi12 = liquids stomach + total small intestine.

Supplementary Table S5.3. Driving variables of the dataset obtained from literature for the independent evaluation of postprandial amino acid absorption and model estimates of digesta passage kinetics in stomach and stomach + small intestine by the digestion model for growing pigs.

			Driving variables[2]									Model estimates of mean retention time (h)[3]			
Ref[1]	Obs	Diets	D_j	RAV	Ds	Ccp_d	Caa_d	Cup_cp	Csp_cp	Kipgs_spgs	Cdpgg_aai	Kdpi_aai	slgs	lqgs	sli12	lqi12
(90)	1	Fishmeal	2.4	0.0	9	13	0	7	24	0.03	49	2.2	3.4	2.0	6.1	4.7
(89)	2	Whey protein isolate	1.3	0.0	100	23	0	5	100	0.00	14	5.0	2.1	0.7	4.8	3.4
(80)	3	Control diet - high CP	2.8	2.8	12	20	6	5	22	0.19	28	1.7	3.9	2.5	6.6	5.2
	4	Control diet – low CP	2.8	1.6	10	16	6	5	25	0.23	31	2.0	3.7	2.3	6.4	5.0
(7)	5	CP16	2.1	0.5	7	16	0	6	13	0.19	29	2.0	3.1	1.7	5.8	4.3
	6	CP12+AA	2.1	0.5	5	12	1	6	13	0.23	32	1.9	3.0	1.6	5.7	4.3
(75)	7	Control diet	2.3	0.6	9	18	0	6	13	0.18	28	2.1	3.3	1.9	6.0	4.6
	8	High fibre diet	2.3	1.1	7	19	0	9	19	0.15	37	1.4	3.3	1.9	6.0	4.5
(79)	9	Native tapioca starch	2.0	0.3	5	15	0	4	12	0.06	26	1.1	2.9	1.5	5.6	4.2
	10	Gelatinised wheat starch	2.0	0.3	14	15	0	3	17	0.15	35	1.1	3.5	2.1	6.2	4.8
(31)	11	Soybean meal	2.5	0.7	26	19	0	0	14	0.12	23	2.3	3.8	2.4	6.5	5.1
	12	Wheat gluten meal	2.5	0.0	20	17	1	6	9	0.63	22	3.5	4.2	2.8	6.9	5.4
	13	Rapeseed meal	2.5	0.0	26	21	0	13	12	0.19	39	4.0	3.7	2.3	6.4	5.0
	14	Dried porcine plasma	2.5	0.0	37	20	0	6	82	0.07	25	2.7	3.3	1.9	6.0	4.6
	15	Black soldier fly larvae	2.5	0.0	22	17	0	14	26	0.13	40	19.4	4.0	2.6	6.7	5.3

[1] References net portal appearance studies: (7; 75; 80; 89; 90); arterial study: (79) ; systemic venous study: (31)

[2] Driving variables estimated based on study information: D_j = feed intake relative to maintenance requirement for metabolisable energy (419 kJ ME/kg $BW^{0.75}$/d; (30)), RAV = real applied viscosity (mL/g; (39)), Ds = diet solubility (as % of soluble protein and glucose in the diet), Ccp_d = crude protein content diet (%), Cup_cp = enzymatically undegradable protein (% of crude protein content), Csp_cp = instantly soluble protein in the stomach (% of crude protein content), Kipgs_spgs = solubilisation rate in the

stomach (/h), Cdpgs_aai = fraction of digestible protein passed from the stomach instantly entering amino acid pool in the small intestine (g/g), Kdpi_aai = hydrolysis rate of protein in the small intestine (/h).

[3] Mean retention times estimated by the model based on dietary input parameters and modelled digesta passage kinetics: slgs = solids in the stomach, lqgs = liquids in the stomach, sli12 = solids stomach + total small intestine, lqi12 = liquids stomach + total small intestine.

Supplementary TableS5.4. List of driving variables required for each simulation (i.e. user defined input) by the digestion model for growing pigs, and example variables based on a practical reference diet (i.e. LS diet [2]) with soybean meal, rapeseed meal, maize and wheat.

Simulation (i.e. user) input	Description	unit	Reference diet
D_j	Feed intake level relative to maintenance requirement for energy	x (419 kJ/kg $BW^{0.75}$/d)	1.9
DMI[1]	dry matter intake	g/d	975
Ds[2]	Fraction of soluble protein and sugars in the diet	g/g	0.09
RAV[1]	diet viscosity	ml/g	0.95
Ccp_d[1]	Fraction total crude protein in the diet	g/g DM	0.22
Cup_cp[3]	Fraction undegradable protein of total crude protein intake	g/g	0.07
Csp_cp[4]	Fraction soluble protein of total crude protein intake	g/g	0.17
Kipgs_spgs[4]	Rate of protein solubilisation in the stomach	/h	0.24
Cdpgs_aai[4]	Fraction of protein emptied from the stomach instantly entering amino acid pool in the small intestine	g/g	0.38
Kdpi_aai[4]	Rate of protein hydrolysis in the small intestine	/h	2.18
Cts_d[1]	Fraction total starch in the diet	g/g DM	0.54
Crs_ts[5]	Fraction resistant starch of total starch intake	g/g	0.01
Cds_ts	Fraction enzymatically degradable starch of total starch intake (i.e. 1-Crs_ts)	g/g	0.99
Kds_gl[5]	Rate of starch hydrolysis *in vitro*	/h	1.52
Ctf_d[1]	Fraction fat in the diet	g/g DM	0.05
Cuf_tf[6]	Fraction undegradable fat of total fat intake	g/g	0.01
Kdfi_fai	Rate of fat hydrolysis in the small intestine	/h	0.13
Ctn_d[6]	Fraction of non-starch polysaccharides in the diet	g/g DM	0.19
Kdtnc[7]	Fermentation rate of non-starch polysaccharides in the colon	/h	0.05

[1] Calculated as weighted average based on feed ingredient chemical composition and content in the diet. Reference value per ingredients based on dry matter, crude protein, starch (amyloglucosidase-method) and fat (hydrolysis method)[12], and RAV [39].

[2] Calculated as fraction of soluble protein (Ccp_d × Csp_cp) + reducing sugar content in the diet.

[3] Calculated as weighted average based on crude protein content of the diet and feed ingredient, where per feed ingredient (i) Cup_cp, i = 0.5 × (1 – (AIDCP+ 1.5 ×BEPL)/CP), AIDCP = apparent ileal crude protein digestibility coefficient (g/g), BEPL = basal endogenous protein losses (i.e. 11.43 g/kg DM), and CP = crude protein content (g/kg DM) [12].

[4] Calculated as weighted average based on crude protein content of the diet and feed ingredient, and in vitro protein hydrolysis kinetics ([4; 33]).

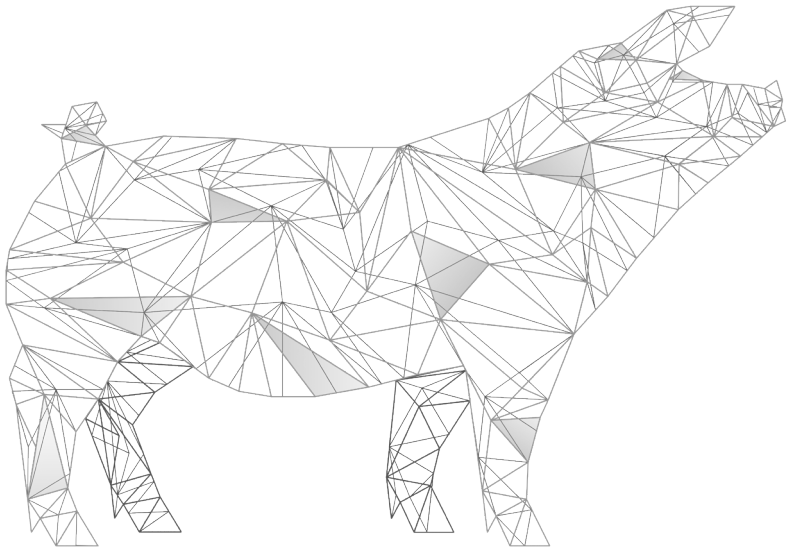
[5] *Calculated as weighted average based on starch content of the diet and feed ingredient, and in vitro starch hydrolysis kinetics [28; 32; 34]. Where per feed ingredient (i) RS(i, t_6) = Dmax × (1- $e^{(-kds\_gl \times t_6)}$), Dmax = maximum fraction of starch hydrolysis, Kds_gl = fraction rate of starch hydrolysis (/h), and t = 6 h.*

[6] *Calculated as weighted average based on fat content of the diet and feed ingredient, where per ingredient (i) Cuf_ft, i = 0.5 × (1-(((fat × DCfat) + BEFL)/ fat)), fat = fat content (g/kg DM), DCfat = digestibility coefficient (g/g) for fat based on equation by [35] if fatty acid composition was available, otherwise based on the Dutch feed evaluation system[12], and BEFL = basal endogenous fat loss (i.e. 4.7 g/kg DM)[37].*

[7] *Calculated as weighted average based on non-starch polysaccharides content of the diet and feed ingredient, where per feed ingredient (i) Kdtnc, i = (-DCNSP ×Kc_o)/(DCNSP-1), DCNSP = digestibility coefficient (g/g) of NSP [12] and Kc_o = passage rate of digesta through the colon (i.e. 0.0298/h).*

Chapter 6

General discussion



As the nutritional value of feed ingredients in pigs is determined by the kinetics of nutrient digestion, the aim of the present thesis was to develop a computer model. Nutrient digestion kinetics is known to affect the nutritional value of feed ingredients due to 1) differences in the nutritional value of digestion products resulting from enzymatic hydrolysis *v.* fermentation of nutrients in the gastrointestinal tract, and 2) effects on post-absorptive metabolism of nutrients resulting from differences in the kinetics of nutrient absorption. Digestion is the aggregated process of digesta passage, nutrient hydrolysis, endogenous secretions, and absorption of nutrients. Nutrient digestion is driven by feed intake and is affected by animal and dietary factors (Chapter 1). To better predict the nutritional value of feed ingredients in pigs in the future, a nutrient-based dynamic mechanistic digestion model was developed (Chapter 5). Focus was on the kinetics of digesta passage and nutrient hydrolysis. Effects of diet viscosity, dietary nutrient solubility, and feed intake level on digesta passage were studied (Chapter 2 and 3), variation in protein hydrolysis kinetics of different feed ingredients (Chapter 4). Together with quantitative knowledge from literature, the *in silico* model is developed and evaluated on the kinetics of nutrient absorption simulated for pigs fed diets varying in constituting feed ingredient and physicochemical properties. In this final chapter, I will discuss i) the framework of the model, ii) integration of the various digestive processes and diet physicochemical properties, and iii) what improvements can be considered for the model to better predict and understand nutrient digestion and absorption kinetics in growing pigs in the future, and iv) finish with an outline of the conclusions drawn from this thesis.

FRAMEWORK OF THE MODEL

Like a map, a model is a simplified representation of reality, otherwise it would be reality itself. Animal and dietary factors are known to influence the kinetics of nutrient digestion (Chapter 1) and more specifically the kinetics of digesta passage (Chapter 2, 3) and nutrient hydrolysis (Chapter 4). While former digestion models have not [4] or to a limited extent taken dietary factors into account [7], we considered them as determinants for variation in the absorption kinetics of nutrients (see Chapter 5). Therefore, we aimed to identify true dietary factors to be used as model input variables, and to develop a model that simulates their interaction with animal factors. In Chapter 4, this was practiced by assessing the maximum potential of protein hydrolysis kinetics in various feed ingredients as a dietary factor. For digesta passage it is more complex to separate animal and dietary factors as, especially in the stomach [8; 9], it is a highly regulated process by the animal. Effects of dietary factors on digesta passage can be caused by effects on the physicochemical properties of digesta [10] but also by affecting the absorption of nutrients, thereby triggering nutrient-sensing feedback mechanisms [11]. Hence, the effects of dietary

treatments on digesta passage, as well as, physicochemical properties of digesta were studied. The latter is discussed in the following section. To summarize, for the model framework focus was on identifying feed ingredient properties, as dietary factors, that influence the kinetics of nutrient digestion.

PHYSICOCHEMICAL PROPERTIES AND THEIR EFFECT ON DIGESTA PASSAGE

In literature often the influence of digesta rheological properties on the passage of digesta through the gastrointestinal tract is mentioned [10; 12; 13; 14; 15], however, only limited studies have actually quantified or presented rheological properties of pig digesta [16; 17]. Or even quantified the relation between digesta rheological properties and passage of digesta [5]. To increase our knowledge, we therefore, studied digesta viscosity and water-binding capacity (WBC), as physicochemical properties, in our *in vivo* passage studies (Chapter 2 and 3).

In Chapter 2, we hypothesized that feeding diets to pigs with incremental levels of dietary viscosity, would increase digesta viscosity in the stomach, and possibly the small intestine. In turn, an increase in digesta viscosity was expected to reduce the fractional passage rate of digesta solids and liquids. In contrast to our hypothesis, however, while digesta viscosity decreased, the fractional passage rates of liquids reduced in pigs fed diets increasing in viscosity. This result coincided with a decrease in dry matter concentration of stomach digesta in pigs fed increasing levels of diet viscosity, which was presumably caused by WBC of the associated dietary oat-β glucans. The decrease in dry matter concentration explains the decrease in digesta viscosity and, as the prior is caused by WBC, it explains the decrease in passage rate of liquids. These results indicate that, dietary hydration properties rather than digesta viscosity affected the fractional passage rate liquids. Moreover, they indicate that digesta viscosity cannot simply be derived from diet viscosity, as in agreement with previous studies [18; 19; 20]. Furthermore, the results indicate that we cannot assume that high viscous digesta passes at a lower fractional rate in the stomach, than low viscous digesta. One might consider that the relationship between diet and digesta viscosity in the stomach is clearer when digesta is sampled directly after a meal, however, as observed by Guerin *et al.* [18] the relationship remained absent when digesta is collected directly instead of 2 h after the meal.

While digesta passage cannot simply be derived from digesta viscosity, a correlation was found for diet viscosity and the fractional passage rate of liquids in the stomach. When diet viscosity increased, the fraction passage rate of liquids decreased, thereby reducing the difference in passage rates of solids and liquids. This relationship is implement in the computer model, however its applicability outside of our study was not assessed yet. Based on data from Martens [5], however, the relationship can be extended. Like in our study, their data also shows that

differences in fractional passage rates of solids and liquids decrease when diet viscous properties, derived from oscillatory rheology measurements, increase. Interestingly, in contrast to our study, they [5] observed that the difference in passage rate reduced through effects on solids rather than liquids. This discrepancy can be explained by the dietary treatment which induced diet viscosity. In their study, extrusion of starch increased diet viscosity, as the prior is related to the particle-associated behaviour of starch, and thereby of solids. It explains why the difference in passage rate of solids and liquids is determined by affecting the solid fraction. In our study, diet viscosity was induced by adding soluble oat β-glucans in the diet which effects are more associated with liquids. Based on the former, the relation between diet viscosity and digesta passage can be extended outside of our study. However, the relationship can also be extended to the effect of dietary hydration properties instead of diet viscosity. As explained earlier, in our study the reduction in differences between the passage rate of solids and liquids might be explained by WBC of the diet, as induced by oat β-glucans. Together with data from Martens [5] we can see in Figure 6.1 that the relationship between hydration properties of the diet and difference in passage rates of solids and liquids exists. As hydration properties are more easily quantified compared to rheological properties, changing over the effect of diet viscosity in the model to the effect of diet hydration properties would make the model more easy to use in practice.

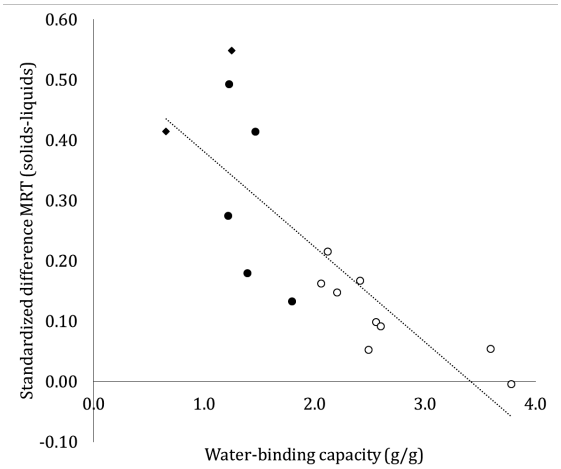


Figure 6.1. Relationship between standardized difference in mean retention time (MRT, i.e. (MRT_{solids} – $MRT_{liquids}$)/ (MRT_{solids} + $MRT_{liquids}$)) of digesta solids and liquids in the stomach, and the water-binding capacity of diets fed to growing pigs (y=0.54-0.16x; r^2=0.66; model P=0.0001). Combined data from [2](●), [3](●) and [5; 6] (○). Water-binding capacity was based on AACC International Method 56-11-02 (i.e. 1 g sample in 50 mL de-ionized water, soaked for 60 min, thereafter centrifuged (10 min × 4 000 g) and drained inverted at 45° angle, everything executed at room temperature).

As hydration properties can be pH depend, a method to assess the solvent-binding capacity (SBC) instead of WBC is proposed to quantify the hydration properties of diets and constituent feed ingredients. In this method the use of a citric-acid buffer (pH 3.0) instead of water is proposed as solvent. Although SBC and WBC are in good correlation (SBC=1.2×WBC–0.3; R^2=0.79), SBC

explains more of the variation in the difference between fraction al passage rates of solids and liquids in the stomach than WBC (Figure 6.2). This likely relates to the effect of pH on hydration properties of the diet in the stomach. Based on the former it is proposed to assess SBC as dietary property used as a model input to explain variation in the difference between the fractional passage rate of solids and liquids in the stomach.

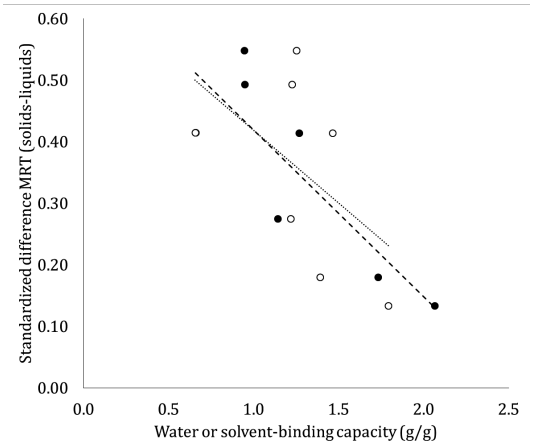


Figure 6.2. Relationship between water-binding capacity (○, y=0.66-0.24x, R^2=0.26; based on the AACC International Method 56-11-02) or solvent-binding capacity (●, y=0.69-0.27x, R^2=0.71; Text box 1) of diets and the standardized difference in mean retention time (MRT) of solids and liquids in the stomach.

In the small intestine, no effects of diet viscosity on digesta viscosity or digesta passage rate were observed. This is presumably caused by the partial degradation of oat-β glucans that were used to induce diet viscosity. However, it may as well be caused by the low dry matter concentration of digesta in the small intestine. The latter being based on the exponential relation between viscosity and the fraction of particles in a suspension [21], and the correlation we observed between digesta dry matter concentration and digesta viscosity (Chapter 2). digesta dry matter concentrations in the small intestine are considered to be low, the effect of diet viscosity on digesta passage in the small intestine in general may be limited. Data from previous studies, in which effects of soluble dietary fibres on the passage kinetics of digesta by the end of the small intestine were observed [22; 23; 24], can't be used to derive whether the effects were caused by affecting passage in the stomach rather than the small intestine. As soluble fibres can affect nutrient digestibility in the small intestine, it is of interest to find out whether they affect the kinetics of digesta passage or nutrient hydrolysis, absorption, or endogenous secretions. This knowledge can give insight where future model improvements on the extent of nutrient digestibility can be focussed.

Text box 1. Estimated solvent-binding capacity of 22 feed ingredients at stomach-like pH

The solvent-binding capacity (SBC) of 22 feed ingredients was studied. Ingredients were ground to pass a 1-mm sieve (Retsch ZM 200, Haan, North Rhine-Westphalia, Germany). Per ingredient, 5 g of material are weighed into pre-weighed Sarsted tubes containing 25 mL of citric acid buffer (0.1 M; pH3). The closed tubes are then submerged in a shaking water-bath (39 °C; 90 rpm) to allow ingredients to soak. After 2 h of soaking, the suspensions are centrifuged at 4 000 *g* for 10 min at room temperature (21 °C), followed by draining of the supernatant through by-hand inverting of the tubes. The solvent retained by the sample is calculated as follows:

$$\text{SBC}\left(\frac{\text{g}}{\text{g}}\right) = \frac{W_D - W_0}{W_s}$$

where, W_D = weighted of the tube incl. drained material (g), W_0 = weight of the tube incl. ingredient sample and soaking solution (g), and W_s = weight of the ingredient sample (g).

Results, showed that SBC ranged from 0.7 g/g in rapeseed to 10.5 g/g in oat β-glucans (35% β-glucans; PromOat Beta Glucan, Tate & Lyle PLC, London, United Kingdom). Cereal ingredients (unprocessed, except for grinding) showed the lowest SBC and smallest variation. The SBC was higher for processed ingredients (i.e. '-meal' ingredients), and highest for fibre-rich feed ingredients. This data can be useful when dietary hydration properties are considered for estimation of the difference in passage rate of solids and liquids through the stomach.

Ingredient	SBC (g/g)
Rapeseed	0.7
Maize starch	0.8
Rye	0.8
Wheat	0.8
Barley	0.9
Maize	1.0
Peas	1.1
Whey powder	1.2
DDGS (maize)	1.2
Oats	1.2
Fish meal	1.3
Maize gluten meal	1.4
Wheat gluten meal	1.6
Rapeseed meal	1.7
Soybean meal	1.8
Sunflower meal	2.4
Wheat middlings	2.5
Potato protein	2.5
Soy hulls	3.8
Sugar beet pulp	4.3
Linseed extracted	5.1
Oat β-glucan (PromOat)	10.5

Relevance and considerations of quantifying diet and digesta rheological properties

As discussed earlier, the effect of diet viscosity on digesta passage kinetics, as mentioned throughout this thesis, might well be related to hydration instead of rheological properties. Variation in digesta passage of liquids might, however, not only be covered by variation in hydration properties of diet and constituent feed ingredients. In the case of casein, for example, it is imaginable that its' water-holding capacity [25] might not reflect its passage behaviour through the stomach [26; 27]. For the latter, agglomeration of proteins in casein that affect the rheological properties of digesta play a role. Hence, it still may be worthwhile to consider the effect of diet rheological properties on the passage of digesta, especially in the stomach. Diets and digesta, however, are complex suspensions that vary in solid and liquid fractions and contain particles that can settle in the suspension. This makes assessment of their rheological properties difficult. Moreover, while feed ingredients and diets are generally fed dry to pigs, their effect on digesta viscosity is induced after mixing with solutions in the stomach. Hence, to assess the rheological properties of diets a soaking procedure should be considered. This has to be done careful, as (part of) the rheological properties of suspensions depend on their dry matter concentration (Chapter 2) or packing volume of particles [21]. In previous studies, the complexity of particles have been excluded by measuring extract viscosity [28]. Although easier, the latter excludes the undeniable effects of particles on the rheological properties of digesta [29]. Therefore, when the relationship between diet rheological properties and digesta passage continues to be studied, viscosity of complete suspensions rather than extracts should be considered.

To summarise this section, digesta viscosity in the stomach cannot be predicted based on diet viscosity. Differences between the fractional passage rates of solids and liquids were explained through effects of diet rheological properties. This effect, however, might be better explained through dietary hydration properties, which are more easily assessed than rheological properties. Hence, to predict effects on the difference between the fractional passage rates of solids and liquids, hydration properties of diets, especially SBC, should be considered instead of rheological properties. Diet viscosity did not cause effects on digesta viscosity and digesta passage in the small intestine. It is questionable whether digesta viscosity will play a role in the passage of digesta through the small intestine, considering the low dry matter concentration of digesta in the small intestine, and the (exponential) relationship between viscosity and the particle fraction in suspensions. Predicting the effects of diet and digesta rheological properties on nutrient digestion in the small intestine, as for example caused by soluble fibres, may need to focus on affecting hydrolysis, absorption and endogenous secretions of nutrients rather than on the passage of digesta.

MODELLING DIGESTA PASSAGE

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 nutrient 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 digesta in our model has been represented by first-order kinetics using fractional rates. This approach has been commonly applied in segments in which complete and instant mixing of digesta occurs, and the absolute rate of digesta passing is determined by the pool size and the fractional rate. For the stomach, digesta passage, like in previous models, has been represented by fixed fractional rates [6; 7]. Stomach digesta, however, consists of different fractions of which solids and liquids differ in passage behaviour [30; 31]. For the model, we therefore applied different fractional passage rates for insoluble and soluble nutrients in the stomach. These different fractional passage rates allowed for simulation of variation in the time of peak of amino acid absorption due to variation in solubility of dietary proteins. For example, increasing the solubility of proteins from 17% in a low soluble protein diet [2] to 100% in an isolated whey protein diet, resulted in a 12 min earlier time of peak of amino acid absorption after a meal (58 *v.* 46 min).

In addition, other dietary factors were included that are known to affect the kinetics of digesta in the stomach, by, for example, triggering feedback mechanisms or by altering the physicochemical properties of digesta. The model accounts for effects of total nutrient/energy intake (i.e. feed intake level), nutrient load in the solid and liquid fraction (i.e. diet solubility: solubility of dietary protein and glucose-equivalents), and resistance to flow (i.e. apparent diet viscosity). As discussed earlier, the latter should be altered to the effects of dietary hydration properties. In contrast to previous digestion models in pigs, including variation in passage kinetics in our model allowed to predict variation in the absorption kinetics of nutrients. However, the current representation of stomach passage still needs to be reconsidered. As explained in the model, applying fractional rates of passage in the stomach causes a large fraction of ingested nutrients to directly enter the small intestine after a meal. This causes the time of peak of nutrient absorption to be drawn close to the moment of feed intake, and may even result in lower variation in time of peak predicted compared to observed. Hence, to improve model predictions on the kinetics of nutrient absorption and to better represent the physiology of stomach emptying, the passage rate of digesta in the stomach, as represented by the model, should be improved. Stomach digesta shows complex flow behaviour [32] as, for example, digesta is emptied in different phases after a meal [5; 31; 33] and passage of solids is affected by particle size [34] and particle strength [35]. While for true liquids fractional passage rates may be applied [31], for solids a power-law model might be considered to represent the temporary storage and/or disintegration of particles[32; 34;

[35]. The latter requires representation of the kinetics of particle disintegration in the stomach for which limited quantitative data exists. The inclusion of the different phases of stomach emptying may be applied by conditional statements using time after a meal as driving variable (e.g.[4]).

For the small intestine and colon, digesta passage was also represented by fractional passage rates, like in the stomach. Fractional passage rates, however, conflicts with the principle of tubular flow in intestinal segments [36]. Direct transfer of digesta into the colon after entering the small intestine is minimized by modelling two small intestinal segments (I1 and I2). Others have avoided direct transfer of digesta by adding a lag phase [7], a location dimension [37], or introducing numerous small intestinal segments [4; 38]. As the impact of digesta passage in the small intestine on the kinetics of nutrient absorption is limited (see Chapter 5), introduction of a location dimension or numerous small intestinal segments likely adds unnecessary complexity to the model compared to introduction of a simple lag phase.

A single fixed fraction passage rate for both insoluble and soluble nutrients was applied in the small intestine and colon. It is generally assumed that digesta becomes more homogenous when it passes along the gastrointestinal tract, whereby differences between the passage of solids and liquids should diminish. However, although no major differences between the fractional passage rates of solids and liquids are observed in the intestines, studies have shown that liquids can be retained longer than solids in the distal small intestine [2; 3; 5] (Table 6.1). Causes might be i) peristaltic movements of the small intestinal gut wall, hereby expelling part of the liquid fraction from the digesta bolus [12], ii) reflux of liquids from the caecum into the distal small intestine [39; 40], or iii) selective retention of liquid markers in (parts of) the gastrointestinal tract. The latter, however, seems unlikely based on the use of various markers [41](solids: TiO_2 [2; 3; 42], Cr_2O_3 [5], YbO_2 [36]; liquids: Cr-EDTA [2; 3; 36; 42], Co-EDTA [5]). While it is remarkable that liquids can be retained longer in the small intestine than solids, its relevance is unknown. In addition, its effect on the time of peak of nutrient absorption is presumably non-existing. The latter is based on the relative small differences between the fractional passage rates of solids and liquids in the small intestine, and low sensitivity of predicted time of peaks of nutrient absorption to changes in the fractional passage rate of digesta in the small intestine.

Table 6.1. Difference in mean retention time (min) of digesta solids and liquids in segments of the small intestine (SI)

Study	Segment[1]	Solids	Liquids	Δsolids-liquids	Δ standardized[2]
Schop *et al.* [2]	SI-1	21	21	0	0.00
	SI-2	89	100	11	0.06
	Total SI	110	121	11	0.05
Schop *et al.* [3]	SI-1	21	21	0	0.00
	SI-2	104	133	29	0.12
	Total SI	125	154	29	0.10
Martens [5]	SI-1	7	6	0	-0.02
	SI-2	22	23	1	0.02
	SI-3	49	64	14	0.13
	SI-4	28	34	5	0.09
	Total SI	107	126	19	0.08
Van Erp [42]	SI-1	21	18	-3	-0.08
	SI-2	69	66	-3	-0.02
	SI-3	33	30	-3	-0.05
	Total SI	123	114	-9	-0.04
Wilfart *et al.* [36]	Total SI	238	246	8	0.02

[1] Small intestine segments were divided based on length [2; 3]: SI-1,2 = 50%; [5]: SI-4 = last 1.5 m, SI-1,2,3 = (total SI minus SI-4)/3; [42]: SI-1 = 50%, SI-3 = last 1.5 m, SI-2=total SI minus (SI-1 plus SI-3)

[2] Standardized difference: (solids – liquids)/(solids + liquids)

NUTRIENT HYDROLYSIS KINETICS

For the model, fractional rates were considered for the hydrolysis of starch, protein, and of fat in the small intestine, and of hydrolysis by bacterial enzymes of starch and non-starch polysaccharides in the colon. These rates are based on *in vitro* analysis of feed ingredients (e.g. for starch, see [43; 44; 45; 46; 47; 48; 49], and for protein, see [50; 51]. For starch, after taking stomach emptying into account, directly using the small intestinal fractional rate of hydrolysis *in vitro* as proxy for that occurring *in vivo,* resulted in a substantial underestimation of ileal starch digestibility. In order to correct for this, the relationship between the *in vitro* and *in vivo* hydrolysis rate was estimated (Chapter 5). As starch digestibility is the results of passage and hydrolysis kinetics of starch in the small intestine, the fractional hydrolysis rates could be deduced using the prior known variables (see **Text box 2**). We assumed that no starch hydrolysis took place in the stomach due to lack of data. There is, however, increasing evidence that starch hydrolysis is initiated in the stomach by effects of (endogenous and microbial) α-amylases [5]. Considering the different digestion products resulting from enzymatic hydrolysis and

Text box 2. Estimating the *in vivo* rate of starch and protein hydrolysis in the small intestine

The rates of *in vivo* starch and protein hydrolysis (Kd) were estimated using the *in silico* framework [52]. The observed data [2; 3; 5; 42; 50] contained digestibility coefficients (DC) of protein and/or starch and cumulative mean retention times (CMRT, see below) of digesta in multiple consecutive segments of the small intestine of pigs fed diets varying in feed ingredients (e.g. Figure 6.3). Kd was estimated by fitting predicted [52] to observed [2; 3; 5; 42; 50] DC's, data on digesta passage (Kp, see below) [2; 3; 5; 42; 50], and pre-estimated hydrolysis rates of protein in the stomach(*in vitro*) [50; 53] and fractions of undegradable protein and resistant starch [52] were used. CMRT and Kp per segment (i) were calculated as follows:

$$\mathrm{CMRT_i} = \sum_{0}^{i-1} \mathrm{MRT_{0-(i-1)}} + 0.5 \times \mathrm{MRT_i}$$

$$\mathrm{kp_i} = \frac{1}{\mathrm{CMRT_i}}$$

Based on the perceived potential inaccuracy of DC and/or MRT estimates in proximal and distal SI segments, Kd was estimated using data of only intermediate SI segments (i; for studies [5; 42; 50] i= 2, for studies [2; 3] i = 2 for protein and 1 for starch). The proximal SI segment, which covered only (part of) the duodenum, was not chosen due to small amounts of digesta harvested as a result of the high passage rate of digesta, thereby potentially affecting the accuracy of DC and MRT estimates in this segment. Whereas, in the most distal SI segments, the observed timing at which the plateaued DC is reached, might not correctly reflect the time of reaching the maximum DC. This could yield underestimated Kd values. Hence, Kd was estimated using DC and CMRT data in intermediate SI segments.

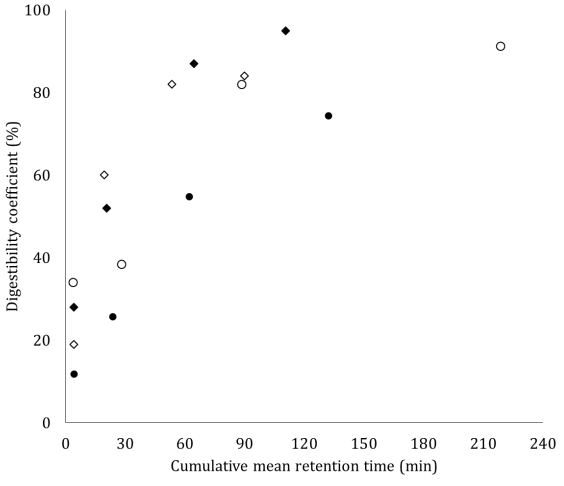


Figure 6.3. In vivo digestibility coefficients (%) of protein (soybean meal: ●; wheat gluten meal: ○) and starch (barley: ◆; maize: ◇) in consecutive small intestinal segments. Data adapted from [5; 54].

fermentation that are of varying nutritional value [42; 55], it is of interest to consider the different types of starch hydrolysis occurring in the stomach and small intestine [42].

Regressing *in vitro* and *in vivo* hydrolysis kinetics of starch yielded a significant relationship which was applied in the model. The model was able to predict variation in time of peak of glucose absorption, thanks to variation in the hydrolysis kinetics of starch, however, model predictions were lower compared to literature (56 *v.* 44 min). As the time of peak of glucose absorption is determined by the kinetics of starch hydrolysis, in addition to passage kinetics in the stomach, the relationship between *in vitro* and *in vivo* hydrolysis kinetics of starch might be reconsidered. This may be done by quantifying the *in vitro* hydrolysis kinetics of starch based on the time of peak glucose absorption instead of on the extent starch digestion, by using part of the dataset for model evaluation.

For protein, assessing the relationship between *in vitro* and *in vivo* fractional hydrolysis rates is more complex than it is for starch. Protein hydrolysis is initiated in the stomach, of which *in vivo* kinetics is not easily quantifiable. Compared to the small intestine, the stomach cannot be divided in multiple segments from which the kinetics of protein hydrolysis can be calculated based on the kinetics of passage and disappearance of protein. Therefore, only for the small intestine, the relationship between *in vitro* and *in vivo* hydrolysis kinetics of proteins was assessed in analogy to that of starch. Again passage of digesta in the stomach was taken into account and, for a lack of better, the hydrolysis kinetics of protein in the stomach as measured *in vitro*. As a consequence of estimating this relationship, all residual variation in the kinetics of protein digestion observed *in vivo* is ascribed to the kinetics of protein hydrolysis in the small intestine. No clear relationship, however, could be established between *in vitro* and *in vivo* fractional hydrolysis rates of protein in the small intestine. Hence, for the model the *in vitro* hydrolysis kinetics of protein were directly used as proxy for that occurring *in vivo*.

To improve the relationship between *in vitro* and *in vivo* protein hydrolysis kinetics the following could be considered. For the stomach, digesta from pigs with a duodenal cannula can be collected in time after the meal. The passage rate of digesta should be assessed, for example using a solid and liquid marker added to the pigs' diet [41]. Combined with information on the initial solubility of dietary protein, and the shift of protein from the insoluble to the soluble phase, i.e. when digesta samples are analysed on protein content in the solid and liquid fractions, this should provide enough data for assessment of protein hydrolysis kinetics in the stomach. After the relationship for the stomach has been established, the relationship for protein hydrolysis kinetics in the small intestine could be reassessed.

NUTRIENT ABSORPTION KINETICS, GUT METABOLISM, AND MODEL EVALUATION

Studies in pigs and humans have shown that the kinetics with which ingested nutrients are absorbed, can affect their metabolic use and, hence, their nutritional value. Intake of 'fast' *v.* 'slow' absorbed protein sources [56; 57; 58], and asynchronous *v.* synchronous feeding of glucose and protein [59], affected amino acid oxidation, protein deposition, and thereby the net metabolic protein balance. With the model developed in Chapter 5, we aimed to simulate the absorption of glucose and amino acids from the gut to assess their post-absorptive availability. As the kinetics of nutrient absorption *in vivo* can only be studied after nutrients are digested and appear in portal blood, data of portal blood studies were used to evaluate the model. One of the shortcomings in this comparison, however, is that the model doesn't take into account metabolism by portal drained viscera [60]. Portal-drained viscera, being the stomach, intestines, pancreas and spleen, comprises of metabolic highly active tissue, ~20-35% of protein metabolism and energy use compared to the whole body [60]. Once amino acids are absorbed by the gut wall, they can pass on into the portal bloodstream or can be used for the synthesis of non-essential amino acids and proteins, or used for oxidation. The proportion of absorbed amino acids passing the portal bloodstream differs between amino acids. For example, glutamine, glutamate, and aspartate are almost completely used upon absorption, whereas newly synthesized non-essential amino acids (e.g. arginine and alanine) can be released [60; 61]. Moreover, differences between the fed and fasted state, and dietary conditions are known to affect the extent of amino acid use in the gut [62]. Similarly to amino acids, glucose can be used and/or produced during gut metabolism and subsequently affect (net) portal glucose appearance [63]. Hence, to evaluate model predictions on the total extent of nutrient absorption, it would be better to use values on starch and protein digestibility at the end of the small intestine, rather than using the extent of portal blood nutrient appearance. For evaluation of model predictions regarding the kinetics of nutrient absorption however, one cannot do without portal blood studies. It is, however, postulated that the gut comprises the body's labile protein pool, and is able to retain and release proteins based on supply and demand of nutrients within the body [62; 64]. Although, gut metabolism might cause discrepancies between the kinetics of nutrient absorption and the appearance in portal blood, quantitative data on the magnitude of this gut function is lacking. Hence, despite gut metabolism not being part of the digestion model, model prediction on the kinetics of nutrient absorption can only be evaluated using data the kinetics nutrient appearance in portal blood.

Comparing predicted nutrient absorption kinetics with observed (net) portal, arterial, or systemic blood nutrient appearance

In previous studies, the absorption kinetics of nutrients has been studied based on the (net) portal appearance of nutrients. Others, however, have also used arterial or systemic blood nutrient appearances as a proxy (e.g. systemic blood glucose in humans for the glycaemic index [65])[54], as it is easier to assess compared to that in portal blood. The validity of using arterial or systemic blood as a proxy for nutrient absorption kinetics is, however, questionable. In Chapter 5, the dataset used for evaluation of model predictions on amino acid absorption covered an interesting range of protein sources. An equally interesting range in time of peak of amino acid absorptions were observed. This range originated from studies in which AA concentrations were analysed in portal, arterial, and systemic (venous) blood of pigs. Remarkably, the outer range of time of peak of amino acid appearance, for which the model was not able to make accurate predictions, was covered by studies measuring arterial and systemic blood nutrient concentrations. At the outer range, the time of peak of amino acid absorption occurred after 120 min. When the model was calibrated to fit such a late peak of amino acid absorption after the meal, a really low fractional rate of protein hydrolysis was required, resulting in unrealistically low values for protein digestibility (i.e. ~10% apparent ileal digestibility). Hence, assuming that on average digesta passage kinetics are adequately represented in the model, variation in the kinetics of protein hydrolysis could not explain such a late time of peak of amino absorption. The kinetics of amino acid appearance in portal and subsequent arterial and systemic blood might be affected by first-pass and/or whole body metabolism [61], therefore it was of interest to study whether differences occur in time of peak of nutrient

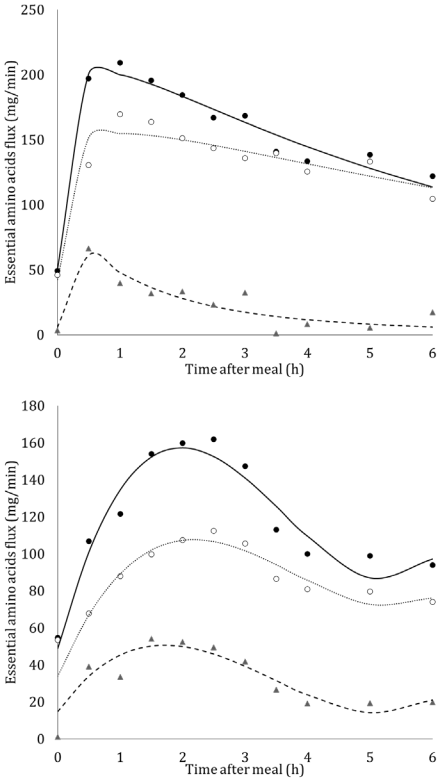


Figure 6.4. Fitted curves to estimate time of peak nutrient appearance of essential amino acids in pigs fed equal amounts of amino acids of soybean meal-based diets either with 12% crude protein + free amino acids (top) or 16% crude protein (bottom) in portal (●, solid) or arterial (○, dotted) blood, or net portal (▲, dashed) appearance. Data adapted from Yen et al. [1].

appearances between (net) portal and arterial or systemic blood. This was assessed using portal blood studies that covered both portal and arterial blood nutrient appearances [1; 66; 67; 68]. Curves were fitted using the derivative of a generalized Michaelis-Menten function [69] or a higher order (i.e. third; fifth) polynomial function, depending on pattern of nutrient appearance (e.g. Figure 6.4; PROC NLIN, SAS 9.4). Results showed that the time of peak of amino acid appearance in arterial blood occur later than in portal blood (PROC TTEST: P-value = 0.025; Table 6.2). In addition, the time of peak of net portal appearance of nutrients is observed to be significantly different from that in portal (P-value = 0.045) or arterial (P-value = 0.040) blood. These results indicate that, the kinetics of nutrients absorption from the gut should be derived from the difference in portal and arterial blood, rather than from portal, arterial or venous blood.

Table 6.2. Absolute and standardized[1] difference in time of peak of amino acid[2] appearance in portal (P), arterial (A), or net portal (P-A) blood in various portal blood nutrient appearance studies.

		Time of peak (min)			Standardized difference[1]		
Ref	Diet	P	A	P-A	PA-P	PA-A	P-A
[66]	Control diet	141	168	91	0.43	0.60	0.17
	High fibre diet	163	157	58-305[3]	0.95-0.61	0.92-0.64	0.04
[67]	High crude protein	24	23	23	0.04	0.00	0.04
	Low crude protein	23	27	53	0.79	0.65	0.16
[1]	Low crude protein + AA	41	60	16	0.88	1.16	0.38
	High crude protein	119	131	103	0.14	0.24	0.10
[68]	Fishmeal	96	104	87	0.10	0.18	0.08
P-value (H_0=0; H_a≠0)[4]					*0.045*	*0.040*	*0.025*

[1] Standardized difference calculated as absolute difference over the average, i.e. |x-y|/average(x,y)
[2] Dependent on the study, amino acid was quantified based on essential amino acids or α-amino nitrogen.
[3] Two peaks, a smaller peak followed by a larger one, were observed after ingestion of a single meal
[4] P-value were calculated on all data excluding the high fibre diet [66] (PROC TTEST, SAS 9.4)

Alternative parameters representing the kinetics of nutrient absorption

To evaluate the absorption kinetics of nutrients as predicted by the model, the time of peak absorption of nutrients was used as a parameter. As shown above, it is an easy to determine parameter using data from portal blood studies. The time of peak of absorption however, doesn't cover the complete kinetics of nutrient absorption, as the latter depends on both the rate and extent of the absorption of nutrients from the gastrointestinal tract. Moreover, while the time of peak absorption of nutrients might be similar for various diets or feed ingredients, the overall kinetics of absorption can be different (e.g. Figure 6.5). It is, however, questionable if the time of peak of nutrient absorption, as such, is physiological relevant, since both the rate and extent of absorption affect post-absorptive metabolism. As for example shown for glucose [42]. Therefore, the kinetics of nutrient absorption should be viewed upon as a whole, rather focussing on the

time of peak or extent of nutrient absorption separately. Classification of nutrient fractions according to the combined time and extent of absorption can be considered, for example, as is done for starch fractions which are classified according to the extent of degradation in time [70] (i.e. rapid starch = fraction degraded within 20 min, slow starch = fraction degraded between 20 and 60 min, and resistant starch = fraction not degraded within 60 min). When such a classification system is applied to represent the absorption kinetics of nutrient fractions, one has to keep in mind, however, that absorption is a result of digestion kinetics. Hence, it can't be used to identify inherent feed ingredient properties, as is done with classification of starch fractions (i.e. rapid, slow, and resistant starch).

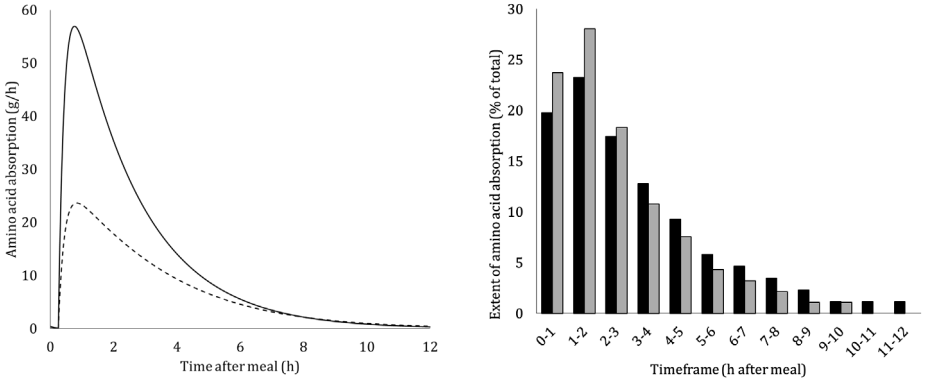


Figure 6.5. Flux (left panel) and time-extent (right panel) of amino acid absorption in pigs fed rapeseed meal (dashed line, black bars: time of peak absorption 43 min after meal, total 89% of ingested amino acids absorbed) or whey protein isolate (solid line, grey bars: time of peak absorption 44 min after meal, 93% of ingested amino acids absorbed) based diets simulated by the model in Chapter 5, by varying model input parameters only regarding protein hydrolysis kinetics.

Relevance of the kinetics of nutrient absorption in growing-finishing pig

In pigs and humans, the kinetics of nutrient digestion and absorption have been shown to affect post-absorptive metabolism (Chapter 1). Post-absorptive metabolism can be affected by 1) (a)synchrony in the availability between absorbed nutrients, e.g. amino acids and glucose [59], and 2) (a)synchrony between the availability of absorbed nutrients and the demand for [1], or capacity of, metabolic processes to use them [57]. Post-absorptive availability of absorbed nutrients is, besides nutrient digestion kinetics, determined by the amount of feed intake and the intake pattern of pigs. The latter is known to vary widely amongst individual pigs [42]. While, nutrient (a)synchrony might be prominent in restricted (meal) fed pigs, it might be less relevant in growing-finishing pigs that are generally fed ad libitum. Ad libitum-fed pigs consume their feed intake in multiple meals over the day thereby having a more continuous absorption of nutrients

compared to meal-fed pigs. It is therefore, less likely that peak or asynchronous between different nutrients occurs [71]. The developed digestion model can be used to simulate the difference in nutrient absorption of both feeding strategies (Figure 6.6). For the ad libitum-fed pigs information was used on average meal size, number, and duration, and intake-pattern over the day of group-housed growing-finishing pigs [42; 72]. Not surprisingly, the absorption of nutrients in meal-fed pigs is steep and transient after a meal, whereas for unrestricted-fed pigs the absorption of nutrients after one meal continues while the next meal is ingested. Due to the gradual absorption of nutrients over the day it is less likely that, in ad libitum-fed pigs, the nutritional value of protein is compromised by amino acid oxidation due to peak or asynchronous absorption between nutrients. However, it is still possible that the metabolic use in ad libitum-fed pigs is affected by asynchrony in the availability of absorbed nutrient and their demand for metabolic processes that are continuous over the day. Studying the effect and importance of meal size and/or frequency on post-absorptive metabolisms remains of interest [73].

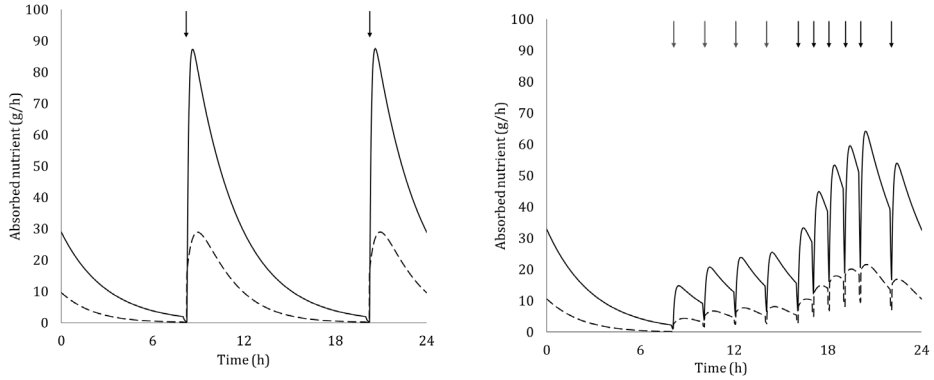


Figure 6.6. Simulated differences in absorption kinetics of amino acids (dashed line) and glucose (solid line) of meal-fed (left panel) v. ad libitum-fed (right panel) pigs. Meal-fed pigs received daily feed allowance in two equal meals at 08.00 h and 20.00 h (50% of daily intake per meal: arrows; 15 min/ meal). Based on [42; 72]*, ad libitum-fed pigs were simulated to ingest their daily intake in 10 meals (6 min/meal) of which 4 small meals in the morning (30% of daily intake; 08.00 to 14.00 h every 2 hours: light arrows) and 6 large meals in the afternoon and evening (70% of daily intake; 17.00 to 20.00 h hourly, and one at 22.00 h: dark arrows). Diet and feed intake levels were equal for both feeding strategies and represented a practical reference diet consisting primarily of maize, wheat, soybean meal, and rapeseed meal (see Chapter 5, Supplementary Table 5.3).*

Combining the current digestion model with a model that covers metabolic processes will allow for the assessment of the quantitative effects of different feeding strategies on the metabolic use of nutrients and consequently the growth performance of pigs. In addition, expanding the model with an age-effect [74; 75] and/ or expanding the dataset of model input variables with wet feed ingredients can be of interest for simulation of sows and liquid-fed pigs that generally receive a restricted number of meals per day (e.g. 2-5 times per day).

CONCLUSIONS

- Increasing diet viscosity, by oat β-glucans, decreases the fractional passage rate of liquids in the stomach and thereby reduces the difference in passage rate of solids and liquids. (Chapter 2)
- Increasing feed intake decreases the fractional passage rate of digesta in the stomach, especially for liquids. (Chapter 3)
- Increasing feed intake increases the fractional passage rate of solids in the distal small intestine. (Chapter 3)
- Increasing dietary nutrient solubility (i.e. protein, glucose-equivalents), causes the fractional passage rate of solids, and to a lesser extent of liquids, to be affected in a non-linear manner. Increasing dietary nutrient solubility from low (8%) to medium (19%) causes a numerical decrease in the fractional passage rate of solids and liquids, followed by a significant increase when nutrient solubility increased from medium (19%) to high (31%). (Chapter 3)
- Protein hydrolysis kinetics (*in vitro*) comprising initial protein solubility and solubilisation in the stomach, and degradation in the small intestine, varies among feed ingredients. (Chapter 4)
- The developed computer simulation model can predict variation in the absorption kinetics of glucose, and to a lesser extent of amino acids, when simulating a pig that is fed diets varying constituting feed ingredients and physicochemical properties. (Chapter 5)
- Variation in the fractional passage rate of digesta in the stomach is of bigger influence on the kinetics of nutrient absorption than the fractional passage rate of digesta in the small intestine (Chapter 5)
- *In vivo* starch hydrolysis kinetics are higher than observed *in vitro* (Chapter 5)
- Variation in the *in vitro* hydrolysis kinetics of proteins cannot adequately represent the variation observed in the *in vivo* absorption kinetics of amino acids (Chapter 5)

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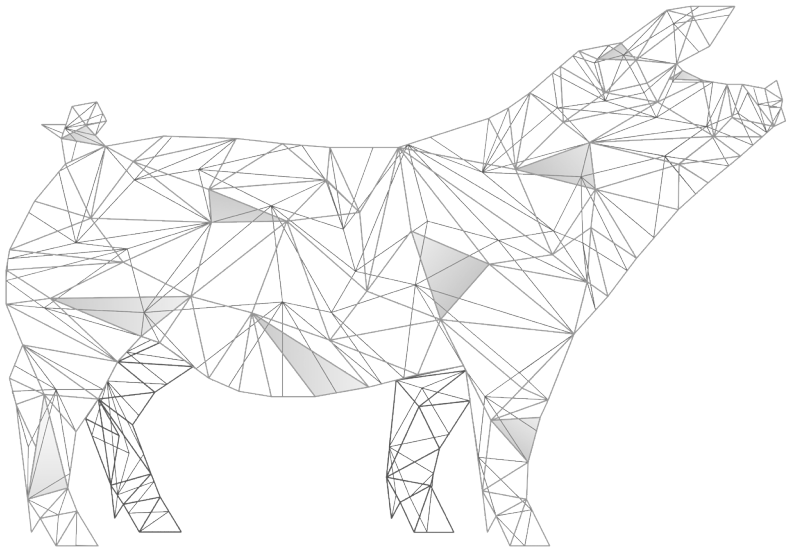
Summary



Due to an increasing world population and wealth per capita, the competition for resources for food, feed, and fuel production increases. In pig production, one of the strategies to cope with this competition is to increase production efficiency, i.e. $\frac{\downarrow \text{resources (input)}}{\uparrow \text{products (output)}}$. In pig production, production efficiency is effectuated by formulating diets that meet the pigs' nutrient requirement for maintenance and production (i.e. growth, reproduction). The amount of nutrients available to the pig, depends on the nutrient content of the diet and on the ability of pigs to digest and absorb these nutrients from their gastrointestinal tract. The availability, but also the utilization of absorbed nutrients for metabolic processes (e.g. heat production, protein and fat synthesis), depends on the kinetics of nutrient digestion after ingestion of feed. Digestion is the aggregated process of passage, hydrolysis, and absorption of nutrients and endogenous secretions by organs and tissues involved. These processes determine at what rate and to what extent (i.e. kinetics) nutrients are digested and absorbed. Current feed evaluation systems, used to formulate pig diets, do not take into account the kinetics of nutrient digestion. To gain insight into the impact of nutrient digestion kinetics on absorption of nutrients in pigs we developed a computer model ('SNAPIG').

To parameterise the model, we studied the kinetics of digesta passage in the stomach and small intestine of growing pigs (**Chapter 2 and 3**). Special focus was on the passage of solids and liquids, and the quantitative impact of diet viscosity (Chapter 2), and nutrient solubility in the diet (further mentioned as diet solubility) and feed intake level (Chapter 3). Two studies were performed in male growing pigs (30-35 kg initial body weight). The pigs were individually housed and assigned to different dietary treatments. Diets contained two indigestibility markers, one insoluble (TiO_2) and one soluble (Cr-EDTA) marker, to quantify the passage of digesta solids and liquids. After a 17-day adaptation period, including a period of feeding to steady-state of digesta passage, the pigs were euthanised for total digesta collection. Digesta was collected from the stomach, small intestine (proximal and distal half), caecum, and colon (proximal and distal half). Digesta was analysed to assess the mean retention time (MRT) of solids and liquids, and the digestibility of starch and protein in the stomach and small intestinal segments, and the apparent viscosity (i.e. measure of resistance to flow) and water-binding capacity of digesta in all segments.

Results presented in **Chapter 2** relate to the study investigating the relation between diet viscosity, induced by oat β-glucans, and the passage and physicochemical properties of digesta. We hypothesized that feeding diets with incremental levels of dietary viscosity would increase digesta viscosity in the stomach and potentially in the small intestine. This increase in digesta viscosity was expected to slow down the passage of digesta in these segments. To this end, twenty pigs were individually assigned to one of five diets with increasing dietary concentrations of oat

β-glucans (BG; from 0% to 10 %), in exchange for maize starch. Results showed that the MRT of liquids, but not of solids, in the stomach increased (from 39 to 99 min) when pigs were fed diets with increasing viscosity. The separation of solids and liquids in stomach digesta was hereby reduced. Concomitantly, the dry matter concentration of digesta in the stomach decreased, as well as, the apparent viscosity of digesta. In contrast to our hypothesis, the results indicate that increasing diet viscosity does not necessarily increases digesta viscosity, and that digesta viscosity is a consequence of, rather than a determinant for, digesta passage in the stomach. Diet viscosity did not influence physicochemical properties of digesta in the proximal small intestine, which might related to low dry matter concentrations for digesta in this segment. The WBC of digesta in the distal small intestine and colon increased when dietary BG level increased, as did apparent digesta viscosity in the proximal colon. This likely reflects the increase in concentration of BG in digesta when moving through the gastrointestinal tract.

In **Chapter 3**, the relationship between diet solubility and feed intake level was studied. It is known that the passage of solids and liquids through the stomach differs and that digesta passage kinetics can be affected by feedback mechanisms based on nutrient sensing in the gastrointestinal tract. As solubility of nutrients in the diet affects the nutrient load of the solid and liquid digesta fractions, we were interested in the effect of diet solubility on digesta passage kinetics in pigs. Forty pigs were individually assigned to one of four dietary treatments consisting of three levels of diet solubility (8, 19 and 31% of soluble protein and sucrose in the diet) and two levels of feed intake (low: 1.9 × maintenance requirement for energy; high: 2.8 × maintenance requirement for energy). Overall, solids were retained 2 h longer in the stomach than liquids. In the stomach, when diet solubility increased from 8 to 19%, the MRT of solids and liquids numerically increased, but it decreased significantly when diet solubility increased from 19 to 31%. Hence, a non-linear relationship was observed between diet solubility and the kinetics of digesta passage in the stomach. No effect of diet solubility was observed in the small intestine. Considering the effects of feed intake level, the MRT of solids and liquids in the stomach increased, depending on solubility of the nutrients provided to increase the level of feed intake. When provided as insoluble nutrients, the MRT of solids and liquids increased by about 45 min, whereas no effect was observed when the level of feed intake increased by soluble nutrients. In contrast, in the small intestine, independent of diet solubility, increasing feed intake level caused the MRT of solids to decrease by 24 min. For MRT over the stomach and small intestine combined, no effects of diet solubility and feed intake were observed. These results show that diet solubility affects digesta passage kinetics in the stomach. Feed intake affects both digesta passage kinetics in the stomach and small intestine, although for the prior it depended on nutrient solubility.

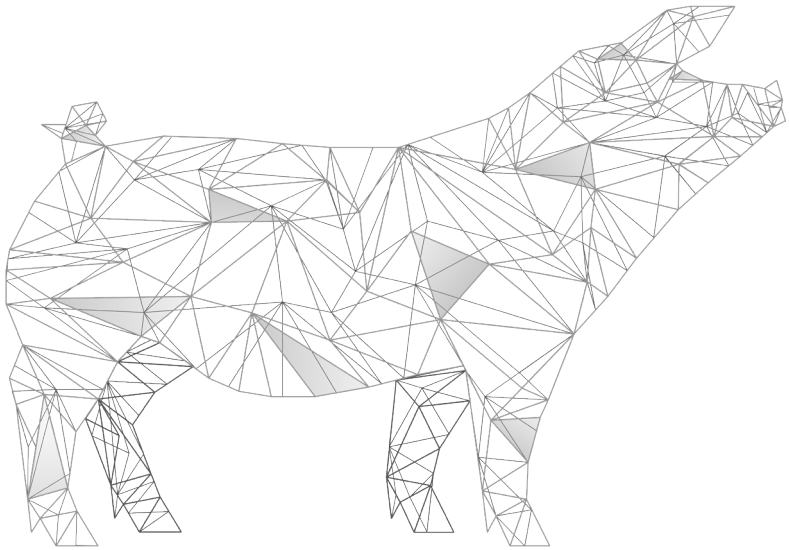
In order to provide input variables for the model presented in Chapter 5, we aimed to quantify the kinetics of protein hydrolysis of feed ingredients using an *in vitro* assay described in **Chapter 4**. The *in vitro* assay was used to simulate stomach and small intestinal enzymatic hydrolysis of protein of nineteen feed ingredients (barley, fishmeal, extracted linseed, maize, maize gluten meal, maize DDGS, oats, peas, potato protein, full-fat rapeseed, rapeseed meal, rye, soy hulls, soybean meal, sunflower meal, wheat, wheat middlings, whey powder, and whey protein isolate). Protein hydrolysis kinetics in the stomach was based on determination of the soluble protein fraction, whereas for the small intestine it was based on the appearance of low-molecular weight (MW) peptides and amino acids (<500 Da). The maximum degradable protein fraction (%) was quantified as total protein (%) minus the undegradable protein fraction in the residue (%) after 6 h of incubation. In the stomach phase, ingredients varied in the fraction (%) of protein that was instantly soluble, i.e. 8% in potato protein and 100% in whey powder and whey protein isolate, and they varied in the fractional solubilisation rate, i.e. 0.031/h in fish meal and 0.43/h in wheat. The maximum degradable protein fraction, determined at the end of the stomach + small intestine incubation, (%) ranged from 55% in soy hulls to 100% in whey powder and whey protein isolate. Part of this fraction was instantly present as low MW peptides at onset of the small intestinal simulation, i.e. 8% in oats and 96% in extracted linseed. At the end of this incubation, the low MW peptide fraction of the degradable protein fraction varied from 60% in soybean meal to >100% in extracted linseed. Data from this study were used as model input variables for the kinetics of protein hydrolysis of diets varying in feed ingredient composition (Chapter 5).

Chapter 5 contains the description and evaluation of SNAPIG, an *in silico* dynamic mechanistic digestion model. The aim of the model was to predict the absorption of nutrients by simulating nutrient digestion kinetics in pigs fed diets varying in feed ingredients and physicochemical properties. Data from own *in vivo* (Chapter 2 and 3) and *in vitro* (Chapter 4) studies, and from literature were used to parameterise the model. The model simulates the kinetics of digesta passage in the gastrointestinal tract, including effects of diet viscosity, diet solubility, and feed intake level on digesta passage of solids and liquids in the stomach; the kinetics of nutrient hydrolysis, varying among nutrients and feed ingredients; the kinetics of endogenous secretions, as affected by feed intake level and flow of organic matter through the gastrointestinal tract; and the kinetics of nutrient absorption. In this way the absorption of nutrients after a meal was simulated. The model is driven by the intake of nutrients originating from different feed ingredients. The model is able to predict variation in the absorption kinetics of glucose and amino acids when simulating the digestive process in pigs fed diets varying in feed ingredients and physicochemical properties. Sensitivity analysis of the model indicated that glucose absorption

kinetics is mainly affected by starch hydrolysis kinetics and by the kinetics of passage of solids in the stomach. Amino acid absorption kinetics is mostly affected by passage of solids in the stomach and by protein hydrolysis kinetics in the small intestine. Apparent protein and fat digestibility are most sensitive to changes in endogenous losses, while for protein also kinetics of hydrolysis in the small intestine is a highly influential factor. Evaluation of model predictions on the kinetics of starch and protein digestion was carried out by comparing predicted glucose and amino acid absorption against data from *in vivo* studies. These studies were obtained from literature and covered the kinetics and extent of nutrient appearance in (net) portal blood of pigs fed diets varying in feed ingredients and physicochemical properties. Model evaluation indicated that the kinetics of glucose absorption can be adequately predicted by the model, albeit a slight underestimation on average. For the kinetics of amino acid absorption, despite an adequate mean prediction, capacity of the model to predict variation in protein digestion kinetics leaves room for improvement. Model evaluation regarding the capacity to predict apparent ileal digestibility of protein and fat, showed that predictions were adequate although somewhat higher compared to observed *in vivo* data. Finally, although not evaluated in the present thesis, the model predicts the fermentation of organic matter in the colon fuelling microbial biomass production and formation of short-chain fatty acids. Combined results of the sensitivity analysis and the model evaluation indicated that the model can be improved by including more details on mechanisms of stomach emptying, and strengthening or improving the relation between *in vitro* and *in vivo* hydrolysis kinetics of protein and starch.

The work described in this thesis provides insight on the quantitative relations between the kinetics of digesta passage and diet viscosity, diet solubility, and feed intake level. In addition, the thesis presents further data on the variation of protein hydrolysis kinetics among feed ingredients used in practice for pigs diets. The computer model, described and evaluated in this thesis, simulates variation in the kinetics of nutrient digestion in growing pigs. It is a promising tool that can be used to predict the kinetics of nutrient absorption in pigs fed diets varying in feed ingredient and physicochemical properties.

Acknowledgements



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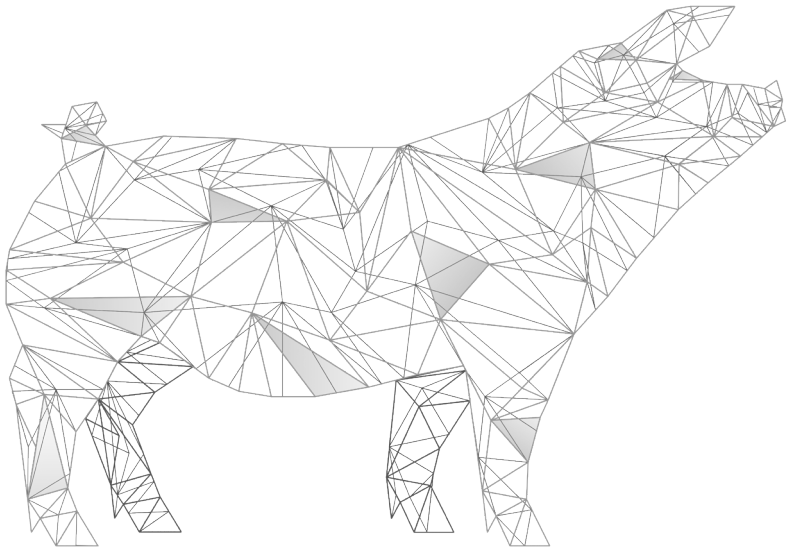
About the author

Curriculum vitae - List of publications - Training and supervision plan



CURRICULUM VITAE

Trijntje Areke (Marijke) Schop was born in 's Hertogenbosch, The Netherlands, on June 11th, 1990. She graduated from secondary education (VWO) at Altena College in Sleeuwijk, The Netherlands, in 2008. After one year of studying Veterinary Sciences at Ghent University, Belgium, she started in 2009 with her studies Animal Sciences at Wageningen University, The Netherlands, from which she obtained her BSc degree in 2012 and MSc degree in 2014. For her major thesis, she modelled the phosphorus flows in veal calves fed milk replacer and solid feed. For her internship Marijke took part in several research projects related to pigs at the Swine Research Centre (VSP, currently known as Seges) in Copenhagen, Denmark. After finishing her Masters, Marijke continued as a PhD candidate at Wageningen University & Research. She focussed on the kinetics of nutrient digestion in growing pigs. The result of her PhD work is presented in this thesis. Starting from August 2019, Marijke continues to work for Wageningen University & Research as a postdoctoral researcher in the Animal Production Systems group.

LIST OF PUBLICATIONS

Peer reviewed scientific publications

Bruun, T.S., C. Amdi, J. Vinther, M. Schop, A.B. Strathe, and C.F. Hansen. 2016. Reproductive performance of "nurse sows" in Danish piggeries. Theriogenology, 86 (4): 981-987. DOI: 10.1016/j.theriogenology.2016.03.023.

Schop M., A. J. M Jansman, S. de Vries, and W. J. J. Gerrits. 2019. Increasing intake of dietary soluble nutrients affects digesta passage rate in the stomach of growing pigs. British Journal of Nutrition 121 (5), 529-537. DOI: 10.1017/S0007114518003756.

Schop M., A. J. M Jansman, S. de Vries, and W. J. J. Gerrits. 2019. Increased diet viscosity by oat β-glucans decreases the passage rate of liquids in the stomach and affects digesta physicochemical properties in growing pigs. Animal: in press.

Conference and symposia proceedings

Schop M., A. J. M Jansman, and W. J. J. Gerrits. 2018. Effects of nutrient solubility and feeding level on digesta passage rate through the proximal gastrointestinal tract of growing pigs. Advances in Animal Biosciences, 9(S2), S160. DOI: 10.1017/S2040470018000225

Schop M., A. J. M Jansman, and W. J. J. Gerrits. 2018. The effects of diet viscosity on the passage rate and physicochemical properties of digesta in the digestive tract of pigs. Advances in Animal Biosciences, 9(S2), S161. DOI: 10.1017/S2040470018000225

Schop M., A. J. M Jansman, and W. J. J. Gerrits. 2018. Passage of digesta through the gastrointestinal tract of pigs: the effect of feeding level and nutrient solubility. Proceedings of the WIAS Science Day, 5 February, 2018, Wageningen, the Netherlands.

Schop M., A. J. M Jansman, and W. J. J. Gerrits. 2018. The effect of dietary viscosity on the passage of digesta through the gastrointestinal tract of pigs. Proceedings of the WIAS Science Day, 5 February, 2018, Wageningen, the Netherlands.

Schop M., A. J. M Jansman, and W. J. J. Gerrits. 2017. Assessing *in vitro* nutrient degradation kinetics of (processed) feed ingredients for pigs. Proceedings of the 42nd Animal Nutrition Research Forum, 7 April, 2017, Ghent, Belgium.

TRAINING AND SUPERVISION PLAN

Completed in fulfilment of the requirements for the Education Certificate of the Wageningen Institute of Animal Sciences

The basic package (2 ECTS[1])	**Year**
WIAS Introduction Day (WIAS)	2014
Course on philosophy of science and/or ethics (WIAS)	2014
Disciplinary competences (16 ECTS)	
Varkensvoeding in de praktijk (Wageningen Academy)	2014
Writing research proposal	2015
Mathematical modelling of biological systems (Virginia Tech, online)	2015
Advances in feed evaluation science (Wageningen acedemy)	2016
Reaction kinetics in food science (VLAG)	2016
Professional competences (5 ECTS)	
Project and time management (Valley Consult)	2017
Scientific writing (Wageningen In'to language)	2017
Career assessment (Meijer en Meijaard adviesbureau)	2017
Presenting with impact (Wageningen In'to language)	2017
Critical thinking and argumentation (WGS)	2018
Presentation skills (4 ECTS)	
Animal Nutrition Research Forum, Belgium (oral)	2017
Advances in Feed Evaluation Science course, The Netherlands (oral)	2017
WIAS Science Day, The Netherlands (oral, 2x)	2018
Digestive Physiology of Pigs, Australia (poster, 2x)	2018
Teaching competences (6 ECTS)	
Supervising MSc students (4x)	2015-2019
Supervising course practicals	
Introduction to Animal Sciences	2016,2017
Principles of Animal Nutrition	2015,2017
Animal Nutrition and Physiology	2015,2017
Nutrient Dynamics	2017

Total 32 ECTS ([1] One ECTS credit equals a study load of 28 hours)

Colophon

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